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1.0 Introduction

This *Risk Assessment (RA) Report for the National Emerging Infectious Diseases Laboratories (NEIDL at the Boston University Medical Center (BUMC))* presents the human health consequences of a potential accidental event or malevolent action resulting in the loss of pathogen or biological containment (biocontainment) at the BUMC NEIDL biological research facility. The purpose of the NEIDL is to provide safe and secure laboratories dedicated to the study of disease-causing microorganisms (pathogens) to research the pathogenesis of emerging infectious diseases (including Centers for Disease Control and Prevention (CDC)/National Institutes of Health (NIH) Category A, B, and C pathogens); develop vaccines, therapeutics, and diagnostics for the pathogens; develop animal models for the comparative study of the pathogens; perform preclinical and clinical research in humans; train scientists and related support personnel in the requirements of the area of research; and support a national response if a biodefense emergency occurs. Tetra Tech, Inc., under a contract to prepare this RA for NIH. The NEIDL (Figure 1-1) is within the BioSquare Research Park, adjacent to the BU Medical Center (BUMC) campus in Boston, Massachusetts (Figure 1-2). Construction of the facility began on March 6, 2006, and was completed in the fourth quarter of 2011. The facility is partially occupied and used for administrative and training purposes.

Pathogen

Pathogens are disease-causing microorganisms or biologically derived infectious or toxic materials that present a potential health risk to humans, animals, or plants. Throughout this RA the term *pathogen* is used as a comprehensive descriptor for the purposes of discussion. Where a more specific or limiting definition is required, more precise terms are used (e.g., select agent). Pathogens include the following:

- Bacteria
- Fungi
- Parasites
- Viruses
- Toxins (bacterial, fungal, plant)
- Other infectious pathogens, such as prions (a disease-causing pathogen that is neither bacterial, fungal, or viral containing no genetic material)

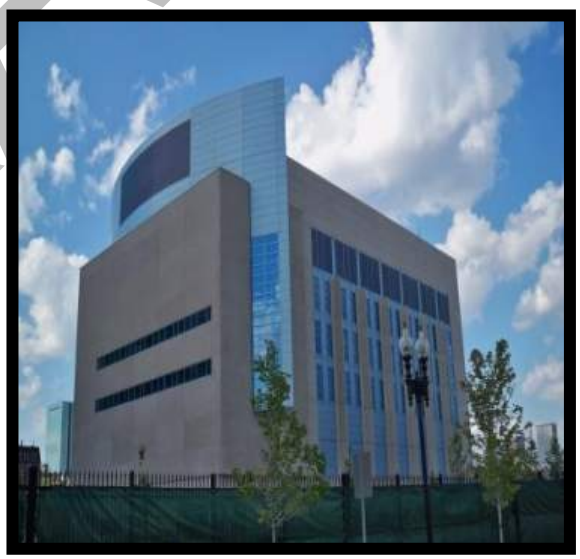

Figure 1-1. NEIDL facility 2008.

Several residents and public interest groups filed federal and state lawsuits challenging the adequacy of earlier reviews of the potential environmental effects of the NEIDL and whether those potential risks would vary depending on the location of the facility in an urban or less densely populated area. In addressing the issues raised in those lawsuits and public comments provided during those previous reviews, the NIH sought the advice of a Blue Ribbon Panel (BRP) of experts in infectious diseases, risk assessment, environmental justice, modeling, biosafety, risk communications and other areas. The NIH also sought guidance from the National Research Council (NRC) committee that was critical of the draft of an earlier risk assessment prepared by the NIH.

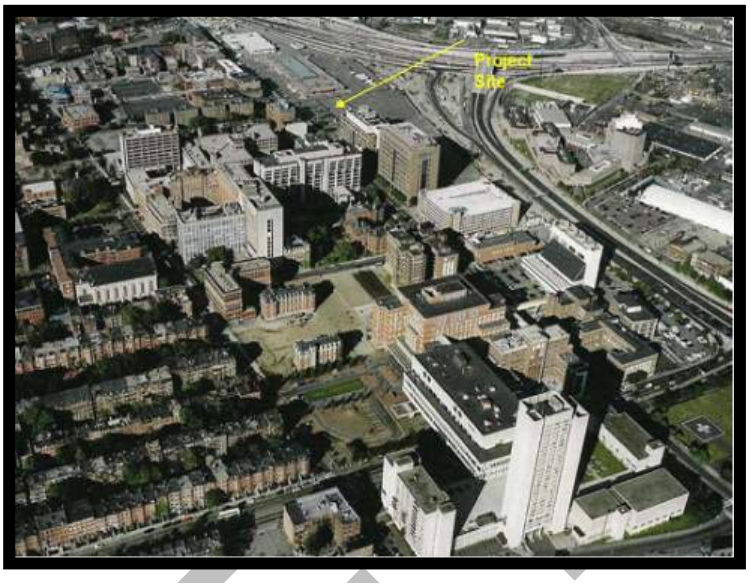

Figure 1-2. Location of NEIDL in Boston

Because of public concerns and state and federal court direction, this RA compares the frequency and public health consequences associated with potential loss of pathogen biocontainment events in a range of population density areas that represent urban, suburban, and rural environments. The urban, suburban, and rural sites were selected for the purposes of the comparative analysis, including the BUMC BioSquare Research Park, Boston, where the NEIDL has been constructed; the former BU Corporate Education Center in Tyngsborough, Massachusetts; and the former BU Sargent Center for Outdoor Education near Peterborough, New Hampshire (Figure 1-3) (NIH and DHHS 2005).

Figure 1-3. Locations of the three NEIDL sites for suitability analysis.

Risk Assessment Chapter Organization

The RA chapters, 1 through 13, are organized to build on the preceding chapter to provide the reader with a basic understanding of the need for the NEIDL; the facility design, equipment, and operations; the environment surrounding the three sites under study; and the RA process and findings. Each chapter's corresponding appendix, as needed, provides detailed and fundamental information, methodology, and analysis. In combination, the RA chapters and their corresponding appendices are designed to provide the courts, scientists, the public and adjacent communities/neighborhoods and interest groups with the information necessary to assess and understand the RA methodology and analysis that is responsive to public concerns and state and federal court direction. Section 1.6 of this chapter provides the organization and brief descriptions of the RA chapters and appendices.

1.1 National and Regional Biosafety Laboratories

The federal government responded to the September 11, 2001, terrorist attacks and the subsequent anthrax bioterrorist attacks with increased focus on and funding for biodefense. In February 2002, NIH convened a BRP on Bioterrorism and Its Implications for Biomedical Research, made up of distinguished scientists representing academia, private industry, and governments to provide guidance to the National Institute of Allergy & Infectious Diseases (NIAID) on its biodefense research agenda. That panel concluded that the insufficient amount of biological safety (biosafety) level (BSL)-3 and BSL-4 laboratory space was a significant barrier to progress in protecting the United States from further bioterrorist attacks (NIAID 2003). BSL designations are differentiated by the degree of protection provided to the integrity of the research, to research personnel, to the community, and the environment when working with infectious microorganisms or biological toxins. BSL-1 requires the most basic level of protection, while BSL-4 requires the most stringent protection (see Section 1.4). The problem of insufficient BSL-3 and BSL-4 laboratory space has been previously documented by the Institute of Medicine of the National Academy of Sciences (NAS) and repeatedly identified in NIAID's strategic planning process (NIAID 2003a). In response, on September 30, 2003, NIAID announced funding for the construction of two National Biocontainment Laboratories (NBLs) and 13 Regional Biocontainment Laboratories (RBLs) to conduct research on biological pathogens that are considered to be of significant research importance. The overall objective of the NBL construction program is to provide funding to design, construct, and commission comprehensive, state-of-the-art BSL-2, BSL-3, and BSL-4 laboratories, as well as associated research and administrative support space. The RBL construction program provided funding for similar facilities containing BSL-2 and BSL-3 laboratories. The NBL and RBL proposals were selected on the basis of multiple factors but primarily on the scientific and technical merit of the applications received from state, university, and private research organizations as assessed by peer review and on the applicant's ability to contribute to the overall NIAID biodefense research mission (Figure 1-4). The biosafety laboratories are also to be available for and prepared to assist national, state, and local public health efforts if a bioterrorism or infectious disease emergency occurs (NIAID 2003).

Regional Biocontainment Labs (BSL-3)

Alabama
University of Alabama at Birmingham School of Medicine, Southeast Biosafety Laboratory Alabama (SEBLAB)

Colorado
Colorado State University (Fort Collins) Regional Biocontainment Laboratory

Hawaii*
University of Hawaii John A. Burns School of Medicine, Pacific Regional Biocontainment Laboratory, Honolulu, Hawaii.

Illinois
University of Chicago The Ricketts Laboratory

Kentucky
University of Louisville The Center for Predictive Medicine

Louisiana
Tulane National Primate Research Center (Covington, Louisiana) Regional Biocontainment Laboratory

Massachusetts
Tufts University, Cummings School of Veterinary Medicine (Grafton, Massachusetts) Regional Biosafety Laboratory-New England

Missouri
University of Missouri-Columbia College of Veterinary Medicine

New Jersey
University of Medicine and Dentistry of New Jersey (Newark), New Jersey Medical School Center for Infectious Disease Research

North Carolina
Duke University Medical Center (Durham), Global Health Research Building (GHRB)

Pennsylvania
University of Pittsburgh The Regional Biocontainment Laboratory at the Bioscience Tower III (BST3)

Tennessee
University of Tennessee Health Science Center (Memphis) University of Tennessee Health Science Center Regional Biocontainment Laboratory

Virginia
George Mason University, George Mason University Biomedical Research Laboratory

National Biocontainment Labs (BSL-4)

Massachusetts
Boston University Medical Center National Emerging Infectious Diseases Laboratories

Texas
University of Texas Medical Branch at Galveston, Galveston National Laboratory

Figure 1-4. National and regional biocontainment laboratories.

Sources: NIAID 2009; University of Louisville 2010
*The Hawaii facility has not begun construction or finalized siting.

BU was selected as an NBL grant recipient in a nationwide competition. The NEIDL is one of only two NBLs in the United States for which the NIAID, an institute within the NIH, awarded partial construction funding in 2003. The other NBL, Galveston National Laboratory (GNL) is at The University of Texas Medical Branch (UTMB) in Galveston, Texas, and is operational. NBLs are designed and constructed to provide comprehensive, state-of-the-art biocontainment laboratories, research support space, and animal facilities to protect public health through developing and evaluating improved diagnostics, therapeutics, and vaccines for protecting against emerging and reemerging diseases, including those that have the potential for bioterrorism.

NBL and RBL activities, operations, and research will be performed solely for scientific research and biodefense purposes (i.e., developing effective vaccines and other countermeasures such as antiviral therapies). No research will occur for developing bioweapons, which is prohibited by international law. The Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, signed in 1972, prohibits the development, production, and stockpiling of biological and toxin weapons (BTWC 2010).

1.2 Federal Agency Roles

A key consideration in the federal government is an increased need for infectious diseases research, as well as for biodefense to address capacity shortages for diagnostic, clinical, and research laboratories.

1.2.1 U.S. Department of Health and Human Services

The U.S. Department of Health and Human Services (DHHS) is the principal agency for protecting the health of all Americans and providing essential human services. DHHS programs are administered by 11 operating divisions, which include the NIH.

1.2.2 National Institutes of Health

The NIH, a part of DHHS, is the primary federal agency for conducting and supporting biomedical research. The NIH is composed of 27 institutes and centers and is the steward of medical and behavioral research for the nation. The NIH annually invests more than $31.2 billion in medical research to more than 325,000 researchers at more than 3,000 universities, medical schools, and other research institutions in every state and around the world (NIH 2011). Its mission is science in pursuit of fundamental knowledge about the nature and behavior of living systems

and applying that knowledge to extend healthy life and reduce the burdens of illness and disability. The goals of the agency are as follows (NIH 2011a):

1. Foster fundamental creative discoveries, innovative research strategies, and their applications as a basis to advance significantly the nation's capacity to protect and improve health;
2. Develop, maintain, and renew scientific human and physical resources that will ensure the nation's capability to prevent disease;
3. Expand the knowledge base in medical and associated sciences to enhance the nation's economic well-being and ensure a continued high return on the public investment in research; and
4. Exemplify and promote the highest level of scientific integrity, public accountability, and social responsibility in the conduct of science.

1.2.3 The National Institute of Allergy and Infectious Diseases

The NIAID (http://www.niaid.nih.gov/) under the NIH conducts and supports basic and applied research to better understand, treat, and ultimately prevent infectious, immunologic, and allergic diseases. To meet the nation's biodefense needs, NIAID, in consultation with other experts in the field, developed a strategic plan for emerging infectious diseases and biodefense research. Key elements of the plan include the following (NIAID 2008):

- Support of medical research on microbes and the human immune response to them;
- Apply such research to the discovery and development of vaccines, drugs, and diagnostic tests designed to protect the general population; and
- Ensure that the United States has enough research facilities to carry out those activities.

NIAID's ultimate goal is to develop new and improved diagnostics, vaccines, and treatments for diseases caused by infectious pathogens. Medical tools such as those can be developed only with a solid understanding of the biology of the disease-causing pathogens and working with the actual microbes or their toxins. Achieving NIAID's research goals requires constructing biocontainment laboratories with facilities and procedures for handling potentially lethal infectious pathogens, including pathogens that have the potential to be used in bioterrorism. Such research must be conducted in special biosafety laboratories and in accordance with the laws, regulations, policies, and well-established guidelines that govern research on those microbes and the design, management, and operation of the laboratories. All the provisions aim to protect not only the laboratory workers, but also the surrounding community from accidental exposure to pathogens (NIAID 2003a).

1.3 NEIDL Owner and Operator—Boston University

BUMC, a consortium of BU and Boston Medical Center, submitted an application to NIAID in response to the NBL Broad Agency Announcement in February of 2003 and, on the basis of the merits of that application, received a grant of $141 million—$128 million in 2003 and $13 million in 2006—to construct the NEIDL. The entity holding legal title to the site is the Biosquare Realty Trust, the sole beneficiaries of which are the Trustees of Boston University, a Massachusetts nonprofit, educational corporation, and Boston Medical Center Corporation, a Massachusetts nonprofit corporation.

BUMC is in the South End of Boston and consists of the BUMC School of Medicine, the BU School of Public Health, the Goldman School of Dental Medicine, and Boston Medical Center.

BU's NEIDL bioresearch operations would be governed by all applicable federal, state, and local laws regulations and guidance. Representative regulatory oversight consists of the following agencies:

- Centers for Disease Control and Prevention
- U.S. Department of Transportation
- Occupational Safety and Health Administration
- U.S. Environmental Protection Agency
- National Institutes of Health
- Nuclear Regulatory Commission
- U.S. Department of Agriculture
- Massachusetts Department of Public Health
- Massachusetts Department of Environmental Protection
- Massachusetts Water Resources Authority
- Boston Public Health Commission
- Boston Fire Department
- Boston Water and Sewer Commission
- Boston Inspectional Services

NEIDL operations would include the use of select agents, which are biohazardous materials that could pose a severe threat to public health and safety; animal or plant health; or animal or plant products. The Bioterrorism Act of 2002 [U.S. Food and Drug Administration (USFDA) P.L 107-188] requires entities to register with the DHHS or the U.S. Department of Agriculture (USDA) if they possess, use, or transfer select agents or toxins. The DHHS secretary has delegated the responsibility for promulgating and implementing select agent or toxin regulations to the CDC. In addition to ensuring that laboratories safely handle such select pathogens or toxins, the Bioterrorism Act of 2002 requires increased safeguards and

security measures for such select agents, including controlling access, screening entities and personnel (i.e., performing a threat assessment [TA]). Before operating, the NEIDL would be registered with the CDC, and the CDC would register those NEIDL personnel who would work with select agents or toxins. Registration with either the CDC or the USDA allows a laboratory to use select agents, organisms or toxins, regulated by either agency according to the level of facility certification. Additionally, NEIDL will obtain a permit from the Boston Public Health Commission prior to beginning BSL-3 and BSL-4 operations.

Biohazardous materials, such as microorganisms and biotoxins, that are not select agents or toxins will be used in the NEIDL laboratories and handled according to CDC and NIH guidance and requirements. The CDC and NIH guidance and requirements also extend to handling genetically altered microorganisms and recombinant DNA (NIH 2011b).

1.4 Biosafety-Related Information and Practices

Biosafety describes practices and policies that serve to protect the integrity of the research, laboratory workers, facility workers, the public, and the environment from exposure to infectious microorganisms and hazardous biological materials. Protection is provided by using four primary controls: engineering; workplace practices; personnel protective equipment (PPE); and administrative (e.g., security clearances, training and supervision, immunizations). Biocontainment laboratories provide for the handling and storage of infectious microorganisms and hazardous biological materials. The NEIDL facility was designed to protect the laboratory workers, staff, the community, and the environment from harmful infectious agents through (1) primary containment including the use of appropriate safety equipment (e.g., biological safety cabinets [BSCs], centrifuges with sealed rotors), administrative controls, and the use of PPE (e.g., respirators, fully encapsulating suits) and (2) secondary containment systems including unidirectional air flow and high-efficiency particulate air (HEPA)-filtered exhaust systems in the facility design.

1.4.1 Biosafety Levels

Four BSLs of operation define the biocontainment conditions under which infectious pathogens can be safely manipulated on the basis of risk to the laboratory worker. BSL-1, BSL-2, and high-biocontainment laboratories, which refer to BSL-3 and BSL-4 laboratories in the United States, which are designed, constructed, and operated in accordance with guidelines from the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) manual, 5th edition (CDC and NIH 2007).

Similarly, four BSLs of operation exist for using experimentally infected animals housed in indoor research facilities or maintaining laboratory animals that could naturally harbor zoonotic infectious pathogens (i.e., a disease that can be transmitted from animals to people). Those levels are organized as Animal Biosafety Levels (ABSL) 1 through 4, which correspond with the BSL pathogen classification system (CDC and NIH 2007).

When arthropods (e.g., insects, spiders, fleas, ticks) are used, facilities, trained staff, and established practices must be in place to ensure appropriate safety and the protection of the health and well-being of workers and the environment. Where an arthropod is infected with a biological pathogen, the Arthropod Containment Level (ACL) will be consistent with or exceed the BMBL BSL containment recommendations. Consistent with BSL and ABSL, four levels of ACLs exist (CDC and NIH 2007).

While the BMBL is a set of guidelines, host facilities have also used it to establish requirements for visiting principal investigators for working with biological pathogens requiring higher biocontainment laboratories.

The BSLs are designated by the degree of protection provided to personnel, the environment, and the community. The principal hazardous characteristics of a biological pathogen used to determine the appropriate BSL are the following (CDC and NIH 2007):

- Its capability to infect and cause disease in a susceptible human or animal host;
- Its virulence as measured by the severity of disease;
- The availability of preventive measures and effective treatments for the disease;
- The origin of the biohazardous material—whether indigenous or exotic—for biological pathogens that cause moderate to severe disease; and
- The nature of the work being conducted.

In addition, the mode of transmission, specifically aerosol transmission which is a common mode of transmission, is part of the hazard characteristics of biologic pathogens that are used to determine BSLs. Both the BSL-3 and BSL-4 definitions indicate that aerosol transmission is possible. Each level of biocontainment describes the administrative controls, safety equipment, and facility features for the corresponding level of health risk associated with handling a biohazardous material. For the purposes of this RA, the BSL is referred to as inclusive of those additionally compartmentalized areas such as ABSL, ACL, or other protection categories. As explained in the following bulleted points, BSL-1 requires the

most basic level of protections (e.g., administrative controls, safety equipment, facility features) and BSL-4 requires the most stringent protections (CDC and NIH 2007):

Figure 1-5. Class III BSC.

- BSL-1: Practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans such as *Bacillus subtilis*, *Naegleria gruberi*, and infectious canine hepatitis virus. BSL-1 represents a basic level of biocontainment that relies on standard microbiological practices, a sink for hand washing, with no special primary or secondary barriers recommended.
- BSL-2: Practices, equipment, and facility design and construction are appropriate to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous (i.e., occurring naturally in a region) moderate-risk pathogens that are present in the community and associated with human diseases of varying severity. Hepatitis B virus, the salmonellae, and *Toxoplasma* spp. are representative of pathogens that can be assigned to this biocontainment level. Primary hazards to personnel working within an area requiring BSL-2 controls are accidental exposure via penetrating wounds, through broken skin or via mucous membranes (i.e., nasal passages), or ingestion of pathogens.
- BSL-3: Practices, safety equipment, and facility design and construction are appropriate to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic (i.e., not naturally occurring in a region) pathogens that can cause serious or potentially lethal disease through inhalation exposure. *Bacillus anthracis* (anthrax), *Francisella tularensis*, (tularemia) and *Yersina pestis* (pneumonic plague) are examples of pathogens that can be assigned to this level. Primary hazards to personnel working with such pathogens are needle sticks or cuts resulting from using sharps, ingestion, and exposure to infectious aerosols.
- BSL-4: Practices, safety equipment, and facility design and construction are appropriate for work with dangerous and exotic pathogens that pose a high individual risk of life-threatening disease, which can be transmitted via the aerosol route and for which there is no available vaccine or therapy. Viruses, such as Ebola and Marburg, and those of the tick-borne encephalitis complex

are manipulated at BSL-4. The primary hazards to personnel working with BSL-4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All work with potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment. The laboratory worker's complete isolation from aerosolized pathogens is accomplished primarily by working in a Class III BSC (see Figure 1-5) and in a full-body, one-piece, positive-pressure suit with externally supplied, HEPA-filtered air (see Figure 1-6). In general, BSL-4 facilities are completely isolated zones with complex, specialized, ventilation requirements and waste management systems to prevent release of viable biohazardous materials to the environment (CDC and NIH 2007, NIH 2008).

The ABSL laboratories can present unique problems and hazards not found in standard microbiological laboratories. Animals can generate aerosols, bite and scratch, and can be infected with a zoonotic pathogen. In general, ABSL laboratories have special engineering and design features, and personnel are specifically trained in animal facility procedures and the handling of infected animals.

The ACLs feature specific practices, procedures, biocontainment equipment, and facility requirements to prevent the escape of infected or uninfected arthropods. ACLs are designed in accordance with the American Committee of Medical Entomology (ACME) of the American Society for Tropical Medicine and Hygiene (ASTMH) recommendations for arthropod laboratory work (Mary Ann Liebert, Inc. 2003).

Figure 1-6. HEPA filtration plenum.

High- and maximum-biocontainment facilities (BSL-3 and BSL-4) are located throughout the nation. The U.S. Government Accountability Office (GAO) conducted a survey in 2006/2007 of U.S. academic, biotechnology, and pharmaceutical facilities to better define the location, capacity, and status of existing and operating U.S. laboratory facilities that incorporate BSL-3 biocontainment precautions. Survey packets were distributed to 2,170 distinct entities with a survey return rate of around 40 percent. Federal government laboratories were not included in the survey. The survey results identified 1,356 CDC or

USDA-registered laboratories in 46 states that had BSL-3 capable laboratories (GAO 2007). That survey has been augmented and updated with additional data in 2008 showing 1,362 BSL-3 laboratories registered with CDC's Division of Select Agents and Toxins. As of March 2009, all 50 states have at least one BSL-3 laboratory).

1.4.2 Biocontainment

The term biocontainment is used to describe safe methods for managing pathogens in the laboratory environment where they are being handled or maintained. The three elements of biocontainment consist of the microbiological practices, biocontainment and safety equipment, and facility design and safeguards that protect laboratory workers, other facility personnel, the public, and the environment from exposure to pathogens that are handled and stored in a biocontainment laboratory.

Primary biocontainment involves protecting personnel and the immediate laboratory environment from exposure to pathogens and is provided by both good microbiological techniques and using appropriate biocontainment equipment. Biocontainment equipment consists of BSCs, enclosed containers, and other engineering controls designed to prevent or minimize exposures to pathogens. The BSC is the principal device used to provide biocontainment of infectious splashes or aerosols generated by many microbiological procedures. An example of another primary barrier is the aerosol containment centrifuge bucket/rotor and cover, an enclosure container designed to prevent aerosols from being released during centrifugation (see Figure 1-7). To minimize the hazard from potentially infectious aerosols, biocontainment controls such as BSCs or aerosol containment centrifuge buckets/rotors must be used when handling pathogens that can be transmitted through the aerosol route of exposure. Safety equipment also can include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Such PPE is often used in combination with BSCs and other devices that contain the pathogen, animals, or materials being handled. In the case of BSL-4 biocontainment, as described above, PPE would consist of a full-body, one-piece, positive-pressure suit with externally supplied, HEPA-filtered air. In some situations in which it is impractical to use a BSC, PPE can form the primary barrier between personnel and the pathogens.

Figure 1-7. Researcher in BSL-4 PPE placing a rotor in a centrifuge.

Secondary biocontainment, protecting the environment external to the laboratory from exposure to pathogens, is provided by a combination of facility design and operational practices. The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects the public and environment from pathogens that could be accidentally released from the laboratory. The recommended secondary barrier(s) will depend on the risk associated with specific pathogens. For example, the exposure risks for most laboratory work in BSL-2 facilities would be potential direct contact with the pathogens or inadvertent contact exposures through contaminated work environments. Secondary barriers in such laboratories can include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., steam sterilizer), and hand washing facilities. When the risk of infection by exposure to an aerosol is present, higher levels of primary biocontainment and multiple secondary barriers might become necessary to prevent infectious pathogens from escaping into the environment. Such design features consist of specialized ventilation systems to ensure directional air flow, air treatment systems to remove pathogens from exhaust air, controlled access zones, airlocks as laboratory entrances (Figure 1-8), or separate buildings or modules to isolate the laboratory.

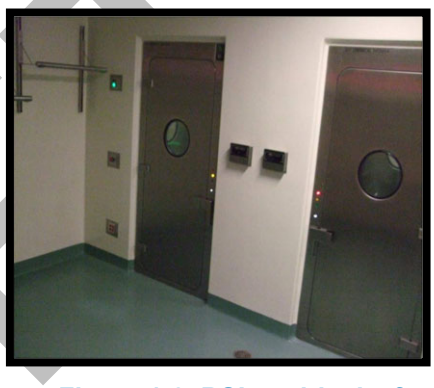

Figure 1-8. BSL-4 airlocks for laboratories.

1.5 Risk Assessment

The risk associated with the probability of becoming infected if exposure to an infectious agent has occurred is defined by the U.S. Environmental Protection Agency (EPA) as the combination of frequency and consequences. An RA is, therefore, a scientific and technical analysis that assembles and synthesizes information to determine the frequency and the extent of possible risk to human health and safety or the environment. For this RA, risk is a result of a potential sequence of unplanned events or malevolent actions that could result in exposure to a laboratory worker, facility worker, a member of the public, or an environment. An event could be caused by equipment malfunction, external events, human error, natural phenomena, or malevolent acts. This section presents the RA purpose and provides an overview of the RA methodology.

1.5.1 Purpose of the Risk Assessment

The purpose of this RA is to address and respond to the human health issues raised in the public and judicial review process and to respond to findings from the BRP and the NRC of the NAS . In a human health RA, the analyses determine what, if any, adverse human health effects would occur from an accidental or malevolent release of a pathogen or infected insects/animals from biocontainment. It also determines whether there are differences in the effects if the facility were in an area with a lower population density than the Boston NEIDL site.

That purpose is accomplished by identifying the characteristics of a known pathogen, the events that can result in an individual's exposure to a pathogen, the likelihood that such exposure will cause an infection, the potential for an infected person to transmit the pathogen to contacts, and the probable health consequences in terms of infections and fatalities attributable to the pathogen. A laboratory-acquired infection (LAI) is a result of laboratory-related activities with a pathogen. Generally an LAI results from contact with an infectious pathogen via inhalation (i.e., aerosol), ingestion, direct contact (i.e., skin or mucous membranes), or puncture wound from a sharp object (e.g., needle, scalpel).

An infection can also occur as a result of contact with an infectious pathogen outside the laboratory setting. For example, an infection could result from exposure to pathogens while they are being transported to/from the NEIDL. In addition, an infection could be acquired from contact with an infected and infectious individual. A chain of such secondary transmissions stemming from NEIDL-related events could occur, resulting in the spread of a pathogen through the community.

1.5.2 Scope of the Risk Assessment

The scope of this RA includes qualitative and quantitative analyses of an array of pathogens and events leading to exposure of individuals to pathogens and probabilistic estimates of initial infections, subsequent secondary transmissions, and fatalities. An analysis of potential differences among the three NEIDL sites in terms of infections and fatalities is also included. This RA follows guidelines established by federal agencies for conducting and reporting risk assessments (EPA 2000) and has been performed by using available scientific data and established methods of analyses. The RA acknowledges the uncertainty associated with the data and the appropriate role of judgment (expert opinion) in estimating key parameters required for risk assessment.

The four essential principles of risk assessment are applied as follows to this RA and are described in the preceding chapters and associated appendices:

1. Transparency: This is achieved by providing details of the assessment approach applied at each step of the analyses; stating the assumptions used for the analyses and the basis for those assumptions; addressing data gaps and the methods used to overcome the data gaps such as expert judgment; the uncertainties in the available data, qualitative discussions and quantitative assessments of the impact of the uncertainties in the data and sensitivity analyses to determine impact of variability in key parameters

2. Clarity: This is achieved by attempting to convey details with brevity; providing lay language summaries and discussions in chapters; providing details in appendices and using tables and graphs where possible to present technical data

3. Consistency: This is achieved by following established guidance and guidelines, following precedence wherever possible and using established and published methods for all analyses

4. Reasonableness: the RA is based on best available scientific information, uses generally accepted scientific knowledge and has been subjected to peer review by the BRP and NRC. Furthermore this RA strives to include reasonableness and realism in the analyses based on 'real world experience'; however, the absence of appropriate operational data poses a significant challenge in that regard and in several cases the event sequence assumptions are expected to overestimate the likelihood or consequences of potential events. This use of conservative assumptions (i.e., overestimations) to account for uncertainty is consistent with National Environmental Policy Act (NEPA) accident analysis guidance (DOE 2002).

The following paragraphs describe the scope of each aspect of the RA.

Pathogens—The BRP considered and selected a total of 13 pathogens for detailed analyses. Those 13 pathogens were chosen on the basis of a review of the federal and state court decisions, the BRP, NRC committee, community concerns, and public documents. These pathogens differ in key characteristics such as their ability to be spread from person to person (transmissibility), the method by which they are spread from one person to the next (either directly or via vectors, such as insects), their ability to cause human disease (pathogenicity) and their ability to cause deaths among those infected (case fatality rate). All the pathogens are qualitatively modeled, and a subset of 5 of the 13 pathogens were chosen for detailed secondary transmission modeling because of representativeness and the availability of published mathematical models and adequate epidemiological data. These pathogens are classified as requiring biological safety level 3 (BSL-3) or BSL-4 biocontainment precautions and are analyzed separately for this RA (Table 11-1).

Table 11-1 Pathogens Selected for Analysis.

Pathogen	Abbreviation
BSL-3	
1. *Bacillus anthracis* (either BSL-2 or BSL-3)	*B. anthracis*
2. *Francisella tularensis*	*F. tularensis*
3. Yersinia pestis	*Y. pestis*
4. 1918 H1N1 influenza virus	1918 H1N1V
5. SARS-associated coronavirus	SARS-CoV
6. Rift Valley fever virus	RVFV
7. Andes virus (either BSL-3 or BSL-4[a])	ANDV
BSL-4	
8. Ebola virus	EBOV
9. Marburg virus	MARV
10. Lassa virus	LASV
11. Junín virus	JUNV
12. Tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus)	TBEV-FE
13. Nipah virus	NIPV

[a] BSL-4 is required when infecting rodent species permissive for (susceptible to) chronic infection.

Event types—This assessment considers internally and externally initiated events. Scenarios were developed that account for NEIDL-specific operations based on NEIDL equipment and standard operating procedures (SOP) such as; equipment malfunctions and worker errors. Externally initiated events such as a loss of off-site power, transportation event, natural phenomena events such as an earthquake, and malevolent acts such as insider and terrorist actions were additionally considered.

Exposed groups—This RA considers potential effects on laboratory workers in the immediate area or laboratory space at the time of the event, facility workers who are elsewhere in the facility, and members of the public, which includes anyone outside the facility and in the surrounding communities. It must be noted that risk to lab workers is independent of site locations as the NEIDL structure, as designed and constructed at the BioSquare Research Park (urban site), is assumed to be the identical structure that would have been constructed at the Tyngsborough, Massachusetts (suburban site)[formerly Boston University Corporate Education Center], or the Boston University Sargent Center for Outdoor Educations at Peterborough, New Hampshire (rural site).

Route and extent of initial exposure—Direct exposure via direct contact is considered, which includes exposure to eyes, mucous membranes, and preexisting breaks in the skin (e.g., accidental needle sticks); ingestion; inhalation; and exposure via arthropods (i.e., insects and arachnids) and mammals (e.g., non-human primates, rodents). The extent of exposure is estimated for both workers and the public.

Secondary transmission—Secondary transmission is considered, which includes the transmission of a pathogen from an initially infected individual to another individual and any subsequent generations of transmission. The RA considers direct, person-to-person transmission and indirect, vector-borne transmission, as well as the possibility of establishing an animal or environmental reservoir of pathogens from which further human exposures could occur.

Infection and fatalities—This RA estimates the number of infections and fatalities that result from the initial exposures and secondary transmissions.

Locations—The NEIDL structure, as designed and constructed at the BioSquare Research Park (urban site), is assumed to be the identical structure that would have been constructed at the Tyngsborough, Massachusetts (suburban site)[formerly Boston University Corporate Education Center], or the Boston University Sargent Center for Outdoor Educations at Peterborough, New Hampshire (rural site), site if either of those locations had been selected. Therefore, the facility description and proposed operations are applicable for all three sites. In addition, the team considered medically vulnerable as well as minority and low-income populations at each location to determine if they would be disproportionately and adversely affected by an accidental event. That approach was necessary to provide a meaningful basis to compare the risks from one site to another.

1.5.3 Risk Assessment Approach

An RA includes the processes, methods, and techniques used to estimate both the frequency (i.e., rate of occurrence within a given period) and consequences of specified adverse events. The RA was initiated by assembling an interdisciplinary team composed of personnel with expertise in medical and veterinary microbiology, medical and veterinary virology, engineering, epidemiology, medicine, biodefense, mathematical modeling, risk analysis, risk modeling, security, and other fields.

The interdisciplinary team then used the following questions to guide the risk analysis:

1. What could go wrong? That is, what might be the sequence of events that could cause an infectious pathogen to escape the laboratory, set up a chain of transmission, and cause infectious disease in the surrounding community?
2. What are the probabilities of such a sequence of events?
3. What would be the consequences of such a sequence of events?

The scope of the guidance was expanded to include consideration of off-site transportation. This RA approach consists of two major analyses that, when combined, provide answers to those questions. The event sequence analysis addresses the first question and part of the second question above. The health effects analysis addresses part of the second question and the third question. Figure 1-9 provides a process overview of the entire RA process. The event sequence analysis consists of the first three steps of the process shown in Figure 1-9. The health effects analysis consists of steps 4 through 6 of the process shown in the figure. The final step is to characterize the risk, which includes a summary of the results of the analyses plus the synthesis of key findings regarding the risk to exposed groups, potential differences between sites, and the potential for disproportionate effects on vulnerable subpopulations. The following subsections describe the event sequence analysis and the health effects analysis, respectively.

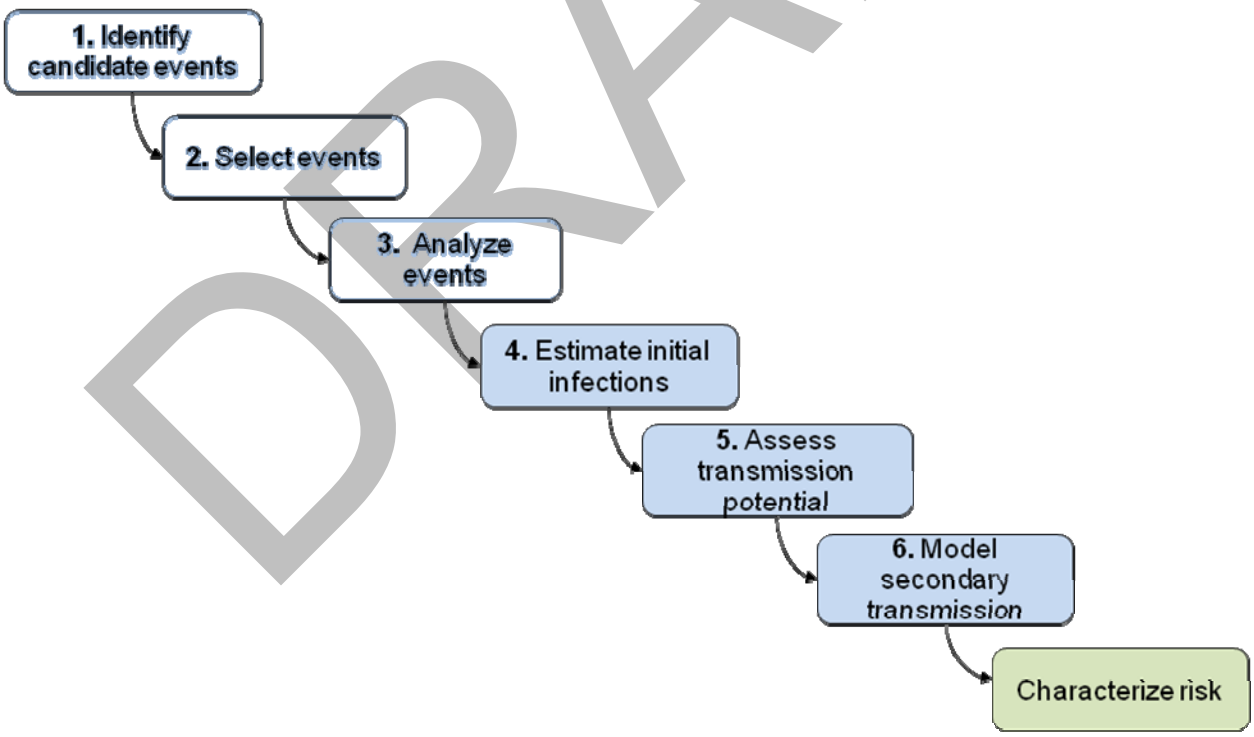

Figure 1-9. Hazard identification process overview.

1.5.3.1 Event Sequence Analysis

The event sequence analysis included a comprehensive identification of candidate events, selecting events for analysis, and detailed analyses of the events selected. Details of the event sequence analysis are presented in Chapter 4. The results of the event sequence analysis are inputs to the human health analysis.

Step 1 – Identify candidate events—This step of the RA process involves the comprehensive identification of candidate events. Candidate events were identified in consideration of known biosafety experience data, BRP guidance, NRC technical input, public comments, NEIDL design and operating plans, site characteristics, the adversary types present in the three locations, previous NEIDL studies, and analyses of similar facilities. The product of this step of the process is the list of candidate events.

Step 2 – Select event—Each of the candidate events identified in the previous step was categorized on the basis of the location, the groups exposed, and the initial route of exposure, as identified in Section 1.5.2. From that set of categorized candidate events, a subset of events was selected for detailed analysis. Events were selected to include high-consequence, low-frequency events as well as a variety of more plausible events. The team considered each of the 13 pathogens for each event to select relevant event-pathogen pairs for analysis. Events were selected to address each route of exposure at each location, for each exposed group. The team reviewed the list of candidate events to ensure that events are included that address potential pathogen and site differences.

Step 3 – Analyze events—The analysis of selected events began by defining the event sequence, which includes the initiating event as well as the failure of preventive and mitigation features, as appropriate. The frequency estimate of the event sequence was then developed on the basis of incident data from similar facilities as interpreted by professional judgment. The number of people potentially exposed by the loss of biocontainment and the route of exposure was identified. An analysis of the release and potential airborne transport provided a pathogen-specific extent of exposure. Those products are inputs for the health effects analysis.

1.5.3.2 Health Effects Analysis

The health effects analysis used exposure information from the event sequence analysis to estimate the number of initial infections and the health consequences, including fatalities, among those initially exposed individuals. It also considered the potential for secondary transmission for each of the 13 pathogens, including a quantitative analysis of potential for spreading within a community for a subset of

pathogens. An estimate of the numbers of infections and fatalities were also considered for secondary transmissions.

Step 4 – Estimate initial infections—This step of the RA addresses factors that influence the probability that an individual will develop an infection after exposure to a pathogen. The pathogen-specific characteristics such as human infectious dose or human experimental infectious dose (HID_{50}) are key concepts and challenging to obtain from the scientific literature. The HID_{50} is the minimum number of infectious particles required to establish infection in 50 percent of exposed, fully susceptible humans. Infectious doses vary between pathogens, hosts, and routes of exposure. Estimates of HID_{50} are available from the literature for only 4 of the 13 pathogens. This RA used data in the literature and an expert consultation approach, which is discussed in greater detail in later chapters of the RA, to estimate HID_{50} for all pathogens and to estimate initial infections after exposure to a pathogen. The team performed a comprehensive literature search to supplement the BRP guidance for initial infection assumptions. NIH convened an expert panel to develop (1) estimates of HID_{10}, HID_{50}, and HID_{90} for all 13 pathogens; (2) concurrence with reproductive numbers derived from the literature for secondary transmission modeling for selected pathogens; (3) estimates of increased vulnerability to infection for specific population subgroups; and (4) estimates of atmospheric decay of pathogens after release into the environment. The team then used those consensus dose-effect relationships to estimate initial infections after exposure to a pathogen.

Step 5 – Assess transmission potential—This step includes a summary of those event sequences that could set up the possibility for secondary transmission, including undetected or unreported laboratory events resulting in an infected NEIDL worker leaving the facility, events resulting in direct exposure and infection of members of the public who subsequently interact with contacts, and events leading to the escape of infected animals or arthropods with potential to transmit to humans. This step also includes a qualitative discussion of transmissibility for each of the 13 pathogens, including a summary of evidence for direct person-to-person transmission, indirect transmission via vectors, and potential establishment of reservoirs for each pathogen.

Step 6 – Model secondary transmission—Quantitative epidemiological modeling was performed on five of the pathogens. The five pathogens cover a range of characteristics, including higher and lower levels of transmissibility, higher and lower mortality rates, direct and indirect (vector-borne) modes of transmission, and BSL-3, BSL-4, Category A and select agents. The quantitative data for the remaining eight pathogens were assessed, and Chapter 5 of this RA describes why the data were insufficient to

support detailed modeling for those pathogens. The quantitative modeling approach is based on models that are well established in peer-reviewed publications and suitable for answering a variety of questions relevant to the NEIDL RA. Results from model simulations were expressed in terms of the total number of infections over the course of simulated outbreaks and projected operating lifetime of the facility. Numerous simulations were run for each scenario to assess the effect of chance events and of varying input values for parameters that are uncertain. The team analyzed and presented the simulation results in a variety of forms, including analysis of low-probability but high-consequence outcomes. Onto the results of the number of infections, the team overlaid the results of the number of fatalities. The team then broke the results down for important subgroups, including the portion of infections/fatalities that were likely to have occurred among members of vulnerable subpopulations. The team ran simulations for each of the three sites by varying the transmission value on the basis of demographic data. The team then performed uncertainty and sensitivity analyses (i.e., parameters were systematically changed in the model to determine the effects of such changes) for all models.

That process is described in greater detail in Chapters 4 through 9.

1.6 Risk Assessment Organization

The content of the remaining RA chapters is as follows:

Chapter 2. Facility Design, Operations, and Site Description

This chapter describes the facility design and the operations and activities that would be conducted within the NEIDL followed by an overview and characterization of the potentially affected environments at the three sites.

Chapter 3. Pathogena

This chapter identifies the pathogens selected for analysis, provides an overview of the pathogen selection process, data collection process, and concludes with a brief description of the pathogen characteristics.

Chapter 4. Event Sequence Analysis

This chapter summarizes the identification of candidate events, selection of events for analysis, the analysis of event sequences and the resulting exposures, and provides a summary of biocontainment elements that prevent and mitigate events.

Chapter 5. Transportation Analysis

This chapter summarizes the results of the off-site pathogen transportation analysis. The impacts of both truck-only and mixed mode (air-truck) shipments in the vicinity of the facility are addressed.

Chapter 6. Threat Assessment

The TA addresses concerns raised by the courts, NRC, BRP, and the public regarding the capability of the facility's security systems (e.g., personnel, policy, procedure) to prevent or withstand a malevolent action against critical systems and assets at the facility that could result in the exposure of personnel or release of a pathogen into the community.

Chapter 7. Potential For Released Pathogens To Become Established In The Environment

This chapter summarizes the estimation of initial infections, the assessment of transmission potential, and the mathematical modeling of secondary transmission.

Chapter 8. Health Effects - Initial Exposure

This chapter provides details of the analysis of initial infections and fatalities, including estimates of dose-effects information for each pathogen and details of the procedure used to estimate the number of initial infections from a given exposure.

Chapter 9. Secondary Transmission

This chapter provides details of the quantitative modeling procedure for secondary transmission and the statistical analyses performed on the results of those simulations.

Chapter 10. Environmental Justice

This chapter identifies the economically and culturally disadvantaged (i.e., low-income and minority) communities in the vicinity of each site and determines whether those communities would be affected any more than other communities by a potential loss of biocontainment.

Chapter 11. Risk Characterization

This chapter summarizes the number of exposures, infections, and fatalities potentially resulting from each event. This chapter also synthesizes the key findings that include a summary of the risk to each exposed group, differences among sites, and potential disproportionate effects on vulnerable subpopulations.

Chapter 12. Authors

This chapter identifies the personnel along with their project responsibilities, employment affiliation, education, and years of experience.

Chapter 13. Abbreviations and Glossary

This chapter lists the abbreviations and the glossary of terms in the report.

The appendices present the following articles or information:

Appendix A. Facility Design and Operations provides details on the NEIDL facility design and operations that affect biocontainment.

Appendix B. Site Characteristics provides the characteristics of the urban, suburban, and rural locations analyzed in the RA.

Appendix C. Pathogen Characteristics provides a comprehensive and cited list of pathogen characteristics, followed by a compilation of the cited references.

Appendix D. A Review of Reported Incidents, Exposures and Infections in BSL-3AND BSL-4 Laboratory Facilities summarizes the operating experience at other high-biocontainment facilities. It also identifies the types of events that have occurred and provides some insight into their frequency.

Appendix E. Identification of Candidate Initiating Events identifies the candidate events and the results of the selection process.

Appendix F. Event Sequence Analyses provides the details of the event sequence analysis, which addresses the biocontainment element role in the event, estimates the frequency of the events, and characterizes the exposure from the events.

Appendix G. Transportation Analysis: is internationally blank because the details have been placed into Chapter 5.

Appendix H. Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment provides the input from an expert panel on topics where the available literature is not sufficient for this RA.

Appendix I. Medically Vulnerable Populations provides details of the choice of specific medically vulnerable subpopulations considered for this RA, the estimates of differential susceptibility to pathogens under study for the subpopulations and the use of subpopulation data in the estimates of initial infection and secondary transmission modeling.

Appendix J. Dose-Response Relationships provides the details for linking estimates of the amount of exposure to pathogens resulting from event sequences to estimates of initial infection in those potentially exposed.

Appendix K. Initial Infection provides details of the analysis of initial infections and fatalities, including estimates of dose-effects information for each pathogen and details of the procedure used to estimate the number of initial infections from a given exposure.

Appendix L. Health Effects - Secondary Transmission provides details of the quantitative modeling procedure for secondary transmission and the statistical analyses performed on the results of simulations.

Appendix M. Environmental Justice identifies the economically and culturally disadvantaged (i.e., low-income and minority) communities in the vicinity of each site and determines whether those communities would be affected any more than other communities by a potential loss of biocontainment.

Appendix N. References Not Cited provides references that were considered in the process of performing the RA but are not cited specifically.

1.7 References

BTWC (Biological and Toxin Weapons Convention). 1972. *Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction*. *Available on the internet at:* <http://www.opbw.org/convention/documents/btwctext.pdf>. Accessed December 29, 2011.

BU 2011. Boston University Medical Center National Emerging Infectious Diseases Laboratories, NEIDL Fact Sheet. Available on the internet at: <http://www.bu.edu/neidl/about/neidl-fact-sheet/>. Accessed December 30, 2011.

CDC (Centers for Disease Control and Prevention) and NIH (National Institutes of Health). 2007. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. U.S. Government Printing Office, Washington, DC.

DOE (U.S. Department of Energy). 2002. *Recommendations For Analyzing Accidents Under The National Environmental Policy Act*, U.S. Department of Energy, Office of Environment, Safety and Health, Environment, Safety and Health, Office of NEPA Policy and Compliance July 2002.

EPA 2000 *Science Policy Council Handbook: Risk Characterization*. Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-002. Available on the internet at: http://www.epa.gov/spc/2riskchr.htm, Accessed December 18, 2009.

GAO (Government Accountability Office). 2007. *High Containment Biosafety Laboratories: Preliminary Observations on the Oversight of the Proliferation of BSL-3 and BSL-4 Laboratories in the United States*, October 4. *Available on the internet at:* <http://www.gao.gov/new.items/d08108t.pdf>. Accessed December 29, 2011.

Mary Ann Liebert, Inc. 2003. *Vector-Borne and Zoonotic Diseases*, Volume 3, Number 2, 2003. *Available on the internet at:* <http://www.liebertonline.com/doi/pdf/10.1089/153036603322163475>. Accessed December 29, 2011.

NIAID (National Institute of Allergy and Infectious Diseases). 2003. NIAID Funds Construction of Biosafety Laboratories. *NIH News*. September 30, 2003. *Available on the internet at:* <http://www.niaid.nih.gov/news/newsreleases/2003/Pages/nblscorrect21.aspx>. Accessed December 29, 2011.

NIAID (National Institute of Allergy and Infectious Diseases). 2003a. National Institutes of Health. Office of Research Facilities. *Design Policy and Guidelines, Biomedical Research Laboratories*. 2003. Available on the internet at: <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/policy-index.htm>. Accessed December 29, 2011.

NIAID (National Institute of Allergy and Infectious Diseases). 2008. NIAID Planning for the 21st Century: 2008 Update. Available on the internet at: <http://www.niaid.nih.gov/about/whoWeAre/Documents/niaidstrategicplan2008.pdf>. Accessed December 29, 2011.

NIAID (National Institute of Allergy and Infectious Diseases). 2009. Current National Biocontainment Laboratories (NBL) and Regional Biocontainment Laboratories (RBL) Available on the internet at: <http://www.niaid.nih.gov/LabsAndResources/resources/dmid/NBL_RBL/Pages/site.aspx>. Accessed December 29, 2011.

NIH (National Institutes of Health) and DHHS (Department of Health and Human Services). 2005. Final Environmental Impact Statement, National Emerging Infectious Disease Laboratory, Boston, MA. Available on the internet at: < http://www.bu.edu/neidl/files/2010/07/NEIDL-Final-Environmental-Impact-Statement.pdf >. Acessed March 18, 2009.

NIH (National Institutes of Health). 2008. *Design Requirements Manual*. NIH, Office of Research Facilities, Division of Technical Resources. Version 1.7. August 27, 2008. Available on the internet at: <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/DesignRequirementsManualPDF.htm>. Accessed November 2, 2009.

NIH (National Institutes of Health). 2011. NIH Budget, updated May 23, 2011 Available on the internet at: <http://www.nih.gov/about/budget.htm>. Accessed December 29, 2011.

NIH (National Institutes of Health). 2011a. NIH Mission, updated March 3, 2011. Available on the internet at: <http://www.nih.gov/about/mission.htm>. Accessed December 29, 2011.

NIH (National Institutes of Health). 2011b. *Notice Pertinent to the October 2011 Revisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines). Available on the internet at: <http://oba.od.nih.gov/rdna/nih_guidelines_oba.html>. Accessed December 29, 2011.

University of Louisville. 2010. <http://louisville.edu/community/biosafetylab/>.

Table of Contents

2.0 Facility Design, Operations, and Site Descriptions

This chapter provides an overview of the NEIDL design, critical systems, and proposed operations. Following that information are descriptions of the environmental settings of the three comparable sites. The chapter concludes with a synopsis of the public safety and emergency response capabilities in the jurisdictions of the urban, suburban and rural site locations.

As stated in Chapter 1, the NEIDL facility, as designed and constructed at BU BioSquare Research Park, is assumed to be the identical structure that would have been constructed at the Tyngsborough, Massachusetts, and the Peterborough, New Hampshire, site if either of those locations had been selected. Similarly, NEIDL proposed bioresearch operations are considered to be the same for all three sites. Those assumptions are necessary to provide meaningful comparisons of the relative risks at the three sites.

The Tetra Tech team having the facility design, operational, and site description discussions, relied primarily on information obtained from review of the NEIDL design specifications, supplementary data provided by BUMC, the NEIDL Environmental Impact Report (EIR) and Environmental Impact Statement, original research and analysis conducted by the RA team, NEIDL tours, and site visits to the urban, suburban and rural sites. Additional details on each topic presented in this chapter are included in Appendices A and B.

2.1 NEIDL Design and Operation

In support of performing basic and clinical research on emerging infectious diseases and developing diagnostic tests, treatments, and vaccines, the NEIDL design and construction is intended to ensure the laboratory workers' protection, provide a barrier to protect persons outside the laboratory, and protect persons or animals in the community from pathogens that could be accidentally or malevolently released from a high-containment laboratory in the facility. The NEIDL was designed and constructed in conformance with the NIH's *Design and Policy Guidelines* (NIH 2008), the NIH's *Design Requirements Manual* (NIH 2003), the BMBL 5th edition (CDC and NIH 2007), the Massachusetts State Building Code 780 Code of Massachusetts Regulation (CMR 6th edition [expired on March 1, 2009]), Executive Office of Public Safety and Security, and NIH Security Guidelines Level 5 (BPHC 2009). Once the NEIDL becomes fully operational, it is anticipated that approximately 271–302 new positions would be established that would include administrative staff, maintenance personnel, research technicians, research scientists, safety officers, and management staff (BUMC 2009a).

The BMBL identifies the required elements of biocontainment as the following (CDC and NIH 2007):

- Facility design and construction;
- Safety equipment; and
- Laboratory practice and technique, referred to here as administrative controls.

A more nebulous but equally important element of biocontainment is the "culture of safety" The culture of safety is the manner in which safety is managed and is reflected in the attitudes and values of the employees and management. In 2010, BU appointed a task force to review its safety program and the task force made the following key recommendations (BUMC 2010):

- Active embracement of a "culture safety" as a core value at every level.
- Inclusion of "safety" as a condition of employment for all those engaged in research and as a key factor in the annual "Performance Appraisals".
- Written confirmation by all individuals engaged in research that they have been adequately trained and that they will follow the safety requirements.
- New procedures for the temporary or permanent removal of the privileges of individuals who violate health and safety requirements.
- Clear indication that while safety is a shared responsibility of each individual working in a laboratory ultimately the Principal Investigator has full responsibility for safety in their laboratories.
- Appointment of a Laboratory Safety Coordinator" with specific responsibility for implementing day-to-day safety requirements in the laboratory.
- Enhancements in the operation of the Institutional Biosafety Committee (IBC) operations and its membership.
- Appointment of a Chief Safety Officer at the NEIDL with full oversight responsibility on all safety aspects of the NEIDL and with the authority to stop any operations that are judged to present a health and safety hazard or in violation of regulatory or policy requirements.
- Appointment of a NEIDL Safety Committee with a specific charge for the review of all aspects of safety the NEDIL.
- Recruitment of a communication specialist to assist in developing campus-wide and NEIDL specific "culture of safety" communication plans.

BUMC has developed the "Implementation Plan for Enhancing the Research Culture of Safety at Boston University and Boston Medical Center" (BUMC 2010), which implements these recommendations and

includes a NEIDL-specific addendum (i.e., Section II). These enhancements have the potential to produce significantly improve safety at the NEIDL, but this analysis does not account for these potential impacts.

The following sections provide an overview of the NEIDL facility, a description of the biocontainment elements it contains, and a description of other facility systems critical to its safe and reliable operation.

2.1.1 NEIDL Overview

This section provides an overview of the NEIDL layout and descriptive information regarding its planned research and maintenance operations.

2.1.1.1 Facility Layout

The NEIDL facility is a structural steel and reinforced concrete seven-story building that houses BSL-2, BSL-3, and BSL-4 laboratories, including ABSL-3 and ABSL-4 animal facilities, ACL-3 and ACL-4 insectaries. It also has offices, conference rooms, and support facilities including an effluent treatment room, secure loading dock, and dedicated mechanical floors to support and maintain the containment features of the building. The superstructure is 139 feet (ft) high by 226 ft long by 120 ft wide, and it is surrounded by an 8-foot-high security fence. NEIDL's approximately 192,000 gross square feet (ft^2) of floor space is allocated as follows (NEIDL Fact Sheet May 2008):

- 48 percent administrative, mechanical, and building management system support space
- 3 percent BSL-2 clinic research facility
- 20 percent BSL-2 laboratories and related support space
- 13 percent BSL-3 laboratories and related support space
- 16 percent BSL-4 laboratory and related support space

The building is designed to meet Massachusetts State Group III Building requirements, seismic performance criteria D (Haidar 2005), which is applicable to buildings considered essential to survive a seismic event, survive a 90 mph wind at 30 ft above the ground, and 30 pounds per square foot (psf) of snow load (CUH2A, Smith Carter, and Hemisphere Engineering 2005 and Boston Public Health Commission [BPHC] 2009). Critical building systems (e.g., biocontainment and life safety systems) were designed with built-in redundancy such that the failure (or removal for maintenance) of any one component would not affect overall system integrity (BUMC 2009b). Emergency backup power is provided by emergency diesel generators (BUMC 2009b). Day tanks provide approximately 15 minutes (min) of additional run time (BUMC 2009b). Loads supported by on-site power include but are not limited to: the fire suppression system components, fire alarms, exit lighting, air supply and exhausts,

cooling towers and chillers, and the building automation system (BAS) (BUMC 2008). The BAS control system is a computerized, intelligent network of electronic devices, designed to monitor and control the mechanical and lighting systems in a building. The BAS core functionality keeps the building climate within a specified range, provides lighting according to an occupancy schedule, and monitors system performance and device failures and provides notification to specified laboratory or engineering/maintenance staff.

The building has a lightning protection system grounded to the base of the structure (BUMC 2008). Liquid nitrogen is plumbed throughout the building for freezers, and natural gas is plumbed into the building for the BSL-2 laboratories (BUMC 2008). Exhaust stacks are on the roof.

The NEIDL's seven floors have the following (BUMC 2008):

- 16 BSL-2 Laboratory Modules;
- 6 BSL-3 General/Assignable Laboratory Modules;
- 1 BSL-3 Insectary;
- 8 ABSL-3 Animal Rooms;
- 3 BSL-4 General Laboratory Modules;
- 7 ABSL-4 Animal Rooms;
- 13 BSL-2/3/4 Scientific Core Technologies;
- 1 BSL-2 Clinical research facility;
- 21 Administrative Offices;
- 1 BSL-4 Training Simulator Laboratory; and
- 1 Multipurpose Meeting Room.

The highest biocontainment BSL laboratories are in the interior spaces of the building. Such a layout provides added protection against, and lowers the likelihood of, a low-frequency but high-consequence accident (e.g., aircraft crash) breaching high-biocontainment areas. In addition, the location reduces the probability of the highest biocontainment areas being breached by a malevolent act.

Rodents and nonhuman primates (NHP) will be the principal mammal species housed in the NEIDL, and NEIDL design features preclude their escape from the laboratory. The design of ABSL-3 and ABSL-4 laboratories comply with recommendations and requirements of the BMBL 5th Edition, NIH *Design Policy and Guidelines – Animal Research Facilities* (NIH 2003 and NIH 2008), and the *Guide for the*

Care and Use of Laboratory Animals (National Research Council 1996). Animal holding rooms and their associated support space will also be provided in conjunction with the BSL-3 and BSL-4 laboratories. The Institutional Animal Care and Use Committee will review and approve all research protocols involving animals in accordance with the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Health Research Extension Act of 1985, Public Law 99-158, November 20, 1985 and NIH 2002b). All animals will be treated according to the applicable rules set forth by the Institutional Animal Care and Use Committee, the USDA Animal Welfare Act regulations, Title 9 of the *Code of Federal Regulations* (CFR) Subchapter A, and the *Guide for the Care and Use of Laboratory Animals* (NRC 1996).

Arthropods, such as mosquitoes and ticks, will be housed in specialized insectarium rooms. The construction and operation of the ACLs meet the recommendations and requirements of the ACME of the ASTMH *Arthropod Containment Guidelines*, Version 3.1 (ACME ASTMH 2002; Mary Ann Liebert, Inc. 2003), as well as the BMBL which is an internationally accepted standard. Uninfected arthropods will be completely segregated from those arthropods that contain vector-borne pathogens. Multiple insectaria barriers are in place that are designed to prevent the escape of any insects, such as manipulation areas where cooler temperatures will be maintained to slow arthropod movement to reduce the potential for escape. The surfaces of all insectaria spaces will be white—to allow for quick identification of arthropods that escape primary containment. Arthropod species will be kept segregated. Infected arthropod work will be conducted in the innermost rooms under negative pressure conditions, and all air supply and exhaust terminal devices will be screened to prevent arthropod escape. Additional room barriers in place will depend on the risk assessment of the pathogen/insect being studied.

Additional overview descriptions of the NEIDL facility are in Section A.1 of Appendix A.

2.1.1.2 Research and Operations

The NEIDL supports comprehensive core studies that enable basic, translational, and clinical research and developing products related to emerging infectious diseases. Administrative offices and support space will be provided to house administrative staff, safety staff, resident principal investigators (PIs), visiting PIs, and facility support staff employed to safely operate and maintain the facility.

The NEIDL will support key research and support operations. Clinical research space will be provided to support clinical research protocols. The clinical research facility will include reception, nursing, administration, and examination rooms. Specific protocols are also being developed to address the

transport of infected individuals from the NEIDL to the existing isolation facilities at BMC, if necessary. In accordance with current practices, the BUMC Institutional Review Board, composed of members of the academic community that the BUMC provost oversees, will approve all human investigation studies to be performed at the NEIDL.

The research and support core operations that require high-biocontainment laboratories (BSL-3 and -4) are listed below (Klempner 2008). Additionally, BUMC is responsible for developing standard operating procedures (SOPs) for those operational services.

- *Aerobiology Core.* Animal research is an essential element of defining the pathogenesis of infectious diseases, and such knowledge is essential for developing diagnostic tests, treatments, therapies, and vaccines for such diseases. Because aerosol exposure is the primary route of infection for many of the most important naturally occurring emerging infectious pathogens, inhalational studies are an essential component for studying pathogenesis and preventing and treating infectious diseases. Inhalation studies would focus primarily on NHP and rodents and would be conducted in BSL-3 and -4 laboratories using aerosol-generating equipment within Class III biological BSCs.
- *Laboratory Animal Science Core.* This support operation is primarily responsible for overseeing the veterinary medical care of study animals. Such activity involves participating in experimental design and setup, assisting in experimental procedures, providing training for core staff, and providing necropsy and pathology services for study animals. Approximately 50–75 rodents and up to 30 NHPs can be used for research in the BSL-3 or BSL-4 spaces at any time and will be housed in single-occupancy cages.
- *Biomolecule Production Core.* This operational service is responsible for developing SOPs for propagation and titration of all BSL-4 pathogens that will be used in the NEIDL. Personnel provide inactivated viral antigens, purified genomic materials expression vectors of viral proteins, and in vitro antiviral screening assays (i.e., samples) from the BSL-2 to BSL-4 facilities. In addition, biomolecule production personnel will conduct simulations of all SOP protocols that will be used for biomolecule production to validate their accuracy and, if necessary, modify the SOPs for greater effectiveness and safety.
- *Cell and Tissue Imaging Core.* This support group offers multiple imaging systems to analyze specimens using state-of-the-art technologies. The availability of different high-resolution microscopy solutions will allow NEIDL investigators to integrate fine-scale topography of fixed tissues gathered from transmission or scanning electron microscopy with information gathered

from multi-probe, live cell analyses using visible light, fluorescence, deconvolution (Deltavision), or laser confocal microscopy.

- *Collaborative Research Core.* The objective of this core is to internally facilitate extramural collaborations with investigators whose research requires high-containment capabilities. In addition, this group is responsible for initiating the development of SOPs for specific domestic and international research collaborations and initiating discussions and proposals with potential collaborators.
- *Immunology Core.* Immunology support services personnel will have the necessary infrastructure and provide support to characterize the innate and adaptive immune cellular response to infectious pathogens and their products. That includes maintaining stocks of needed reagents, providing consultation services and technical assistance, performing cell separation (via magnetic beads), and other immunological analyses as needed.
- *Multimodal Whole Animal Imaging Core.* A unique operational objective of the NEIDL is to provide the means to perform in vivo, whole-animal, multimodal imaging in a BSL-4 biocontainment setting. BSL-4 multimodal instrument configuration includes a small animal Magnetic Resonance Imaging scanner, an optical imaging device, an ultrasound, and X-ray/computerized tomography configuration. This core aims to serve as a central service facility for experimental imaging in emerging infectious diseases, using the most recent and anticipated advances in multimodal imaging.
- *Specimen Processing Core.* This service supports NEIDL investigators in studying emerging infectious diseases by handling the collection and storage of animal specimens and cultures in the appropriate biocontainment setting (e.g., BSL-2, BSL-3). In addition, the core provides a system of centralized laboratory diagnostic testing.
- *Vector Transmitted Infectious Diseases Core.* This core (1) houses ACL-3 and ACL-4, which are required for research on many of the emerging vector-borne infectious diseases; (2) breeds and maintains arthropod colonies including mosquitoes and ticks; (3) conducts natural infection and transmission studies; (4) conducts vector competence experiments to determine what insects are capable of transmitting the microorganism, (5) develops novel strategies to interrupt pathogen transmission at the vector level to eliminate the vector or pathogen and eliminate the vector competence for pathogen transmission; and (6) is involved with new pathogen discovery in potential vectors.

2.1.2 Facility Design and Construction

The facility design and construction contributes to laboratory and facility worker protection and provides a barrier that protects the public from potential accidental loss of biocontainment. Additional discussion of the facility design and construction is in Section A.2 of Appendix A.

2.1.2.1 Building Control Systems

The NEIDL has a BAS that consists of a dedicated operator work station with alarm status and trend logging printers. The BAS consists of a personal computer with graphic displays and the capability to monitor and control the operation of the building systems. Individual operator workstations allow human/machine interface with the BAS through visual displays in a dynamic graphic format of the laboratories. The BAS controls or monitors environmental and other operational parameters (temperature, humidity, flow, and pressure values) for individual areas or rooms, fire suppression, and liquid waste treatment. It also monitors enunciators for entry control systems. In addition to the graphic displays of laboratories, the central supply air and exhaust air systems dedicated to the individual spaces are also managed through the BAS. All control systems are equipped with an uninterruptable power supply. A satellite control center external to the NEIDL facility (i.e., in a nearby facility on the BUMC campus) provides redundant indication and control of NEIDL systems (BUMC 2009c).

2.1.2.2 HVAC Systems

The integrated heating, ventilation, and air conditioning (HVAC) system maintains a consistent and controlled indoor environment throughout the facility. The system allows the ability to adjust temperature and humidity (within selected laboratories) to parameters required by individual research activities. The NEIDL facility's HVAC has redundancies incorporated . Each air-handling system and corresponding exhaust system in the high-biocontainment laboratories (i.e., the BSL-3 and BSL-4 spaces) have multiple air handlers and exhaust fans. Some systems are sized to operate at full capacity with any individual unit out of service (Kajunski 2009). BSL-3 and BSL-4 laboratories are equipped with HEPA filtration. The HEPA filters meet the design criteria of removing at least 99.97 percent of particles having a diameter of 0.3 micrometer. Their efficiency is greater than 99.97 percent at particle sizes larger or smaller than 0.3 micrometer (CDC and NIH 2007). Figure 2-1 shows relative sizes and scale of particles. All BSL-3 exhaust passes through a single HEPA filter at the point of exit from biocontainment. The BSL-4 exhaust passes through double HEPA filters in series at the point of exit from biocontainment. The BSL-4 portion of the facility is supplied air from a common BSL-4 supply header through a single HEPA filter at the biocontainment barrier for each laboratory space.

The mechanical support space for BSL-4 biocontainment areas includes individual HVAC plenums for each BSL-4 laboratory room. The HEPA filter banks are above the laboratory suites and are connected to the individual suite rooms below using stainless steel ducts that are embedded in the concrete floor to minimize the potential for duct related issues. Each filter system is equipped with a damper system at the outlet, which is designed to close if an emergency occurs, maintaining negative airflow, and isolating the airflow path to and from the laboratory (Kajunski 2009; BUMC 2009c). Air flow is monitored through the filter houses via the BAS, and reduction of flow is used as an indicator of filter loading or breach.

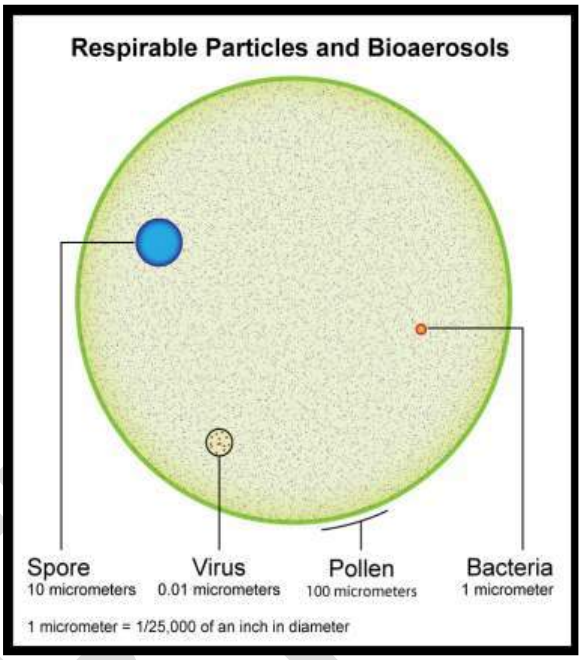

Figure 2-1. Graphic representation of respirable particles.

2.1.2.3 Other Facility Features

The fundamental NEIDL public safety barriers are the high-biocontainment laboratories themselves. Other NEIDL features help prevent the accidental release of a pathogen from the laboratories. The BSL-3 and BSL-4 laboratories within the NEIDL were designed and built to the applicable federal standards, incorporating safety barriers and safeguards to prevent or mitigate the accidental release of a pathogen. Redundant, critical systems are incorporated in the utility and building infrastructure to ensure full operations. A networked electrical service provides four separate incoming feeds such that if any one feed is disrupted, the remaining electrical feeds provide the necessary service (BUMC 2009a).

Each BSL-4 laboratory is supported by multiple functional areas including a locker room, showers, suit room, air lock, and the laboratory itself—all surrounded by a non-containment corridor. Biological pathogens on the interior of the containment area will be isolated from exterior spaces by interlocked doors with the interlocking mechanism allowing only one door to open at a time to ensure proper function of the barrier (BUMC 2009b).

All electrical conduit, plumbing, piping, supply and exhaust ducts and miscellaneous penetrations are sealed at the point of penetration into the high biocontainment laboratories (BSL-3 and BSL-4) (BUMC 2009d). The high-biocontainment laboratories, animal laboratories, and insectary are separated by access controls (i.e., electronic recognition devices) and electronic recognition devices for overall building

access control. The high-biocontainment BSL-4 laboratory environment employs the concept of a *box-within-a-box* principle, whereby the laboratory is built within a pressure-controlled buffer. Laboratory air flows from areas with the lowest potential for contamination (e.g., office areas) to areas with the highest potential for contamination (e.g., the BSL-3 and BSL-4 laboratories), which helps to restrict pathogens to the laboratory environment. Ventilated airlocks separate the common corridors from the high-biocontainment laboratories. The airlock doors are interlocked to prevent multiple doors between the outside corridors and the high-biocontainment areas opening simultaneously. Directional airflow is provided through the airlock with differential pressure monitoring. The air pressure control system for maintaining the required pressure differentials is capable of being monitored inside and outside the BSL-4 laboratory. That direction of airflow into the BSL-4 laboratories is verifiable by gauges and an audible alarm system that will notify personnel of HVAC problems or a total or partial system failure. BSL-3 laboratories do not have those gauges or audible alarm system. All BSL-3 and BSL-4 laboratories will operate under negative air pressure. All high-biocontainment laboratory exhaust air is HEPA filtered before discharge through the NEIDL roof air emission discharge stacks. All surfaces in the high-biocontainment laboratories were designed and constructed to be easily cleaned and decontaminated. Seams, floors, walls, and ceiling surfaces are sealed to facilitate fumigation and are resistant to liquids and chemical used for cleaning and decontamination. No expansion joints or cracks are at the wall/floor interfaces (BUMC 2009c, 2009d).

One floor of the NEIDL is dedicated to BSL-4 laboratories and associated BSL-4 support space (e.g., access to ventilation system, instrument air compressors) directly above on the next upper floor level. The floor of the BSL-4 biocontainment areas is structurally isolated from the rest of the building, thereby minimizing the transfer of energy (i.e., vibration and shaking) from the building to the BSL-4 laboratories. The BSL-4 laboratories are also physically and functionally independent from other laboratory functions. The BSL-4 laboratories use airlocks for entry and exit, have dedicated supply and exhaust ventilation, and laboratory personnel will use positive pressure suits. Before exiting a BSL-4 laboratory, personnel will decontaminate the suit's outer surface via a chemical shower. Exhaust air will pass through dual HEPA filters mounted in series in a dedicated, sealed exhaust system before discharge through the NEIDL roof air emission stacks (BUMC 2009c).

2.1.3 Equipment

The NEIDL contains extensive equipment common to a biological laboratory. Much of the equipment and engineering controls are designed to remove or minimize exposures to hazardous biological materials.

The following list illustrates the major types of equipment that could be in the NEIDL (NIH and CDC 2007; BUMC 2009c):

- Autoclaves
- Biosafety Cabinets
- Centrifuges
- Flammable Storage Cabinets
- Freezers
- Incubators and Bioreactors
- Furnishings

The list is not intended to be comprehensive because NEIDL operations and BSL equipment can evolve. Thus, other equipment could be installed to meet research and safety considerations. In the following discussions, photos are provided as representative of the type of equipment.

2.1.3.1 Autoclaves

An autoclave is a device that uses steam, pressure, and heat to sterilize equipment, culture media, and contaminated wastes (Figure 2-2). Bacteria, fungi, and viruses are killed via autoclave sterilization. Two methods are used to determine the proper operation of an autoclave. Indicators can be biological or chemical (thermal). Biological indicators are used to ensure autoclaves reach the correct temperature and pressure for the specified length of time. Biological indicators contain innocuous bacterial spores that will grow when the proper operating temperature has not been achieved. In general, biological indicator vials are placed inside biocontainment bags containing the material to be processed in the autoclave. If the autoclave does not reach the programmed temperature, the spores will subsequently grow, and change the color of a pH-sensitive chemical in the growth medium. Colorimetric tape can also be used to verify the proper operation of the autoclaves. The indicator changes color when exposed to the correct temperature. Those steps provide multiple, independent, positive records to indicate the correct functioning of the autoclaves (BUMC 2009c).

Figure 2-2. Autoclave.

2.1.3.2 Biological Safety Cabinets

BSCs are the principal devices used to provide biocontainment of biohazardous aerosols that can be generated from laboratory operations (CDC and NIH 2007). There are three main classes (and multiple subclasses) of BSCs.

Class II BSC. Class II BSCs (Figure 2-3) are available in several types. All types use HEPA filtration to filter exhaust air and incoming air, such that they provide personal, environmental, and sample protection (i.e., protection against contamination of the samples being processed).

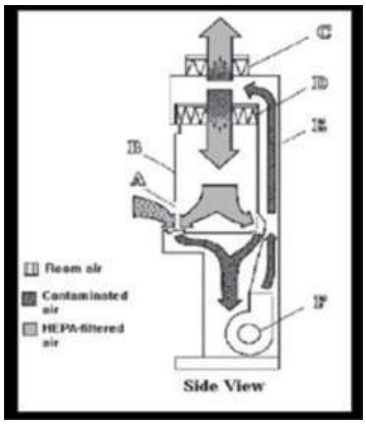

Figure 2-3. A non-ducted Class II BSC. The parts are identified as follows: A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. common plenum, F. blower.

Class III BSC. A Class III BSC, is illustrated in Figure 2-4. The purpose of a Class III BSC is to provide an additional level of safety for workers. A Class III BSC has two chambers: the pass-through chamber and the working chamber. The pass-through chamber allows safe transfer of materials into and out of the working chamber. The pass-through chamber could consist, for example, of a steam sterilizer featuring interlocked double doors. The NEIDL Class III BSC is a detachable, mobile, HEPA-filtered rapid transfer cart which attaches on the ABSL-4 side of the BSL-4 cabinet line or the ABSL-3 side of the BSL-3 cabinet line. In BSL-3, samples can be removed via a sample (canister) port (under construction). Materials are passed between the pass-through chamber and the working chamber. The working chamber provides a maximum biocontainment enclosure for manipulating biohazardous materials. As required by the BMBL, the supply air for the Class III BSC is HEPA filtered; the exhaust air is double-HEPA filtered (with at least one filter integral to the BSC); and the BSC is directly connected (hard ducted) to a dedicated, independent, exhaust system. Air-tight dampers are on the supply and exhaust ducts of the BSC to allow gas or vapor decontamination of the interior. Long, heavy-duty gloves are attached in an air-tight manner to ports in the Class III BSC and allow direct manipulation of the materials isolated inside. Although the gloves restrict movement, they prevent the laboratory worker's direct contact with the biohazardous materials, maximizing personal safety (CDC and NIH 2007). Within the working chamber is a drain to allow liquids used in decontamination to be captured and processed in an autoclave before their removal from the laboratory.

Figure 2-4. Centrifuge.

2.1.3.3 Centrifuges

A centrifuge is an instrument used for separating particles of different masses in liquid suspension (Figure 2-4) by centrifugal force. It is used to concentrate particles, to separate insoluble molecules or whole cells from the suspension. When a centrifuge's motor is activated, the

spindle revolves at high speed, and centrifugal force drives the heavier materials toward the bottom of the centrifuge tube. Centrifuges come in various specifications to accommodate different applications. Some centrifuges (e.g., the tabletop model as shown in Figure 2-4) have multiple safety features, for example, gasket-sealed caps on each rotor bucket that holds each sealed centrifuge tube. Some centrifuges incorporate aerosol-containment technology. All modern centrifuges have automatic shutdown electronic sensors and indicators for unbalanced or disturbed operation. Sections F.2 and F.6 of Appendix F provide additional details on equipment and protocol used for centrifugation.

2.1.3.4 Flammable-Storage Cabinets

Flammable-storage cabinets provide for the safekeeping of flammable liquids close to the work area in an organized manner. All flammable-storage cabinets in the NEIDL comply with Occupational Safety and Health Administration regulations, are designed in accordance with applicable National Fire Protection Act (NFPA) standards, and have appropriate markings. The NEIDL will limit the amount of chemicals to be used in BSL-4 laboratories to quantities needed for the duration of the experiments.

2.1.3.5 Freezers

Ultra-low temperature freezers (Figure 2-5) provide long-term protection and storage for valuable samples of biohazardous materials. Many biological samples must be stored at below-freezing temperatures to prevent degradation and preserve them for future reference, analysis, or use. Protecting the integrity of biological samples is extremely important and can be achieved by combining rapid temperature recovery, temperature stability and operational efficiency. Ultra-low temperature freezers can maintain their internal compartments at temperatures as low as –86 degrees Celsius (°C) (–123 degrees Fahrenheit [°F]) to ensure the long-term preservation and storage of biological samples. Such sub-zero temperatures help maintain extended viability of preserved biological samples by dramatically reducing metabolic activity. Minimizing temperature fluctuations (achieved by temperature programming and alarms) is critical to preserving the viability of biological samples.

Figure 2-5. Ultra-low temperature freezers.

2.1.3.6 Incubators and Bioreactors

Biological incubators (Figure 2-6) come in a variety of sizes and types to serve a broad range of applications. They are designed to maintain optimal conditions to achieve growth or desired chemical reaction by controlling environmental factors such as temperature, humidity, and atmospheric composition. Incubators can be large, like a standard refrigerator, or small, as shown in Figure 2-6.

Figure 2-6 Incubator

Bioreactors, also known as fermenters, are highly specialized incubators designed for specialized liquid culture of microbes. Bioreactors allow continuous, precise control and monitoring of temperature, acidity, oxygen concentration, air input, air output, agitation, and allow for continuous input of fresh nutrients.

2.1.3.7 Furnishings

Laboratory furnishings are capable of supporting anticipated loading and use. Bench tops are impervious to water and resistant to moderate heat, chemicals, and disinfection solutions. Laboratory furnishings were selected to meet BMBL requirements.

2.1.3.8 Other Laboratory Equipment

Other laboratory equipment includes small, electric incinerators (dry heat), pipettes, refrigerators, ice makers, microscopes, mixers, shakers, and vacuum pumps. All instruments or devices will be used with the appropriate combinations of PPE and other physical biocontainment as required for biocontainment laboratory operations per BMBL guidance. The use of sharp instruments, also called sharps, will be avoided whenever practical.

Safety equipment also encompasses many of the items used for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, and safety glasses or goggles. PPE is often used in combination with BSCs and other devices that contain the pathogens, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, PPE might form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, pathogen production activities, and activities relating to maintenance, service, or support of the laboratory facility.

2.1.4 Administrative Controls

In addition to the NEIDL structure, biosafety is achieved by implementing various administrative controls. Administrative controls are a cornerstone to ensuring protection of the involved worker, the uninvolved worker, the public, and the environment. Administrative controls include programs, policies, and procedures designed to guide, direct, and assist employees with the safe handling of potentially hazardous biological materials. Additional details on administrative controls are provided in Section A.4 of Appendix A.

2.1.4.1 Laboratory Safety Program

The BUMC Office of Environmental Health and Safety (OEHS) oversees a laboratory safety program that emphasizes preventing illness and injury, promoting safe work practices, and protecting the environment while working with chemical, biological, and radioactive agents. Through the program, safety staff provide RA, consultation, and support to workers, supervisors, and management. Laboratory safety services include specialized safety training in biological, chemical, and radiation safety; routine laboratory inspections; new laboratory setups; emergency response; and laboratory decommissioning services in collaboration with the BUMC Facilities Department (BUMC 2009c).

2.1.4.2 Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) is a university-wide committee responsible for reviewing and approving recombinant DNA (rDNA) research and biohazard research projects throughout the BU system. The IBC is responsible for overseeing the Biosafety Program at BU and BMC. The committee sets biocontainment levels in accordance with the NIH and the CDC guidelines. It also periodically reviews previously approved research projects for changes that will necessitate changes to experimental protocols and SOPs or increasing the required BSL. The IBC evaluates research projects that use rDNA; agents that are infectious to humans, animals, and plants; other potentially infectious materials; select agents and biological toxins; human materials including blood, cells, unfixed human tissues, and other body fluids; xenotransplant; and gene transfer clinical studies. The IBC coordinates its application procedures with two other offices, Research Occupational Health Program (ROHP), to ensure that research personnel have adequate occupational health monitoring, training on safe work practices, exposure control emergencies, and use of PPE. The IBC carries out those functions pursuant to requirements set forth by federal, state, and local agencies as well as BU. The IBC is composed of faculty investigators (with expertise in rDNA and biohazards research) from both campuses, non-scientist and community members, and a biosafety officer. IBC responsibilities include the review and approval of all new research involving rDNA and biohazards; continued review of approved research projects; review of

laboratory inspection reports; investigation of complaints and concerns, and review of training and a medical surveillance programs. During and following approval from the IBC and before operations, NEIDL staff indicate that they will develop SOPs outlining the appropriate safety equipment, research equipment, safety processes and procedures, and the BSL in which the research must be conducted (BUMC 2009e).

2.1.4.3 Standard Operating Procedures and Training

Biocontainment administrative controls include strict adherence to standard microbiological practices and techniques, personnel training, and safety oversight and management. SOPs are under development for the NEIDL staff that will establish, implement, and control laboratory activities for BSL-2, BSL-3, and BSL-4 laboratories. SOPs provide direction to ensure that laboratory activities will be performed within the biological safety requirements pursuant to the BMBL guidance, CDC and USDA regulations, and BUMC policies pertaining to SOPs, laboratory practices, and training programs. The key elements of a successful administrative controls program are the direct involvement of laboratory workers in controlling the risks and the accountability of BUMC line management for safety, security, and environmental protection. The NEIDL director, line management, lab workers, and the biosafety officer are responsible for ensuring that NEIDL research activities are conducted in accordance with BUMC policies and procedures, and federal, state, and local regulations. The NEIDL training regimen being developed is intended to ensure that all personnel engaging in research or clinical activities have received the appropriate information about the biohazardous and hazardous materials in their work environment, the nature of the risks associated with handling such materials, and the conditions under which the materials can be harmful. Training programs will be integrated, comprehensive, and multidisciplinary and identify job-specific and task-specific training necessary to ensure safe and effective BSL-2, BSL-3, and BSL-4 laboratory techniques (BUMC 2009c, 2009e).

BUMC has policies and procedures in place to monitor and prevent worker exposure. They consist of a detailed medical surveillance training program, serum banking, and other procedures effective at preventing and monitoring worker exposures. The NEIDL will have a comprehensive medical surveillance program that will be integrated into the BU medical monitoring system. If a pathogen-specific immunization is available, laboratory personnel will receive immunizations for the pathogen handled or present in the laboratory. BUMC provides annual laboratory training as a minimum standard and increases training frequencies depending on the type of work being done in each laboratory. BUMC will determine the levels of training necessary to ensure that all NEIDL employees are compliant with and fully knowledgeable of all regulations. The laboratory safety program requires all personnel who work

with chemical or biological hazardous materials to be aware of the hazards that are present, use appropriate PPE, and be trained in emergency response procedures. All persons who plan to work in the BSL-3 and BSL-4 laboratories will be required to undergo additional specialized training. A training program still under development for laboratory practices and safety protocols in high-containment laboratories will be conducted in the NEIDL seminar room, mock BSL-4 training simulator (a fully functional laboratory operated in a non-contaminated state), and use of viewing windows into the operating, high-containment suite (BUMC 2009c and 2009e).

2.1.5 Waste Management

Waste management practices at the NEIDL are laboratory-specific and dependent on the wastestreams generated by individual BSL-2, BSL-3, and BSL-4 laboratory activities. In general, disposal of waste is specific to the organisms or SOPs of the research group, but in general the following principles apply:

- Rigid containers labeled with the universal biohazard symbol and lined with red biohazard bags are provided in every clinical and research facility in the NEIDL for all biohazard waste.
- Sharp containers are also provided, which are specifically designated for disposing of needles, syringes, and scalpel blades.

Solid waste is the classification given to all nonhazardous waste generated from offices and maintenance areas, including recyclable materials, in the NEIDL. In addition to normal solid waste, it is anticipated that the NEIDL will generate three types of special waste: biological waste, radioactive waste, and hazardous-chemical waste. The NEIDL will have chemical/biological mixed waste (and could also have radiological/biological). The biological (pathogen) component will be inactivated by the appropriate (and compatible) chemical disinfectant, and the material will then be disposed of properly as chemical or radiological waste. The use, storage, and disposal of all solid and special waste will be performed in accordance with state and local regulations.

Additional details on waste management are in Section A.5 of Appendix A.

2.1.5.1 Biological Waste

No waste materials will be removed from the high-containment laboratories without first being processed in an autoclave or decontaminated by a method approved and managed by BU's OEHS (BUMC 2009f). Several materials require special decontamination methods to assure safe removal from the BSL-3 and BSL-4 laboratories. Such materials are biological samples needing further analysis, laboratory equipment, and laboratory clothing (BUMC 2009f). BSL-3 and BSL-4 laboratories use a disinfectant that is particular

to each pathogen used as outlined in the research-specific SOPs and an autoclave to further disinfect solid biological and nonbiological waste. The BSL-2 laboratories will use the conventional system of bagging biohazardous waste and shipping the material off-site for incineration using a licensed third-party contractor (BUMC 2009f).

The liquid effluent disposal system will include a sterilization system BSL-4 facilities and a dedicated liquid effluent decontamination system. All liquid waste from the BSL-4 laboratories will first be decontaminated with a chemical disinfectant and then be piped to a biowaste processor and heated under pressure until the temperature reaches 121 ºC (249 ºF) where it will be maintained for a minimum of 60 minutes to ensure that two decontamination (BUMC 2009f) processes are completed. Decontamination will be verified using biological indicators and electronic monitoring and charting of the process (BUMC 2009c). Once cooled and verified to meet acceptable discharge limits, the liquid waste effluent will be discharged to the Boston Water and Sewer Commission sanitary sewer system. Ventilation from plumbing system gases pass through a HEPA filter before discharge to the atmosphere. The filters will be decontaminated and disposed of as appropriate (NIH and DHHS 2005).

The decontamination system for the BSL-3 and BSL-4 laboratories will include 5 large autoclaves and 11 medium autoclaves. Animal carcass materials will be placed on rack sterilizers for easy introduction or removal of large materials, while smaller autoclave models will be used for general laboratory waste. Once waste material has been processed in an autoclave in and removed from the BSL-3 and BSL-4 biocontainment space, all animal carcass waste will be placed in the tissue digestion system and undergo alkaline hydrolysis for final processing and disposal (mineral oil could be added to the tissue digester to aid in removal of the autoclave bags following processing) (BUMC 2009f).

2.1.5.2 Radioactive Waste

Radioactive waste generated at the NEIDL will consist primarily of solid waste such as paper, plastic and glass contaminated with trace amounts of radioactive isotopes (radioisotopes). The wastes will be limited to those materials that meet the definition of low-level radioactive waste as defined by the Nuclear Regulatory Commission. The radioisotopes that are anticipated to be used at the NEIDL include both long-lived and short-lived radioisotopes. Long-lived radioisotopes must be disposed of off-site. However, waste contaminated with short-lived radioisotopes will be held on-site in the BUMC decay-in-storage facility for periods ranging anywhere from one week to not more than 2 years and 9 months, depending on the radioisotope's half-life, to allow sufficient decay and subsequently disposed of as nonradioactive sanitary waste (BUMC 2009c).

2.1.5.3 Hazardous Chemical Waste

Hazardous-chemical waste generated at the NEIDL could include the following:

- Flammable liquids
- Flammable, toxic liquids
- Corrosive liquids
- Oxidizing liquids
- Ethidium bromide
- Liquid effluents
- Chemical disinfectants

Such wastes will be disposed of at off-site, permitted, commercial disposal facilities (BUMC 2009c) in accordance with the requirements of 40 CFR 260..

2.1.6 Security Risk Assessment

In accordance with federal regulations implementing the Bioterrorism Act of 2002, BUMC has completed a NEIDL site-specific security RA. The security RA identified and characterized the threat posed to the NEIDL-postulated inventory of biological toxins and agents, evaluated inventory protection from theft, loss, or release of the biological toxins and agents, assessed the current or necessary safeguards and security to protect the inventory, and provided recommendations for security enhancements to mitigate vulnerabilities.

In addition, an independent TA was conducted as a component of this RA. Chapter 6 of this document provides a synopsis of the TA because the actual TA contains Restricted Distribution Security Information, making it a controlled document under the provisions in the Bioterrorism Act of 2002. Therefore, Chapter 6 presents an overview of the TA and its findings to provide a description of the linkages between the TA and the overall RA process.

The primary objective of the TA was to identify and evaluate credible scenarios that would involve the internal or external breach of the NEIDL security systems due to a malevolent action, such as a disgruntled laboratory worker spreading a biological agent in the community or terrorist action. As such, the TA provides an evaluation of *initiating events* associated with malevolent acts that could result in the release of a pathogen. In the context of security, the term *system* is used to define an integrated set of building design, security policies and procedures, programs, activities, and equipment for protecting the NEIDL mission, personnel, operations, inventory, and sensitive information. The TA analysis was conducted specific to each of the three sites under evaluation using the *as built* condition of the NEIDL to model the security systems. Initiating event information was then provided to the RA team to integrate into the RA human health and environmental effects analysis.

2.1.6.1 Physical Security

Physical security protection consists of barriers, electronic surveillance systems, and intrusion detection systems that form a comprehensive site-wide network of monitored alarms. Various types of barriers are used to delay or channel personnel or to deny access to sensitive materials, and vital areas. Barriers are also used to direct the flow of vehicles through designated entry control portals and to deter and prevent penetration by motorized vehicles where vehicular access could significantly enhance the likelihood of a malevolent act being carried out.

NEIDL researchers working with select agents will be registered with the CDC before possessing, transferring, or using a select agent. The security layers will be operationally assured using security officers, biometric and card access devices, closed circuit television cameras, automatic door locking systems, and access alarms assigned or installed at each layer's barrier (BUMC 2009c).

Systems will monitor work being performed in high-level biocontainment areas to ensure that two authorized persons are in each area to minimize risk. Access to high-containment laboratories will be restricted to people whose presence is required and authorized. Strict operational protocols will be imposed on laboratory personnel including specific training and background checks before working in the facility. The security system will also monitor and control the Gasketed APR (air-pressure resistant doors) in BSL-4 and BSL-3 entry and exit doors. The Access Control Office will maintain a log of persons entering and exiting the laboratory that will include their name and the time, date, and reason for entering the lab. BUMC's OEHS professionals and security officers (i.e., BU public safety officers) assigned to the laboratory will periodically audit the log (BUMC 2009c).

BUMC security staff will provide building security. Public safety officers assigned to the NEIDL will receive training about the nature of the research, the risk associated with the building's unique emergency response protocols, and enhanced police academy training in addition to the significant, ongoing training program already in place (BUMC 2009c).

2.1.7 Facility Commissioning, Registration, and Operation Process

Commissioning is the process of ensuring that all building systems are installed and perform interactively according to the design intent, that the systems meet the user's operational needs, that the installation is adequately documented, and that the operators are adequately trained. Commissioning begins in the planning phase and proceeds through design, construction, startup, acceptance, and training. Ongoing and

routine inspections of the facility and systems performance assessments continue through the life of the NEIDL (NIH 2008).

Commissioning of biocontainment laboratories includes verifying the design and operational parameters established in the design documents are implemented, as outlined in the BMBL guidelines, NIH *Facility Design Guidelines*, and other applicable guidelines and regulations. As stated in the BMBL, the NEIDL building design and operational procedures must be documented, and the facility must be evaluated before operation to verify that the design and operational parameters have been met (CDC and NIH 2007).

In addition, the operation of the NEIDL must comply with various city, state, and federal regulations must be met before operations with pathogens begin. The NEIDL is subject to the requirements of the Select Agent Program, which requires that the Secretary for HHS issue a certificate of registration.

CDC is responsible for the registration and oversight of laboratories that possess, use, or transfer select agents that could pose a threat to human health. The Animal and Plant Health Inspection Service is responsible for the registration and oversight of laboratories that possess, use, or transfer select agents that could pose a threat to animal or plant health or products. For facilities registered with CDC or USDA that possess, use, or transfer select agents, the Select Agent regulations require the following (GAO 2007a):

- An FBI security RA for a number of individuals involved in the operation of the facility, including each person who is authorized to have access to select agents;
- Written biosafety, security and incident response plans;
- Training of individuals with access to select agents and individuals who will work in or visit areas where select agents are handled and stored;
- A security plan sufficient to safeguard the select agent and toxin against unauthorized access, theft, loss, or release, designed according to a site-specific RA, and that provides protection in accordance with the risk of the select agent or toxin;
- Inspection by CDC or USDA of the facility and its records before the certificate of registration is issued; and
- Maintaining and retaining records relating to the activities covered by the Select Agent regulations.

In addition to the certification from HHS, an inspection of the NEIDL by CDC, USDA, or both, is required. Inspections by CDC or USDA may be conducted without advance notice at any time during the

registration period. Each year thereafter, inspections by the designated Responsible Official for the Facility are required. Facilities must be re-verified and documented at least annually to comply with BMBL and Select Agent guidelines (CDC and NIH 2007). Facility registration with CDC or USDA includes the following (GAO 2007a):

- A list of each select agent and toxin the facility intends to possess, use, or transfer;
- The objectives of the work for each select agent and toxin, including a description of the methodologies or laboratory procedures to be used;
- A description of the physical security, biosafety, and incident response plans;
- Policies on review, authorization, and monitoring of biological research, including public participation in review process;
- Policies on the humane care and use of laboratory animals; and
- Assurance of security awareness and biosafety training for individuals who have access to areas where select agents are handled and stored.

2.2 Site Descriptions

This RA considers the impacts of potential events for urban, suburban, and rural settings for the facility. The same facility design and operations are assumed for all three settings. The following subsections describe the general population, environmental justice, public safety, and emergency response characteristics of the three settings.

Additional details of the sites (i.e., Boston, Tyngsborough, and Peterborough) are presented in Appendix B.

2.2.1 General Population

To evaluate the relative risk (i.e., frequency and consequence) of an accidental or malevolent act that results in a release of a pathogen in the three settings, a basic understanding of the characteristics and demographics—including environmental justice communities, emergency response capability, and medical infrastructure—is necessary for each of the alternate sites. The site characterization data presented in this section such as site location information, utilities descriptions, transportation access, and health care infrastructure began with consideration of the information presented in the *Draft Supplementary Risk Assessment and Site Suitability Analyses* (NIH 2007). Those data were augmented by site visits, additional research, and data-gathering activities.

Interviews were conducted the week of January 26, 2009, with public safety and emergency services officials whose jurisdictions include the three sites under analysis. The interview team used a standardized set of data-collection and analysis tools to assure consistency in the manner in which questions were presented to officials and their responses were recorded.

For the purposes of assessing general populations near the three sites, a 10-kilometer (km) (6.2-mile) radius was defined around each site centered on the actual or hypothetical location of the laboratory. Section F.4 of Appendix F present the resident and non-resident population estimates associated with each of the three sites.

Detailed environmental justice population data are presented in Chapter 10 and Appendix M.

2.2.2 Public Safety and Emergency Response

The following is a general overview and comparison of the public safety and emergency response capabilities at the three sites.

2.2.2.1 Law Enforcement

Law enforcement agencies provide a variety of services that are broadly intended to prevent criminal acts and to identify those responsible for committing crimes. Services include uniformed patrol, criminal investigation and attribution, intelligence gathering and data analysis, crime prevention, and specialized services such as special weapons and tactics (SWAT) teams and explosive ordnance disposal (EOD) teams or bomb squads. Rural and suburban police agencies typically have fewer resources than agencies serving urban areas. That held true when the law enforcement capabilities of Boston were compared those of Tyngsborough and Peterborough. More than 2,000 officers staff the Boston Police Department, while both of the smaller agencies had fewer than 25 officers. Unlike Boston, neither Tyngsborough nor Peterborough police agencies have the capabilities to conduct intelligence gathering nor do they directly participate in an intelligence fusion center. Established by many states and large cities, intelligence fusion centers share information and intelligence within their jurisdictions and with the federal government. Both Tyngsborough and Peterborough police agencies depend on external mutual aid for SWAT and EOD resources (BUMC 2009g).

In addition to the three cities, the BU Police Department (BUPD) has approximately 50 police officers dedicated to the campus. BUPD's 50 police officers have primary jurisdiction over the campus including the NEIDL facility. The BUPD is supplemented by armed public safety officers and non-sworn security

officers. The BUPD officers are also trained to operate within health care and biomedical research facilities (BUMC 2009g).

2.2.2.2 Fire Protection

Fire protection services generally include emergency response to extinguish fires, conduct rescues, encouraging fire prevention through public education programs, fire code enforcement through plan review and site inspections, and fire cause investigation. Specialized fire department services include hazardous material (HazMat) response, marine firefighting, high angle rescue, and trench rescue. Fire protection in the rural and suburban areas of the United States is largely provided by volunteer firefighters (BUMC 2009g).

The Boston Fire Department (BFD) is one of the oldest fire departments in the nation and is staffed by more than 1,600 full-time personnel operating from 35 fire stations. BFD provides specialized response services including a dedicated HazMat team. Personnel have received training to address incidents that involve biomedical research facilities. Boston has also adopted building and fire codes that address the unique characteristics and needs associated with biomedical research facilities. Tyngsborough and Peterborough, with 38 and 50 firefighters, respectively, both depend on volunteer and on-call firefighters. Both departments also depend upon mutual aid from nearby jurisdictions for fire suppression and specialized services including HazMat response (BUMC 2009g) this limits response capabilities respectively.

Successful fire suppression activities depend on access to a reliable water supply system. Of the three sites in the comparative analysis, only the Boston water system has the capacity to support the fire protection needs required to protect the seven-story, 192,000 ft^2 facility (BUMC 2009g).

2.2.2.3 Emergency Medical Services

Emergency medical services (EMS) involve the delivery of Basic Life Support (BLS) and Advanced Life Support (ALS) treatment and the transport of patients by ambulance to a hospital emergency department. EMS is typically a function of the fire department with firefighters cross trained as emergency medical technicians (EMTs) and paramedics (EMT-P). Fire department EMS can be a *first responder* service operating only from a HazMat vehicle. A HazMat vehicle is a vehicle specifically designed for responding to HazMat incidents. It is basically a truck filled with all the tools and supplies required for such situations. Many fire departments also provide ambulance response and patient transport services.

EMS can also be delivered by an independent government agency or a commercial provider (BUMC 2009g).

In addition to fire fighting, Tyngsborough and Peterborough fire department personnel provide EMS within their response areas. Tyngsborough provides BLS first responder services that are supported by a third-party ambulance provider. The Peterborough Fire Department provides both BLS and ALS first responder and ambulance transport services. EMS in Boston are provided by a division of the Public Health Commission. Boston EMS uses more than 350 EMTs and paramedics to staff an average of 20 ambulances at all times (BUMC 2009g).

2.2.2.4 Emergency Management

Emergency management functions fall into the categories of prevention, preparedness, response, and recovery. They include tasks such as developing contingency plans, coordinating resources and information during an emergency, processing disaster declarations to initiate external assistance, and guiding recovery operations. Each jurisdiction appoints an emergency management coordinator who is responsible for administering the preparedness program. Larger jurisdictions normally staff an Office of Emergency Management, while smaller cities typically assign the emergency management function to the fire department. Larger jurisdictions might also construct a dedicated Emergency Operation Center (EOC) that serves as a centralized location from which large-scale emergency incidents are managed (BUMC 2009g).

The mayor's Office of Emergency Preparedness is responsible for administering the homeland security and emergency management activities for Boston. A full-time emergency management coordinator leads a staff that is based out of Boston's EOC. The fire chiefs of both Tyngsborough and Peterborough also serve as the emergency management coordinator within their jurisdictions. Neither town has dedicated space for use as an EOC nor full-time dedicated Office of Emergency Management staff to support emergency preparedness functions (BUMC 2009g).

2.2.2.5 Public Health Preparedness

Public health agencies are responsible for providing services intended to safeguard the health and welfare of the general population. Public health agencies deliver a number of services including providing public health education, administering vaccination programs, identifying and monitoring disease trends, and assuring the safety of the food and water supplies. The threat of bioterrorism has significantly expanded the role of public health agencies in most communities. Since 2002 the CDC has provided funding to state

and local health departments to enhance public health preparedness measures. New public health initiatives include the delivery of mass prophylactic medication, enhanced disease monitoring through active surveillance and atmospheric sampling, and direct involvement in traditional first responder activities (BUMC 2009g).

Medical Response System. Boston was an early adopter of the Metropolitan Medical Response System concept developed after the terrorist attacks in 1995. The Boston Public Health Commission has developed a robust public health preparedness program that is administered by the Office of Public Health Preparedness. That office is responsible for a number of tasks including developing plans to deliver mass prophylactic care in response to disease outbreak such as pandemic influenza. Tyngsborough and Peterborough each have one individual assigned the duties of health officer. They are primarily tasked with providing basic services such as restaurant inspections and approving the installation of septic systems. Through the use of Assistant Secretary for Preparedness and Response (ASPR) and CDC grant funding, plans are developed by state public health departments and local jurisdictions responsible for preparedness efforts needed to protect the residents of the towns (BUMC 2009g).

Emergency Services. The capabilities of the public safety and emergency service agencies vary significantly among each of the three sites. Since 2001 the capabilities of emergency service agencies in the nation's urban areas have been enhanced through grant-funded U.S. Department of Homeland Security (DHS) initiatives. Boston public safety agencies possess a greater number of resources and capabilities because of the size of the agencies, collocation with other agencies, and participation in national homeland security initiatives. Boston area agencies formed the nine-city Metro-Boston Homeland Security Region (MBHSR) under the DHS Urban Area Security Initiative. The MBHSR has implemented regional initiatives to enhance coordination among emergency response organizations through training, acquiring compatible equipment, and creating a regional radio interoperability program. Regional terrorism and criminal analysis activities are coordinated through the Boston Regional Intelligence Center. Heavily populated metropolitan areas, such as Boston, have developed the public safety infrastructure and capabilities necessary to provide services across the spectrum of prevention, preparedness, response, and recovery (BUMC 2009g).

2.3 References

The American Committee of Medical Entomology (ACME) of the American Society of Tropical Medicine and Hygiene (ASTMH) 2002. Arthropod Containment Guidelines version 3.1. Accessed online at <http://www.astmh.org/AM/Template.cfm?Section=ACME&Template=/CM/ContentDisplay.cfm&ContentID=1444>. Accessed January 3, 2012.

BUMC (Boston University Medical Center). 2008a. *Boston Public Health Commission Biological Laboratory Safety Permit Application*: Boston University Medical Center National Emerging Infectious Diseases Laboratories, September 3, 2008 Draft.

Boston University Medical Center (BUMC) 2008. Site Visit and Data Collection Plan to Support the Risk Assessment. December 1-4 2008.

_____. 2009a. Kevin Tuohey to Chuck Pergler. Tetra Tech Inc. Request for Information. Letter. January 28, 2009.

_____. 2009b. Telephone Meeting Notes, Uninterruptible Power Supply (UPS), Emergency On-Site Power, and Spill Response. Conversation with Tetra Tech, Inc. June 19, 2009.

_____. 2009c. Tetra Tech Site Visit. December 4, 2008 and June 1-4, 2009.

_____. 2009d. Telephone Meeting Notes, HVAC/ Ventilation system overview. Conversation with Tetra Tech, Inc. June 16, 2009.

_____. 2009e. Telephone Meeting Notes, IBC and change management and Source Term. Conversation with Tetra Tech, Inc. July 31, 2009.

_____. 2009f. Telephone Meeting Notes, Tissue Digester, Liquid Waste, and Solid Waste. Conversation with Tetra Tech, Inc. July 1, 2009.

_____. 2009g. Tetra Tech Site Visit for Threat Assessment. January 26-30, 2009.

Boston University Medical Center (BUMC) 2010. Implementation Plan for Enhancing the Research Culture of Safety at Boston University and Boston Medical Center. Draft dated August 15, 2010.

Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH). 1999. *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. U.S. Government Printing Office, Washington, DC. http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf

_____. 2007. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Government Printing Office, Washington, DC.

CUH2A, Smith Carter, and Hemisphere Engineering 2005. National Emerging Infectious Disease Laboratory, Basis of Design, 100 percent Construction Drawings. November.

Government Accounting Office (GAO), 2007. High Containment Biosafety Laboratories: Preliminary Observations on the Oversight of the Proliferation of BSL-3 and BSL-4 Laboratories in the United States, October 4. Available online at, http://www.gao.gov/new.items/d08108t.pdf

Haidar, Mohammad 2005. Memo to Kevin Jelinek, CUH2A, BU_NEIDL Structural Seismic Design of Containment. 10 Nov. 2005.

Health Research Extension Act of 1985, Public Law 99-158, November 20, 1985. Accessed at: http://grants.nih.gov/grants/olaw/references/hrea1985.htm

Kajunski, Joe 2009. Telephone Meeting Notes, HVAC. Conversation with Tetra Tech, Inc. June 19, 2009.

Klempner, Mark S. M.D. 2008. National Emerging Infectious Diseases Laboratories Overview- Tetra Tech Briefing. December 3, 2008.

Mary Ann Liebert, Inc. 2003. Vector-Borne and Zoonotic Diseases Volume 3, Number 2, 2003 http://www.liebertonline.com/doi/pdf/10.1089/153036603322163475

National Emerging Infectious Diseases Laboratory (NEIDL) 2008. Fact Sheet May - http://www.bu.edu/neidl/data/pdf/NEIDL-Fact-Sheet.doc

National Institutes of Health (NIH) 2002b. Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals. August 7, 2002. Reprinted on Office of Extramural Research website at http://www.grants.nih.gov/grants/olaw/references/phspol.htm

_____. **2003.** NIH Design Policy and Guidelines. Office of Research Facilities. Spring 2003.Accessed online at http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/policy-index.htm

_____. **2007.** Model Commissioning Guide and Plan. Office of Research Services, Division of Engineering Services, Design, Construction and Alteration Branch, Technical Resource Group. 2007. Accessed online at http://des.od.nih.gov/eWeb/research/farhad2/Commissioning/nih_cx_guide/ComGuideTitle.htm.

_____. **2008.** Design Requirements Manual. NIH, Office of Research Facilities, Division of Technical Resources. Version 1.7. August 27 2008. Accessed online at

_____. **2010a.** http://www.nih.gov/about/NIHoverview.html

National Institutes of Health (NIH) and Department of Health and Human Services (DHHS) 2005. NIH and DHHS (National Institutes of Health and Department of Health and Human Services), 2005. Final Environmental Impact Statement, National Emerging Infectious Disease Laboratory, Boston, Massachusetts. December. NIAID 2003a National Institutes of Health. "NIAID Funds

Construction of Biosafety Laboratories." *NIH News*. September 30, 2003.
http://www2.niaid.nih.gov/Newsroom/Releases/nblscorrect21.htm (website no longer found)

National Research Council (NRC) 1996. Guide for the Care and Use of Laboratory Animals. National Academies Press. ISBN 0-309-05377-3. July 2006.

Table of Contents

3. Pathogens

3.1 Introduction

NIAID is the primary Institute at the NIH for emerging infectious disease research. The mission of NIAID is to carry out research needed to understand the pathogenesis of these microbial pathogens and the host responses to them, and to translate this knowledge into useful interventions and diagnostic tools for an effective response to natural outbreaks, accidental releases, or bioterrorist events. For the latter, NIAID is committed to an agendum of basic and translational research for bioterrorism defense, working with partners in academia, industry, and other private and public-sector agencies. NIAID has developed a Strategic Plan for Biodefense Research to guide the implementation of the necessary research and development program (US Department of Health and Human Services 2011).

Key elements of the NIAID Strategic Plan for Biodefense Research are:

- Support biomedical research on microbes and the human immune response to them
- Apply such research to the discovery and development of vaccines, drugs, and diagnostic tests designed to protect the general population
- Ensure that the United States has enough research facilities to carry out these activities.

To meet these key elements, research is needed on a range of microbial pathogens.

3.2 Selected Pathogens

This RA addresses 13 pathogens that are currently considered likely research areas of interest at NEIDL:

Pathogens	**Abbreviation**
BSL-3	
1. *Bacillus anthracis* (either BSL-2 or BSL-3)	*B. anthracis*
2. *Francisella tularensis*	*F. tularensis*
3. *Yersinia pestis*	*Y. pestis*
4. 1918 H1N1 influenza virus	1918 H1N1V
5. SARS-associated coronavirus	SARS-CoV
6. Rift Valley fever virus	RVFV
7. Andes virus (either BSL-3 or BSL-4[a])	ANDV
BSL-4	
8. Ebola virus	EBOV
9. Marburg virus	MARV
10. Lassa virus	LASV

Pathogens	Abbreviation
11. Junín virus	JUNV
12. Tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus)	TBEV-FE
13. Nipah virus	NIPV

3.3 Pathogen Characteristics

Characteristics of each of the 13 pathogens and the diseases they cause can be used to place them (Table) among four hazard categories for humans that were identified by the Advisory Committee to the Director of the NIH (Mahmoud 2008). The categories are:

- Highly transmissible pathogen, highly pathogenic, with a high case fatality ratio (CFR).
- Highly transmissible pathogen, pathogenic, with a lower CFR.
- Poorly transmissible pathogen, highly pathogenic, with a high CFR.
- Vector-borne pathogen that is relevant to the particular sites under evaluation.

These four categories serve to describe the general nature of risks from infectious pathogens to laboratory workers and to the public at large, and to describe in broad terms the range of potential effects from the release of such pathogens. The first category describes characteristics of pathogens that can be easily spread from person to person during casual contact, potentially resulting in large numbers of infections among the general population. This category also specifies a relatively high number (based on comparisons between the 13 pathogens) of deaths due to infection. As indicated in Table 3-1, only SARS-associated coronavirus is placed into this category.

The second category likewise describes characteristics of pathogens that can be easily spread from person to person during casual contact. However, it differs based on a relatively lower specified fatality rate that would be expected to result in fewer deaths as a result of infection. As indicated in Table 3-1, only the 1918 H1N1 influenza virus is concluded as meeting these two criteria.

The third category describes characteristics of pathogens that have very little or no ability to spread from person to person during casual contact, and therefore have very little or no potential to spread among the general population. However, infection from a pathogen in this category can pose a high or relatively high risk of death for an infected laboratory worker if the infection is not treated. As indicated in Table 3-1, 10 of the 13 pathogens meet these two criteria.

The fourth category recognizes that some of the 13 pathogens exhibit the additional characteristic of a vector (such as a mosquito, tick, or other arthropod) being involved in the spread of infections among populations of humans and animals. This refers to spread of these pathogens that can occur apart from the direct person to person pathway. As a result, such pathogens, if established in vector species in the environment, could be particularly difficult if not impossible to eradicate. As indicated in Table 3-1, 4 of the 13 pathogens fit this characterization. The environmental concerns posed by vector-borne pathogens are considered in detail in Chapter 7.

Table 3-1. Pathogen categorization by pathogen and disease characteristics

Highly transmissible, highly pathogenic, and high CFR	Highly transmissible, pathogenic, and lower CFR	Poorly transmissible, highly pathogenic, and high CFR	Vector-borne relevant to the sites to be assessed
		Bacillus anthracis[c]	*Francisella tularensis*[c]
		Francisella tularensis[c]	*Yersinia pestis*[c]
		Yersinia pestis[c]	Rift Valley fever virus[a,e]
		Andes virus[d]	TBEV-FE [b]
SARS-associated coronavirus[a]	1918 H1N1 Influenza virus[a]	Ebola virus[b]	
		Marburg virus[b]	
		Lassa virus[b]	
		Junín virus[b]	
		TBEV-FE[b]	
		Nipah virus[b]	

a. BSL-3 biocontainment precautions with enhancements are recommended (CDC and NIH 2007)
b. BSL-4 biocontainment precautions recommended (CDC and NIH 2007)
c. BSL-3 biocontainment precautions are recommended (CDC and NIH 2007)
d. BSL-4 biocontainment is required when infecting rodent species permissive for chronic infection (CDC and NIH 2007)
e. RVFV will be assessed also as Poorly transmissible (person-to-person), pathogenic, and lower CFR

Several parameters, including potential consequences of release of pathogen, were considered in placement of the pathogens into the above hazard categories:

- Susceptible host species (host range)
- Infectivity (infectious dose, primary routes of human infection, primary infection rate)
- Incubation period
- Infectious period

- Transmissibility (including secondary transmission)
- Reproduction ratio (R_0)
- Pathogenesis
- Morbidity
- Mortality (case fatality ratio)
- Pathogen concentrations
- Pathogen stability
- Reservoirs
- Vectors
- Availability/effectiveness of antiviral/antibacterial agents and vaccines

3.4 Literature Review

To compile representative data for the thirteen pathogens, literature searches were conducted which resulted in review of more than 2,500 publications from a variety of sources, with primary emphasis being placed on data published in peer-reviewed scientific journals. Additional sources included reference and textbooks, government documents, conference proceedings, and personal communications with leading authorities. Most of these sources are available through the internet and in printed copy. Data are representative of the open literature as of January 2009, but are not necessarily exhaustive. A brief update from the open literature was performed in April, 2010 and May 2011. It is acknowledged that additional relevant data may exist under classified status; these data were not available for review for this RA. Internet-based sources used for this RA included:

- PubMed
- CDC fact sheets
- BMBL 5th Edition, 2007
- The International Catalog of Arboviruses
- Terrestrial Animal Health Code
- ProMed-mail
- Defense Technical Information Center
- Community Strategy for Pandemic Influenza Mitigation, 2007
- Public Health and Biosafety Practices for Research with 1918 H1N1 Influenza Virus

PubMed is a free service of the U.S. National Library of Medicine and the NIH that comprises an internet platform for electronic access to more than 19 million citations for biomedical articles from MEDLINE

and life science journals. It may be freely accessed at http://www.ncbi.nlm.nih.gov/pubmed/. Citations may include links to full-text articles from PubMed central or publisher web sites. Many of these articles are freely available while others can be accessed through a subscriber such as a university library. CDC Fact Sheets for a variety of topics are freely available online from the DHSS, CDC. CDC documents may be freely accessed at http://www.cdc.gov/.

Biosafety in Microbiological and Biomedical Laboratories, 5th Edition (BMBL) 2007: BMBL, is the cornerstone of biosafety practice and policy in the United States. Historically, the information in this publication has been advisory in nature even though legislation and regulation, in some circumstances, have overtaken it and made compliance with the guidance provided mandatory. The 5th edition of the BMBL remains an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories, from a biosafety perspective and is not intended as a regulatory document; though it is recognized that it will be used that way by some. BMBL is available at http://www.cdc.gov/OD/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf.

The International Catalog of Arboviruses Including Certain Other Viruses of Vertebrates originally began publishing data from a continuously revised, confidentially-communicated working document entitled *The Catalog of Arthropodborne and Selected Vertebrate Viruses of the World*. The first published edition in 1967 contained information regarding 204 viruses; two supplements describing 37 and 14 additional viruses were published in the *American Journal of Tropical Medicine and Hygiene* in 1970 and 1971, and the second edition describes 359 viruses. The Catalog currently describes more than 573 registered viruses. These catalogs are results of efforts of the Subcommittee on Information Exchange of the American Committee on Arthropod-borne Viruses. The history of the origin and development, and the operation of the Working Catalogue are described in this second edition, as are the two other Subcommittees whose activities relate directly to the Catalogs (Interrelationships Among Catalogued Arboviruses formed in 1966, and Evaluation of Arthropod-borne Status started in 1971). The Catalog is now freely available online through the CDC at www.ncid.cdc.gov/arbocat.

The Terrestrial Animal Health Code 2009 is freely available from the World Organization for Animal Health (OIE) on the internet at www.oie.int/eng/normes/mcode/en_sommaire.htm. The purpose of the Code is to assure the sanitary safety of international trade in terrestrial animals (mammals, birds, and bees) and their products by detailing health measures to be used by veterinary authorities. Information contained in the Code is relevant to some of the diseases under consideration in the RA (i.e. tularemia, anthrax, Rift Valley fever, and Nipah virus).

ProMed-mail (Program for Monitoring Emerging Diseases) is a free internet-based reporting system dedicated to rapid global dissemination of information on outbreaks of infectious diseases and operates as an official program of the International Society for Infectious Diseases. It is freely available at http://promedmail.oracle.com/pls/otn/f?p=2400:1000.

Defense Technical Information Center (DTIC): DTIC serves the Department of Defense (DoD) community as a central resource for DoD- and government-funded scientific, technical, engineering, and business-related information. All visitors can search DTIC's publicly accessible collections and read or download scientific and technical information, using DTIC Online service. DTIC also makes available sensitive and classified information to eligible users who register for DTIC services. http://www.dtic.mil/dtic/index.html

Community Strategy for Pandemic Influenza Mitigation (US Department of Health and Human Services 2011) provides interim planning guidance for State, territorial, tribal, and local communities that focuses on several measures other than vaccination and drug treatment that might be useful during an influenza pandemic to reduce its harm. The document is available at http://www.flu.gov/professional/community/commitigation.html.

"Public Health and Biosafety Practices with 1918 H1N1 Influenza Virus", Safety symposium, NIH RAC Dec 2, 2008. An agenda, audio recording, and slide presentations are freely available at http://oba.od.nih.gov/rdna_rac/rac_past_meetings_2000.html#RAC2008 and http://oba.od.nih.gov/oba/RAC/meetings/dec2008/FINAL%20AGENDA.pdf

Non-internet sources reviewed included textbooks and reference books such as:

- Principles and Practice of Infectious Diseases 7th edition, 2010, GL Mandell, JE Bennett, R Dolin editors, ISBN 978 0 4430 6839 3
- Topley & Wilson's Microbiology and Microbial Infections 19th edition, 2005, ISBN 0 340 614706
- Infectious Disease Epidemiology: Theory and Practice 2nd edition, 2007, ISBN 978 0 7637 2879 3
- Biological Safety: Principles and Practices 4th edition, 2006, ISBN 978 1 55581 339 0
- The Arboviruses: Epidemiology and Ecology, CRC Press, 1989, ISBN 0849343852 (v.1)
- An Introduction to Experimental Aerobiology, 1969, SBN: 471 21558 9
- Bioaerosols Handbook, 1995, ISBN: 0 87371 615 9
- Bioaerosols, 1995, ISBN: 0 87371 724 4

3.5 Abridged Literature Search Results of Pathogen Characteristics

The following are brief synopses of literature search results for each pathogen, sorted by biosafety level (BSL) designation for fully virulent, wild-type strains. The goals of providing the synopses are to introduce the 13 pathogens to the reader and provide a basis for their selection for analysis in this RA. Pathogen characteristics that are highlighted include a general description of the diseases caused and extent of knowledge regarding infectious doses and potential for spread from person to person. The history and potential for laboratory associated infections is also discussed. All 13 pathogens have the potential to cause to cause life-threatening and fatal illnesses and are the subjects of on-going research in high bio-containment laboratories around the world. Full search results of the available literature on these pathogens are contained in Appendix C Pathogen Characteristics.

Data concerning infectious doses for humans for these 13 pathogens are minimal or absent in the literature. Accordingly, opinion elicited from a panel of experts as described in Appendix H was used to supplement data from the literature for infectious doses. All other data is this chapter were obtained from the sources listed above and are individually referenced below.

3.5.1 BSL-3 Biocontainment Precautions: Pathogens

3.5.1.1 Bacillus anthracis

Introduction

Bacillus anthracis is a bacterium that causes the animal and human disease known as anthrax. For this RA, *B. anthracis* was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). Anthrax is contracted largely by exposure to *B. anthracis* spores from the environment. Based on a review of the existing epidemiologic literature, there is no evidence for direct spread of *B. anthracis* vegetative forms from a person infected with anthrax to another person. The human infectious dose of *B. anthracis* is not known. The potential for laboratory acquired infections (LAI) from *B. anthracis* exists and LAI have been reported. There were no secondary cases of anthrax in the close contacts of the laboratory worker or in the community as a result of the LAI.

The name of the pathogen is derived from the Greek word for coal, *anthrakis,* as the disease typically causes black, coal-like skin lesions. Reports of suspected anthrax outbreaks in animals and humans have

been reported for centuries throughout the world, with the earliest reports dating back to 1250 BC. Recently, *B. anthracis* has been of interest to the public and scientific community after an intentional release of spores caused disease and deaths in humans in the United States in 2001 (Greene et al. 2002; Inglesby et al. 2002)

Outbreaks of anthrax in animals occur routinely throughout the world. Grazing herbivores, especially cattle, sheep, goats, and horses are particularly susceptible to anthrax, while pigs are more resistant. Birds are resistant with some exceptions such as ostrich (Beyer and Turnbull 2009) The disease is endemic among animals in the Middle East, equatorial Africa, Mexico and Central America, Argentina, Cambodia, Chile, China, India, Myanmar, Papua New Guinea, Peru, Thailand, and Vietnam. Recent outbreaks have been described among cattle and horses in Canada (Center for Infectious Disease Research & Policy 2006; Kumor et al.) and non-human primates in Cameroon and Ivory Coast (Leendertz et al. 2004; Leendertz et al. 2006; Leendertz et al. 2006). In the US, anthrax is seen in animals in several counties in southwestern Texas near the border of Mexico (Johnson), and in pockets of infection in Nebraska, Oklahoma and South Dakota (McBride et al. 1998) During the period1996-2001, 21 outbreaks in animals in the US accounted for 1,862 animal deaths (Johnson 2008).

The natural reservoir for anthrax spores is the soil with reports of spores in soil samples from around the world (Turnbull et al. 1998; Hugh-Jones and Blackburn 2009) (Dragon et al. 2001) (Titball et al. 1991) Naturally occurring anthrax infections in humans occur after contact with the spores, an anthrax-infected animal, or anthrax-contaminated animal products.

This pathogen is studied in laboratories around the world, including high biocontainment laboratories in the US. BSL-3 practices, biocontainment equipment, and specially designed biocontainment facilities are recommended for work involving production quantities or high concentrations of cultures, for screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. *B. anthracis* is a Select Agent requiring registration with CDC and/or US Department of Agriculture for possession, use, storage, and/or transfer (Chosewood et al. 2009).

Human Disease and Outbreaks

Human anthrax is classified based on the natural route of infection. Cutaneous anthrax is acquired by contact with *B. anthracis* through a skin lesion; gastrointestinal anthrax is contracted via the oral route, usually by ingesting contaminated food, primarily meat from an herbivore animal that died from the

disease; and inhalational anthrax which is acquired by breathing in airborne spores via the respiratory tract (Inglesby et al. 2002) (Chosewood et al. 2009). Naturally occurring anthrax is also classified by the occupation of the exposed individual. This differentiates non-industrial exposure in butchers, farmers, and veterinarians from industrial exposure in those employed in the processing of wool (woolsorter's disease), bones, and hides. The number of cases of natural disease in humans is directly correlated to the level of animal disease and to the level of exposure to affected animals, which occurs mostly in developing countries.

Cutaneous disease accounts for approximately 95 percent of naturally occurring human cases. The skin lesions develop 1-12 days after infection and are characterized by ulceration and the characteristic black eschar that is reminiscent of coal. Patients who are not treated early may progress to a systemic disease with sepsis and death. Ingestion of *B. anthracis* spores may result in oropharyngeal anthrax that is localized to the oral cavity, tongue, tonsils, or pharyngeal wall. Ingestion may also lead to gastrointestinal anthrax. This is the next most common form and accounts for the majority of the remainder of naturally occurring cases. Symptoms such as nausea, vomiting, and diarrhea appear 3-7 days after ingestion of contaminated food. Symptoms may be mild at first and, if untreated, can lead to bleeding in the gastrointestinal tract resulting in vomiting blood or blood in the stools. Inhalational anthrax in humans is rare, is infrequently seen in industrial settings and has been noted as a result of accidental or intentional release of spores (Meselson et al. 1994) (Greene et al. 2002). The incubation period in inhalational anthrax is typically 1-6 days; with a wide range of 2-43 days noted in an accident at Sverdlovsk, USSR. Germination of spores is followed by rapid hemorrhage, edema, and necrosis of surrounding tissue from release of bacterial toxins. The case fatality rate is highest for inhalational anthrax, especially in untreated cases or where the treatment was initiated late. Meningitis is a frequent complication of inhalational disease and contributes to poor outcomes (Sejvar et al. 2005).

The true numbers of cases of naturally occurring anthrax in countries where the disease is endemic are not known. This is due to lack of surveillance systems to track the disease, lack of facilities to diagnose the disease, and under-reporting. It is estimated that there have been several hundred thousand cases of cutaneous anthrax over the past several centuries (World Health Organization. 2008). Numbers of cases of gastrointestinal anthrax are estimated to be in the several thousands and naturally occurring inhalational anthrax is rare.

An oft-cited accidental release of *B. anthracis* spores was noted in 1979 from a military facility in the former Soviet Union (Meselson et al. 1994; Wilkening 2006). It is noted that *B. anthracis* is the only

pathogen examined in this RA for which there is a documented historical example of a large-scale aerosol release from a biological research facility that caused infections in members of the public downwind of the release point (the Sverdlovsk incident, discussed in Appendix J). The exact quantity of *B.anthracis* that was released from the facility is not known. There were a total of 77 likely human cases with 66 deaths from this release. In a factory that was 2.8 km downwind of the facility, the attack rate was calculated to be approximately 1-2% with 18 out of about 1500 employees infected, including 10 out of 450 employees working in a single unpartitioned building (Meselson et al. 1994).

Naturally occurring anthrax in all three forms is rare in the US and has been infrequently seen since the first half of the 20th century. Most of the cutaneous anthrax cases in the US are linked to the processing of imported goat hair, hides, and other animal products.

More recently, there have been reports of anthrax infections from accidental exposure to animal hides used in drums. Cases of inhalational anthrax were confirmed in a drum-maker from New York City (Nguyen et al. 2010),London, England (Anaraki et al. 2008) and from Scotland (2006). There were two cases of cutaneous anthrax in a drum maker and his child in Connecticut in the US (Centers for Disease Control and Prevention (U.S.) 2008; Guh et al. 2010). A rare presentation of gastrointestinal anthrax was noted in a woman in New Hampshire in the US who participated in a drumming circle (Centers for Disease Control and Prevention (U.S.) 2010). In August 2011, a case of inhalational anthrax was confirmed in a patient who was hospitalized in Minnesota (The Center for Infectious Disease Research and Policy (CIDRAP) 2011). Preliminary evidence suggests that this person may have contracted the disease from a natural source (soil and animal remains) while traveling through the states of North Dakota, Montana, Wyoming, and South Dakota.

In the past decade, there have been outbreaks of anthrax associated with intravenous drug users in Scotland, prompting the suggestion of including 'injectional' anthrax as a type of disease (Ringertz et al. 2000; Booth et al. 2010; Jallali et al. 2011). The heroin they used was reportedly contaminated with *B. anthracis*, presumably spores. The disease was characterized by skin lesions, sepsis and meningitis with a total of 31 cases and 11 deaths in the 2010 outbreak.

The 'anthrax attacks' in the US in October 2001 are a notable example of the potential for *B. anthracis* spores to be used for malevolent purposes. The intentional release of spores through the US postal system resulted in a total of 22 cases of anthrax (11 inhalational, 11 cutaneous); 5 of the inhalational cases were fatal (Jernigan et al. 2002). Twenty of these cases (91%) were associated closely with the US Postal

Service in that those affected were either mail handlers or were exposed at worksites where mail contaminated with *B. anthracis* spores was processed or received. The attack rate at one postal sorting facility was estimated to be 1.2% (Greene et al. 2002; Inglesby et al. 2002).

The diagnosis of anthrax is based on the clinical picture along with a strong epidemiological history of appropriate occupation and exposure that could include travel and contact with animal products from developing countries A positive diagnosis of anthrax is made by using the Gram stain to demonstrate square-ended, encapsulated, Gram-positive chains of bacilli in blood smears and tissues (World Health Organization. 2008). The laboratory confirmation of anthrax is made from microbiologic cultures. Cutaneous anthrax is often diagnosed with skin biopsies.

Anthrax can be prevented or treated effectively with several different anti-bacterial medications if the medications are given early in the course of disease. Delays in diagnosis and institution of appropriate therapy, especially in inhalational anthrax, contribute to the high case fatality rate in human anthrax. The CDC recommends the use of intravenous or oral doxycycline or fluoroquinolones depending on the type and severity of disease (2001). Often, combinations of appropriate antibiotics by the intravenous route are used for severe cases, especially with suspected meningitis. Duration of therapy is often guided by clinical improvement and may be prolonged in cutaneous anthrax. For those who have been exposed, prophylaxis is recommended with oral doxycycline or fluoroquinolones for 60 days to prevent the disease. There is also a recommendation to add a 3-dose regimen of the available vaccine to the oral medications (2001).

There is an FDA-licensed vaccine for anthrax available in the US. The vaccine is produced by Emergent BioSolutions, Inc., Rockville, MD (BioThrax®, Anthrax Vaccine Absorbed). A precursor to the currently available vaccine was shown to be 92.5% effective in a clinical trial (Brachman et al. 1962) The pre-exposure use of this vaccine is based on quantifiable risk of exposure and is recommended for workers in settings in which repeated exposure to aerosolized *B. anthracis* spores might occur (Prevention 2002; CDC and NIH 2007). These guidelines do not recommend the vaccine for members of the general public who do not engage in work that places them at risk for repeated exposures. There is a recommendation to consider pre-exposure vaccination in emergency first responders under the supervision of an occupational health program for purposes of maintaining a workforce ready and prepared to respond to agents of bioterrorism such as *B. anthracis (Wright et al. 2010)*

For non-vaccinated individuals who may have been exposed to aerosolized *B. anthracis* spores, the CDC recommends the following post-exposure prophylaxis: 60 days of selected oral antibiotics in conjunction with a 3-dose regimen of BioThrax® (AVA) vaccine, a combination that has proven effective in nonhuman primates exposed to *B. anthracis* (CDC, 2000). Antibiotics taken by exposed individuals may prevent infection if applied before inhaled spores germinate and reproduce, while the vaccine may promote the immune system to neutralize disease-causing toxins produced by active *B. anthracis* vegetative cells.

B. anthracis occurs in two forms. One is the vegetative form that occurs within the low-oxygen environment of the human or animal host. Once outside the host (for example by means of shedding from the blood at death of an infected host), sporulation commences on exposure to air and *B. anthracis* persists in the environment in the spore form. Anthrax is contracted by animals and humans largely by the uptake of spores from the environment (World Health Organization. 2008). Thus, based on a review of the available epidemiologic literature, human anthrax disease is generally considered to be non-communicable in the classical sense of the vegetative form of the pathogen spreading directly from a person infected with anthrax to another person. There are no data to indicate that patient -to-patient transmission of anthrax occurs (Inglesby et al. 2002). Direct person-to-person transmission of anthrax has not been described for inhalational or gastrointestinal anthrax.

There are rare reports of person-to-person transmission of cutaneous anthrax. There are reports of humans acting as vectors in physically carrying spores on hands or inanimate items such as clothing to close contacts resulting in infection in the close contact (World Health Organization. 2008). In one epidemiologic study in Gambia, the transmission of spores was mediated by inanimate objects such as shared grooming instruments (Heyworth et al. 1975). The most recent example of this is the cutaneous anthrax that developed in a 7-month old infant who most likely came into contact with *B. anthracis* spores while being held by co-workers of his mother at her workplace in New York City that was contaminated with spores during the 2001 intentional release (Freedman et al. 2002).

Disease in Medically Vulnerable Subpopulations

Children are infected by anthrax, though the disease among children is reported rarely in the literature. This likely indicates under-reporting or under-diagnoses rather than a true lower incidence (Bravata et al. 2006). There are no published reports of increased susceptibility to anthrax among children, the elderly, or those among disadvantaged socio-economic status in the US. Similarly, there are no specific reports of increased susceptibility to anthrax among those with immune-compromised conditions such as diabetes or

HIV/AIDS. It is postulated that the infectious dose required to cause anthrax infections among those with immune-compromised conditions and lung disease may be lower, based on the experience from the Sverdlovsk accidental release (World Health Organization. 2008). The worldwide literature on anthrax in pregnancy is limited with a total of 6 cases reported (Kadanali et al. 2003; Jamieson et al. 2006). In two cases reported in 2003, both women were successfully treated, though they both experienced pre-term labor and delivery (Kadanali et al. 2003). There are no published reports on possible associations of asthma and anthrax.

Human Infectious Dose

The human infectious dose for *B. anthracis* is not known. With regard to industrial exposures and infections, the body of human evidence with regard to inhalational anthrax suggests that humans have a moderate level of resistance to exposure, given the relative rarity of human cases among animal workers who likely inhaled spores repeatedly (World Health Organization. 2008). No human experimental data are available for inhalational anthrax and it was not possible to determine the infectious doses for the patients affected in the anthrax attacks of 2001 (Greene et al. 2002; Jernigan et al. 2002) or in the Sverdlovsk facility release in 1979 (Meselson et al. 1994). Estimates of the dose needed to cause infection in 50% of exposed individuals (HID_{50}) range from 8,000 to 55,000 spores (Franz 2009) (Wilkening 2006). One study estimated the HID_{50} dose to be as low as 100 spores (Peters and Hartley 2002). Data from experiments with non-human primates for inhalational anthrax provide an estimate in the range of 4,130 to 27,000 spores required to cause lethal infection in 50% of cynomolgus monkeys exposed to *B. anthracis* (LD50) (Glassman 1958) (Brachman et al. 1966). A study in rhesus monkeys noted this LD_{50} estimate to be in the range of 96,800 spores.

Though inhalational route is considered the most dangerous, many human infections occur by the cutaneous or intestinal route due to consumption of infected meat from terminal near-death or recent-death slaughter of livestock in developing countries. The human infectious dose for *B. anthracis* by these routes is also not known.

Laboratory Acquired Infections

Anthrax infections were frequently reported among laboratory accidents prior to 1965 (Pike et al. 1965). In the past few decades, there have been very few laboratory-acquired anthrax infections, though accidents and exposures have occurred. Of the 15 recently reported laboratory-related incidents involving *B. anthracis* detailed in the Biosafety Appendix, only one incident has resulted in anthrax infection (Biosafety Review, Appendix D).

In the aftermath of the October 2001 intentional release, a laboratory worker in Texas was confirmed to have cutaneous anthrax (Centers for Disease Control and Prevention (U.S.) 2002; Centers for Disease Control and Prevention (U.S.) 2002). The investigation revealed that the possible source of *B. anthracis* spores may have been the surfaces of vials containing *B. anthracis* isolates that the worker had handled while placing them in the freezer. These isolates were from environmental samples taken during the intentional release investigation.

There are several lessons learned from a review of these laboratory incidents. Laboratory-associated infections can occur from laboratory accidents leading to clinical infection in the worker. Only cutaneous anthrax has been confirmed from laboratory exposure in the past 10 years. There were no secondary cases of anthrax in the close contacts of the laboratory worker or in the community as a result of the laboratory-acquired infection. These incidents reinforce the importance of biosafety practices and laboratory worker education and training.

Summary

B. anthracis causes anthrax, an ancient disease that continues to be seen in animals throughout the world, with very few naturally occurring human infections. The pathogen is, in general, poorly transmissible and humans can acquire the disease when they come into contact with spores, anthrax-infected animals, or anthrax-contaminated animal products such as hides. There is a potential for *B. anthracis* to be highly pathogenic and cause a high fatality rate among humans if not recognized or treated early. Based on the October 2001 intentional release incident, there is high potential for this pathogen to be used in intentional malevolent release scenarios, especially via the inhalational route of exposure. Isolated laboratory-acquired infections have been reported.

There is a possibility of infection if an individual is exposed to *B. anthracis* spores. Of the three types of anthrax, based on a review of the literature, there does not appear to be any evidence of person-to-person transmission of inhalational or gastrointestinal anthrax. Person-to-person transmission may occur from cutaneous anthrax; this may result from contact with discharges from the lesions that are potentially infectious (Centers for Disease Control and Prevention (U.S.) 2009) or carriage of spores via inanimate objects (Heyworth et al 1975). Cutaneous anthrax resulting from contact with spores from either inanimate objects in the laboratory (Centers for Disease Control and Prevention (U.S.) 2002; Centers for Disease Control and Prevention (U.S.) 2002) or from humans acting as vectors in carrying the spores during close contact has occurred in the US (Freedman et al. 2002).

Anthrax can be treated with currently available anti-bacterial medications. There is a vaccine available that is offered to those individuals engaged in laboratory work or other high-risk occupations involving *B. anthracis.* This pathogen is expected to be studied in BSL-3 high biocontainment laboratories worldwide, including NEIDL.

For the purposes of this RA, *B. anthracis* will be analyzed in detail with regard to (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to *B. anthracis*; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences; (3) probabilistic estimates of initial infection in those exposed to those amounts of *B. anthracis*; and (4) discussion of potential scenarios by which laboratory workers may act as physical vectors for transmission of *B. anthracis* spores to another person. As there is no possibility of secondary transmission of inhalational anthrax and very low possibility of secondary spread in cutaneous anthrax, secondary transmission modeling of spread of infection in the community will not be performed for *B. anthracis*.

3.5.1.2 Francisella tularensis (F. tularensis)

Introduction

Francisella tularensis is the causative pathogen of tularemia, which is an infectious disease of animals that also affects humans. For this RA, *F. tularensis* was selected for analysis based on its characteristic of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). This pathogen can be transmitted to humans via arthropod vectors and also by close contact with infected animals. Based on a review of the epidemiologic literature, there is no evidence for direct spread of *F. tularensis* from a person infected with this pathogen to another person. The human infectious dose for *F. tularensis* is not known, though there appears to be evidence that the infectious dose is extremely low for *F. tularensis* subsp. *tularensis.* The potential for laboratory acquired infections (LAI) from *F. tularensis* exists and LAI have been reported. There were no secondary cases of tularemia in the close contacts of the laboratory workers or in the community as a result of the laboratory-acquired infections.

The common name of 'rabbit fever' indicates one of the natural reservoirs of this pathogen. Tularemia was first described as a plague-like disease of rodents in California in 1911 and soon after was recognized as a potentially fatal illness in humans (Dennis et al. 2001; Ellis et al. 2002). The disease occurs worldwide and in the US in animals. There are a significant number of naturally occurring cases reported in the US annually (Centers for Disease Control and Prevention 2002).

F. tularensis is small Gram-negative cocco-bacillus of the *Francisella* genus within the *Francisellaceae* family (Dennis et al. 2001; Ellis et al. 2002). The pathogen is a hardy non–spore-forming microorganism that survives for weeks at low temperatures in water, moist soil, hay, straw, and decaying animal carcasses. There are two major subspecies or biovars that are differentiated by virulence testing, biochemical reactions, and epidemiological features. **Francisella tularensis** biovar *tularensis* (type A) is highly virulent and is the most common biovar seen in North America. Type B (biovar *palaearctica*) is relatively benign.

The natural reservoirs of *F. tularensis* include small animals such as squirrels, rabbits, hares, voles, mice, and water rats (Dennis et al. 2001; Ellis et al. 2002). The pathogen is widely distributed in nature and can be found in diverse animal hosts and habitats and can be recovered from contaminated water, soil, and vegetation. Animals acquire the pathogen through bites by ticks, flies, and mosquitoes, and by contact with contaminated environments.

BSL-3 is required for work the fully virulent strain of *Francisella tularensis* in high biocontainment laboratories (Chosewood et al. 2009).

Human Disease and Outbreaks

The exact numbers of worldwide human cases is not known as tularemia is not an internationally notifiable disease. In the US, the incidence of human cases has decreased from the thousands in prior years to several hundred in the 1990s (Centers for Disease Control and Prevention 2002).

Francisella tularensis can infect humans through the skin, mucous membranes, gastrointestinal tract, and lungs (Dennis et al. 2001; Ellis et al. 2002). The route of entry and virulence of the pathogen influence the clinical manifestations. The most common presentation of tularemia is the ulceroglandular form that consists of a skin lesion and infection of the regional lymph node. Tularemia often presents abruptly with fevers, chills, body aches, and sore throat. The illness can progress rapidly to more serious forms such as septicemic and pneumonic forms; these are the clinical forms of most concern with regard to management and mortality. Untreated, the severe forms of tularemia had a fatality rate of 30-60 percent in the pre-antibiotic era. With the advent of antibiotics and modern hospital and intensive care, the overall mortality from tularemia is less than 2 percent (Evans et al. 1985).

There are several routes of transmission of *F. tularensis* to humans (Dennis, Inglesby et al. 2001; Ellis, Oyston et al. 2002). An infection from the bite of an arthropod vector which has previously fed on an

infected animal is considered a major route; this leads to the ulceroglandular or septicemic type of tularemia. Direct contact with infected animals during hunting and skinning is also considered a common route of infection in the US; this route may also lead to pneumonic form, apart from ulceroglandular or septicemic type. Pneumonic tularemia results from inhalation of aerosolized pathogen or as a complication of other forms of tularemia such as septicemia. Ingestion of infected animals or contaminated water may lead to oropharyngeal or gastrointestinal tularemia.
There are naturally occurring cases of human tularemia in the US; the numbers have dramatically decreased over the past 5 decades. Currently reported cases are generally limited to those involving hunting and trapping of animals.

In the past decade, there have been two outbreaks of tularemia reported in the US. In 2000, there were 15 cases of tularemia (11 were pneumonic, 1 death) reported from Martha's Vineyard in Massachusetts. The risk factor was mowing lawns or cutting brushes (weed whacking). It was postulated that the exposed individuals had inhaled aerosolized *F. tularensis* from an infected small animals (Feldman et al. 2001; Feldman et al. 2003; Matyas et al. 2007). More recently, there were 14 cases of tularemia reported from Utah (Petersen et al. 2008; Calanan et al. 2010). The risk factor was noted to be visiting a lodge and participating in outdoor activities near Utah Lake; the pathogen was transmitted by the bite of the deerfly.

There are no reports of direct transmission of *F. tularensis* from a person infected with this pathogen to another person, even from the pneumonic form. It is important to note that there were no secondary cases of tularemia reported in contacts of patients described in the outbreaks from Martha's Vineyard and Utah Lake. There is one published report that suggests that bacteria are aerosolized from patients and in animal models of pneumonic tularemia and this could potentially cause secondary human infections (Jones et al. 2005); these conclusions have not been validated by other authors or experts

The diagnosis of tularemia is based on a high index of suspicion, clinical presentation, epidemiological history of exposure or travel to endemic areas, and laboratory confirmation. Rapid testing is not commercially available. Identification of the bacterium by direct examination of secretions, exudates, or biopsy specimens using direct fluorescent antibody or immunohistochemical stains is possible. Bacterial culture is considered the gold standard. Other specialized testing is available only in reference and research laboratories (Dennis et al. 2001).

Management of patients with tularemia consists of specific anti-bacterial medications and supportive therapy (Dennis et al. 2001; Ellis et al. 2002). The aminoglycoside antibiotics, streptomycin and

gentamicin, are the drugs of choice. In the US, the availability of streptomycin is limited. Another class of antibiotics, the fluoroquinolones have been shown to be active *in vitro*, and ciprofloxacin has been used in outbreaks in Europe. Tetracycline and chloramphenicol are bacteriostatic against *F. tularensis* and have been used to treat tularemia. In mass casualty settings, oral ciprofloxacin and doxycycline are recommended for adults and children.

A vaccine was available for both military and civilian use that was offered to laboratory workers; however, currently, there are no FDA-approved vaccines available for *F. tularensis.*

Disease in Medically Vulnerable Subpopulations

In general, all age groups and both genders are susceptible to *F. tularensis*. There are no reports of increased susceptibility to the pathogen in medically vulnerable Subpopulations. The limited published literature of tularemia is from the 1930s and there are no reports of increased incidence, susceptibility, or worse outcomes in pregnant women.

Human Infectious Dose

The exact human infectious dose of *F. tularensis* is not known. There is strong evidence that the infectious dose is extremely low for *F. tularensis* subsp. *tularensis* and that potentially one to 10 organisms could cause infection in a human. The amount required to cause disease in 50% of humans exposed to the Schu4 strain of *F. tularensis* is estimated to be between 10-50 cells. As few as 10 microorganisms of *F. tularensis* by skin inoculation or 15 microorganisms by aerosol were determined to be sufficient to cause infections in human challenge of immunity studies (Saslaw et al. 1961; Franz et al. 1997). These estimates are also supported by data from laboratory-acquired infection rates that are estimated to be 1 infection/1,000 at-risk worker years in vaccinated laboratory workers (Overholt et al. 1961; Pike 1976; Burke 1977; Shapiro and Schwartz 2002).

Laboratory Acquired Infections

Laboratory-acquired infections (LAI) with *F. tularensis* have been reported from the early days of the study of this pathogen in laboratories; it was consistently in the top three of the lists of LAI from the 1930s to the 1970s both in the US and worldwide (Pike et al. 1965; Pike 1976; Pike 1979). A recent review of select agent incidents reported by the CDC to the National Research Council indicates that of 7 LAI reported to the CDC's Division of Select Agents and Toxins during the period 2003-2009, two were from *F. tularensis* (NRC (National Research Council) 2011; NRC (National Research Council) 2011). The incidence of *F. tularensis*-related LAI have decreased with modern biosafety practices and

recognition of the importance of adhering to these precautions; this was especially noted in US maximum biocontainment laboratories such as the USAMRIID (National Research Council (U.S.). Committee to Review the Health and Safety Risks of High-Biocontainment Laboratories at Fort Detrick. 2010).
In the past decade, there have been several laboratory incidents involving *F. tularensis*, with very few infections. As noted in the Biosafety Review (Appendix D), during the period 2000-2010, there have been at least 11 incidents reported to the NIH involving this pathogen.

In 2004, there was an incident involving laboratory researchers at Boston University. Researchers were working under BSL-2 biocontainment protocols with what was believed to be a non-infectious vaccine strain of the *F. tularensis* bacterium. Later, it was determined the bacterial culture also contained the infectious wild-type strain that requires BSL-3 biocontainment precautions. There were a total of 3 infections and no deaths. Subsequent investigations resulted in revising standard operating protocols to prevent such incidents from occurring again (Lawler 2005).

More recently, in November 2009, a laboratory worker at USAMRIID was diagnosed with tularemic pneumonia (National Research Council (U.S.). Committee to Review the Health and Safety Risks of High-Biocontainment Laboratories at Fort Detrick. 2010). She had previously had a non-laboratory related clinical case of tularemia and was found to have positive hemagglutinin titers suggesting immunity to the bacterium. She was working with *F. tularensis* and subsequent investigation concluded that she most likely had an aerosol exposure to the pathogen in the laboratory.

Laboratory-associated incidents and infections with *F. tularensis* remain a concern. The recent incidents reinforce the importance of biosafety practices and laboratory worker education and training. The incidents also underscore the importance of prompt reporting of laboratory incidents to supervising authorities so that appropriate prophylactic and mitigative strategies can be promptly instituted.

Summary

Francisella tularensis is the pathogen that causes tularemia, a serious human illness. Human infection results from transmission of *F. tularensis* from the animal reservoir via arthropod vectors; by direct contact with infected animals, or by ingesting contaminated food or water. The human mortality was higher in the pre-antibiotic era; available antibiotics and modern hospital care have decreased the mortality rate to below 2%. There is concern that the pathogen could be used for malevolent purposes. There is no known direct person-to-person transmission of *F. tularensis.*

For the purposes of this RA, *F. tularensis* will be analyzed in detail with regard to: (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to *F. tularensis*; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of initial infection in those exposed to those amounts of *F. tularensis* . As there is no risk of direct person-to-person transmission of *F. tularensis*, secondary transmission modeling of the spread of this bacterium in the community following an initial infection will not be performed.

3.5.1.3 Yersinia pestis (Y. pestis)

Introduction

Yersinia pestis is a bacterium that causes the animal and human disease known as plague. . For this RA, *Y. pestis* was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). This pathogen can be transmitted to humans via arthropod vectors and also by close contact with infected animals. *Y. pestis* can be transmitted directly from a person infected with this pathogen to another person in the setting of pneumonic plague affecting the lungs. The human infectious dose for *Y. pestis* is considered to be low, though there are no direct human dose-response data available in the literature. The potential for laboratory acquired infections (LAI) from *Y. pestis* exists and LAI have been reported. Laboratory-acquired infections involving *Y. pestis* are rare in the era of modern biosafety practices. The recent case of death from an LAI from an attenuated strain resulted in septicemic plague with no features of pneumonic plague, thus posing minimal risk to the public.

Plague is one of the oldest diseases known to humanity, with the first pandemic dating back to AD 541. There continues to be interest in this pathogen with several thousand human cases of *Y. pestis* infection reported in several countries around the world in recent years and its potential as a biologic weapon (Inglesby et al. 2000; Butler 2009). More recently, this pathogen has been in the news with the report of the death of a wildlife biologist who contracted the disease from the carcass of a mountain lion (Wong et al. 2009) and the death of a researcher exposed to an attenuated strain of *Y. pestis* in the laboratory (Centers for Disease Control and Prevention (U.S.) 2011).

Primarily a disease of wild rodents, plague is spread from one rodent to another by fleas, cannibalism, or possibly from contaminated soil. Wild plague exists in its natural foci of rodents independent of human activity. Domestic plague is associated with rodents living in close proximity to humans and can cause

disease in both rodents and humans (Dennis et al. 1999). *Yersinia pestis* occurs in 17 of the contiguous western U.S. states (Dennis and Mead, 2010).

Y. pestis is maintained in nature through endless cycles between fleas and reservoir hosts, such as rodents. Wildlife biologists have increasingly realized that certain wild mammal species also are highly susceptible to plague (Gage and Kosoy 2005). More recently, it has been recognized that cats and mountain lions could be infected with plague and transmit to humans (Gage et al. 2000) (Wong et al. 2009). The pathogen has also been shown to survive and persist in soil, especially in the presence of blood, thus raising the possibility of another reservoir for human infections (Eisen et al. 2008). *Y. pestis* is a Gram–negative cocco–bacillus that belongs to the group of bacilli with low resistance to environmental factors. Sunlight, high temperatures, and desiccation have a destructive effect on the pathogen.

This pathogen is studied in laboratories around the world, including in maximum biocontainment laboratories in the US. Virulent *Y. pestis* is a BSL-3 pathogen and also is a Select Agent requiring registration with CDC for the possession, use, storage, and transfer of the bacterium (Chosewood et al. 2009).

Human Disease and Outbreaks

Humans are extremely susceptible to plague and may be infected either directly or indirectly. Indirect transmission through the bite of a flea is the most common route of transmission between plague–infected rodents and humans. People can be infected directly from a plague–infected rodent or other animal while handling, skinning, or cutting up the meat. *Y. pestis* enters humans through skin lesions or through the mucous membranes of the mouth, nose, or eyes. More recently, it was postulated that human primary pneumonic plague was caused by inhaling aerosolized bacteria during the post-mortem examination of an infected mountain lion (Wong et al. 2009).

Human disease occurs primarily in three forms (Dennis et al. 1999). Bubonic plague is the most common and classical form. This condition is characterized by enlarged regional lymph nodes (the ‘buboes’) resulting from exposure to the pathogen through the skin or mucous membrane. Primary septicemic plague is an overwhelming blood stream infection with the pathogen following exposure through the skin. Primary pneumonic plague occurs when *Y. pestis* aerosols are inhaled through the respiratory tract. Less common plague syndromes include plague meningitis which follows the seeding of the pathogen into the central nervous system from the bloodstream (from septicemic plague) Plague pharyngitis follows inhalation or ingestion of the pathogen.

Of these forms, bubonic plague is not transmissible directly from person-to-person. Based on a review of the available epidemiologic literature, pneumonic plague where the pathogen is in the lungs can be transmitted directly person-to-person. This may be a result of primary pneumonic plague or secondary pneumonic plague resulting from spread of *Y. pestis* to the lungs from the bloodstream (from septicemic plague). The pathogen is not truly airborne and so transmission of pneumonic plague from person-to-person requires close face-to-face contact within 3-6 feet of a person with pneumonic plague who is actively coughing. From historical accounts and recent experiences, the risk of transmission is considered lower than previously claimed (Kool 2005).

The first certain pandemic, known as Justinian's plague, was recorded in the sixth century AD in Africa, Asia, and Europe and claimed large numbers of victims. The second pandemic in the 14th century is well known as the 'black death' or 'great pestilence' that caused the death of a third of Europe's population at that time. The third pandemic occurred in the 19th century, causing similar havoc in Asia. Plague remains endemic in many natural foci around the world. The decrease in the incidence of plague today is due primarily to the improvement of living standards and health services in many countries. Despite the general decline in the incidence of plague worldwide, the numbers of countries affected by plague remains substantial. Human cases continue to be reported from several countries, with most of them being in developing countries (Butler 2009).

In the US, 415 cases were reported from 1970-2007 (Dennis and Mead 2010). Recent cases that received attention were in two New Mexico residents who became ill and were diagnosed with plague in New York City (2003) and a case of fatal pneumonic plague in a wildlife biologist who contracted the pathogen while performing a necropsy examination on an infected mountain lion (Wong et al. 2009).

Historically, plague has been considered in biologic warfare with reports of the Japanese dropping plague-infected fleas over China during World War II (Inglesby et al. 2000). Following this, several countries including the US and the former Soviet Union initiated programs to look at plague as a biologic weapon. There are no reports of accidental or intentional release of plague. There is one report of a scientist with suspect motives ordering *Y. pestis* in the mail (Inglesby et al. 2000) and another report of samples of this pathogen being 'mishandled' (Malakoff and Drennan 2004).

The diagnosis of plague is based on the clinical presentation along with a strong epidemiological history of appropriate exposure that includes contact with small wild animals. A positive diagnosis may be made

from blood, sputum, or lymph-node samples by the characteristic bipolar staining of the bacteria using Wright-Giemsa stain (safety pin appearance). The laboratory confirmation is made from microbiologic cultures. Other diagnostic methods such as immunodiagnosis and PCR-based tests that were previously only available through state public health laboratories, the CDC, or military laboratories (Inglesby et al. 2000) are now more widely available (Butler 2009).

Untreated, mortality, particularly from pneumonic plague, may reach high levels. In the pre-antibiotic era, nearly 100% of the cases were reported to be fatal. When rapidly diagnosed and promptly treated, plague may be successfully managed with antibiotics reducing mortality from 60% to less than 15%.

Plague can be treated using several classes of antibiotics. Streptomycin was the mainstay of treatment for several decades and remains the drug of choice (though availability of this medication is limited in the US). Monotherapy with gentamicin has been used in the US. Doxycycline or tetracycline has been used as an alternative for treatment. Cephalosporins and other beta-lactams, along with fluoroquinolones, have been shown to be effective in animal models. Chloramphenicol is also effective, though it is rarely used in the US due to toxicities. The 1995 appearance in Madagascar of a strain of *Y pestis* showing resistance to routinely used antibiotics is a matter of much concern (Butler 2009). In that situation, combinations of medications have been used.

Doxycycline and trimethoprim/sulfamethoxazole have been recommended as prophylactic medication for those exposed to pneumonic plague. Plague vaccines have been administered to high-risk workers in the past; however the vaccine is no longer available in the US.

Disease in Medically Vulnerable Subpopulations

Individuals of all ages are susceptible to plague. In recent years, there were many cases of plague reported in children, including in the 1995 outbreak in Madagascar (Butler 2009). There is an increased incidence of plague among those in lower socio-economic strata in developing countries due to their closer proximity to rodents and fleas. However, there are no published reports of increased susceptibility to plague among children, the elderly, or those among disadvantaged socio-economic status in the US. The literature on plague in pregnancy is extremely limited with only three published reports (Mann and Moskowitz 1977; Coppes 1980; Wong 1986). There are no reports of increased susceptibility to plague in pregnancy although there is a mention of septic abortion. There are no published reports on possible associations of plague and asthma.

Human Infectious Dose

The human infectious dose for *Y. pestis* is considered to be low, though there are no direct human dose-response data available in the literature. The infectious dose for humans exposed to *Y. pestis* aerosols has been stated to be between 100-500 organisms although from the reference, it is not clear whether those numbers were derived from animal or human data (Franz et al. 1997). Epidemiological information from human outbreaks of pneumonic plague have lead to estimates of a low attack rate, with approximately 8% of close, unprotected contacts of symptomatic primary cases becoming secondarily infected (Begier et al. 2006) (Ratsitorahina et al. 2000). This likely is due to the low transmissibility of the pathogen rather than a higher infectious dose (Kool 2005).

Laboratory Acquired Infections

Plague infections have been reported from laboratory incidents prior to 1965 with at least four known cases; the last was reported in 1959 (Pike et al. 1965) (Burmeister et al. 1962). Of the 7 recently reported laboratory-related incidents involving *Y. pestis* detailed in the Biosafety Review (Appendix D), there have been no reports of plague infection. Several laboratory workers received prophylactic medications.

In 2010, there was a report of the death of a senior laboratory researcher at the University of Chicago who was found to be infected with an attenuated strain of *Y. pestis* that had to date not been reported to cause any laboratory-acquired infections or fatalities (Centers for Disease Control and Prevention (U.S.) 2011). The route of transmission was not clearly identified and the researcher developed primary septicemic plague. A detailed investigation revealed that the researcher had underlying co-morbidities that included diabetes and hitherto undiagnosed hemachromatosis, which could have contributed to his increased susceptibility to the attenuated strain. *Y. pestis* has an inherent need for iron and it is postulated that in the setting of iron overload, the researcher had increased susceptibility to the attenuated strain. The CDC concluded that under certain environmental and host conditions, even infection with attenuated strains of pathogens may result in severe disease and death.

Laboratory-acquired infections involving *Y. pestis* are rare in the era of modern biosafety practices. The single case of death from an attenuated strain resulted in septicemic plague with no features of pneumonic plague, thus posing minimal risk to the public. Laboratory worker education and training remain important components of biosafety, as well as prompt reporting and seeking of medical attention when symptomatic.

Summary

Y pestis causes plague, an ancient disease that continues to be seen in animals throughout the world, and in humans, mostly in developing countries. The pathogen is in general, poorly transmissible and humans acquire the disease from the bite of infected fleas or when handling infected animals. It is widely distributed in the tropics and subtropics and in warmer areas of temperate countries. Several thousand cases of human disease have been reported from several countries during the period 1993-2004, with a case fatality rate of 7.1% (Butler 2009). Naturally occurring human infections in the US are rare and limited geographically to the western states. There is a potential for *Y. pestis* to be highly pathogenic and cause a high fatality rate, especially if the diagnosis and treatment are delayed. There have been no intentional releases in modern times, though the potential for its use as a biological weapon remains. Only one laboratory-acquired infection has been reported in the US since 1959; the circumstances were unique in that the researcher had an underlying undiagnosed condition that likely contributed to his increased susceptibility to an attenuated strain of *Y. pestis.*

There is a possibility of infection if an individual is exposed to *Y. pestis*. Of the three types of plague, bubonic plague is not known to be transmitted directly from person-to-person. There is a possibility of transmission of pneumonic plague directly from person-to-person. Plague can be treated with currently available anti-bacterial medications, though resistance has been reported. There is no plague vaccine available for use in the US at this time. This pathogen is expected to be studied in BSL-3 high biocontainment laboratories, including NEIDL.

For the purposes of this RA, *Y. pestis* will be analyzed in detail with regard to (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to *Y. pestis* including routes of exposure ; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences; (3) probabilistic estimates of initial infection in those exposed to those amounts of *Y. pestis,* including the type of infection (bubonic, septicemic, or pneumonic); and (4) transmission modeling of the potential for secondary transmission of *Y. pestis* among the general public in the event of a laboratory worker or member of the public experiencing an initial infection of pneumonic plague.

3.5.1.4 1918 H1N1 Influenza Virus (1918 H1N1V)

Introduction

The 1918 H1N1 Influenza Virus (1918 H1N1V) is the prototypical pandemic strain of influenza and was the etiology of the 1918-1919 "Spanish Flu" pandemic (reviewed in (Taubenberger and Morens 2006; Morens and Fauci 2007). For this RA, 1918 H1N1V was selected for analysis based on its characteristics of being highly transmissible and highly pathogenic (Mahmoud 2008). The case fatality rate for this pathogen is considered high, though relatively lower compared to pathogens such as SARS-associated corona virus. This pathogen is easily transmitted directly from person-to-person. The human infectious dose for 1918 H1N1V is not known. Though there have been no LAI reported with 1918 H1N1V, the potential for LAI with influenza viruses exists and these have been reported. The risk of secondary transmission in the community exists from LAI resulting from influenza viruses.

The 1918-1919 "Spanish Flu" is the deadliest influenza pandemic known with estimates of 675,000 deaths in the US and 50-100 million deaths worldwide. Contrary to popular opinion, this was not the first influenza pandemic to occur; there are reports of the first recorded pandemic from 1510 AD (500 years ago) (Morens et al. 2010). With many unique characteristics in terms of the biology of the virus, the disease it caused, and the death toll among healthy young adults, the 1918 H1N1V remains an important pathogen to study.

The impact of the 1918 H1N1V is not limited to just this pandemic. From analysis of circulating influenza viruses and pandemics since then, it is postulated that all influenza A cases worldwide have been caused by descendants of the 1918 virus, literally earning this pathogen the moniker of "the Mother of All Pandemics" for reasons beyond its virulence (Taubenberger and Morens 2006). The only exceptions are the avian influenza viruses H5N1 and H7N7.

1918 H1N1V is a spherical or pleomorphic, single-stranded, negative-sense RNA enveloped virus of the genus *Influenzavirus A* belonging to family *Orthomyxoviridae* The virus was recovered from lung tissue from victims of the Spanish Flu andhas been studied extensively; the sequencing of the entire coding region was completed and the fully reconstructed virus was generated for the first time in 2005 (Taubenberger et al. 1997; Reid et al. 2000; Tumpey et al. 2005). Much of the laboratory work on pathogenicity of the virus has been performed on 'reconstructed' or recombinant influenza viruses (Tumpey et al. 2004; Tumpey et al. 2005; Tumpey et al. 2005). These 1918 recombinant viruses exhibit pathogenicity, can infect laboratory animals, and behave in essence as the original virus. There is variability in the transmission potential of the recombinant viruses when they contain less than the entire

complement of 8 gene segments (reviewed in (Tumpey and Belser 2009). An interesting observation was that the 1918 H1N1V did not arise from genetic re-arrangement between a human and animal virus as has been the case for most other pandemic influenza viruses, but rather it was gene adaptation.

This RA considers both the original 1918 H1N1V and the recombinant viruses used in laboratory work to be equivalent with regard to their potential for causing human disease and ability to be transmitted from person-to-person. The pathogen description is focused on what is known of the 1918 H1N1V; additional information gleaned from the study of other circulating influenza viruses and more recent pandemics is also provided for context as to how influenza would be managed in the 21st century.

The origins of 1918 H1N1V are not known, though the ancestral sources of its genes are considered avian (Reid et al. 2004; Taubenberger 2006). There is no known natural reservoir for 1918 H1N1V (Taubenberger 2006). Influenza A in general can infect several different species, including humans, ducks, chickens, pigs, whales, horses, and seals.

BSL-3 is required for work 1918 H1N1Vin high biocontainment laboratories (Chosewood et al. 2009).

Human Disease and Outbreaks

The 1918 H1N1V was the cause of the 1918-1919 Spanish Flu pandemic which is the most devastating of known influenza pandemics. It is estimated that roughly one third of the world's population at that time was affected by the disease; there were an estimated 675,000 deaths in the US and 50-100 million worldwide (Taubenberger and Morens 2006; Morens and Fauci 2007).

One key feature of this pandemic was the high case fatality rate among healthy young adults; in reviewing the data for excessive deaths due to influenza and pneumonia from 1918-1919, the less than 65 year age group accounted for 99% of those deaths (Taubenberger and Morens 2006). There appeared to be a relative sparing of older adults who were born before 1889 and had experienced or lived through the prior influenza pandemic. The high fatality rate caused by this pathogen has been a subject of great debate and was likely a combination of its biology and the high incidence of post-influenza pneumonia noted in victims (Morens et al. 2008). It is interesting to note that all influenza A descendents of the 1918 H1N1V have only a fraction of the parent pathogen's virulence or case fatality rate.

The attack rates for 1918 H1N1V were high in the younger age groups, with incidence rates of 300-400/100,000 individuals noted for the 5-25 age groups (Taubenberger and Morens 2006). Older adults had attack rates that were two-fold lower. The overall fatality rate of 1918 H1N1V in the US was

estimated to be nearly 2.5 percent, with higher rates in the age group 20-40 (Taubenberger and Morens 2008). In comparison, all other influenza A pandemics have a fatality rate of less than 0.1 percent. Based on CDC statistics, seasonal influenza attack rates in the US typically range between 5-20% of the population and there are, on average, 36,000 fatalities attributable to influenza every year (Fiore et al. 2010).

The reproductive number (R_0), the average number of secondary cases caused by an index human case with 1918 H1N1V, has been estimated for different countries and settings. Overall, the range for R_0 was in the range 1.5 to 3.5 for large US cities (Mills et al. 2004) (Bootsma and Ferguson 2007) (Chowell et al. 2007) Estimates have been in that range for most other locations such as Iceland (2.2) (Dowell and Bresee 2008) England and Wales (2.1) (Viboud et al. 2006) and Switzerland (spring wave 1.49, fall wave 3.75) (Chowell et al. 2006)

Since the 1918 Spanish Flu, there have been several influenza pandemics (all caused by influenza A) that have affected humans worldwide, including the US. Each of the viral strains responsible for pandemics has, by definition, been described to be a new or previously unknown strain of influenza virus. The 1977 outbreak has several features that make it unique. The disease was noted in children and young adults generally less than 23 years old and some consider this to be a Russian Flu outbreak and not a true pandemic (US Department of Health and Human Services 2011). The virus was shown to be genetically and antigenically similar to the H1N1 virus that had circulated in 1950 (Nakajima et al. 1978) and this has raised the question of the origin of the 1977 virus. It seems unlikely that the virus remained unchanged in human hosts or animal reservoirs between 1950 and 1977; some speculate that the virus may have been accidently re-introduced into nature in 1977 from a frozen state, possibly a laboratory freezer (Ennis 1978; Kendal et al. 1978; Webster et al. 1992; Zimmer and Burke 2009).

The most recent was the 2009 H1N1 influenza pandemic that originated in Mexico and caused a worldwide pandemic (Peiris et al. 2009; Schuchat et al. 2011; Swerdlow et al. 2011). Though there was concern for high morbidity and mortality from this novel influenza A virus with human, avian, and swine ancestry, the pandemic, in the end, was milder than expected.

Seasonal influenza is the classical febrile illness with fever, cough or sore throat, accompanied by chills, severe myalgias, fatigue, and headaches. The incubation period of seasonal influenza is short, typically 2 days, with a range of 1-4 days (Bridges et al. 2003). Complications include primary viral pneumonia, secondary bacterial pneumonia, or combined bacterial and viral pneumonia (Barnard 2009). Pandemic

influenza caused by 1918 H1N1V or a new strain would likely follow a similar pattern with likely variations in the incubation period and severity of illness.

Influenza viruses in general and 1918 H1N1V, in particular, can be transmitted directly from person-to-person. The routes of transmission include large droplet, aerosol, direct contact with virus-laden secretions, and via fomites (Bridges et al. 2003; Tellier 2006). The exact contribution of these modes in transmission of influenza is not known; however droplet mode appears to be the most common with aerosol transmission posing a concern for infection control and biosafety.

The diagnosis of 1918 H1N1V would be based on clinical presentation and laboratory confirmatory testing using molecular methods such as PCR. With the experience of 2009 H1N1 pandemic (2009), it is very likely that currently available rapid influenza antigen tests would have suboptimal test characteristics for diagnosing 1918 H1N1V . Other modalities of testing for influenza in general include direct fluorescent antibody testing; the gold standard would be viral culture and sequencing.

Management of patients with 1918 H1N1V would consist of supportive therapy including intensive care and treatment with specific anti-viral medications such as neuraminidase inhibitors or adamantanes. Circulating influenza strains often have variable and varying anti-viral medication susceptibility profiles; as such, the use of anti-viral medications for an outbreak of 1918 H1N1V would have to be based on testing of the circulating strain and its specific anti-viral susceptibility profile. In an outbreak situation with 1918 H1N1V, it would be very likely that treatment and prophylaxis with neuraminidase inhibitors and/or adamantanes would be offered until more information was available; if the circulating strains were sensitive to these medications, there would be good efficacy in treating or preventing illness. There is, of course, the risk of the pathogen developing resistance to these medications as has been noted for seasonal and pandemic influenza, especially in those on treatment or with an immunocompromised condition (2009; 2009; Couturier et al. 2010; Gubareva and Fry 2010; Gubareva et al. 2010).

There is no specific vaccine for 1918 H1N1V. Recent observations, however, provide some support for optimism with regard to existing vaccines and immunity in the population. The population today, as compared to 1918, has pre-existing immunity to the influenza antigens such as H1N1, and other related viruses including H3N2; all humans older than 2–3 years have immunity to both H1N1 and H3N2 viruses (Murphy 2008). There is evidence of cross-protection among vaccines (seasonal influenza vaccine & 2009 H1N1 and 2009 H1N1 pandemic vaccine & 1918 H1N1V) (Johns et al. 2010; Manicassamy et al. 2010; Medina et al. 2010). The search for a ‘universal’ influenza vaccine that would cross-protect against

several different strains is an active area of research (Epstein and Price 2010; Henderson 2010; Rudolph and Ben Yedidia 2011). With this background, it is possible that vaccination to protect or at least mitigate disease due to 1918 H1N1V would be available at some point after an outbreak.

During the 1918 Spanish Flu pandemic, there was an interesting set of observations from a few studies that blood transfusions or blood products from convalescent patients were of benefit in decreasing risk of death in patients (Luke et al. 2006). There are parallels in modern day medicine of using pathogen-specific immune globulin for post-exposure prophylaxis for conditions such as hepatitis A, hepatitis B, and varicella zoster virus infection. The 'passive immunization' protocol for 1918 H1N1V would have to be modified and updated to 21st century scientific and ethical standards before there is any consideration of application to patients today.

Disease in Medically Vulnerable Subpopulations

In general, influenza affects all age groups. For seasonal influenza, morbidity and mortality is noted at extremes of ages, those with medical co-morbidities, and pregnant women. Pandemic influenza has the potential to preferentially affect Subpopulations; the 1918 H1N1V was especially severe and fatal for younger age groups. {Rasmussen, 2011 #2231;Mosby, 2011 #13693;Louie, 2011 #2228;Patel, 2010 #2276;Labant, 2009 #2285;Jamieson, 2009 #10568}Pregnant women are considered to be immune-suppressed and they are at high risk of adverse events from seasonal and pandemic influenza (Jamieson et al. 2009; Siston et al. 2010; Mosby et al. 2011; Rasmussen et al. 2011). Seasonal influenza related hospitalization rates from selected acute cardiopulmonary conditions were found to nearly 5-fold higher in pregnant women in their third trimester (Neuzil et al. 1998). Specifically with respect to pandemic influenza, the historical mortality rates of pregnant women have been noted to be in the range of 20 to 51% for the 1918 and 1957 influenza pandemics (reviewed in (Callaghan et al. 2010)). In reviewing observations from the 2009 H1N1 influenza pandemic, pregnancy was associated with an increased risk of hospital and intensive care unit admission and of death (Mosby et al. 2011). Pregnant women were disproportionately represented in hospitalizations and deaths; pregnancy-specific mortality rates were not provided.

Those with co-morbidities such as HIV/AIDS are considered at increased susceptibility to seasonal influenza, however, a recent review of the literature has found no evidence of increased susceptibility to pandemic influenza in the setting of HIV infection. However, it would appear that more studies are needed (Sheth et al. 2011). Diabetes is considered a risk factor for adverse outcomes in influenza infection; this has been noted for seasonal and pandemic influenza (Diepersloot et al. 1990; Valdez et al.

1999; 2000; Allard et al. 2010). Applying observations from seasonal and prior pandemics including the 1918 Spanish Flu, with regard to 1918 H1N1V, it would be likely that the pathogen would preferentially affect younger age groups, the elderly would likely be relatively spared, diabetics would experience worse outcomes, HIV/AIDS patients may experience worse outcomes, and pregnant women would be severely affected.

Preliminary reports from the 2009 H1N1 influenza pandemic have suggested a disproportionate impact of the pandemic on racial and ethnic minorities (Kwan-Gett et al. 2009; Centers for Disease Control and Prevention 2010; Truelove et al. 2011; Uscher-Pines et al. 2011; Wenger et al. 2011), indicating higher rates of hospitalization, morbidity and mortality among racial/ethnic minorities. No clear reason was noted for the increased hospitalization and mortality, though it was postulated that underlying chronic diseases may have played a role. A detailed study based on survey responses of minorities during the 2009 influenza pandemic has also expressed concern of the disproportionate impact of the pandemic, however, the authors were unable to demonstrate an increased risk of susceptibility when controlled for socioeconomic status and demographics (Quinn et al. 2011).

Human Infectious Dose

The human infectious dose of 1918 H1N1V is not known. There are experimental data from the early 1970s that looked at infections in human volunteers after exposing them to a related H1N1 strain of influenza virus (Carrat et al. 2008). In these volunteers, doses ranging from ten thousand to one million $CCID_{50}$ result in approximately 80% to 95% infection probability. In other experiments (Alford et al. 1966), the low-dose inhalational data reveal that roughly 50% of unprotected humans were infected after inhaling doses of 1–5 $CCID_{50}$ Thus the human infectious dose is presumed to be low for 1918 H1N1V.

Laboratory Acquired Infections

During the period 2000-2010, there were 7 incidents reported in government and academic laboratories as noted in the Biosafety Review (Appendix D). These included a centrifuge leak and other incidents leading to potential exposures of several different strains of influenza virus. There were no influenza virus infections reported and no deaths from these incidents.

From a review of the published literature, there are at least 3 laboratory-acquired infections reported for influenza viruses (Wentworth et al. 1997; Harding 2006). Of these, symptomatic influenza infections were reported in two laboratory workers who were involved in taking nasal swabs from laboratory pigs infected with a swine influenza virus. The influenza viruses isolated from the laboratory workers were

antigenically identical to the inoculum swine influenza virus used in the experiments. Partial gene sequencing of the viruses isolated from humans indicated that they were direct descendants of the inoculum swine virus; they were 99.7% identical with regard to the hemagglutinin genes.

Summary

The 1918 H1N1V is a pathogen that has caused one of the deadliest influenza pandemics known. Recovery of the pathogen and complete sequencing has allowed scientists to construct 'recombinant' influenza viruses that contain some or all of the genes from 1918 H1N1V. This pathogen is extensively studied in high biocontainment laboratories around the world, including the US. The pathogen caused severe illness and death in healthy young individuals in the 1918-1919 pandemic. There are antiviral medications that could potentially be effective against this pathogen. There is no vaccine specifically for 1918 H1N1V. There is a possibility of infection if an individual is exposed directly to this pathogen; secondary cases are likely to occur as the pathogen can be transmitted directly from person-to-person. Laboratory-acquired infections with influenza virus have been reported.

For the purposes of this RA, 1918 H1N1V will be analyzed in detail with regard to: (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to 1918 H1N1V; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of initial infection in those exposed to those amounts of 1918 H1N1V and (4) secondary transmission modeling of the spread of 1918 H1N1V in the community following an initial infection.

3.5.1.5 SARS-associated Coronavirus (SARS-CoV)

Introduction

The Severe Acute Respiratory Syndrome-associated coronavirus (SARS-CoV) is an emerging pathogen that was first discovered and described in 2003. For this RA, SARS-CoV was selected for analysis based on its characteristics of being highly transmissible and highly pathogenic with a high case fatality rate (Mahmoud 2008). This pathogen is easily transmitted directly from person-to-person. The human infectious dose for SARS-CoV is not known. The potential for LAI with SARS-CoV exists and such infections have been reported. The risk of secondary transmission in the community exists from SARS-CoV laboratory acquired infections and this has been reported from China; the secondary infections were in a limited number of close contacts.

The disease caused by SARS-CoV, severe acute respiratory syndrome (SARS), was first noted as an atypical pneumonia in patients and their health care providers in a rural province in China; the pathogen spread within weeks to several countries around the world. Overall, there were nearly 8000 confirmed cases and 800 deaths.

SARS-CoV is an RNA virus classified in the genus *Coronavirus*, family *Coronaviridae*, order *Nidovirales* (Weiss and Navas-Martin 2005). The virus was first isolated from patient samples from the worldwide outbreak in 2003 by viral culture and identified by electron microscopy and complete genomic sequencing (Parashar and Anderson, *International Journal of Epidemiology* 2004;33:628–634).

The natural reservoirs appear to be horseshoe bats, *Rhinolophus* species (Shi and Hu 2008). It is hypothesized that human infections occurred via numerous mammalian species used for food in Eastern Asian cultures (Guan et al. 2003; Feng and Gao 2007).

SARS-CoV propagation in cell culture and the initial characterization of viral pathogens recovered in cultures of SARS-CoV specimens must be performed in a BSL-3 facility using BSL-3 practices and procedures. SARS-CoV is currently not on the list of HHS and USDA Select Agents and Toxins.

Human Disease and Outbreaks

SARS-CoV causes the clinical disease called severe acute respiratory syndrome (SARS). This is a highly infectious viral disease of humans first described in 2003 and retrospectively recognized earlier in November 2002. The disease is highly transmissible among humans and is characterized by fever, malaise, and headache and progressing rapidly to frequently fatal atypical pneumonia. The highest attack rate was noted in Beijing (25% among males aged 20-39) (Liang and Xue 2004). Secondary household attack rates were 10% in Toronto, 8% in Hong Kong , 6.2% in Singapore and 4.6% in China (Goh et al. 2004) (Lau et al. 2004) (Wilson-Clark et al. 2006)The disease was mild in children and severe in adult age groups with an overall *case fatality rate* (CaseFR, percentage of those individuals who were diagnosed with SARS and died as a result of SARS) worldwide of 9.6% during the 2003 outbreak, although the fatality rate reached 50 percent among patients >60 years old (Anderson et al. 2004; Liang and Xue 2004; Zhong and Wong 2004; Gillim-Ross and Subbarao 2006). Initially, SARS-CoV was transmitted rapidly and mortality rate was high. As the epidemic continued and prevention/control methods were implemented, CaseFR fell to 0 in many countries (Chan-Yeung and Xu 2003) as a result of prompt identification and institution of supportive care in a hospital setting.

The estimates of the incubation period from the China/Hong Kong data were in the range of 2-12 days (Anderson et al. 2004). In Toronto, the estimate was a mean of 5 days (median 4 days; range 2-10 days) (Varia et al. 2003); overall worldwide data consistently estimated the range to be 4 to 6 days (Donnelly et al. 2004). The infectious period estimated from the Amoy Gardens, Hong Kong cluster of cases was in the range of 5-15 days with peak viral loads from naso-pharyngeal washes noted at day 10 and correlated with symptoms (Peiris et al. 2003).

The 2003 spread of SARS-CoV across several continents in a shortspan of days to weeks represented a modern day outbreak of an emerging infection. One of the important lessons learned from this outbreak was that air travel by individuals exposed to the pathogen who were in their incubation period can lead to outbreaks in their destination location when they start exhibiting symptoms of the disease. Another lesson was that efficient direct person-to-person transmission of SARS can occur, leading to large outbreaks in the community. Worldwide, there were a total of 8096 confirmed cases reported to the WHO as of December 31, 2003 Mainland China, followed by Hong Kong Special Administrative Region, Taiwan ROC, Canada and Singapore accounted for the majority of cases. There were a total of 27 confirmed cases reported from the US.

There is a large body of literature on the transmission of SARS with the majority of the work describing the large outbreaks in China, Hong Kong, Canada and Singapore. Overall, it was estimated that one person infected with SARS transmitted the disease to 2 to 3 other contacts in a population that had not yet instituted control (Lipsitch et al. 2003). There was a phenomenon described as “super spreaders” that referred to the observation that some individuals spread the disease to many other contacts. In Singapore, it is hypothesized that individual patients may have transmitted the virus to >10 persons (2003) Though large outbreaks occurred, it is also important to note that most SARS patients transmitted the virus to no others. In Singapore it was noted that after the institution of intensive infection-control measures, 81% of probable SARS patients had no evidence of transmission to other persons (Peck et al. 2004). Despite numerous unprotected exposures, there was no serologic evidence of healthcare-related SARS-CoV transmission in the US, which may have been related to the relative absence of high-risk procedures or patients and prompt institution of bio-safety practices (Peck et al. 2004). The transmissibility of SARS-CoV is comparable to that of 1918 pandemic influenza (Mills et al. 2004; Dowell and Bresee 2008) and lower than that of the measles virus (each patient with measles spread it to an average of 15–18 other individuals prior to wide scale immunization) (Anderson et al. 2004).

An important lesson learned from the SARS outbreak was the high occupational risk posed to healthcare workers from patients that were not yet recognized to have SARS and inadequate infection prevention practices. Sixty three percent of SARS cases in Hanoi hospitals, 46% in Hong Kong, 76% in Singapore and equally high rates in Toronto were in healthcare workers (Low and Wilder-Smith 2005). An increased awareness of SARS among the public and healthcare workers coupled with a high index of suspicion for possible cases led to the prompt institution of universal bio-safety practices; this resulted in dramatically reduced transmission and infections during the course of the epidemic from Asia to North America and Europe. The most effective public health and bio-safety strategies were respiratory isolation of symptomatic patients, quarantine of exposed asymptomatic individuals, appropriate personal protective equipment for healthcare workers and good hand hygiene practices.

The natural route of SARS-CoV transmission to humans is initially thought to have occurred by inhalation of aerosolized blood and body fluids from infected mammals, primarily *Paguma larvata*, the Himalayan palm civet cat (Feng and Gao 2007; Shi and Hu 2008). Direct human-to-human transmission is thought to have occurred via respiratory droplets, infected bodily fluids, and fomites (Weiss and Navas-Martin 2005; Lo et al. 2006). Aerosol transmission was considered responsible for rapid human-to-human transmission in medical facilities, high density residential facilities, and aircraft, with rapid spread internationally (McDonald et al. 2004).

There is no specific medication for prophylaxis or treatment available for SARS-CoV infection at the present time; there is also currently no vaccine available for this virus. Several pharmacologic and biologic regimens had been tried during the 2003 outbreak, though there are no controlled trials to judge their usefulness; similarly researchers are working on several vaccine candidates and approaches against the virus to either prevent and treat the disease (Groneberg 2005 Lancet Infectious Diseases, 5:147-155).

Disease in Medically Vulnerable Subpopulations

In reviewing the data from the 2003 worldwide outbreak of SARS, young adults in the age group 20-39 years and older individuals greater than 75 years of age were the most affected in terms of illness and fatalities. Children less than 10 years of age accounted for 0.9% of probable cases of SARS. The median age of those who became ill was 33 years. The relative risk (RR) of becoming ill with SARS was highest in those 20–39 years of age, with their risk being nearly twice as high as younger and older age groups. Overall, male patients had similar rates of illness as female patients, but the risk differed significantly in certain age groups: among those 10–19 years of age and in those greater than 75 years of age, the RR for SARS in male patients was nearly twice as compared to females (Liang and Xue 2004).

There are no peer-reviewed published data on differential vulnerability or disease severity to SARS-CoV in those with diabetes, HIV/AIDS or other immune-compromised conditions. There are no published studies discussing the differential impact of this virus on lower socio-economic groups.

Several countries reported confirmed cases of SARS among pregnant women in 2003. The largest case series from Hong Kong reported a case fatality rate of 25% among 12 pregnant women admitted to hospital ((Wong et al. 2004). In the US, two pregnant women were among the 8 laboratory confirmed cases (Robertson et al. 2004; Stockman et al. 2004). The numbers are too small to make any inferences regarding susceptibility or disease severity of SARS in pregnancy.

With regard to asthma, there is one report that concludes that there was no increase in exacerbations of asthma among children during the SARS outbreak (Van Bever et al. 2004). There are no published reports of any association of SARS and asthma in adults.

Human Infectious Dose

The human infectious dose for SARS-CoV is not known. There are no direct human dose response data for SARS-CoV. A review of the published literature reveals one detailed dose response modeling study of SARS-CoV (Watanabe, 2010). This study consists of an analysis of multiple data sets generated by others from experiments on mice that have been proposed as relevant models for human SARS-CoV infection. The best fitted dose response model to these data results in an estimated ID_{50} of 280 PFU (95% confidence interval 130 to 530 PFU), ID_{10} of 43 PFU (95% confidence interval 20 to 81 PFU), and ID_1 of 4 PFU (95% confidence interval not reported, but estimated 2 to 8 PFU).

Laboratory Acquired Infections

There is a potential for transmission of the virus to laboratory workers during routine laboratory activity (Normile 2004; Orellana 2004). It is important to note that during the 2003 outbreak, clinical and research laboratories around the world handled large numbers of specimens from confirmed SARS patients and there were no reports of any transmission among laboratory workers during that time. Once the large human epidemic subsided, several clinical and research laboratories retained SARS-CoV isolates that had been adapted to human transmission.

There have been three laboratory accidents reported involving SARS-CoV. In 2003, in Singapore, the first incident was reported in a BSL-3 laboratory at a research institute in Singapore (Ayats et al. 2011). A

graduate student working on a virulent New York strain of West Nile Virus (WNV) became sick with fever and myalgia after making several passages of the WNV in Vero E6 cells which were also used to grow SARS-CoV. The student had minimal training and help from an Institute technician. On Sept. 3, he was admitted to the hospital with a dry cough and signs of respiratory distress. He was placed in isolation precautions and subsequently developed a moderately severe case of confirmed SARS. The technician was not infected. Surveillance and quarantine was maintained on several dozen contacts of the graduate student; no secondary infections occurred. An investigation of the laboratory proved that the WNV was contaminated with the SARS-CoV.

Also in 2003, in Taiwan, a senior research scientist working with SARS in a Class III bio-safety cabinet at The National Defense University in Taipei, Taiwan, cleaned up waste fluid that leaked from a tightly docked transfer chamber connected to the main cabinet (Lim et al. 2006). From the main cabinet, he sprayed alcohol into the chamber, waited 10 minutes, opened the chamber to spray more and finally physically cleaned it up. The next day he attended a SARS meeting in Singapore. On December 10, 2003, he noted fever and fatigue, which progressed to a dry cough and severe muscle aches. He was hospitalized on December 16, and experienced moderately severe clinical illness which was confirmed to be SARS. Contacts, especially plane co-passengers, were monitored or quarantined; no secondary infections occurred. An investigation of the laboratory revealed that SARS-CoV nucleic acid was on the handle of an alcohol bottle in the transfer chamber and on the light switch in the Class III cabinet.

In 2004, after the worldwide epidemic had subsided, two researchers working with SARS at The National Institute of Virology in Beijing, China, became ill and were diagnosed with SARS 2 weeks apart in April 2004 (World Health Organization 2004, Cooper 2009). The significance and identity of these infections was not recognized until the mother of one of the researchers became ill as well. The mother died and six other persons in contact with the two index researchers were also subsequently diagnosed with SARS. An intense laboratory investigation revealed that two other workers had experienced SARS-compatible illnesses in Feb. 2004 and were found to have antibodies to SARS-CoV in their blood, indicating that they had been exposed to the virus. The investigation also revealed that the infections did not occur in BSL-3; however, the infections involved failure of BSL-3 policy and practice. Viral material was released to lower level labs without testing the effectiveness of the inactivation procedure used by the BSL-3 lab. This laboratory accident is the only reported incident that resulted in the initial laboratory workers secondarily transmitting SARS to contacts in the community.

There are several lessons learned from a review of these laboratory accidents. Laboratory associated infections can occur from laboratory accidents leading to clinical infection in the worker. There is a potential for spread in the community from direct person-to-person spread from the index patient (laboratory worker). Laboratory worker education and training is an important component of SARS-CoV bio-safety, as well as encouraging prompt reporting and seeking medical attention when symptomatic.

Summary

SARS-CoV is a virus that emerged to cause a newly recognized human infection in 2003. The virus is highly transmissible from person-to-person via respiratory aerosols and direct contact. The disease it causes, SARS, was responsible for a large number of illnesses and an overall high death rate of nearly 10% among those confirmed to have the illness. There was a rapid spread across countries and continents due to air travel of exposed individuals and lack of knowledge among medical personnel of the pathogen and the way it was spread (transmission characteristics). The worldwide epidemic was extinguished due to sharing of knowledge of the virus and prompt institution of infection prevention and mitigation strategies in hospitals and the community, despite the fact that there were no specific medications or vaccines against the virus. Laboratory accidents have occurred with this pathogen, resulting in infections in the laboratory workers and secondary transmission reported in a limited number of close contacts following one accident.

There are currently no medications or vaccines to prevent or treat the infection caused by SARS-CoV. This virus is expected to be studied in BSL-3 high containment laboratories worldwide, including NEIDL, with the objective of understanding its biology and to develop countermeasures for future outbreaks.

For the purposes of this RA, SARS-CoV will be analyzed in detail with regard to (1) Possible event sequences that could lead to loss of bio-containment at NEIDL resulting in exposure of laboratory workers and general public to SARS-CoV; (2) Estimates of the amount of virus the laboratory workers and general public would be exposed to as a result of those event sequences; (3) Probabilistic estimates of initial infection in those exposed to those amounts of SARS-CoV and (4) Transmission modeling of the potential for secondary transmission of SARS-CoV among the general public in the case of a laboratory worker or member of the public experiencing an initial infection.

3.5.1.6 Rift Valley Fever Virus (RVFV)

Introduction

Rift Valley fever virus (RVFV) is the pathogen that causes Rift Valley fever (RVF), which is potentially a severe disease among humans and animals. For this RA, RVFV was selected for analysis based on its characteristic of transmission by vectors that are relevant to potential NEIDL sites (Mahmoud 2008). The primary route of transmission of RVFV to humans is via arthropod vectors. Transmission of the pathogen can also occur after contact and/or exposure to infected animal products, especially products of conception, blood or other fluids (White et al. 1996). Based on a review of the epidemiologic literature, there is no evidence of direct spread of RVFV from a person infected with this pathogen to another person. The human infectious dose for RVFV is not known. The potential for LAI involving RVFV exists and infections have been reported; all were prior to 1980 and all of them occurred in the absence of appropriate bio-containment precautions (Meadors et al. 1986).

RVFV was first described in 1930 from an outbreak of sudden death and abortions in sheep along the Lake Naivasha in the greater Rift Valley of Kenya (reviewed in (Pepin et al. 2010)). It was described in humans soon after. The geographic distribution of the virus has increased over the years to currently include all of Africa and the Arabian Peninsula. The pathogen causes widespread morbidity and mortality among livestock and can be devastating to the areas affected. With the availability of competent arthropod vectors in many part of the world including Europe and the US and movement of animals, there is concern for importation of this virus to RVFV-free areas.

Rift Valley fever virus is an enveloped RNA virus of the genus *Phlebovirus*, family *Bunyaviridae* (Shimshony and Barzilai 1983; Meegan 1989; Murphy et al. ; Bird et al. 2009). The virus family *Bunyaviridae* includes viral hemorrhagic viruses in other genera such as Crimean-Congo hemorrhagic fever (genus *Nairovirus*) and Hantaan viruses (genus *Hantavirus)* (LeDuc 1989).

The natural reservoirs of RVFV are animals, mostly ruminants which have a high mortality from the disease. The host range for RVFV is broad and many species of vertebrates are affected by this pathogen, including rodents, domesticated animals such as dogs and cats, to non-human primates (Shimshony and Barzilai 1983).

RVFV has adapted to a large array of arthropod vectors including mosquitoes and ticks. This is in contradistinction to many arboviruses that are adapted to a narrow range of vectors. Transmission of

RVFV to animals is via biological vectors in whose bodies the pathogenic organism develop and multiply before being transmitted to the next host. . Many species of mosquitoes are competent to carry RVFV; more than 23 species in 5 genera including Aedes, Anopheles, Culex, Coquillettidia, Eretmapodites, Mansonia have been described (Meegan and Bailey 1989; Bird et al. 2009). *Rhipicephalus* ticks have also been noted to be biologic vectors (Meegan and Bailey 1989). RVFV has been isolated from mechanical vectors such as *Culicodes* (biting gnats or midges), *Simulium* (black flies); these vectors are not essential to the life cycle of the parasite. RVFV contaminates the mouth parts of these arthropods when they feed on a viremic host, the pathogen does not reproduce in the arthropod, but may be transmitted via pathogen-contaminated mouth parts when the arthropod subsequently feeds on another host.

During interepidemic periods, the virus is maintained in nature via transovarial transmission in mosquitoes, as was shown in *Aedes lineatopennis* in Kenya and in *Aedes vexans* in Senegal (Flick and Bouloy 2005).

BSL-3 is required for work RVFV (Chosewood et al. 2009). RVFV possession in the United States requires a USDA permit, and is a USDA restricted, USDA high consequence agent. A Department of Commerce permit is required, and vaccination is recommended (Weinbren 2008).

Human Disease and Outbreaks

Rift Valley fever in humans has been reported from an ever increasing geographic area (Pepin et al. 2010). Within Africa, it has now reported from almost all nations. The pathogen crossed a large natural barrier (Sahara desert) and appeared in Egypt in 1977. Later, in 1979, it was first reported outside continental Africa in the island nation of Madagascar across the Indian Ocean where the virus is now endemic. In 2000, the virus crossed over the Red Sea to the Arabian Peninsula and caused a large outbreak of RVF in both animals and humans. There is concern that RVF will spread even wider in the future.

The reported outbreaks of RVF have involved large numbers of humans, estimated to be in the thousands. In 1977, in the Egypt outbreak, it is estimated that there were 20,000 human clinical cases with 598 deaths; the overall case fatality rate was 3 percent, in hospitalized patients, the fatality rate was 14 percent. The true prevalence of disease was estimated to be many fold higher than reported as there are sub-clinical infections (Meegan and Bailey 1989).

An outbreak in 1987 in Senegal and Mauritania was reported to have a higher mortality rate, with 400 confirmed cases and 224 deaths (Meegan 1989; Swanepoel and Coetzer 1994; Flick and Bouloy 2005).

The largest outbreak to date was reported from Kenya during 1997–1998. It was estimated that there were nearly 90,000 human cases, fortunately with a very low case fatality rate (478 deaths) (Centers for Disease Control and Prevention 1998)Recent outbreaks have been smaller; in 2007, Sudan reported 125 human cases including 60 deaths (Gerdes 2002; Gerdes 2004; Bird et al. 2009).

Overall human fatality rate from RVF has been estimated at 0.5–2.0 percent of those infected; the rate is much higher among those with severe disease (Pepin et al. 2010).

The disease is considered to be mild and sub-clinical in a majority of patients and presents frequently as a self-limiting influenza-like syndrome without any severe sequelae (Bird et al. 2008) (Pepin et al. 2010).

The manifestations of severe RVF are variable. After a short incubation period, humans may experience hepatitis, retinitis, delayed-onset encephalitis and, in the most severe cases, hemorrhagic disease.

The primary route of transmission of RVFV to humans is via arthropod vectors. Transmission of the pathogen can also occur after contact and/or exposure to infected animal products, especially products of conception, blood or other fluids (White et al. 1996). The viral load among these animal products is exceedingly high and aerosolization of pathogen is considered to be very common. There are anecdotal reports of human cases of RVF with no clear history of exposure, other than being in the vicinity of an animal that was being slaughtered (Hoogstraal 1979). There is also a potential of transmission via ingestion of raw milk from infected animals (Flick and Bouloy 2005).

Based on a review of the available literature from epidemiologic investigations on outbreaks of RVFV, there does not appear to be evidence for direct spread of RVFV from a person infected with this pathogen to another person; especially during an epidemic (Centers for Disease Control and Prevention (U.S.) 1998; Centers for Disease Control and Prevention (U.S.) 2007a; Centers for Disease Control and Prevention (U.S.) 2007b). Recent studies have shown a high prevalence of seropositivity to RVFV among populations during inter-epidemic periods; the natural reservoir for RVFV and the mechanism by which humans become infected during interepidemic periods are unknown (LaBeaud et al. 2008; LaBeaud et al. 2010).

The diagnosis of RVF is based on clinical presentation and epidemiological history involving appropriate exposure or travel to endemic areas. The differential diagnosis is large and includes other viral hemorrhagic fevers; hence laboratory confirmation is required for diagnosis of RVF caused by RVFV. Laboratory tests include PCR-based rapid tests, including virus isolation, antigen detection and detection of specific antibodies to the pathogen (Pepin et al. 2010). The availability of these different modalities of testing varies, especially in resource poor endemic regions.

Supportive therapy remains the mainstay of the management of patients with RVF. There are no specific anti-viral medications for RVFV. The utility of ribavirin is not proven; however, there is a recommendation of its use in mass casualty situations (Sidwell and Smee 2003).

Vaccines are widely used for livestock in endemic areas. There is an experimental human vaccine that is available for use in at-risk laboratory workers and military personnel (LaBeaud et al. 2007). Recently, researchers from the US have developed two recombinant RVFV vaccines using vaccinia virus (VACV) as a vector for use in livestock (Papin et al. 2011). Testing in animal models showed that the vaccines are safe and efficacious; protective levels of antibody titers were noted in vaccinated mice and baboons.

Disease in Medically Vulnerable Subpopulations

There are no specific reports of adverse outcomes or increased susceptibility in medically vulnerable Subpopulations. There are concerns of RVFV causing abortions in humans as it occurs in animals (Abdel-Aziz et al. 1980; Niklasson et al. 1987). In the 2000 RVF outbreak in Saudi Arabia, among 683 patients reported with disease, it was noted that young children, pregnant women and neonatal infants were relatively spared of the disease and there were no deaths reported in any patient younger than 10 years (Balkhy and Memish 2003).

Human Infectious Dose

There are no human dose-response data available. Animals of several species have been infected by low doses of RVFV by the inhalational route, as detailed in Appendix J. These data, as well as cases of humans acquiring the disease in the vicinity of animal products and in laboratories (prior to implementation of modern bio-safety precautions) suggest that RVFV infection by the aerosol route in humans might be a concern.

Laboratory Acquired Infections

There have been no reports of accidental or intentional exposures to RVFV. Laboratory-acquired infections were frequently reported from exposure to RVFV, all of them prior to 1980 and all of them in the absence of appropriate bio-containment precautions (Meadors et al. 1986). There were at least 47 infections in laboratory workers described with 1 death during this period (Hanson et al. 1967; Pike 1979) (Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses 1980)

There have been limited reports of laboratory incidents involving RVFV after institution of appropriate biosafety practices (NRC (National Research Council) 2011)As noted in the Biosafety Review (Appendix D), during the period 2000-2010, there was one incident reported of a laboratory spill involving RVFV in 2009.

Summary

Rift Valley fever virus is an RNA virus in the larger family of viral hemorrhagic fevers and causes Rift Valley fever. The disease causes high morbidity and mortality in animals and is a potentially serious illness of humans. There have been large outbreaks of RVF in humans reported from Africa and Arabian Peninsula involving several hundreds to nearly 90,000 cases. The pathogen is transmitted to humans via arthropod vectors or direct contact/exposure to infected animal products. There is no specific treatment for RVF and case fatality rates are generally less than 2 percent. For severe cases of RVF involving bleeding, the mortality is higher. The geographic area for RVF is ever expanding and this pathogen is expected to be continued to be studied in laboratories around the world, including the US.

For the purposes of this RA, RVFV virus will be analyzed in detail with regard to: (1) Possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to RVFV virus; (2) Estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) Probabilistic estimates of initial infection in those exposed to those amounts of RVFV virus.

There is no direct person-to-person transmission of RVFV; transmission to humans is via arthropod vectors or by contact with infected animal products. There is a limited literature on disease transmission models of RVFV involving animals, arthropod vectors and human hosts. Several models have addressed other complex variables such as climate conditions and livestock in the prediction and transmission of RVFV in endemic areas (Clements et al. 2006; Favier et al. 2006; Anyamba et al. 2009; Metras et al.

2011; Mpeshe et al. 2011). The applicability of these published models and epidemiologic, climate and livestock data to conditions in the US, specifically to NEIDL sites under study in the RA is unknown. Furthermore, epidemiologic data on ruminants and mosquito vectors are not uniformly available in a format suitable for use in secondary transmission modeling. The risk analysis for RVFV will include a qualitative discussion of transmission of RVFV and will not include detailed mathematical modeling of transmission of RVFV in the community.

3.5.1.7 Andes Virus (ANDV)

Introduction

Andes virus (ANDV) is the major etiological pathogen of Hantavirus Pulmonary Syndrome (HPS) that occurs in South America. For this RA, ANDV was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). Inhalation of aerosolized virus by humans from contact with contaminated rodent feces, saliva, and urine, and contact with contaminated fomites are considered the primary routes of transmission of ANDV (CDC 2008). Direct person-to-person transmission of ANDV has been documented; though the extent of this transmission is limited to close family contacts and has not resulted in large outbreaks of HPS. The human infectious dose for ANDV is not known. The potential for LAI with ANDV exists though no reports of LAI have been reported with this pathogen. LAI have been reported with related hantaviruses, all reported prior to 1994 (reviewed in (Schmaljohn and Hjelle 1997)).

ANDV is maintained in rodent reservoirs and causes 50-80 cases of this potentially fatal disease annually, mostly in Chile and Argentina, when humans come into contact with rodent excrement (Mertz et al. 2006). ANDV is the only hantavirus that has been shown to be transmitted directly from person-to-person.

Andes virus is one of nearly 40 hantaviruses that have been described to date, of the Genus *Hantavirus*, family *Bunyaviridae* (CDC 2008). Hantaviruses are classified as either New World strains that cause HPS or Old World strains that cause Hemorrhagic Fever with Renal Syndrome. Apart from Andes virus, other New World hantaviruses are Choclo, Laguna Negra, and Sin Nombre viruses which are distributed in North and South America. Old World strains include the prototypic Hantaan, Puumala, and Seoul hantaviruses (Jonsson et al. 2010). The natural reservoir of Andes virus is the long-tailed pygmy rice rat, *Oligoryzomys longicaudatus*, and other species of genus *Oligoryzomys* (CDC 2008). The rodents are in the sub-family *Sigmodontinae* within the family *Muridae*, order *Rodentia* with which the hantaviruses have evolved for thousands of years (Toro et al. 1998; McCaughey and Hart 2000; Mertz et al. 2006). The

natural reservoir is maintained by chronic infection of the rodent with the virus, with no overt signs in the rodent.

BSL-4 biocontainment precautions are recommended for ANDV when infecting rodent species permissive for or susceptible to chronic infection. Otherwise, BSL-3 biocontaiment precautions are recommended. (Chosewood et al. 2009).

Human Disease and Outbreaks

HPS caused by Andes virus occurs in regions where the rodent reservoir is common, namely in Argentina and Chile. Several hundred cases have been reported in these countries since the disease was first described (Mertz et al. 2006). Between the years 2001 and 2005, the number of cases of HPS in Chile ranged from a low of 56 cases in 2004 to a peak of 81 cases in 2001.

HPS is characterized by a prodromal phase after a variable incubation period that includes headache, backache, abdominal pain, nausea, and diarrhea (CDC 2008). The cardiopulmonary phase starts abruptly and usually results in respiratory failure, requiring ventilator support. Most patients also develop cardiogenic shock. Overall case fatality rates for HPS patients is in the range of 30-40 percent as reported in most outbreaks, with one report from Argentina reporting a higher case fatality rate of 55 percent (Lazaro et al. 2007).

The route of transmission of ANDV to humans was by exposure to rodent excrements, not necessarily direct contact with the rodent. Inhalation of aerosolized virus from contaminated rodent feces, saliva, and urine, and contact with contaminated fomites are considered the primary routes of transmission of ANDV (CDC 2008). Human activities that increase the chances of coming into contact with rodent excrements such as cleaning out old, unused sheds or cabins are associated with infection with New World hantaviruses in general, thus accounting for the spring and summer time increase in reports of cases. There are rare reports of rodent bites transmitting ANDV (Merino et al. 2002). There are no known arthropod vectors in the transmission of ANDV.

A unique feature of ANDV is that direct person-to-person transmissions of ANDV have been reported. There are reports of HPS in close contacts with genetic evidence of person-to-person transmission, from earlier outbreaks in the 1990s to more recent cases. In these circumstances, transmission generally occurred in close family contacts who exchanged bodily fluids, with evidence of ANDV RNA found in saliva of patients. (Enria et al. 1996; Padula et al. 1998; Martinez et al. 2005; Castillo et al. 2007; Lazaro

et al. 2007). A recent study that prospectively studied 476 household contacts of 76 index patients with HPS in Chile found 16 contacts developed confirmed HPS (3.4%) (Ferres et al. 2007). A third of all the cases occurred in family clusters. Person-to-person transmission was definite in only 3 household contacts and probable in another 9. Sexual contacts were at the highest risk for HPS in this study. In summary, direct person-to-person transmission of ANDV has been documented; though the extent of this transmission is limited to close family contacts and has not resulted in large outbreaks of HPS.

In other reports from Argentina, 16 cases of HPS were suspected to be due to person-to-person transmission, though contact or exposure to rodents could not be completely ruled out for a majority of those patients (Wells et al. 1997; Cantoni et al. 2001).
There did not appear to be any hospital- or healthcare-associated person-to-person transmission in one outbreak in Chile (Castillo et al. 2004). Other reports have described cases in health care workers (Lopez et al. 1996; Wells et al. 1997; Toro et al. 1998; Mertz et al. 2006).

The diagnosis of ANDV is based on clinical presentation, epidemiological history of exposure to rodent excrements, travel to endemic areas, and laboratory confirmation. Serological assays for hantavirus specific IgG and IgM are the most commonly used tests for confirmation. Rapid PCR-based tests are also available in select laboratories. Immunohistochemistry on pathologic specimens is also performed in select cases to confirm hantavirus infection (Mertz et al. 2006; Jonsson et al. 2008).

Supportive therapy remains the mainstay of the management of patients with HPS caused by ANDV (Mertz et al. 2006; Jonsson et al. 2008). Intensive care support along with specialized procedures such as extracorporeal membrane oxygenation has been shown to be beneficial to patients. There is no specific antiviral that has been approved or is effective against ANDV.

Disease in Medically Vulnerable Subpopulations

In general, all age groups are affected. There are no specific reports of adverse outcomes or increased susceptibility to ANDV in medically vulnerable Subpopulations. There are reports of hantavirus infection in children, including those in Argentina; some have reported worse outcomes, others have reported clinical courses and outcomes being similar to those in adults (Pini et al. 1998; Ramos et al. 2001; Ferres and Vial 2004; Overturf 2005). There are no specific reports of ANDV infection in pregnancy. There appeared to be no difference in maternal or fetal outcomes in HPS caused by Sin Nombre virus in one study (Howard et al. 1999) and concern for worse outcomes in 2 patients (Gilson et al. 1994). .

Human Infectious Dose

There are no human dose-response data available for ANDV. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing ANDV, but the amount of virus inhaled by humans who became infected is not known. Data from animal models suggests that the ANDV infectious dose for humans is low.

Laboratory Acquired Infections

There have been no laboratory incidents or laboratory-acquired infections reported with ANDV (Biosafety Review, Appendix D). Laboratory-acquired infections have been reported with related hantaviruses, all reported prior to 1994 (reviewed in (Schmaljohn and Hjelle 1997)). These were reported prior to institution of modern biosafety practices and involved laboratory workers handling infected rats obtained from breeders, wild-caught, naturally-infected rodents, or experimentally infected rodents. Asymptomatic seroconversions have been reported among workers using cell-culture adapted hantaviruses. There is one report of occupationally acquired hantavirus infection in a utility company employee (Jay et al. 1996). He developed HPS from the Sin Nombre virus.

Summary

Andes virus is a New World hantavirus that causes a severe cardio-pulmonary syndrome (HPS) and is restricted to Argentina and Chile. The risk factor for humans is exposure to rodent excrements that are contaminated with ANDV. Inhalation of aerosolized virus is the primary route of transmission to humans. The mortality from HPS can be significant and in the range of 30-55 percent. There are no specific treatment modalities for HPS; supportive therapies including intensive care are the main stay of management of HPS patients. ANDV is unique among hantaviruses in that there is documented direct person-to-person transmission; the extent of this transmission is limited to close family contacts and has not resulted in large outbreaks of HPS.

There is a possibility of infection if an individual is exposed directly to ANDV. Direct person-to-person transmission of ANDV is possible; based on natural outbreaks, this risk is limited to close household contacts and the attack rate is low.

For the purposes of this RA, Andes virus will be analyzed in detail with regard to: (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to Andes virus; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of

initial infection in those exposed to those amounts of Andes virus. As there is a low risk of direct-to-person transmission of Andes virus amongst members of the public and low risk for such transmission to cause large outbreaks, secondary transmission modeling of the spread of this virus in the community following an initial infection will not be performed. Moreover, there are limited studies available to provide epidemiologic data for detailed secondary transmission modeling and no published mathematical models for this pathogen.

3.5.2 BSL-4 Biocontaiment Precautions: Pathogens

3.5.2.1 Ebola Virus (EBOV)

Introduction

Ebola virus is the common designation for a group of closely related viruses that cause Ebola hemorrhagic fever (EHF). For this RA, EBOV was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). The exact mode and route of transmission of Ebola viruses are not known. The pathogen is believed to be transmitted to humans via contact with infected animals. Direct person-to-person transmission of Ebola viruses from index cases to family and community contacts has been described via close contact with bodily fluids from infected patients. The human infectious dose of EBOV is not known. The potential for LAI with EBOV exists. There have been several laboratory incidents involving Ebola viruses, though no LAI have been reported.

EHF is characterized by a severe illness with bleeding and very high mortality. The first description of these viruses was from two natural outbreaks in 1976 from the Democratic Republic of the Congo (DRC, formerly Zaire) and southern Sudan (Hartman et al. 2010; Feldmann and Geisbert 2011). The viruses are named for the Ebola River located in northwestern DRC where the first outbreak occurred.

Ebola viruses are RNA viruses of the genus *Ebolavirus* of the family *Filoviridae.* These pathogens are closely related to Marburg virus and there are concerns of co-circulation of these viruses in their reservoir hosts (Bausch et al. 2006; Pourrut et al. 2009; Hartman et al. 2010). Currently, there are 5 known closely-related strains of Ebola virus; Ebola-Zaire, Ebola-Sudan, Ebola-Côte d'Ivoire, Ebola-Reston and Ebola-Bundibugyo (Towner et al. 2008; Normile 2009; 2009; Wamala et al. 2010; Feldmann and Geisbert 2011).

The natural reservoir of Ebola viruses is thought to be bats of various species, namely fruit bats, Old world fruit bats, and flying foxes in three genera of *Pteropus* (Leroy et al. 2005; Leroy et al. 2009). There are no known insect vectors for these pathogens. Several laboratory animals including mice (requires

adapted viral strains), guinea pigs, and non-human primates can be infected with the pathogen and have been used as animal models of Ebola virus infections. BSL-4 biosafety practices are required for all work with Ebola viruses (Chosewood et al. 2009).

Human Disease and Outbreaks

The human disease caused by Ebola viruses is the prototypical hemorrhagic fever that has received considerable media attention and is of concern to the public (Leffel and Reed 2004). EHF is characterized by an abrupt start of the illness with non-specific flu like symptoms such as fever, chills, muscle aches, and general malaise. This is often followed by gastro-intestinal symptoms and more severe illness with low blood pressure. Contrary to popular belief, hemorrhagic manifestations are variable among patients and usually occur at the peak of the illness. These include bleeding from all mucous membranes and other body sites.

Of the five related strains, the Ebola-Zaire virus strain has been responsible for the largest number of outbreaks. There have been 12 outbreaks from 1976 through to 2008, with large numbers of cases and a fatality rate between 25-90% (Hartman et al. 2010). Attack rates in these outbreaks have varied between 5 percent among the community and 20 percent in close relatives of patients (Feldmann et al. 1996). The Zaire strain is generally considered the most virulent of the Ebola virus strains.

The Ebola-Sudan virus strain was responsible for the largest outbreak described to date in Uganda during 2000-2001, with 425 cases and an overall case fatality rate of 53% (2001; Okware et al. 2002). Attack rates in this and prior outbreaks have varied between 2.5 and 12 percent.

The Ebola-Cote d'Ivoire virus strain has only been reported to cause a single nonfatal infection acquired during the necropsy of a dead chimpanzee (Formenty et al. 1999; Le Guenno et al. 1999).

The Ebola-Reston virus strain was discovered in 1989 from cynomolgus macaques imported from the Philippines for medical research in the United States in Reston, Virginia. An unusually large number of monkeys died in quarantine and investigations led to the isolation of the new strain of Ebola virus. There were no human cases; however, 21 animal handlers at the Philippine exporter and four employees of the quarantine facility were found to have antibodies to the virus, indicating that they had been infected (Normile 2009). Reston virus strain can infect humans but no serious illness or death in humans have been reported to date (Towner et al. 2008; Normile 2009; 2009; Wamala et al. 2010; Feldmann and Geisbert 2011).

The Ebola-Bundibugyo virus strain is the most recent to be described from an outbreak in Uganda, with over 100 persons ill and a 40% fatality rate (Towner et al. 2008; MacNeil et al. 2010).

The exact mode and route of transmission of Ebola viruses are not known. The pathogen is believed to be transmitted to humans via contact with infected animal hosts (Pourrut et al. 2005; Swanepoel et al. 2007). Some recent outbreaks been have attributed to the consumption or handling of bush meat (Leffel and Reed 2004). Human infections have also been documented via handling of infected dead and living chimpanzees, gorillas, and forest antelopes (Peterson et al. 2004). Recent evidence suggests that fruit bats might have a reservoir role; it is unclear as to whether other species of bats are involved or how transmission to humans or apes occurs (Groseth et al. 2007). Fruit bats are eaten by local populations in outbreak areas and could be the source of human infections (Leroy et al. 2005). One route of infection is presumably to be by inhalation of the aerosolized virus, though this has only been noted in experimental conditions (Leffel and Reed 2004). There have been no insect vectors reported in the transmission of Ebola viruses.

Direct person-to-person transmission of Ebola viruses from index cases to family and community contacts has been described. Secondary attack rates have varied among different outbreaks and strains. It has been shown that the viruses are shed in a wide variety of bodily fluids during the acute period of illness and this contributes to the person-to-person spread in the setting of the cultural practice of touching and close contact with ill patients in Africa (Bausch et al. 2007). Though this is postulated to be the major route of transmission, other routes are possible such as by droplets, airborne particles, or contaminated objects (fomites); these came to light after several patients reported no direct contact or exposure during a large outbreak in the Congo (Roels et al. 1999).

An important aspect of filovirus outbreaks in general (Ebola and Marburg viruses) is the amplification that often occurs in hospitals by transmission of the viruses from patients to family or health care workers (Fisher-Hoch 2005; Hartman et al. 2010) . This is postulated to have occurred in the setting of poor sanitary conditions, lack of personal protective equipment, and reuse of injection needles. Close contact with a severely ill patient, during care at home or in hospital, and certain burial practices are common routes of infection in Africa. Transmission via contaminated injection equipment or through needle-stick injuries is associated with more severe disease, rapid deterioration, and possibly higher fatality. The risk of transmission of Ebola viruses from fomites in an isolation ward and from convalescent patients is low when recommended infection control guidelines for the viral hemorrhagic fevers are followed (Bausch et al. 2007).

Based on analysis of the larger outbreaks, several estimates of the reproductive number (R_0) for Ebola viruses have been generated. Estimates range from a low of 1.34 to as high as 2.7 (Chowell et al. 2004; Lloyd-Smith et al. 2005; Lekone and Finkenstadt 2006). This indicates that in the right social and community setting where there is close contact with severely ill patients and the dead, especially in hospital settings, there is a possibility of direct person-to-person transmission and this could lead to large outbreaks. This situation is considered unlikely in the US.

The diagnosis of Ebola virus infection is based on epidemiological history of exposure to a patient during a known outbreak or travel history. Laboratory diagnostic methods include antibody detection by ELISA, antigen detection by PCR, and virus isolation by culture. Electron microscopy is also employed for detection and identification of the viruses. Laboratory confirmation is available only in specialized laboratories such as the CDC (Hartman et al. 2010).

There are no effective medications or vaccines for the treatment or prophylaxis of Ebola virus infection (Hartman et al. 2010). Vaccine and therapeutic drug candidates are an active area of research for both Ebola and Marburg viruses (Geisbert et al. 2010; Falzarano et al. 2011).

Disease in Medically Vulnerable Subpopulations

EHF has been diagnosed in all age groups; though the large African outbreaks were noted to be predominantly in the elderly. Children are thought to be relatively resistant to infection (Dowell 1996). There are no specific reports of increased susceptibility to Ebola virus infection in medically vulnerable Subpopulations. There are reports of increased attack rates among women in one outbreak (Okware et al. 2002). In one large outbreak in the Congo (Mupapa et al. 1999), it did not appear the incidence of EHF was increased in pregnant women; there was a loss of nearly all pregnancies and the mortality rate among these women was higher, though not statistically higher, than that in the general population (95% vs 77%).

Human Infectious Dose

The human infectious doses of Ebola viruses are not known. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing EBOV, but the amount of virus inhaled by humans who became infected is not known. From animal data, the infectious dose for Ebola viruses it is postulated to be low (Pratt et al. 2010).

Laboratory Acquired Infections

There have been several laboratory incidents involving Ebola viruses (Biosafety Review, Appendix D). A fatal Ebola infection occurred in a laboratory worker in Russia in 2004. The worker had suffered a needle stick injury with the Ebola-Zaire strain while working with a guinea pig model of the pathogen. Another accident in Germany in 2009 involved a laboratory researcher wearing protective gloves who experienced a needle stick from a syringe suspected to contain Ebola virus (Tuffs 2009). The researcher was isolated and provided an experimental vaccine along with supportive measures. There was no evidence of clinical infection.

In the US, a significant incident occurred in 2004 at U.S. Army Medical Research Institute For Infectious Diseases (USAMRIID) when a researcher sustained a needle stick injury through a gloved hand when handling and injecting mice with an antibody (Kortepeter et al. 2008). The exposure to the virus was considered probable and the risk of infection was low as the mice did not yet have Ebola virus in their blood. The worker was placed in isolation and subsequently did not develop either asymptomatic disease or clinical illness. Another incident in 2005 involved both Ebola and Marburg viruses where a sharp object punctured a boot with no skin penetration.

As with other high-containment pathogens, laboratory-acquired infections with Ebola virus are a concern and laboratory personnel education and training should be reinforced while dealing with this BSL-4 pathogen.

Summary

Ebola viruses cause a hemorrhagic fever that is highly fatal to humans. The virus was discovered as a pathogen in 1976 and since then, three major strains have been responsible for the large outbreaks, all in Africa. The natural reservoir of the pathogen appears to be fruit bats in Africa. There is no known insect vector. There is a possibility of infection if an individual is exposed to Ebola viruses. There is evidence of direct person-to-person transmission via close contact with bodily fluids from infected patients. There are no effective medications or vaccines for these pathogens. These pathogens are expected to be continued to be studied in BSL-4 maximum biocontainment laboratories worldwide, including the US. There have been laboratory-acquired infections involving Ebola viruses, though none in the US.

For the purposes of this RA, Ebola viruses will be analyzed in detail with regard to (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to Ebola viruses; (2) estimates of the amount of pathogen the laboratory workers

and general public would be exposed to as a result of those event sequences; (3) probabilistic estimates of initial infection in those exposed to those amounts of Ebola viruses and (4) estimates of secondary transmission of Ebola viruses in the community in the event of an initial infection in a laboratory worker.

3.5.2.2 Marburg Virus MARV

Introduction

Marburg virus is a member of a group of hemorrhagic fever viruses that was first described in laboratory workers, as opposed to a natural outbreak. For this RA, MARV was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). The mode of transmission of Marburg virus is direct exposure to bats and/or their secretions or excrement. Direct person-to-person transmission of MARV from index cases to family and community contacts has been described via close contact with bodily fluids from infected patients. The human infectious dose of MARV is not known. MARV was first described as a laboratory acquired infection and thus the potential exists. There have been no reports of laboratory incidents or infections involving Marburg virus in the US.

The first cases involving this virus were described in Marburg, Germany (thus the name) and Belgrade, Yugoslavia in 1967 in workers who had handled infected African green monkeys (*Chlorocebus sabaeus* [formerly, *Cercopithecus aethiops*]), imported from Uganda. There were primary and secondary infections in these incidents and detailed studies led to the discovery of this new pathogen. Since then, through 2005, there have been several natural outbreaks reported, with two large ones reported from Africa.

Marburg virus is an RNA virus of the genus *Marburgvirus* of the family *Filoviridae.* This pathogen is closely related to Ebola virus and there are concerns of co-circulation of these viruses (Bausch et al. 2006; Pourrut et al. 2009; Hartman et al. 2010).

Despite the discovery of this pathogen in 1967, the natural reservoir was not known till recently, when it was shown to be a common species of fruit bat, *Rousettus aegyptiacus,* and possibly the insectivorous bats *Miniopterus inflatus* and *Rhinolophus eloquens* (Swanepoel et al. 2007; Towner et al. 2007; Kuzmin et al. 2010).

Since its discovery, Marburg virus has been studied extensively in maximum biocontainment laboratories around the world, including the US. BSL-4 is required for all work with Marburg virus (Chosewood et al. 2009).

Human Disease and Outbreaks

Marburg virus causes a rapidly progressive illness that starts abruptly with severe headache, malaise, and muscle pains. Fever is followed by other non-specific symptoms and within a week, patients develop the characteristic features of 'hemorrhagic fever' with bleeding from body openings, often from multiple sites. Case fatality rates are generally high, though there appears to be some variation between strains. The Angolan strain is reported to have the highest case fatality rates approaching 100% with other strains being less pathogenic (Mahanty and Bray 2004; Jeffs 2006; Bausch et al. 2008; Hartman et al. 2010).

Human outbreaks were rare between the discovery of the virus in 1967 and 1998 with only three cases reported, each involving either a single person (Kenya, 1987) or an index case and the infection of a traveling companion, medical personnel, or both (Zimbabwe–South Africa, 1975, and Kenya, 1980) (Feldmann 2006; Towner et al. 2006). Between 1998 and 2005, there have been two large natural outbreaks in Africa. All outbreaks have been associated with humans coming into contact with bats and their secretions/excreta either by visiting caves (which is a popular tourist attraction), working in mines, or sleeping in areas with bats. In all outbreaks, there have been secondary infections in family members and in the local community from the index cases.

One large outbreak occurred in the Democratic Republic of the Congo during the period 1998-2000, which involved gold miners. A total of 154 cases (with 83% case fatality rate) were reported with multiple genetic variants of the virus, indicating that there was ongoing introduction of the virus to humans over a period of time. In this outbreak, the index cases were in miners and with secondary cases in their family members and in the general community (Bausch et al. 2006; Feldmann 2006). An interesting point to note was the low numbers of infections in health care workers caring for these patients, which is unusual for a filovirus infection.

The largest natural outbreak of Marburg virus occurred in Angola in 2004-2005 (Feldmann 2006; Towner et al. 2006). This was an extension from the usual territory of filoviruses to Western Africa and is a matter of concern. It is postulated that this outbreak started with one index case, with secondary transmission to family and community members. The outbreak was further amplified in hospital settings as is typical for filoviruses. The Angolan outbreak was unique in several ways and this may represent a new strain of

Marburg virus: in the illness which has a shorter incubation period, many children were affected and the 100% case fatality rate was higher than reported even in the Zaire outbreak of Ebola virus.

The most recent outbreak was reported in 2007, with Ugandan miners being affected; the likely source was fruit bats in the cave mine (2007).

The mode of transmission of Marburg virus is direct exposure to bats and/or their secretions or excrement; presumably by inhalation of the aerosolized virus, though this has only been noted in experimental conditions (Leffel and Reed 2004). There have been no insect vectors reported in the transmission of Marburg virus.

Direct person-to-person transmission of Marburg virus from index cases to family and community contacts has been described in the 1967 outbreak and the two large outbreaks in Africa. Secondary transmission is associated with close contact with the ill patient or their bodily fluids, mainly blood (Bausch et al. 2006; Feldmann 2006; Towner et al. 2006). Other body fluids from infected humans (feces, vomitus, urine, saliva, and respiratory secretions) with high virus concentrations, especially when these fluids contain blood, have also been implicated in transmission. Transmission is known to occur only during the symptomatic phase of the illness and not during the incubation period.

An important aspect of filovirus outbreaks in general (Ebola and Marburg) is the amplification that often occurs in hospitals by transmission of the virus from patients to family or health care workers (Fisher-Hoch 2005; Hartman et al. 2010) . This is postulated to have occurred in the setting of poor sanitary conditions, lack of personal protective equipment, and reuse of injection needles. Close contact with a severely ill patient, during care at home or in hospital, and association with certain burial practices, are common routes of infection in Africa. Transmission via contaminated injection equipment or through needle-stick injuries is associated with more severe disease, rapid deterioration, and possibly higher case fatality rates.

There have been two recent reports of travel-related Marburg virus infection in those who visited the same mine in Uganda. The first case was discovered in the Netherlands (Timen et al. 2009) and the second was diagnosed in Colorado (2009). These cases highlight the importance of increasing awareness of the risks associated with travel and specific activities such as cave exploration in areas where filovirus outbreaks have been reported.

The diagnosis of Marburg virus is based on epidemiological history of exposure to a patient during a known outbreak or travel history. Laboratory diagnostic methods include antibody detection by ELISA, antigen detection by PCR, and virus isolation in culture. Electron microscopy is also employed for detection and identification of the virus. Laboratory confirmation is available only in specialized laboratories such as the CDC (Hartman et al. 2010).

There are no effective medications or vaccines for the treatment or prophylaxis of Marburg virus infection (Hartman et al. 2010). Vaccines and medications are an active area of research for filoviruses (Ebola and Marburg virus).

Disease in Medically Vulnerable Subpopulations

All age groups are susceptible to infection, but most cases have occurred in adults. Prior to the 2004 outbreak in Angola, pediatric cases were considered extremely rare. In the large outbreak that occurred in the Democratic Republic of the Congo from late 1998 to 2000, only 12 (8%) of the cases were under the age of 5 years (Feldmann 2006; Towner et al. 2006). There are no reports of increased susceptibility in medically vulnerable Subpopulations including pregnant women.

Human Infectious Dose

The human infectious dose of Marburg virus is not known. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing the pathogen, but the amount of virus inhaled by humans who became infected is not known. From animal data, the human infectious dose for Marburg virus is postulated to be low.

Laboratory Acquired Infections

Marburg virus was first described as a laboratory-acquired infection in 1967 in Marburg, Germany and Belgrade, Yugoslavia, when laboratory workers handled infected African green monkeys or their tissues. The infected monkeys consigned from Uganda to Europe were caught on the shores of Lake Victoria and on islands where fruit bats are prevalent (Towner et al. 2007). The outbreaks involved 25 primary infections, with 7 deaths, and 6 secondary cases, with no deaths. The primary infections were in laboratory staff exposed to Marburg virus while working with monkeys or their tissues. The secondary cases involved two doctors, a nurse, a post-mortem attendant, and the wife of a veterinarian. All secondary cases had direct contact, usually involving blood, with a primary case. Both doctors became infected through accidental skin pricks when drawing blood from patients.

There have been 4 incidents reported from a maximum biocontainment laboratory in Johannesburg, South Africa involving Marburg virus (Biosafety Review, Appendix D); these resulted in no infections. In two separate incidents in a BSL-4 laboratory in Russia involving exposure of workers to Marburg virus, both resulted in infections, with one death. There have been no reports of laboratory incidents involving Marburg virus in the US.

As with other high-containment pathogens, laboratory-acquired infections with Marburg virus are a concern and reinforce laboratory personnel education and training while dealing with this BSL-4 pathogen.

Summary

Marburg virus causes a hemorrhagic fever that is highly fatal to humans. The virus was discovered as a pathogen in 1967 from laboratory primates and did not pose a major threat to humans till large outbreaks were noted starting in 1998. There appears to have been a shift in the natural history of the disease with the Angolan outbreak in 2004. The natural reservoir of the pathogen appears to be fruit bats in Africa. There is no known insect vector. There is evidence of direct person-to-person transmission via close contact with bodily fluids from infected patients that contain high concentrations of the virus, such as blood.

There is a possibility of infection if an individual is exposed to Marburg virus. There are no effective medications or vaccines for this pathogen. This pathogen is expected to be continued to be studied in BSL-4 maximum biocontainment laboratories worldwide, including the US. There have been laboratory-acquired infections involving Marburg virus, though none in the US.

For the purposes of this RA, Marburg virus will be analyzed in detail with regard to (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to Marburg virus; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of initial infection in those exposed to those amounts of Marburg virus.

There is a possibility of person-to-person transmission of Marburg virus via close contact; however the risk is low and requires close contact that may be culture- and region-specific. The closely related Ebola virus is being analyzed by secondary transmission modeling for the spread of infection in the community, the results of which will be broadly applicable to the risk analysis of Marburg virus. For these reasons,

secondary transmission modeling of the spread of Marburg virus infection in the community will not be performed as part of this RA.

3.5.2.3 Lassa Virus (LASV)

Introduction

Lassa viruses are the causative pathogens of Lassa fever, which is a viral hemorrhagic fever. For this RA, LASV was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). The mode of transmission of Lassa viruses to humans is from direct contact with *Mastomys natalensis*, and African rodent species. Direct person-to-person transmission of Lassa viruses has been described, especially in hospital settings and is associated with direct contact with the blood or other bodily fluids containing virus particles from infected individuals. The human infectious dose of LASV is not known. The potential for LAI with LASV exists; though there are no reports of laboratory-acquired infections with Lassa viruses after institution of appropriate biosafety practices.

Lassa viruses were first described in 1970 after a small outbreak in Nigeria and were named for a village there (Geisbert and Jahrling 2004; Gunther and Lenz 2004). The disease is endemic in several countries of West Africa. A staggering number of cases and deaths are reported annually; estimates of cases are in the range of 300,000 to 500,000, with 5000 deaths yearly across West Africa (Ogbu et al. 2007; Idemyor 2010). It is concerning that the geographic region for these pathogens is considered to be expanding (Gunther and Lenz 2004).

Lassa viruses are members of the genus *Arenavirus*, family *Arenaviridae* and are RNA viruses (Gunther and Lenz 2004). There are four strains of Lassa virus described to date; these are *Josiah*— Sierra Leone, *Nigeria, LP*-Nigeria, and *AV,* imported to Germany by a traveler who had visited Ghana, Côte D'Ivoire, and Burkina Faso (Ogbu et al. 2007).

Lassa viruses are considered to be in the Old World complex of the *Arenaviridae* family that includes the prototype *arenavirus* lymphocytic choriomeningitis virus (LCMV), Mopeia virus, Mobala virus, and Ippy virus. The larger New World (Tacaribe) complex of the family of viruses includes Tacaribe virus, Pichinde virus, Junín virus, Machupo virus, Sabia virus, and Guanarito virus. It is interesting to note that the majority of viruses in this family are not known to cause disease in humans. However, Lassa virus, Junín virus, Machupo virus, Guanarito virus, and Sabia virus can cause a viral hemorrhagic fever (VHF) upon transmission to humans.

The natural reservoir of Lassa viruses is the rodent of the genus *Mastomys* (multimammate rat) that is endemic to sub-Saharan Africa. The infection in rodents is considered life-long (Fisher-Hoch 2005) and are likely infected *in-utero* (McCormick and Fisher-Hoch 2002). In Guinea and Sierra Leone, the reservoir is likely to be *M. natalensis* where there is evidence of vertical (rodent to offspring) as well horizontal (rodent-to-rodent) transmission of the virus (Lecompte et al. 2006). BSL-4 biosafety practices are required for all work with Lassa viruses (Chosewood et al. 2009).

Human Disease and Outbreaks

The human disease caused by Lassa viruses is a type of hemorrhagic fever (Lassa fever). The disease is endemic in several countries of West Africa, including Sierra Leone, Guinea, Liberia, and Nigeria. The territory is expanding and disease has been reported also in Ghana, Ivory Coast, and Burkina Faso (Drosten et al. 2003). Estimates of annual disease cases range from 100,000 (Ehichioya et al. 2010), to between 300,000 – 500,000 (Ehichioya et al. 2010) , to over a million (Idemyor 2010). Fatalities are estimated to be between 5,000 and 10,000 annually. The peak season for Lassa fever is the dry season from February through May and it is estimated that 5–20 percent of susceptible persons are infected each year (McCormick and Fisher-Hoch 2002), thus accounting for the large and varying estimates of burden of disease. The rodent reservoirs are present both in agricultural and domestic settings. This increases the possibility of coming into contact with rodents, contributing to the high incidence of disease among the population.

Lassa fever is characterized by a wide range of clinical manifestations (Gunther and Lenz 2004). Fever is a hallmark, accompanied by flu-like illness and gastrointestinal symptoms. These features make Lassa fever indistinguishable from other fevers in West Africa, including other hemorrhagic fevers. Pharyngitis is an early sensitive indicator and bleeding is a late specific symptom in Lassa fever patients.

Lassa fever is mild in about 80 percent of people infected with the viruses**.** Fatality rates from Lassa viruses are significant at 10-20 percent in hospitalized patients and low for the general population at 1-2 percent. These are lower than that of other hemorrhagic fever viruses such as Ebola and Marburg viruses. In untreated cases, however, mortality from Lassa fever may approach 60 percent.
There have been cases of Lassa fever reported in returning travelers from Africa from the 1970s in Europe and in the US. A recent case in a traveler was the sixth imported case diagnosed and reported in the US (Macher and Wolfe 2006; Amorosa et al. 2010).

The mode of transmission of Lassa viruses is from direct contact with rodents. Primary infection is postulated to occur via breaks in the skin, by mucosal contact, ingestion of food contaminated by rodent feces, and by consumption of the infected rodent (Curtis 2006). Though it is possible that aerosolization of viruses from rodent droppings may cause human infection via the respiratory tract, this is considered an infrequent event (McCormick and Fisher-Hoch 2002). The aerosol route has been shown to cause infections in laboratory animals (Peters et al. 1987). Lassa viruses are not known to be vector-borne.

Direct person-to-person transmission of Lassa viruses occurs, especially in hospital settings. Person-to-person transmission is associated with direct contact with the blood or other bodily fluids containing virus particles from infected individuals. Airborne transmission has also been postulated to occur. Contact with objects contaminated with virus, such as medical equipment (reused needles), is also associated with transmission in healthcare settings (Simonsen et al. 1999).The viruses are generally not known to be spread through casual contact, including skin-to-skin contact without exchange of bodily fluids (Ogbu et al. 2007). These hospital-associated transmissions can be prevented with modern day practices such as patient isolation, strict barrier precautions, personal protective equipment, sterilization of medical equipment, and appropriate disposal of contaminated supplies.

The diagnosis of Lassa fever is based on epidemiological history of exposure to a patient during a known outbreak or travel. Rapid diagnostic testing includes PCR-based methods (Drosten et al. 2002; Panning et al. 2010). Other methods include serologic testing for IgM and IgG antibodies by enzyme immunoassay or immunofluorescent antibody assay and antigen detection by enzyme immunoassay. Viral isolation by cell culture remains the gold standard. The availability of these different modalities of testing is variable, with some restricted to national and international reference laboratories.

The management of Lassa fever includes supportive therapy and the anti-viral medication, ribavirin, has been shown to be effective, especially if given early in the course of the illness (Bausch et al. 2010; Charrel et al. 2011). There is currently no vaccine available for Lassa fever and vaccine development remains an active area of research (Geisbert et al. 2005; Kotturi et al. 2009).

Disease in Medically Vulnerable opulations

In general, all age groups are affected. The clinical presentation and outcomes in children are different from those of adults, though there is no indication of increased incidence among children (Monson et al. 1987). There are no reports of increased incidence or susceptibility in specific medically vulnerable

Subpopulations. Lassa fever in pregnancy results in loss of the fetus in over 90% of patients. The risk of death is also higher for pregnant women, especially in their third trimester (Price et al. 1988).

Human Infectious Dose

There are no human dose-response data available for Lassa viruses. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing Lassa viruses; however, the amount of virus inhaled by humans who became infected is not known. The human infectious dose is presumed to be low.

Laboratory Acquired Infections

Prior to 1979, there were three occupational infections reported with Lassa viruses (Pike 1979). One was a fatal infection in the researcher who described the first outbreak. She contracted it while doing an autopsy on a victim. There were two infections in laboratory workers in 1969 in the US, one was fatal.

There have been several laboratory incidents involving Lassa viruses, including three incidents at U.S. Army Medical Research Institute For Infectious Diseases (USAMRIID) (Biosafety Review, Appendix D). One of these incidents involved a needle and syringe loaded with a Lassa virus strain and posed a significant exposure (reported in 1979). None of the incidents resulted in clinical infection or asymptomatic disease (seroconversion).

There are no reports of laboratory-acquired infections with Lassa viruses or any other BSL-4 pathogens after institution of appropriate biosafety practices; this is a reflection of modern biosafety practices and awareness of risks associated with maximum biocontainment settings (National Research Council (U.S.). Committee to Review the Health and Safety Risks of High-Biocontainment Laboratories at Fort Detrick. 2010).

Laboratory-acquired infections with Lassa viruses are a concern and laboratory personnel education and training should be reinforced while dealing with this BSL-4 pathogen.

Summary

Lassa virus strains cause a hemorrhagic fever that is fatal to humans, especially if untreated. The incidence of Lassa fever is high in West Africa, where 5-20 percent of the population is at risk and numerous people contract the disease annually. The virus strains are transmitted to humans by close contact with rodents, their excretions, consuming food contaminated by rodents, or consuming the

infected rodents. The illness is characterized by a wide variety of symptoms including fever, flu-like illness, gastro-intestinal symptoms, severe illness, and bleeding. Supportive and specific therapy with ribavirin is effective. The overall case fatality rate is 1-2 percent in the general population and 10-20 percent in hospitalized patients. The case fatality rate may approach 60 percent in untreated patients. There have been no recent laboratory-acquired infections with Lassa virus strains. Infections in travelers returning from endemic areas have been reported.

There is a possibility of infection if an individual is exposed directly to Lassa virus strains. There is also a possibility of person-to-person transmission of Lassa viruses; though the risk is low and requires close contact with blood or bodily fluids with the viruses, especially in hospital settings.

For the purposes of this RA, Lassa viruses will be analyzed in detail with regard to: (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to Lassa viruses; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of initial infection in those exposed to those amounts of Lassa viruses. As the risk of direct person-to-person transmission of Lassa viruses is low and there are limited published mathematical models of such transmission, secondary transmission modeling of Lassa viruses in the community will not be performed for this RA.

3.5.2.4 Junín virus (JUNV*)*

Introduction

Junín virus is the causative pathogen of Argentine hemorrhagic fever (AHF) and was first described in the 1950s in Argentina. For this RA, JUNV was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio despite its poor transmissibility (Mahmoud 2008). The primary route of transmission to humans is inhalation of aerosolized virus through the respiratory tract from rodent urine, feces, saliva, and contaminated fomites). There is potential for direct person-to-person transmission of JUNV; however it is to be noted that there have been no reports of person-to-person transmission of this pathogen. The human infectious dose of JUNV is not known. The potential for LAI with JUNV exists and several such infections have been reported, all prior to 1980 and the institution of modern biosafety practices.

JUNV is endemic to central Argentina, where there are annual outbreaks of AHF during the agricultural season when humans come into contact with rodents that harbor the virus.

Junín virus is a member of the family *Arenaviridae*, genus **Arenavirus** , and belongs to the Tacaribe complex (New World arenaviruses) (LeDuc 1989).

The natural reservoir hosts for Junín virus are different species of vesper field mice, mainly *Calomys musculinus* (dry lands vesper mouse) and *Calomys laucha* (small vesper mouse). Virus antigens have been found in other species of mice including Bolomys obscurus (dark bolo mouse), *Mus musculus* (house mouse), and *Oligoryzomys flavescens* (yellow pigmy rice rat) (Carballal et al. 1988; LeDuc 1989; Ambrosio et al. 2006) (Mills et al. 1994). Experimental infection and animal models for Junín virus include wild rodents such as *Calomys musculinus* and laboratory-bred animals such as mice, rats, guinea pigs, and non-human primates. The guinea pig model most closely resembles human disease (Gomez et al. 2011).

Since its discovery, Junín virus has been studied extensively in maximum biocontainment laboratories, including in the US. BSL-4 is required for all work with Junín virus (Chosewood et al. 2009).

Human Disease and Outbreaks

Argentine hemorrhagic fever is endemic in a large area of fertile land in Argentina, termed the "humid pampas". An estimated 200 to 2,000 cases of Argentine hemorrhagic fever are reported annually in this

region, with a distinct seasonal peak in the fall (February to May) during the agricultural harvest when humans come into contact with the rodent reservoir (Jay et al. 2005). It is important to note that there have been uninterrupted annual outbreaks noted since the discovery of the virus in 1950. The geographic region from which AHF is reported has been expanding in north-central Argentina, such that today, nearly 5 million individuals are considered at risk for the disease (Gomez et al. 2011).

Human disease is characterized by fever, malaise, and headache, progressing to vascular, renal, and/or hematologic/hemorrhagic disease and death. The hallmarks of the condition are impaired hemostasis, endothelial cell dysfunction, and low platelet counts resulting in bleeding and death; hence AHF is considered one of the 'hemorrhagic fever viruses'. Overall, the case fatality rate is reported to be in the range of 15-30 percent (Harrison et al. 1999; Colebunders et al. 2002; Enria and Barrera Oro 2002; Enria et al. 2008; Gomez et al. 2011).

Junín virus is transmitted to humans from bodily secretions and excretions from persistently-infected rodents. This could occur via aerosol, ingestion, fomites, and bites. The primary route of transmission to humans is inhalation of aerosolized virus through the respiratory tract from rodent urine, feces, saliva, and contaminated fomites (Carballal et al. 1988; LeDuc 1989; Enria and Barrera Oro 2002; Enria et al. 2008).

As with other hemorrhagic fever viruses in the Family *Arenaviridae* (Lassa fever virus and the New World arenaviruses), there is potential for direct person-to-person transmission of Junín virus, postulated to occur via close contact with infectious blood and body fluids (Borio et al. 2002). It is to be noted that there have been no reports of person-to-person transmission of Junín virus from patients to health care workers, despite the several hundred patients with hemorrhages cared for each year in Argentina (Charrel and de Lamballerie 2003). The closely related Machupo virus has been confirmed to be responsible for outbreaks in hospitals.

The diagnosis of Junín virus infection is based on the clinical presentation, laboratory data, and an epidemiological history including appropriate travel (Colebunders et al. 2002). Laboratory confirmation is by serology and PCR-based tests for rapid diagnosis (Gomez et al. 2011).

General supportive measures are an important component of the care of patients with viral hemorrhagic fevers. The individual patient level management of patients with Junín virus infection has been greatly improved with the use of immune plasma (or convalescent serum) containing antibodies to the virus. This has resulted in decrease of mortality rates to less than 1% from an average of 20% in those who have no

treatment. Ribavirin has also been shown to be effective for this pathogen. At the population level, a live attenuated vaccine, Candid-1, has been shown to be effective in decreasing incidence of Argentine hemorrhagic fever (Enria and Barrera Oro 2002; Enria et al. 2008; Gomez et al. 2011).

Disease in Medically Vulnerable Subpopulations

There are no reports of any particular age groups being affected, nor any increased susceptibility in medically vulnerable Subpopulations, including pregnant women, specifically for Junín virus. In general, for viral hemorrhagic fevers, the presentation of illness is similar in adults and children and there is an increase in fetal loss if pregnant women get infected.

Human Infectious Dose

The human infectious dose of Junín virus is not known. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing the pathogen, but the amount of virus inhaled by humans who became infected is not known. From animal data, the human infectious dose for Junín virus it is postulated to be low.

Laboratory Acquired Infections

There are several reports of laboratory-acquired infections (LAI) with Junín virus. The potential for aerosolization of the virus is postulated to be the reason behind infections acquired in the laboratory. There were at least 21 cases of LAI with Junín virus with one death up to 1980 (Scherer et al. 1980). There was one incident reported from the USAMRIID in 1982 where during autopsy, a bone fragment of a monkey infected with Junin virus punctured the finger of the worker. Immune plasma was used and no clinical or subclinical infection ensued. In another incident in 2009 at the University of Texas Medical Branch in Galveston, a scientist dropped a plate containing Junín virus on the floor. The spill was decontaminated and reported after proper procedures were performed. There appeared to be no exposure to virus and no infections were noted. (Biosafety Review, Appendix D).

From these incidents, it is concluded that laboratory-acquired infections are a concern with Junín virus. All infections though were noted to be prior to 1980 when knowledge of biology and transmission of the virus and biosafety precautions were rudimentary.

Summary

Junín virus is the causative pathogen of Argentine hemorrhagic fever and was first described in the 1950s. Several hundred cases continue to occur in an endemic area of Argentina every year and an expanding

geographic region of the rodent reservoir places a large number of individuals at risk. The virus is transmitted from secretions and excretions of rodents via aerosolization to humans. A vaccine and the availability of specific treatment measures such as immune plasma and ribavirin have greatly reduced the risk, incidence, and mortality due to this virus. There is a potential for direct person-to-person transmission of viral hemorrhagic fevers; however, there are no specific reports of this occurring with Junín virus.

There is a possibility of infection in an individual exposed to Junín virus. There are effective treatment modalities available for this pathogen, along with supportive measures. A vaccine is available (in Argentina) that is used for prophylaxis. This pathogen is expected to be studied in BSL-4 maximum biocontainment laboratories worldwide, including the US. There have been reports of laboratory incidents and infections involving Junín virus; though all of these were from before 1980 when laboratory procedures and safety protocols were less rigorous.

For the purposes of this RA, Junín virus will be analyzed in detail with regard to (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to Junín virus; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of initial infection in those exposed to those amounts of Junín virus.

Though the potential exists, there is a very low possibility of person-to-person transmission of Junín virus and there have been no specific reports documenting this; thus secondary transmission modeling of the spread of Junín virus infection in the community will not be performed as part of this RA.

3.5.2.5 Tick-borne Encephalitis Virus, Far Eastern Sub-type (TBEV-FE)

Introduction

Tick-borne encephalitis virus, Far Eastern sub-type (TBEV-FE) was formerly known as Russian spring-summer encephalitis. This virus is one member of the tick-borne encephalitis virus complex. For this RA, TBEV-FE was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio and transmitted via arthropod vectors (Mahmoud 2008). The virus is transmitted to humans through the bite of an infected tick. There is no known direct person-to-person transmission of TBEV-FE other than the rare cases of vertical transmission described from mother to fetus and via breast milk. The human infectious dose of TBEV-FE is not known. The potential for LAI with TBEV-FE exists

and several such infections have been reported, all prior to 1980 and the institution of modern biosafety practices.

TBEV-FE is one of the causative pathogens of tick-borne encephalitis (TBE) (Lindquist and Vapalahti 2008). As the name implies, the disease is transmitted to humans by ticks, causes encephalitis and isendemic in a large area from Western Europe to the eastern coast of Japan, and. The disease is characterized by acute meningoencephalitis with or without myelitis, with varying morbidity and mortality. The first description of TBE as a clinical entity was in Austria in 1931 and the causative pathogen was isolated in the far eastern region of Russia in 1937.
Once considered a local health issue in certain regions of Russia and Central/Eastern Europe, TBE is now an international health problem with expansion of endemic areas and rapidly increasing numbers of cases due to travel to and pursuit of leisure activities into high-risk areas (Banzhoff et al. 2008).

The TBE complex of viruses are members of the genus *Flavivirus*, family *Flaviviridae* (Lindquist and Vapalahti 2008; Mansfield et al. 2009). Medically important flaviviruses include mosquito-borne viruses such as yellow fever, dengue Japanese encephalitis, and West Nile viruses; tick-borne flaviviruses include those in the mammalian group such as tick-borne encephalitis virus, Central European encephalitis virus, louping ill virus, Powassan virus, Kyasanur Forest disease virus, Omsk hemorrhagic fever virus, and Langat virus (Lasala and Holbrook 2010). There are three subtypes of TBEV: (1) European (TBEV-Eu); (2) Siberian (TBEV-Sib) and (3) Far Eastern (TBEV-FE). This risk analysis will focus on the Far Eastern sub-type (TBEV-FE).

The natural reservoir of TBEV-FE is the tick *Ixodes persulcatus*, which also acts as the primary vector for the virus (Lasala and Holbrook 2010). This tick is endemic to a large area extending from eastern Europe to China and Japan. The virus can chronically infect ticks and is transmitted both transtadially (from larva to nymph to adult ticks) and transovarially (from adult female tick through eggs. Ticks remain infected throughout their life cycle and transmit the virus to uninfected ticks when co-feeding on small wild rodents, such as the red vole (*M. rutilus* Pallas) which is indigenous to the area (Gritsun et al. 2003). Currently, there are no effective animal models of the disease.

Since its discovery, virus has been studied extensively in maximum biocontainment laboratories around the world, including the US. BSL-4 is required for all work with TBEV-FE virus (Chosewood et al. 2009).

Human Disease and Outbreaks

Several thousand human cases of TBE occur in Europe annually with Russia bearing the largest burden. As the older name suggests, the disease occurs during the summer and spring time when the ticks are active. During the period 1990-2006, Russia reported an average of 6,000 cases every year (adapted from data from The International Scientific Working Group on Tick-borne Encephalitis reported in (Lindquist and Vapalahti 2008)). The country with the next highest reported cases in the year 2006 was the Czech Republic with nearly 1000 cases. During this period, the annual incidence has been steadily increasing in all European countries except Austria, with an ever-expanding geographic area where the ticks are found. Humans are accidental hosts for TBEV-FE in that they can be infected by the virus and the virus can be detected in the blood; however, they do not participate in the circulation of the virus (Suss 2003). The virus is transmitted to humans through the bite of an infected tick. Following the tick bite and a median incubation period of 8 days, the first stage of illness appears. The prominent symptoms at this stage are fever, fatigue, general malaise, and headache/body pain (Lindquist and Vapalahti 2008). Following a symptom-free period, the second stage of the illness causes the most morbidity. The spectrum of illness includes mild meningitis to severe encephalitis, with or without spinal paralysis or myelitis. Altered consciousness or seizures may also be noted. One of the most concerning features is a flaccid poliomyelitis-like paralysis that preferentially affects the upper part of the body.

There is a long-term morbidity associated with TBE that includes residual spinal paralysis, a post-encephalitic syndrome, and cognitive problems. The case fatality rate is highest among patients with TBEV-FE, and of up to 20-40 percent.

Other than via ticks, TBEV has been reported to be transmitted to humans following consumption of raw milk from infected goats, sheep, or cows; consumption of imported goat cheese; vertical transmission from mother to fetus; and via breast milk (Randolph 2008).

There is no known direct person-to-person transmission of TBEV-FE other than the rare cases of vertical transmission from mother to fetus and via breast milk mentioned above. There have been cases of TBE reported in the US from travelers returning from Europe, especially Russia (2010). There were 2 cases reported prior to the year 2000 and between 2000-2009, there were 5 confirmed cases. Risk of acquiring TBEV while traveling to endemic areas remains a risk for tourists.

The diagnosis of TBEV-FE is based on clinical presentation and laboratory confirmation (Lindquist and Vapalahti 2008; Lasala and Holbrook 2010; Ruzek et al. 2010). A clinical and epidemiological history is

important along with an awareness of travel history to endemic areas. Laboratory testing includes TBEV-FE specific IgM and IgG that is detected during the second phase of the illness when central nervous system symptoms are prominent. The virus can also be detected by PCR from the blood during the first phase of the illness. Antibody detection in cerebrospinal fluid and antigen detection in the blood by enzyme immunoassay are also available. There is some cross-reactivity of these tests with other flaviviruses and this has to be noted while interpreting results.

There are no specific treatment modalities for TBEV-FE. The management of TBEV-FE infection is largely supportive; with intensive care being an important component. There is an effective vaccine available to prevent TBE by active immunization. There are Russian vaccines and also 2 vaccines based on the Siberian sub-type that are licensed in Europe. The success of mass vaccination campaigns in decreasing TBE has been well demonstrated in Austria, where the rate of protection is estimated to be 95 percent (Kunz 2002; Kunz 2003). Passive immunization using hyperimmune IgG against TBEV has fallen out of favor due to the lack of documented efficacy.

Disease in Medically Vulnerable Subpopulations

In general, all age groups are affected. The extremes of ages are affected adversely with a higher severity of illness noted in preschool children and a substantial increase in morbidity is seen in elderly people (Lindquist and Vapalahti 2008). There are no reports of increased susceptibility to TBEV-FE in specific medically vulnerable Subpopulations or in pregnant women.

Human Infectious Dose

There are no human dose-response data available for TBEV-FE virus. Animal models do not effectively reproduce human aspects of disease and so it is a challenge to interpret and extrapolate animal infectious dose data to humans. The human infectious dose for TBEV-FE is not known.

Laboratory Acquired Infections

Laboratory infections with TBEV were common before the advent of modern biosafety practices that prevented exposure to aerosols and vaccines. A total of 26 infections and 2 deaths have been reported in the literature, all prior to 1980. Risk factors appeared to be needle stick injuries and aerosolization of virus from breakage of glass- ware (Hanson et al. 1967; Pike 1979; Scherer et al. 1980; Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses 1980; Pedrosa and Cardoso 2011). Laboratory-acquired infections with TBEV-FE virus are a concern and reinforce laboratory personnel education and training while dealing with this BSL-4 pathogen.

Summary

The TBEV-FE sub-type causes TBE and is transmitted to humans via the bite of infected ticks of the species *Ixodes persulcatus*. Several thousand cases of TBE are reported from Europe every year; the bulk of the disease burden is in Russia. The disease has a case fatality rate of 20-40 percent and a high morbidity in terms of neurological sequelae. There is no specific treatment for TBE, however, it can be prevented with effective vaccines that are available in Europe. There is a possibility of infection if an individual is exposed directly to TBEV-FE. There is no direct person-to-person transmission of TBEV-FE, other than rare cases of transmission from mother to child and via breast milk (Randolph 2008).

For the purposes of this RA, TBEV-FE virus will be analyzed in detail with regard to: (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to TBEV-FE virus; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences and (3) probabilistic estimates of initial infection in those exposed to those amounts of TBEV-FE virus. As there is no direct-to-person transmission of TBEV-FE, secondary transmission modeling of the spread of this virus in the community following an initial infection will not be performed.

3.5.2.6 Nipah Virus (NIPV)

Introduction

Nipah virus is an emerging pathogen that was first described in 1998 from an outbreak of encephalitis in Malaysia and Singapore (1999). For this RA, NIPV was selected for analysis based on its characteristics of being highly pathogenic with a high case fatality ratio (Mahmoud 2008). Humans contract the virus when they come into contact with animals infected with the virus (such as pigs or domestic animals) or items contaminated with the virus by bats that serve as a reservoir for NIPV. Evidence from epidemiologic investigations of outbreaks in Bangladesh and India indicates that NIPV can be transmitted directly from person-to-person. The human infectious dose of NIPV is not known. The potential for LAI with NIPV exists; there are no reports of laboratory-acquired infections with Nipah virus.

Infections with Nipah virus were noted in workers with exposure to pigs such as pig farmers and abattoir workers. It was initially thought to be Japanese encephalitis that is known to occur in the region; subsequent detailed investigations and virologic studies revealed a new virus in the family *Paramyxoviridae*. The virus was named for the Nipah village in Malaysia where a fatal human case was first described in 1999 (Chua et al. 2000). Since the descriptions of the initial outbreaks, there have been several human outbreaks noted in Bangladesh (Hsu et al. 2004; 2004; Luby et al. 2009; Homaira et al.

2010). There are two outbreaks reported from areas in India that are close to the border with Bangladesh (Luby et al. 2009).

The Nipah virus, and its close relative, Hendra virus, belong to the family of paramyxoviruses, which have a single stranded non-segmented RNA virus that is fully encapsulated by protein (Lo and Rota 2008; Weingartl et al. 2009).

The natural reservoir hosts for Nipah virus appear to be several species of fruit bats of the genus *Pteropus,* as well as non-*Pteropus* species (Lo and Rota 2008; Blum et al. 2009; Luby et al. 2009; Weingartl et al. 2009; Chua 2010). Apart from bats in Malaysia, there is evidence for Nipah or Nipah-like viruses in bats from Cambodia and Thailand (Reynes et al. 2005; Wacharapluesadee et al. 2005). Nipah virus is able to infect a range of hosts, including swine, humans, and, to a minor extent, cats and dogs (Middleton et al. 2002; Mills et al. 2009; Weingartl et al. 2009).

Since its discovery in 1998 and description in 1999, Nipah virus has been studied extensively in maximum biocontainment laboratories, including in the US. BSL-4 is required for all work with Nipah and Hendra viruses (Chosewood et al. 2009).

Human Disease and Outbreaks

There appear to be two distinct clinical presentations of patients infected with Nipah virus. Neurological manifestations of encephalitis were noted in Malaysian pig farmers (Chua et al. 1999; Goh et al. 2000). Abattoir workers in Singapore demonstrated neurological and respiratory manifestations including pneumonia (Chew et al. 2000). Patients in the Bangladesh and Indian outbreaks primarily exhibited respiratory symptoms with evidence of pneumonia (Hossain et al. 2008). The case fatality rate is also different between the Malaysian and Bangladeshi outbreaks; higher in Bangladesh at 73%, as compared with 39% from Malaysia. This is postulated to be due to differences in availability of modern ICU care in Malaysia, rather than true differences in the biology and pathogenicity of the virus in the two outbreaks (Luby et al. 2009).

There have been no further outbreaks in Malaysia and Singapore after the initial outbreaks described in 1998 and 1999. Since then, there have been at least 10 large outbreaks described in Bangladesh and India in the period 2001-2008 and 17 minor transmission events involving one to four human cases (Luby et al. 2009).

The mode of transmission of Nipah virus has changed between the Malaysian/Singapore outbreaks and those in Bangladesh/India. In the Malaysian/Singapore outbreaks, it is postulated that the virus was transmitted from bats (natural reservoir) to pigs, causing an outbreak in pigs, which subsequently led to an outbreak in humans in close contact with the pigs (abattoir workers and pig farmers).

In Bangladesh, the transmission from bats to humans appears to be ongoing and via at least three different routes (Luby et al. 2006; Luby et al. 2009). The most frequent mode is food-borne through ingestion of Nipah-virus-contaminated date palm sap which is a staple food source in that region. The sap from the date palm tree is harvested in pots left hanging overnight on the trees after 'tapping' the tree. There is evidence that bats that harbor Nipah virus in their saliva and urine lick the sap at night and Nipah virus is known to survive in raw sap for several days.

A second mode of transmission appears to be via domestic animals that feed on contaminated fruits or date palm sap that have been licked or partially eaten by fruit bats infected with Nipah virus. Cows, pigs, and goats have been implicated in transmission of the virus to humans. A third route of transmission is when humans come directly into contact with Nipah virus-infected bat secretions or excretions (saliva, urine, feces). There do not appear to be any arthropod vectors in the transmission of Nipah virus.

Evidence from epidemiologic investigations of outbreaks in Bangladesh and India indicates that Nipah virus can be transmitted directly from person-to-person. This has occurred in patients with respiratory illness. Close physical contact with a known Nipah virus patient who later died was found to be the strongest risk factor for direct person-to-person transmission (Gurley et al. 2007). Nipah virus has been found in respiratory secretions of infected patients (Chua et al. 2001). Though direct transmissions have occurred and are known to be responsible for many of the Bangladeshi outbreaks, the risk of direct transmission appears to be low and requires close contact that may be culture- and region-specific (to Bangladesh) (Luby et al. 2009). Given the limitations of conducting field investigations in rural areas of developing countries, this study (Luby et al. 2009) estimated that only a few individuals transmitted to others and the generations of transmissions rarely exceeded two. The overall number of secondary cases resulting from an infected person was expected to be less than 0.5, indicating that it was unlikely that any one chain of transmission would result in a large outbreak.

With regard to accidental or occupational exposure to Nipah virus and risk of transmission and infection, there are differences between the Malaysian/Singapore outbreaks and those in Bangladesh and India.

From a study in Singapore, looking at Nipah virus infection in several different exposed groups including abattoir workers and health care workers, there were no illnesses reported, however serologic studies suggested evidence of asymptomatic infection in those directly exposed to pigs (Chan et al. 2002). Similarly, in a study of 1412 military personnel who were involved in culling of pigs in Malaysia, 6 (0.4%) were noted to be seropositive (Ali et al. 2001). Of these, 4 were reported to be well with no symptoms.

In a study of 338 health care workers who cared for Nipah patients at 3 Malaysian hospitals and, a combined 89 episodes of exposure to Nipah virus from patient blood or body fluid directly contacting bare skin, 39 splash exposures of blood or bodily fluid into their eyes, nose, or mouth, and 12 needle stick injuries were reported and none developed clinical illness associated with Nipah virus infection (Mounts et al. 2001).

In a Bangladesh hospital, there was no evidence of transmission of Nipah virus within the hospital, despite substantial exposures and minimal use of personal protective equipment (Gurley et al. 2007).

In an outbreak in India, a patient not previously identified as having Nipah virus was admitted to a hospital in Siliguri District. This patient transmitted the virus to 11 other patients who were then transferred to other facilities. In two of those other facilities, a total of 25 staff and 8 visitors were infected with Nipah virus from the transferred patients (Chadha et al. 2006).
There are differences noted in outcomes of accidental and occupational exposure to Nipah virus. Variations in the virus and contact patterns between patients and health care workers are possible factors leading to these differences.

The diagnosis of Nipah virus infection is based on epidemiological history of exposure or travel history. Laboratory confirmation is by acute and convalescent serum antibody tests by ELISA (IgG and IgM), real time polymerase chain reaction (RT-PCR) from various body fluids, and virus isolation attempts in specialized laboratories. (Centers for Disease Control and Prevention (U.S.) 2011).

There are no effective medications for the treatment or prophylaxis of Nipah virus infection. Ribavirin has been shown to be effective *in vitro*. There are currently no vaccines available for this pathogen (Centers for Disease Control and Prevention (U.S.) 2011).

Disease in Medically Vulnerable Subpopulations

The initial outbreaks of Nipah virus infection occurred in male abattoir workers in Malaysia and Singapore. Subsequent outbreaks have been described in families and close contacts in Bangladesh and India. There are no reports of any particular age groups being preferentially affected, nor of any increased susceptibility in medically vulnerable subpopulations, including pregnant women.

Human Infectious Dose

The infectious dose of Nipah virus for humans is not known. From data from animal models and epidemiological data from outbreaks, it is postulated that the human infectious dose of Nipah virus is low.

Laboratory Acquired Infections

There are no reports of laboratory-acquired infections with Nipah virus.

Summary

Nipah virus is an emerging pathogen that causes a highly fatal disease in humans. There is a reservoir of this pathogen in fruit bats and though there are no further reports of outbreaks from Malaysia and Singapore where it was first discovered, there are reports of human outbreaks in Bangladesh and India as recently as 2007. There is a potential for this pathogen to be used in malevolent, intentional release scenarios as it is viable in certain high-sugar foods for several days. There is evidence of direct person-to-person transmission via respiratory secretions among close contacts who share food and/or bodily fluids with ill patients.

There is a possibility of infection in an individual is exposed to Nipah virus. There are no effective medications or vaccines for this pathogen. This pathogen is expected to be studied in BSL-4 maximum biocontainment laboratories worldwide, including the US. There have been no reports of laboratory incidents involving Nipah virus.

For the purposes of this RA, Nipah virus will be analyzed in detail with regard to (1) possible event sequences that could lead to loss of biocontainment at NEIDL resulting in exposure of laboratory workers and the general public to Nipah virus; (2) estimates of the amount of pathogen the laboratory workers and general public would be exposed to as a result of those event sequences, and (3) probabilistic estimates of initial infection in those exposed to those amounts of Nipah virus.

There is a possibility of person-to-person transmission of Nipah virus via respiratory secretions; however the risk is low and requires close contact that may be culture- and region-specific (Luby et al. 2009). Moreover, there are limited studies available to provide epidemiologic data for detailed secondary transmission modeling and no published mathematical models for this pathogen. For these reasons, secondary transmission modeling of the spread of Nipah virus infection in the community will not be performed as part of this RA.

3.6 References

Abdel-Aziz, A. A., J. M. Meegan, et al. (1980). "Rift Valley fever as a possible cause of human abortions." Transactions of the Royal Society of Tropical Medicine and Hygiene **74**(5): 685-686.

Alford, R. H., J. A. Kasel, et al. (1966). "Human influenza resulting from aerosol inhalation." Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine **122**(3): 800-804.

Ali, R., A. W. Mounts, et al. (2001). "Nipah virus among military personnel involved in pig culling during an outbreak of encephalitis in Malaysia, 1998-1999." Emerg Infect Dis **7**(4): 759-761.

Allard, R., P. Leclerc, et al. (2010). "Diabetes and the severity of pandemic influenza A (H1N1) infection." Diabetes Care **33**(7): 1491-1493.

Ambrosio, A. M., L. M. Riera, et al. (2006). "Immune response to vaccination against Argentine hemorrhagic Fever in an area where different arenaviruses coexist." Viral Immunol **19**(2): 196-201.

Amorosa, V., A. MacNeil, et al. (2010). "Imported Lassa fever, Pennsylvania, USA, 2010." Emerg Infect Dis **16**(10): 1598-1600.

Anaraki, S., S. Addiman, et al. (2008). "Investigations and control measures following a case of inhalation anthrax in East London in a drum maker and drummer, October 2008." Euro Surveill **13**(51).

Anderson, R. M., C. Fraser, et al. (2004). "Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic." Philosophical transactions of the Royal Society of London. Series B, Biological sciences **359**(1447): 1091-1105.

Anyamba, A., J. P. Chretien, et al. (2009). "Prediction of a Rift Valley fever outbreak." Proceedings of the National Academy of Sciences of the United States of America **106**(3): 955-959.

Ayats, J., E. Martin-Mazuelos, et al. (2011). "[Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) guidelines for the diagnosis of invasive fungal infections. 2010 update]." Enfermedades infecciosas y microbiologia clinica **29**(1): 39 e31-15.

Balkhy, H. H. and Z. A. Memish (2003). "Rift Valley fever: an uninvited zoonosis in the Arabian peninsula." International journal of antimicrobial agents **21**(2): 153-157.

Banzhoff, A., M. Pellegrini, et al. (2008). "MF59-adjuvanted vaccines for seasonal and pandemic influenza prophylaxis." Influenza and other respiratory viruses **2**(6): 243-249.

Barnard, D. L. (2009). "Animal models for the study of influenza pathogenesis and therapy." Antiviral Res **82**(2): A110-122.

Bausch, D. G., C. M. Hadi, et al. (2010). "Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever." Clin Infect Dis **51**(12): 1435-1441.

Bausch, D. G., S. T. Nichol, et al. (2006). "Marburg hemorrhagic fever associated with multiple genetic lineages of virus." N Engl J Med **355**(9): 909-919.

Bausch, D. G., A. G. Sprecher, et al. (2008). "Treatment of Marburg and Ebola hemorrhagic fevers: a strategy for testing new drugs and vaccines under outbreak conditions." Antiviral Res **78**(1): 150-161.

Bausch, D. G., J. S. Towner, et al. (2007). "Assessment of the risk of Ebola virus transmission from bodily fluids and fomites." J Infect Dis **196 Suppl 2**: S142-147.

Begier, E. M., G. Asiki, et al. (2006). "Pneumonic plague cluster, Uganda, 2004." Emerg Infect Dis **12**(3): 460-467.

Beyer, W. and P. C. Turnbull (2009). "Anthrax in animals." Mol Aspects Med **30**(6): 481-489.

Bird, B. H., C. G. Albarino, et al. (2008). "Rift valley fever virus lacking the NSs and NSm genes is highly attenuated, confers protective immunity from virulent virus challenge, and allows for differential identification of infected and vaccinated animals." Journal of virology **82**(6): 2681-2691.

Bird, B. H., T. G. Ksiazek, et al. (2009). "Rift Valley fever virus." Journal of the American Veterinary Medical Association **234**(7): 883-893.

Blum, L. S., R. Khan, et al. (2009). "In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies." Am J Trop Med Hyg **80**(1): 96-102.

Booth, M. G., J. Hood, et al. (2010). "Anthrax infection in drug users." Lancet **375**(9723): 1345-1346.

Bootsma, M. C. and N. M. Ferguson (2007). "The effect of public health measures on the 1918 influenza pandemic in U.S. cities." Proceedings of the National Academy of Sciences of the United States of America **104**(18): 7588-7593.

Borio, L., T. Inglesby, et al. (2002). "Hemorrhagic fever viruses as biological weapons: medical and public health management." JAMA **287**(18): 2391-2405.

Brachman, P. S., H. Gold, et al. (1962). "Field Evaluation of a Human Anthrax Vaccine." Am J Public Health Nations Health **52**(4): 632-645.

Brachman, P. S., A. F. Kaufman, et al. (1966). "Industrial inhalation Anthrax." Bacteriol Rev **30**(3): 646-659.

Bravata, D. M., E. Wang, et al. (2006). "Pediatric anthrax: implications for bioterrorism preparedness." Evid Rep Technol Assess (Full Rep)(141): 1-48.

Bridges, C. B., S. A. Harper, et al. (2003). "Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP)." MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control **52**(RR-8): 1-34; quiz CE31-34.

Burke, D. S. (1977). "Immunization against tularemia: analysis of the effectiveness of live Francisella tularensis vaccine in prevention of laboratory-acquired tularemia." J Infect Dis **135**(1): 55-60.

Burmeister, R. W., W. D. Tigertt, et al. (1962). "Laboratory-acquired pneumonic plague. Report of a case and review of previous cases." Ann Intern Med **56**: 789-800.

Butler, T. (2009). "Plague into the 21st century." Clin Infect Dis **49**(5): 736-742.

Calanan, R. M., R. T. Rolfs, et al. (2010). "Tularemia outbreak associated with outdoor exposure along the western side of Utah Lake, Utah, 2007." Public health reports **125**(6): 870-876.

Callaghan, W. M., S. Y. Chu, et al. (2010). "Deaths from seasonal influenza among pregnant women in the United States, 1998-2005." Obstetrics and gynecology **115**(5): 919-923.

Cantoni, G., P. Padula, et al. (2001). "Seasonal variation in prevalence of antibody to hantaviruses in rodents from southern Argentina." Tropical medicine & international health : TM & IH **6**(10): 811-816.

Carballal, G., C. M. Videla, et al. (1988). "Epidemiology of Argentine hemorrhagic fever." Eur J Epidemiol **4**(2): 259-274.

Carrat, F., E. Vergu, et al. (2008). "Time lines of infection and disease in human influenza: a review of volunteer challenge studies." American journal of epidemiology **167**(7): 775-785.

Castillo, C., C. Nicklas, et al. (2007). "Andes Hantavirus as possible cause of disease in travellers to South America." Travel medicine and infectious disease **5**(1): 30-34.

Castillo, C., E. Villagra, et al. (2004). "Prevalence of antibodies to hantavirus among family and health care worker contacts of persons with hantavirus cardiopulmonary syndrome: lack of evidence for nosocomial transmission of Andes virus to health care workers in Chile." The American journal of tropical medicine and hygiene **70**(3): 302-304.

CDC. (2008). "All about hantaviruses." CDC Fact Sheet 1-4, 2008 Retrieved 11, Oct., 2009.

CDC and NIH (2007). Biosafety in Microbiological and Biomedical Laboratories. H. H. Services.

Center for Infectious Disease Research & Policy. (2006). "Anthrax in Canada kills 149 animals, infects man." from http://www.cidrap.umn.edu/cidrap/content/bt/anthrax/news/jul1706anthrax.html.

Centers for Disease Control and Prevention (1998). "Rift Valley Fever--East Africa, 1997-1998." MMWR. Morbidity and mortality weekly report **47**(13): 261-264.

Centers for Disease Control and Prevention (1999). "Update: outbreak of Nipah virus--Malaysia and Singapore, 1999." MMWR Morb Mortal Wkly Rep **48**(16): 335-337.

Centers for Disease Control and Prevention (2000). "From the Centers for Disease Control and Prevention. Influenza and pneumococcal vaccination rates among persons with diabetes mellitus--United States, 1997." JAMA : the journal of the American Medical Association **283**(1): 48-50.

Centers for Disease Control and Prevention (2001). "Outbreak of Ebola hemorrhagic fever Uganda, August 2000-January 2001." MMWR Morb Mortal Wkly Rep **50**(5): 73-77.

Centers for Disease Control and Prevention (2002). "Tularemia--United States, 1990-2000." MMWR Morb Mortal Wkly Rep **51**(9): 181-184.

Centers for Disease Control and Prevention. (2007a). "Questions and Answers about Rift Valley Fever." Retrieved November 20, 2011, 2011, from http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/rvf/rvf_qa.htm.

Centers for Disease Control and Prevention (2007b). "Rift Valley fever outbreak--Kenya, November 2006-January 2007." MMWR. Morbidity and mortality weekly report **56**(4): 73-76.

Centers for Disease Control and Prevention. (2009). "Anthrax." Retrieved November 21, 2011, 2011, from http://www.cdc.gov/nczved/divisions/dfbmd/diseases/anthrax/technical.html#transmission.

Centers for Disease Control and Prevention (2009). "Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) Virus - United States, 2009." MMWR. Morbidity and mortality weekly report **58**(30): 826-829.

Centers for Disease Control and Prevention (2009). "Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis--North Carolina, 2009." MMWR. Morbidity and mortality weekly report **58**(35): 969-972.

Centers for Disease Control and Prevention (2009). "Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients - Seattle, Washington, 2009." MMWR. Morbidity and mortality weekly report **58**(32): 893-896.

Centers for Disease Control and Prevention. (2010). "Information on 2009 H1N1 impact by Race and Ethnicity (Historical Archive) from February 24, 2010." Retrieved September 21, 2011, 2011, from http://www.cdc.gov/h1n1flu/race_ethnicity_qa.htm.

Centers for Disease Control and Prevention (U.S.) (2002). "Suspected cutaneous anthrax in a laboratory worker--Texas, 2002." MMWR Morb Mortal Wkly Rep **51**(13): 279-281.

Centers for Disease Control and Prevention (U.S.) (2002). "Update: Cutaneous anthrax in a laboratory worker--Texas, 2002." MMWR Morb Mortal Wkly Rep **51**(22): 482.

Centers for Disease Control and Prevention (U.S.) (2008). "Cutaneous anthrax associated with drum making using goat hides from West Africa--Connecticut, 2007." MMWR Morb Mortal Wkly Rep **57**(23): 628-631.

Centers for Disease Control and Prevention (U.S.) (2010). "Gastrointestinal anthrax after an animal-hide drumming event - New Hampshire and Massachusetts, 2009." MMWR Morb Mortal Wkly Rep **59**(28): 872-877.

Centers for Disease Control and Prevention (U.S.) (2011). "Fatal laboratory-acquired infection with an attenuated Yersinia pestis Strain--Chicago, Illinois, 2009." MMWR Morb Mortal Wkly Rep **60**(7): 201-205.

Centers for Disease Control and Prevention (U.S.) (2011). Hendra Virus Disease and Nipah Virus Encephalitis. Atlanta.

Chadha, M. S., J. A. Comer, et al. (2006). "Nipah virus-associated encephalitis outbreak, Siliguri, India." Emerg Infect Dis **12**(2): 235-240.

Chan-Yeung, M. and R. H. Xu (2003). "SARS: epidemiology." Respirology **8 Suppl**: S9-14.

Chan, K. P., P. E. Rollin, et al. (2002). "A survey of Nipah virus infection among various risk groups in Singapore." Epidemiol Infect **128**(1): 93-98.

Charrel, R. N., B. Coutard, et al. (2011). "Arenaviruses and hantaviruses: From epidemiology and genomics to antivirals." Antiviral Res **90**(2): 102-114.

Charrel, R. N. and X. de Lamballerie (2003). "Arenaviruses other than Lassa virus." Antiviral Res **57**(1-2): 89-100.

Chew, M. H., P. M. Arguin, et al. (2000). "Risk factors for Nipah virus infection among abattoir workers in Singapore." J Infect Dis **181**(5): 1760-1763.

Chosewood, L. C., D. E. Wilson, et al. (2009). Biosafety in microbiological and biomedical laboratories. Washington, D.C., U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health.

Chowell, G., C. E. Ammon, et al. (2006). "Transmission dynamics of the great influenza pandemic of 1918 in Geneva, Switzerland: Assessing the effects of hypothetical interventions." Journal of theoretical biology **241**(2): 193-204.

Chowell, G., N. W. Hengartner, et al. (2004). "The basic reproductive number of Ebola and the effects of public health measures: the cases of Congo and Uganda." J Theor Biol **229**(1): 119-126.

Chowell, G., H. Nishiura, et al. (2007). "Comparative estimation of the reproduction number for pandemic influenza from daily case notification data." Journal of the Royal Society, Interface / the Royal Society **4**(12): 155-166.

Chua, K. B. (2010). "Epidemiology, surveillance and control of Nipah virus infections in Malaysia." Malays J Pathol **32**(2): 69-73.

Chua, K. B., W. J. Bellini, et al. (2000). "Nipah virus: a recently emergent deadly paramyxovirus." Science **288**(5470): 1432-1435.

Chua, K. B., K. J. Goh, et al. (1999). "Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia." Lancet **354**(9186): 1257-1259.

Chua, K. B., S. K. Lam, et al. (2001). "The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia." J Infect **42**(1): 40-43.

Clements, A. C., D. U. Pfeiffer, et al. (2006). "Application of knowledge-driven spatial modelling approaches and uncertainty management to a study of Rift Valley fever in Africa." International journal of health geographics **5**: 57.

Colebunders, R., M. Van Esbroeck, et al. (2002). "Imported viral haemorrhagic fever with a potential for person-to-person transmission: review and recommendations for initial management of a suspected case in Belgium." Acta Clin Belg **57**(5): 233-240.

Control, E. C. f. D. P. a. (2006). "Probable human anthrax death in Scotland." Euro Surveill **11**(8): E060817 060812.

Coppes, J. B. (1980). "Bubonic plague in pregnancy." J Reprod Med **25**(2): 91-95.

Couturier, B. A., J. M. Bender, et al. (2010). "Oseltamivir-resistant influenza A 2009 H1N1 virus in immunocompromised patients." Influenza and other respiratory viruses **4**(4): 199-204.

Curtis, N. (2006). "Viral haemorrhagic fevers caused by Lassa, Ebola and Marburg viruses." Adv Exp Med Biol **582**: 35-44.

Dennis, D., K. L. Gage, et al. (1999). Plague Manual: Epidemiology, Distribution, Surveillance and Control, World Health Organization.

Dennis, D. T., T. V. Inglesby, et al. (2001). "Tularemia as a biological weapon: medical and public health management." JAMA **285**(21): 2763-2773.

Dennis, D. T. and P. S. Mead (2010). *Yersinia* species, Including Plague. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. G. L. Mandell, J. E. Bennett and R. Dolin. Philadelphia, Churchill Livingstone**:** 2943-2953.

Diepersloot, R. J., K. P. Bouter, et al. (1990). "Influenza infection and diabetes mellitus. Case for annual vaccination." Diabetes Care **13**(8): 876-882.

Donnelly, C. A., M. C. Fisher, et al. (2004). "Epidemiological and genetic analysis of severe acute respiratory syndrome." The Lancet infectious diseases **4**(11): 672-683.

Dowell, S. F. (1996). "Ebola hemorrhagic fever: why were children spared?" Pediatr Infect Dis J **15**(3): 189-191.

Dowell, S. F. and J. S. Bresee (2008). "Pandemic lessons from Iceland." Proceedings of the National Academy of Sciences of the United States of America **105**(4): 1109-1110.

Dragon, D. C., R. P. Rennie, et al. (2001). "Detection of anthrax spores in endemic regions of northern Canada." J Appl Microbiol **91**(3): 435-441.

Drosten, C., S. Gottig, et al. (2002). "Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR." J Clin Microbiol **40**(7): 2323-2330.

Drosten, C., B. M. Kummerer, et al. (2003). "Molecular diagnostics of viral hemorrhagic fevers." Antiviral Res **57**(1-2): 61-87.

Ehichioya, D. U., M. Hass, et al. (2010). "Lassa fever, Nigeria, 2005-2008." Emerg Infect Dis **16**(6): 1040-1041.

Eisen, R. J., J. M. Petersen, et al. (2008). "Persistence of Yersinia pestis in soil under natural conditions." Emerg Infect Dis **14**(6): 941-943.

Ellis, J., P. C. Oyston, et al. (2002). "Tularemia." Clin Microbiol Rev **15**(4): 631-646.

Ennis, F. A. (1978). "Influenza A viruses: shaking out our shibboleths." Nature **274**(5669): 309-310.

Enria, D., P. Padula, et al. (1996). "Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission." Medicina **56**(6): 709-711.

Enria, D. A. and J. G. Barrera Oro (2002). "Junin virus vaccines." Curr Top Microbiol Immunol **263**: 239-261.

Enria, D. A., A. M. Briggiler, et al. (2008). "Treatment of Argentine hemorrhagic fever." Antiviral Res **78**(1): 132-139.

Epstein, S. L. and G. E. Price (2010). "Cross-protective immunity to influenza A viruses." Expert review of vaccines **9**(11): 1325-1341.

Evans, M. E., D. W. Gregory, et al. (1985). "Tularemia: a 30-year experience with 88 cases." Medicine (Baltimore) **64**(4): 251-269.

Falzarano, D., T. W. Geisbert, et al. (2011). "Progress in filovirus vaccine development: evaluating the potential for clinical use." Expert Rev Vaccines **10**(1): 63-77.

Favier, C., K. Chalvet-Monfray, et al. (2006). "Rift Valley fever in West Africa: the role of space in endemicity." Tropical medicine & international health : TM & IH **11**(12): 1878-1888.

Feldman, K. A., R. E. Enscore, et al. (2001). "An outbreak of primary pneumonic tularemia on Martha's Vineyard." N Engl J Med **345**(22): 1601-1606.

Feldman, K. A., D. Stiles-Enos, et al. (2003). "Tularemia on Martha's Vineyard: seroprevalence and occupational risk." Emerg Infect Dis **9**(3): 350-354.

Feldmann, H. (2006). "Marburg hemorrhagic fever--the forgotten cousin strikes." N Engl J Med **355**(9): 866-869.

Feldmann, H. and T. W. Geisbert (2011). "Ebola haemorrhagic fever." Lancet **377**(9768): 849-862.

Feldmann, H., W. Slenczka, et al. (1996). "Emerging and reemerging of filoviruses." Arch Virol Suppl **11**: 77-100.

Feng, Y. and G. F. Gao (2007). "Towards our understanding of SARS-CoV, an emerging and devastating but quickly conquered virus." Comparative immunology, microbiology and infectious diseases **30**(5-6): 309-327.

Ferres, M. and P. Vial (2004). "Hantavirus infection in children." Current opinion in pediatrics **16**(1): 70-75.

Ferres, M., P. Vial, et al. (2007). "Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in chile." The Journal of infectious diseases **195**(11): 1563-1571.

Fiore, A. E., T. M. Uyeki, et al. (2010). "Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010." MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control **59**(RR-8): 1-62.

Fisher-Hoch, S. P. (2005). "Lessons from nosocomial viral haemorrhagic fever outbreaks." Br Med Bull **73-74**: 123-137.

Flick, R. and M. Bouloy (2005). "Rift Valley fever virus." Current molecular medicine **5**(8): 827-834.

Formenty, P., C. Hatz, et al. (1999). "Human infection due to Ebola virus, subtype Cote d'Ivoire: clinical and biologic presentation." J Infect Dis **179 Suppl 1**: S48-53.

Franz, D. R. (2009). "Preparedness for an anthrax attack." Mol Aspects Med **30**(6): 503-510.

Franz, D. R., P. B. Jahrling, et al. (1997). "Clinical recognition and management of patients exposed to biological warfare agents." JAMA **278**(5): 399-411.

Freedman, A., O. Afonja, et al. (2002). "Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old infant." JAMA **287**(7): 869-874.

Gage, K. L., D. T. Dennis, et al. (2000). "Cases of cat-associated human plague in the Western US, 1977-1998." Clin Infect Dis **30**(6): 893-900.

Gage, K. L. and M. Y. Kosoy (2005). "Natural history of plague: perspectives from more than a century of research." Annu Rev Entomol **50**: 505-528.

Geisbert, T. W., D. G. Bausch, et al. (2010). "Prospects for immunisation against Marburg and Ebola viruses." Rev Med Virol **20**(6): 344-357.

Geisbert, T. W. and P. B. Jahrling (2004). "Exotic emerging viral diseases: progress and challenges." Nat Med **10**(12 Suppl): S110-121.

Geisbert, T. W., S. Jones, et al. (2005). "Development of a new vaccine for the prevention of Lassa fever." PLoS Med **2**(6): e183.

Gerdes, G. H. (2002). "Rift valley fever." The Veterinary clinics of North America. Food animal practice **18**(3): 549-555.

Gerdes, G. H. (2004). "Rift Valley fever." Revue scientifique et technique **23**(2): 613-623.

Gillim-Ross, L. and K. Subbarao (2006). "Emerging respiratory viruses: challenges and vaccine strategies." Clinical microbiology reviews **19**(4): 614-636.

Gilson, G. J., J. A. Maciulla, et al. (1994). "Hantavirus pulmonary syndrome complicating pregnancy." American journal of obstetrics and gynecology **171**(2): 550-554.

Glassman, H. N. (1958). "World incidence of anthrax in man." Public Health Rep **73**(1): 22-24.

Goh, D. L., B. W. Lee, et al. (2004). "Secondary household transmission of SARS, Singapore." Emerg Infect Dis **10**(2): 232-234.

Goh, K. J., C. T. Tan, et al. (2000). "Clinical features of Nipah virus encephalitis among pig farmers in Malaysia." N Engl J Med **342**(17): 1229-1235.

Gomez, R. M., C. Jaquenod de Giusti, et al. (2011). "Junin virus. A XXI century update." Microbes Infect **13**(4): 303-311.

Greene, C. M., J. Reefhuis, et al. (2002). "Epidemiologic investigations of bioterrorism-related anthrax, New Jersey, 2001." Emerg Infect Dis **8**(10): 1048-1055.

Gritsun, T. S., V. A. Lashkevich, et al. (2003). "Tick-borne encephalitis." Antiviral Res **57**(1-2): 129-146.

Groseth, A., H. Feldmann, et al. (2007). "The ecology of Ebola virus." Trends Microbiol **15**(9): 408-416.

Guan, Y., B. J. Zheng, et al. (2003). "Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China." Science **302**(5643): 276-278.

Gubareva, L. V. and A. M. Fry (2010). "Current challenges in the risk assessment of neuraminidase inhibitor-resistant influenza viruses." The Journal of infectious diseases **201**(5): 656-658.

Gubareva, L. V., A. A. Trujillo, et al. (2010). "Comprehensive assessment of 2009 pandemic influenza A (H1N1) virus drug susceptibility in vitro." Antiviral therapy **15**(8): 1151-1159.

Guh, A., M. L. Heyman, et al. (2010). "Lessons learned from the investigation of a cluster of cutaneous anthrax cases in Connecticut." J Public Health Manag Pract **16**(3): 201-210.

Gunther, S. and O. Lenz (2004). "Lassa virus." Crit Rev Clin Lab Sci **41**(4): 339-390.

Gurley, E. S., J. M. Montgomery, et al. (2007). "Person-to-person transmission of Nipah virus in a Bangladeshi community." Emerg Infect Dis **13**(7): 1031-1037.

Gurley, E. S., J. M. Montgomery, et al. (2007). "Risk of nosocomial transmission of Nipah virus in a Bangladesh hospital." Infect Control Hosp Epidemiol **28**(6): 740-742.

Hanson, R. P., S. E. Sulkin, et al. (1967). "Arbovirus infections of laboratory workers. Extent of problem emphasizes the need for more effective measures to reduce hazards." Science **158**(806): 1283-1286.

Harding, A. L., Byers, K.B. (2006). Epidemiology of Laboratory-Associated Infections, ASM Press.

Harrison, L. H., N. A. Halsey, et al. (1999). "Clinical case definitions for Argentine hemorrhagic fever." Clin Infect Dis **28**(5): 1091-1094.

Hartman, A. L., J. S. Towner, et al. (2010). "Ebola and marburg hemorrhagic fever." Clin Lab Med **30**(1): 161-177.

Henderson, D. A. (2010). "Universal influenza vaccination: an optimal goal--but how and when?" Biosecurity and bioterrorism : biodefense strategy, practice, and science **8**(3): 219-221.

Heyworth, B., M. E. Ropp, et al. (1975). "Anthrax in the Gambia: an epidemiological study." Br Med J **4**(5988): 79-82.

Homaira, N., M. Rahman, et al. (2010). "Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007." PLoS One **5**(10): e13570.

Hoogstraal, H. (1979). "The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa." Journal of medical entomology **15**(4): 307-417.

Hossain, M. J., E. S. Gurley, et al. (2008). "Clinical presentation of nipah virus infection in Bangladesh." Clin Infect Dis **46**(7): 977-984.

Howard, M. J., T. J. Doyle, et al. (1999). "Hantavirus pulmonary syndrome in pregnancy." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **29**(6): 1538-1544.

Hsu, V. P., M. J. Hossain, et al. (2004). "Nipah virus encephalitis reemergence, Bangladesh." Emerg Infect Dis **10**(12): 2082-2087.

Hugh-Jones, M. and J. Blackburn (2009). "The ecology of Bacillus anthracis." Mol Aspects Med **30**(6): 356-367.

Idemyor, V. (2010). "Lassa virus infection in Nigeria: clinical perspective overview." J Natl Med Assoc **102**(12): 1243-1246.

Inglesby, T. V., D. T. Dennis, et al. (2000). "Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense." JAMA **283**(17): 2281-2290.

Inglesby, T. V., T. O'Toole, et al. (2002). "Anthrax as a biological weapon, 2002: updated recommendations for management." JAMA **287**(17): 2236-2252.

Jallali, N., S. Hettiaratchy, et al. (2011). "The surgical management of injectional anthrax." J Plast Reconstr Aesthet Surg **64**(2): 276-277.

Jamieson, D. J., J. E. Ellis, et al. (2006). "Emerging infectious disease outbreaks: old lessons and new challenges for obstetrician-gynecologists." Am J Obstet Gynecol **194**(6): 1546-1555.

Jamieson, D. J., M. A. Honein, et al. (2009). "H1N1 2009 influenza virus infection during pregnancy in the USA." Lancet **374**(9688): 451-458.

Jay, M., B. Hjelle, et al. (1996). "Occupational exposure leading to hantavirus pulmonary syndrome in a utility company employee." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **22**(5): 841-844.

Jay, M. T., C. Glaser, et al. (2005). "The arenaviruses." J Am Vet Med Assoc **227**(6): 904-915.

Jeffs, B. (2006). "A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa." Trop Doct **36**(1): 1-4.

Jernigan, D. B., P. L. Raghunathan, et al. (2002). "Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings." Emerg Infect Dis **8**(10): 1019-1028.

Johns, M. C., A. A. Eick, et al. (2010). "Seasonal influenza vaccine and protection against pandemic (H1N1) 2009-associated illness among US military personnel." PLoS One **5**(5): e10722.

Johnson, R. Epizootiology and ecology of anthrax. A. a. P. H. I. S. United States Department of Agriculture, Veterinary Service**:** 1-44.

Johnson, R. (2008). Differentiation of naturally occurring from non-naturally occurring epizootics of anthrax in livestock populations. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Service,**:** 1-16.

Jones, R. M., M. Nicas, et al. (2005). "The Infectious Dose of Francisella tularensis (Tularemia)." Applied Biosafety **10**(4): 227-239.

Jonsson, C. B., L. T. Figueiredo, et al. (2010). "A global perspective on hantavirus ecology, epidemiology, and disease." Clinical microbiology reviews **23**(2): 412-441.

Jonsson, C. B., J. Hooper, et al. (2008). "Treatment of hantavirus pulmonary syndrome." Antiviral research **78**(1): 162-169.

Kadanali, A., M. A. Tasyaran, et al. (2003). "Anthrax during pregnancy: case reports and review." Clin Infect Dis **36**(10): 1343-1346.

Kendal, A. P., G. R. Noble, et al. (1978). "Antigenic similarity of influenza A (H1N1) viruses from epidemics in 1977--1978 to "Scandinavian" strains isolated in epidemics of 1950--1951." Virology **89**(2): 632-636.

Kool, J. L. (2005). "Risk of person-to-person transmission of pneumonic plague." Clin Infect Dis **40**(8): 1166-1172.

Kortepeter, M. G., J. W. Martin, et al. (2008). "Managing potential laboratory exposure to ebola virus by using a patient biocontainment care unit." Emerg Infect Dis **14**(6): 881-887.

Kotturi, M. F., J. Botten, et al. (2009). "A multivalent and cross-protective vaccine strategy against arenaviruses associated with human disease." PLoS Pathog **5**(12): e1000695.

Kumor, L., L. Bates, et al. (2006). "2005 Anthrax outbreak in Manitoba." from http://www.gov.mb.ca/agriculture/livestock/anhealth/pdf/jaa02s00c.pdf.

Kunz, C. (2002). "Vaccination against TBE in Austria: the success story continues." Int J Med Microbiol **291 Suppl 33**: 56-57.

Kunz, C. (2003). "TBE vaccination and the Austrian experience." Vaccine **21 Suppl 1**: S50-55.

Kuzmin, I. V., M. Niezgoda, et al. (2010). "Marburg virus in fruit bat, Kenya." Emerg Infect Dis **16**(2): 352-354.

Kwan-Gett, T. S., A. Baer, et al. (2009). "Spring 2009 H1N1 influenza outbreak in King County, Washington." Disaster medicine and public health preparedness **3 Suppl 2**: S109-116.

LaBeaud, A. D., Y. Ochiai, et al. (2007). "Spectrum of Rift Valley fever virus transmission in Kenya: insights from three distinct regions." The American journal of tropical medicine and hygiene **76**(5): 795-800.

LaBeaud, A. D., J. W. Kazura, et al. (2010). "Advances in Rift Valley fever research: insights for disease prevention." Curr Opin Infect Dis **23**(5): 403-408.

LaBeaud, A. D., E. M. Muchiri, et al. (2008). "Interepidemic Rift Valley fever virus seropositivity, northeastern Kenya." Emerg Infect Dis **14**(8): 1240-1246.

Lasala, P. R. and M. Holbrook (2010). "Tick-borne flaviviruses." Clinics in laboratory medicine **30**(1): 221-235.

Lasala, P. R. and M. Holbrook (2010). "Tick-borne flaviviruses." Clin Lab Med **30**(1): 221-235.

Lau, J. T., M. Lau, et al. (2004). "Probable secondary infections in households of SARS patients in Hong Kong." Emerg Infect Dis **10**(2): 235-243.

Lawler, A. (2005). "Biodefense labs. Boston University Under Fire for Pathogen Mishap." Science **307**(5709): 501.

Lazaro, M. E., G. E. Cantoni, et al. (2007). "Clusters of hantavirus infection, southern Argentina." Emerging infectious diseases **13**(1): 104-110.

Le Guenno, B., P. Formenty, et al. (1999). "Ebola virus outbreaks in the Ivory Coast and Liberia, 1994-1995." Curr Top Microbiol Immunol **235**: 77-84.

Lecompte, E., E. Fichet-Calvet, et al. (2006). "Mastomys natalensis and Lassa fever, West Africa." Emerging Infectious Diseases **12**(12): 1971-1974.

LeDuc, J. W. (1989). "Epidemiology of hemorrhagic fever viruses." Rev Infect Dis **11 Suppl 4**: S730-735.

Leendertz, F. H., H. Ellerbrok, et al. (2004). "Anthrax kills wild chimpanzees in a tropical rainforest." Nature **430**(6998): 451-452.

Leendertz, F. H., F. Lankester, et al. (2006). "Anthrax in Western and Central African great apes." Am J Primatol **68**(9): 928-933.

Leendertz, F. H., S. Yumlu, et al. (2006). "A new Bacillus anthracis found in wild chimpanzees and a gorilla from West and Central Africa." PLoS Pathog **2**(1): e8.

Leffel, E. K. and D. S. Reed (2004). "Marburg and Ebola viruses as aerosol threats." Biosecur Bioterror **2**(3): 186-191.

Lekone, P. E. and B. F. Finkenstadt (2006). "Statistical inference in a stochastic epidemic SEIR model with control intervention: Ebola as a case study." Biometrics **62**(4): 1170-1177.

Leroy, E. M., A. Epelboin, et al. (2009). "Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007." Vector Borne Zoonotic Dis **9**(6): 723-728.

Leroy, E. M., B. Kumulungui, et al. (2005). "Fruit bats as reservoirs of Ebola virus." Nature **438**(7068): 575-576.

Liang, H. and Y. Xue (2004). "Investigating public health emergency response information system initiatives in China." International journal of medical informatics **73**(9-10): 675-685.

Lim, W., K. C. Ng, et al. (2006). "Laboratory containment of SARS virus." Annals of the Academy of Medicine, Singapore **35**(5): 354-360.

Lindquist, L. and O. Vapalahti (2008). "Tick-borne encephalitis." Lancet **371**(9627): 1861-1871.

Lipsitch, M., T. Cohen, et al. (2003). "Transmission dynamics and control of severe acute respiratory syndrome." Science **300**(5627): 1966-1970.

Lloyd-Smith, J. O., S. J. Schreiber, et al. (2005). "Superspreading and the effect of individual variation on disease emergence." Nature **438**(7066): 355-359.

Lo, A. W., N. L. Tang, et al. (2006). "How the SARS coronavirus causes disease: host or organism?" The Journal of pathology **208**(2): 142-151.

Lo, M. K. and P. A. Rota (2008). "The emergence of Nipah virus, a highly pathogenic paramyxovirus." J Clin Virol **43**(4): 396-400.

Lopez, N., P. Padula, et al. (1996). "Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina." Virology **220**(1): 223-226.

Low, J. G. and A. Wilder-Smith (2005). "Infectious respiratory illnesses and their impact on healthcare workers: a review." Annals of the Academy of Medicine, Singapore **34**(1): 105-110.

Luby, S. P., E. S. Gurley, et al. (2009). "Transmission of human infection with Nipah virus." Clin Infect Dis **49**(11): 1743-1748.

Luby, S. P., M. J. Hossain, et al. (2009). "Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007." Emerg Infect Dis **15**(8): 1229-1235.

Luby, S. P., M. Rahman, et al. (2006). "Foodborne transmission of Nipah virus, Bangladesh." Emerg Infect Dis **12**(12): 1888-1894.

Luke, T. C., E. M. Kilbane, et al. (2006). "Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment?" Annals of internal medicine **145**(8): 599-609.

Macher, A. M. and M. S. Wolfe (2006). "Historical Lassa fever reports and 30-year clinical update." Emerg Infect Dis **12**(5): 835-837.

MacNeil, A., E. C. Farnon, et al. (2010). "Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda." Emerg Infect Dis **16**(12): 1969-1972.

Mahanty, S. and M. Bray (2004). "Pathogenesis of filoviral haemorrhagic fevers." Lancet Infect Dis **4**(8): 487-498.

Mahmoud, A., D. Burke, S. Eubank, V.S. Freimuth, G Friedman-Jimenez, P. Hamburg, K.A. Holbrook, D.L. Kasper, J. Lewis, W.I. Lipkin, T.H. Murray, M.E. Northridge, J. Patterson, M. Robson, S. Stanley, W. Thomann, S. Bennett, P. Highnam, and R. Khabbaz. (2008). NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratory at Boston University Medical Center, Finding and Recommendations, Part I: Risk Assessment; Briefing of the Advisory Committee to the Director, NIH, June 6, 2008., Department of Health and Human Services.

Malakoff, D. and K. Drennan (2004). "Texas bioterror case. Butler gets 2 years for mishandling plague samples." Science **303**(5665): 1743-1745.

Manicassamy, B., R. A. Medina, et al. (2010). "Protection of mice against lethal challenge with 2009 H1N1 influenza A virus by 1918-like and classical swine H1N1 based vaccines." PLoS pathogens **6**(1): e1000745.

Mann, J. M. and R. Moskowitz (1977). "Plague and pregnancy. A case report." JAMA **237**(17): 1854-1855.

Mansfield, K. L., N. Johnson, et al. (2009). "Tick-borne encephalitis virus - a review of an emerging zoonosis." J Gen Virol **90**(Pt 8): 1781-1794.

Martinez, V. P., C. Bellomo, et al. (2005). "Person-to-person transmission of Andes virus." Emerging infectious diseases **11**(12): 1848-1853.

Matyas, B. T., H. S. Nieder, et al. (2007). "Pneumonic tularemia on Martha's Vineyard: clinical, epidemiologic, and ecological characteristics." Ann N Y Acad Sci **1105**: 351-377.

McBride, B. W., A. Mogg, et al. (1998). "Protective efficacy of a recombinant protective antigen against Bacillus anthracis challenge and assessment of immunological markers." Vaccine **16**(8): 810-817.

McCaughey, C. and C. A. Hart (2000). "Hantaviruses." J Med Microbiol **49**(7): 587-599.

McCormick, J. B. and S. P. Fisher-Hoch (2002). "Lassa fever." Curr Top Microbiol Immunol **262**: 75-109.

McDonald, L. C., A. E. Simor, et al. (2004). "SARS in healthcare facilities, Toronto and Taiwan." Emerg Infect Dis **10**(5): 777-781.

Meadors, G. F., 3rd, P. H. Gibbs, et al. (1986). "Evaluation of a new Rift Valley fever vaccine: safety and immunogenicity trials." Vaccine **4**(3): 179-184.

Medina, R. A., B. Manicassamy, et al. (2010). "Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus." Nature communications **1**: 28.

Meegan, J. M., and C.L. Bailey (1989). " Rift Valley Fever." Epidemiology and Ecology **4**: 51-76.

Meegan, J. M. and C. L. Bailey (1989). Rift Valley Fever. The Arboviruses:Epidemiology and Ecology. T. P. Monath. Boca Raton, CRC Press, Inc. **4:** 51-76.

Merino, C., A. Arias, et al. (2002). "First case of hantavirus cardiopulmonary syndrome secondary to a rodent bite." Rev Chil Enf Respir **18**: 199-205.

Mertz, G. J., B. Hjelle, et al. (2006). "Diagnosis and treatment of new world hantavirus infections." Current opinion in infectious diseases **19**(5): 437-442.

Meselson, M., J. Guillemin, et al. (1994). "The Sverdlovsk anthrax outbreak of 1979." Science **266**(5188): 1202-1208.

Metras, R., L. M. Collins, et al. (2011). "Rift Valley Fever Epidemiology, Surveillance, and Control: What Have Models Contributed?" Vector borne and zoonotic diseases.

Middleton, D. J., H. A. Westbury, et al. (2002). "Experimental Nipah virus infection in pigs and cats." J Comp Pathol **126**(2-3): 124-136.

Mills, C. E., J. M. Robins, et al. (2004). "Transmissibility of 1918 pandemic influenza." Nature **432**(7019): 904-906.

Mills, J. N., A. N. Alim, et al. (2009). "Nipah virus infection in dogs, Malaysia, 1999." Emerg Infect Dis **15**(6): 950-952.

Mills, J. N., B. A. Ellis, et al. (1994). "Prevalence of infection with Junin virus in rodent populations in the epidemic area of Argentine hemorrhagic fever." Am J Trop Med Hyg **51**(5): 554-562.

Monson, M. H., A. K. Cole, et al. (1987). "Pediatric Lassa fever: a review of 33 Liberian cases." Am J Trop Med Hyg **36**(2): 408-415.

Morens, D. M. and A. S. Fauci (2007). "The 1918 influenza pandemic: insights for the 21st century." The Journal of infectious diseases **195**(7): 1018-1028.

Morens, D. M., J. K. Taubenberger, et al. (2008). "Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness." The Journal of infectious diseases **198**(7): 962-970.

Morens, D. M., J. K. Taubenberger, et al. (2010). "Pandemic influenza's 500th anniversary." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **51**(12): 1442-1444.

Mosby, L. G., S. A. Rasmussen, et al. (2011). "2009 Pandemic influenza A (H1N1) in pregnancy: a systematic review of the literature." Am J Obstet Gynecol.

Mounts, A. W., H. Kaur, et al. (2001). "A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999." J Infect Dis **183**(5): 810-813.

Mpeshe, S. C., H. Haario, et al. (2011). "A Mathematical Model of Rift Valley Fever with Human Host." Acta biotheoretica.

Mupapa, K., W. Mukundu, et al. (1999). "Ebola hemorrhagic fever and pregnancy." J Infect Dis **179 Suppl 1**: S11-12.

Murphy, B. (2008). Pandemic potential of the 1918 H1N1 Virus -Is it the same in 2008? Safety symposium on public health and biosafety practices for research with 1918 H1N1 influenza virus. Bethesda, NIH-RAC.

Murphy, F. A., E. P. J. Gibbs, et al. (1999). Rift Valley Fever. San Diego, Academic Press.

Nakajima, K., U. Desselberger, et al. (1978). "Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950." Nature **274**(5669): 334-339.

National Research Council (U.S.). Committee to Review the Health and Safety Risks of High-Biocontainment Laboratories at Fort Detrick. (2010). Evaluation of the health and safety risks of the new USAMRIID high containment facilities at Fort Detrick, Maryland. Washington, D.C., National Academies Press.

Neuzil, K. M., G. W. Reed, et al. (1998). "Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women." American journal of epidemiology **148**(11): 1094-1102.

Nguyen, T. Q., N. Clark, et al. (2010). "Public health and environmental response to the first case of naturally acquired inhalational anthrax in the United States in 30 years: infection of a new york city resident who worked with dried animal hides." J Public Health Manag Pract **16**(3): 189-200.

Niklasson, B., J. Liljestrand, et al. (1987). "Rift Valley fever: a sero-epidemiological survey among pregnant women in Mozambique." Epidemiology and infection **99**(2): 517-522.

Normile, D. (2004). "Infectious diseases. Second lab accident fuels fears about SARS." Science **303**(5654): 26.

Normile, D. (2009). "Emerging infectious diseases. Scientists puzzle over Ebola-Reston virus in pigs." Science **323**(5913): 451.

NRC (National Research Council) (2011). Protecting the Frontline in Biodefense Research: The Special Immunizations Program. Washington DC, National Academies Press.

NRC (National Research Council) (2011). Review of Risk Assessment Work Plan for the Medical Countermeasures Test and Evaluation Facility at Fort Detrick:
A Letter Report. Washington DC.

Ogbu, O., E. Ajuluchukwu, et al. (2007). "Lassa fever in West African sub-region: an overview." J Vector Borne Dis **44**(1): 1-11.

Okware, S. I., F. G. Omaswa, et al. (2002). "An outbreak of Ebola in Uganda." Trop Med Int Health **7**(12): 1068-1075.

Orellana, C. (2004). "Laboratory-acquired SARS raises worries on biosafety." The Lancet infectious diseases **4**(2): 64.

Organization, W. H. (2007). "Outbreak of Marburg haemorrhagic fever: Uganda, June-August 2007." Wkly Epidemiol Rec **82**(43): 381-384.

Organization, W. H. (2009). "Outbreak news. Ebola Reston in pigs and humans, Philippines." Wkly Epidemiol Rec **84**(7): 49-50.

Overholt, E. L., W. D. Tigertt, et al. (1961). "An analysis of forty-two cases of laboratory-acquired tularemia. Treatment with broad spectrum antibiotics." Am J Med **30**: 785-806.

Overturf, G. D. (2005). "Clinical sin nombre hantaviral infections in children." The Pediatric infectious disease journal **24**(4): 373-374.

Padula, P. J., A. Edelstein, et al. (1998). "Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus." Virology **241**(2): 323-330.

Panning, M., P. Emmerich, et al. (2010). "Laboratory diagnosis of Lassa fever, liberia." Emerg Infect Dis **16**(6): 1041-1043.

Papin, J. F., P. H. Verardi, et al. (2011). "Recombinant Rift Valley fever vaccines induce protective levels of antibody in baboons and resistance to lethal challenge in mice." Proceedings of the National Academy of Sciences of the United States of America.

Peck, A. J., E. C. Newbern, et al. (2004). "Lack of SARS transmission and U.S. SARS case-patient." Emerg Infect Dis **10**(2): 217-224.

Pedrosa, P. B. and T. A. Cardoso (2011). "Viral infections in workers in hospital and research laboratory settings: a comparative review of infection modes and respective biosafety aspects." International

journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **15**(6): e366-376.

Peiris, J. S., C. M. Chu, et al. (2003). "Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study." Lancet **361**(9371): 1767-1772.

Peiris, J. S., L. L. Poon, et al. (2009). "Emergence of a novel swine-origin influenza A virus (S-OIV) H1N1 virus in humans." Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **45**(3): 169-173.

Pepin, M., M. Bouloy, et al. (2010). "Rift Valley fever virus(Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention." Veterinary research **41**(6): 61.

Peters, C. J. and D. M. Hartley (2002). "Anthrax inhalation and lethal human infection." Lancet **359**(9307): 710-711.

Peters, C. J., P. B. Jahrling, et al. (1987). "Experimental studies of arenaviral hemorrhagic fevers." Curr Top Microbiol Immunol **134**: 5-68.

Petersen, J. M., J. K. Carlson, et al. (2008). "Multiple Francisella tularensis subspecies and clades, tularemia outbreak, Utah." Emerging infectious diseases **14**(12): 1928-1930.

Peterson, A. T., J. T. Bauer, et al. (2004). "Ecologic and geographic distribution of filovirus disease." Emerg Infect Dis **10**(1): 40-47.

Pike, R. M. (1976). "Laboratory-associated infections: summary and analysis of 3921 cases." Health Lab Sci **13**(2): 105-114.

Pike, R. M. (1979). "Laboratory-associated infections: incidence, fatalities, causes, and prevention." Annu Rev Microbiol **33**: 41-66.

Pike, R. M., S. E. Sulkin, et al. (1965). "Continuing Importance of Laboratory-Acquired Infections." Am J Public Health Nations Health **55**: 190-199.

Pini, N. C., A. Resa, et al. (1998). "Hantavirus infection in children in Argentina." Emerging infectious diseases **4**(1): 85-87.

Pourrut, X., B. Kumulungui, et al. (2005). "The natural history of Ebola virus in Africa." Microbes Infect **7**(7-8): 1005-1014.

Pourrut, X., M. Souris, et al. (2009). "Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in Rousettus aegyptiacus." BMC Infect Dis **9**: 159.

Pratt, W. D., D. Wang, et al. (2010). "Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector." Clinical and vaccine immunology : CVI **17**(4): 572-581.

Prevention, C. f. D. C. a. (2001). "Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001." MMWR Morb Mortal Wkly Rep **50**(42): 909-919.

Prevention, C. f. D. C. a. (2002). "Use of Anthrax Vaccine in Response to Terrorism: Supplemental Recommendations of the Advisory Committee on Immunization Practices " Morbidity and Mortality Weekly Report **51**(45): 1024-1026.

Prevention, C. f. D. C. a. (2003). "Imported plague--New York City, 2002." MMWR Morb Mortal Wkly Rep **52**(31): 725-728.

Prevention, C. f. D. C. a. (2003). "Severe acute respiratory syndrome--Singapore, 2003." MMWR. Morbidity and mortality weekly report **52**(18): 405-411.

Prevention, C. f. D. C. a. (2004). "Nipah virus outbreak(s) in Bangladesh, January-April 2004." Wkly Epidemiol Rec **79**(17): 168-171.

Prevention, C. f. D. C. a. (2009). "Imported case of Marburg hemorrhagic fever - Colorado, 2008." MMWR Morb Mortal Wkly Rep **58**(49): 1377-1381.

Prevention, C. f. D. C. a. (2010). "Tick-borne encephalitis among U.S. travelers to Europe and Asia - 2000-2009." MMWR. Morbidity and mortality weekly report **59**(11): 335-338.

Price, M. E., S. P. Fisher-Hoch, et al. (1988). "A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy." BMJ **297**(6648): 584-587.

Quinn, S. C., S. Kumar, et al. (2011). "Racial disparities in exposure, susceptibility, and access to health care in the US H1N1 influenza pandemic." American journal of public health **101**(2): 285-293.

Ramos, M. M., G. D. Overturf, et al. (2001). "Infection with Sin Nombre hantavirus: clinical presentation and outcome in children and adolescents." Pediatrics **108**(2): E27.

Randolph, S. E. (2008). "Tick-borne encephalitis virus, ticks and humans: short-term and long-term dynamics." Curr Opin Infect Dis **21**(5): 462-467.

Rasmussen, S. A., D. M. Kissin, et al. (2011). "Preparing for influenza after 2009 H1N1: special considerations for pregnant women and newborns." Am J Obstet Gynecol.

Ratsitorahina, M., S. Chanteau, et al. (2000). "Epidemiological and diagnostic aspects of the outbreak of pneumonic plague in Madagascar." Lancet **355**(9198): 111-113.

Reid, A. H., T. G. Fanning, et al. (2000). "Characterization of the 1918 "Spanish" influenza virus neuraminidase gene." Proceedings of the National Academy of Sciences of the United States of America **97**(12): 6785-6790.

Reid, A. H., J. K. Taubenberger, et al. (2004). "Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus." Nature reviews. Microbiology **2**(11): 909-914.

Reynes, J. M., D. Counor, et al. (2005). "Nipah virus in Lyle's flying foxes, Cambodia." Emerg Infect Dis **11**(7): 1042-1047.

Ringertz, S. H., E. A. Hoiby, et al. (2000). "Injectional anthrax in a heroin skin-popper." Lancet **356**(9241): 1574-1575.

Robertson, C. A., S. A. Lowther, et al. (2004). "SARS and pregnancy: a case report." Emerging infectious diseases **10**(2): 345-348.

Roels, T. H., A. S. Bloom, et al. (1999). "Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: risk factors for patients without a reported exposure." J Infect Dis **179 Suppl 1**: S92-97.

Rudolph, W. and T. Ben Yedidia (2011). "A universal influenza vaccine: Where are we in the pursuit of this "Holy Grail"?" Human vaccines **7**(1): 10-11.

Ruzek, D., G. Dobler, et al. (2010). "Tick-borne encephalitis: pathogenesis and clinical implications." Travel Med Infect Dis **8**(4): 223-232.

Saslaw, S., H. T. Eigelsbach, et al. (1961). "Tularemia vaccine study. II. Respiratory challenge." Arch Intern Med **107**: 702-714.

Scherer, W. F., G. A. Eddy, et al. (1980). "Laboratory safety for arboviruses and certain other viruses of vertebrates." American Journal of Tropical Medicine and Hygiene **29**((6)): 1359-1381.

Scherer, W. F., G. A. Eddy, et al. (1980). "Laboratory safety for arboviruses and certain other viruses of vertebrates " Am. J.Trop. Med. Hyg. **29**: 1359–1381.

Schmaljohn, C. and B. Hjelle (1997). "Hantaviruses: a global disease problem." Emerging infectious diseases **3**(2): 95-104.

Schuchat, A., B. P. Bell, et al. (2011). "The science behind preparing and responding to pandemic influenza: the lessons and limits of science." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **52 Suppl 1**: S8-12.

Sejvar, J. J., F. C. Tenover, et al. (2005). "Management of anthrax meningitis." Lancet Infect Dis **5**(5): 287-295.

Shapiro, D. S. and D. R. Schwartz (2002). "Exposure of laboratory workers to Francisella tularensis despite a bioterrorism procedure." J Clin Microbiol **40**(6): 2278-2281.

Sheth, A. N., K. N. Althoff, et al. (2011). "Influenza susceptibility, severity, and shedding in HIV-infected adults: a review of the literature." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **52**(2): 219-227.

Shi, Z. and Z. Hu (2008). "A review of studies on animal reservoirs of the SARS coronavirus." Virus research **133**(1): 74-87.

Shimshony, A. and R. Barzilai (1983). "Rift Valley fever." Advances in veterinary science and comparative medicine **27**: 347-425.

Sidwell, R. W. and D. F. Smee (2003). "Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control." Antiviral research **57**(1-2): 101-111.

Simonsen, L., A. Kane, et al. (1999). "Unsafe injections in the developing world and transmission of bloodborne pathogens: a review." Bulletin of the World Health Organization **77**(10): 789-800.

Siston, A. M., S. A. Rasmussen, et al. (2010). "Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States." JAMA : the journal of the American Medical Association **303**(15): 1517-1525.

Stockman, L. J., S. A. Lowther, et al. (2004). "SARS during pregnancy, United States." Emerging infectious diseases **10**(9): 1689-1690.

Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses (1980). "Laboratory safety for arboviruses and certain other viruses of vertebrates. The Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses." The American journal of tropical medicine and hygiene **29**(6): 1359-1381.

Suss, J. (2003). "Epidemiology and ecology of TBE relevant to the production of effective vaccines." Vaccine **21 Suppl 1**: S19-35.

Swanepoel, R. and J. A. W. Coetzer (1994). Rift Valley Fever. Infectious Diseases of Livestock with Special Reference to Southern Africa. G. T. JAW Coetzer, & RC Tustin. New York, Oxford University Press. **1:** 688-717.

Swanepoel, R., S. B. Smit, et al. (2007). "Studies of reservoir hosts for Marburg virus." Emerg Infect Dis **13**(12): 1847-1851.

Swerdlow, D. L., L. Finelli, et al. (2011). "2009 H1N1 influenza pandemic: field and epidemiologic investigations in the United States at the start of the first pandemic of the 21st century." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **52 Suppl 1**: S1-3.

Taubenberger, J. K. (2006). "The origin and virulence of the 1918 "Spanish" influenza virus." Proceedings of the American Philosophical Society **150**(1): 86-112.

Taubenberger, J. K. and D. M. Morens (2006). "1918 Influenza: the mother of all pandemics." Emerging infectious diseases **12**(1): 15-22.

Taubenberger, J. K. and D. M. Morens (2008). "The pathology of influenza virus infections." Annual review of pathology **3**: 499-522.

Taubenberger, J. K., A. H. Reid, et al. (1997). "Initial genetic characterization of the 1918 "Spanish" influenza virus." Science **275**(5307): 1793-1796.

Tellier, R. (2006). "Review of aerosol transmission of influenza A virus." Emerging infectious diseases **12**(11): 1657-1662.

The Center for Infectious Disease Research and Policy (CIDRAP). (2011). "Minnesota officials confirm case of inhalational anthrax." Retrieved August 30, 2011, 2011, from http://www.cidrap.umn.edu/cidrap/content/bt/anthrax/news/aug0911anthrax.html.

Timen, A., M. P. Koopmans, et al. (2009). "Response to imported case of Marburg hemorrhagic fever, the Netherland." Emerg Infect Dis **15**(8): 1171-1175.

Titball, R. W., P. C. Turnbull, et al. (1991). "The monitoring and detection of Bacillus anthracis in the environment." Soc Appl Bacteriol Symp Ser **20**: 9S-18S.

Toro, J., J. D. Vega, et al. (1998). "An outbreak of hantavirus pulmonary syndrome, Chile, 1997." Emerging infectious diseases **4**(4): 687-694.

Towner, J. S., M. L. Khristova, et al. (2006). "Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola." J Virol **80**(13): 6497-6516.

Towner, J. S., X. Pourrut, et al. (2007). "Marburg virus infection detected in a common African bat." PLoS One **2**(1): e764.

Towner, J. S., T. K. Sealy, et al. (2008). "Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda." PLoS Pathog **4**(11): e1000212.

Truelove, S. A., A. S. Chitnis, et al. (2011). "Comparison of patients hospitalized with pandemic 2009 influenza A (H1N1) virus infection during the first two pandemic waves in Wisconsin." The Journal of Infectious Diseases **203**(6): 828-837.

Tuffs, A. (2009). "Experimental vaccine may have saved Hamburg scientist from Ebola fever." BMJ **338**: b1223.

Tumpey, T. M., C. F. Basler, et al. (2005). "Characterization of the reconstructed 1918 Spanish influenza pandemic virus." Science **310**(5745): 77-80.

Tumpey, T. M. and J. A. Belser (2009). "Resurrected pandemic influenza viruses." Annual review of microbiology **63**: 79-98.

Tumpey, T. M., A. Garcia-Sastre, et al. (2004). "Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus." Proceedings of the National Academy of Sciences of the United States of America **101**(9): 3166-3171.

Tumpey, T. M., A. Garcia-Sastre, et al. (2005). "Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice." Journal of virology **79**(23): 14933-14944.

Turnbull, P. C., P. M. Lindeque, et al. (1998). "Airborne movement of anthrax spores from carcass sites in the Etosha National Park, Namibia." J Appl Microbiol **84**(4): 667-676.

US Department of Health and Human Services. (2011). "Biodefense Strategic Plan." Retrieved August 30, 2011, 2011, from http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/about/pages/strategicplan.aspx.

US Department of Health and Human Services. (2011). "Community Strategy for Pandemic Influenza Mitigation." Retrieved August 30, 2011, 2011, from http://flu.gov/professional/community/commitigation.html.

US Department of Health and Human Services. (2011). "History of Flu Pandemics." Retrieved September 29, 2011, 2011, from http://www.flu.gov/individualfamily/about/pandemic/history.html.

Uscher-Pines, L., J. Maurer, et al. (2011). "Racial and ethnic disparities in uptake and location of vaccination for 2009-H1N1 and seasonal influenza." American journal of public health **101**(7): 1252-1255.

Valdez, R., K. M. Narayan, et al. (1999). "Impact of diabetes mellitus on mortality associated with pneumonia and influenza among non-Hispanic black and white US adults." American journal of public health **89**(11): 1715-1721.

Van Bever, H. P., S. Y. Chng, et al. (2004). "Childhood severe acute respiratory syndrome, coronavirus infections and asthma." Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology **15**(3): 206-209.

Varia, M., S. Wilson, et al. (2003). "Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada." CMAJ **169**(4): 285-292.

Viboud, C., T. Tam, et al. (2006). "1951 influenza epidemic, England and Wales, Canada, and the United States." Emerging infectious diseases **12**(4): 661-668.

Wacharapluesadee, S., B. Lumlertdacha, et al. (2005). "Bat Nipah virus, Thailand." Emerg Infect Dis **11**(12): 1949-1951.

Wamala, J. F., L. Lukwago, et al. (2010). "Ebola hemorrhagic fever associated with novel virus strain, Uganda, 2007-2008." Emerg Infect Dis **16**(7): 1087-1092.

Webster, R. G., W. J. Bean, et al. (1992). "Evolution and ecology of influenza A viruses." Microbiological reviews **56**(1): 152-179.

Weinbren, M. P. (2008). "Rift Valley Fever." International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates. **CDC On-line Edition**.

Weingartl, H. M., Y. Berhane, et al. (2009). "Animal models of henipavirus infection: a review." Vet J **181**(3): 211-220.

Weiss, S. R. and S. Navas-Martin (2005). "Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus." Microbiology and molecular biology reviews : MMBR **69**(4): 635-664.

Wells, R. M., S. Sosa Estani, et al. (1997). "An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia." Emerg Infect Dis **3**(2): 171-174.

Wenger, J. D., L. J. Castrodale, et al. (2011). "2009 Pandemic influenza A H1N1 in Alaska: temporal and geographic characteristics of spread and increased risk of hospitalization among Alaska Native and Asian/Pacific Islander people." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **52 Suppl 1**: S189-197.

Wentworth, D. E., M. W. McGregor, et al. (1997). "Transmission of swine influenza virus to humans after exposure to experimentally infected pigs." The Journal of infectious diseases **175**(1): 7-15.

White, D. J., R. G. Means, et al. (1996). "Human and rodent hantavirus infection in New York State: public health significance of an emerging infectious disease." Archives of internal medicine **156**(7): 722-726.

Wilkening, D. A. (2006). "Sverdlovsk revisited: modeling human inhalation anthrax." Proc Natl Acad Sci U S A **103**(20): 7589-7594.

Wilson-Clark, S. D., S. L. Deeks, et al. (2006). "Household transmission of SARS, 2003." CMAJ **175**(10): 1219-1223.

Wong, D., M. A. Wild, et al. (2009). "Primary pneumonic plague contracted from a mountain lion carcass." Clin Infect Dis **49**(3): e33-38.

Wong, S. F., K. M. Chow, et al. (2004). "Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome." American journal of obstetrics and gynecology **191**(1): 292-297.

Wong, T. W. (1986). "Plague in a pregnant patient." Trop Doct **16**(4): 187-189.

World Health Organization. (2008). Anthrax in humans and animals. Geneva, Switzerland, World Health Organization.

Wright, J. G., C. P. Quinn, et al. (2010). "Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009." MMWR Recomm Rep **59**(RR-6): 1-30.

Zhong, N. S. and G. W. Wong (2004). "Epidemiology of severe acute respiratory syndrome (SARS): adults and children." Paediatric respiratory reviews **5**(4): 270-274.

Zimmer, S. M. and D. S. Burke (2009). "Historical perspective--Emergence of influenza A (H1N1) viruses." The New England journal of medicine **361**(3): 279-285.

Contents

Abbreviations and Acronyms

µm	micrometers
1918 H1N1V	1918 H1N1 influenza virus
ANDV	Andes virus
B. anthracis	*Bacillus anthracis*
BDB	beyond design basis
BRP	Blue Ribbon Panel
BSC	biological safety cabinet
BSL	biosafety level
BUMC	Boston University Medical Center
$CCID_{50}$	median cell culture infective doses
CFU	colony forming units
DOE	U.S. Department of Energy
EBOV	Ebola virus
F. tularensis	*Francisella tularensis*
FAA	Federal Aviation Administration
FFU	fluorescent focus unit
HVAC	heating, ventilation, and air conditioning
JUNV	Junín virus
km	kilometer
LAI	laboratory-associated infection
LASV	Lassa virus
m	meter
MACCS2	MELCOR Accident Consequence Code System, version 2
MARV	Marburg virus
MEI	maximally exposed individual
mi	mile
$MICLD_{50}$	median mouse intracerebral lethal doses
MID_{50}	median mouse infective dose
MRF	maximum reasonably foreseeable
NEIDL	National Emerging Infectious Diseases Laboratory
NEPA	National Environmental Protection Agency
NIH	National Institutes of Health
NIPV	Nipah virus
NRC	National Research Council of the National Academies
PAPR	powered air-purifying respirator
PFU	plaque forming units
PPE	personal protective equipment
RA	*Risk Assessment (RA) of the National Emerging Infectious Diseases Laboratories (NEIDL) at Boston University Medical Center (BUMC)*
RVFV	Rift Valley fever virus
SARS-CoV	SARS-associated coronavirus
SECPOP	Sector Population program
TBEV-FE	tick-borne encephalitis virus, Far Eastern sub-type, formerly known as Tick-borne encephalitis complex (Russian spring-summer encephalitis virus)
$TCID_{50}$	median tissue culture infective dose
Y. pestis	*Yersinia pestis*

4. Event Sequence Analysis

***Chapter Highlights**: This chapter explains the process of identifying, selecting, and analyzing postulated events that have the potential to expose workers or the public to pathogens. The events selected and analyzed in this chapter are:*

- *Aerosol release from a centrifuge*
- *Needlestick*
- *Earthquake*
- *Aircraft crash*
- *Malevolent acts*

Worker and public infections and fatalities resulting from direct exposure and secondary transmission are analyzed in Chapters 8 and 9 for the centrifuge release, needlestick, and earthquake postulated events. These analyses were performed for the three sites (i.e., urban, suburban, and rural) and all 13 pathogens being evaluated.

Transportation accidents, malevolent act, and environmental persistence in the environment were also selected for analysis and these analyses are reported in Chapters 5, 6, and 7, respectively.

Section 4.1 describes the methodology that was employed to select events for analysis and identifies the selected events. Section 4.2 presents the results of the analysis for the events selected. Appendices E and F provide the details that support this chapter.

4.1 Selection of Events for Analysis

4.1.1 Introduction and Scope

One of the key recommendations provided by the NRC in its advice on the development of the NEIDL RA is to identify: *What can go wrong, what are the probabilities, and what would be the consequences?* (NIH 2009). This RA began with a broad consideration of what can go wrong and then selected a smaller set of potential events for analysis that involve a compromise of biocontainment. This RA analyzed multiple potential loss of biocontainment events to provide insights into the following:

1. What are the risks to the workers?
2. What are the risks to the public?
3. Are there differences in risks if NEIDL were located at a less-densely populated site?

This analysis follows the guidance for analyses of this type provided by the U.S. Department of Energy (DOE) *Recommendations for Analyzing Accidents under the National Environmental Policy Act* (DOE

2002, referred to as the DOE NEPA Guidance). The DOE NEPA Guidance was used for this analysis because it is the most relevant and detailed guidance available for this type of analysis. Use of the DOE NEPA Guidance is consistent with the recommendations of the NRC, which reviewed the DOE NEPA Guidance and concluded the following (NRC 2010):

> U.S. Department of Energy's (DOE) recommendations for the preparation of EISs [Environmental Impact Statements] contain some of the most detailed explanations and guidelines for discussing human health impacts in an EIS. Although DOE's recommendations for analyzing human health effects are limited to exposure to radiation and chemicals, they also are relevant to pathogen exposures.

DOE NEPA Guidance recommends that the analyses consider the spectrum of reasonably foreseeable events,[1] "including low probability/high consequence accidents and higher probability/(usually) lower consequence accidents" (DOE 2002). To ensure that a broad range of events is comprehensively addressed, reasonably foreseeable events were considered for the following three types of events:

- **Maximum reasonably foreseeable (MRF) event**—The state courts and the NRC have expressed interest in analysis of a *worst-case* scenario, but the NRC noted that that is a nebulous term that is not well defined (NIH 2009). The DOE NEPA Guidance is used here to define the worst-case scenario as (DOE 2002):

  > A maximum reasonably foreseeable accident is an accident with the most severe consequences that can reasonably be expected to occur for a given proposal. … *Reasonably foreseeable* events include events which may have catastrophic consequences, even if their probability of occurrence is low, provided that the analysis of the impacts is supported by credible scientific evidence, is not based on pure conjecture, and is within the rule of reason.

  For this RA, the MRF event was defined solely in terms of the accident with the maximum pathogen release from the facility.

[1] The term *reasonably foreseeable* extends to events that may have catastrophic consequences, even if their probability of occurrence is low, provided that the analysis of the impacts is supported by credible scientific evidence, is not based on pure conjecture, and is within the rule of reason (DOE 2002).

- **Representative events**—To better understand the nature of the risks posed by each pathogen, representative events (i.e., an event selected as a surrogate for multiple other events because it has greater or similar frequency and consequences as a group of other events) were analyzed for each pathogen, route of exposure, and exposed group.

- **Unique events—**As appropriate, additional events were selected to explore unique characteristics of a given pathogen or differences between the sites being considered. Those unique events could address such factors as differences in natural phenomena at the sites.

The following events were considered: (1) accidental loss of biocontainment at the facility, which includes internal hazards (e.g., equipment failures and personnel errors), external hazards (e.g., aircraft crash and loss of offsite power), and natural phenomena (e.g., earthquake and strong wind hazards that could result in partial or complete loss of biocontainment); and (2) transportation accidents. Operating experience at similar other BSL-3 and BSL-4 laboratories was used as one input for the identification and evaluation of these events.

The scope includes the spectrum of reasonably foreseeable events. Reasonably foreseeable events are defined as events "including low probability/high consequence accidents and higher probability/(usually) lower consequence accidents." (DOE 2002) The analysis of reasonably foreseeable events is generally limited to events with a frequency greater than 10^{-6}/yr (a frequency equivalent to once in a million years), but this is not an absolute cutoff. Lower frequency events are included if the resulting consequences could be disproportionately greater than those with a higher frequency (DOE 2002).

Operating Experience at BSL-3 and BSL-4 Laboratories*: This RA relies on past experience at similar laboratories to the extent data are available and useful. Appendix D summarizes various sources of this operating experience. Appendix D includes the recent CDC report of 395 "potential release events" and 7 laboratories associated infections (LAIs) from 2003 to 2009 nationwide at laboratories working with select agents (see Section D.1.1 of Appendix D).*

The operating experience was used to identify potential initiating events, develop scenarios, and estimate the scenario frequencies. While helpful for qualitative analyses, the data were not used for quantitative analyses as details of appropriate measures of operating time (e.g. researcher-hours), descriptions of individual incidents leading to loss of biocontainment and biosafety protocols in place at the time of the events are not specified in available reports.

Therefore, past BSL-3 and BSL-4 experience was used to support the RA wherever appropriate, but they are not suitable for quantitative use.

The scope of this analysis was limited to the 13 pathogens selected for study by the NIH BRP and NRC in a teleconference (see NIH 2009). The scenarios analyzed include consideration of NEIDL-specific preventive and mitigative features, which tend to reduce the frequency and severity of events.

4.1.2 Methodology

The event identification and selection process began with a comprehensive identification of candidate initiating events. The candidate initiating events were then evaluated to determine the applicable routes of exposure, NEIDL locations, and potentially exposed groups. Then, a multi-step process incorporating frequency (probability of occurrence) and exposure categories (the number of people potentially exposed) was used to select events for more detailed analysis. Each of those steps is described in the following subsections.

4.1.2.1 Identification of Candidate Initiating Events

The first step in the process was the comprehensive identification of candidate initiating events that could lead to loss of biocontainment. The process of tabulating candidate events included the following activities:

- Operating experience at other high biocontainment facilities was reviewed because incidents that have occurred at other facilities might also occur at the NEIDL.
- Previous analyses of the NEIDL were reviewed to identify the postulated events considered appropriate for analyses in those documents.
- The NRC list of scenarios to be studied (NIH 2009) was reviewed to ensure that all events identified were considered.
- NEPA documents for other BSL-3 and BSL-4 facilities were reviewed to identify the postulated events considered for analysis.
- The NEIDL facility design and operating plans were reviewed to identify potential scenarios.
- The site characteristics for the three sites were reviewed to consider the potential for unique external and natural phenomenon hazards.

On the basis of those reviews, a preliminary list of candidate initiating events was developed. The candidate initiating events were then evaluated, as explained in Section 4.1.2.2, to select the events to be analyzed in detail.

4.1.2.2 Evaluation of Candidate Initiating Events

Each candidate initiating event that could result in worker or public exposure was evaluated to determine the relevant routes of potential exposure, NEIDL locations, potentially exposed groups, frequency categories, and exposure categories (i.e., number of people exposed). Those evaluations are explained in sections 4.1.2.2.1 through 4.1.2.2.5.

4.1.2.2.1 Routes of Exposure

This analysis considered exposures to pathogens via inhalation, ingestion, direct contact, puncture, and animal-related exposure (including arthropod vectors). Inhalation exposure is typically only considered for particles with an aerodynamic equivalent diameter no greater than 10 micrometers (μm) (DOE 2000). Ingestion can occur via a liquid contaminated with a pathogen or by contaminated hand-to-mouth exposure. Direct contact is exposure to broken skin, eyes, mucous membranes, or nasal passages. Puncture is a general term used to address a condition whereby microorganisms are potentially placed below the outermost layer of the skin through a mechanical means such as a syringe needle or other sharp object. Animal-related exposures via one the other routes that is animal-related (i.e., associated with mammals or arthropods).

4.1.2.2.2 NEIDL Locations

Each event was assigned to each of the NEIDL locations that are relevant for the event. The NEIDL locations are as follows: (1) BSL-3 laboratories; (2) BSL-4 laboratories; and (3) other locations (e.g., the loading dock or off-site).

4.1.2.2.3 Potentially Exposed Groups

As recommended by DOE NEPA Guidance (DOE 2002), this analysis considers impacts associated with the following potentially exposed groups: (1) laboratory worker (e.g., people working in the laboratory room when the event occurs); (2) facility worker (e.g., people working in the NEIDL but not in the laboratory room when the event under consideration occurs. For example, they might work in the administrative areas); and (3) the public (e.g., any person outside the NEIDL-controlled perimeter, specifically referring to the population in the surrounding communities).

4.1.2.2.4 Frequency Categories

The likelihood of events can be described and calculated in several ways. Table 4-1 compares several equivalent ways of describing (using numbers and measures) the likelihood of events.

Table 4-1 Measures of likelihood.

Average Return Period[a] (years)	Average Frequency[b] (per year)	Probability / Chance of Occurrence in Facility Lifetime[c] (in 50 years)	
1	1	Virtually 100%	Virtually 100-in-100
10	0.1	99%	99-in-100
100	0.01	39%	1-in-2.5
1,000	0.001	4.9%	1-in-21
10,000	0.0001	0.5%	1-in-200
100,000	0.00001	0.05%	1-in-2000
1,000,000	0.000001	0.005%	1-in-20,000
10,000,000	0.0000001	0.0005%	1-in-200,000

[a] **Average return period in years**: This is the average time, in years, before the event would be expected to occur. If the event was to occur multiple times, this would be the average time between occurrences. This way of describing events is often used in characterizing flood levels; for example, a "1000-year flood" is a water level that is estimated to occur with a 1,000-year average return period or "once per 1,000 years".

[b] **Average frequency per year:** This is the average number of occurrences of the event per year.

[c] **Probability / chanceof occurrence in facility lifetime (50 years)**: This is the chance that the event would occur at least once in a given 50-year period.

The operational data from research laboratories like the NIEDL (see Appendix D) are not adequate for development of quantitative frequency estimates (e.g., mean rate plus uncertainties) for the events analyzed, so alternate approaches were used. A technique commonly used when quantitative estimates of frequency are not possible is the use of categories (i.e., ranges of values), which is used by the U.S. Environmental Protection Agency (EPA), U.S. Department of Energy (DOE), and National Aeronautics and Space Administration (NASA). (EPA 1987, NASA 2005, NASA 2009, DOE 1994) Each event sequence is assigned to a frequency category based the initiating event, and the number and nature of concurrent failures of preventive and mitigative features. The assignment of frequency categories often relies on comparison with events in other industries and use of judgment. For this analysis, when it was not clear which of two frequency categories should be assigned, the higher frequency category was used to avoid underestimating the risk. Table 4-2 identifies the frequency categories (i.e., A, B, C, and D) used in this RA and provides a verbal description and average return period for each category.

Table 4-2 Frequency categories

Category	Verbal description	Average return period (once in "this many" years)
A	An event sequence was assigned to this category if its likelihood is sufficiently high to assume that it will occur during the operational lifetime of the NEIDL (i.e., during 50 years of operation).	1 to 100 years
B	An event sequence was assigned to this category if one or more of the events in this category could occur during the NEIDL's operating life, but any specific event sequence in the group is not expected to occur.	100 to 10,000 years
C	An event sequence was assigned to this category if collectively none of the events in this category is expected to occur during the operating life of the facility, but the events in this category are still reasonably foreseeable.	10,000 to 1 million years
D	An event sequence was assigned to this category if it does not meet the criteria for *reasonably foreseeable*. An event sequence is categorized as category D if it is impossible or highly improbable (i.e., beyond reasonably foreseeable).	>1 million years

4.1.2.2.5 Exposure Categories

The exposure categories were defined in terms of the number of people potentially exposed to a pathogen by an event sequence. The number of people potentially exposed can vary depending on the type of event and the operational parameters at the time of the event. For example, the number of people in a room can vary from event to event depending on the activities involved. In addition, some events, such as a needle stick, might affect only one person, while another event such as an aerosolized pathogen release could affect multiple people in the room. Therefore, the exposure categories were generally defined as a range in the number of people potentially exposed. Table 4-3 defines the exposure categories for each potentially exposed group because the total population and potential for exposure is different for each group.

Table 4-3. Exposure categories

Exposure category	Laboratory workers	Facility workers	Members of the public (number of people within the radius)
NONE	0	0	≤ 30 m
LOW	A single individual (1)	A few individuals on the same floor (≤10)	> 30 m to ≤ 300 m
MODERATE	Most individuals in the room (1 to 4)	Most individuals on the floor (≤ 87 for BSL-3 and ≤ 30 for BSL-4)	> 300 m to ≤3 km
HIGH	An atypical number of individuals (>4)[a]	Most individuals in the building (≤ 300)	> 3 km

a Although the possibility exists, it is not expected that activities will involve more than four workers in a room (BUMC 2009).

4.1.2.3 Selection of Events for Analysis

From the list of candidate events, a subset was selected for detailed analysis as the MRF event, representative events, and unique events. The objective of the selection process was to provide broad coverage of the risk profile by judicious select of events. The events were selected to ensure that all routes of exposure, NEIDL locations, and potentially exposed groups are addressed. Selections were based on the frequency and exposure categories of the events and their applicability to multiple routes of exposure, NEIDL locations, and potentially exposed groups.

4.1.3 Results

The following results are based on the methodology presented in Section 4.1.2.

4.1.3.1 Candidate Initiating Events

More than 300 candidate incidents and postulated events were identified (see Section 4.1.2.1). Numerous incidents and postulated events are similar to others in the list, so common incidents were consolidated into more than 30 candidate initating event groups presented in Table 4-4. Table 4-4 also identifies the relevant NEIDL locations, exposed groups, and routes of exposure for each candidate event group.

1 **Table 4-4. Candidate events for the NEIDL RA**

Candidate event group	NEIDL location			Exposed group			Route of exposure				
	BSL-3	BSL-4	Other	Laboratory worker	Facility worker	Public	Inhalation	Ingestion	Direct contact	Puncture	Animal-related
Aircraft crash	X	X	X	X	X	X	X	X	X		X
Animal bite/scratch	X	X		X						X	X
Animal escape or pathogen release to environment	X	X	X	X	X	X					X
Animal-related infectious aerosol in the laboratory	X	X		X							X
Breach of containment (wall cracks or open doors)	X	X	X	X	X	X	X				
Centrifuge release	X	X		X			X	X	X		
Container leak/spill/open	X	X		X			X	X	X		
Contaminated waste (e.g., not inactivated)	X	X	X	X	X	X	X	X	X		
Contamination inside laboratory	X	X		X			X	X	X		
Contamination outside laboratory			X		X	X	X	X	X		
Deflagration	X	X	X	X			X				
Fire	X	X	X	X	X	X	X				
Flooding inside laboratory	X	X		X					X		
Fomite/vector	X	X		X					X	X	X
Hand to mouth/eyes/nose contamination	X	X		X				X	X		
HVAC[a] failure	X	X	X	X	X	X	X				
Inadequate animal control	X	X		X							X
Inadequate pathogen accountability	X	X		X			X	X	X		
Inadequate PPE[b] use	X	X		X			X	X	X	X	X
Liquid waste leak	X	X	X	X	X	X		X	X		
Loss of power	X	X	X	X	X	X	X				
Malevolent act	X	X	X	X	X	X	X	X	X	X	X
NPH—earthquake	X	X	X	X	X	X	X	X	X	X	X
NPH[c]—tornado and strong wind	X	X	X	X	X	X	X				
NPH—other (flooding, snow, etc.)	X	X	X	X	X	X	X				
PAPR[d] failure (addressed separately from other PPE)	X	X		X			X				
Pathogen not inactivated	X	X	X	X	X		X	X	X	X	
Pathogen used with inappropriate biocontainment	X	X		X			X	X	X	X	
PPE failure (excluding PAPR[c])	X	X		X			X	X	X	X	
Puncture—during necropsy	X	X		X						X	
Puncture—needlestick	X	X		X						X	
Puncture—general	X	X		X						X	
Spill/splash	X	X		X			X	X	X		
Transportation mishap			X		X	X	X	X	X	X	

2 a Heating, ventilating, and air conditioning system (HVAC)

3 b Personal protective equipment (PPE). Powered air-purifying respirator (PAPR) are PPE, but PAPR are addressed separately
4 here because of their unique role in the BSL-3 centrifuge release scenarios.

5 c Natural phenomena hazards

6 d PAPR are addressed separately because of their significance in the BSL-3 centrifuge release scenarios.

4.1.3.2 Evaluation of Candidate Events

The next step in the selection process was to assign frequency and exposure categories (see Sections 4.1.2.2.4 and 4.1.2.2.5) to each candidate event. Table 4-5 provides the results of that categorization of candidate event groups. A dash is shown for cells where the exposure category is not appropriate for an exposed group. For example, the public is not at risk of a direct primary exposure from a needlestick event. The assignment of frequency and exposure categories were assigned on the basis of the operational incidents described in Appendix D with the selection of the next higher category in cases where there was a question of which of two categories to use. The assignments were also reviewed by the Tetra Tech team and reviewers from the NIH and BRP. Adjustments to the category assignments were made in consideration of these reviews. As the detailed analyses were being completed, the assignments were again reviewed and adjusted where appropriate.

Table 4-5. Event and exposure categories for candidate events

Candidate event group	Frequency category	Exposure category		
		Laboratory worker	Facility worker	Public
Aircraft crash	C	High	High	Moderate
Animal bite/scratch	A	Low	--[a]	--
Animal escape or pathogen release to environment	B	Moderate	Low	Low
Animal-related infectious aerosol	B	Moderate	--	--
Breach of containment (wall cracks or open doors)	B	Low	Low	Low
Centrifuge release	A	Moderate	--	--
Container leak/spill/open	A	Moderate	--	--
Contaminated waste (e.g., not inactivated)	B	Moderate	Low	Low
Contamination inside laboratory	B	Moderate	--	--
Contamination outside laboratory	B	-	Low	Low
Deflagration	C	High	Moderate	Low
Fire	C	High	Low	Low
Flooding inside laboratory	C	Moderate	Low	--
Fomite/vector	B	Moderate	Low	Low
Hand to mouth/eyes/nose contamination	B	Low	--	--
HVAC[b] system failure	B	Moderate	Low	Low
Inadequate animal control	B	Moderate	--	--
Inadequate pathogen accountability	A	Moderate	Low	Low
Inadequate PPE[c] use	A	Low	--	--
Liquid waste leak	B	Moderate	Low	Low
Loss of power	B	Moderate	Low	Low
Malevalent act	not defined	High	High	Moderate

Candidate event group	Frequency category	Exposure category		
		Laboratory worker	Facility worker	Public
NPH[d]—earthquake	C	High	High	Moderate
NPH—tornado and strong wind	C	High	Low	Low
NPH—other (flood, snow, etc.)	C	High	Low	Low
PAPR[e] failure (addressed separately from other PPE)	A	Low	--	--
Pathogen not inactivated	A	Moderate	Low	--
Pathogen used with inappropriate biocontainment	A	Moderate	--	--
PPE failure (excluding PAPR[c])	A	Low	--	--
Puncture—during necropsy	A	Low	--	--
Puncture—needlestick	A	Low	--	--
Punctured—general	A	Low	--	--
Spill/splash	A	Moderate	--	--
Transportation mishap	C	--	Low	Low

a -- indicates that the cell is not applicable.
b Heating, ventilating, and air conditioning system (HVAC)
c Personal protective equipment (PPE). Powered air-purifying respirator (PAPR) are PPE, but PAPR are addressed separately here because of their unique role in the BSL-3 centrifuge release scenarios.
d Natural phenomena hazards
e PAPR are addressed separately because of their significance in the BSL-3 centrifuge release scenarios.

4.1.3.3 Events Selected for Analysis

4.1.3.3.1 Maximum Reasonably Foreseeable (MRF) Event

The MRF event selected for analysis was a severe earthquake. A severe earthquake was selected for the following reasons:

- Has the potential to affect the entire facility inventory of pathogens;
- Has the potential to compromise all biocontainment features;
- Can occur under any meteorological conditions;
- Results in the potential for higher airborne concentrations than tornadoes and hurricanes, which result in much greater mixing, thereby resulting in much lower concentrations;
- Is less likely to have advanced warning than tornadoes or hurricanes;
- Has the potential for ground level unfiltered release as opposed to the filtered exhaust stack releases;
- Can result in escape of animals (mammals and arthropods); and
- Typically *bounds* (i.e., is at least as severe as) other natural phenomena events (DOE-HDBK-3010).

Because of the importance of the MRF event, an evaluation of an aircraft crash was performed to confirm the expectation that the severe earthquake bounds (i.e., has consequences and frequencies that are not exceeded by) an aircraft crash. Appendix F presents this comparison and demonstrates that the severe earthquake bounds an aircraft crash in terms of both frequency and consequences.

Malevolent acts were not considered in the selection of the MRF event, "because the potential number of scenarios is limitless and the likelihood of attack is unknowable" (DOE 2002). As recommended by the DOE NEPA Guidance, malevolent acts were evaluated by comparison to accidents with similar consequences (see Chapter 6).

Therefore, a severe earthquake was selected as the MRF event for BSL-3, BSL-4, and other areas.

4.1.3.3.2 Representative Events

A subset was selected for analysis that represents or bounds a large number of events. This process consisted of selecting candidate events were qualitatively assessed to represent or bound the frequency and exposure category for each potentially exposed group. Table 4-6 provides the results of this process with the selected events in bold at the top of each subsection and the events considered adequately addressed by the selected event indented in normal font.

Table 4-6. Events selected for analysis

Candidate event group	Frequency category	Exposure category for potentially exposed groups		
		Laboratory worker	Facility worker	Public
Centrifuge release	A	Moderate	Low	-[e]
Container leak/spill/open	A	Moderate	--	--
Spill/splash	A	Moderate	--	--
Inadequate pathogen accountability	A	Moderate	Low	Low
Pathogen not inactivated	A	Moderate	Low	--
Pathogen used with inappropriate biocontainment	A	Moderate	--	--
Inadequate PPE[a] use	A	Low	--	--
PAPR[b] failure (addressed separately from other PPE)	A	Low	--	--
PPE failure (excluding PAPR)	A	Low	--	--
HVAC[c] failure	B	Moderate	Low	Low
Animal--related infectious aerosol	B	Moderate	--	--
Breach of containment (wall cracks or open doors)	B	Moderate	Low	Low
Hand to mouth/eyes/nose contamination	B	Moderate	--	--
Contamination inside laboratory	B	Moderate	--	--
Loss of power	B	Moderate	Low	Low
Liquid waste leak	B	Moderate	Low	Low
Puncture—needlestick	A	Low	--	--
Animal bite/scratch	A	Low	--	--
Puncture—during necropsy	A	Low	--	--
Punctured—general	A	Low	--	--
NPH[d]—earthquake	C	High	High	Moderate
Aircraft crash	C	High	High	Moderate
Fire	C	High	Low	Low
Deflagration	C	High	Moderate	Low
NPH—tornado and strong wind	C	High	Low	Low
NPH—other (flooding, snow, etc.)	C	High	Low	Low
Flooding inside laboratory	C	Moderate	Low	--
Contaminated waste not inactivated	B	Moderate	Low	Low
Contamination outside laboratory	B	--	Low	Low
Animal escape or pathogen release to environment	B	Moderate	Low	Low
Inadequate animal control	B	Moderate	Low	Low
Fomite/vector release	B	Moderate	Low	Low
Transportation mishap	C	--	Low	Low
Malevolent act	not defined	High	Moderate	Moderate

a Personal protective equipment (PPE). Powered air-purifying respirator (PAPR) are PPE, but PAPR are addressed separately here because of their unique role in the BSL-3 centrifuge release scenarios.
b PAPR are addressed separately because of their significance in the BSL-3 centrifuge release scenarios.
c Heating, ventilating, and air conditioning system (HVAC)
d Natural phenomena hazards
e -- indicates that the cell is not applicable.

Table 4-7 provides a brief textual rationale for the selection of the events being carried forward for further analysis and dismissal of others. The events selected tended to achieve the following: (1) highest

frequency, (2) highest exposure category for each exposed group, and (3) events that could incorporate or bound other events.

Table 4-7. Rationale for event selection

Candidate event group	Discussion
Centrifuge release	This event was selected for analysis because it has occurred relatively frequently in research laboratories and results in one of the higher airborne aerosol concentrations for relevant events (Bennett and Parks 2006). A PAPR failure and HVAC failure were considered as part of this scenario.
Container leak/spill/open	The centrifuge event has the potential for a larger release (Bennett and Parks 2006) and has a similar frequency.
Spill/splash	The centrifuge event has the potential for a larger release (Bennett and Parks 2006) and has a similar frequency.
Inadequate pathogen accountability	A review of incidents at other facilities shows that although there have been several incidents, they seldom have resulted in exposures or infections. Therefore, the risk from the centrifuge release and needlestick events is at least as great as the risk of this event.
Pathogen not inactivated	The concern here is that the pathogen is used with inappropriate biocontainment features because it was incorrectly thought to be inactivated. In terms of exposure, the effect operating in a lower BSL level facility can be similar to a failure of biocontainment features. The centrifuge release includes consideration of multiple biocontainment failures and is expected to be in the same frequency category.
Pathogen used with inappropriate biocontainment	The absence of a biocontainment feature has an effect similar to a failure of the same biocontainment feature. The centrifuge release includes consideration of multiple biocontainment failures and is expected to be in the same frequency category.
Inadequate PPE use	The inadequate use of PPE is similar to the failure of the PPE; however, both generally require a loss of biocontainment in order to result in an exposure. For example, failure to wear mesh gloves when changing knife blades can only result in exposure if there is a mishap when changing the blade. The centrifuge release and needlestick events include consideration of some PPE failures.
PAPR failure (addressed separately from other PPE)	This is included as part of the centrifuge release event.
PPE failure (excluding PAPR)	The centrifuge release and needlestick events include consideration of some PPE failures.
HVAC failure	HVAC failure will not result in exposure by itself and a concurrent release is require. Animals or incidents could produce aerosols in the room, but the release is unlikely to be spread to other parts of the building because of the HVAC isolation dampers. Alarms would notify workers of the HVAC failure and would have been trained to take appropriate action. The centrifuge release includes consideration of HVAC failure, so there is a concurrent release.
Animal-related aerosol	The centrifuge release is expected to result in comparable or greater aerosol release and it can occur in non-animal laboratories. While animals can be a continuous source of aerosol generation, the HVAC in the animal holding areas is designed with about double the air exchange rate to purge these release more rapidly and dilute the release. Therefore, the centrifuge event is selected.
Breach of containment (wall cracks or open doors)	This condition does not result in potential exposure without an initial release. The centrifuge release event includes loss of HVAC.

Candidate event group	Discussion
Hand to mouth/eyes/ nose contamination	The potential for ingestion and direct contact exposure are included in the centrifuge release event.
Contamination inside laboratory	The centrifuge release has the potential to contaminate the laboratory and the potential for ingestion and direct contact exposure are included in the centrifuge release event.
Loss of power	By itself, total loss of power could result in a loss of biological safety cabinet (BSC) flow and a release to a room; however, it is improbable that it would result in significant exposure outside the room because the isolation dampers and limited-leakage rooms would retard release. The centrifuge release includes consideration of HVAC failure.
Liquid waste leak	Liquid leaks inside the laboratory are readily detected and do not pose as great a risk as an aerosol release. Contaminated liquid released to the sewage system from the facility would be diluted to extremely low concentrations and would likely be inactivated.
Puncture – needlestick	This event was selected for analysis.
Animal bite/scratch	The needlestick has the potential for a greater level of exposure and is at least as frequent.
Puncture—during necropsy	The needlestick has the potential for a greater level of exposure and is at least as frequent.
Punctured—general	The needlestick has the potential for a greater level of exposure and is at least as frequent.
NPH – earthquake	This event was selected for analysis.
Aircraft crash	Both frequency and consequences are expected to be no greater than those of the earthquake. However, because this is a topic of special interest following the 9/11 attack, this event will be considered further to confirm the expectations.
Fire	The temperatures associated with a fire will tend to inactivate a release, and the consequences will be less than those of the earthquake.
Deflagration	A deflagration is unlikely to fail the walls or compromise as many biocontainment features as an earthquake.
NPH—strong wind	A strong wind is unlikely to fail the walls or compromise as many biocontainment features as an earthquake.
NPH—other	Other NPH (e.g., snow and flooding) are unlikely to fail the walls or compromise as many biocontainment features as an earthquake.
Flooding inside laboratory	Flooding inside the laboratory does not have the potential for public exposure.
Contaminated waste	The earthquake has the potential to spread contamination to more people, though likely at a lower level of exposure. In addition, solid waste is double bagged, so any internal contamination is not likely to result in exposure.
Contamination outside laboratory	The earthquake includes the potential for public exposure and is likely to affect more people.
Animal escape or pathogen release to environment	This candidate was selected for analysis
Inadequate animal control	The centrifuge release considers inhalation, ingestion, and direct contact potential; needlestick considers puncture potential; and animal escape a loss to the environment. Therefore, analysis of this event is not likely to provide additional insights.
Transportation mishap	This event was selected for analysis.
Malevolent act	This event was selected for analysis.

1

Note: It has been noted that fomites (i.e., inanimate objects or substances capable of carrying infectious pathogens) were identified as one of the more significant risks for the National Bio and Agro-Defense Facility (NBAF) (DHS 2008) but fomites were not selected as one of the events analyzed in detail for the NEIDL. Fomites are considered a lesser risk for NEIDL than for NBAF for several reasons.

BSL-3 and BSL-4 biocontainment precautions for NBAF and NEIDL are highly similar. However, much of NBAF research involves BSL-3Ag (agricultural) biocontainment precautions, which pertain to agricultural pathogens. BSL-3Ag precautions are used when infected animals cannot be readily housed in a primary biocontainment device, as in the case of very large animals such as swine, cattle, and horses. In this circumstance, it is the room itself that provides primary biocontainment of the pathogen. Much of NBAF research will involve foot and mouth disease virus (FMDV). Cattle and swine that are infected with FMDV shed enormous amounts of the virus into room air during the course of the infection (as much as $10^{8.6}$ $TCID_{50}$ in a 24 hour period) (Donaldson 2002). As a result, all objects and personnel in the room routinely become contaminated with the virus. Moreover, FMDV has significant stability under favorable ambient conditions, as shown by airborne infections that have occurred as far as 250 km downwind from a release point (Kitching 2005). Furthermore, FMDV is highly infectious for susceptible animal species. As a result, the risk of an FMDV release via fomite became a leading event for consideration in the NBAF risk assessment.

In contrast, BSL-3 work at NEIDL will use primary containment devices, such as bioaerosol containment caging systems, for infected animals. NEIDL will not use BSL-3Ag biocontainment precautions. As a result, pathogens routinely are contained within the primary device rather than being released into room air. Accordingly, the potential for fomite contamination in laboratory rooms at the NEIDL is not comparable to that of NBAF BSL-3Ag facilities. Therefore, although fomites were considered in this RA for the NEIDL, they were not selected for detailed analysis.

4.1.3.3.3 Unique Events

Potential exposure of the laboratory workers, facility workers, and the public was analyzed for the MRF event and representative events for each of the 13 pathogens at each of the three sites. The earthquake and representative event analyses provide a basis for examination of both pathogen and site differences from direct exposure and human-to-human exposure (initial infection is addressed in Chapter 8, and secondary transmission is addressed in Chapter 9); however, those analyses do not address the potential persistence of a pathogen in the environment as a result of an animal escape or a pathogen release. Therefore, each of the 13 pathogens was evaluated in Chapter 7 to determine if it possibly could persist or replicate in air,

soil, water, or in arthropods or other animal species in the local environments outside the biocontainment space.

4.2 Analysis of Selected Events

4.2.1 Introduction

This section provides the analysis of potential loss of biocontainment events selected in Section 4.1 for analysis. Scenarios that account for NEIDL-specific biocontainment features were developed for the potential initiating events selected for analysis. The potential initiating events addressed in this section are as follows:

- *Centrifuge release*—The centrifuge release event provides representative results for aerosol release events.
- *Needlestick*—The needlestick event provides representative results for puncture events.
- *Earthquake*—An earthquake was selected as the MRF event. To investigate the severity of the MRF earthquake, an earthquake that is slightly beyond the design basis was also analyzed.
- *Aircraft crash*—The aircraft crash was expected to pose less risk than the earthquake, and this analysis confirms that expectation.
- *Malevolent acts*—Malevolent act scenarios are identified in Chapter 6.

In addition, transportation events are addressed in Chapter 5, and the potential for environmental persistence is addressed in Chapter 7.

The analysis has been conducted in accordance with the DOE NEPA Guidance discussed in Section 4.1.1. A key element of the DOE NEPA Guidance for the accident analyses is the application of a *sliding scale*. The sliding scale allows for adjustment of the level of detail of an accident analysis in accordance with the frequency and consequences of the accident and the level of information available. While realism is important, the DOE NEPA Guidance also supports use of *bounding* (i.e., analyses based on conservative assumptions that envelope potential factors) when its use is consistent with the sliding scale approach. Bounding approaches can have several potential benefits including streamlining the analysis and potentially being more defensible than more rigorous approaches because they are unlikely to underestimate potential accident consequences (DOE 2002).

The analysis in this section summarizes the detailed information presented in Appendix F. That appendix describes the additional analyses that were performed to develop inputs to determine the potential impacts

from the postulated events that could result in a loss of biocontainment. The input analyses included the following:

- *Biocontainment features*—The frequency and consequence of a potential loss of biocontainment event is dependent on the biocontainment features.
- *Inventory*—The pathogen and animal inventories are a required input for all loss of biocontainment analyses.
- *Airborne dispersion analysis*- —An analysis of the airborne dispersion is required for all potential infectious aerosol release events (e.g., an earthquake).
- *Population estimates*—Population estimates are required to determine the population consequences for all potential infectious aerosol release events (e.g., an earthquake).

Appendix F also discusses the MELCOR Accident Consequence Code System, Version 2 (MACCS2), POSTMAX, and SECPOP 2000 computer codes that were used in the analysis. MACCS2 is a DOE/Nuclear Regulatory Commission-sponsored code that has been used widely in support of probabilistic RAs (PRAs) for the nuclear power industry and for consequence analyses for safety documentation throughout the DOE complex. The MACCS2 module used performs all the calculations pertaining to atmospheric transport, dispersion, and deposition. POSTMAX is a code developed at Los Alamos National Laboratory to facilitate calculation of site-specific consequence metrics from MACCS2 output files. The SECPOP 2000 code formats U.S. Census Bureau population data to be consistent with the MACCS2 code. The codes and the parameters used in the analysis are explained in Appendix F.

4.2.2 Centrifuge Events

4.2.2.1 Introduction

Centrifuges use centrifugal force to separate mixtures of materials having differing densities and will be used in the NEIDL to concentrate pathogen particles in suspensions. A pathogen aerosol release resulting from a centrifuge-related event sequence has been selected as a representative event sequence to be analyzed for LAIs. A centrifuge-related initiating event was selected because it is one of the more frequent sources of infectious aerosol releases in the laboratory setting and infectious aerosol releases pose a threat to the laboratory workers, as demonstrated by Appendix D, which reports several centrifuge incidents.

4.2.2.2 Methodology

This analysis focuses on centrifuge operations and potential exposures to a pathogen (or pathogens) that could result. Operations associated with centrifuges have the potential to expose workers as a result of

infectious aerosol formation and release. The aerosol has the potential of exposing laboratory workers via inhalation to the lungs, direct contact with mucous membranes and eyes, and ingestion via an open mouth while breathing or speaking.

The laboratory worker exposure is dependent on the aerosol concentration in the room, which was based on experimental data for this type of event (Bennett and Parks 2006), and the effectiveness of the powered air-purifying respirator (PAPR) at removing the aerosol. The laboratory worker and public exposures are dependent on the quantity of the aerosol released and the effectiveness of the HVAC system.

4.2.2.3 Results

This section presents the results of the analyses for the BSL-3 event sequence. There are a number of biocontainment features that prevent and/or mitigate the consequences of this event sequence (for a description of several biocontainment features, see Section F.2 of Appendix F). Modern centrifuges have safety features that minimize the risk of an aerosol release. These features could include imbalance detection and shutdown circuitry, certified aerosol-tight rotor/bucket seals, incorporation of a fluid containment annulus in the rotor, HEPA-filtered air evacuation systems, and automatic rotor identification systems that prevent operation at speeds beyond the recommendations for the rotor/bucket. Other biocontainment features important to this scenario include respiratory protection (i.e., PAPR for BSL-3) and the HVAC system. The role of each biocontainment feature for this event sequence is shown in Table 4-8. Section F.2 in Appendix F provides a description of the biocontainment features.

Table 4-8. Exposed group protected by each biocontainment feature—BSL-3 centrifuge infectious aerosol release with full respiratory protection

Exposed group	Admin. controls		Safety equipment							Facility	
	Procedures	Training	Container[e]	Rotor/buckets[e]	Centrifuge chamber[a]	BSC[f]	PPE[g]	Respiratory protection	Positive-pressure suit	HVA[h]C system	Sealed walls, ceilings, and floors
Laboratory worker	M[b]/P[c]	M/P	M[e]	M/P	--[d]	---	--	M	--	M	--
Facility worker	P	P	M	M/P	--	--	--	--	--	M	M
Public	P	P	M	M/P	--	--	--	--	--	M	M

a Centrifuge chambers are not necessarily aerosol-tight and could provide only partial mitigation even when closed. Centrifuge lids provide no mitigation when open, so this is a minor mitigating effect and, therefore, not considered in this evaluation.

b M – signifies a mitigative feature, which reduces the consequences

c P – signifies a preventive feature, which reduces the likelihood

d This biocontainment feature is either not relevant for this event sequence or it does not have a preventive or mitigative role for this exposed group.

e The cells with cross-hatching indicate a biocontainment feature that is assumed to fail or have partial performance for this event sequence.

f Biological safety cabinet (BSC)

g Personal protective equipment (PPE). Powered air-purifying respirators (PAPR) are PPE, but PAPR are addressed separately here because of their unique role in the BSL-3 centrifuge release scenarios.

h Powered air-purifying respirator

The postulated event sequences analyzed involve an undetected/unreported release because a detected and reported event has very limited potential to result in secondary transmission to the public due to medical intervention. Centrifuge release events are analyzed for the BSL-3 laboratories with laboratory workers receiving both full respiratory protection and partial respiratory protection, as well as centrifuge events in BSL-4 laboratories. The results of those scenarios are addressed below.

4.2.2.3.1 BSL-3 Centrifuge Infectious Aerosol Release with Full Respiratory Protection

Frequency Category. This event sequence requires three distinct events or conditions to occur: (1) a pathogen leakage into the rotor; (2) failure of the aerosol-tight rotor seal to contain the aerosol; and (3) a failure to detect or report the incident. If those events or conditions were all independent, the event sequence would be considered to be in the frequency category B (1 in 100 to 10,000 years). However, because the laboratory worker plays a significant role in the prevention and identification of this event sequence, this sequence is conservatively assigned to frequency category A (1 in 1 to 100 years).

Exposure Category and Exposures. It is assumed that one to four laboratory workers are in the room at the time of the release, and they could all be exposed to the aerosolized pathogen.

Laboratory worker. The laboratory workers could be exposed via various routes, but the analysis focused on the inhalation route as the surrogate for all other routes because it is expected to be the most likely and result in the highest exposure. All laboratory workers in the room (i.e., from one to four) could be exposed to the aerosolized pathogen, so the exposure category is MODERATE.

Facility worker. No reasonable mechanism for exposure of facility worker was identified and the facility worker exposure category for a centrifuge aerosol release event is NONE.

Public. Any aerosolized release from a BSL-3 centrifuge would be drawn into the HVAC system, diluted with air from other portions of the facility (i.e., non-contaminated) and HEPA-filtered before discharge. The BSL-3 HEPA filter is at least 99.97 percent efficient at removing airborne particles 0.3µm in diameter (NIH 2008), with higher efficiencies for all other particle sizes, thereby removing nearly all the aerosol particles. The HEPA-filtered air from the HVAC system is ultimately discharged through the stack, where any particles not filtered out will undergo atmospheric dispersion. Atmospheric conditions (e.g., sunlight) will inactivate some infectious particles over time (Bozzette 2011), but that inactivation is conservatively ignored for this analysis. As a result of the dilution, filtration, and dispersion, the public exposure category for a centrifuge aerosol release event is NONE.

Table 4-9 summarizes the results of the analysis of a BSL-3 centrifuge infectious aerosol release with full respiratory protection. The inhalation exposures are assumed to also be applicable for direct contact and ingestion exposure to laboratory workers because inhalation exposures are expected to exceed exposures from the other routes and the data necessary to estimate the exposure is not available. The risks associated with centrifuge events would be the same for the three sites being evaluated (i.e., urban, suburban, and rural).

Table 4-9. Summary of results—BSL-3 centrifuge infectious aerosol release with full respiratory protection

Frequency category	Exposed group: category	Route of exposure	Pathogen[a]	Exposure range[b]
A (1 in 1 to 100 years)	Laboratory workers: MODERATE (1-4)	Direct contact Ingestion Inhalation	*B. anthracis*[c]	0–2 CFU
			F. tularensis	0–2 CFU
			Y. pestis	0–0.09 CFU
			1918 H1N1V	0–0.9 PFU
			SARS-CoV	0–0.09 PFU
			RVFV	0–0.9 PFU[d] 0–9 $CCID_{50}$[d]
			ANDV	0–0.009 $CCID_{50}$
	Facility worker: NONE (0)	--[e]	--	--
	Public: NONE (0)	--	--	--

a *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b Exposures are given in terms of colony forming units (CFU) for bacteria. For viruses, exposures are given in terms of plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$).

c Spores in a liquid suspension

d Two values are reported for RVFV using different units as reported in the literature. The $CCID_{50}$ value is an order of magnitude greater because this measurement is more sensitive that the PFU measurement. The units of $MICLD_{50}$ also apply to the $CCID_{50}$ value.

e -- indicates that the cell is not applicable.

4.2.2.3.2 BSL-3 Centrifuge Infectious Aerosol Release with Partial Respiratory Protection

This scenario is similar to the one addressed in the preceding section, with the exception that it includes the potential for one laboratory worker to have a PAPR operating with only partial efficiency. The PAPR efficiency can be reduced by such conditions as a cracked filter housing, defective filter, filter installation error, or a breached filter. A two order of magnitude reduction in the filtration efficiency (i.e., the aerosol passing through the filter increases from 0.1 to 10 percent) is assumed, which is considered to be a very conservative estimate of the reduction in respiratory protection (i.e., overstates the risk).

Frequency Category. The frequency for this scenario is reduced by one category from the previous scenario to account for the likelihood that a reduced PAPR efficiency occurs coincident with the centrifuge aerosol release. A one-category reduction in the frequency (i.e., from category A to B) is considered appropriate because a two order of magnitude reduction in PAPR efficiency is an extreme reduction in efficiency that is unlikely to occur without detection. Therefore, this sequence is conservatively assigned to frequency category B (1 in 100 to 10,000 years). (Note: the results of

Appendix K show that this potential reduction in PAPR effectiveness does not significantly affect worker risk.)

Exposure Category and Exposures. The only difference between this scenario and the previous scenario is that one of the laboratory workers in the room has a PAPR with reduced respiratory protection. Table 4-10 summarizes the results of the analysis of a BSL-3 centrifuge aerosol release with partial respiratory protection. The risks associated with the centrifuge events would be the same for the three sites being evaluated (i.e., urban, suburban, and rural).

Table 4-10. Laboratory worker exposures—BSL-3 centrifuge aerosol release with partial respiratory protection

Frequency Category	Exposed group: category	Route of exposure	Pathogen[a]	Exposure range[b]
B (1 in 100 to 10,000 years)	Laboratory workers—full respiratory protection: MODERATE (0-3)[c]	• Direct contact • Ingestion • Inhalation	*B. anthracis*[d]	0–2 CFU[d]
			F. tularensis	0–2 CFU
			Y. pestis	0–0.09 CFU
			1918 H1N1V	0–0.9 PFU
			SARS-CoV	0–0.09 PFU
			RVFV	0–0.9 PFU[e] 0–9 $CCID_{50}$[e]
			ANDV	0–0.009 $CCID_{50}$
	Laboratory workers—partial respiratory protection: LOW (1)	• Direct contact • Ingestion • Inhalation	*B. anthracis*[d]	0–200 CFU[d]
			F. tularensis	0–200 CFU
			Y. pestis	0–9 CFU
			1918 H1N1V	0–90 PFU
			SARS-CoV	0–9 PFU
			RVFV	0–90 PFU[e] 0–900 $CCID_{50}$[e]
			ANDV	0–0.9 $CCID_{50}$
	Facility worker: NONE (0)	--[f]	--	--
	Public: NONE (0)	--	--	--

a *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b Exposures are given in terms of colony forming units (CFU) for bacteria. For viruses, exposures are given in terms of plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$).

c Accounts for the one worker with only partial protection.

d Spores in a liquid suspension

e Two values are reported for RVFV using different units as reported in the literature. The $CCID_{50}$ value is an order of magnitude greater because this measurement is more sensitive that the PFU measurement. The units of $MICLD_{50}$ also apply to the $CCID_{50}$ value.

f -- indicates that the cell is not applicable.

4.2.2.3.3 BSL-4 Centrifuge Infectious Aerosol Release

This event sequence is similar to the event sequence analyzed in Section 4.2.2.3.1 with the exception that it occurs in a BSL-4 laboratory rather than in a BSL-3 laboratory. One-piece, totally encapsulating positive-pressure suits are required for NEIDL BSL-4 laboratories, whereas PAPRs are required for NEIDL BSL-3 laboratories. The positive-pressure suits are supplied with air from an external source, so infectious aerosol releases will not result in laboratory worker exposures. Scenarios involving the compromise in respiratory protection from positive-pressure suits were also considered, but no credible scenarios were identified. Therefore, no credible BSL-4 centrifuge release scenarios were identified that result in exposure to laboratory workers. As with the BSL-3 scenario, facility workers and the public are not at risk. Therefore, further analysis is not warranted.

4.2.3 Needlestick Events

4.2.3.1 Introduction

A needlestick is an inadvertent penetration of the skin with by a syringe needle. Needlestick and other puncture events involving sharp objects are some of the more common potential exposure incidents in BSL-3 and BSL-4 laboratories, as discussed in Section F.7.3.2 of Appendix F. If the needle contains any infectious material, a needlestick in a BSL-3 or BSL-4 laboratory could result in an LAI of a laboratory worker. It is reasonable to expect that the majority of the 13 pathogens evaluated could be used to inoculate animals; however, some pathogens are more likely to be used in syringes than others. Wild-type SARS-associated coronavirus (SARS-CoV) and 1918 H1N1 influenza virus (1918H1N1V) are not expected to be used as injectable live inocula. However, it is possible that blood samples could be taken from animals infected with the pathogens using needle and syringe, and a needlestick would be possible in that case. If those viruses are used, the pathogen concentration in the animal blood is taken to be the same as the maximum working concentration of the pathogen. There is also a risk of exposure to SARS-CoV and 1918H1N1V via other sharp objects (such as a scalpel), and a needlestick scenario is used as a surrogate for all sharps exposure.

4.2.3.2 Methodology

The laboratory worker experiencing the needlestick is the only person with the potential to be directly exposed, so facility workers and the public are considered for secondary transmission only (see Appendix L).

Estimating the extent of exposure (e.g., the number of bacteria or virions received) is speculative for needlestick events because it could result in a broad range of pathogen exposures for the laboratory

worker. The extent of exposure depends on such factors as the extent to which the needle penetrates the skin, whether pathogen is present in the needle/syringe, the amount and concentration of pathogen present in the needle/syringe, and whether the syringe plunger is depressed in conjunction with the needlestick. If no pathogen is present, the extent of exposure is zero. Conversely, if a pathogen is present and the syringe plunger is depressed, the exposure could be very large. Instead of speculating on the extent of exposure, it is conservatively assumed that an infectious dose was administered for each needlestick event. That conservatism will result in an overestimate of the number of infections that result from needlestick events.

4.2.3.3 Results

Needlestick events can occur in both BSL-3 and BSL-4 laboratories. Prompt detection and reporting of a needlestick event can maximize the effectiveness of medical intervention for some pathogens; thus, potentially lowering the likelihood that an initial exposure results in infection to the worker and lowering the likelihood of secondary transmissions to other workers or the public. Needlesticks that are promptly detected and reported were analyzed in Appendix F, but they do not pose a significant threat to the public because of NEIDL medical intervention. Medical interventions could include quarantine of the potentially exposed worker if appropriate. Therefore, only those scenarios involving a failure to promptly detect and report the incident are addressed here.

A needlestick scenario with a failure to promptly detect and report the incident could be representative of a worker who does not feel comfortable reporting the injury for fear of reprisal, believes it was a near-miss event that does not require reporting, or does not notice the needlestick, for example. The risks associated with needlestick events would be the same for the three sites being evaluated (i.e., urban, suburban, and rural).

4.2.3.3.1 BSL-3 Needlestick without Prompt Detection and Reporting

Frequency Category. The operational incident data (see Attachment C in Appendix F) identifies some incidents that have occurred at other BSL-3 and BSL-4 facilities and provide insights into the types of incidents that might occur at NEIDL. Needlestick events that are promptly detected and reported frequency category A (1 in 1 to 100 years) Needlestick events in BSL-3 that are not promptly detected and reported are assigned to frequency category B (1 in 100 to 10,000 years). This frequency category assignment is appropriate and even conservative because: (1) the historic estimate is on the boundary of frequency categories A and B, (2) historic values likely overstate the value for current facilities due to enhanced practices, equipment, and facilities (see Section D.1.1 of Appendix D), and (3) the NEIDL is expected to have lower incident rates due to its attention to sharps safety (see Section F.7.2.1 of Appendix

F) and the enhancement of safety (see Section 2.1 of Chapter 2). Section F.7.3.2 of Appendix F provides additional details.

Exposure Category and Exposures. Because this analysis is limited to the point of primary exposure, exposure categories to facility workers and members of the public are not applicable. For laboratory workers, at most one worker will be exposed from a needlestick event. Therefore, an exposure category of LOW is chosen.

Table 4-11 summarize the results for a needlestick in a BSL-3 laboratory, assuming the incident is not promptly detected and reported.

Table 4-11. Summary of results—Needlestick in BSL-3 laboratory without prompt detection and reporting

Frequency category	Exposure group category	Pathogen[a]	Exposure range
B (1 in 100 to 10,000 years)	Laboratory workers: LOW (1)	*B. anthracis*	Infection assumed
		F. tularensis	Infection assumed
		Y. pestis	Infection assumed
		1918 H1N1V[b]	Infection assumed
		SARS-CoV[b]	Infection assumed
		RVFV	Infection assumed
		ANDV	Infection assumed
	Facility worker: NONE (0)	--[c]	--
	Public: NONE (0)	--	--

a *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b SARS-associated coronavirus, and 1918 H1N1 influenza virus are not expected to be used in syringes as wild type inocula but are analyzed here for completeness. Additionally it is assumed that if these viruses are used, then the pathogen concentration in the animal blood concentration is taken to be the same as the maximum working concentration of the pathogen.

c -- indicates that the cell is not applicable.

4.2.3.3.2 BSL-4 Needlestick without Prompt Detection and Reporting

A needlestick incident is also possible in a BSL-4 laboratory. This scenario is the same as the incident addressed in Section 4.2.3.3.1 with the exception that it occurs in a BSL-4 laboratory with BSL-4 pathogens. Table 4-12 summarizes the results for a needlestick in a BSL-4 laboratory assuming the incident is not promptly detected and reported.

Table 4-12. Summary of results—Needlestick in BSL-4 laboratory without prompt detection and reporting

Frequency category	Exposure group category	Pathogen[a]	Exposure range
B (1 in 100 to 10,000 years)	Laboratory workers: LOW (1)	EBOV	Infection assumed
		MARV	Infection assumed
		LASV	Infection assumed
		JUNV	Infection assumed
		TBEV-FE	Infection assumed
		NIPV	Infection assumed
	Facility worker: NONE (0)	--[b]	--
	Public: NONE (0)	--	--

a Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as Tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b -- indicates that the cell is not applicable.

4.2.4 Earthquake Events

4.2.4.1 Introduction

The NEIDL facility was designed and constructed in compliance with strict seismic criteria. Attachment D of Appendix F provides an overview of the relevant design criteria and compliance of the NEIDL design with those criteria. As required by the Massachusetts State Building Code, the effective peak velocity-related acceleration and the effective peak acceleration are each 0.12 g (g is the standard acceleration due to gravity). This 0.12 g peak acceleration cannot be related to a Richter magnitude because the Richter magnitude is a measure of energy involved and the acceleration is affected by factors such as the depth, ground conditions, and focusing. For Seismic Performance Category C, the building structure must stay functional after a seismic event. (Massachusetts 1997). The BSL-4 suites are structurally separated from the adjoining floors. Such a structural separation allows for movement if an earthquake occurs, while maintaining structural integrity of the BSL-4 suites. BSL-4 suites have 12-inch-thick, reinforced concrete walls with special epoxy covering that acts as a sealant. All fixtures for the BSL-4 suite were designed specifically for the facility and are Underwriters Laboratories tested to ensure that the facility retains its air-tightness.

4.2.4.2 Methodology

Two earthquake scenarios were selected for analysis; one was an MRF (total collapse) earthquake, which was selected as the MRF event, and another less severe earthquake referred to as a slightly beyond design basis (BDB) earthquake. The initial conditions for both earthquakes event sequences are as follows:

- There are no warnings of potential seismicity and the facility is operating without forewarning of the earthquake.

- Any or all pathogen(s) could be in use in the facility at its maximum volumes and maximum concentrations in a liquid suspension. The pathogen(s) could be in one or more containers at the time of the earthquake.
- The facility contains infected animals (mammals and arthropods) at the time.

A severe earthquake is postulated to occur that results in the following events and conditions:

- It is assumed that a fire does not result from the earthquake. A fire would inactivate most pathogens and would tend to loft releases over the immediately surrounding population, so this assumption results in the highest potential consequences.
- As a result of building motion or falling debris from the earthquake, an aerosolized pathogen release results from the container(s) of pathogens in liquid suspension. Containers of frozen pathogen suspensions could be breached, but their release would be minimal because they are initially frozen and a large-scale, prompt release is unlikely; therefore, they are dismissed from further consideration for this analysis.
- Per guidance, this event occurs during median meteorological conditions.
- Infected animals (mammals and arthropods) could escape from the facility.

For both accident scenarios, the level of exposure is dependent on the amount of pathogen released and how the pathogen is dispersed. Both the airborne dispersion factors and the number of people are site-specific, and they have been developed using the same radial (or polar) grid with sixteen 22.5°-sectors.

For the earthquake analysis, each annulus is 100 m wide (i.e., outer radius minus inner radius), and the grid extends to a maximum radius of 1 km. While a radius of 1 km (0.6 mile) captures the majority of the impacts, the expected number of infections would be somewhat higher if the calculation were performed for a larger radius.

The maximum radius of 1 km was selected for several reasons including the following:

- The 1-km radius is consistent with U.S. Nuclear Regulatory Commission recommendations for the NEPA environmental justice evaluation of proposed actions in cities (Nuclear Regulatory Commission 2003b).
- The highest density of nonresidents surrounding the urban site is within 0.5 km of NEIDL and is included in the 1-km radius (see Section F.4 of Appendix F).
- There are no high-population communities just beyond 1-km radius that would significantly affect the results at any of the sites (see Section F.4 of Appendix F).

- The average exposure levels are extremely low at 1 km and would be even lower for greater distances. The MRF (total collapse) earthquake analysis (as shown later) estimates the average exposure level to be less than one one-thousandth (1/1,000) of a unit for all but one pathogen and less than one one-hundredth (1/100) of a unit for all pathogens at 1 km. The calculated exposures at even greater distances would result in even lower exposure levels.

After release from the facility, the aerosolized pathogen would be transported and dispersed as a result of winds and meteorological conditions. The computer code and parameters used to estimate the airborne dispersion are consistent with guidance (DOE 2002, DOE 2004a) and are addressed in Section F.5.2 of Appendix F. The input parameters for the analyses were the recommended or conservative values (i.e., overestimate aerosol concentrations). For example, wet deposition the dry deposition and building wake effect parameters were the recommended default values. Conservative parameters include the suppression of wet deposition, buoyant plume rise, building wake effect plume rise, and plume meander. The results of these dispersion analyses were compared with the results of other dispersion analyses and wind tunnel tests for NEIDL and were found to be in good agreement. In the case of the MRF earthquake, the assumption of a ground-level release is very conservative and compensates for uncertainties in the analyses. For example at the urban site, concentrations are over 200 times greater at the 30-m exclusion fence for a ground-level release than for an elevated release (see Table F.5.3-1 of Appendix F). See Section F.5 of Appendix F for additional details.

The methodologies for analysis of both earthquake events are described in the following sections.

4.2.4.2.1 MRF Earthquake

A bounding analysis was performed for the MRF (total collapse) earthquake event sequence. *American National Standard Categorization of Nuclear Facility Structures, Systems, and Components for Seismic Design* (ANSI 2004), provides guidance specifically for analysis of earthquake events. That document is based on the methodology developed by DOE and is intended for facility design purposes; however, this methodology is frequently used in safety analyses. The relevant guidance provided for the impact analysis includes the following recommendations:

1. "The unmitigated consequence analysis shall be performed considering only the inherent physical or chemical characteristics of the hazardous material and the energy sources for dispersing the material."
2. The "…engineered mitigating features shall be assumed not to function unless the robustness of each mitigating feature can be demonstrated to survive the postulated event."

3. "ANSI/ANS-5.10-1998, *Airborne Release Fractions at Non-Reactor Nuclear Facilities* provides guidance concerning mechanisms for release of the hazardous material into the air or water and shall be used to support similar calculations required by this standard." Because the document referred to is based on DOE-HDBK-3010 (DOE 2000) and because DOE-HDBK-3010 is more comprehensive, this analysis uses DOE-HDBK-3010 as its basis.
4. The "…consequence analysis shall strive to use mean values for the parameters related to material release, dispersal in the environment, and health consequences."

In summary, that guidance results in development of bounding scenarios with the use of median factors when analyzing the bounding scenarios. The assumption that engineered mitigation to fail means that the building effectively collapses with no walls or HVAC to mitigate release. This is an extremely severe scenario that is extremely unlikely to occur and whose airborne releases are highly unlikely to be exceeded.

4.2.4.2.2 Beyond Design Basis (BDB) Earthquake

DOE NEPA Guidance stresses the importance of realism in the analysis, and the MRF (total collapse) earthquake is an extremely unlikely event. To put the risks associated with the MRF earthquake into perspective, an earthquake that is less severe and more likely than the MRF event is also analyzed. Earthquakes that are within the seismic design basis of the facility were not analyzed because the NEIDL structures, systems, and components were designed to perform their functions during and following design basis earthquakes, thereby not resulting in a significant loss of biological containment. A BDB (minor damage) earthquake that is more likely than the MRF earthquake but still slightly beyond the design basis was selected for analysis. The BDB earthquake is postulated to result in partially mitigated releases. The NEIDL building is not expected to lose structural integrity until well beyond the design basis, as discussed in Section F.8.3.2 of Appendix F. Therefore, a stack release with some degradation in HEPA filtration is assumed to occur. After release from the stack, the aerosolized pathogen would be transported and dispersed as a result of winds and meteorological conditions.

4.2.4.3 Results

This section documents the analysis results for both the MRF earthquake and the BDB earthquake. The seismic design information available does not distinguish between the BSL-3 and BSL-4 areas when addressing the design criteria or seismic capacity, so there is no basis for distinction of the two events, even though it is expected that the BSL-4 has a higher seismic capacity. Because there is no basis for

distinguishing between the two areas and all biocontainment features are assumed to fail, the event sequence for both BSL-3 and BSL-4 are the same and are addressed together in the following section.

4.2.4.3.1 MRF Earthquake Affecting BSL-3 and BSL-4

Frequency Category. The NEIDL structure was designed to withstand an earthquake with a peak acceleration of 0.12g (g is the acceleration of gravity), per the requirements of the Massachusetts Building Code. The fundamental period of the NEIDL structure is 2 seconds and seismic shaking at the fundamental period has the potential of causing the greatest damage. Based on the U.S. Geological Survey (USGS) seismic hazard maps, the annual exceedance probability for a 2-second 0.12 earthquake is estimated to be 1×10^{-5}, which corresponds to frequency category C (1 in 10,000 to 1 million years). The MRF earthquake is assigned to frequency category C, but this is considered conservative because a significantly more severe, hence less likely, earthquake would be required to result in a total collapse of the NEIDL structure. See Attachment E of Appendix F for details of this frequency category assignment.

Exposure Category. The exposure category for the laboratory workers is HIGH because all people in all rooms have the potential to be exposed. The exposure category for the facility workers is HIGH because all people in the facility have the potential to be exposed. The estimated exposure category for the public is expected to be MODERATE. Aerosolized pathogen particles could be dispersed beyond 300 m, but concentrations would be extremely low beyond 3 km.

Extent of Exposure. The DOE NEPA Guidance (DOE 2002) provides the following guidance:

...in many cases the acceleration forces associated with extremely rare earthquakes (e.g., frequencies of less than 10^{-6} per year) may be so great that destructive impacts unrelated to the proposed action or alternatives would overwhelm impacts associated with the proposed action or alternatives. Such an analysis would not be informative regarding the proposed action or alternatives because a decision maker would be unable to distinguish the consequences resulting from the proposed action or alternatives from the general destructive effects of the earthquake.

That caution is certainly applicable for the MRF earthquake because of the low frequency of the event and the extremely conservative assumptions used for the analyses. However, the exposures are estimated for this event because this scenario provides insight into the maximum biological consequences that could reasonably be expected from operation of the facility and also provide insight into potential site differences.

Laboratory Worker. If a severe earthquake were to result in total structural failure of the NEIDL, it is unlikely that laboratory workers would survive if they were in the BSL-3 or BSL-4 laboratories. Estimation of exposures to workers in a collapsed building provides no insight into worker risk from such an event. Therefore, the laboratory workers are assumed to have escaped the building and congregate at the NEIDL exclusion fence for the duration of the release. In such a case, the laboratory workers are no different than any other facility worker and are included in the following exposure estimate.

Facility Worker. For this analysis, it is assumed that facility workers exit the building promptly and congregate at the NEIDL exclusion fence, which is at least 30 m from the NEIDL. A person at the exclusion fence is defined as the maximally exposed individual (MEI), so the MEI exposure will be used for laboratory workers. The MEI exposure is calculated in the public exposure section below. Approximately 300 workers are present at NEIDL about 25 percent of the year (about 50 weeks at 40 hours per week out of an 8,760 hour year), which results in an average worker population of 75 people for all 16 radial sectors combined. The average population for a single sector would be about five workers. Because of the very low levels of exposure and the small number of workers potentially exposed, a facility worker infection is considered to be in frequency category D (1 in more than 1 million years), which is beyond reasonably foreseeable and is dismissed from further consideration.

Public. Airborne dispersion of the pathogen organisms can result in inhalation, ingestion, and direct contact routes of exposures. Tables 4-13a, 4-13b, and 4-13c present a composite of exposures results to BSL-3 pathogens resulting from a MRF earthquake for the urban, suburban, and rural sites for each annulus. The average RVFV exposure is 0.66 $CCID_{50}$ for people located at the NEIDL exclusion fence for the urban site, which is approximately the same as two people receiving 1 $CCID_{50}$ and one person having no exposure if three people where located at the fenceline. The average exposure at the urban site is 1.6×10^{-3} $CCID_{50}$ for people located 1 km from the release, which approximately the same as one person receiving 1 $CCID_{50}$ and 625person having no exposure. Tables 4-14a, 4-14b, and 4-14c present a composite of exposures results to BSL-4 pathogens resulting from a MRF earthquake for the urban, suburban, and rural sites for each annulus. The average segment population in each of the 16 radial sectors is shown in Table 4-15 for each of the three sites.

Escaped Animals. The NEIDL could contain a number of infected animals (mammals and arthropods) at any time. In the event of a MRF earthquake, it is likely that enclosures would be breached and there is the potential that infected animals (mammals and arthropods) would survive and escape to the environs. There is also the potential for contaminated inanimate objects to be released from the facility. As a result,

there is the potential for some pathogens to become established in the environment. The animals and the pathogen with which they could be infected are identified in Section F.3 of Appendix F. The potential impact of the infected escaped animals is analyzed in Chapter 7.

Table 4-13a. Average public exposures to BSL-3 pathogens resulting from a MRF earthquake for the urban site

Pathogen[a]	Units[b]	Annulus (km)[b]										
		0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
B. anthracis[d]	CFU	0.053	0.029	$3.1x10^{-3}$	$1.1x10^{-3}$	$6.2x10^{-4}$	$4.1x10^{-4}$	$3.0x10^{-4}$	$2.3x10^{-4}$	$1.8x10^{-4}$	$1.5X10^{-4}$	1.2E-04
F. tularensis	CFU	$8.8x10^{-3}$	$4.8x10^{-3}$	$5.2x10^{-4}$	$1.8x10^{-4}$	$1.0x10^{-4}$	$6.8x10^{-5}$	$5.0x10^{-5}$	$3.8x10^{-5}$	$3.0x10^{-5}$	$2.5X10^{-5}$	2.1E-05
Y. pestis	CFU	$4.4x10^{-4}$	$2.4x10^{-4}$	$2.6x10^{-5}$	$9.2x10^{-6}$	$5.2x10^{-6}$	$3.4x10^{-6}$	$2.5x10^{-6}$	$1.9x10^{-6}$	$1.5x10^{-6}$	$1.2X10^{-6}$	1.0E-06
1918H1N1V	PFU	0.066	0.036	$3.9x10^{-3}$	$1.4x10^{-3}$	$7.8x10^{-4}$	$5.1x10^{-4}$	$3.7x10^{-4}$	$2.8x10^{-4}$	$2.3x10^{-4}$	$1.9X10^{-4}$	1.6E-04
SARS-CoV	PFU	$6.6x10^{-3}$	$3.6x10^{-3}$	$3.9x10^{-4}$	$1.4x10^{-4}$	$7.8x10^{-5}$	$5.1x10^{-5}$	$3.7x10^{-5}$	$2.8x10^{-5}$	$2.3x10^{-5}$	$1.9X10^{-5}$	1.6E-05
RVFV[e]	PFU	0.066	0.036	$3.9x10^{-3}$	$1.4x10^{-3}$	$7.8x10^{-4}$	$5.1x10^{-4}$	$3.7x10^{-4}$	$2.8x10^{-4}$	$2.3x10^{-4}$	$1.9X10^{-4}$	1.6E-04
	$CCID_{50}$ or $MICLD_{50}$	0.66	0.36	0.039	0.014	$7.8x10^{-3}$	$5.1x10^{-3}$	$3.7x10^{-3}$	$2.8x10^{-3}$	$2.3x10^{-3}$	$1.9X10^{-3}$	1.6E-03
ANDV	$CCID_{50}$	$6.6x10^{-4}$	$3.6x10^{-4}$	$3.9x10^{-5}$	$1.4x10^{-5}$	$7.8x10^{-6}$	$5.1x10^{-6}$	$3.7x10^{-6}$	$2.8x10^{-6}$	$2.3x10^{-6}$	$1.9X10^{-6}$	1.6E-06

a *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b Exposures are plume centerline values and are given in units of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 of Appendix F provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

d In spore form in liquid suspension.

e Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

Table 4-13b. Average public exposures to BSL-3 pathogens resulting from a MRF earthquake for the suburban site

Pathogen[a]	Units[b]	Annulus (km)[b] 0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
B. anthracis[d]	CFU	0.19	0.10	$8.7x10^{-3}$	$2.7x10^{-3}$	$1.2x10^{-3}$	$6.7x10^{-4}$	$4.9x10^{-4}$	$3.9x10^{-4}$	$3.1x10^{-4}$	$2.4x10^{-4}$	$2.0x10^{-4}$
F. tularensis	CFU	0.032	0.017	$1.5x10^{-3}$	$4.5x10^{-4}$	$2.0x10^{-4}$	$1.1x10^{-4}$	$8.1x10^{-5}$	$6.5x10^{-5}$	$5.2x10^{-5}$	$4.0x10^{-5}$	$3.4x10^{-5}$
Y. pestis	CFU	1.6x10-3	$8.6x10^{-4}$	$7.3x10^{-5}$	$2.3x10^{-5}$	$1.0x10^{-5}$	$5.6x10^{-6}$	$4.0x10^{-6}$	$3.2x10^{-6}$	$2.6x10^{-6}$	$2.0x10^{-6}$	$1.7x10^{-6}$
1918H1N1V	PFU	0.24	0.13	0.011	$3.4x10^{-3}$	$1.5x10^{-3}$	$8.4x10^{-4}$	$6.1x10^{-4}$	$4.8x10^{-4}$	$3.9x10^{-4}$	$3.0x10^{-4}$	$2.5x10^{-4}$
SARS-CoV	PFU	0.024	0.013	$1.1x10^{-3}$	$3.4x10^{-4}$	$1.5x10^{-4}$	$8.4x10^{-5}$	$6.1x10^{-5}$	$4.8x10^{-5}$	$3.9x10^{-5}$	$3.0x10^{-5}$	$2.5x10^{-5}$
RVFV[e]	PFU	0.24	0.13	0.011	$3.4x10^{-3}$	$1.5x10^{-3}$	$8.4x10^{-4}$	$6.1x10^{-4}$	$4.8x10^{-4}$	$3.9x10^{-4}$	$3.0x10^{-4}$	$2.5x10^{-4}$
	$CCID_{50}$ or $MICLD_{50}$	2.4	1.3	0.11	0.034	0.015	$8.4x10^{-3}$	$6.1x10^{-3}$	$4.8x10^{-3}$	$3.9x10^{-3}$	$3.0x10^{-3}$	$2.5x10^{-3}$
ANDV	$CCID_{50}$	2.4x10-3	$1.3x10^{-3}$	$1.1x10^{-4}$	$3.4x10^{-5}$	$1.5x10^{-5}$	$8.4x10^{-6}$	$6.1x10^{-6}$	$4.8x10^{-6}$	$3.9x10^{-6}$	$3.0x10^{-6}$	$2.5x10^{-6}$

a *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b Exposures are plume centerline values and are given in units of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose (CCID50), or median mouse intracerebral lethal dose (MICLD50) for viruses. Section F.3.1.2 of Appendix F provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

d In spore form in liquid suspension.

e Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

Table 4-13c. Average public exposures to BSL-3 pathogens resulting from a MRF earthquake for the rural site

Pathogen[a]	Units[b]	Annulus (km)[b]										
		0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
B. anthracis[d]	CFU	0.25	0.13	0.011	$3.4x10^{-3}$	$1.7x10^{-3}$	$1.1x10^{-3}$	$8.1x10^{-4}$	$6.1x10^{-4}$	$4.9x10^{-4}$	$4.0x10^{-4}$	$3.3x10^{-4}$
F. tularensis	CFU	0.041	0.022	$1.8x10^{-3}$	$5.7x10^{-4}$	$2.8x10^{-4}$	$1.9x10^{-4}$	$1.3x10^{-4}$	$1.0x10^{-4}$	$8.1x10^{-5}$	$6.6x10^{-5}$	$5.5x10^{-5}$
Y. pestis	CFU	$2.1x10^{-3}$	$1.1x10^{-3}$	$9.0x10^{-5}$	$2.8x10^{-5}$	$1.4x10^{-5}$	$9.4x10^{-6}$	$6.7x10^{-6}$	$5.1x10^{-6}$	$4.1x10^{-6}$	$3.3x10^{-6}$	$2.8x10^{-6}$
1918H1N1V	PFU	0.31	0.17	0.013	$4.3x10^{-3}$	$2.1x10^{-3}$	$1.4x10^{-3}$	$1.0x10^{-3}$	$7.7x10^{-4}$	$6.1x10^{-4}$	$5.0x10^{-4}$	$4.1x10^{-4}$
SARS-CoV	PFU	0.031	0.017	$1.3x10^{-3}$	$4.3x10^{-4}$	$2.1x10^{-4}$	$1.4x10^{-4}$	$1.0x10^{-4}$	$7.7x10^{-5}$	$6.1x10^{-5}$	$5.0x10^{-5}$	$4.1x10^{-5}$
RVFV[e]	PFU	0.31	0.17	0.013	$4.3x10^{-3}$	$2.1x10^{-3}$	$1.4x10^{-3}$	$1.0x10^{-3}$	$7.7x10^{-4}$	$6.1x10^{-4}$	$5.0x10^{-4}$	$4.1x10^{-4}$
	$CCID_{50}$ or $MICLD_{50}$	3.1	1.7	0.13	0.043	0.021	0.014	0.010	$7.7x10^{-3}$	$6.1x10^{-3}$	$5.0x10^{-3}$	$4.1x10^{-3}$
ANDV	$CCID_{50}$	$3.1x10^{-3}$	$1.7x10^{-3}$	$1.3x10^{-4}$	$4.3x10^{-5}$	$2.1x10^{-5}$	$1.4x10^{-5}$	$1.0x10^{-5}$	$7.7x10^{-6}$	$6.1x10^{-6}$	$5.0x10^{-6}$	$4.1x10^{-6}$

a *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b Exposures are plume centerline values and are given in units of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 of Appendix F provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

d In spore form in liquid suspension.

e Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

Table 4-14a. Average public exposures to BSL-4 Pathogens resulting from a MRF earthquake for the urban site

Pathogen[a]	Release[b]	Annulus (km)[b] 0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
EBOV	$CCID_{50}$	0.033	0.018	1.9E-03	6.9E-04	3.9E-04	2.6E-04	1.9E-04	1.4E-04	1.1E-04	9.3E-05	7.8E-05
MARV	$CCID_{50}$	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
LASV	$TCID_{50}$ or FFU (PFU)	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
JUNV	PFU	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
TBEV-FE	MID50	0.066	0.036	3.9E-03	1.4E-03	7.8E-04	5.1E-04	3.7E-04	2.8E-04	2.3E-04	1.9E-04	1.6E-04
NIPV	$TCID_{50}$ or PFU	0.013	7.2E-03	7.8E-04	2.8E-04	1.6E-04	1.0E-04	7.4E-05	5.7E-05	4.5E-05	3.7E-05	3.1E-05

a Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as Tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b Exposures are plume centerline values for the midpoint of the segment and are given in units of plaque forming units (PFU), median tissue culture infective dose (TCID50), fluorescent focus units (FFU), median cell culture infective dose (CCID50), or median mouse infective dose (MID50).b Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), or median cell culture infective dose (CCID50). Section F.3.1.2 of Appendix F provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

Table 4-14b. Average public exposures to BSL-4 pathogens resulting from a MRF earthquake for the suburban site

Pathogen[a]	Units[b]	Annulus (km)[b] 0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
EBOV	$CCID_{50}$	1.2E-01	6.4E-02	5.5E-03	1.7E-03	7.6E-04	4.2E-04	3.0E-04	2.4E-04	1.9E-04	1.5E-04	1.3E-04
MARV	$CCID_{50}$	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
LASV	$TCID_{50}$ or FFU (PFU)	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
JUNV	PFU	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
TBEV-FE	MID_{50}	2.4E-01	1.3E-01	1.1E-02	3.4E-03	1.5E-03	8.4E-04	6.1E-04	4.8E-04	3.9E-04	3.0E-04	2.5E-04
NIPV	$TCID_{50}$ or PFU	4.8E-02	2.6E-02	2.2E-03	6.8E-04	3.0E-04	1.7E-04	1.2E-04	9.7E-05	7.8E-05	6.0E-05	5.0E-05

a Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as Tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b Exposures are plume centerline values for the midpoint of the segment and are given in units of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).b Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), or median cell culture infective dose ($CCID_{50}$). Section F.3.1.2 of Appendix F provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

Table 4-14c. Average public exposures to BSL-4 pathogens resulting from a MRF earthquake for the rural site

Pathogen[a]	Units[b]	Annulus (km)[b] 0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
EBOV	$CCID_{50}$	1.5E-01	8.3E-02	6.7E-03	2.1E-03	1.1E-03	7.0E-04	5.0E-04	3.8E-04	3.0E-04	2.5E-04	2.1E-04
MARV	$CCID_{50}$	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
LASV	$TCID_{50}$ or FFU (PFU)	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
JUNV	PFU	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
TBEV-FE	MID_{50}	3.1E-01	1.7E-01	1.3E-02	4.3E-03	2.1E-03	1.4E-03	1.0E-03	7.7E-04	6.1E-04	5.0E-04	4.1E-04
NIPV	$TCID_{50}$ or PFU	6.2E-02	3.3E-02	2.7E-03	8.5E-04	4.3E-04	2.8E-04	2.0E-04	1.5E-04	1.2E-04	9.9E-05	8.3E-05

a Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as Tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b Exposures are plume centerline values for the midpoint of the segment and are given in units of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).b Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), or median cell culture infective dose ($CCID_{50}$). Section F.3.1.2 of Appendix F provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

Table 4-15. Segment-averaged population by annular ring for the three sites

Site	Annulus (km) 0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0	Total
Urban	31	108	196	372	376	178	165	250	310	215	2,201
Suburban	0.1	0.4	4.4	0.9	1.2	12.6	3.2	12.2	4.6	11.1	50.6
Rural	0.03	1.37	0.18	0.25	0.32	0.40	0.47	0.54	1.55	0.69	5.8

4.2.4.3.2 BDB Earthquake Affecting BSL-3 and BSL-4

Frequency Category. The NEIDL structure was designed to withstand an earthquake with a peak acceleration of 0.12g (g is the acceleration of gravity), per the requirements of the Massachusetts Building Code. The fundamental period of the NEIDL structure is 2 seconds and seismic shaking at the fundamental period has the potential of causing the greatest damage. (BUMC 2005) Based on the U.S. Geological Survey (USGS) seismic hazard maps, the annual exceedance probability for a 2-second 0.12 earthquake is estimated to be 1×10^{-5}, which corresponds to frequency category C (1 in 10,000 to 1 million years). See Attachment E of Appendix F for details of this frequency category assignment.

Note: The risks associated with a BDB earthquake are beyond reasonably foreseeable (see Section 8.6.2) and an increase in the frequency category from C (1 in 10,000 to 1 million years) to B (1 in 100 to 10,000 years) would not alter that conclusion.

Exposure Category. The exposure category for the laboratory workers is HIGH because all people in all rooms have the potential to be exposed. The HVAC system, including its dampers, sealed walls, and the room airlocks confine the release and protect laboratory workers from potential expose during their prompt evacuation, so the exposure category for the facility worker is HIGH. The estimated exposure category for the public is expected to be LOW. Dispersion calculations indicate that pathogens could be transported beyond the 30-m exclusion zone, but that are unlikely to spread beyond 300 m. It is possible for pathogen particles to be spread beyond 300 m, but the concentrations would be so low that they would be a minimal risk to health.

Extent of Exposure. Airborne dispersion of the pathogen organisms can result in inhalation, ingestion, and direct contact routes of exposures. Inhalation exposures are calculated for the laboratory worker and the general public because that is the most likely route of exposure and there is very limited dose-response information for the other routes. The extent of exposure was calculated for the laboratory worker, facility worker, and the public. The laboratory worker exposures are site-independent because the same facility design and operations are assumed for all three sites. However, the facility worker and public exposures are site-dependent and are calculated separately for each site.

Laboratory Worker. The laboratory worker's potential exposure to pathogenic airborne aerosols is presented in Section F.8.3.2.4.1 of Appendix F. The laboratory worker exposures resulting from a BDB earthquake are considerably less than the exposures resulting from the centrifuge release event (see Section 4.2.3.3). For example, the highest exposure level is for RVFV where the BDB earthquake

exposure is 0.3 $CCID_{50}$ and the centrifuge release event exposure to RVFV is 0 to 9 $CCID_{50}$ with full respiratory protection, which is as much as a factor of thirty times greater than the exposure from the BDB earthquake. The centrifuge release event is in frequency category A (1 in 1 to 100 years) and the BDB earthquake is in frequency category C (once in 10,000 to 1 million years). The number of infections and secondary transmissions is not calculated for laboratory worker for the following reasons:

- On the basis of the results in Chapter 8 (Health Effects–Initial Exposure) for the centrifuge release, the frequency of a laboratory worker infection caused by a BDB earthquake is expected to be either in or on the cusp of frequency category D (1 in more than 1 million years). Events that are in frequency category D are considered beyond reasonably foreseeable and are dismissed from further consideration.
- The analysis would not provide additional insight into either laboratory worker or public risk because the risk associated with the centrifuge release event is much greater (nominally four orders of magnitude based on the frequency of the release sequence).

For the BSL-4 laboratories, the positive-pressure suits provide clean external air to the BSL-4 laboratory workers so the laboratory workers in the room would not be exposed the aerosolized pathogen released into the room. For an exposure to result, the following must all occur concurrently:

1. A BDB earthquake, which is assigned to frequency category C (1 in 10,000 to 1 million years)
2. A large release of aerosolized pathogen in a room
3. A large breach in a positive-pressure suit in the specific room containing the aerosolized pathogen release, and
4. The laboratory worker with the breached suit remains in the room long enough to receive a significant exposure

The combination of all those events occurring is in frequency category D (1 in more than 1 million years), which is beyond reasonably foreseeable and, therefore, the exposure for the BSL-4 laboratory worker is dismissed from further consideration.

Facility worker. Following an earthquake of this magnitude, it is expected that all facility workers in BSL-3 and BSL-4 laboratories would promptly exit the facility and intuitively move away from the building. Facility workers that would remain in the building would be protected from aerosolized pathogen because the HVAC system, including its dampers, sealed walls, and the room airlocks would confine the release if still operational. The HVAC is assumed to be operational because this maximizes the potential for public exposure, which is the primary focus of this RA.

For this analysis, it is conservatively assumed that facility workers and laboratory workers exit the building promptly and congregate at the NEIDL exclusion fence (at least 30 m from the NEIDL). A person at the exclusion fence is defined as the MEI, so the MEI exposure will be used for facility workers. The MEI exposure is calculated in the public exposure section below. Approximately 300 workers at NEIDL are present about 25 percent of the year (about 50 weeks at 40 hours per week out of an 8,760 hour year), which results in an average worker population of 75 people for all sectors combined. The average population for a single sector would be about five workers. Because of the very low levels of exposure (less than one-millionth of a unit, as shown in the subsequent results for public exposures), a facility worker (and laboratory workers able to exit promptly) infection is considered to be in frequency category D (1 in more than 1 million years), which is beyond reasonably foreseeable and is dismissed from further consideration.

Public Exposure. Following an aerosolized pathogen release inside a laboratory room, the HVAC system would purge aerosol from the room, filter out aerosol particles, and exhaust the air through the stack. Members of the general public could be exposed to aerosol particles that are not filtered out of the building exhaust. Tables 4-16a, 4-16b, and 4-16c present a composite of exposures results to BSL-3 pathogens resulting from a BDB earthquake for the urban, suburban, and rural sites for each annulus. For the BSL-4 laboratories, all stack source term values are much less than 1 unit. This. That means that less than one unit of pathogen would be expected to be released from the BDB earthquake. As a result, the average public exposures would be extremely small, and a significant exposure is in frequency category D (1 in more than 1 million years), which is beyond reasonably foreseeable and is dismissed from further consideration.

Escaped Animals. The NEIDL could contain many infected animals (mammals and arthropods) at any time (see Section F.3.3.4 of Appendix F). The NEIDL structure is only expected to experience minor damage as a result of the BDB earthquake and the enclosures would not be expected to breach, though it is possible that one or more enclosures would be breached. For an animal to escape the facility in the event of an enclosure breach, it would need to escape through interlocking laboratory doors and escape the building through exterior doors or other openings. In an animal were to escape, there is the potential for some pathogens to become established in the environment. The potential for an animal escape is addressed in Chapter 7 (Environmental Persistence of Pathogens).

Table 4-16a. Average public exposures to BSL-3 pathogens resulting from a BDB earthquake for the urban site

Pathogen[a]	Units[b]	Annulus (km)[b]										
		0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
B. anthracis[d]	CFU	4.4E-08	4.4E-08	4.3E-08	4.0E-08	3.4E-08	2.8E-08	2.4E-08	2.1E-08	1.9E-08	1.7E-08	1.6E-08
F. tularensis	CFU	7.3E-09	7.3E-09	7.2E-09	6.7E-09	5.7E-09	4.7E-09	4.0E-09	3.5E-09	3.1E-09	2.9E-09	2.6E-09
Y. pestis	CFU	3.7E-10	3.7E-10	3.6E-10	3.3E-10	2.9E-10	2.3E-10	2.0E-10	1.8E-10	1.6E-10	1.4E-10	1.3E-10
1918H1N1V	PFU	5.5E-08	5.5E-08	5.4E-08	5.0E-08	4.3E-08	3.5E-08	3.0E-08	2.6E-08	2.3E-08	2.2E-08	2.0E-08
SARS-CoV	PFU	5.5E-09	5.5E-09	5.4E-09	5.0E-09	4.3E-09	3.5E-09	3.0E-09	2.6E-09	2.3E-09	2.2E-09	2.0E-09
RVFV[e]	PFU	5.5E-08	5.5E-08	5.4E-08	5.0E-08	4.3E-08	3.5E-08	3.0E-08	2.6E-08	2.3E-08	2.2E-08	2.0E-08
	$CCID_{50}$ or $MICLD_{50}$	5.5E-07	5.5E-07	5.4E-07	5.0E-07	4.3E-07	3.5E-07	3.0E-07	2.6E-07	2.3E-07	2.2E-07	2.0E-07
ANDV	$CCID_{50}$	5.5E-10	5.5E-10	5.4E-10	5.0E-10	4.3E-10	3.5E-10	3.0E-10	2.6E-10	2.3E-10	2.2E-10	2.0E-10

a *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b Exposures are plume centerline values for the midpoint of the segment and are given in units of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Attachment C provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

d In spore form in liquid suspension.

e Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

Table 4-16b. Average public exposures to BSL-3 pathogens resulting from a BDB earthquake for the suburban site

Pathogen[a]	Units[b]	Annulus (km)[b]										
		0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
B. anthracis[d]	CFU	6.6E-08	6.6E-08	6.3E-08	5.7E-08	5.1E-08	4.5E-08	4.1E-08	3.9E-08	3.6E-08	1.9E-08	1.6E-08
F. tularensis	CFU	1.1E-08	1.1E-08	1.0E-08	9.5E-09	8.6E-09	7.6E-09	6.8E-09	6.5E-09	6.0E-09	3.2E-09	2.7E-09
Y. pestis	CFU	5.5E-10	5.5E-10	5.2E-10	4.8E-10	4.3E-10	3.8E-10	3.4E-10	3.2E-10	3.0E-10	1.6E-10	1.3E-10
1918H1N1V	PFU	8.2E-08	8.2E-08	7.8E-08	7.1E-08	6.4E-08	5.7E-08	5.1E-08	4.8E-08	4.5E-08	2.4E-08	2.0E-08
SARS-CoV	PFU	8.2E-09	8.2E-09	7.8E-09	7.1E-09	6.4E-09	5.7E-09	5.1E-09	4.8E-09	4.5E-09	2.4E-09	2.0E-09
RVFV[e]	PFU	8.2E-08	8.2E-08	7.8E-08	7.1E-08	6.4E-08	5.7E-08	5.1E-08	4.8E-08	4.5E-08	2.4E-08	2.0E-08
	$CCID_{50}$ or $MICLD_{50}$	8.2E-07	8.2E-07	7.8E-07	7.1E-07	6.4E-07	5.7E-07	5.1E-07	4.8E-07	4.5E-07	2.4E-07	2.0E-07
ANDV	$CCID_{50}$	8.2E-10	8.2E-10	7.8E-10	7.1E-10	6.4E-10	5.7E-10	5.1E-10	4.8E-10	4.5E-10	2.4E-10	2.0E-10

a *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b Exposures are plume centerline values for the midpoint of the segment and are given in units of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Attachment C provides background information on the methods and units associated with the concentration measurements.

c The maximally exposed individual (MEI) is a person assumed to be at the exclusion fence (i.e., the point of highest exposure) for the duration of the plume travel.

d In spore form in liquid suspension.

e Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

Table 4-16c. Average public exposures to BSL-3 pathogens resulting from a BDB earthquake for the rural site

Pathogen[a]	Units[b]	Annulus (km)[b]										
		0.03 (MEI)[c]	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
B. anthracis[d]	CFU	7.3E-08	7.3E-08	7.1E-08	6.3E-08	5.5E-08	4.9E-08	4.5E-08	4.3E-08	4.2E-08	4.0E-08	3.4E-08
F. tularensis	CFU	1.2E-08	1.2E-08	1.2E-08	1.0E-08	9.1E-09	8.2E-09	7.5E-09	7.2E-09	7.1E-09	6.7E-09	5.7E-09
Y. pestis	CFU	6.1E-10	6.1E-10	5.9E-10	5.2E-10	4.6E-10	4.1E-10	3.8E-10	3.6E-10	3.5E-10	3.3E-10	2.8E-10
1918H1N1V	PFU	9.1E-08	9.1E-08	8.9E-08	7.9E-08	6.8E-08	6.2E-08	5.7E-08	5.4E-08	5.3E-08	5.0E-08	4.2E-08
SARS-CoV	PFU	9.1E-09	9.1E-09	8.9E-09	7.9E-09	6.8E-09	6.2E-09	5.7E-09	5.4E-09	5.3E-09	5.0E-09	4.2E-09
RVFV[e]	PFU	9.1E-08	9.1E-08	8.9E-08	7.9E-08	6.8E-08	6.2E-08	5.7E-08	5.4E-08	5.3E-08	5.0E-08	4.2E-08
	$CCID_{50}$ or $MICLD_{50}$	9.1E-07	9.1E-07	8.9E-07	7.9E-07	6.8E-07	6.2E-07	5.7E-07	5.4E-07	5.3E-07	5.0E-07	4.2E-07
ANDV	$CCID_{50}$	9.1E-10	9.1E-10	8.9E-10	7.9E-10	6.8E-10	6.2E-10	5.7E-10	5.4E-10	5.3E-10	5.0E-10	4.2E-10

a *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b Exposures are plume centerline values for the midpoint of the segment and are given in units of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Attachment C provides background information on the methods and units associated with the concentration measurements.

c In spore form in liquid suspension.

d Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

4.2.5 Aircraft Crash

4.2.5.1 Introduction

An accidental aircraft crash into the NEIDL is a postulated externally initiated accident scenario for the potential release of pathogens from the NEIDL facility to the public. This analysis demonstrates that the risk of an aircraft crash is bounded by the risk of other analyzed accident scenarios, namely the MRF earthquake event, and therefore does not necessitate a detailed analysis. Risk is a function of (1) the frequency of an adverse event, and (2) the consequences of the adverse event. Thus, the frequency and consequence of a postulated aircraft crash scenario will be compared to that of the MRF earthquake event.

This scenario involves an accidental aircraft crashing into the NEIDL facility resulting in an aerosol release of a pathogen and potentially exposing the public. Only the Boston site is analyzed because the large Boston Logan International Airport has many more flights than the municipal airports near the two comparable sites and, therefore, a higher anticipated frequency for such an event. An aircraft crash scenario initiated by a malevolent act is not specifically addressed by this section.

4.2.5.2 Methodology and Results

Frequency. The DOE has detailed guidance for estimating the frequency of an aircraft crash probability at a given location, as opposed to crash frequencies on a per flight basis. The DOE guidance, *Accident Analysis for Aircraft Crash into Hazardous Facilities* (DOE-STD-3014-2006, DOE 2006), was used in this evaluation.

DOE 2006 uses a four-factor formula to estimate the annual aircraft crash frequency at a given location. The four factors are (1) number of aircraft operations; (2) the probability that an aircraft will crash; (3) given a crash, the probability that the aircraft will crash into a one-square-mile (mi^2) area where a facility is; and (4) the size of the facility.

As discussed in Appendix F (Section F.9), the potential crash frequency was calculated to be approximately 6×10^{-5}/yr. The frequency of 6×10^{-5}/yr (return period of approximately 16,700 years) places the aircraft crash into frequency category C (1 in 10,000 to 1 million years). However, this is the frequency of an aircraft crashing into NEIDL, and it does not take into account the conditional probability of conditions that must exist for a pathogen release to occur. For an aircraft crash to result in a pathogen release, the following conditions are necessary:

1. The aircraft crashing must have sufficient energy (speed and mass of a projectile) to penetrate the

building. It is not known which aircraft would be capable of penetrating the walls of the building exterior and the laboratory walls (BSL-3 or BSL-4), but not all are likely to be capable of penetrating both walls. That is especially true of the general aviation flights, which dominate the frequency for non-airport operations, because those flights include small planes.

2. The angle of impact must be sufficiently perpendicular for penetration to result. Impacts at lower angles could result in the aircraft ricocheting off the building or hitting with a grazing blow, without penetrating the interior laboratory spaces.
3. The impact must be at a location that results in a pathogen release. An aircraft impact into administrative areas is not likely to result in a pathogen release. The BSL-3 and BSL-4 areas compose 29 percent of the facility floor space (13 percent BSL-3 and 16 percent BSL-4) (BUMC 2011). The laboratory rooms will contain pathogens in a releasable form (i.e., a liquid suspension) for only a portion of the time. Rooms where pathogens are stored are not in an easily releasable form (i.e., frozen).

On the basis of the conditional probabilities, the frequency of an aircraft crash that results in a pathogen release is judged to be in frequency category C (1 in 10,000 to 1 million years), but it is likely in the low frequency (high return period) end of that category. The MRF earthquake scenario frequency category is also in frequency category C (1 in 10,000 to 1 million years). However, given the multiple extreme conservative assumptions for the calculation of the aircraft crash frequency (e.g., all airport operations are considered take-offs or landings) and the conditional probabilities stated above, it is judged that that aircraft crash frequency is considered comparable to or lower than the MRF earthquake frequency.

Consequences. While a structural evaluation of aircraft crash per the guidance of DOE-STD-3014-2006 has not been performed, the consequences (i.e., the number and extent of potential exposures) to the public are qualitatively judged to be less than that of a MRF earthquake event for the following reasons:

1. The MRF earthquake event assumes total collapse of the NEIDL building [i.e., all available material at risk (MAR) has the potential to be release and appropriate release factors applied to the entire inventory]. An aircraft crashing into the building would likely affect only the immediate portion of the building that is involved at point of impact, and to a lesser degree, the surrounding areas. That means that only a portion of the available MAR has the potential to be released in any given crash. In the unlikely event of a total facility collapse after an aircraft collision (similar to that from the September 11, 2001, terrorist strikes on the World Trade Center and the Pentagon) could affect all the available MAR. In such a case, the amount of MAR affected would be equal to, but not exceed, that assumed in the MRF earthquake event.

2. An aircraft provides a considerable fuel source and an ignition potential when crashed into a building; thus, the potential of a fire exists. A fire would raise the local and surrounding temperatures of the crash site (i.e., areas where MAR is affected), thus likely inactivating the pathogens before or during their release. An inactive pathogen presents no hazard to a public receptor.
3. NEIDL rooms containing pathogens are in the interior of the facility, and there is at least one exterior wall plus one primary containment wall protecting the pathogens. Therefore, an aircraft projectile would have to penetrate two walls to affect the pathogens and result in a potential airborne release. In addition, the BSL-4 area is constructed as a box-within-a-box with interior walls that are more robust than (and seismically independent from) the rest of the building structure.
4. The BSL-3 and BSL-4 laboratories are several stories above ground level, so any release would be an elevated release that would allow dilution as the plume is dispersed, while the MRF earthquake scenario was analyzed on the basis of a ground-level release. Because a ground-level release would tend to result in higher concentrations in the respirable zones/altitudes than an elevated release, the MRF earthquake scenario will tend to bound the aircraft crash scenario.

Therefore, the consequences of an aircraft crash are expected to be less than the consequence estimates for the MRF earthquake.

In summary, both the frequency and consequences of an aircraft crashing would be less than or no greater than the frequency and consequences of the MRF earthquake as analyzed. Therefore, the MRF earthquake analysis is considered bounding for the aircraft crash and further detailed aircraft crash analysis is not deemed to be necessary.

4.3 Summary

The following conclusions are drawn from the analyses reported above.

- Operational data appropriate for informing estimates for the frequency and extent of exposures from events were found to be inadequate. To compensate for inadequate data, frequency categories spanning wide ranges were employed and conservative assumptions (i.e., assumptions that tend to overestimate the frequency or consequences) were made. The overall risk estimates are sensitive to these assumptions, as they contribute significantly to the uncertainty in overall estimates. Better operational data with respect to past and potential accidents would serve to significantly decrease uncertainty for risk assessment of biocontainment laboratories.

- It was determined that undetected exposure of 1-4 laboratory workers from a centrifuge bioaerosol release could credibly occur under BSL-3 laboratory conditions. The amount of exposure estimated from this scenario could result in infection of at least one laboratory worker from any of the BSL-3 pathogens analyzed. Some pathogens are estimated to be much more likely than others to cause infection because of differences in bioaerosol concentration and dose-response.
- Because of the requirement for the use of positive pressure encapsulating suits in BSL-4 laboratories, no credible scenario was found in which a bioaerosol exposure of a laboratory worker to a BSL-4 pathogen would go undetected. A detected exposure may occur, but is not considered a risk to the public because operating procedures would prevent the exposed laboratory worker from interacting with public contacts until risk of transmission is ruled out.
- It was determined that undetected exposure and subsequent infection of a laboratory worker from a needlestick could credibly occur for any BSL-3 or BSL-4 pathogen. It was estimated that, for most pathogens, undetected needlestick infections would occur more frequently than infections resulting from an undetected centrifuge bioaerosol release. However, this comparison could change if the conservative assumptions that were used to compensate for incomplete data were altered.
- Airborne dispersion calculations for the MRF earthquake show that individual members of the public beyond the NEIDL exclusion fence (i.e., at least 30 m from the facility) would receive an average exposure that is smaller than any dose proven to cause infection in humans or animals via inhalation, with the possible exception of RVFV. While this is an extremely severe event that includes the loss of all biocontainment features and results in the maximum credible release amount, the public exposure estimates are still small due to the small quantities of pathogen in the laboratory, the limited potential for release of this inventory, and the dilution of any release in the atmosphere.
- A threat assessment was performed that included a comparison of the three sites (see Chapter 6). The security systems (e.g. electronic systems, personnel, policy, procedure, etc.) are assumed to be the same at each of the three sites, so the threat from any malevolent action(s) was the same at all three sites. Therefore, the analysis provides no basis for discerning any differences in the frequency among the three sites. The consequences may be slightly different for malevolent acts that involve release of pathogens from the facility; however, the analysis determined that any exposures resulting from such releases would be no greater than the exposures from the earthquake event.

4.4 References

ANSI (American National Standards Institute). 2004. American National Standard Categorization of Nuclear Facility Structures, Systems, and Components for Seismic Design, prepared by the American Nuclear Society Standards Committee Working Group, ANS-2.26-2004.

Bennett, A., and S. Parks. 2006. Microbial aerosol generation during laboratory accidents and subsequent risk assessment. *Journal of Applied Microbiology* 100(2006):658-663. ISSN 1364-5072. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2005.02798>. Accessed August 18, 2010.

Bozzette, S. 2011. Final Project Report: Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment, February 28, 2011. Interdisciplinary Health Sciences Advisors, Inc., San Diego, CA.

BUMC (Boston University Medical Center). 2005. NEIDL Seismic Design, file BU_NEIDL01R5 Design Notes 01.doc.pdf.

BUMC (Boston University Medical Center). 2009. Estimated Total Workforce.pdf, Item #16 of BUMC Files Received July 13, 2009.

BUMC (Boston University Medical Center). 2011. Boston University Medical Campus Fact Sheet. <http://www.bumc.bu.edu/FactSheet.html>. Assessed June 7, 2011.

DHS (U.S. Department of Homeland Security) 2008. National Bio And Agro-Defense Facility Final Environmental Impact Statement. http://www.dhs.gov/files/labs/gc_1187734676776.shtm#1 Accessed April 13, 2009.

DOE (U.S. Department of Energy). 1994. DOE STD 3009-1994, Preparation Guide For U.S. Department Of Energy Nonreactor Nuclear Facility Safety Analysis Reports, U.S. Department of Energy, with Change Notices 1, 2, and 3 dated through March 2006. <http://hss.energy.gov/enforce/docs/std/DOE_STD_3009.pdf>.

DOE (U.S. Department of Energy). 2000. DOE Handbook—Airborne Release Fractions/Rates and Respirable Fractions for Nonreactor Nuclear Facilities. DOE-HDBK-3010-94, U.S. Department of Energy. <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/hdbk3010/h3010v1.pdf>. Accessed 09/11/2010. Also, Change Notice 01 dated March 2000 <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/hdbk3010/hdbk301094_cn.pdf>. was reviewed but it does not affect the portions used in this analysis.

DOE (U.S. Department of Energy) 2002. Recommendations For Analyzing Accidents Under The National Environmental Policy Act, U.S. Department of Energy, Office of Environment, Safety and Health, Environment, Safety and Health, Office of NEPA Policy and Compliance July 2002. <http://nepa.energy.gov/nepa_documents/TOOLS/GUIDANCE/Volume2/2-10-greenbook-recommendations.pdf>. Accessed 8/16/2010.

DOE (U.S. Department of Energy). 2006. DOE STD 3014-2006, Accident Analysis for Aircraft Crash into Hazardous Facilities, U.S. Department of Energy. <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/std3014/std3014.pdf>.

Donaldson AI, Alexandersen S. Predicting the spread of foot and mouth disease by airborne virus. Rev Sci Tech Off Int Epiz 21: 569-575; 2002. Available on the internet at <http://bvs1.panaftosa.org.br/local/file/textoc/DonaldsonPredicting2002.pdf > Accessed November 28, 2011.

EPA (U.S. Environmental Protection Agency) 1987. "Technical Guidance for Hazards Analysis—Emergency Planning for Extremely Hazardous Substances," U.S. EPA, FEMA, U.S. DOE, December 1987. Available on the internet at: http://www.epa.gov/oem/docs/chem/tech.pdf . Accessed on June 25, 2010.

Kitching RP, Hutber AM, Thrusfiled MV 2005. A review of foot-and-mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease. Vet J 169: 197-209; 2005 <http://www.sciencedirect.com/science/article/pii/S1090023304001315> Accessed November 28, 2011.

Massachusetts. 1997. Massachusetts State Building Code. 6th ed. 780 CMR 1612.0 Earthquake Loads. <http://www.mass.gov/Eeops/docs/dps/BuildingCode/780016PT4.pdf>. Accessed October 31, 2010.

NASA (National Aeronautics and Space Administration) 2005. Development of Risk Assessment Matrix for NASA Engineering and Safety Center, Kelly D. Moses. Futron Corporation, and Roy W. Malone, Jr. NASA Marshall Space Flight Center, 2005. Available on the web at: http://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20050123548_2005093494.pdf . Accessed 6/4/2010.

NASA (National Aeronautics and Space Administration) 2009 Guidelines for Risk Management, S3001, Revision B, NASA Independent Verification & Verification Program, Effective Date March 25, 2009. Available on the web at: http://www.nasa.gov/centers/ivv/pdf/209213main_S3001.pdf . Accessed 6/15/2010.

NIH (National Institutes of Health). 2008. Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities (DRM), 2008. <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilities DesignPoliciesandGuidelines/DesignRequirementsManualPDF.htm>. Accessed November 2, 2009.

NIH (National Institutes of Health). 2009. NIH Blue Ribbon Panel to Advise on the Risk Assessment for the BU National Emerging Infectious Diseases Laboratories—Teleconference with the National Research Council on Technical Input, presentation on April 7, 2009, slide 21. <http://nihblueribbonpanel-bumc-neidl.od.nih.gov/docs/2009/April/BRP_NRC_Teleconf_April_7.pdf>. Accessed July 27, 2009.

NRC (National Research Council). 2010. *Evaluation of the Health and Safety Risks of the New USAMRIID High Containment Facilities at Fort Detrick, Maryland,* Committee to Review the Health and Safety Risks of High Biocontainment Laboratories at Fort Detrick Board on Life Sciences Division on Earth and Life Studies, National Research Council of the National Academies <http://www.nap.edu/openbook.php?record_id=12871>. Accessed August 26, 2010.

Nuclear Regulatory Commission. 2003. Environmental Review Guidance for Licensing Actions Associated with NMSS Programs (NUREG-1748). Available on the internet at: <http://www.nrc.gov/reading-rm/doc-collections/nuregs/staff/sr1748/>. Accessed June 14, 2011.

Chapter 5: TRANSPORTATION ANALYSIS

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5. Transportation Analysis

Chapter Highlights: Chapter 4 identified transportation mishaps as events warranting analysis. This chapter describes the analysis of public impacts associated with transportation of pathogen samples to and from the laboratory by truck and air. The public impacts analyzed are crash-related injuries and fatalities and potential exposure to pathogen releases as a result of truck and aircraft crashes.

This analysis has determined that crash-related injuries and fatalities are more likely than public exposure to infectious pathogens.

5.1 Introduction

NEIDL operations will include both incoming and outgoing shipments of infectious BSL-3 and BSL-4 pathogen samples throughout the life of the facility. In addition to pathogen samples, there is also the potential for infrequent shipment of specimens (e.g., blood, plasma, serum, tissue, urine, and respiratory secretions). These shipments may involve both the ground (truck) and mixed mode (combination of truck and air) transportation. This chapter addresses the public risks (i.e., frequency and consequences) to members of crash-related injuries and fatalities and a potential release of an infectious BSL-3 or BSL-4 pathogen resulting from a postulated transportation mishap. These risks are estimated for members of the public within the vicinity of the three sites being evaluated (i.e., the urban, suburban, and rural sites).

5.1.1 Transportation Analysis Guidance

No directly applicable guidance has been published that pertains to the analysis of accidents associated with transportation of infectious pathogens to and from the NEIDL. The National Research Council of the National Academies has observed this lack of guidance for facilities similar to NEIDL and concluded the following (NRC 2010):

> *U.S. Department of Energy's (DOE) recommendations for the preparation of EISs* [(Environmental Impact Statements)] *contain some of the most detailed explanations and guidelines for discussing human health impacts in an EIS. Although DOE's recommendations for analyzing human health effects are limited to exposure to radiation and chemicals, they also are relevant to pathogen exposures.*

DOE provides detailed guidance for transportation analyses in *A Resource Handbook on DOE Transportation Risk Assessment* (DOE 2002). This guidance was written specifically for transportation of

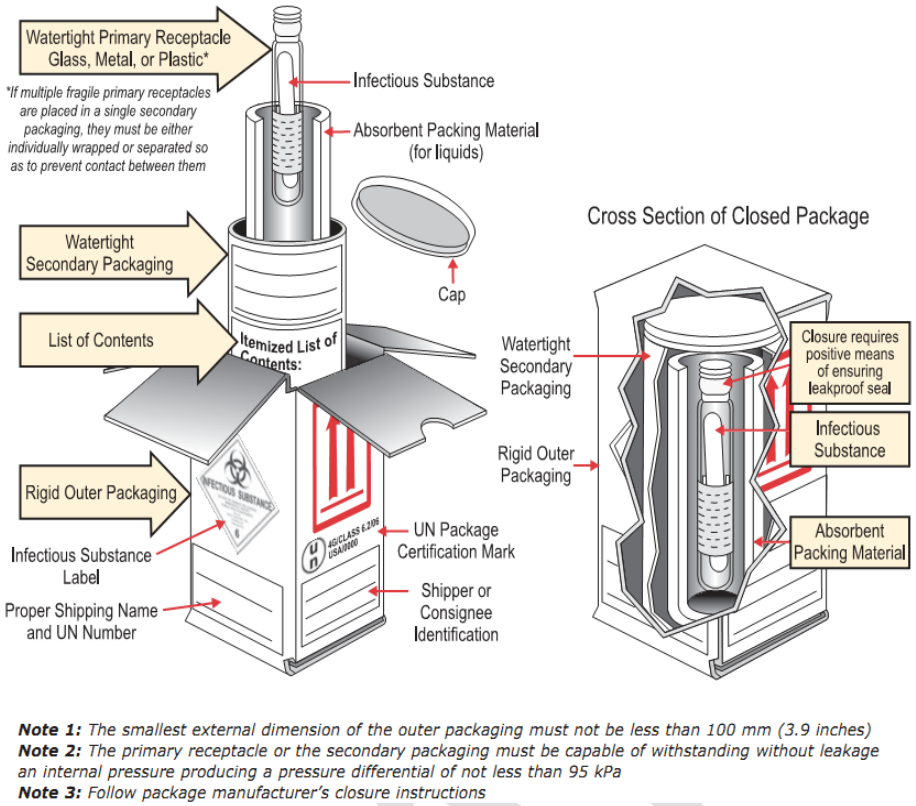
radioactive material, but the general methodology is applicable for any transportation analysis and was used to guide this analysis.

5.1.2 Packaging Requirements

U.S. Department of Transportation (DOT) Hazardous Material Regulations (49 CFR Parts 171-180) govern the transportation of infectious substances. The BSL-3 and BSL-4 pathogens addressed in this analysis are categorized as Class 6, Division 6.2 hazardous materials (i.e., biohazards) (49 CFR 173.134) subject to the Category A Infectious Substances packaging requirements in 49 CFR 173.196, *Category A infectious substances*. Category-A infectious substances must be triple-packed, which includes

- *A leakproof primary receptacle;*
- *A leakproof secondary packaging. If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either wrapped individually or separated to prevent contact between them;*
- *A rigid outer packaging of adequate strength for its capacity, mass and intended use. The outer packaging must measure not less than 100 mm (3.9 inches) at its smallest overall external dimension.* (49 CFR 173.196(a))

An example of the triple-packaging required by 49 CFR 171.196 for infectious substance shipments is shown in Figure 5-1.



Note 1: *The smallest external dimension of the outer packaging must not be less than 100 mm (3.9 inches)*
Note 2: *The primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa*
Note 3: *Follow package manufacturer's closure instructions*

Figure 5-1: Example Packaging and Markings for Category-A Pathogens (DOT 2006)

DOT testing requirement for infectious substance packaging are provided in 49 CFR 178.609. Some of the requirements are dependent upon the materials of construction for the packaging. Assuming that the primary and secondary containers are constructed of plastic, which is expected to be the case for NEIDL, the requirements include the following:

- Impact test: Samples must be subjected to free-fall drops onto a rigid, non-resilient, flat, horizontal surface from a height of 9 m (30 ft) in various orientations with no leakage from the primary container. This drop test must be performed promptly after conditioning the container at -18°C (0°F).

- Puncture test: Samples must be subjected to steel rod puncture test with a 38 mm (1.5 in) diameter cylindrical steel rod. The mass for this test must be 7 kg (15 lb) or the mass of the sample, whichever is greater. The test must be conducted with a vertical free fall from a height of 1 m (3 ft). One sample must be placed on its base and a second sample must be placed in an orientation perpendicular to that used for the first. In each instance, the steel rod must be aimed to impact the primary receptacle(s). For a successful test, there must be no leakage from the primary receptacle(s) following each impact. (40 CFR 178.609)

5.1.3 Description of NEIDL Shipments

The likelihood and consequences of a transportation mishap are dependent upon the number of shipments, the packaging used, and other details of the shipments. This section describes the NEIDL shipments and identifies key assumptions for this analysis. The following assumptions have been accepted by NIH and Tetra Tech, based on information provided by BUMC (Murphy 2011).

While some shipments will be truck-only and others will be mixed mode (truck and air), all shipments will arrive at or depart from the NEIDL via trucks. While all NEIDL shipments must comply with the DOT regulations addressed in Section 5.1.2, BUMC imposes additional shipment requirements. The following sections provide descriptions that apply to all shipments, as well as those that are unique to truck and air portions of shipments.

Pathogen configuration: Bacteria may be shipped in a liquid, frozen, or solid form (e.g., agar in a tube). For this analysis, all bacteria are assumed to be shipped in a liquid suspension, such as a 10–15% glycerol (aqueous) at ambient temperature. The liquid form is assumed here for bacteria because this form is more vulnerable to release, which is a conservative assumption (i.e., tends to over-estimate the risk). If bacteria are shipped in solid form, the consequences of any packaging breach will be lower because a solid form (e.g., agar in a tube or frozen liquid) will have lower release fractions.

Viruses will be shipped in a tissue culture broth after harvesting and it is likely that they will be shipped in a frozen form. A frozen sample will have a lower release fraction than a liquid, so releases could be higher if viruses are shipped in liquid form. This analysis considers both the liquid and the frozen forms.

Specimen (e.g., blood, plasma, serum, tissue, urine, and respiratory secretions) shipments are not expected to be frequent but could occur. The specimens may be shipped in various forms, but are assumed to all be in a liquid suspension since that is the most releasable form.

Pathogen concentration: Bacteria and virus sample concentrations are expected to be comparable to the master/seed/working-stock concentrations identified in Appendix F, Section F.3.

If specimen shipments do occur, the pathogen concentrations will generally be less than or approximately the same as the seed stock concentrations; but in some cases the pathogen concentrations in specimens could exceed those in the seed stock by an order of magnitude.

Pathogens per shipment: A shipment may contain multiple strains of a pathogen and/or multiple infectious pathogens. All known strains of a pathogen could potentially be included in a single shipment.

Primary container: The DOT-compliant primary (inner-most) containers for sample shipments to and from NEIDL are assumed to be sealed, shatter-resistant 2-ml containers similar to the one shown in Figure 5-2. Experience at similar facilities confirms that 2-ml tubes are the typically used for shipments. This example container is made of polypropylene with an approximate length of 50 mm and a diameter of about 11 mm. This container has a screw cap, which will be reinforced with adhesive tape in accordance with 49 CFR 173.196(b)(1). Containers of this type are extremely robust and can withstand forces associated with accelerations of 20,000 g (g is the acceleration of gravity). (Sarstedt 2011)

Figure 5-2: Example Primary Container (Sarstedt 2011)

Secondary container: The DOT-compliant secondary container serves multiple functions: it contains absorbent material that will absorb all of the liquid potentially leaking from the primary container, provides a leak-proof secondary container, shields the primary container from external forces, and, when multiple primary containers are shipped in the same secondary container, includes a means of separating primary containers from each other. Either the primary or the secondary container must be capable of withstanding an internal pressure of at least 95 kPa (approximately 14 psi) without leaking. (10 CFR 173.196)

Tertiary container: The DOT-compliant rigid, tertiary packaging must be at least 100 mm (4 in) in its smallest external dimension. Substances shipped frozen must carry the refrigerant outside the secondary container. If dry ice is used to keep the infectious pathogen frozen, then the packaging must permit the release of carbon dioxide gas. (10 CFR 173.196).

Over-pack case: The DOT-compliant triple packaging will be placed in a "non-crushable," liquid-tight, solid container for an added layer of safety (Murphy 2011). A Pelican™ 0370, 24″ Cube Case, shown in Figure 5-3, meets these criteria and is used as the basis for this analysis. The case is watertight,

"crushproof," and dust proof. The Pelican™ case has an open cell with an O-ring seal between the lid and the base and is equipped with a pressure-equalization valve, so it is not air-tight with respect to internal pressurization. The case has been independently tested for vibration, low temperature, dry heat, impact, dust, and water immersion and has been found to meet various international specifications (Pelican 2011); however, the crush-resistance of the case is not known, as discussed below. Use of the over-pack case is above and beyond the requirements of 49 CFR, so any crush protection provided by the case is an additional defense-in-depth layer of protection.

Figure 5-3: Pelican™ Case

Number of shipments: The number of shipments in any given year will vary throughout the course of the facility's lifetime; however, 50 shipments per year are expected on average. Of these shipments, the majority (about 95%) of shipments will involve BSL-3 pathogens, the majority (about 90%) will involve non-Select Agents, and the majority (about 90%) will be shipments to the NEIDL. Based on BUMC policy, all shipments to or from locations in the USA are assumed to be made via truck transport. It is assumed that the majority (i.e., 90%) will be truck-only shipments because of the commitment to use trucks for shipments within the USA and Canada. Table 5-1 provides the assumed number of shipments of each type. (Murphy 2011)

Table 5-1: Assumed Number of Annual Shipments by Transportation Mode

Mode of Transport	BSL-3 Pathogens	BSL-4 Pathogens	Total
Truck-only	42	3	45
Truck-Air	4	1	5
Total	**46**	**4**	**50**

All air segments of the shipments are assumed to be to/from Boston Logan International Airport for each of the three sites.

Truck carrier: Shipments will be made via carriers specializing in expedited, increased-security shipping services accustomed to handling Division 6.2, Category A hazardous materials. BUMC will require these carriers to provide exclusive-use vehicles (i.e., vehicles that do not contain cargo from any other shipper) (Murphy 2011). FedEx Custom Critical is one example of a carrier that may be used. The shipping agent (i.e., the shipper) would fully comply with all of the provisions of the federal and international regulations and requirements for transportation.

Vehicle: Shipments will be made via a "large truck" (Murphy 2011) with an enclosed cargo box. "Large trucks," which are defined by DOT as trucks with a gross vehicle weight rating (GVWR) of greater than 10,000 lbs. Large trucks can either be single-unit or combination vehicles. A single-unit truck has the engine, cab, drive train, and cargo area on one chassis. A combination truck is a truck tractor pulling any number of trailers or a straight truck pulling at least one trailer. Because the NEIDL packages are small (approximately a 2-ft cube) and light (less than 100 lb), a single-unit truck would likely be used rather than a much larger combination truck. An example of a single-unit large truck includes trucks based on a Ford Super Duty 350/450/550/650/750/800 chassis (DOT 2010a).

Loading: The over-pack case would be secured to the middle of the truck bed away from the walls (Murphy 2011), thereby protecting it from impacts with the walls or from other vehicles that may impact the walls in a collision.

Global positioning system (GPS) tracking: Shippers must have the ability to provide GPS tracking of packages or vehicles as determined appropriate and approved by BUMC. In addition, the over-pack case will include a BU-monitored GPS tracking device (Murphy 2011). GPS tracking provides for detection of interruptions in transit, thereby enhancing the opportunity to support emergency response, including guidance to the responders.

Driver: Trained, professional drivers dedicated to these shipments will operate the cargo vehicles. Drivers will be HAZMAT trained.

Transfer to other BUMC facilities: Transfer of infectious BSL-3 and BSL-4 pathogens between the NEIDL and other BUMC facilities will be performed with the same triple-packaging and over-pack case requirements imposed on external shipments; however, BUMC courier services may be used rather than commercial carriers.

5.1.4 Department of the Army Packaging Testing

Triple-packaged containers similar to those required for all Category A pathogen shipments were tested by the Department of the Army in the 1960s (DOA 1969). The tests were conducted to support development of packaging regulations and consisted of a series of drops from heights of 500 and 1,000 feet with varying packaging configurations including glass and plastic bottles. These drop heights (i.e., 500 and 1,000 ft) are well beyond the 30-ft (9-m) drop test requirements for triple-packaging (49 CFR 178.609). The bottles were over-packed in two outer containers (metal and fiberboard) with either cotton or vermiculite as packaging between the containers. The packages were dropped onto soil, concrete, and macadam surfaces. The purpose of the tests was to determine which packaging configurations could survive such drops. Despite the extreme test conditions, half of the packaging configurations did not leak after the 500-ft drop and a third did not leak after the 1,000-ft drop. In addition, the Department of the Army report states that "containers similar to those tested in May 1961 later withstood the crash of a C-119 aircraft at 138 miles per hour" into a concrete wall. (DOA 1969)

The packages tested by the DOA differ in several important respects from the packages used for shipping pathogens to NEIDL. The effects of these differences are discussed below:

The primary container volumes in the DOA tests ranged from 450 ml to 1,300 ml, while the NEIDL primary containers are expected to be 2-ml tubes. The 2-ml tubes have much less mass, so the impact loads would be much smaller than those used in the DOA tests. In addition to the lower impact loads, the 2-ml containers will tend to have greater strength-to-weight ratio.

The plastic primary containers in the DOA tests were made of polyethylene while the containers expected to be used for NEIDL are made of polypropylene. Polyethylene is softer and not as strong as polypropylene, so the NEIDL containers are less likely to eject caps or breach. Also, the caps are required to be sealed with adhesive tape.

The DOA tests did not include the over-pack case, which is required for infectious BSL-3 and BSL-4 pathogen shipments to or from NEIDL (Murphy 2011). The shell and foam liner of the case would absorb and distribute much of the impact loads.

Based on the partial survival of the DOA packages, the NEIDL packaging (a 2-ml vial triple packaged plus the over-pack case) are expected to survive both the 500-ft and 1,000-ft DOA drop tests, as well as the aircraft crash test, without leakage from the primary container.

5.1.5 Hypothetical Accident Conditions

Triple packaging must pass the requirement of 49 CFR 173.196, Section G.1.2; however, this does not ensure that the packaging will survive all potential accident conditions. The requirements for testing of Type B radioactive material shipping containers are provided in 10 CFR 71.73 and are intended to simulate hypothetical accident conditions. These tests go beyond the requirements of 49 CFR 173.196. While the NEIDL packaging is not required to comply with them, the requirements of 10 CFR 71.73 do provide insight into the type of severe hypothetical conditions that may occur and their potential to breach the packaging. These tests include consideration of fire, impact loads, puncture, and crush loads on the package. The following paragraphs identify the 10 CFR 71.73 testing requirements and discuss expected performance of NEIDL packages for each test to provide some insights into the survivability of the NEIDL packaging.

Free drop: This test consists of a free drop of the package from a height of 9 m (30 ft) onto a flat, essentially unyielding, horizontal surface striking in an orientation that is expected to produce the maximum damage. This test addresses the survivability of the package for the hypothetical impact loads. This 10 CFR 71.71 requirement is equivalent to the requirement for packaging of infectious substances (i.e., 49 CFR 173.196), as discussed in Section 1.2. The over-pack case may or may not be damaged in the test, but the case will distribute and absorb much of the impact load. The NEIDL packaging is expected to pass this test because the triple-packaging must pass the equivalent 49 CFR 173.196 test and the over-pack case will likely enhance its ability to withstand the free drop.

Crush: This test consists of the drop of a 500-kg (1,100-lb) plate that has a cross section of 1 m (40 in) by 1 m (40 in) from a height of 9 m (30 ft) onto the package. This test is not required for all radioactive material packages but would be required for containers that are the size of the NEIDL package. The over-pack case is unlikely to survive this test without extensive damage. The survivability of the primary container depends upon the amount of dynamic energy absorbed by the over-pack case and the extent to which the case distributes the load. The static load resulting from this test would average less than 1 pound per square inch, which is not likely to breach the primary container. While the survival of the primary container from the dynamic loading of this test is unknown, any aerosol or liquid release is unlikely because the over-pack foam liner would compress around the inner packaging and prevent any leakage. Therefore, the NEIDL packaging may be breached, but any release would be unlikely.

Puncture: This test consists of the free drop of a package 1 m (40 in) in the position of maximum expected damage onto the upper end of a 15-cm (6-in) diameter bar. The size of the bar is smaller in the 49 CFR 178.609 test, so the triple packaging is expected to survive this test. The over-pack case may or may not survive this test.

Thermal: This test consists of a fully engulfing fire with a flame temperature of at least 800°C (1,475°F) for 30 minutes. The NEIDL packaging would not withstand this test; however, this test is not relevant for infectious pathogen shipments because the pathogens would be inactivated at temperatures much lower than these. Thus, the public nor the environment would be at risk to infectious pathogens. In addition, the plastic over-pack case and its foam lining would likely melt and encapsulate the primary container.

Immersion: The package must be subjected to water pressures of 150 kPa (21.7 lb/in^2) gauge, which is equivalent to immersion in 15 m (50 ft) of water. 49 CFR 173.196 requires a test for water spray that simulates rainfall, but does not require a similar test for packaging of infectious substances. The primary or secondary container is required to withstand an internal pressure of 95 kPa (approximately 14 psi), which is about 60% of this criterion. The 2-ml primary containers are expected to survive this test based on their ability to withstand 20,000 g of acceleration (see Section G.1.3.1).

Based upon the 10 CFR 71.73 criteria for severe hypothetical accident conditions, free drop, puncture, immersion, and thermal conditions are of minimal concern for NEIDL package survival. The greatest concern for the primary container is a crushing load (e.g., the truck rolls over onto the package). However, the foam lining of the over-pack case would confine any release from a pure crushing load. Therefore, the only postulated loads that can result in a significant release are a series of loads that first eject the triple-package from the over-pack case and then crush the triple packaging.

5.2 Methodology

Truck and aircraft crash rates were necessary inputs for this analysis. The following sections identify the truck and aircraft crash data sources used for this analysis and provide the basis for their selection. The population for which impacts were considered is also described.

5.2.1 Truck-Crash Data

Crash rates for large trucks have been declining significantly over the past two decades. Figure 5-4 shows the injury and fatality rates for single unit large trucks over the past two decades. Over that period, the injury rate has decreased by 60%, and the fatality rate has decreased by 40% (DOT 2010); therefore, use of older crash data (e.g., the data provided in the DOE transportation analysis guide) would overstate the

risk. The most recent report that contains data on injuries and fatalities related to large trucks is the *Large Truck and Bus Crash Facts – 2008*, published by the U.S. Department of Transportation, Federal Motor Carrier Safety Administration (DOT 2010), and is used as the basis for this analysis. While the 2008 data are the most recent detailed dataset, an overview of the 2009 data shows the trend of decreasing accident rates has continued with an 18% decrease in injury rate and a 20% decrease in fatality rate from 2008 to 2009 (DOT 2011). Therefore, use of the most recent detailed dataset (i.e., the 2008 data) is appropriate and is expected to be slightly conservative for future NEIDL shipments.

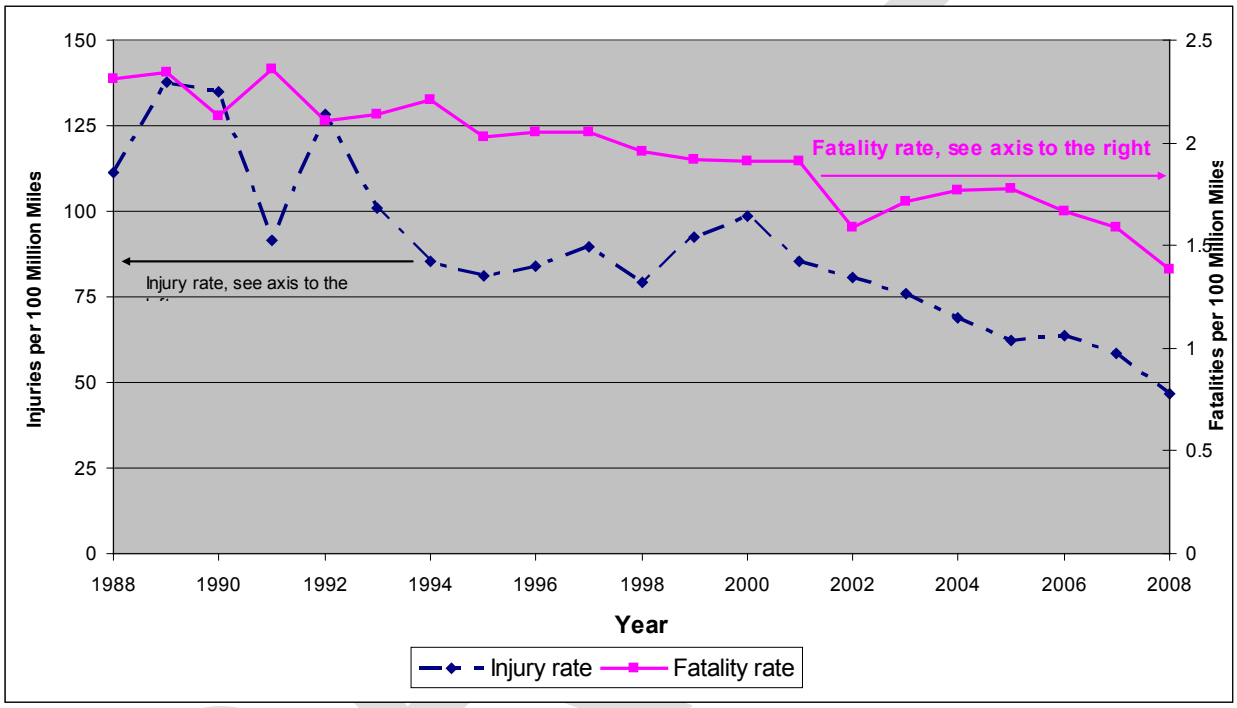


Figure 5-4: Historic Injury and Fatality Rates (DOT 2011, Table 14 and 17)

In addition to containing the most recent detailed data, *Large Truck and Bus Crash Facts – 2008* was selected because it consolidates the results of four databases into a single document and provides a data quality review function on the data from the various sources.

5.2.2 Aircraft-Crash Data

The guidance provided in *Accident Analysis for Aircraft Crash into Hazardous Facilities*, DOE STD 3014-2006 (DOE 2006) is used as the basis for this analysis of aircraft crash. Infectious pathogens are assumed to be transported via certified commercial air carrier (i.e., a plane with 30 seats or more or a maximum payload of 3,401 kg [7,500 lb] or more).

5.2.3 Population

This analysis addresses the impacts on the population within 10 km of the three sites being evaluated (urban, suburban, and rural). This 10 km distance is consistent with the distance used for the environmental justice analysis (see Chapter 10) and is greater than the distance recommended by the U.S. Nuclear Regulatory Commission to define the vicinity of a site for environmental justice purposes (Nuclear Regulatory Commission 2003). The population within 10 km is reported in Appendix F, Section F.4.

5.3 Results

Consistent with the recommendations of the DOE transportation analysis guidance (DOE 2002), this section estimates both the crash-related risks (e.g., injuries and fatalities from directly resulting from the crash) and the pathogen-related risks (potential exposure from released pathogens) of NEIDL shipments. Additionally, the analysis estimates the pathogen-related risks of potential crashes of aircraft carrying NEIDL shipments.

5.3.1 Crash-Related Risks

The following sections address the crash-related risk to members of the public near each of the three sites from the truck portion of shipments, specifically injuries and fatalities that result directly from the potential crashes.

5.3.1.1 Crash-Related Injuries

The 2008 injury rate for single-unit large trucks is 46.9 injuries per 100 million vehicle miles traveled (2.91×10^{-7} fatalities per km) (DOT 2010). The distance traveled within 10 km of the facility is assumed to be 20 km per shipment because the truck must travel both to and from the facility. Table 5-2 provides the estimate for the frequency and period for injuries associated with BSL-3 and BSL-4 shipments. Crash-related injuries include those involving occupants of the other vehicle, non-occupant members of the public, and occupants of the truck.

Table 5-2: Estimate of Crash Injuries within 10 km of the Facility Due to NEIDL Truck Shipments

Parameter	BSL-3 Shipments	BSL-4 Shipments
Shipments per year[a]	46	4
Distance traveled (km/yr)[b]	920	80
Injury rate (injuries/km)[c]	2.91×10^{-7}	2.91×10^{-7}
Frequency of injuries (injuries/yr)[d]	2.7×10^{-4}	2.3×10^{-5}
Period (yrs) between injuries[e]	3,700	43,000

[a] The number of shipments are based on Section 5.1.
[b] Distance traveled within 10 km of the facility is assumed to be 20 km to account for arrival and departure.
[c] Source: 2008 value from Table 17 of DOT 2010 (converted to km).
[d] The average frequency equals the distance traveled times the injury rate.
[e] The average period between injuries is the reciprocal of the average frequency of injuries.

5.3.1.2 Crash-Related Fatalities

The 2008 fatality rate for single-unit large trucks is 1.38 fatalities per 100 million vehicle miles traveled (8.57×10^{-9} fatalities per km) (DOT 2010). Similar to crash-related injuries (above), the distance traveled within 10 km of the facility is assumed to be 20 km per shipment because the truck must travel both to and from the facility. Table 5-3 provides the estimate for the frequency and period between fatalities for BSL-3 and BSL-4 shipments. Crash-related fatalities include those involving occupants of the other vehicle, non-occupant members of the public, and occupants of the truck.

Table 5-3: Estimate of Crash Fatalities within 10 km Due to NEIDL Truck Shipments

Parameter	BSL-3 Shipments	BSL-4 Shipments
Shipments per year[a]	46	4
Distance traveled (km/yr)[b]	920	80
Fatality rate (fatalities/km)[c]	8.57×10^{-9}	8.57×10^{-9}
Frequency of fatalities (fatalities/yr)[d]	7.9×10^{-6}	6.9×10^{-7}
Period (yrs) between fatalities[e]	130,000	1,500,000

[a] The number of shipments are based on Section 5.1.
[b] Distance traveled within 10 km of the facility is assumed to be 20 km to account for arrival and departure.
[c] Source: 2008 value from Table 14 of DOT 2010 (converted to km).
[d] The average frequency equals the distance traveled times the fatality rate.
[e] The average period between fatalities is the reciprocal of the average frequency of fatalities.

5.3.2 Likelihood of Pathogen Release for Truck Transport

This section estimates the pathogen-related risk to the public associated with NEIDL shipments. The section begins with the consideration of various event types and then estimates the frequency and consequence of such events.

5.3.2.1 Events Considered

Multiple preventive and mitigative biocontainment features protect the shipper and the public from potential release of infectious pathogens during transport (see Section 5.1.3); however, there are also

multiple mechanisms for compromise of one or more of these features. The DOE transportation analysis guidance (DOE 2002) focuses solely on vehicle crashes and does not address non-crash releases. For completeness, this section considers both crash and non-crash pathogen releases. The non-crash events may either be initiated by actions prior to transport (e.g., placing dry ice inside a sealed container) or during transport. Therefore, this section addresses events that can occur prior to transport, non-crash events during transport, and crash events. The discussion applies intra-campus shipments (i.e., between the NEIDL and another BUMC laboratory) with the exception that BUMC vehicles would be used rather than a commercial carrier.

Pathogen Shipping Experience

Two potential pathogen release incidents during shipping are identified in Appendix D. In one incident, a shipment included two vials with no caps and a third vial with a loose cap. This incident resulted in possible laboratory worker exposures. In the other incident, a container burst due to improper packaging of dry ice. One or more shipping employees were potentially exposed, but the risk of infection was characterized as low.

5.3.2.1.1 Release Due to Events Prior to Transport

Two types of events— errors in the preparation of the package for shipment and errors in the loading of the package into the truck—are considered below:

Package Preparation Errors

Events prior to transport (e.g., in the preparation of samples for shipment) may result in the compromise of one or more of the biocontainment features. The following paragraphs discuss events that have the potential to compromise each of the biocontainment features. Most of these events only have the potential for a liquid release and have limited potential for a significant aerosol release. It should be noted that many of the shipments will originate at other laboratories and it may be difficult for BUMC to control the procedures and training used by those laboratories; however, all shippers must comply with the packaging requirements of DOT.

Primary container: The innermost container could fail to provide containment as a result of either personnel error or a faulty container. Personnel could fail to place caps on all vials, to appropriately tighten the cap (either under or over tightening), to appropriately tape the cap, or to use the correct cap (e.g., use a cap with a different thread pitch). Procedures and personnel training at the shipping laboratory have the potential to reduce the likelihood of such failures. A faulty container (e.g., cracked container or leaky cap) could also cause a leak.

Absorbent material: For liquids, absorbent material sufficient to absorb the entire volume of liquid must be placed between the primary and secondary containers (10 CFR 173.196). In the event of a leak from

the primary container, this absorbent material should absorb the leakage and limit any release. Personnel could fail to place any absorbent or a sufficient absorbent into the secondary container. Additionally, even if sufficient absorbent is present, a pool of liquid could accumulate at the bottom of the secondary container; however, such a pool of liquid would be contained by the secondary container.

Secondary container: The leak-proof secondary container could fail to provide containment as a result of either personnel error or a faulty container. Personnel could fail to install the cap, use the wrong cap (e.g., one with a different thread pitch), or fail to appropriately tighten the cap (either under or over tightening). Procedures and personnel training at the shipping facility will reduce the likelihood of such failures. A faulty container (e.g., cracked container or leaky cap) could also cause a leak.

When frozen samples are shipped, they are typically kept frozen with dry ice, which is required to be positioned outside the secondary container (49 CFR 173.196). If dry ice is inadvertently placed inside the sealed secondary container, the secondary container will become pressurized as the dry ice sublimates, leading to over-pressurization and breach of the container. Over-pressurization is not credible for intra-campus shipments because of the short time periods involved. This pressurization is also unlikely to breach the primary container, which is expected to be able to withstand higher pressures because (1) the secondary container would experience tensile stresses while the primary container would experience compressive stresses and the compressive strength of plastics is generally greater than the tensile strength, and (2) the surface area of the primary container is much less so the forces are much smaller on the primary container.

Over-pack foam lining: The containment features of the over-pack case include the foam lining and the watertight shell. The open-cell foam core can absorb many times more liquid than the liquid volume contained within the triple-packaging. In addition, the foam would provide a degree of "filtration" for any aerosol released. The foam would continue to perform these absorbing and "filtration" roles even if worn or damaged by normal use.

Over-pack shell: The plastic shell of the over-pack case has a liquid-tight molded bottom that contains any liquids not absorbed by the foam lining. The O-ring sealed lid would impede any liquid or aerosol release, but the case is not air-tight for internal pressurization because of the pressure equalization valve.

Summary: Multiple personnel errors and/or equipment failures prior to shipment would be required for a liquid or aerosol release due to a failure of the primary container, secondary container, over-pack foam core, and over-pack shell. While potential human errors or equipment defects/failures can fail each of these barriers, no common-causes (i.e., single events that fail multiple barriers) prior to shipment were

identified. No published data exists on which to base an estimate of frequency of a release due to events prior to transport (one incident is identified in the text box of Section 5.3.2.1, but the number of shipments is not known); nevertheless, a release caused by such events is highly unlikely. Furthermore, events of this type are very low energy events, including the over-pressuring of the secondary container, so there is a high likelihood that the over-pack case would confine any release. Therefore, this type of event is dismissed from further consideration.

Package Loading Errors

A NEIDL package could be breached prior to transport if it is inadvertently left on the driveway and the truck drives over it. In order for this event to occur, several failures and conditions must exist:

The requirement to secure the over-pack case to the middle of the bed must be violated in order for the NEIDL package to be left in the path of the truck.

1. The driver and the laboratory personnel must overlook the hazard. The large over-pack case (approximately a 2-foot cube) is less likely to be overlooked than the triple-packaging, which is only required to be larger than 100 mm (4 in).

2. The NEIDL over-pack case is (approximately a 2-foot cube) would tend to slide because the case is probably higher than the middle of the tire; however, it is possible that an obstruction would prevent the case from sliding and allow it to be crushed.

The driver would need to fail to be alerted by the sound of the case either scooting on the pavement or beginning to be crushed and stop the vehicle.

No data have estimated the frequency of this event, which is highly dependent upon a failure to implement the NEIDL protocol for securing the case to the middle of the truck bed. However, in the event that all of these failures and conditions occurred and the primary container is breached, then the multiple layers of packaging, especially the foam lining, would confine any release. Therefore, this scenario is not considered further.

5.3.2.1.2 Package Drops from the Truck during Transport

A package could topple from the truck onto the roadway and be crushed by another vehicle. In order for this event to occur, the following sequence of events must transpire:

- Shipper fails to secure the NEIDL package in the middle of the vehicle: Though required by BUMC procedures (see Section 5.1.3.2), personnel could neglect to secure the package properly.

- Shipper fails to close the cargo door on the truck: An unsecured package cannot topple from the cargo bed unless the cargo door is open. It is possible that the driver neglects to properly close the cargo door, which may then fully open in transit.
- Package drops from the truck: An unsecured package in a cargo bed with an open door would not necessarily drop from the truck. Severe braking might exert enough force on the package to cause it to slide, but it would slide to the front of the bed rather than to the back. However, bumps in the roadway could cause an unsecured package to bounce to the rear of the bed and fall from the truck if the cargo door is open.
- Another vehicle crushes the package: If a NEIDL package falls onto the highway, the over-pack case will absorb most of the dynamic energy and the tipple-packaging is expected to remain intact. However, if the package is struck by another vehicle, the NEIDL package could be crushed. Because of its size (a 2-ft cube), the package would likely be noticed and evasive action would be attempted by other vehicles, but they may not be successful. If an automobile were to strike the 24-in over-pack case, the over-pack case might be deflected out of the pathway rather than being crushed (automobile bumpers are typically 16–20 in from the highway). A large truck bumper would not necessarily deflect the over-pack case and the case could be crushed. The GPS tracking ensures that the package is not left on or beside the roadway for prolonged time periods.

This scenario requires multiple personnel errors (failure to secure and failure to close the cargo door) and chance events (toppling from the truck and being crushed). This scenario is unlikely because it requires a series of unrelated events and these events are not likely to occur within 10 km radius of the laboratory. This scenario is not considered further.

5.3.2.1.3 Crash Events During Transport

The term "crash" is used by the National Highway Traffic Safety Administration (NHTSA) to include both collision and non-collision events. Collision events include those involving a motor vehicle and fixed objects (poles, walls, barriers, bridge supports, etc.) and those involving a motor vehicle and non-fixed objects (pedestrians, animals, pedal cyclists, other motor vehicles, etc). Non-collision events include a single vehicle that catches fire, a single vehicle that runs off the roadway and is immersed in water, injuries due to shifting cargo, and damages due to pavement irregularities. (DOT 2010a)

Crash events are analyzed here because (1) they are foreseeable, highly energetic events capable of failing all biocontainment barriers concurrently with the potential of causing an aerosol release of a pathogen; (2)

the DOE transportation analysis guidance (DOE 2002) recommends that they be analyzed; and (3) estimates of their frequency can be made because of the availability of truck-crash data.

5.3.2.2 Frequency of Releases Due to Truck Crashes

The DOE transportation analysis guidance (DOE 2002) provides severity categories for truck accidents involving radioactive materials, as well as the fraction of occurrences for each severity category. Unfortunately, those severity categories were developed for the massive casks used to transport radioactive materials and are not applicable to the low-mass, energy absorbing NEIDL packages. Therefore, alternate approaches were used to relate the crash severity to package failure. The frequency for pathogen release events was estimated based on two approaches: (1) crashes that involve an occupant fatality since the NEIDL packages are far more robust than a human, and (2) identification of the types of crashes that are most likely to involve crushing forces capable of breaching all layers of packaging. Though not directly relevant to NEIDL shipments, data on the frequency of Class 6 hazardous material releases during crashes is also presented to provide a frame of reference.

5.3.2.2.1 Frequency Based on Occupant-Fatal Crashes

The primary containers have a high strength-to-weight ratio and are surrounded with energy absorbing and liquid absorbing material. As discussed in Section 5.1.5, only severe truck crashes involving crushing forces have the potential to breach the packaging. There are no analytical or test estimates of the forces necessary to breach the NEIDL packaging nor are there data on the fraction of crashes that involve such forces, so the likelihood of a NEIDL package breach from a crash cannot be estimated directly. However, the packaging is clearly able to survive forces more severe than the human occupants can survive. Therefore, this analysis uses occupant-fatal (i.e., an occupant of the truck), single-unit, large truck crashes as a means of estimating the rate of NEIDL package breach and pathogen release. Crashes that are fatal to occupants of the other vehicle or to non-occupants do not provide any indication of the forces that would be exerted on the NEIDL package and are not considered in this calculation.

The occupant-fatal, single-unit, large truck crash rate is 2.44×10^{-9}/mile (205 occupant fatalities in 83.9 billion miles) or 1.52×10^{-9}/km, which is the rate used for this analysis (DOT 2010). Table 5-4 provides the frequency calculation for occupant-fatal crashes. The NEIDL package is able to withstand much greater forces than the occupant, so the pathogen release frequency is expected to be less than this frequency.

Table 5-4: Estimate of the Frequency of Pathogen Releases within 10 km Based on Occupant-Fatal, Single-Unit, Large Truck Crashes

Parameter	BSL-3	BSL-4
Occupant-fatal crash rate for single-unit trucks (/km)[a]	1.52×10^{-9}	1.52×10^{-9}
Annual truck shipments (/yr)[b]	46	4
Distance per shipment (km)[c]	10	10
Total distance per year (km/yr)[d]	460	40
Frequency (/yr) of pathogen release events[e]	**7.0×10^{-7}**	**6.1×10^{-8}**
Period (yrs) between pathogen release events[f]	**1.4×10^{6}**	**1.6×10^{7}**

[a] Source: Table 17, DOT 2010.
[b] The number of shipments per year are based on Section 5.1.
[c] Distance traveled per shipment is assumed to be 10 km per within the 10 km radius.
[d] Total distance per year is the product of the number of shipments per year times the distance per shipment.
[e] The frequency equals the distance traveled times the occupant-fatal crash rate.
[f] The period between events is the reciprocal of the frequency.

5.3.2.2.2 Frequency Based on the Types of Occupant-Fatal Crash

Impact loads due to collisions with other vehicles and objects may result in fatalities and severe damage to the vehicle but are unlikely to breach the primary container secured in the over-pack case secured in the middle of the bed (see Section 5.1.5). Therefore, crashes that have the potential to involve large crushing loads are considered the most likely crashes to breach the NEIDL packaging. A review of the most harmful event characteristics of truck accidents (Table 53, DOT 2010) identified only two categories of accidents that are judged to have the potential to result in the types of crushing forces necessary to breach all layers of packaging: namely, (1) overturns (rollover) and (2) collisions involving trains. Events that were dismissed include collisions (with vehicles in transport, fixed objects, pedestrians, pedal-cycles, other objects and animals), jackknives, and fire/explosions. Severe rollovers could fail the truck's cargo box, crush the NEIDL packaging, and eject the triple-package from the over-pack case, which leaves the primary container vulnerable to further crushing. Train collisions also have the potential to breach the NEIDL package because they can involve multiple severe crushing and shear forces. Rollover and train collision crashes are addressed below.

Rate of rollovers: Rollovers are defined by DOT as any rotation of 90° or more (DOT 2010a). The fraction of fatal, large-truck crashes in which rollover is the most harmful event is 7.0% (Table 53, DOT 2010); however, this value is likely to overstate the number of rollovers that have the potential to breach the NEIDL packaging for the following reasons:

This 7% includes 90° rollovers, which are likely to be a large percentage of the rollovers. A 90° rollover does not have the potential to crush the NEIDL package (a 180° rollover is required to crush the package). Therefore, 7% is a conservative value for the fraction of crashes that can breach all layers of the packaging.

This value reflects all large trucks, both single-unit and combination trucks. Compared to single-unit trucks, combination trucks tend to have a higher center of gravity, which is a prime factor in rollovers. In addition, the absence of any significant cargo load in the NEIDL shipments also creates a lower center of gravity. Therefore, this factor would tend to make the 7% factor conservative for a single-unit large truck with a very light load.

This value reflects all fatalities (truck occupant and as well as other vehicle occupants and non-occupants). It is not known whether the more severe truck damage crashes (i.e., those involving a truck-occupant fatality) would tend to have a higher or lower fraction of rollovers than all fatal large truck crashes. Rollover is the most harmful event in 9.8% of injury crashes and 1.3% of the property-damage-only crashes (DOT 2010). If this factor is non-conservative, the conservatism of the previous two factors is judged to more than compensate.

Based upon the above rationale, the 7% rollover fraction was used for this analysis as the approximate rate of greater than 90° rollovers for single-unit large trucks in severe crashes where an occupant of the truck is killed.

Rate of train collisions: A collision with a train is the most harmful event for 0.3% of all fatal, large-truck crashes. As with the rollover rate, the train collision rate is not directly applicable for this analysis for the following reasons:

The "cattle catcher" at the front of the train would tend to prevent a truck from being pulled under the train and crushed. The impact loads may be fatal to the occupant and severely damage the truck, but the crash is unlikely to compromise the packaging. From this perspective, the 0.3% value is likely a conservative value.

The driver of a vehicle may exit before the collision, so crashes that are non-fatal may still have the potential of breaching the packaging. The train collision rate for injury crashes and property-damage-only crashes are 0.2% and 0.1%, respectively (DOT 2010).

The 0.3% value reflects both single-unit and combination trucks collisions. It is assumed that the rates are not significantly different for single-unit and combination large trucks.

Based upon the above rationale, the 0.3% fraction was used for this analysis as the approximate rate of collisions with a train for single-unit large trucks in severe crashes where an occupant of the truck is killed.

As discussed above, it is expected that, on average, crashes that have the potential to breach all packaging will also result in a truck occupant fatality and that the loads that have the greatest potential to breach the packaging are crushing loads, such as those associated with rollovers or train collisions. The crash rate for truck occupant fatalities is 1.52×10^{-9}/km (see Section 5.3.2.2.1). The discussion above shows the fraction of fatal crashes that involve rollover is 7.0% and the fraction that involves train collisions is 0.3%. The most likely crashes to breach the NEIDL package are occupant-fatal truck crashes where the most harmful events are rollovers or train collisions, and the frequency of a pathogen release is estimated in Table 5-5.

Table 5-5: Estimate of the Frequency of Pathogen Release Crashes within 10 km Due to Occupant-Fatal Rollover and Train Collision Crashes for NEIDL Shipments

Parameter	BSL-3	BSL-4
Frequency of occupant-fatal crashes (/yr) (from Table 5-4)	7.0×10^{-7}	6.1×10^{-8}
Period (yrs) between occupant-fatal crashes (from Table 5-4)	1.4×10^{6}	1.6×10^{7}
Fraction of crashes that involve rollover or train collision[a]	7.3%	7.3%
Frequency of pathogen release events (/yr)[b]	**5.1×10^{-8}**	**4.4×10^{-9}**
Period (yrs) between pathogen release event[c]	**2.0×10^{7}**	**2.3×10^{8}**

[a] Source: Table 53, DOT 2010.
[b] Value is 7.3% of the frequency of occupant-fatal crashes.
[c] The period between events is the reciprocal of the frequency of pathogen release events.

5.3.2.2.3 Comparison with Historic Release Data

The DOT report (DOT 2010) contains data on truck crashes, including data on the number of hazardous material releases that have occurred as a result. However, those data are not directly relevant for NEIDL shipments for the following reasons:

1. The data include all Class 6 hazardous materials: The DOT data are presented for "poisonous and infectious substance," effectively Class 6 hazardous materials as defined by 49 CFR 173. Class 6 hazardous materials include Division 6.1 (poisonous materials) and Division 6.2 (infectious materials). Division 6.1 includes chemicals transported in relatively large quantities (e.g., acids and gasoline additives) with different packaging requirements than Division 6.2 materials. Because larger containers routinely have a lower strength-to-weight ratio than smaller containers, Division 6.1 materials are likely to be more vulnerable to release than Division 6.2 materials in crashes. In addition, the NEIDL shipments evaluated are Division 6.2, Category A materials, which have more stringent packaging requirements than Division 6.2, Category B materials. Because the Division 6.2, Category A shipments materials have more robust packaging than the other Class 6 materials, they are less likely to involve releases as a result of crashes and, therefore, these data are likely to overestimate the frequency for release crashes.

2. Unique NEIDL shipment requirements: NEIDL shipments are likely to have lower frequencies of releases because the BUMC shipping requirements (see Section 5.1.3) go well beyond those required for Division 6.2, Category A shipments. The additional requirements for NEIDL shipments includes the following:

The required over-pack case protects the triple-package from impact loads.

The requirement that the over-pack case be secured to the middle of the truck bed reduces the chance that the package is ejected from the vehicle and crushed.

The exclusive-use vehicles eliminate the potential for other cargo to topple onto and crush the NEIDL package.

As a result, NEIDL shipments are expected to have a much lower rate of releases from crashes than other Division 6.2, Category A shipments.

Therefore, the average rate for crashes resulting in release of Class 6 hazardous material is expected to be greater than the rate for NEIDL shipments and the conservatism is judged to be in excess of an order of magnitude.

As stated previously, humans are more vulnerable in a crash than the NEIDL package, so the rate of fatal occupant crashes is considered to be an upper bound on the rate of release-causing crashes for NEIDL shipments. DOT reports that in 2008, one fatal crash (fatality in either vehicle) involving "poisonous and infectious substances" (i.e., Class 6 hazardous materials) occurred and did not result in a release (DOT 2010). DOT also reports that there were 11 nonfatal crashes involving "poisonous and infectious substances" (i.e., Class 6 hazardous materials) where the outcome is known, and there was a release in only one of those crashes (DOT 2010). The DOT data on Class 6 hazardous material releases provide only limited insight into the rate of release from NEIDL packages; however, the DOT data do support the expectation that NEIDL packages are likely to survive crashes that do not involve truck-occupant fatalities since the less robust Class 6 packaging survived in 11 of the 12 non-fatal crashes and the one fatal crash.

5.3.3 Likelihood of Pathogen Release from Air Transport

As discussed in Section 5.1.3, pathogen shipments may include air transport. Because BUMC policy requires ground transport for shipments to/from locations in the USA and Canada (Murphy 2011), any air transport would be to or from foreign locations. Foreign shipments are likely to be made through the

Boston Logan International Airport for all three sites being evaluated. If an aircraft carrying a NEIDL pathogen crashes on takeoff or landing, there is a potential for a release and this analysis estimates the likelihood of such an event. The potential for a NEIDL package being dropped from the aircraft during takeoff or landing is not considered because the packaging is expected to survive such drops (see Section 5.1.4).

The guidance provided in *Accident Analysis for Aircraft Crash into Hazardous Facilities*, DOE STD 3014-2006 (DOE 2006) is used as the basis for the rate of aircraft crashes on takeoff and landing. The infectious pathogen is assumed to be transported via a certified commercial air carrier (i.e., a plane with 30 seats or more or a maximum payload of 3,401 kg [7,500 lb] or more). The crash rate for certified commercial air carrier is 1.9×10^{-7} for takeoffs and 2.8×10^{-7} for landings. The crash rate for these specific aircraft while in flight over the rural and suburban sites much lower and are not addressed. (DOE 2006)

An aircraft crash does not necessarily result in an infectious pathogen release. Based on the Army tests results discussed in Section 5.1.4, the NEIDL package is expected to survive drops from both 500-ft and 1,000-ft, as well as an aircraft crash into a concrete wall without leakage from the primary container (see Section 5.1.4). While the NEIDL packages themselves have not been tested for aircraft crash survival, it is conservatively judged that the conditional probability of pathogen leakage given an aircraft crash is less than 10% because: (1) packaging used in the 1960s tests survived a plane crash into a concrete wall; (2) the triple-packaging used for NEIDL shipments will be more robust than the packaging used in the 1960s test (the 2-mL vials are much more resistant to breaching); and (3) the over-pack case for NEIDL shipments protects the triple-packaging from impacts. Table 5-6 provides the estimated likelihood of a pathogen release due to an aircraft crash.

Table 5-6: Estimate of the Likelihood of a Pathogen Release from the Takeoff or Landing Crash of Aircraft Carrying a NEIDL Pathogen

Parameter	BSL-3	BSL-4
Crash rate for commercial aviation - air carrier (takeoff)[a]	1.9×10^{-7}	1.9×10^{-7}
Crash rate for commercial aviation - air carrier (landing)[a]	2.8×10^{-7}	2.8×10^{-7}
Number of takeoffs per year[b]	2	0
Number of landings per year[b]	2	1
Conditional probability of release given a crash	0.1	0.1
Frequency of pathogen release events (/yr)[c]	**9.4×10^{-8}**	**2.8×10^{-8}**
Period (yrs) between pathogen release event[d]	**1.1×10^{7}**	**3.6×10^{7}**

[a] Source: Table B-1, page B-3, of DOE STD 3014-2006 (DOE 2006).
[b] From Table 5-1 in Section 5.1.
[c] The frequency of pathogen release is the sum of the crash rate times the number of events for takeoff and landing crashes, times the conditional probability of a release given a crash.
[d] The period is the reciprocal of the frequency.

5.3.4 Consequences of a Potential Pathogen Release

If an infectious pathogen release were to occur, the public or responders at the scene of the truck or aircraft crash could be exposed to the release. The DOE transportation analysis guidance (DOE 2002) recommends use of a "sliding scale" where "the preparer should analyze issues and impacts with the amount of detail that is commensurate with their importance." The frequency of a pathogen release from either truck or air transport is very low (i.e., less than 1 in 1 million years), so per the guidance, detailed consequence analyses are not warranted. Consistent with this guidance, detailed analyses of exposures in close proximity were not performed, but a qualitative analysis is provided.

The maximum reasonably foreseeable release earthquake estimated the average exposures to each of the 13 pathogens evaluated for a person 30 m from a ground-level release (see Appendix F, Section F.8.3.4). Exposures to people 30 m or more from the point of a transportation release can be approximated by scaling the earthquake results on the basis of the smaller volumes associated with transportation. It is reasonable to use the maximum reasonably foreseeable release earthquake exposure calculations because they (1) are based on meteorological and terrain conditions appropriate for each of the three sites; (2) did not include any mitigative features; and (3) were based on a ground-level release, which would be consistent with a truck or aircraft crash. While reasonable, this scaling approach is likely to be conservative (i.e., overestimate the exposures) because (1) virus samples are likely to be shipped in frozen form (see Section 5.1.3), which has a lower release fraction than the liquid form used for the earthquake analyses; and (2) the small vials and over-pack foam associated with transportation are likely to provide a degree of confinement even if breached.

The pathogen with the highest exposure levels and the greatest probability of infections for the maximum reasonably foreseeable release earthquake was Rift Valley fever virus (RVFV). The RVFV volume was assumed to be 150 ml for the earthquake. The transportation packages contain duplicate samples in 2 ml vials which would be only partially full (approximately half full). There may be multiple strains of a given virus in a given shipment. It is assumed here that the transportation volume is 8 ml of liquid, which would be the equivalent of duplicate samples for 4 strains of a given virus, which is likely to be greater than the number of strains in the average shipment. The exposure levels would scale approximately proportionally to the volume, so the 8 ml release would result in exposures that are about 5.3% of the value for the maximum reasonably foreseeable release earthquake. The earthquake average RVFV exposures at a distance of 30 m from the point of release and the associated base case probability of infections occurring at that distance or greater are presented in Table 5-7 for each of the three sites. Table 5-7 also presents the scaled transportation exposures and the corresponding probability of infection for each of the three sites. As shown, the greatest probability of infection given a transportation release is 0.093 (i.e., 9.3%) at the urban site. The exposure and probability of one or more infection is lower for all other pathogens.

Table 5-7: Transportation RVFV Exposures and Probabilities of Infection Given Exposure

Parameter	Earthquake	Transportation
Urban site:		
Exposure at 30 m ($CCID_{50}$ or $MICLD_{50}$)	0.66[a]	0.035[b]
Probability of one or more infections in the population given exposure[d]	0.84[c]	0.093
Suburban site:		
Exposure at 30 m ($CCID_{50}$ or $MICLD_{50}$)	2.4[a]	0.13[b]
Probability of one or more infections in the population given exposure[d]	0.038[c]	2.1×10^{-3}
Rural site:		
Exposure at 30($CCID_{50}$ or $MICLD_{50}$)	3.1[a]	0.17[b]
Probability of one or more infections in the population given exposure[d]	0.019 [c]	1.1×10^{-3}

[a] These values were taken from Table 4-13 of Chapter 4.
[b] These values are 5.3% of the corresponding value for the earthquake.
[c] These values are taken from Tables 5-3-14a, -16a, and -17a of Chapter 8, respectively, for the urban, suburban, and rural sites.
[d] Probabilities are one or more infection are for the population 30 m to 1,000 m from the point of release.

In the event of a prompt pathogen aerosol release to the atmosphere due to a transportation crash, the aerosol would likely be transported 30 m in less than ½ minute (the 95th percentile wind speed is 1.5 m/sec at all three sites, as shown in Appendix F, Section F.5.3.2). First responders and potential Good Samaritans are unlikely to arrive at the scene within ½ minute and are unlikely to be exposed to a prompt release. The cargo bed may retain an aerosol release, but Good Samaritans would have no reason to search the cargo bed because it would not contain crash victims. The packaging is required to have the appropriate DOT labeling to warn first responders and Good Samaritans of the risk associated with

handling of the packaging, so a liquid leaking from the package is unlikely to result in contamination exposures. In the event of a crash and package breach, responders can be identified, contacted, and appropriate medical action taken, but that is less certain in the case of a Good Samaritan. Although not likely, the potential for public exposure due to a transportation accident cannot be ruled out.

5.3.5 Variability and Uncertainty

Numerous factors result in variability and uncertainty associated with the above analyses. Table 5-8 identifies the key factors responsible for variability and uncertainty and provides a qualitative assessment of their potential impact on the results.

Table 5-8: Evaluation of Variability and Uncertainty

Variability/ uncertainty	Discussion	Potential effect[a]
Number of truck shipments	The number of truck shipments is based on conservative projections by BUMC, which were confirmed from NIH. The number of shipments could be somewhat greater or less than these estimates. The likelihood of truck-related injuries and fatalities and pathogen releases would be affected proportional to the change in the number of shipments.	Minor conservatism or non-conservatism, which would not affect conclusions
Distance traveled per truck shipment	The analysis is based on a travel distance of 10 km within a 10-km radius of the laboratory. The actual distance traveled could be somewhat greater, depending upon the route taken. The frequency of crash-related injuries/fatalities and pathogen releases would scale proportionally. The potential non-conservatism is expected to be small (e.g., 10%).	Potential non-conservatism
Number of air shipments	The number of air shipments is based on the BUMC policy of using truck transport for shipments within the USA and Canada. The number of shipments could be somewhat greater or less than these estimates. The likelihood of air-related pathogen releases would be affected proportionally.	Minor conservatism or non-conservatism, which would not affect conclusions
Use of over-pack case	It is assumed that all NEIDL shipments will include use of the over-pack case. If the case is not used, the triple-package is much more vulnerable.	Highly non-conservative, if the over-pack case is not used
Use of exclusive-use truck	If the shipment is not exclusive-use, then other cargo could topple onto the NEIDL package and crush it.	Non-conservatism, if other cargo shipped concurrently
Package secured to the middle of the truck bed	If the package is not secured, it is more likely to drop from the truck if the cargo door is left open. If the package is next to the outer edge of the bed, it is more vulnerable to being punctured.	Non-conservatism, if this requirement is not implemented
Single-unit truck	Use of a light truck could increase the likelihood of crushing loads and the accident rates would differ.	Unknown effect, if lighter trucks are used
Single-unit-truck injury rate	The injury rates have declined 60% over the past two decades, so the rates are likely conservative. If the rate continues to decline at this pace, then the injury estimate would be overestimated by about 60% over the facility lifetime of 50 years.	Conservatism (potentially 1.6x over facility lifetime)
Single-unit-truck fatality rate	The fatality rates have declined 40% over the past two decades, so the rates are likely conservative. If the rate continues to decline at this pace, then the fatality estimate would be overestimated by about 40% over the facility lifetime of 50 years	Conservatism (potentially 1.4x over facility lifetime)

Variability/ uncertainty	Discussion	Potential effect[a]
Use of occupant-fatal crash rate for release rate	The NEIDL package is likely to survive many of the occupant-fatal crashes, so this assumption is likely conservative. It is not possible to estimate the degree of conservatism.	Conservatism, unknown extent
Use of rollover and train-collision rate for release rate	The NEIDL package is likely to survive many of the occupant-fatal rollovers and train collisions; however, there may be other types of crashes that result in the package being ejected and crushed. It is not possible to determine whether this assumption is conservative or non-conservative.	Unknown
Use of single crash rates	The crash frequency and severity is likely to be different for different types of roadways (e.g., city streets, country roads, and interstate highways). DOT 2010 does not provide the necessary crash rates for different road types and is a compilation that accounts for the total distance of each type, the vehicle used of each type, and the crash rate for each type. The differences are not expected to change the conclusions.	Unknown
Use of overall crash rates	The crash rates used for this analysis (i.e., DOT 2010) are national average values and specific rates for different types of roadway for these types of crashes are not available. There are certainly differences in the rates for different roadways such as rural versus urban roadways, single-lane versus interstate roadways, and high-speed versus low-speed roadways. Use of the average rates is likely to be conservative for low-speed roadway segments near the sites because the severity of low-speed crashes would be much lower. Use of the average rates may be non-conservative for high-speed non-divided roadways. Overall, the average rates provide reasonable estimates for this analysis and more specific rates are unlikely to alter the conclusions.	Potentially conservative for some roadways and potentially non-conservative for other roadways
Sample volume	The total volume of the samples for a pathogen is assumed to be 8 ml, which accounts for shipment of 4 strains of the virus (duplicate 2 ml vials that are half full). For cases where only one strain is shipped, this value is conservative by a factor of 4.	Expected conservatism of up to 4x for consequences
Sample concentration	The sample concentrations are assumed to be the same as the maximum master/seed/working-stock values. Since these are maximum concentrations, it is expected that they overestimate the actual concentrations, but it is possible that concentrations may occasionally exceed these values. If the estimated concentration is exceeded, the extent of exposure could increase proportionally to this increase.	Likely conservatism but potential non-conservatism, unknown magnitude
Virus samples assumed to be liquid form	The consequence scaling is based on the samples being liquid suspensions. The viruses are expected to be frozen, which results in a lower release fraction. There is a large conservatism of unknown magnitude.	Conservatism, unknown magnitude

[a] This is a qualitative indication of the direction and magnitude of the conservative or non-conservative effect of this factor on risk. Quantitative effects are noted where possible.

While there are many variabilities and uncertainties associated with these calculations, the frequency and consequence estimates are judged to be conservative overall. It is not possible to estimate the degree of

this conservatism. As noted in Table 5-8, the results of this analysis are based specifically on the NEIDL shipping protocol as defined in Section 5.1.3. Changes to those protocols may invalidate these results.

5.3.6 Conclusions

The previous sections estimated the likelihood of crash-injuries, crash-fatalities, and pathogen release due to truck and aircraft crashes. As discussed in Section 5.3.5, there is a large variability and uncertainty associated with these estimates. As discussed in Section 4.1.2.2.4 of Chapter 4, this RA uses frequency categories to address these uncertainties when they cannot be addressed quantitatively. Table 5-9 presents the frequency categories associated with each event for BSL-3 shipments and BSL-4 shipments.

Table 5-9: Frequency Category for Transportation Impacts with 10 km of the Laboratory

Event	Frequency category	
	BSL-3	BSL-4
Crash-related injuries (including the public and driver of the truck)	⦿	○
Crash-related fatalities (including the public and driver of the truck)	○	·
Public infections due to the crash of a truck carrying NEIDL pathogens	·	·
Public infections due to the crash of an aircraft carrying NEIDL pathogens	·	·

Frequency categories: ● = A (1 in 1 to 100 years) ⦿ = B (1 in 100 to 10,000 years)
○ = C (1 in 10,000 to 1,000,000 years) · = D (1 in > 1 million years)

The conclusion of this transportation analysis can be summarized as follows:

- Crash-related injuries and fatalities from NEIDL shipments are more likely than pathogen-related infections or fatalities in the public. A crash-related injury due to truck or air transport of NEIDL pathogens has an estimated frequency of one in 100 to 10,000 years and a crash-related fatality has an estimated frequency of one in 10,000 to 1 million years. An infectious pathogen release from a truck or aircraft crash resulting in an infection has an estimated frequency of one in more than 1 million years, which is considered beyond reasonably foreseeable.
- In the event of an infectious pathogen release from a transportation crash, the exposure levels are expected to be no greater than 5% of the exposures resulting from the maximum reasonably foreseeable release earthquake. The probability of one or more infections from a pathogen release due to a transportation crash is also smaller than the probability for the maximum reasonably foreseeable release earthquake.

These conclusions are valid for all three sites being evaluated (i.e., urban, suburban, and rural), since it is assumed the protocols followed for pathogen shipments would be similar for all sites.

5.4 References

DOA 1969 Department of the Army, Fort Detrick, Technical Study 67, Containers for Chemical/Biological Agents Drop-Tested from Aircraft, M. Barbeito and A. Wedum, March 1969. Available on the web at <http://www.dtic.mil/cgi-bin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=AD0686313>. Accessed 05/20/2011.

DOE 2002 "A Resource Handbook on DOE Transportation Risk Assessment," DOE/EM/NTP/HB-01, July 2002. Available on the web at: <http://nepa.energy.gov/documents/DOETransportationRiskAssessment.pdf>. Accessed 05/20/2011.

DOE 2006 DOE STD 3014-2006, Accident Analysis for Aircraft Crash into Hazardous Facilities, U.S. Department of Energy, available online at <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/std3014/std3014.pdf>. Accessed 03/26/2011.

DOT 2006 Transporting Infectious Substances Safely, Guide to Changes, Effective October 1, 2006. Available on the web at: http://www.aps.anl.gov/Safety_and_Training/Hazardous_Materials/Transporting%20Infectious%20Substances%20Safely.pdf.pdf . Accessed May 24, 2011.

DOT 2010 U.S. Department of Transportation, Federal Motor Carrier Safety Administration Analysis Division, *Large Truck and Bus Crash Facts-2008*, FMCSA-RRA-10-043, March 2010. Available on the internet at: <http://www.fmcsa.dot.gov/facts-research/LTBCF2008/LargeTruckandBusCrashFacts2008.pdf>. Accessed 10/12/2011.

DOT 2010a U.S. Department of Transportation, National Highway Traffic Safety Administration, *2009 FARS Coding and Validation Manual*, DOT HS 811 353, July 2010. Available on the internet at: <http://www-nrd.nhtsa.dot.gov/Pubs/811353.pdf>. Accessed 10/11/2011.

DOT 2011 U.S. Department of Transportation, National Highway Traffic Safety Administration (NHTSA), *Traffic Safety Facts-2009 Data for Large Trucks*, DOT HS 811 388, Updated September 2011. Available on the internet at: <http://www-nrd.nhtsa.dot.gov/pubs/811387.pdf >. Accessed 10/12/2011.

Murphy 2011 J. R. Murphy, BUMC, e-mail to N. Boyd, NIH dated Sept. 20, 2011 with attachment.

NRC 2010 "Evaluation of the Health and Safety Risks of the New USAMRIID High Containment Facilities at Fort Detrick, Maryland." Committee to Review the Health and Safety Risks of High Biocontainment Laboratories at Fort Detrick Board on Life Sciences Division on Earth and Life Studies, National Research Council of the National Academies, 2010. Available on the web at <http://www.nap.edu/openbook.php?record_id=12871>. Accessed August 26, 2010.

Nuclear Regulatory Commission 2003 "Environmental Review Guidance for Licensing Actions Associated with NMSS Programs," NUREG-1748. Available on the internet at:

	<http://www.nrc.gov/reading-rm/doc-collections/nuregs/staff/sr1748/>. Accessed April 2011.
Pelican 2011	Pelican 0340 Protector 18" Cube Case, Description and Specifications, available on-line at <http://www.pelican-case.com/0340.html>. Accessed May 14, 2011.
Sarstedt 2011	Sarstedt Screw Cap Micro Tube Description, available online at <http://www.sarstedt.com/php/tube.php?artnr=72.694.006&lnr=>. Accessed May 10, 2011.

Contents

6. Threat Assessment Methodology Overview

6.1 Introduction

The objective of this overview of the methodology for the Threat Assessment (TA) conducted for this RA is 1) to inform the reader of the need for the TA; 2) to provide the detailed, in-depth review of the process utilized in determining the threat, consequence and vulnerabilities of the security systems in place at NEIDL; and 3) to summarize the consequences of a malevolent act to NEIDL at the urban, suburban, and rural site.

6.1.1 Security Systems and Critical Assets

The TA was developed as a component of the RA in response to concerns raised by the public regarding the capability of the facility's security systems inclusive of personnel, policies, and procedures in place to prevent or withstand a malevolent action (e.g., disgruntled or unbalanced lab worker, terrorist action) against critical systems and assets at NEIDL that could result in the exposure of personnel or release of a pathogen into the community. It is important to note that for the context of the TA, the term *security system* is used to define an integrated approach to security that includes

- Security and police personnel and procedures (stationary and patrol);
- Electronic systems (such as access control, alarms, and cameras);
- Programs (including personnel hiring practices, two-person rules, select agent clearance);
- Facility design and construction; and
- Policy and procedures designed to protect NEIDL's critical assets (the facility, systems, personnel, and sensitive information).

One of the primary goals of a well designed security system, as well as one of the overarching philosophies in security and threat assessment practice, is the understanding that by protecting the high-value assets against a sophisticated adversary, the systems in place will also provide sufficient protection of all assets against lesser threats (i.e., adversaries).

As the TA was developed, the security risks and effective mitigation strategies for ensuring the secure operation of NEIDL at the urban, suburban, and rural sites were also validated by the following:

- Identifying and evaluating threats (i.e., adversaries) at each of the three comparable sites;
- Determining the likelihood of attack of those adversaries on NEIDL;
- Identifying the critical assets associated with NEIDL;

- Assessing the potential consequences associated with the impact or loss of critical assets (as defined by the previous Threat and Risk Assessment (TRA) conducted by Applied Risk Management, LLC (ARM) and validated by the Tetra Tech Team during this TA);
- Quantifying vulnerabilities of physical and operational security;
- Calculating the cumulative risks associated with the threats and consequences with respect to each comparable site being evaluated; and
- Providing effective mitigation measures to ensure secure operations against the identified threats.

In order to evaluate the security system in relation to the protection of the critical and non-critical assets, the TA provides an evaluation of *initiating events* associated with malevolent acts that could result in a pathogen release or loss. The TA developed and evaluated numerous malevolent act scenarios that had the possibility and capability of breaching engineering and security systems within the facility resulting in an exposure of laboratory workers or the public. Additionally, based on the results and outcomes of the scenarios developed and conducted against the security systems, the Tetra Tech team identified and recommended mitigation strategies to reduce the effects of deliberate efforts by terrorists to destroy, incapacitate, or exploit the facility's mission, pathogens, and technology (i.e., high-value, critical assets, the loss of which would have significant negative effects on the operation of the facility and possibly on the public).

6.1.2 Restricted Distribution of Security Information

Based on the information contained within the TA (including but not limited to security system's design and implementation, response capabilities, and system upgrades) the TA is required to be maintained as a "Controlled Document" under the provisions in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (the Bioterrorism Act). The Bioterrorism Act was passed by Congress following the events of September 11 in order to enhance the security of the United States and improve, prevent, prepare for, and respond to bioterrorism public health emergencies.

6.2 Threat Assessment Methodology

The following is an overview of the methodology that was employed to quantify risks associated with malevolent acts against the critical assets at NEIDL. Additional information pertaining to risk evaluation in security applications can be found in references cited throughout this chapter. The TA accomplished this objective by answering the following questions:

- What needs to be protected (i.e., what are the high-value, critical assets)?

- What are their vulnerabilities (i.e., what are the gaps in the security system protecting those assets)?
- What are the threats (i.e., who are the potential adversaries with the capability to defeat the security system to destroy or illegally obtain those assets)?
- What can be done to minimize the risk of a malevolent act (i.e., how can the high-value critical assets be protected more effectively)?

The evaluation of risks associated with physical and operational security elements for NEIDL was based on the standard risk equation, the most basic form of which is represented as follows (Andrews 2002):

$$\text{Risk} = P \times C$$

where

P is probability, referring to the likelihood or chances of some adverse event;

C is consequence, referring to the effect of the adverse event.

In the security application, the generalized risk equation as defined in DHS's National Infrastructure Protection Plan (NIPP) is represented as follows (NIPP 2008):

$$\text{Risk} = f(T, V, C)$$

where

T is the *threat* which represents the likelihood of an adversary mounting a successful attack against a specific target;

V is the *vulnerability* of the security system's ability to protect an identified asset against a specific adversary (i.e., threat);

C is the *consequence* associated with the loss of a specific asset or component based on a specific threat (i.e., attack) scenario.

Therefore, the analysis associated with developing the TA primarily involved the definition and comparison of the T, V, and C variables in NEIDL's location at the urban site and its theoretical risks at the suburban and rural sites.

The following sections present an overview of the methods employed to define each of those primary variables to estimate the risks associated with NEIDL in its location at the urban site and its theoretical risks at the suburban and rural sites.

6.3 Review of Previous Threat and Risk Assessments

To accurately assess the risk to NEIDL associated with internal and external malevolent acts and to evaluate those same risks to similar facilities at the comparable sites, it was necessary to first conduct a thorough assessment of the effectiveness of NEIDL's security systems as constructed at the urban location. The effectiveness of the security system is typically determined by evaluating how effective system components are at deterring, detecting and assessing, and delaying and responding to malevolent acts. The assessment of those security systems was required to provide the baseline condition (i.e., to define the starting point). The process began by reviewing the previous TA conducted by ARM to determine the baseline condition of the facility.

The initial NEIDL threat risk assessment (TRA) was conducted as part of the conceptual design process and published in a limited-distribution report entitled *Risk and Vulnerability Assessment, Boston University, BU Medical Center, National Bio-Containment Laboratory* (NBL), dated August 5, 2004 (ARM 2004). In addition, a report update was issued on June 30, 2008, that included updated threat information (ARM 2008). The Tetra Tech TA team reviewed both of those documents and agreed with the identified targets (i.e., the high-value critical assets) and the consequences of their loss. However, because the initial assessment was performed before the building's final design and ultimate construction (i.e., the initial assessment evaluated security systems and operations at the conceptual phase), it lacked specific information related to the *as-built* condition of the facility. Additionally, the initial TRA did not analyze the risk associated with NEIDL at the suburban and rural sites. Therefore, an additional analysis (i.e., this TA) was required to accurately characterize the security of the built facility at the urban location, in addition to compare the threat and risks at the suburban and rural sites

6.4 Defining Threat

The threat variable in the risk equation, *T*, represents the relationship between a defined adversary with specific capabilities, motives and objectives, and a specified target (i.e., something the adversary identifies as having value to his specific motives and objectives). The threat (i.e., *T*) is therefore determined by combining specific adversary abilities with identified NEIDL critical assets to arrive at an estimate of the likelihood that a specific adversary would target a specific asset. The following steps were required to accomplish that task:

1. Analyzing crime statistics: This analysis was performed by use of the CAP Index, Inc., CRIMECAST© tool to identify specific crimes and, therefore, specific types of criminals within each of the three regions (urban, suburban and rural);

2. Determining the local threat environment: This step was accomplished by conducting site visits and interviews with federal, state, and local law enforcement agencies to define the history and current intelligence and capabilities related to adversary actions within the regions of the three comparable sites;
3. Collecting and evaluating threat intelligence: This step entailed research to identify threats against and attacks on similar facilities worldwide to develop the general and relevant threat information for each of the three comparable sites;
4. Determining the target *attractiveness* (i.e., the target's suitability to an adversary's primary goal) to each identified adversary.

Each of those components, described in the following subsections, was used to define the threat component of risk for NEIDL at each of the three locations.

6.4.1 CAP Index Analysis

The TA team used the services of CAP Index, Inc., to collect and categorize crime statistics by using the CRIMECAST® data analysis and assessment tool, which is continuously maintained by a team of criminologists, statisticians, and security and mapping professionals. This analytical technology enabled the TA team to generate predictive reports and graphics that provided precise information for each of the study locations and by crime and loss history via a scoring mechanism designed to objectively measure risk of criminal events.

Using regional crime data, the CRIMECAST® system provided a risk profile for each of the regions around the three comparable sites and an assessment of the crime in the surrounding areas. The regions were defined on the basis of the mailing addresses for NEIDL in downtown Boston (at 650 Albany Street, Boston, Massachusetts), the former BU Corporate Education Center (at 72 Tyng Road in Tyngsboro, Massachusetts), and the BU Sargent Center for Outdoor Education (at 36 Sargent Camp Road, Hancock, New Hampshire). In addition, global positioning system coordinates were determined (via Google Earth) for the geographical center of each site and validated against the mailing address before calculating the CAP indices.

The crimes against persons and crimes against property (CAP) profiles generated for each of the three comparable sites included a breakdown of offenses, along with a rating for the three regions. All CAP scores reported are based on a scale of 0–2,000, with 0 representing the lowest risk and 2,000 representing the highest. The rating scale is normalized to national and state averages (i.e., 100 is defined as the

average score). For example, using this scale, a score of 200 would indicate that the frequency of a crime in a region is at twice the normalized national or state average rate (depending on the data being compared in the tool) for that specific crime category. Similarly, a score of 600 would mean a risk level is 6 times higher than average. Conversely, a CAP score of 25 would indicate that the risk would be 0.25 times (i.e., one-quarter) the national or state average.

The CAP scores were calculated using two different radial distances from the geographical center of each actual and comparable location of NEIDL. The first analysis (i.e., the CRIMECAST® Standard Report) used a radius of 3 miles to calculate the index. The second analysis (i.e., the CRIMECAST® Expansion Report) applied the methodology to a radius of 6 miles. Producing and evaluating both reports allowed the TA team to determine the effect of the regional setting on the overall indices. For example, in a more rural setting, the CAP Index would be anticipated to increase in the expansion report analysis if neighboring communities with more dense populations are between 3 to 6 miles from the center of the observation area (i.e., the location of the comparable site).

Because of the sensitive nature of the crime statistical information collected and analyzed during this portion of the TA, that information is not included in this document. However, generally the CAP indices (i.e., the crime rates) decrease as the distance from downtown Boston increases.

6.4.2 Determining the Local Threat Environments

The local threat environment was defined by conducting interviews with federal (i.e., Federal Bureau of Investigation's Joint Terrorism Task Force representatives), state, and local law enforcement entities at each of the three comparable sites. In addition, representatives from the BU Police Department and the BU executive director of Public Safety were interviewed to obtain an overview of the crime history at the BUMC campus. Interviews were conducted during November 2008 and January 2009 by using a standardized set of data collection forms and threat definitions. Standardization assures consistency in the manner in which questions were presented to officials, and responses were recorded. The data collection forms were used to identify site-specific threat and crime information for each site. The information gathered during each interview was broken down into five categories:

1. History of threat, activity, or attacks in the region and area;
2. Identifiable threat intelligence;
3. Potential adversarial or threat groups present in the region;
4. Motivation, intent, and capabilities of identified threats;
5. Public safety response resources.

The results of the interviews and a compilation of the data collection forms are included in the TA.

6.4.3 Determining Threat Information from National and International Intelligence

The next phase of the threat definition involved developing a threat intelligence report to be used in supporting and creating a threat definition and threat spectrum for evaluating the potential NEIDL security risks at each of the three comparable sites from national and international terrorist organizations. The focus of the research was on the potential threat levels determined to be applicable to the three sites within the context of the threat faced, both past and present, by similar domestic and foreign facilities. The task consisted of the identification and detail compilation of any and all threats, attacks, and other adversarial actions against animal bio-research laboratories or similar facilities (whether government, private, or academic), both domestically and internationally. The task included the identification as to whether those types of facilities had been targeted in the past by terrorists, extremists, or other threat or adversarial groups to determine the current NEIDL threat environment at each of the three comparable sites. The intelligence review effort, therefore, focused on the following areas of research and analysis:

1. The general biosecurity environment worldwide;
2. Past events and current threats against 13 domestic BSL-3 and two domestic BSL-4 facilities identified by NIH's NIAID;
3. The general threat environment at each of the NEIDL comparable sites.

Those tasks were accomplished by using thorough research methods, including both analyst- and software-based techniques to research open-source materials. By using predefined screening criteria and keyword searches, the process allowed the accumulation of a considerable amount of documentation pertaining to topics of interest and aided in the efficient analysis to determine the relevance of the material collected.

Because of the uncertain nature of potential threats, the analysis considered both official and unofficial information sources to gain an understanding of the overall threat environment, the perspective of policy makers, and the opinions of the general population, including dissenting groups within the communities where BSLs existed or were being considered for future sites of similar facilities. Those sources included transnational media, foreign state-run media, local media, government sites (including DHS, FBI, White House, Government Accountability Office, Congressional Research Service, Interpol, United Nations, World Health Organization, state, county, and local law enforcement agencies, state civilian government, county civilian government, and local civilian government), university sites, nongovernment players, blogs, chat-rooms, and online forums.

The analyses used the same threat categories that were used during interviews to identify and categorize individuals and groups that could pose an identifiable threat to the facility specific to each of the three locations being investigated.

6.4.4 Target Attractiveness

An assessment of a target's attractiveness was performed to determine how likely an adversary would be to plan and carry out an attack on an asset. The target's attractiveness, therefore, becomes a function of the adversary's assessment that the target is critical to their mission, it is easily accessible, it has some identifiable vulnerability, and its destruction or loss would have the desired effect (e.g., panic among the public, death of innocent victims, draw attention to the terrorist's cause). For those reasons, a target's attractiveness is an integral part of the determination of the likelihood of an attack against a specific high-value critical asset.

The TA team performed an analysis of the targets (i.e., the high-value, critical assets) that the identified adversaries in each of the three regions would be most likely to attack. That analysis was used to identify the areas within the NEIDL that must be considered as vital (i.e., high-consequence, critical assets) and how they should be best protected against the identified adversaries. The outcome of the target analysis was used to define the target portion of the threat variable in the risk equation.

Both the CARVER and MSHARPP methodologies were used to evaluate the attractiveness of specific features and components of the NEIDL facility as potential targets from the perspective of the adversaries identified in each of the three regions. Both of those methodologies were developed by the Department of Defense for offensive target analysis based on military objectives. MSHARPP is a targeting analysis tool geared more closely to assessing personnel vulnerabilities relative to terrorist scenarios, whereas the CARVER method is used by Special Forces and commandos to target enemy infrastructure including public works facilities such as bridges and power plants. Those methodologies were appropriate for use as potential adversaries, both overt and covert, would employ similar methods to select and target identified assets (e.g., facilities, personnel).

In addition to the CARVER and MSHARPP methods, target attractiveness was evaluated relative to other critical assets in each of the three regions by use of the CAP Index's Proximity Analysis Tool. Each of those methodologies and their individual evaluation criteria are described in the detail in the TA document.

6.5 Defining Consequences

To quantify the variable *C* in the risk equation, consequences were defined in terms of the value of the loss of an asset (e.g., such as in the loss of NEIDL's mission or reputation, the intentional release of a pathogen). The value of the loss was assigned to the identified high-value assets for purposes of providing reasonably credible bounding threat scenarios for detailed analysis. The consequence of loss of an asset was defined by determining its criticality (i.e., the impact from the loss). NEIDL's mission statement was used to establish the list of assets for consideration in determining criticality (i.e., the high-value critical assets). Subsequently, a list of high-value critical assets was developed, compared, and integrated with the previous list compiled for the initial TA. The list was then used in the baseline risk calculation.

6.6 Determining Vulnerabilities

After all the threats and critical assets (and the consequences of their loss) were defined, the TA team began the process of determining the vulnerabilities of the security systems protecting those assets at NEIDL at each of the three locations to ultimately calculate the baseline risk at each of the three locations. In security assessments, vulnerabilities are defined as the measure of the likelihood of a specific adversary and its ability to cause damage to an identified asset or target. The method for determining vulnerabilities, therefore, involved developing specific scenarios that tested the ability of NEIDL security systems (which included hardware, software, procedures, and personnel) to prevent an adversary from accomplishing their mission against a specific target. The process involved using the threat spectrum developed for each of the three regions and the targeting analysis for the high-value, critical assets to evaluate the probability of success. The higher the vulnerability (i.e., the less effective the systems in place are at protecting a specific asset), the higher the risk to the facility.

The current NEIDLs' security system (which included both physical and operational elements) was used to simulate the vulnerabilities and calculate the risk of occurrence for a specified adversary, target, and consequence (i.e., for each identified scenario). The security system advantage of the security system in place allowed the team to quantify the vulnerabilities identified to provide the basis for mitigation strategies to reduce the risk to the facility. That evaluation identified two potential mitigations stategies:

- Vulnerabilities that must be mitigated with NEIDL at its actual location;
- Vulnerabilities that would need to be mitigated if the facility were at one of the other two comparable sites.

A *baseline* operational security environment (i.e., the security system in place at NEIDL) was defined to evaluate the threat-consequence scenarios and to identify necessary risk mitigation features on the basis of vulnerabilities identified during the analysis of the scenarios. The baseline conditions are outlined in the TA and provide the framework for measuring the effectiveness of NEIDL controls and barriers against the defined threats and the potential consequences related to the loss of critical assets. The same NEIDL-specific baseline security system components were used to model the conditions at the comparable sites.

The baseline model for developing the NEIDL TA defines the operational security configuration on the basis of existing features at the facility. The individual components of the baseline security system model are categorized by the physical protection system functions of deterrence, detection, delay, and response. The system effectiveness was determined for each scenario and then used to calculate a failure probability for each scenario.

The TA includes the analysis of threat-consequence scenarios against the identified high-consequence assets with site-specific responses to determine risk from the facility being at any one of the three sites. In addition, as vulnerabilities were identified that increased the risk, mitigation strategies were developed and additional risk calculations performed to demonstrate a lower mitigated risk.

6.6.1 Overview of Threat-Consequence Scenarios

The BRP concluded that additional studies were needed to adequately address judicial requests and concerns of the public and as a result made general and specific recommendations with regard to the agents and scenarios to be studied and the methodologies for risk assessment. The BRP also indicated that the scenarios evaluated should include the possibility of exposures resulting from malevolent actions. The following types of scenarios involving malevolent actions were recommended for study by the BRP:

- Internal breach of security, such as release/exposure due to malevolent actions;
- External breach of security, such as a terrorist attack.

The TA team developed realistic, reality-based scenarios keeping in mind the *worst-case* possibility, as required by the BRP as the framework for NIH BRP deliberations.

To identify vulnerabilities associated with the NEIDL security systems at the three comparable sites, adversary- and target-specific scenarios were developed to represent the theoretical risks associated with a site location and its physical and operation security systems. The number of site-specific, threat-consequence scenarios developed was based on the range of adversaries identified, the critical assets'

value, and the identified security system vulnerabilities. The vulnerability variable, *V*, in the security risk equation was ultimately determined by specifically modeling the effectiveness of the in-place physical and operational security systems and subsystems for specific threat-consequence scenarios. The identified vulnerabilities then become the best estimate of the security system's effectiveness in terms of a probability of an adversary attack being a success (Garcia 2006).

The adversary-consequence scenarios were reviewed by BU Police Department personnel and the BU executive director of Public Safety and determined to be credible bounding scenarios. In addition, developing NEIDL's baseline security system model (i.e., the description of the systems planned and in place) was developed and based on on-site observations and research of the security systems' ability to deter, detect, delay, and respond to a threat.

The potential for terrorists, extremists, criminals, malicious employees, and persons with psychopathic tendencies were all considered in developing specific threat-consequence scenarios for analysis. The evaluation of those scenarios was based on the site-specific adversaries in the three comparable geographic areas. The intended target (i.e., the high-value, critical asset determined to be most attractive to each specific adversary by the target attractiveness evaluation) was assigned to the scenarios with regional relevance on the basis of the adversaries' capabilities in the region and the relative value of the various targets for each adversary.

Tables 6-1 and 6-2 provide a general description of the scenarios that were developed for insider and outsider threat analysis, respectively. Table 6-3 provides a general description of the scenarios that involved insiders in collusion with an outsider. Those scenarios were reviewed by the BU executive director of Public Safety and NIH security specialists within the Office of Research Services and were determined to be *realistic and credible bounding.*

Table 6-1. Insider Scenarios Relative to BRP Requirements for Evaluation

Scenario	Adversary–Objective–Tactic
Scenario 1. Threatened or Actual Release of Pathogen into the Environment	Adversary: Disgruntled employee Objective: Intentional release of pathogen to cause harm Tactic: Surreptitious removal and release of pathogen
Scenario 2. Loss of Mission	Adversary: Disgruntled employee Objective: To cause financial harm or facility destruction Tactic: Sabotage the facility containment system(s) or lab work areas
Scenario 3. Simple Theft	Adversary: Employee (criminal) Objective: Monetary gain Tactic: Using deceit or stealth entry to gain access to the facility and steal a pathogen
Scenario 4. Loss of Laboratory Personnel	Adversary: Disgruntled employee (psychotic active shooter) Objective: Kill researchers at the NEIDL facility Tactic: Smuggle a weapon into the NEIDL facility
Scenario 5. Loss of Laboratory Personnel	Adversary: Disgruntled employee (psychotic insider) Objective: Cause maximum number of deaths Tactic: Introduce a weapon into the NEIDL facility

Table 6-2. Outsider Scenarios Relative to BRP Requirements for Evaluation

Scenario	Adversary–Objective–Tactic
Scenario 6. Extremist Attack	Adversary: External attack (extremist group) Objective: Rendering the NEIDL facility unusable and possible secondary release of pathogen Tactic: Use an improvised explosive device (IED) to attack the NEIDL
Scenario 7. Release of Pathogen into the Environment	Adversary: External attack (international terrorists) Objective: Panic and the disruption of the food chain Tactic: Acquire and release pathogen off-site
Scenario 8. Loss of Laboratory Personnel	Adversary: External attack (extremist group) Objective: Wound or kill key NEIDL personnel Tactic: Introduce an IED into the NEIDL facility

Table 6-3. Insider, in Collusion with Outsiders, Scenarios

Scenario	Adversary–Objective–Tactic
Scenario 9. Theft of a Select Agent from the Delivery Vehicle	Adversary: Outsider (insider assisted by means of mail delivery schedule) Objective: Intentional theft of Select Agent for the purpose of resale or release into the public Tactic: Theft of a Select Agent, by the takeover of a common carrier (e.g., mail service, FedEx) delivery vehicle
Scenario 10. Loss of Pathogen	Adversary: Employee (criminal) Objective: Obtain a pathogen for foreign entity Tactic: Foreign intelligence agency, using deceit or coercion, develops a relationship with an employee to remove a pathogen from the NEIDL facility
Scenario 11. Loss of Technology	Adversary: Employee (criminal in collusion with foreign intelligence agency) Objective: Theft of sensitive technology Tactic: Stealing sensitive technology for a foreign intelligence agency

6.6.2 Scenario Analyses

To evaluate each of these scenarios, the TA team, accompanied by NEIDL subject matter experts, toured the facility gathering information pertaining to effectiveness of the various components of the security system to the threat-consequence scenarios created. In addition, the TA team evaluated system component specifications and performance data of various systems either in place at NEIDL or anticipated to be in place before beginning research with select agents. The security systems were then evaluated against each adversary-consequence scenario on the basis of their ability to deter, detect and assess, delay and respond in a timely and effective manner to each malevolent act. The analyses provided data in each of those categories that were ultimately used to determine the success or failure of the combined systems to interdict an adversary before they could successful accomplish their mission. The following paragraphs define and describe each of those features of a security system that were evaluated.

Deterrence is customarily viewed as an implementable measure that is perceived by potential adversaries as an indication that it would be too difficult to defeat the security system either from the perception of detection, the difficulty of defeat, or the timeliness of a robust and interdicting response (Garcia 2001). Deterrence afforded by a physical security system is generally recognized as a difficult function to measure or observe. However, deterrence features (e.g., visible guards, a substantial fence delineating the plant boundary) are generally credited with defining the controlled areas in and around a site. The basis for deterring any incursion into NEIDL begins with discouraging and preventing unauthorized personnel

from gaining access to the facility site. To that end, the facility's visible guard presence, signage, landscaping, and lighting were evaluated.

Detection is typically defined as a system's (or a component of a system's) ability to discover an adversary's presence or unauthorized actions. To initiate a response, detection must also include an assessment that validates the incursion as a potentially malevolent act. Therefore, as used in the context of the TA, assessment was defined as the ability to detect and receive the intrusion information, to determine what actions are required, to determine if those actions are within the capabilities of the response resources, and to communicate the information in near real-time (Garcia 2001). Each of the components of NEIDL's security system that contributed to the detection of a malevolent act was evaluated to determine its effectiveness relative to each scenario evaluated.

To determine a system component's ability to detect and assess the initiation of a malevolent act, all the systems and subsystems in place were evaluated on the basis of each of NEIDL-specific scenarios. Each of the system components with detection and assessment capabilities were included along with their respective effectiveness for the calculation of risk for each scenario. The highest effectiveness value was chosen for each scenario as representative of the point of most probable detection because that would be indicative of the point with the highest probability the malevolent action or intent would be detected.

Delay, as used to describe a component of a security system, is the slowing of an adversary's progress. The design of an effective security system requires that the delay features incorporated into the system are sufficient to allow the response force appropriate time to interdict the adversary (Garcia 2001). Delay is accomplished at NEIDL by using physical techniques and processes such as fencing, locks, reinforced walls, clear zones (i.e., open areas on both sides of the perimeter barrier to provide an unobstructed view of the barrier and the ground adjacent to it), and procedures. Each element of the NEIDL security system (i.e., physical techniques and processes) was evaluated to determine the total delay afforded by the system relative to each scenario evaluated.

Until detected, an adversary would have an infinite amount of time to accomplish the mission; however, once an adversary is detected, the amount of time available to accomplish the mission becomes an amount bounded by the time required for a response force to interrupt or stop the activity. To evaluate delay for each threat-consequence scenario as it relates to the calculation of risk, it must first be recognized that, only those system components in place that would be capable of providing delay after the most probable

point of detection along the path to the objective being reached were evaluated on the basis of each NEIDL scenario.

The delay values for security system features were derived from the tabulated values for the effectives of security system components in the Vulnerability and Assessment Methodology (January 2005). In addition to the delay afforded by specific security system components, there is also a quantifiable delay associated with the distances an adversary must traverse to reach a target. To determine the delay time associated with an adversary traversing a defined distance, the TA team developed average walking and running speeds [i.e., average walking and running rates expressed in feet per second (ft/sec)] using literature values to determine the time it would take for the adversary to traverse the total distance from the point of most probable detection to the targeted critical asset for each scenario. The total delay, (total time associated with security features and distances traversed) were then compared to the times from responding forces to reach and interdict an adversary.

Response, in the security arena, is defined by two components: (1) the ability for suitably trained personnel to arrive at the scene of an unauthorized action (i.e., incident) in time to challenge the person(s) attempting the unauthorized activity; and (2) the capabilities and effectiveness of a responding force.

Those two components were evaluated in each of the three regions to determine the effectiveness of a response to each specific threat-consequence scenario. The response time was evaluated by comparing the time between receipt of a detection and assessment of an adversary action and the mobilization and interruption of that action by a responding entity. The effectiveness of the response was determined using a combination of on-site and off-site response forces according to their level of training, equipment, and other specialized capabilities that could be required against a scenario (e.g., explosive ordinance disposal, SWAT).

6.7 Evaluating Security System Vulnerabilities

To determine the overall effectiveness for the security systems in place at each location, the TA team determined the most ineffective component of the security systems ability to detect, delay, and respond to a scenario as being representative of the overall effectiveness of the security system. That approach is appropriate because the lowest of those elements represents lowest effectiveness (i.e., the highest vulnerable) of the security system components associated with each scenario. That value was then used in the risk equation as a numerical representation of the overall vulnerability of the protectiveness of the

security system and the likelihood of success of the adversary. Where those results were determined to be unacceptable, system improvements (i.e., mitigations) were recommended.

Table 6-4 presents an example of a complete analysis of an adversary-consequence scenario and demonstrates the relationship between the probabilities of failure of the security system to the risk. In other words, as the probability of a security system (or component) failure decreases, so does the risk from a scenario. In the example of the baseline condition, the extremist group would be successful 421 times out of every 1,000 attempts it made to wound or kill key personnel. After mitigations were implemented (i.e., features, personnel or additional procedures) to address the identified vulnerabilities, their success rate would decrease to only being successful 3.5 times out of every 1,000 attempts.

Table 6-4. Example Scenario Analysis

Scenario: Extremist group **Objective: Wound or kill key personnel** **Tactic: Use deceit to introduce and detonate an IED**	**T**	**C**	**V**	**Risk per 1,000**
Baseline analysis results based on site-specific threat spectrum, and vulnerabilities associated with the security systems' ability to deter, detect, delay, and respond to a malevolent act	0.78	0.90	0.6	421
Mitigated analysis results based on site-specific threat spectrum, and vulnerabilities associated with the security systems' ability to deter, detect, delay, and respond to a malevolent act *after incorporating mitigation strategies*	0.78	0.90	0.005	3.5

6.8 Threat Assessment Results in Relation to the RA

The Threat Assessment identifies 11 scenarios that are described in detail, but it does not provide an analysis of potential consequences of those scenarios. DOE NEPA Guidance (DOE 2002) acknowledges the difficulty of analyzing malevolent acts and suggests that the consequences could be compared to consequences of severe accidents because the forces resulting in releases of hazardous materials could be similar. The scenarios evaluated as part of the TA were assigned a probability and a determination was made as to whether a release of a pathogen could be reasonably expected to occur if the adversary was successful in accomplishing their mission.

Section 4.2.6 of Chapter 4 identifies accidents that are similar to a malevolent act scenario and provides a discussion of the potential similarities and differences in consequences. It was concluded that the consequences resulting from the malevolent act scenarios associated with a pathogen release from NEIDL are bounded by the MRF earthquake consequences. Therefore, consequences were not calculated specifically for malevolent acts.

The consequences of malevolent acts could be highly variable depending on the intent and success of the adversary. Malevolent acts that attempt to disperse the pathogen to members of the public can be grouped into two types: (1) those that attempt to release directly from the building; and (2) those that attempt to remove the pathogen from the facility for release at a later time in the location of their choosing. Each of those types is discussed below.

Releases directly from the facility could be compared to accidental releases because the release locations are the same, the inventory is limited, and the mechanism of release can be characterized. For example, malevolent act scenarios of this type could involve the use of an improvised explosive device to damage the containment boundary and the HVAC system, including the HEPA filters. The consequences of the MRF earthquake are expected to exceed those of these malevolent scenarios the following reasons:

1. The viable pathogen inventory in the HEPA filters is expected to be much lower than the maximum working volume, which is the inventory used for the earthquake analysis. Not only is the pathogen inventory in the HEPA filter expected to be less, but the continual flow of dry air through the filter could inactivate much of the pathogen inventory that might accumulate in a filter.
2. Shock to a HEPA filter is approximately 2×10^{-6} (DOE 2000), which is less than the release fraction for the MRF earthquake (i.e., 2.8×10^{-5}, as shown in Section F.8.3.4.4.3 of Appendix F).
3. If the HVAC blowers continue to operate, and/or there is a breach of containment and structure, then the release would likely be through the stack or above ground level, rather than the ground-level release assumed for the MRF earthquake. An above-ground-level release results in large dilution of the pathogens, especially for people near the point of release, which results in low exposure levels.

For those reasons, the consequences of the malevolent scenarios are expected to be much less than the consequences of the MRF earthquake and could be closer to the consequences of the BDB earthquake.

Malevolent acts that attempt to remove the pathogen from the facility cannot be readily compared to accidental releases because the locations are different and the release mechanisms could be different. For example, if an adversary were successful and removed a pathogen from the facility, it might be possible to use a nebulizer and fans in a highly populated area to deliver high exposure levels to a large number of people. However, the release could be attempted at any location of the adversary's choosing. While the likelihood of success of this type of scenario is low, the potential consequences of such a release, after the

fact at an unknown location, would be speculative and is beyond the scope of this RA to attempt to characterize the consequences of this type of scenario.

Table 6-5 addresses each malevolent scenario, identifies a similar accident scenario where one exists, and provides a discussion.

Table 6-5. Relationship of the Consequences of Threat Assessment Scenarios and Accident Scenarios

Malevolent Act Scenario	Accident Scenario	Discussion
1. Threatened or actual release of pathogen into the public	No similar events	As discussed in Section G.10.2.2, there are no similar events for scenarios involving the removal of pathogens from the laboratory. Also, the release location is an unknown.
2. Loss of mission	Bounded by the MRF earthquake	This malevolent scenario involves an explosive device. As discussed in Section G.10.2.1, the consequences are bounded by the MRF earthquake
3. Simple theft	No similar events	See discussion for malevolent scenario 1.
4. Loss of laboratory personnel (first scenario)	No similar events	If successful, this malevolent act results in personnel death(s) from weapons and does not involve pathogens. The accident analyses addressed only pathogen exposures.
5. Loss of laboratory personnel (second scenario)	No similar events	See the discussion for malevolent scenario 4.
6. Extremist attack	Bounded by the MRF earthquake	See the discussion for malevolent scenario 2.
7. Release of pathogen into the environment	No similar events	See the discussion for malevolent scenario 1.
8. Loss of laboratory personnel (third scenario)	No similar events	See the discussion for malevolent scenario 4.
9. Theft of a Select Agent from the delivery vehicle	No similar events	See the discussion for malevolent scenario 1.
10. Loss of pathogen	No similar events	See the discussion for malevolent scenario 1.
11. Loss of technology	No similar events	There are no direct consequences from the loss of technology. Secondary consequences are speculative because they depend on the nature of the technology stolen and the foreign agency's or commercial entity's ability to use it.

As shown in Table 6-5, the consequence of each malevolent scenario that results in a release directly from the facility is bounded by the MRF earthquake. Malevolent scenarios involving personnel deaths are not addressed by the accident scenarios. It would be speculative to attempt to provide an estimate of the consequences of malevolent scenarios involving the removal of pathogens from the facility.

6.9 References

ARM 2004; 2008, (Risk and Vulnerability Assessment, Boston University Medical Center, National Bio-Containment Laboratory (NBL)). August 5, 2004, updated June 30, 2008.

DOE (U.S. Department of Energy). 2000. DOE Handbook—Airborne Release Fractions/Rates and Respirable Fractions for Nonreactor Nuclear Facilities. DOE-HDBK-3010-94, U.S. Department of Energy. <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/hdbk3010/h3010v1.pdf>. Accessed 09/11/2010. Also, Change Notice 01 dated March 2000 <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/hdbk3010/hdbk301094_cn.pdf>. was reviewed but it does not affect the portions used in this analysis.

DOE (U.S. Department of Energy) 2002. Recommendations For Analyzing Accidents Under The National Environmental Policy Act, U.S. Department of Energy, Office of Environment, Safety and Health, Environment, Safety and Health, Office of NEPA Policy and Compliance July 2002. <http://nepa.energy.gov/nepa_documents/TOOLS/GUIDANCE/Volume2/2-10-greenbook-recommendations.pdf>. Accessed 8/16/2010.

Garcia 2001, Garcia, M. L., *The Design and Evaluation of Physical Protection Systems*, Sandia National Laboratories, Butterworth-Heinemann, 2001.

Garcia 2006, Garcia, M. L., *Vulnerability Assessment of Physical Protection Systems*, Elsevier Publishers, 2006.

NIPP 2008, *National Infrastructure Protection Plan*, Department of Homeland Security, Executive Summary, 2008. Available at: www.dhs.gov/nipp.

Chapter 7.

Potential For Released Pathogens To Become Established In The Environment

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7. Potential for Released Pathogens to Become Established in the Environment

7.1 Introduction

One of the four hazard categories identified by the Advisory Committee to the Director of the NIH for evaluation in any subsequent risk analysis for the NEIDL (Chapter 3) is that of a "vector-borne pathogen that is relevant to the particular sites under evaluation" (Mahmoud et al. 2008). The intention of that category is to address whether, as a result of biocontainment operations at the NEIDL, a pathogen could find its way into the environment, either by a direct release route or indirectly in the form of an escaped infected vector or infected animal, and become established in the environment as a result. This chapter considers all 13 pathogens to decide whether any has potential to become established in the environments near the sites under evaluation, following a loss of biocontainment at the NEIDL.[1] Consideration is given to whether there is any basis to conclude that any of the pathogens could persist or replicate in air, soil, vegetation, water, or in an insect, other arthropod, or other animal species in the local environments outside the biocontainment space.

7.2 Approach

Each of the 13 pathogens was considered with regard to its potential to persist in the environment, using data compiled primarily from peer-reviewed scientific literature. The data were supplemented with additional information published by scientific authorities and leading scientific experts, information from documented personal communications, and data published by state and federal agencies. Information was collected on wildlife and livestock populations for the vicinities of the three proposed sites. The information was considered together with regional climatic factors associated with the three sites. For more complete information concerning data methodology and findings, see Chapter 3 and Appendix C of this document. One of the 13 pathogens, RVFV, previously was studied by a specially assembled working group. Summarized findings from that study are included in this chapter, and a complete copy of the working group's report is presented in Appendix H.

Fundamental considerations in evaluating the potential for pathogens to become established in the environment of proposed NEIDL sites include the following:

- Could the pathogen exist in the natural environment in the absence of a permissive vector or a susceptible host?
- Is the pathogen known to require a vector to spread in the environment?
- If a vector is required for spread in the environment, does the vector exist in New England, and, in particular, in proximity to the sites under consideration for the NEIDL?

- Does the pathogen have a known animal host?
- If the pathogen has a known animal host, does the host exist in New England and, in particular, in proximity to the sites under consideration for NEIDL operation?

Each of the five preceding topics requires consideration of related follow-on questions. Examples of such questions include:

- *Could the pathogen exist in the natural environment in the absence of a permissive vector or a susceptible host?* If a pathogen were accidentally released to the exterior of the facility, could it be expected to survive in ambient atmospheric conditions, including temperature, relative humidity, solar irradiation, and open air factors such as ozone, and olefins? Could the pathogen be expected to survive after coming into contact with soil, vegetation, or water? If the pathogen would be expected to survive in the environment, could it be able to replicate independently (e.g., could it grow in soil or water) or would it require a host (as in the case of viruses)?
- *Is the pathogen known to require a vector to spread in the environment?* Some of the 13 pathogens are not known to spread in nature, except via an insect or arthropod vector. If it were possible for a given pathogen to survive its initial release from the facility, would it require a vector to spread, or is it possible for the pathogen to spread by another means?
- *If a vector is required for spread in the environment, does the vector exist in New England and the sites under consideration for the NEIDL?* In the case of tick-borne encephalitis virus, Far Eastern sub-type (TBEV-FE), formerly known as Russian spring-summer encephalitis virus in the tick-borne encephalitis complex, a vector is required for natural spread, and the vector species are not present in North America. However, a closely related vector species is present in North America. Is it possible for that species to serve as a vector for the virus?
- *Does the pathogen have a known animal host?* [If so] *does the host exist in New England and, in particular, in proximity to the sites under consideration for NEIDL operation?* In the case of Junín virus (JUNV), primary hosts are field mice species not found in North America. However, serologic testing of mice in areas where JUNV is endemic shows that other mouse species have been exposed to the virus. One of those species is common in the Americas, including the northeastern United States. Is it possible for that species to serve as a host for the virus?

Conclusions for each topic were developed by systematic consideration of data in Chapter 3 and Appendix C. Particular attention was given to data availability and relevance for the following parameters for each of the 13 pathogens:

- Pathogen stability (outside of host/vector, if any)

- Vectors (if any)
- Host species naturally (field) or experimentally (laboratory) susceptible to infection
- Infectivity of the pathogen (infectious doses and primary routes of infection)
- Transmissibility potential (secondary transmission in the environment)
- Reservoirs (in the environment)
- Other relevant epidemiological/ecological data

Key points and conclusions regarding whether any of the 13 pathogens has potential to become established in the environments near proposed NEIDL sites are presented below and in Tables 7-1 and 7-2. Pathogens are organized according to biosafety level.

7.3 BSL-3 Pathogens

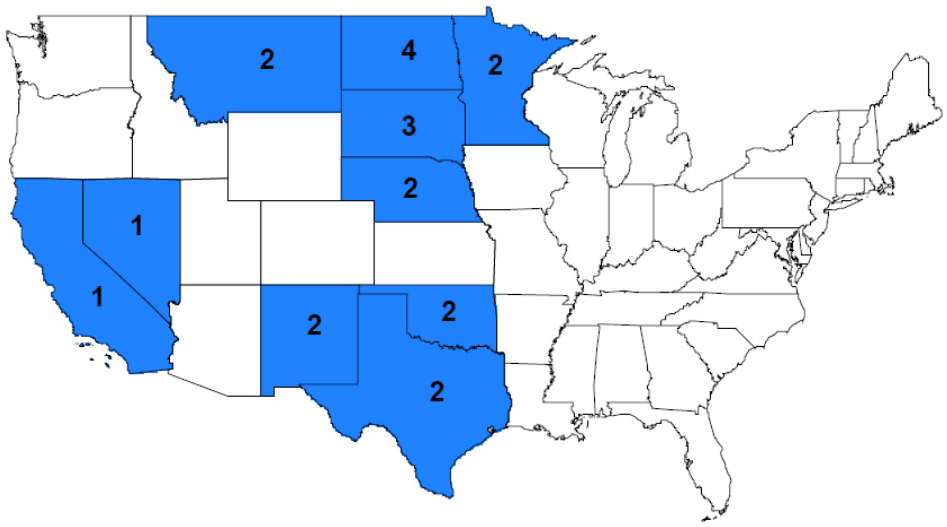
7.3.1 Bacillus anthracis (B. anthracis)

B. anthracis is not vector-borne, although tabanid flies have been associated with rare cases of cutaneous disease (Turell and Knudson 1987). Human infection from B. anthracis is acquired by three main routes: cutaneous, inhalational, and gastrointestinal. Human anthrax is uncommon in the United States, but generally occurs as inhalational or cutaneous anthrax associated with contact with infected animals or contact with aerosols generated from contaminated hair, hides, and skins of infected animals; a singular case of gastrointestinal anthrax associated with this latter exposure source was reported in the United States in 2009 (Goodnough 2009; Brooks 2010; Centers for Disease Control and Prevention 2010). In contrast, anthrax infection in livestock is enzootic (i.e., affecting or peculiar to animals of a specific area) in some geographic regions in the United States. Ten states reported at least one epizootic (i.e., an event attacking a large number of animals simultaneously) of anthrax from January 1996 through October 2001 (Figure 7-1) (Johnson 2008). All states with epizootics are west of the Mississippi River, specifically in the Midwest, the Southwest, and the western United States. Reports of epizootics might not be complete because sporadic cases of anthrax might have gone undiagnosed, or were diagnosed but not reported. Livestock producers in enzootic areas generally recognize the signs of anthrax and respond by vaccinating their animals, without reporting the disease to appropriate authorities (Johnson 2008). Anthrax epizootics in livestock and wildlife are restricted to specific geographic regions, regardless of continent, country, or geopolitical unit within a country.

B. anthracis spores can remain viable in soil for decades under favorable conditions (Titball, Turnbull, and Hutson 1991). Accordingly, there has been speculation that locations of epizootics of anthrax that occur in the United States are geographically correlated to anthrax infections that occurred during the

historic movement of large numbers of cattle during cattle drives and the migration of pioneers and their livestock westward (Johnson 2008). Most outbreaks occur in grazing herbivores, especially cattle, sheep, goats, and horses; pigs are more resistant to infection with B. anthracis. The number of livestock deaths due to anthrax in any year is typically one per one million susceptible range animals.

Figure 7-1. States reporting at least one animal anthrax outbreak, January 1996 to October 2001 (Johnson 2008)



Numbers = Number of outbreaks, January 1996 to October 2001

Observations on the role of climatic factors such as season of the year, ambient temperature, and drought in promoting anthrax epizootics have been made for decades. The commonality of summer months, high ambient temperatures, and drought with anthrax epizootics has been documented. The role of environmental factors such as soil types and soil disturbances via excavation are poorly defined despite attempts to evaluate these potential factors. However evidence suggests that soils rich in organic matter, with high calcium levels and a pH above 6.1 foster spore survival (Hugh-Jones and Blackburn 2009). Two microenvironments of geographic regions have been described in which repeated outbreaks of anthrax have occurred (Van Ness 1971). Those microenvironments or incubator areas are characterized by (1) low-lying depressions, where standing water has collected and devitalized plant life remains, and (2) rocklands, which are dried watercourses or hillside seeps where organic matter accumulates during runoff. Anthrax epizootics occur during the summer months in which there are dry periods punctuated by prolonged periods of intense rain; the hot, dry summer months of June through September are usually preceded by a wet spring. A proposed role of water in anthrax epizootics is the collection (aggregation)

and concentration of spores in storage areas or incubator areas (Dragon and Rennie 1995). Prolonged rainfall promotes runoff and pooling of standing water. The surface of *B. anthracis* spores is highly hydrophobic. The spores are resistant to dissolution by water and might be transported in clumps of organic matter by runoff to standing pools of water. Dry weather causes evaporation of standing water and concentration of floating anthrax spores as the water pools shrink. The high buoyant density of *B. anthracis* spores provides an opportunity for the spores to adhere to vegetation as the vegetation resurfaces during evaporation. The effects of water on anthrax spores have been summarized in three steps: (1) successive cycles of run-off and evaporation concentrates anthrax spores in storage areas, (2) evaporation redistributes the spores from soil onto vegetation, and (3) susceptible herbivores consume the contaminated vegetation (Dragon and Rennie 1995). On the basis of the foregoing geographical and ecological data, as well as the absence in recent decades of *B. anthracis* in the area of the proposed sites, it is concluded that *B. anthracis* is not likely to have an undetected current presence in the vicinities of the three proposed NEIDL sites. That conclusion is supported by ecological niche modeling, which has been conducted on the basis of known or suspected parameters that support survival of *B. anthracis* spores in soil (Blackburn et al. 2007). Findings from this modeling do not predict a current distribution of *B. anthracis* spores in any of the three regions of the proposed NEIDL laboratory sites (Blackburn et al. 2007).

Although livestock populations in the vicinity of the three proposed sites are low or negligible (U.S. Department of Agriculture 2002), ruminants, including wild ruminants, are present in the vicinities of the Tyngsborough and Peterborough sites. It also is possible that residents in metropolitan cities such as Boston harbor ruminants as backyard livestock, and livestock might be present in zoos, or as free-ranging wild ruminants in city parks, reserves, refuges, and woodlands (Rotenberk 2010). Approximately 15,169 deer (*Odocoileus virginianus*) are within a 40km (25-mile) radius of Peterborough, and if the New Hampshire Fish and Game Department reaches its Wildlife Management Unit objectives, the deer population would increase by 27 percent within 5 to 10 years (New Hampshire Fish and Game Department 2005). Approximately 269 moose (*Alces alces*) are in the 2-county area within a 40km (25-mile) radius of Peterborough. The Tyngsborough, Massachusetts, site in Upper Middlesex County is 3km (2-mile) from the New Hampshire border, Hillsborough County. Approximately 9,702 deer and approximately 173 moose populate this adjacent area in New Hampshire.

B. anthracis infection in an animal involves multiplication and dissemination of the vegetative bacterial cells within the animal. Those vegetative cells are exposed to oxygen after the animal dies, thereby stimulating the formation of spores that eventually are deposited near the carcass. That is the natural mechanism by which *B. anthracis* can multiply and spread in the environment. Experience has shown that

it is possible for aerosolized anthrax spores from a laboratory release to be distributed to livestock or wildlife populations and to cause fatal infections in those animals (Meselson et al. 1994; Coleman et al. 2008). That incident, however, involved a Russian military research complex where an estimated 10,000 times the number of spores proposed for work at the NEIDL were accidentally released in a highly dispersable, presumably powdered form (Wilkening 2006). In contrast, calculations that were performed as part of this RA, and that are specific to proposed operations of the NEIDL, indicate that it is beyond reasonably foreseeable (category D, once in more than 1 million years) for aerosolized spores from a maximum reasonably foreseeable release event at the NEIDL to cause infection in the human population at any of the three proposed sites (Chapter 9, Table 3-5-14c). Those calculations also can be used to characterize potential risk to animal populations.

The likelihood of an infection occurring in an animal population increases as the population size increases (just as it does for humans). For a given species, the true number of animals that are present within 1 km of a proposed site at a given time cannot be known. Nor are the infectious doses for the various animal species known. However, if one assumes that the dose-response relationships derived in Appendix J are adequate estimates for a given animal species, the derived initial infections estimates for humans can be related to potential infections among animals by comparing population sizes. It was calculated that an average of about 1,200 residents and 1,000 nonresidents would be in one 22.5-degree sector within 1 km of the urban site and could be exposed to an aerosol release. The number of people potentially exposed at the other sites would be less than 10 percent of the number at the urban site (Table G.4-3), The frequency of a maximum reasonably foreseeable earthquake release leading to *B. anthracis* infection among these people was estimated to be in category D (once in more than 1 million years) and is beyond reasonably foreseeable. It was further calculated that for this estimated frequency to increase to category C (once in 10,000 to 1,000,000 years), the number of people in one 22.5-degree sector within the 1-km radius would have to increase by about a factor of 2,000. By extension, on the basis of the stated assumption about infectious doses in animal species, the population in one 22.5-degree sector within the 1-km radius (about 0.196 km^2 or about 48.5 acres) for a given animal species would have to be at least 4,400,000 (i.e., 2,000 x 2,200) for the frequency of infection in that population to be in category C (once in 10,000 to 1,000,000 years). In the case of natural hosts—ruminants, horses, and swine—such a concentration of animals is not credible, and on the basis of the stated assumptions, it can be surmised that the frequency of at least one anthrax infection occurring within the 1-km radius as a result of the maximum reasonably foreseeable aerosol release would be in category D (once in more than 1 million years) and is beyond reasonably foreseeable.

Consideration also was given to the potential for re-aerosolization of spores that might settle onto environmental substrates following a possible laboratory release, and that might remain infectious for a long period. Studies in conjunction with *B. anthracis* contamination of a U.S. Senate office building demonstrated that there is potential for re-aerosolization of B. anthracis spores from contaminated surfaces in an indoor environment (Weis et al. 2002). However, regarding the potential re-aerosolization of spores from exterior environmental surfaces such as soil and vegetation, findings of the U.S. Department of the Army that were reported in 1996 might have some relevance. As summarized by the Working Group on Civilian Biodefense, the Army findings showed no significant threat to personnel from re-aerosolization of settled spores in the out-of-doors setting (Inglesby et al. 1999). It is possible, though, that these findings might have limited applicability to potential infection of animals subsequent to an accidental release from a NEIDL facility. It might be significant that no data concerning the Russian incident were found to suggest that spores persisted in the environment and caused an increase in the number of animal anthrax cases in the months following the release.

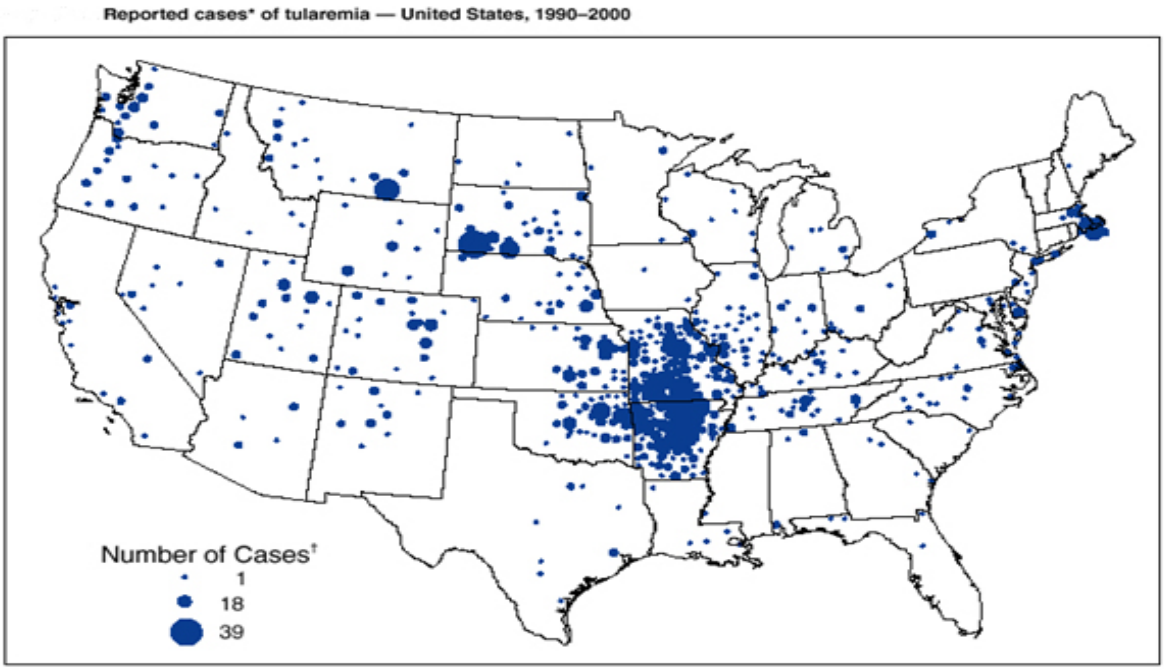
Because *B. anthracis* spores are resistant to adverse environmental conditions, it is possible that spores released from the laboratory remain viable until deposited to a favorable environmental surface, where they could germinate to form vegetative cells and subsequently increase in number by cellular division (Saile and Koehler 2006). However, the available data suggest that the ability of vegetative cells to survive outside an animal is low, particularly in areas other than the microenvironments previously described (Titball, Turnbull, and Hutson 1991; Saile and Koehler 2006). The factors that allow prolonged spore survival in those microenvironments are incompletely defined, but such environments are not found in New England. The inability of *B. anthracis* to become established in New England is evidenced by the cessation of animal and human anthrax in the decades following the demise of tanning and textile industries that imported contaminated materials to the area (Van Ness 1971)(Blackburn, 2007). Furthermore, this evidence is supported by results from ecological niche modeling, which do not predict that *B. anthracis* spores would be capable of long-term survival in the environment of the regions at the urban, suburban, or rural locations (Blackburn et al. 2007). On the basis of the foregoing information, it is surmised that any foreseeable release of spores would not cause *B. anthracis* to become established in the environments in the vicinities of the three proposed sites, either by shedding from the carcass of an infected animal or by direct deposition to a favorable environmental substrate.

7.3.2 Francisella tularensis (F. tularensis)

Distribution of *F. tularensis* appears to be ubiquitous. Tularemia was first reported in the United States in 1911 and has been reported from all states but Hawaii. Except for a 6-year period from 1994 to 2000 when there was no federal requirement for reporting, cases of tularemia have required reporting to the

CDC (Centers for Disease Control and Prevention 2002). From 1990 to 2000, four states accounted for 56 percent of reported cases: Arkansas (315 cases [23 percent]), Missouri (265 cases [19 percent]), Oklahoma (90 cases [7 percent], and South Dakota (96 cases [7 percent]). The county of residence was available for 1,357 reported cases. Among the 3,143 U.S. counties, 543 (17.3 percent) reported at least one case during 1990–2000. The counties with the highest numbers of reported cases were throughout Arkansas and Missouri, in the eastern parts of Oklahoma and Kansas, in southern South Dakota and Montana, and in Dukes County, Massachusetts (Martha's Vineyard island) (Figure 8-3) (Centers for Disease Control and Prevention 2002). From 2001 through 2010, an additional 1,191 cases were reported nationally (Centers for Disease Control and Prevention 2011).

Figure 7-2



In the United States, most persons with tularemia acquired the infection from arthropod bites, particularly tick bites from the dog tick, *Dermacentor variabilis*, or from contact with infected mammals, particularly rabbits. Outbreaks of tularemia in the United States have been associated with tick bites, muskrat handling, deerfly bites, and lawn mowing or cutting brush (Centers for Disease Control and Prevention 2002). Outside the United States (in Italy, Turkey, Russia, and Scandavia), infection also has been reported in association with contaminated water and mosquito bites (Greco et al. 1987; Willke et al. 2009; Meric et al. 2008; Sjostedt 2007).

Tularemia is endemic on Martha's Vineyard, Massachusetts, and *F. tularensis* is present there in mammalian hosts and in ticks (Matyas, Nieder, and Telford 2007; Goethert, Shani, and Telford 2004; Goethert and Telford 2009; Goethert, Saviet, and Telford 2009). Two outbreaks of pneumonic tularemia have been reported in the United States, and both occurred on Martha's Vineyard (1978 and 2000). Fifteen people were infected in these two outbreaks and 11 of those had pulmonary disease (Feldman et al. 2003). A survey conducted on the island to determine the exposure among landscapers (who have a higher risk of infection) found a seroprevalence (indicated by serologic testing) of 9.1 percent (12 of 132) as compared to a seroprevalence of 0.4 percent (1 of 263) among control groups (Feldman et al. 2003). On the basis of the foregoing geographical and ecological data, it is considered likely that *F. tularensis* is already present in wildlife populations, including arthropods, in the vicinities or surrounding areas of all three proposed NEIDL sites.

Animal populations in the vicinities of all three proposed sites include host species that can serve as vectors for, or be infected by, *F. tularensis*. In the case of dense urban areas such as Boston, those species are fewer in number. However, as noted by the Working Group on Civilian Biodefense, and others, tularemia can occur naturally in the urban areas (Dvorak 2005; Martone et al. 1979; Halsted and Kulasinghe 1978; Dennis et al. 2001). In addition to the many animal and arthropod host species, *F. tularensis* subspecies have been detected from water (Keim, Johansson, and Wagner 2007; Morner 1992) and implicated in non-chlorinated municipal water supplies (Greco et al. 1987), and an association with water-associated protozoans has been surmised (Keim, Johansson, and Wagner 2007). The half-life of the bacterium within artificially created aerosols reportedly ranged from about 12 to 45 minutes under experimental conditions (Hood 2009). On the basis of the foregoing information, it is concluded that it is possible for *F. tularensis* to persist in protozoa or become established in susceptible animals or arthropods following release by an aerosol route or by a liquid waste route, or accidental release of an infected vector(s) or escape of an experimentally infected animal(s).

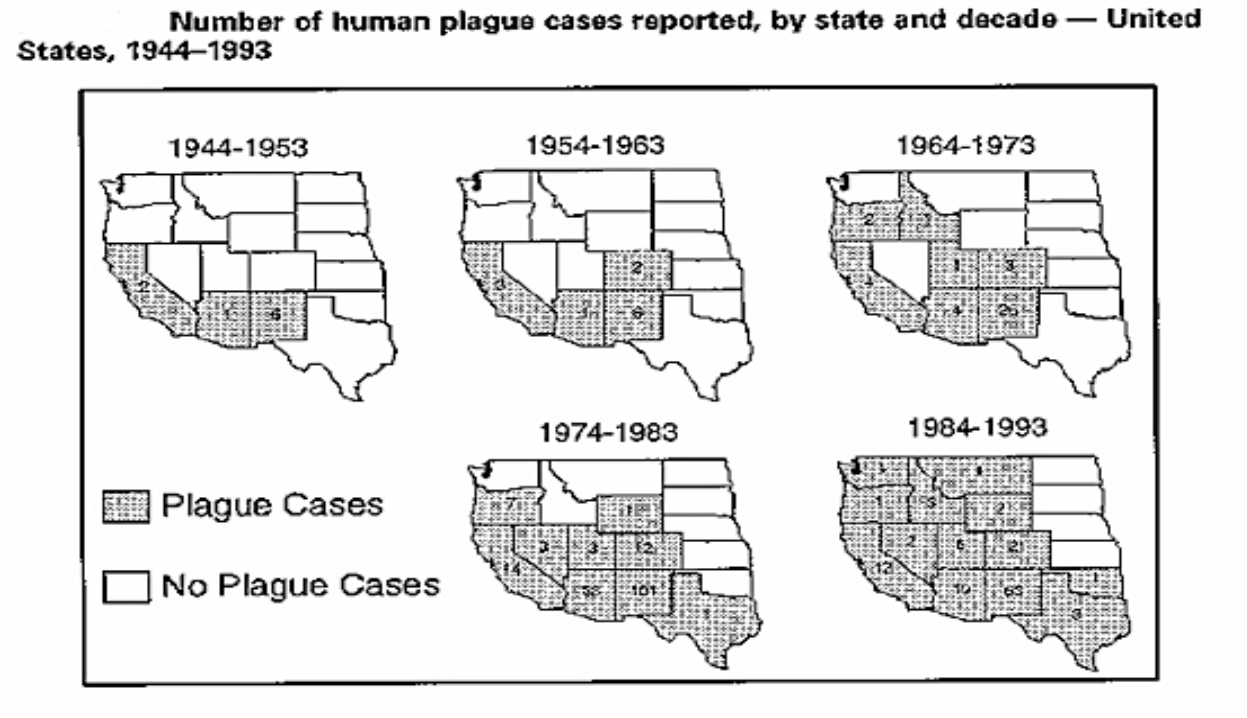
7.3.3 Yersinia pestis (Y. pestis)

Human infection from *Y. pestis* usually is acquired through the bites of infected rodent fleas. Approximately 30 flea species have been documented as vectors of *Y. pestis*, but the primary species in the United States is *Oropsylla montanus* (the squirrel flea). Rodents are the most important hosts for the bacterium, and fleas become infected by taking blood meals from the infected host (Butler 1991; Gabastou et al. 2000; Dennis and Meier 1997; Gage 1998). Globally, natural infections have been reported in approximately 215 mammalian species from 73 genera. Although many mammals have high susceptibility and high case fatality ratios, others are more resistant (Christie 1980; Gage et al. 2000; Dennis and Meier 1997; Reed et al. 1970; von Reyn et al. 1976; Wild, Shenk, and Spraker 2006).

Carnivores appear to be highly resistant to infection (Salkeld et al. 2007; Boone, Kraft, and Stapp 2009). However, orally infected cats develop buboes (inflamed or enlarged lymph nodes) and bacteremia and transmit the infection via scratches, bites, and close contact (Gage et al. 2000).

From 1944 to 1993, 362 cases of human plague were reported in the United States. Approximately 90 percent of those occurred in Arizona, California, Colorado, and New Mexico, where disease is endemic in certain restricted environments that are associated with prairie dog (*Cynomys* spp.) habitats (Centers for Disease Control and Prevention). During each successive decade of that period, the number of states reporting cases increased from 3 during 1944–1953 to 13 during 1984–1993 (Figure 8-4), indicating the spread of human plague infection eastward to areas where cases previously had not been reported. In 1993 health departments in four states reported 10 confirmed cases of human plague to CDC. In addition, one case was confirmed during 1994, and five were confirmed in 1996. As of 2010, confirmed cases in the United States totaled 410 (Centers for Disease Control and Prevention 1994, 2011).

Figure 7-3



As noted by the CDC, "the findings in this report (Centers for Disease Control and Prevention) emphasize the increasing importance of two related trends in the epidemiology of human plague in the U.S.: 1) increased peridomestic transmission [transmissions associated with animals living around human habitations (e.g., cats)] and 2) the role of domestic cats as sources of human infection. Peridomestic transmission is especially important in the most highly plague-endemic states of Arizona, Colorado, and

New Mexico, where rapid suburbanization has resulted in increasing numbers of persons living in or near active plague foci. Domestic cats that are permitted to roam freely in areas where plague occurs in rodents are at increased risk for infection and, therefore, increase the risk for peridomestic transmission to humans."

"Surveillance for plague in rodent and rodent-consuming carnivore populations during the 1990s indicates that plague has spread eastward to counties in areas (e.g., eastern Montana, western Nebraska, western North Dakota, and eastern Texas) believed to be free of this disease since widespread animal surveillance began in the 1930s. The continued expansion of human plague in the U.S. (Figure 8-2) underscores the need to enhance plague surveillance and to increase efforts to prevent, detect, and control human plague"(Centers for Disease Control and Prevention 1994).

In this RA, one laboratory scenario entails release of the bacterium in aerosol form, as an unintended consequence of routine research investigations. Aerosolized *Y. pestis* cells have been found to have a half-life of 9 minutes under laboratory conditions (Won and Ross 1966). Bacterial cells, released by that route, that do not come into contact with a susceptible host ultimately would be expected to be rendered inactive by ambient environmental conditions (Won and Ross 1966; Rose et al. 2003). Furthermore, calculations that were performed as part of this RA, and that are specific to proposed operations of the NEIDL, indicate that aerosolized *Y. pestis* cells from a maximum reasonably foreseeable release event at the NEIDL causing infection in the human population at any of the three proposed sites falls in category D (once in more than 1 million years) and is beyond reasonably foreseeable (Chapter 9, Table 3-5-14c). Those calculations also can be used to characterize potential risk to animal populations.

The likelihood of an infection occurring in an animal population increases as the population size increases (just as it does for humans). For a given species, the true number of animals that are present within 1 km of a proposed site at a given time cannot be known. Nor are the infectious doses for the various animal species known. However, if one assumes that the dose-response relationships derived in Appendix J are adequate estimates for an animal species, the derived initial infections estimates for humans can be related to potential infections among animals by comparing population sizes. It was calculated that an average of about 1,200 residents and 1,000 nonresidents would be in one 22.5-degree sector within 1 km of the urban site and could be exposed to an aerosol release. The number of people potentially exposed at the other sites would be less than 10 percent of the number at the urban site (Table G.4-3). The frequency of a maximum reasonably foreseeable earthquake release leading to *Y. pestis* infection among those people was estimated to be in category D (once in more than 1 million years) and is beyond reasonably foreseeable. It was further calculated that for this estimated frequency to increase to category C (once in

10,000 to 1,000,000 years), the number of people in one 22.5-degree sector within the 1-km radius would have to increase by about a factor of 8,000. By extension, on the basis of the stated assumption about infectious doses in animal species, the population in one 22.5-degree sector within the 1-km radius (about 0.196 km^2 or about 48.5 acres) for an animal species would have to be at least 18,000,000 (i.e. 8,000 x 2,200) for the frequency of infection in that population to be in category C (once in 10,000 to 1,000,000 years). In the case of natural hosts such as mice, such a concentration is not credible, and on the basis of the stated assumptions, it can be surmised that the likelihood of at least one *Y. pestis* infection occurring within the 1-km radius as a result of the maximum reasonably foreseeable aerosol release would be in category D (once in more than 1 million years) and is beyond reasonably foreseeable.

A more plausible laboratory scenario for *Y. pestis* to become established in the environment is accidental release of an infected vector(s) or escape of an experimentally infected animal(s) that survives and then transmits the bacterium to others. On the basis of the foregoing information and the presence of rodents and rodent fleas in the vicinities of the proposed sites, the potential for *Y. pestis* to become established in susceptible animal populations present in the vicinities of the three proposed NEIDL sites cannot be ruled out.

7.3.4 1918 H1N1 Influenza Virus (1918 H1N1V)

1918 H1N1V is not known to be vector-borne. The origin of the 1918 strain of H1N1 virus is unknown (Taubenberger and Morens 2006). However, the available data suggest that natural infections occurred widely in swine during the 1918 pandemic, and the virus has been shown to replicate in experimentally infected pigs (Weingartl et al. 2009). The amount of inoculum used to experimentally infect the pigs was $10^{5.4}$ $TCID_{50}$ for each animal. Experimental infections also are possible in mice (*Mus musculus*), but the available data show that the virus does not spread from inoculated mice to uninfected cage mates, and there is no evidence that infection in mice can occur naturally (Lowen et al. 2006; Tumpey 2008; Tumpey et al. 2004; Kong et al. 2006). Ferrets (*Mustela putorius furo*) are better suited for the study of influenza virus transmission, and researchers have found that 1918 H1N1V transmits efficiently between ferrets by respiratory droplets (Tumpey et al. 2007). Wild ferrets, though, are not present in the three sites under consideration for the NEIDL and are not given further consideration here. Data concerning stability of influenza A virus (not 1918 H1N1 strain) in the environment indicate that the virus half-life can be as short as 18 minutes, but replication competence can retained for as long as 1–2 days (Lytle and Sagripanti 2005; Sagripanti and Lytle 2007; Loosli et al. 1943; Bridges, Kuehnert, and Hall 2003). Isolation of avian strains of influenza virus from fresh waterbodies suggests that influenza A viruses have a measure of stability in water{Lebarbenchon, 2011 #16486}.

Uncertainty calculations that were performed as part of this RA, and that are specific to proposed operations of the NEIDL, indicate that most (more than 90 percent) but not all estimates for the frequency of one or more infections from 1918 H1N1V occurring in the human population at the urban site as a result of a maximum reasonably foreseeable release event at the NEIDL fall in category D (once in more than 1 million years) and are beyond reasonably foreseeable (Chapter 9, Table 3-5-14C). All results for multiple (greater than or equal to 2) infections at the urban site, and any number of infections at the suburban and rural sites, fall in category D (once in more than 1 million years) and are beyond reasonably foreseeable. Those calculations also can be used to characterize potential risk to animal populations. The concentration of susceptible animal hosts at the rural site, and perhaps at the suburban site, could be greater than the concentration of humans. Also, the infectious dose and host susceptibility could vary between species. Assuming the same dose-response models apply for susceptible animal species near the three sites, a given animal species population would have to be comparable to or higher than the estimated urban population for any estimates of infection frequency to fall in category C (once in 10,000 to 1,000,000 years).

Wild birds are hosts to all known subtypes of influenza A virus (Centers for Disease Control and Prevention 2005), and starlings (*Sturnus vulgaris*)—a species that can flock in large numbers—appear to be naturally capable of carrying influenza virus (Qin et al. 2011). In addition, domestic birds can be infected with influenza viruses from wild birds. Recent experimentation has demonstrated that 1918 H1N1V has low pathogenicity in domestic birds (Babiuk et al. 2010). Data from that experiment indicate that 1918 H1N1V does not replicate efficiently in experimentally infected chickens and, although the virus does replicate in ducks (as shown by serological testing), the level of replication in most ducks was below the limit of detection (for nucleic acid-based detection tests). The amount of inoculum used in that experiment was 10^5 PFU per animal (Babiuk et al. 2010).

Potentially susceptible swine populations might be in the vicinity of the rural and suburban sites, and potentially susceptible avian populations are expected to be present in the vicinity of the three proposed NEIDL sites. On the basis of the foregoing information, the potential for released1918 H1N1V to retain infectivity long enough to become established in susceptible animal populations present in the vicinities of the three proposed sites is low but cannot be ruled out.

7.3.5 SARS Corona Virus (SARS-CoV)

SARS-CoV is not known to be vector-borne. Genetic evidence has shown that initial infections in humans were acquired from non-domesticated animals (Wang and Eaton 2007). The reservoirs appear to be horseshoe bats of the genus *Rhinolophus*, and the secondary host appears mainly to be the palm civet (Shi

and Hu 2008; Wang and Eaton 2007). The virus was reported as being detected in raccoon dogs (*Nyctereutes* sp.), Chinese ferret badgers (*Melogale moschata*), and domestic cats (*Felis cattus*) (Wang and Eaton 2007; Shi and Hu 2008). Of those natural hosts and reservoirs, only the domestic cat is native to North America. Experimental infections are possible in mice (*M. musculus*), hamsters (*Mesocricetus auratus*), and cats when using high concentrations of inocula ($10^{3.6}$ to 10^{6} $TCID_{50}$), but the course of infection in those animals is abbreviated and the animals remain asymptomatic (Roberts et al. 2007; Martina et al. 2003). Experimental data show the virus can be transmitted between cats that are housed together. Naturally occurring infections in cats are believed to have resulted from prolonged contact with their infected owners. There is no evidence that the virus can be transmitted to humans by domestic animals. The available experimental data indicate that SARS-CoV is not able to survive in the environment outside a host for more than 2–3 weeks, so viruses accidentally released by an aerosol route, and that do not contact a susceptible host, ultimately would be expected to be rendered inactive by ambient environmental conditions (Lytle and Sagripanti 2005; Darnell et al. 2004; Wang et al. 2005; Rabenau et al. 2005; McKinney, Gong, and Lewis 2006; Wong and Yuen 2005). Calculations that were performed as part of this RA, and that are specific to proposed operations of the NEIDL, indicate that it is beyond reasonably foreseeable for aerosolized SARS-CoV from a maximum reasonably foreseeable release event at the NEIDL to cause infection in the human population at any of the three proposed sites (Chapter 9, Table 3-5-14c). Those calculations also can be used to characterize potential risk to animal populations.

The likelihood of an infection occurring in an animal population increases as the population size increases (just as it does for humans). For a given species, the true number of animals that are present within 1 km of a proposed site at a given time cannot be known. Nor are the infectious doses for the various animal species known. However, if one assumes that the dose-response relationships derived in Appendix J are adequate estimates for an animal species, the derived initial infections estimates for humans can be related to potential infections among animals by comparing population sizes. It was calculated that an average of about 1,200 residents and 1,000 nonresidents would be in one 22.5-degree sector within 1 km of the urban site and could be exposed to an aerosol release. The number of people potentially exposed at the other sites would be less than 10 percent of the number at the urban site (Table G.4-3). The frequency of a maximum reasonably foreseeable earthquake release leading to SARS-CoV infection among those people was estimated to be in category D (once in more than 1 million years) and is beyond reasonably foreseeable. It was further calculated that for this estimated frequency to increase to category C (once in 10,000 to 1,000,000 years), the number of people in one 22.5-degree sector within the 1-km radius would have to increase by about a factor of 200. By extension, on the basis of the stated assumption about

infectious doses in animal species, the population in one 22.5-degree sector within the 1-km radius (about 0.196 km^2 or about 48.5 acres) for an animal species would have to be at least 440,000 (i.e., 200 x 2,200) for the frequency of infection in that population to be in category C (once in 10,000 to 1,000,000 years). In the case of potential hosts such as mice or cats, such a concentration is not credible, and on the basis of the stated assumptions, it can be surmised that the likelihood of at least one SARS-CoV infection occurring within the 1-km radius as a result of the maximum reasonably foreseeable aerosol release would be in category D (once in more than 1 million years) and is beyond reasonably foreseeable.

Cats will not be used as experimental animals at the NEIDL, which should preclude a scenario in which an experimentally infected cat escapes and transmits the virus to other cats. On the basis of those observations, SARS-CoV is not considered able to survive or multiply in the vicinities of the three proposed NEIDL sites.

7.3.6 Rift Valley Fever Virus (RVFV)

The possibility that RVFV could become established in North America, and its potential environmental, economic, and public health effects, recently were considered by a panel of subject matter experts (Rift Valley Fever Virus Working Group 2004). When considering potential impact of the virus to public health, agriculture, and the environment in the United States, the Rift Valley Fever Virus Working Group (RVFVWG) found it useful to compare RVFV to West Nile virus (WNV). In brief, the working group concluded that endemic RVFV would constitute a much greater ecologic, economic, and public health threat to the United States than does WNV (see Appendix I of this RA). Their report envisioned a scenario of a malevolent deliberate release at three stockyards in the United States, with subsequent shipment of infected asymptomatic animals to multiple locations nationally, which would result in high numbers of initial infections and rapid geographic spread of the virus. That scenario is quite different from accidental release scenarios involving laboratory operations but, as the RVFVWG points out, if the virus were to become established in the environment, the effects would be the same regardless of how it had been introduced. The RVFVWG notes that spraying can be an effective strategy to control mosquito vectors in defined areas. Effective mosquito vector control, however, would require immediacy of detection, and there are no programs in place to support rapid detection of Rift Valley fever.

RVFV can be carried by at least 30 mosquito species in 5 genera, notably *Aedes* (Meegan and Bailey 1989). At least one *Aedes* species (*Aedes japonicus*) is known to exist in Massachusetts (Centers for Disease Control and Prevention 2010). A major characteristic in the epidemiology of RVF is that infected mosquitoes transmit the virus to their eggs, and mosquitoes that develop from the eggs become carriers of the virus. This phenomenon, known as transovarial vertical transmission, allows the mosquito to function

as a reservoir for the virus over years or decades and to propagate the virus over indefinite generations of mosquitoes. Infected mosquitoes and their eggs are capable of surviving through winter conditions. Overwintering of arbovirus-carrying mosquito species in northern climates has been documented in the case of WNV (Nasci et al. 2001). In addition, significant mosquito populations have been known to occur in large cities (New York City Department of Health and Mental Hygiene 2010).

In this RA, one laboratory scenario entails release of the virus in aerosol form, as an unintended consequence of routine research investigations. Viruses released by an aerosol route, and that do not contact a susceptible host, would be expected to have a half-life ranging from 7 to 77 minutes, and ultimately would be rendered inactive by ambient environmental conditions (Brown, Dominik, and Larson 1982; Miller et al. 1963). However, the possibility that a susceptible mammalian host could become infected by exposure to accidentally released aerosolized RVFV cannot be ruled out. Calculations that were performed as part of this RA, and that are specific to proposed operations of the NEIDL, indicate that up to about five infections in the human population at the urban site might be expected in the event of a maximum reasonably foreseeable release event at the NEIDL, which is estimated to occur in category C (once in 10,000 to 1,000,000 years) (Chapter 9, Table 3-5-14C). At the suburban and rural sites, most estimates suggest that a maximum reasonably foreseeable release resulting in any infections would be placed in category D (once in more than 1 million years). However, some of the estimates place the likelihood in the Low frequency category and, as a result, the possibility of an infection occurring in an animal in the event of a maximum reasonably foreseeable release event cannot be excluded. If an infected animal were to become viremic, it could be possible for indigenous mosquitoes to become infected by feeding on the host, and subsequently spread the virus to other hosts in the area. Another, perhaps more plausible, laboratory scenario for RVFV becoming established in the environment is accidental release of an infected vector(s) or escape of an experimentally infected viremic mammal. In that scenario, it is possible for the infected vector to transmit the infection to a susceptible animal(s), or for the escaped mammal to transmit the virus to indigenous mosquitoes, thereby creating potential for the virus to become established in the environment.

Ruminants such as cattle, sheep, and goats are the animals most often recognized in outbreaks of Rift Valley fever in endemic areas. Although livestock populations in the vicinity of the three proposed sites are low or negligible (U.S. Department of Agriculture 2002), ruminants, including wild ruminants, are present in the vicinities of the Tyngsborough and Peterborough sites. It also is possible that residents in metropolitan cities such as Boston harbor ruminants as backyard livestock, and livestock could be present in zoos, or as free-ranging wild ruminants in city parks, reserves, refuges, and woodlands (Rotenberk 2010). The RVFVWG also expressed concern that white-tailed deer (*Odocoileus virginianus*) and bats

would be susceptible to infection. Approximately 15,169 deer are found within a 3km (2-mile) radius of Peterborough. If the New Hampshire Fish and Game Department is successful in reaching all the Wildlife Management Unit objectives identified in its plan, the deer population would increase by 27 percent in 5 to 10 years (New Hampshire Fish and Game Department 2005). Approximately 269 moose (*A. alces*) are in the 2-county area within a 3km (2-mile) radius of Peterborough. The Tyngsborough, Massachusetts, site in Upper Middlesex County is 3km (2-mile) from the New Hampshire border, Hillsborough County. Approximately 9,702 deer and approximately 173 moose populate this adjacent area in New Hampshire.

Importantly, and sometimes overlooked in discussion of permissive animal hosts for RVFV, is the fact that a range of non-ruminant mammals has been shown to become infected when experimentally challenged. Kittens and puppies reportedly are highly susceptible to experimental infection, and rodents such as mice, rats, and gray squirrels also are susceptible to the virus under experimental conditions (Shimshony and Barzilai 1983). Rodents are expected to be present in the vicinities of the three sites proposed for the NEIDL, and feral cats could be present as well. Accordingly, on the basis of the foregoing information, the potential for RVFV to become established in susceptible animal populations present in the vicinities of the three proposed NEIDL sites cannot be ruled out and, under favorable conditions, it is possible for the virus to become endemic in the United States as a result.

7.4 BSL-4 Pathogens

7.4.1 Andes Virus (ANDV)

ANDV is not known to be vector-borne. No aerosol stability data for this species were found, but predictions for a different hanta virus showed a half-life of about 28 minutes, which indicates that the virus ultimately would not be not able to survive in the environment outside a host reservoir (Lytle and Sagripanti 2005). The main natural reservoirs for ANDV are rodents in the sub-family Sigmodontinae, namely, *Oligoryzomys longicaudatus* and other species of *Oligoryzomys* (Centers for Disease Control and Prevention 2008; Wells et al. 1997; McCaughey and Hart 2000; Padula et al. 2004). Experimental infections are possible in the Syrian hamster (*M. auratus*). Because none of those animals occur naturally in the United States, any escaped experimentally infected animal would not be able to transmit the virus to a known susceptible host species. Ecological studies to enumerate host species in the South American endemic area showed no infections among rodent species commonly found in New England (*R. rattus, R. norvegicus, M. musculus*) (Toro et al. 1998). As a result, ANDV is not considered able to survive or multiply in the environments in the vicinities of the three proposed NEIDL sites.

7.4.2 Ebola Virus (EBOV)

EBOV is not known to be vector-borne. The natural reservoirs are fruit bats, namely, *Hypsignathus monstrosus, Epomops franquetti,* and *Myonycteris torquata* (Leroy et al. 2005; Warfield, Deal, and Bavari 2009). Experimental infections are possible in the Syrian hamster (*M. auratus*) and in newborn and suckling mice (*M. musculus*) (Pattyn, Bowen, and Webb 2008). Because none of the natural reservoir species are found in the United States and because natural infections have not been found in mice, EBOV is not considered able to survive or multiply in the environments in the vicinities of the three proposed NEIDL sites.

7.4.3 Marburg Virus (MARV)

MARV is not known to be vector-borne. The reservoir appears to be a fruit bat, *Rousettus aegyptiacus,* and possibly insectivorous bats, *Miniopterus inflatus* and *Rhinolophus eloquens* (Swanepoel et al. 2007; Towner et al. 2007). Experimental infections are possible in newborn and weanling mice (*M. musculus*), guinea pigs (*Cavia porcellus*), and hamsters (*M. auratus*) (Siegert and Simpson 2008; Paragas and Geisbert 2006; Leffel and Reed 2004). Because none of the natural reservoir species are found in the United States and because natural infections have not been found in mice, MARV is not considered able to survive or multiply in the environments in the vicinity of the three proposed NEIDL sites.

7.4.4 Lassa Virus (LASV)

LASV is not known to be vector-borne. The natural host for LASV is the rodent *Mastomys natalensis* (Fisher-Hoch 2005). Reported associations with other species of *Mastomys* are unconfirmed, and are disputed because of the possibility of incorrect identifications (Demby et al. 2001). Species of *Mastomys* are not endemic to North America. Experimental infections are possible in guinea pigs (*C. porcellus*), and marmosets (*Callithrix jacchus*) and other NHPs (Carrion, Brasky, et al. 2007; Carrion, Patterson, et al. 2007; Peters et al. 1987). Because those host species are not endemic to North America, and because there is no reported evidence from endemic areas of infection in *M. musculus* (a common mouse in the New England region), LASV is not considered able to survive or multiply in the environments in the vicinity of the three proposed NEIDL sites.

7.4.5 Junín Virus (JUNV)

There is no field evidence of a vector for JUNV. Primary reservoirs and the source of most human infections with JUNV are vesper mice *Calomys musculinus* and *C. laucha*, of the family Muridae, subfamily Sigmodontinae (Carballal, Videla, and Merani 1988; LeDuc 1989; Ambrosio et al. 2006; Parodi 2008). *M. musculus* (a common mouse in the New England region) has been reported as a less important natural host. On the basis of combined tests for antibody and antigen detection, the most recent

field data from the endemic area in Argentina showed a prevalence of JUNV in *C. musculinus* of 11 percent (Mills et al. 1994). In contrast, the data showed no evidence, either by antibody or antigen detection, of JUNV in *M. musculus.* Earlier field studies from the endemic area reported antigen detection in 66 of 1,727 *C. musculinus* (3.8 percent) versus only 2 of 503 *M. musculus* (0.39 percent) (Mills, Ellis, et al. 1991; Mills, Calderon, et al. 1991). Those data indicate that *M. musculus* is not a capable host species for JUNV. Host species for the arenaviruses are very specific. Presence of viral-specific antibodies in a given mammalian species indicates past exposure to and infection with the virus but does not necessarily indicate ability of that mammalian species to become viremic or to transmit the virus. That explains why antibody-based tests detect the virus at a higher rate than antigen-based tests. Presence of anti- JUNV antibodies in South American populations of *M. musculus* does not indicate that members of that species in North America could be successful hosts. (Lytle and Sagripanti 2005)

A prediction of viral stability for aerosols of this pathogen, on the basis of a model that was validated using experimental data from other viruses, estimates the half-life of aerosolized JUNV to be 30 minutes (Lytle and Sagripanti 2005). Because of the instability of aerosolized JUNV, and because *Calomys* species are not endemic to North America, and because available data indicate that *M. musculus* is not a capable host for the virus, JUNV is not considered able to survive or multiply in the environments in the vicinity of the three proposed NEIDL sites.

7.4.6 Tick-borne Encephalitis Virus, Far Eastern Sub-type (TBEV-FE), formerly known as Russian spring-summer encephalitis virus of the tick-borne encephalitis complex

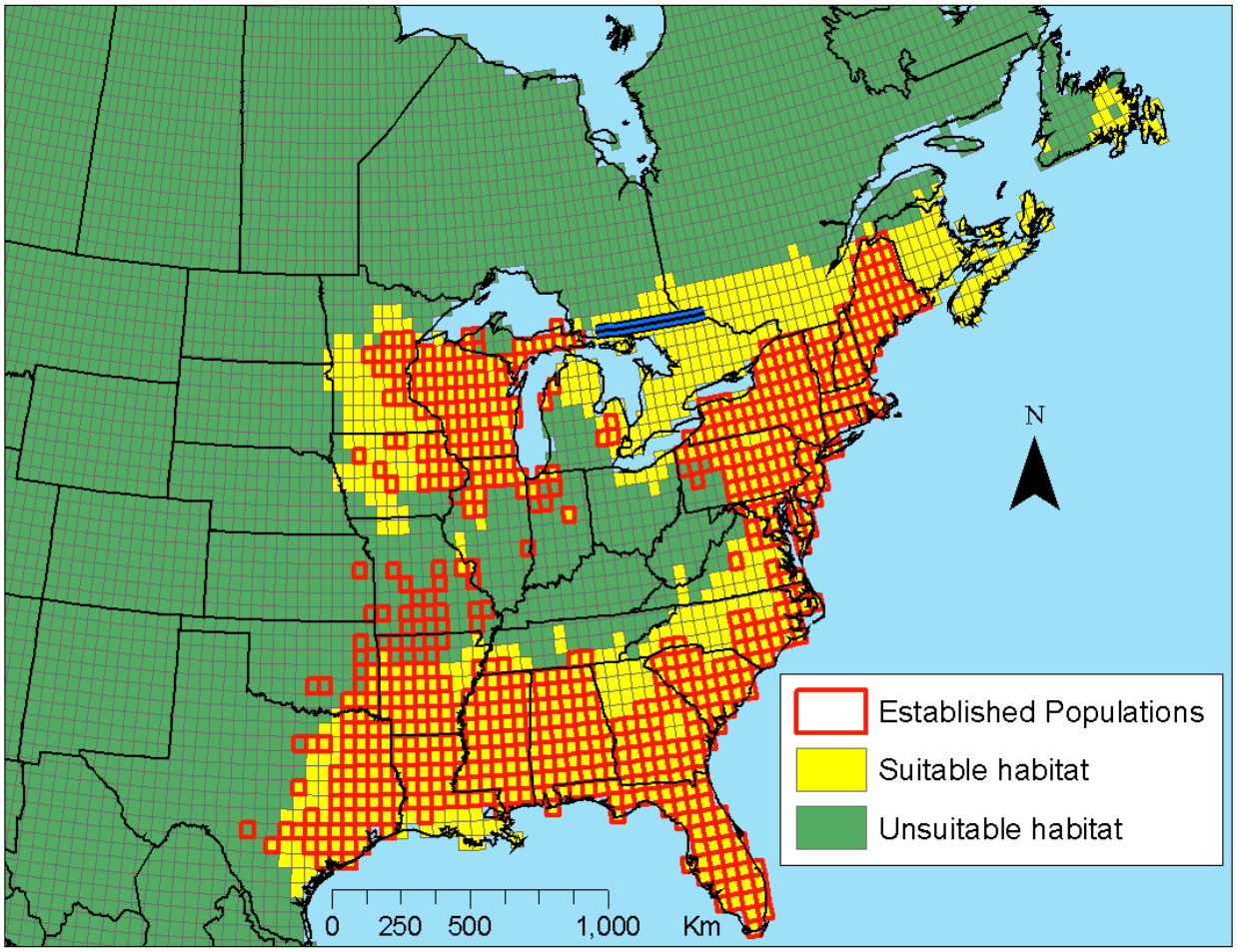
TBEV-FE is a highly infectious, zoonotic, tick-borne virus disease of humans (Gresikova 1989; Calisher 1988). *Ixodes persulcatus* ticks are the primary vectors and reservoirs for TBEV-FE (Gresikova 1989; Alciati et al. 2001; Chumakov 2008; Ruzek et al. 2008; Gritsun, Nuttall, and Gould 2003). The virus chronically infects ticks and is transmitted transstadially (passed from one life cycle stage to the next), transovarially (transmitted from one generation to the next), and between ticks during co-feeding on host mammals (Gritsun, Lashkevich, and Gould 2003; Suss 2003). In additional to passage of the virus between ticks as they co-feed on a host, an infected mammal such as a mouse can serve as a source of infection for ticks, but only over the very limited period during which it is viremic (Turell 2008). Mice do not serve as reservoirs and cannot transmit infection to humans. The tick is the reservoir and the vector. In endemic regions, the northern red-backed vole (*Myodes rutilus*) can serve as a reservoir and a host. The northern red-backed vole is endemic to Alaska but to no other U.S. states (U.S. Department of Agriculture Forest Service). Other major rodent hosts for TBEV-FE are not native to North America. However, goats,

cattle, and sheep can become infected, and goats can transmit the virus in their milk while viremic (Gritsun, Nuttall, and Gould 2003; Heinz and Kunz 2004; Suss 2003).

Aerosolized virus reportedly is stable for at least 6 hours at room temperature (Gritsun, Lashkevich, and Gould 2003). As a result, it can be surmised that aerosols released from the facility could have significant stability. Historically, though, the aerosol route has not been satisfactory in initiating infection with this virus in experimental animals, and the subcutaneous route of infection is preferred. Therefore, in this RA, a more plausible scenario for the virus to become established in the environment would be accidental release of an infected vector that could transmit the virus to a permissive host.

I. persulcatus is a tick that is not endemic to North America. However, the related species *Ixodes cookie* and *I. scapularis* are the primary vectors of Powassan virus, which is closely "related" to viruses of the TBE complex (Turell 2008). *I. scapularis* is the main vector of Lyme disease in North America and is widespread along the eastern half of the United States (Figure 8-5) (Brownstein, Holford, and Fish 2005). Although there is no known experimental work to assess whether the viruses of TBE complex can survive in tick species indigenous to North America, it should be expected that ticks indigenous to New England could be efficient reservoirs and vectors for the TBEV-FE and Central European virus (TBEV-CE) members of the TBE complex (Turell 2008). Also, it is expected that indigenous mice would be suitable hosts for amplification of TBE complex viruses (Turell 2008).

Figure 7-4. Established populations and suitable habitat for *I. scapularis* (Brownstein, Holford, and Fish 2005)



On the basis of the foregoing information, the potential for TBEV-FE to become established in susceptible animal populations present in the vicinities of the three proposed NEIDL sites cannot be ruled out.

7.4.7 Nipah Virus (NIPV)

There are no known arthropod vectors of NIPV. NIPV is able to infect a range of hosts, including swine, humans, and, to a minor extent, cats and dogs (Chua 2003; Eaton, Broder, and Wang 2005; Lo and Rota 2008; McEachern et al. 2008; Torres-Velez et al. 2008; Aljofan et al. 2009; Weingartl, Berhane, and Czub 2009). However, those are regarded as dead end hosts (considered to be non-transmitting and non-amplifying hosts) (Chua 2003; van der Poel, Lina, and Kramps 2006). The natural reservoir hosts for NIPV appear to be several species of fruit bats of the genus *Pteropus*, including *P. giganteus, P. vampyrus, P. hypomelanus, P. lylei,* and *P. poliocephalus* (Chua et al. 2002; Blum et al. 2009; Luby et al.

2009; Weingartl, Berhane, and Czub 2009). The half-life of NIPV under laboratory conditions (applied to a plastic substrate) was determined to be 1.5 minutes at 72 degrees Fahrenheit (Fogarty et al. 2008). Because the natural reservoir host species for NIPV are not endemic to the United States, NIPV is not considered able to survive or multiply in the environments in the vicinity of the three proposed NEIDL sites.

7.5 Summary

On the basis of the available evidence, it is concluded that five of the pathogens, *F. tularensis, Y. pestis,* 1918 H1N1V, RVFV, and TBEV-FE, are regarded as environmentally relevant to the sites under consideration for operation of the NEIDL. That means that those five pathogens all have potential, at least theoretically, to become established in the environments in the vicinity of the three proposed NEIDL sites (Tables 7-1 and 7-2). Four of those are vector-borne. On the basis of the available data, it is concluded that one of these pathogens, *F. tularensis,* might already be present in some areas in the vicinity of proposed NEIDL sites. Another of the pathogens, TBEV-FE, is regarded as environmentally relevant, even though its vectors and reservoirs are not endemic to North America, because it is thought that endemic arthropod species could be capable of serving as competent vectors and reservoirs if the virus were to become established in populations of those arthropods.

Theoretical potential exists for any of the five pathogens to become established in the environments associated with all three sites proposed for the NEIDL. The means by which this might occur involve animals (including arthropods) that could be present in those environments. It can be surmised that the intensively urbanized nature of the BioSquare Research Park site supports smaller populations of such animals and, as a result, would be expected to present a less favorable immediate environment for any such potential to be realized. However, although a quantitative difference regarding that potential can be surmised, no qualitative difference is found between the BioSquare Research Park site, the Tyngsborough site, and the New Hampshire site.

7.6 REFERENCES

Alciati, S., E. Belligni, S. Del Colle, and A. Pugliese. 2001. Human infections tick-transmitted. *Panminerva Med* 43 (4):295-304.

Aljofan, M., S. Saubern, A. G. Meyer, G. Marsh, J. Meers, and B. A. Mungall. 2009. Characteristics of Nipah virus and Hendra virus replication in different cell lines and their suitability for antiviral screening. *Virus Res* 142 (1-2):92-9.

Ambrosio, A. M., L. M. Riera, C. Saavedra Mdel, and M. S. Sabattini. 2006. Immune response to vaccination against Argentine hemorrhagic Fever in an area where different arenaviruses coexist. *Viral Immunol* 19 (2):196-201.

Babiuk, S., R. Albrecht, Y. Berhane, P. Marszal, J. A. Richt, A. Garcia-Sastre, J. Pasick, and H. Weingartl. 2010. 1918 and 2009 H1N1 influenza viruses are not pathogenic in birds. *J Gen Virol* 91 (Pt 2):339-42.

Blackburn, J. K., K. M. McNyset, A. Curtis, and M. E. Hugh-Jones. 2007. Modeling the geographic distribution of Bacillus anthracis, the causative agent of anthrax disease, for the contiguous United States using predictive ecological [corrected] niche modeling. *Am J Trop Med Hyg* 77 (6):1103-10.

Blum, L. S., R. Khan, N. Nahar, and R. F. Breiman. 2009. In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. *Am J Trop Med Hyg* 80 (1):96-102.

Boone, A., J. P. Kraft, and P. Stapp. 2009. Scavenging by mammalian carnivores on prairie dog colonies: implications for the spread of plague. *Vector Borne Zoonotic Dis* 9 (2):185-90.

Bridges, C. B., M. J. Kuehnert, and C. B. Hall. 2003. Transmission of influenza: implications for control in health care settings. *Clin Infect Dis* 37 (8):1094-101.

Brooks, D. 2010. Anthrax, Human - USA (08): (New Hampshire). *ProMED-mail*, http://promedmail.oracle.com/pls/otn/f?p=2400:1001:14713408319931 85::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000,82333.

Brown, J.L., J. W. Dominik, and E. W. Larson. 1982. Airborne survival of Rift Valley fever virus. edited by U.S. Army Medical Research Institute of Infectious Diseases Aerobiology Division. Frederick: U.S. Department of Defense.

Brownstein, J. S., T. R. Holford, and D. Fish. 2005. Effect of Climate Change on Lyme Disease Risk in North America. *Ecohealth* 2 (1):38-46.

Butler, T., ed. 1991. *Plague*. Edited by G. T. Strickland, *Tropical medicine*. Philadelphia: WB Saunders.

Calisher, C. H. 1988. Antigenic classification and taxonomy of flaviviruses (family Flaviviridae) emphasizing a universal system for the taxonomy of viruses causing tick-borne encephalitis. *Acta Virol* 32 (5):469-78.

Carballal, G., C. M. Videla, and M. S. Merani. 1988. Epidemiology of Argentine hemorrhagic fever. *Eur J Epidemiol* 4 (2):259-74.

Carrion, R., Jr., K. Brasky, K. Mansfield, C. Johnson, M. Gonzales, A. Ticer, I. Lukashevich, S. Tardif, and J. Patterson. 2007. Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues. *J Virol* 81 (12):6482-90.

Carrion, R., Jr., J. L. Patterson, C. Johnson, M. Gonzales, C. R. Moreira, A. Ticer, K. Brasky, G. B. Hubbard, D. Moshkoff, J. Zapata, M. S. Salvato, and I. S. Lukashevich. 2007. A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity. *Vaccine* 25 (20):4093-102.

Centers for Disease Control and Prevention. 1994. Human plague--United States, 1993-1994. *MMWR Morb Mortal Wkly Rep* 43 (13):242-6.

———. 2002. Tularemia--United States, 1990-2000. *MMWR Morb Mortal Wkly Rep* 51 (9):181-184.

———. 2005. Transmission of Influenza A Viruses Between Animals and People. http://www.cdc.gov/flu/avian/gen-info/transmission.htm.

———. 2009. *All about hantaviruses*. Centers for Disease Control and Prevention, National Center for Infectious Diseases, Special Pathogens Branch 2008 [cited 11, Oct. 2009].

———. 2010. Gastrointestinal anthrax after an animal-hide drumming event -- New Hampshire and Massachusetts, 2009. *MMWR - Morbidity & Mortality Weekly Report* 59 (28):872-877.

———. *Information on Aedes japonicus* 2010 [cited July 20, 2010. Available from http://www.cdc.gov/ncidod/dvbid/arbor/japonicus.htm.

———. 2011. Notifiable Disease and Mortality Tables. *Morbidity and Mortality Weekly Report* 59 (5):51-55.

———. 2011. Notifiable Diseases and Mortality Tables. *Morbidity and Mortality Weekly Report* 60 (3):65-92.

Christie, A.B., Chen, T.H., Elberg, S.S. 1980. Plague in camels and goats: their role in human epidemics. *J Infect Dis* 141 (6):724-726

Chua, K. B. 2003. Nipah virus outbreak in Malaysia. *J Clin Virol* 26 (3):265-75.

Chua, K. B., C. L. Koh, P. S. Hooi, K. F. Wee, J. H. Khong, B. H. Chua, Y. P. Chan, M. E. Lim, and S. K. Lam. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4 (2):145-51.

Chumakov, M.P., Levkovich, E.N., . *Russian Spring Summer Encephalitis*. CDC 2008. Available from <http://www.ncid.cdc.gov/arbocat/catalog-listing.asp?VirusID=404&SI=1>.

Coleman, M. E., B. Thran, S. S. Morse, M. Hugh-Jones, and S. Massulik. 2008. Inhalation anthrax: dose response and risk analysis. *Biosecur Bioterror* 6 (2):147-60.

Darnell, M. E., K. Subbarao, S. M. Feinstone, and D. R. Taylor. 2004. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. *J Virol Methods* 121 (1):85-91.

Deboosere, N., S. V. Horm, A. Pinon, J. Gachet, C. Coldefy, P. Buchy, and M. Vialette. 2011. Development and Validation of a Concentration Method for the Detection of Influenza A Viruses from Large Volumes of Surface Water. *Appl Environ Microbiol*.

Demby, A. H., A. Inapogui, K. Kargbo, J. Koninga, K. Kourouma, J. Kanu, M. Coulibaly, K. D. Wagoner, T. G. Ksiazek, C. J. Peters, P. E. Rollin, and D. G. Bausch. 2001. Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne Zoonotic Dis* 1 (4):283-97.

Dennis, D. , and F. Meier. 1997. Plague. In *Pathology of emerging infections*, edited by C. R. Horsburgh and A. M. Nelson. Washington, D.C.: ASM Press.

Dennis, D. T., T. V. Inglesby, D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. D. Fine, A. M. Friedlander, J. Hauer, M. Layton, S. R. Lillibridge, J. E. McDade, M. T. Osterholm, T. O'Toole, G. Parker, T. M. Perl, P. K. Russell, and K. Tonat. 2001. Tularemia as a biological weapon: medical and public health management. *JAMA* 285 (21):2763-73.

Dragon, D. C., and R. P. Rennie. 1995. The ecology of anthrax spores: tough but not invincible. *Can Vet J* 36 (5):295-301.

Dvorak, P. 2005. Health Officials Vigilant for Illness After Sensors Detect Bacteria on Mall. *The Washington Post*, October 2, 2005.

Eaton, B. T., C. C. Broder, and L. F. Wang. 2005. Hendra and Nipah viruses: pathogenesis and therapeutics. *Curr Mol Med* 5 (8):805-16.

Feldman, K. A., D. Stiles-Enos, K. Julian, B. T. Matyas, S. R. Telford, 3rd, M. C. Chu, L. R. Petersen, and E. B. Hayes. 2003. Tularemia on Martha's Vineyard: seroprevalence and occupational risk. *Emerg Infect Dis* 9 (3):350-4.

Fisher-Hoch, S. P. 2005. Lessons from nosocomial viral haemorrhagic fever outbreaks. *Br Med Bull* 73-74:123-37.

Fogarty, R., K. Halpin, A. D. Hyatt, P. Daszak, and B. A. Mungall. 2008. Henipavirus susceptibility to environmental variables. *Virus Res* 132 (1-2):140-4.

Gabastou, J. M., J. Proano, A. Vimos, G. Jaramillo, E. Hayes, K. Gage, M. Chu, J. Guarner, S. Zaki, J. Bowers, C. Guillemard, H. Tamayo, and A. Ruiz. 2000. An outbreak of plague including cases with probable pneumonic infection, Ecuador, 1998. *Trans R Soc Trop Med Hyg* 94 (4):387-91.

Gage, K. L., D. T. Dennis, K. A. Orloski, P. Ettestad, T. L. Brown, P. J. Reynolds, W. J. Pape, C. L. Fritz, L. G. Carter, and J. D. Stein. 2000. Cases of cat-associated human plague in the Western US, 1977-1998. *Clin Infect Dis* 30 (6):893-900.

Gage, K.L. 1998. Plague. In *Topley and Wilson's Microbiology and Microbial Infections*, edited by L. Collier, A. Balows, M. Sussman and W. J. Hausler. London: Arnold.

Goethert, H. K., B. Saviet, and S. R. Telford, 3rd. 2009. Metapopulation structure for perpetuation of Francisella tularensis tularensis. *BMC Microbiol* 9:147.

Goethert, H. K., I. Shani, and S. R. Telford, 3rd. 2004. Genotypic diversity of Francisella tularensis infecting Dermacentor variabilis ticks on Martha's Vineyard, Massachusetts. *J Clin Microbiol* 42 (11):4968-73.

Goethert, H. K., and S. R. Telford, 3rd. 2009. Nonrandom distribution of vector ticks (Dermacentor variabilis) infected by Francisella tularensis. *PLoS Pathog* 5 (2):e1000319.

Goodnough, A. 2009. Anthrax case linked to drumming circle, New Hampshire officials say. *New York Times*, December 29, 2009.

Greco, D., G. Allegrini, T. Tizzi, E. Ninu, A. Lamanna, and S. Luzi. 1987. A waterborne tularemia outbreak. *Eur J Epidemiol* 3 (1):35-8.

Gresikova, M., and C.H.Calisher. 1989. Tick-borne Encephalitis. *The Arboviruses: Epidemiology and Ecology* 4:177-202.

Gresikova, M., Calisher,C.H. . 1989. Tick-borne Encephalitis. In *Epidemiology and Ecology*, edited by T. P. Monath. Boca Raton: CRC Press Inc.

Gritsun, T. S., V. A. Lashkevich, and E. A. Gould. 2003. Tick-borne encephalitis. *Antiviral Res* 57 (1-2):129-46.

Gritsun, T. S., P. A. Nuttall, and E. A. Gould. 2003. Tick-borne flaviviruses. *Adv Virus Res* 61:317-71.

Halsted, C. C., and H. P. Kulasinghe. 1978. Tularemia pneumonia in urban children. *Pediatrics* 61 (4):660-2.

Heinz, F. X., and C. Kunz. 2004. Tick-borne encephalitis and the impact of vaccination. *Arch Virol Suppl* (18):201-5.

Hood, A. M. 2009. The effect of open-air factors on the virulence and viability of airborne Francisella tularensis. *Epidemiol Infect* 137 (6):753-61.

Hugh-Jones, M., and J. Blackburn. 2009. The ecology of Bacillus anthracis. *Mol Aspects Med* 30 (6):356-67.

Inglesby, T. V., D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. M. Friedlander, J. Hauer, J. McDade, M. T. Osterholm, T. O'Toole, G. Parker, T. M. Perl, P. K. Russell, and K. Tonat. 1999. Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 281 (18):1735-45.

Johnson, R. 2008. Differentiation of naturally occurring from non-naturally occurring epizootics of anthrax in livestock populations. edited by Department of Agriculture: Animal and Plant Health Inspection Service, Veterinary Service,.

Keim, P., A. Johansson, and D. M. Wagner. 2007. Molecular epidemiology, evolution, and ecology of Francisella. *Ann N Y Acad Sci* 1105:30-66.

Kong, W. P., C. Hood, Z. Y. Yang, C. J. Wei, L. Xu, A. Garcia-Sastre, T. M. Tumpey, and G. J. Nabel. 2006. Protective immunity to lethal challenge of the 1918 pandemic influenza virus by vaccination. *Proc Natl Acad Sci U S A* 103 (43):15987-91.

LeDuc, J. W. 1989. Epidemiology of hemorrhagic fever viruses. *Rev Infect Dis* 11 Suppl 4:S730-5.

Leffel, E. K., and D. S. Reed. 2004. Marburg and Ebola viruses as aerosol threats. *Biosecur Bioterror* 2 (3):186-91.

Leroy, E. M., B. Kumulungui, X. Pourrut, P. Rouquet, A. Hassanin, P. Yaba, A. Delicat, J. T. Paweska, J. P. Gonzalez, and R. Swanepoel. 2005. Fruit bats as reservoirs of Ebola virus. *Nature* 438 (7068):575-6.

Lo, M. K., and P. A. Rota. 2008. The emergence of Nipah virus, a highly pathogenic paramyxovirus. *J Clin Virol* 43 (4):396-400.

Loosli, C.G., H.M. Lemon, O.H Roberstson, and E. Appel. 1943. Experimental airborne influenza infection. I. Influence of humidity on survival of virus in air. *Proc. Soc. Exp. Biol.* 53:205-206.

Lowen, A. C., S. Mubareka, T. M. Tumpey, A. Garcia-Sastre, and P. Palese. 2006. The guinea pig as a transmission model for human influenza viruses. *Proc Natl Acad Sci U S A* 103 (26):9988-92.

Luby, S. P., M. J. Hossain, E. S. Gurley, B. N. Ahmed, S. Banu, S. U. Khan, N. Homaira, P. A. Rota, P. E. Rollin, J. A. Comer, E. Kenah, T. G. Ksiazek, and M. Rahman. 2009. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* 15 (8):1229-35.

Lytle, C. D., and J. L. Sagripanti. 2005. Predicted inactivation of viruses of relevance to biodefense by solar radiation. *J Virol* 79 (22):14244-52.

Mahmoud, A., D. Burke, S. Eubank, V.S. Freimuth, G Friedman-Jimenez, P. Hamburg, K.A. Holbrook, D.L. Kasper, J. Lewis, W.I. Lipkin, T.H. Murray, M.E. Northridge, J. Patterson, M. Robson, S. Stanley, W. Thomann, S. Bennett, P. Highnam, and R. Khabbaz. 2008. NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratory at

Boston University Medical Center, Finding and Recommendations, Part I: Risk Assessment; Briefing of the Advisory Committee to the Director, NIH, June 6, 2008.

Martina, B. E., B. L. Haagmans, T. Kuiken, R. A. Fouchier, G. F. Rimmelzwaan, G. Van Amerongen, J. S. Peiris, W. Lim, and A. D. Osterhaus. 2003. Virology: SARS virus infection of cats and ferrets. *Nature* 425 (6961):915.

Martone, W. J., L. W. Marshall, A. F. Kaufmann, J. H. Hobbs, and M. E. Levy. 1979. Tularemia pneumonia in Washington, DC. A report of three cases with possible common-source exposures. *JAMA* 242 (21):2315-7.

Matyas, B. T., H. S. Nieder, and S. R. Telford, 3rd. 2007. Pneumonic tularemia on Martha's Vineyard: clinical, epidemiologic, and ecological characteristics. *Ann N Y Acad Sci* 1105:351-77.

McCaughey, C., and C. A. Hart. 2000. Hantaviruses. *J Med Microbiol* 49 (7):587-99.

McEachern, J. A., J. Bingham, G. Crameri, D. J. Green, T. J. Hancock, D. Middleton, Y. R. Feng, C. C. Broder, L. F. Wang, and K. N. Bossart. 2008. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* 26 (31):3842-52.

McKinney, K. R., Y. Y. Gong, and T. G. Lewis. 2006. Environmental transmission of SARS at Amoy Gardens. *J Environ Health* 68 (9):26-30; quiz 51-2.

Meegan, J.M., and C.L. Bailey. 1989. Rift Valley Fever. In *The Arboviruses:Epidemiology and Ecology*, edited by T. P. Monath. Boca Raton: CRC Press, Inc.

Meric, M., M. Sayan, A. Willke, and S. Gedikoglu. 2008. [A small water-borne tularemia outbreak]. *Mikrobiyol Bul* 42 (1):49-59.

Meselson, M., J. Guillemin, M. Hugh-Jones, A. Langmuir, I. Popova, A. Shelokov, and O. Yampolskaya. 1994. The Sverdlovsk anthrax outbreak of 1979. *Science* 266 (5188):1202-8.

Miller, W.S., C.R. Demchak, C.R. Rosenberger, J.W. Dominik, and J.L. Bradshaw. 1963. Stability and infectivity of airborne yellow fever and Rift Valley fever viruses. *American Journal of Hygiene* 77:114-121.

Mills, J. N., G. E. Calderon, B. A. Ellis, K. T. McKee, T. G. Ksiazek, J. G. Oro, C. J. Peters, J. E. Childs, and J. I. Maiztegui. 1991. [New findings on Junin virus infection in rodents inside and outside the endemic area of hemorrhagic fever in Argentina]. *Medicina (B Aires)* 51 (6):519-23.

Mills, J. N., B. A. Ellis, J. E. Childs, K. T. McKee, Jr., J. I. Maiztegui, C. J. Peters, T. G. Ksiazek, and P. B. Jahrling. 1994. Prevalence of infection with Junin virus in rodent populations in the epidemic area of Argentine hemorrhagic fever. *Am J Trop Med Hyg* 51 (5):554-62.

Mills, J. N., B. A. Ellis, K. T. McKee, Jr., T. G. Ksiazek, J. G. Oro, J. I. Maiztegui, G. E. Calderon, C. J. Peters, and J. E. Childs. 1991. Junin virus activity in rodents from endemic and nonendemic loci in central Argentina. *Am J Trop Med Hyg* 44 (6):589-97.

Morner, T. 1992. The ecology of tularaemia. *Rev Sci Tech* 11 (4):1123-30.

Nasci, R. S., H. M. Savage, D. J. White, J. R. Miller, B. C. Cropp, M. S. Godsey, A. J. Kerst, P. Bennett, K. Gottfried, and R. S. Lanciotti. 2001. West Nile virus in overwintering Culex mosquitoes, New York City, 2000. *Emerg Infect Dis* 7 (4):742-4.

New Hampshire Fish and Game Department. 2005. New Hampshire Big Game Plan; Species Management Goals and Objectives 2006-2015.

New York City Department of Health and Mental Hygiene. 2010. *Health Department reports high level of West Nile virus activity in the city* 2010 [cited July 20 2010 2010]. Available from http://www.nyc.gov/html/doh/html/pr2010/pr034-10.shtml.

Padula, P., R. Figueroa, M. Navarrete, E. Pizarro, R. Cadiz, C. Bellomo, C. Jofre, L. Zaror, E. Rodriguez, and R. Murua. 2004. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. *J Virol* 78 (21):11972-9.

Paragas, J., and T. W. Geisbert. 2006. Development of treatment strategies to combat Ebola and Marburg viruses. *Expert Rev Anti Infect Ther* 4 (1):67-76.

Parodi, A. S. 2008. Junin virus. *International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates. CDC On-line Edition.*

Pattyn, SR, ETW Bowen, and PA Webb. *Ebola virus.* (CDC On-line Edition.) 2008. Available from http://wwwn.cdc.gov/arbocat/catalog-listing.asp?VirusID=137&SI=1.

Peters, C. J., P. B. Jahrling, C. T. Liu, R. H. Kenyon, K. T. McKee, Jr., and J. G. Barrera Oro. 1987. Experimental studies of arenaviral hemorrhagic fevers. *Curr Top Microbiol Immunol* 134:5-68.

Qin, Z., T. Clements, L. Wang, M. Khatri, S. P. Pillai, Y. Zhang, J. T. Lejeune, and C. W. Lee. 2011. Detection of influenza viral gene in European starlings and experimental infection. *Influenza Other Respi Viruses* 5 (4):268-75.

Rabenau, H. F., J. Cinatl, B. Morgenstern, G. Bauer, W. Preiser, and H. W. Doerr. 2005. Stability and inactivation of SARS coronavirus. *Med Microbiol Immunol* 194 (1-2):1-6.

Reed, W. P., D. L. Palmer, R. C. Williams, Jr., and A. L. Kisch. 1970. Bubonic plague in the Southwestern United States. A review of recent experience. *Medicine (Baltimore)* 49 (6):465-86.

Rift Valley Fever Virus Working Group. 2004. Rift Valley Fever Virus Working Group 24-26 August 2004 Summary Report and Recommendations. Arlington, VA: ANSER.

Roberts, A., D. Deming, C. D. Paddock, A. Cheng, B. Yount, L. Vogel, B. D. Herman, T. Sheahan, M. Heise, G. L. Genrich, S. R. Zaki, R. Baric, and K. Subbarao. 2007. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathog* 3 (1):e5.

Rose, L. J., R. Donlan, S. N. Banerjee, and M. J. Arduino. 2003. Survival of Yersinia pestis on environmental surfaces. *Appl Environ Microbiol* 69 (4):2166-71.

Rotenberk, L. 2010. A small addition to backyard farms. *Chicago News Cooperative*.

Ruzek, D., L. Bell-Sakyi, J. Kopecky, and L. Grubhoffer. 2008. Growth of tick-borne encephalitis virus (European subtype) in cell lines from vector and non-vector ticks. *Virus Res* 137 (1):142-6.

Sagripanti, J. L., and C. D. Lytle. 2007. Inactivation of influenza virus by solar radiation. *Photochem Photobiol* 83 (5):1278-82.

Saile, E., and T. M. Koehler. 2006. Bacillus anthracis multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Appl Environ Microbiol* 72 (5):3168-74.

Salkeld, D. J., R. J. Eisen, P. Stapp, A. P. Wilder, J. Lowell, D. W. Tripp, D. Albertson, and M. F. Antolin. 2007. The potential role of swift foxes (Vulpes velox) and their fleas in plague outbreaks in prairie dogs. *J Wildl Dis* 43 (3):425-31.

Shi, Z., and Z. Hu. 2008. A review of studies on animal reservoirs of the SARS coronavirus. *Virus Res* 133 (1):74-87.

Shimshony, A., and R. Barzilai. 1983. Rift Valley fever. *Adv Vet Sci Comp Med* 27:347-425.

Siegert, R. , and D.I.H. Simpson. 2008. Marburg virus. *International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates.* (CDC On-line Edition.).

Sjostedt, A. 2007. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann N Y Acad Sci* 1105:1-29.

Suss, J. 2003. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine* 21 Suppl 1:S19-35.

Swanepoel, R., S. B. Smit, P. E. Rollin, P. Formenty, P. A. Leman, A. Kemp, F. J. Burt, A. A. Grobbelaar, J. Croft, D. G. Bausch, H. Zeller, H. Leirs, L. E. Braack, M. L. Libande, S. Zaki, S. T. Nichol, T. G. Ksiazek, and J. T. Paweska. 2007. Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis* 13 (12):1847-51.

Taubenberger, J. K., and D. M. Morens. 2006. 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis* 12 (1):15-22.

Titball, R. W., P. C. Turnbull, and R. A. Hutson. 1991. The monitoring and detection of Bacillus anthracis in the environment. *Soc Appl Bacteriol Symp Ser* 20:9S-18S.

Toro, J., J. D. Vega, A. S. Khan, J. N. Mills, P. Padula, W. Terry, Z. Yadon, R. Valderrama, B. A. Ellis, C. Pavletic, R. Cerda, S. Zaki, W. J. Shieh, R. Meyer, M. Tapia, C. Mansilla, M. Baro, J. A. Vergara, M. Concha, G. Calderon, D. Enria, C. J. Peters, and T. G. Ksiazek. 1998. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. *Emerg Infect Dis* 4 (4):687-94.

Torres-Velez, F. J., W. J. Shieh, P. E. Rollin, T. Morken, C. Brown, T. G. Ksiazek, and S. R. Zaki. 2008. Histopathologic and immunohistochemical characterization of Nipah virus infection in the guinea pig. *Vet Pathol* 45 (4):576-85.

Towner, J. S., X. Pourrut, C. G. Albarino, C. N. Nkogue, B. H. Bird, G. Grard, T. G. Ksiazek, J. P. Gonzalez, S. T. Nichol, and E. M. Leroy. 2007. Marburg virus infection detected in a common African bat. *PLoS ONE* 2 (1):e764.

Tumpey, T. 2008. *1918 influenza virus: an overview of the pathogenicity of the H1N1 and virulence factors*. Bethesda: NIH-RAC.

Tumpey, T. M., A. Garcia-Sastre, J. K. Taubenberger, P. Palese, D. E. Swayne, and C. F. Basler. 2004. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A* 101 (9):3166-71.

Tumpey, T. M., T. R. Maines, N. Van Hoeven, L. Glaser, A. Solorzano, C. Pappas, N. J. Cox, D. E. Swayne, P. Palese, J. M. Katz, and A. Garcia-Sastre. 2007. A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. *Science* 315 (5812):655-9.

Turell, M. 2008. Personal communication record regarding TBE-complex viruses and Congo-Crimean viruses. Arlington, October 30, 2008.

Turell, M. J., and G. B. Knudson. 1987. Mechanical transmission of Bacillus anthracis by stable flies (Stomoxys calcitrans) and mosquitoes (Aedes aegypti and Aedes taeniorhynchus). *Infect Immun* 55 (8):1859-61.

U.S. Department of Agriculture. 2002. The Census of Agriculture.

U.S. Department of Agriculture Forest Service.

van der Poel, W. H., P. H. Lina, and J. A. Kramps. 2006. Public health awareness of emerging zoonotic viruses of bats: a European perspective. *Vector Borne Zoonotic Dis* 6 (4):315-24.

Van Ness, G. B. 1971. Ecology of anthrax. *Science* 172 (990):1303-7.

von Reyn, C. F., A. M. Barnes, N. S. Weber, T. Quan, and W. J. Dean. 1976. Bubonic plague from direct exposure to a naturally infected wild coyote. *Am J Trop Med Hyg* 25 (4):626-9.

Wang, L. F., and B. T. Eaton. 2007. Bats, civets and the emergence of SARS. *Curr Top Microbiol Immunol* 315:325-44.

Wang, X. W., J. S. Li, M. Jin, B. Zhen, Q. X. Kong, N. Song, W. J. Xiao, J. Yin, W. Wei, G. J. Wang, B. Y. Si, B. Z. Guo, C. Liu, G. R. Ou, M. N. Wang, T. Y. Fang, F. H. Chao, and J. W. Li. 2005. Study on the resistance of severe acute respiratory syndrome-associated coronavirus. *J Virol Methods* 126 (1-2):171-7.

Warfield, K. L., E. M. Deal, and S. Bavari. 2009. Filovirus infections. *J Am Vet Med Assoc* 234 (9):1130-9.

Weingartl, H. M., R. A. Albrecht, K. M. Lager, S. Babiuk, P. Marszal, J. Neufeld, C. Embury-Hyatt, P. Lekcharoensuk, T. M. Tumpey, A. Garcia-Sastre, and J. A. Richt. 2009. Experimental infection of pigs with the human 1918 pandemic influenza virus. *J Virol* 83 (9):4287-96.

Weingartl, H. M., Y. Berhane, and M. Czub. 2009. Animal models of henipavirus infection: a review. *Vet J* 181 (3):211-20.

Weis, C. P., A. J. Intrepido, A. K. Miller, P. G. Cowin, M. A. Durno, J. S. Gebhardt, and R. Bull. 2002. Secondary aerosolization of viable Bacillus anthracis spores in a contaminated US Senate Office. *JAMA* 288 (22):2853-8.

Wells, R. M., S. Sosa Estani, Z. E. Yadon, D. Enria, P. Padula, N. Pini, J. N. Mills, C. J. Peters, and E. L. Segura. 1997. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia. *Emerg Infect Dis* 3 (2):171-4.

Wild, M. A., T. M. Shenk, and T. R. Spraker. 2006. Plague as a mortality factor in Canada lynx (Lynx canadensis) reintroduced to Colorado. *J Wildl Dis* 42 (3):646-50.

Wilkening, D. A. 2006. Sverdlovsk revisited: modeling human inhalation anthrax. *Proc Natl Acad Sci U S A* 103 (20):7589-94.

Willke, A., M. Meric, R. Grunow, M. Sayan, E. J. Finke, W. Splettstosser, E. Seibold, S. Erdogan, O. Ergonul, Z. Yumuk, and S. Gedikoglu. 2009. An outbreak of oropharyngeal tularaemia linked to natural spring water. *J Med Microbiol* 58 (Pt 1):112-6.

Won, W. D., and H. Ross. 1966. Effect of diluent and relative humidity on apparent viability of airborne Pasteurella pestis. *Appl Microbiol* 14 (5):742-5.

Wong, S.S.Y., and K.Y. Yuen. 2005. The severe acute respiratory syndrome (SARS). *J Neurovirol* 11:455-468.

Table 7-1. BSL-3 pathogens* and their environmental relevance to proposed NEIDL sites

	Natural vector species	Vectors Species endemic to New England	Natural reservoirs	Reservoirs endemic to New England	Potential[a] to become established in environments surrounding proposed NEIDL sites
B. anthracis	none[b]	N/A	Soil (in endemic states)	No	No
F. tularensis	Ticks, deer flies, other arthropods	Yes	Rabbits (*Sylvilagus* or *Oryctolagus* spp.), hares (*Lepus* spp.)	Yes	Yes
Y. pestis	Fleas (*Oropsylla montanus*), ~ 30 other flea species	Yes	Mice (*Mus* spp.), water rats (*Scapteromys* spp.), squirrels (*Sciurus* spp.), voles (*Microtus* spp.), rabbits (*Sylvilagus* or *Oryctolagus* spp.)	Yes	Yes
1918 H1N1V	None	N/A	Avian species, swine[c]	Yes[c]	Yes
SARS-CoV	None	N/A	Horsehoe bat (*Rhinolophus* spp.)	No	No
RVFV	Mosquitoes (~ 23 species), other hematophagous arthropods	Yes	Mosquitoes *Aedes* spp.	Yes	Yes
ANDV[d]	None	N/A	Long-tailed pygmy rice rat *Oligoryzomys longicaudatus*	No	No

Notes:
* *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), Andes virus (ANDV)
a. Potential does not signify likelihood.
b. Tabanid flies have been reported infected rarely; these are endemic in New England.
c. Birds and swine are natural reservoirs for influenza A viruses. There currently is no reservoir for reconstructed 1918 H1N1V.
d. ANDV is a BLS-3 pathogen except when inoculated into a permissive host, which then requires BSL-4
N/A – not applicable

Table 7-2. BSL-4 pathogens* and their environmental relevance to proposed NEIDL sites

	Natural vector species	Vectors species endemic to New England	Natural reservoirs	Reservoirs endemic to New England	Potential[a] to become established in environments surrounding proposed NEIDL sites
ANDV[b]	None	N/A	Long-tailed pygmy rice rat *Oligoryzomys longicaudatus*	No	No
EBOV	None	N/A	Fruit bats (*Hypsignathus monstrosus, Epomops franquetti, Myonycteris torquata*)	No	No
MARV	None	N/A	Fruit bats (*Rousettus aegyptiacus, Miniopterus inflatus, Rhinolophus eloquens*)	No	No
LASV	None	N/A	Multimammate rats (*Mastomys natalensis*)	No	No
JUNV	None	N/A	Vesper mouse (*Calomys musculinus, C. laucha*); possibly other sigmodontine species & house mouse (*Mus musculus*)	No	No
TBEV-FE	Ticks (*Ixodes persulcatus*)	No	Ticks, (*Ixodes persulcatus*); red voles (*Myodes rutilis*	No	Yes
NIPV	None	N/A	Flying fox (*Pteropus* spp.)	No	No

Notes:
* Andes virus (ANDV), Ebola virus (EBOV), Marburg virus (MARV), Lassa virus (LASV), Junín virus (JUNV), Tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus (TBEV-FE), Nipah virus (NIPV)
a. Potential does not signify likelihood.
b. ANDV is a BLS-3 pathogen except when inoculated into a permissive host, which then requires BSL-4
N/A – not applicable

Table of Contents

8. Health Effects – Initial Infection

8.1 Introduction

The goals of the event sequence analyses in Chapter 4 are to answer these questions: What could go wrong at NEIDL? How often would those events be expected to occur, and what would be the immediate consequences of those events? In other words, Chapter 4 estimates the effects of a loss of biocontainment in terms of the frequency of those events and the amount of pathogen an individual would be exposed to as a result of those events. In the next step of this RA, the goal is to answer the question, What would happen if an individual is exposed to a pathogen? This chapter examines the effects of exposure to a pathogen in terms of initial infections and deaths from that exposure. A distinction is made with regard to initial infection addressed in this chapter as that occurring after direct exposure to a pathogen as a result of a release from the laboratory and secondary infection that results from exposure to an already infected individual. Secondary infections are addressed in Chapter 9 and Appendix L.

This chapter is organized as follows. First, the dose-response section discusses probabilities or estimates of how likely it is for an infection to occur after exposure to different amounts of each pathogen through the respiratory route. Second, the event-specific initial infections sections describe how the dose-response estimates are linked with the exposure estimates from the event sequence analyses to provide estimates for the frequency or how often initial infections would be expected to occur as a result of loss of biocontainment at NEIDL. Those sections also include discussion of potential health effects, including deaths (mortalities) that might result in those who become infected. Finally, the potential implications of the presence of medically vulnerable sub-populations among members of the public are discussed in terms of how their vulnerability would affect their chances of infection after exposure to a pathogen. In this chapter, this is discussed in the context of a potential event that would directly expose members of the public to pathogen at the three alternate NEIDL sites.

8.2 Dose Response Assessment

8.2.1 Methodology – Introduction

Dose-response assessment is used to estimate the relationship between a dose, or amount of pathogen to which an individual is exposed, and a given response such as the establishment of infection, sickness (morbidity), or death (mortality). For this RA, dose-response assessment is an important component in converting estimates of the frequency and amount of exposure to a pathogen from the event sequence analysis to estimates of initial infection for the health effects analysis.

When an individual is exposed to a dose of viable pathogens, there is a likelihood that the pathogen will replicate or multiply in the individual and cause an infection. The infection could lead to a disease in that individual. There is also a probability that the pathogens will die off or be eliminated by the individual's immune system before infection can be established. The outcome after a given exposure depends on characteristics of both the pathogen and the individual. For each pathogen, this RA considers evidence for the ability of doses of various sizes to infect humans, and, because a dose of a given size might affect one human differently from another, this RA also considers differences in susceptibility to a pathogen among different people.

Dose-response is assessed by estimating a functional relationship between the amount of exposure, or dose, and the probability of that dose resulting in a response of interest in a randomly chosen individual from a given population. This section describes the general methodology for assessing the relationship between dose and response for each pathogen.

An exposure could result in no detectable response if the initial organisms to which the individual or host is exposed die off, are eliminated from the host, cannot attach to host cells because of lack of or imperfect receptor sites, or are inactivated by the host's immune system. Otherwise, the infection caused by a pathogen in humans is the response of interest at this stage of the RA. Infection is a necessary first step for disease and death to occur. Those are considered in subsequent sections as estimates of the percentage of infections that eventually lead to symptomatic disease and death, respectively. Infection is an important response to consider because it is also a necessary first step for potential secondary transmission. It is important to note that for certain pathogens, the person transmitting the pathogen to another person might not show symptoms of the disease at the time secondary transmission occurs.

Each pathogen has a natural route of infection that is dependent on the biology of the pathogen, natural reservoirs, and typical modes of transmission. In a laboratory setting such as the NEIDL, some experiments would attempt to simulate the natural route of infection, but it is possible that events occurring during culturing, manipulating, transporting, and storing the pathogen could lead to potential exposures that differ from those that would occur naturally. In such circumstances, it is possible that exposure to a pathogen in a laboratory setting from a route different from its natural route of exposure could result in an infection. For this RA, it is assumed that any route of exposure resulting from a NEIDL-related event could potentially lead to infection in the exposed individual.

The probability of infection resulting from a given dose of a pathogen can vary according to the route of exposure. The above routes of infection are considered on a pathogen-by-pathogen basis. Generally, the bulk of the dose-response assessment focuses on the inhalation route of exposure, for the following reasons. Some of the most important event sequences for this RA result in inhalational exposure, and most of the relevant animal dose-response data for many of the pathogens were derived from inhalational exposures. Other routes of exposure are considered, as appropriate, for each pathogen and compared with the estimates generated for dose-response by the inhalational route.

The results of each event sequence analysis are provided in terms of exposures for one or more of the following groups:

- Laboratory worker—People working in the biocontainment area where the event might be initiated.
- Facility worker—People working in the NEIDL but not in the biocontainment area where the event under consideration occurs. For example, they might work in other laboratories or in administrative areas.
- Public—Any person outside the NEIDL-controlled perimeter, specifically referring to the population in the surrounding communities.

For the dose-response assessment, estimates of the probabilities of human infection if exposure occurs are assumed to be equal across all three groups, with the following exceptions.

- Vaccine status—As part of training and preparation for work in a high biocontainment laboratory, it is possible that laboratory workers working with certain pathogens for which a vaccine is available would have received that vaccine to prevent infection. Those vaccines might or might not be available to facility workers or the general public. That possibility is considered on a pathogen-by-pathogen basis.
- Post-exposure prophylaxis—The above three groups could have differential access to prophylactic regimens, if available, after being exposed. The availability and effectiveness of medication or vaccine prophylaxis are discussed on a pathogen-by-pathogen basis. Note that many of the release scenarios examined in this RA assume that the incident leading to the release is either undetected or unreported. In such situations, the issue of post-exposure prophylaxis might not be applicable.
- Population susceptibility—The above three groups likely have different profiles of susceptibility to disease because of differences in age, immune status, and preexisting health conditions. Those differences might or might not be uniform between members of the public near the three sites

compared in this RA. In some cases, where relative susceptibility estimates for a specific population are available, adjustments to dose-response estimates are made.

8.2.2 Methodology – Quantitative Assessment

The relationship between the dose received and the probability of infection can be quantified using mathematical dose-response curves or assessment. For this RA, three different types of assessments were considered (log-probit, exponential and Beta Poisson). The details pertaining to those three types are described in Appendix J.

Two sets of dose-response assessments were derived for use in this RA, termed literature-based and *expert-based* dose-response assessments.

- **Literature-based dose-response assessments**
  The three candidate assessments described in Appendix J were evaluated in light of the dose-response data and information available in the published literature for each of the pathogens. The assessments found in the literature for each pathogen were evaluated, and, in some cases, fit to published experimental data. The techniques are described in Appendix J.
- **Expert-based dose-response assessments**
  The three candidate assessments described in Appendix J were also used to fit curves to the information obtained from the expert panelists who were asked via the Delphi method to provide estimates of infectious doses for the 13 pathogens (see the Delphi Panel Report). The process for fitting curves to the expert-provided values is described in Appendix J. In this context, the three candidate dose-response assessments were evaluated and compared with each other in terms of their ability to match as closely as possible to the estimates provided by each expert for each pathogen.

The results of the literature-based and expert-based dose-response assessment each consist of a central estimate and an uncertainty range. The two alternate ranges of estimates were compared, especially for low doses at which most of the exposure estimates for the event sequences occur. In some cases, the literature-based range is more conservative (estimates higher risk) than the expert-based range, and in other cases, the opposite is true. All results are presented and the differences discussed in conjunction with each pathogen.

8.2.3 Results – BSL-3 Pathogens

This section documents the dose-response assessment for each of the seven BSL-3 pathogens. A detailed dose-response literature review and quantitative description and derivation of dose-response assessments for each pathogen are provided in Appendix J. This section provides a brief summary of dose-response information for each pathogen and the basis for the literature-based dose-response assessment, if applicable. The ranges of numerical infectious dose estimates to be used for this RA are provided in summary form in Section 8.2.5.

8.2.3.1 *Bacillus anthracis*

Bacillus anthracis (B. anthracis) is a bacterial organism that causes anthrax (Chapter 3 and Appendix C). No human experimental dose-response data are available for inhalational anthrax. However, quantitative studies have been performed using available information from historical inhalational exposures and infections that have occurred in human populations. In the absence of human data to further refine potential quantitative dose-response models, experimental studies involving NHPs provide the best available data from which to gain insights into potentially appropriate dose-response relationships for humans. Among the NHP data and models described in Appendix J, a model fit (Haas 2002) to data from cynomolgus monkeys (Brachman 1966) was selected as the literature-based dose-response assessment to be used in this RA.

8.2.3.2 *Francisella tularensis*

Francisella tularensis (F. tularensis) is a bacterial organism that causes tularemia (Chapter 3 and Appendix C). There is strong evidence that small inhaled doses of *F. tularensis* can result in human infections. It was determined that the human experimental data relevant to this RA (Saslaw 1961; McCrumb 1961) are not extensive or wide-ranging enough to serve as the sole basis for a statistically acceptable dose-response assessment. However, dose-response data from NHPs (Day 1972) are consistent with the human data (as confirmed by statistical tests described in Appendix J) and pooling these data with the human data results in a larger data set that serves as the literature-based dose-response assessment for use in this RA.

8.2.3.3 *Yersinia pestis*

Yersinia pestis (Y. pestis) is a bacterial organism that causes plague (Chapter 3 and Appendix C). No direct human dose-response data for *Y. pestis* are available in the literature. In the absence of human dose-response data for *Y. pestis*, experimental studies involving NHPs provide the best available data from which to gain insights into potentially appropriate dose-response relationships for humans. Results from

exposure of rhesus monkeys to aerosolized *Y. pestis* (Speck 1957) appear to be the only inhalational dose-response data that have been fit to dose response assessments (Huang 2010), and the method that was used deliver inhalational doses to the animals is more consistent with exposures that might occur as a result of releases studied in this RA than the dose delivery mechanisms of other studies, as described in Appendix J. Therefore, the best fit model (Huang 2010) to the rhesus monkey data (Speck 1957) was chosen as the literature-based assessment for this RA.

8.2.3.4 1918 H1N1 influenza virus

1918 H1N1 influenza virus (1918 H1N1V) is a particular strain of influenza A virus that caused a worldwide human influenza pandemic in 1918-1919 (Chapter 3 and Appendix C). No direct human dose-response data exist for 1918 H1N1V. Detailed review of the data sets available in the literature for other strains of influenza has determined that these data sets do not support quantitative dose-response estimates for 1918 H1N1V that would provide useful insight for this RA. Therefore, only the expert-based dose-response estimates were applied to exposure data from the event sequence analyses.

8.2.3.5 SARS-associated coronavirus

SARS-associated coronavirus (SARS-CoV) causes severe acute respiratory syndrome (SARS) (Chapter 3 and Appendix C). No direct human dose-response data exist for SARS-CoV. There was one dose-response assessment study of SARS-CoV found in the literature (Watanabe 2010), which consisted of an analysis of multiple data sets from the literature. Those are applied as the literature-based dose-response assessment for this RA. The estimates are based on data from experimental exposures of mice. The derived estimates appeared to be consistent with data from human volunteers exposed to various doses of a human coronavirus related to SARS-CoV.

8.2.3.6 Rift Valley fever virus

Rift Valley fever virus (RVFV) causes Rift Valley fever (Chapter 3 and Appendix C). No human dose-response data are available for RVFV. A dose response curve fit to a pooled data set from exposures of dogs and cats was chosen as the literature-based assessment for this RA.

8.2.3.7 Andes virus

Andes virus (ANDV) is a hantavirus that causes hantavirus pulmonary syndrome (HPS) (Chapter 3 and Appendix C). No human dose-response data are available for ANDV. A data set derived from ANDV exposures of Syrian hamsters (Hooper 2008) appears to be the only intranasal or inhalational dose-

response animal data available in the literature. Syrian hamsters had previously been shown to be an animal model that closely mimics human HPS (Hooper 2001). A dose-response curve fit to this data set is used as the literature-based dose-response assessment for this RA.

8.2.4 Results – BSL-4 Pathogens

This section documents the dose-response assessment for each of the six BSL-4 pathogens. A detailed dose-response literature review and quantitative description and derivation of dose-response assessments for each pathogen are provided in Appendix J. This section provides a brief summary of dose-response information for each pathogen and the basis for the literature-based dose-response assessment, if applicable. The ranges of numerical infectious dose estimates to be used for this RA are provided in summary form in Section 8.2.5.

8.2.4.1 Ebola virus

Ebola virus (EBOV) causes Ebola hemorrhagic fever (EHF) (Chapter 3 and Appendix C). No human dose-response data are available for EBOV. Unimmunized NHPs *(Macaca mulatta or M. fascicularis)* have been infected after inhalational exposure to EBOV in a number of studies, as reviewed in Appendix J. A 100 percent infection and death rate was observed across a range of doses in all studies except one (P'iankov 1995), in which a portion of monkeys survived exposure to the lower dose groups. A data set including results across all the relevant studies was amenable to dose-response curve fitting, and the best fit curve to this consolidated data set is used as the literature-based assessment for this RA

8.2.4.2 Marburg virus

Marburg virus (MARV) causes Marburg hemorrhagic fever (MHF) (Chapter 3 and Appendix C). No human dose-response data are available for MARV. Unimmunized NHPs (*M. mulatta or M. fascicularis)* have been infected after inhalational exposure to MARV in a number of studies, as reviewed in Appendix J. A 100 percent infection and death rate was observed across a range of doses in all studies except one (Bazjutin 1992), in which a portion of monkeys survived exposure in the lower dose group. However, the amounts of virus administered in this low dose group were reported imprecisely and in units that are difficult to reconcile with exposure estimates for this RA. Therefore, it was determined that dose-response curve fitting to the MARV data would not be appropriate.

EBOV is closely related to MARV, and a literature-based dose response curve for EBOV was described in the previous section. The estimates derived from that assessment were found to be consistent with the MARV dose-response data. In the absence of solid quantitative data for MARV and given the numerous

similarities between EBOV and MARV, it was decided to apply the EBOV literature-based dose-response curve to MARV for this RA.

8.2.4.3 Lassa virus

Lassa virus (LASV) causes Lassa fever (Chapter 3 and Appendix C). No human dose-response data are available for LASV. One dose-response curve (Tamrakar 2008) fit to data from guinea pigs exposed to inhalational doses of LASV (Stephenson 1984) was found in the literature. This curve is applied as the literature-based dose-response assessment in this RA.

8.2.4.4 Junin virus

Junin virus (JUNV) causes Argentine hemorrhagic fever (AHF) (Chapter 3 and Appendix C). No human dose-response data are available for JUNV. An NHP, the rhesus macaque *(M. mulatta),* was shown to be susceptible to infection with JUNV after inhalational exposure to a range of doses and developed disease symptoms similar to those observed in humans (Kenyon 1992). Because a 100 percent infection rate was observed, it is not possible to fit a dose-response curve to these data, so a literature-based dose-response assessment is not applied for JUNV in this RA. However, the NHP data are used to estimate upper bounds for various ID levels, for purposes of assessing whether the expert-based dose-response estimates are consistent with the NHP data. The median estimates from the expert-based estimates are close to the literature-based upper bounds estimated from NHP data, which means that about half the weight of the expert-based range is above that estimated upper bound. Those estimates above the median are not necessarily inaccurate because it is possible that humans are not as susceptible to infection from JUNV aerosols as are *M. mulatta*. The part of the distribution that lies below the literature-based upper bound can serve to represent the possibility that human susceptibility to JUNV is consistent with what was observed among NHPs.

8.2.4.5 Tick-borne encephalitis virus, Far Eastern subtype

[Formerly called Tick-borne encephalitis complex (Russian spring summer encephalitis virus)]
The Far Eastern subtype of tick-borne encephalitis virus (TBEV-FE) causes encephalitis (Chapter 3 and Appendix C). No human dose-response data are available for TBEV-FE. A review of the available literature determined that literature-based dose-response assessments were not appropriate for use for this RA. Hence expert-based dose-response estimates are applied.

8.2.4.6 Nipah virus

Nipah virus (NIPV) causes encephalitis and/or respiratory disease (Chapter 3 and Appendix C). No human dose-response data are available for NIPV. Bossart et al. (2009) exposed ferrets to oral-nasal doses of NIPV and generated a data set that is amenable to dose-response curve fitting. The best fit curve to this data set is applied as the literature-based assessment for this RA.

8.2.5 Results – Summary of quantitative estimates

The following tables summarize the range of infectious dose estimates used for this RA for each pathogen. Details are provided in Appendix J.

Table 8–1. Infectious dose (ID) estimates and associated ranges for BSL-3 pathogens

Pathogen	Dose-response assessment	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
B. anthracis	Literature-based	**27,000 spores** (15,000–47,000)	**4,100** (2,200–7,100)	**390** (210–680)	**38** (21−68)	**3.8** (2.1−6.8)
	Expert-based	**8,600 org.** (2,500–29,000)	**690** (360–4,400)	**73** (33–420)	**10** (3.9–56)	**2.4** (0.39–22)
F. tularensis	Literature-based	**11 org.** (6.1–18)	**1.7** (0.93−2.7)	**0.16** (0.089−0.25)		
	Expert-based	**42 org.** (12–460)	**6.0** (1.9–120)	**1.0** (0.18–46)		
Y. pestis	Literature-based	**15,000 org.** (8,200−36,000)	**1400** (530−4,000)	**120** (44−370)	**12** (4.4−37)	**1.2** (0.44−3.7)
	Expert-based	**3,100 org.** (530–27,000)	**280** (80–4,100)	**37** (5.0–390)	**5.6** (0.32–39)	**0.63** (0.034–10)
1918 H1N1V	Expert-based	**700 org.** (110–290,000)	**42** (8.6–52,000)	**3.3** (0.64–15,000)	**0.52** (0.063–6,300)	**0.11** (0.0063–3,000)
SARS-CoV	Literature-based	**280 PFU** (120−580)	**43** (18−88)	**4.1** (1.7−8.4)	**0.41** (0.17−0.84)	**0.041** (0.017−0.084)
	Expert-based	**2900 org.** (280–12,000)	**200** (43–1,800)	**17** (4.1–180)	**3.9** (0.41–18)	**0.67** (0.041–5.0)
RVFV	Literature-based	**9.7 MICLD$_{50}$** (2.9–18)	**1.5** (0.44–2.8)	**0.14** (0.042–0.27)	**0.014** (0.0042–0.026)	
	Expert-based	**1000 org.** (49–7,900)	**100** (7.6–1,200)	**15** (0.71–180)	**3.9** (0.071–56)	
ANDV	Literature-based	**63 PFU** (24−140)	**9.6** (3.6−21)	**0.91** (0.35−2.0)	**0.091** (0.034−0.20)	**0.0091** (0.0034−0.020)
	Expert-based	**520 org.** (10–1,100)	**44** (1.0–160)	**7.1** (0.15–15)	**0.79** (0.039–6.3)	**0.079** (0.013–3.0)

Table 8–2. Infectious dose (ID) estimates and associated ranges for BSL-4 pathogens

Pathogen	Dose-response assessment	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$	$ID_{0.01}$
EBOV	Literature-based	**4 PFU** (1–10)	**0.7** (0.2–2)	**0.06** (0.02–0.2)		
	Expert-based	**300 org.** (120–1,000)	**40** (19–100)	**5.1** (1.8–31)		
MARV	Literature-based	**4 PFU** (1–10)	**0.7** (0.2–2)	**0.06** (0.02–0.2)		
	Expert-based	**200 org.** (100–1,000)	**19** (10–100)	**2.4** (0.74–15)		
LASV	Literature-based	**18 PFU** (6.7−40)	**2.7** (1.0−6.1)	**0.26** (0.098−0.58)		
	Expert-based	**100 org.** (18–30,000)	**11** (2.8–300)	**1.5** (0.27–7.0)		
JUNV	Expert-based	**96 org.** (14–1,000)	**14** (2.1–100)	**1.6** (0.20–15)	**0.19** (0.020–3.9)	
TBEV-FE	Expert-based	**200 org.** (71–10,000)	**20** (10–1,200)	**3.1** (1.0–180)	**0.78** (0.10–56)	**0.25** (0.010–22)
NIPV	Literature-based	**500 $CCID_{50}$** (100–1,000)	**80** (20–200)	**8** (2–20)	**0.8** (0.2–2)	
	Expert-based	**1,000 org.** (500–25,000)	**100** (75–1,400)	**15** (7.2–180)	**3.9** (0.72–56)	

8.3 Initial Infections Resulting from Needlesticks in the Laboratory

8.3.1 Methodology

This section describes the methodology for generating estimates for initial infections resulting from Needlestick events described in Chapter 4 and Appendix F.

Chapter 4 described four distinct sub-events, depending on the setting of the event (BSL-3 or BSL-4 laboratories) and whether the event is detected and reported. The initial infections analyses are primarily concerned with consequences from potential LAI without prompt detection and reporting, because those events pose potential risk to the public through secondary transmission.

As described in Chapter 4 and further detailed in Appendix F, it is conservatively assumed that every needlestick would deliver a sufficiently high dose to cause an infection in the laboratory worker. It is important to note that, on the basis of a review of existing literature on needlestick injuries in the laboratory, not every needlestic results in an infection. However, without data to show how much

pathogen is introduced through the needlestick, the most conservative approach would be to assume that every needlestick will result in an infection. That likely overestimates the risk of needlestick injuries.

The results section for Needlestick events (Section 8.4.2) includes descriptions of potential health consequences for a laboratory worker infected via needlestick for each pathogen. This section also includes estimates for the frequency of mortalities among laboratory workers infected via needlestick without prompt detection and reporting.

8.3.2 Results

The health consequences resulting from exposure to a pathogen via needlestick are dependent on several pathogen characteristics and host factors. This RA assumes that the infectious dose of the pathogen delivered via needlestick is sufficient to cause disease (Chapter 4), so no probabilistic calculations for initial infections are performed. Further, the RA assumes that the pathogen is viable in the needle at the time of the needlestick. An important characteristic is the pathogen's ability to cause infection by this route. As with all infections, the health consequences are also dependent on the immune status and general health of the host. For this RA, for needlestick injuries involving laboratory workers, the assumption is that the laboratory worker is a healthy young adult. For needlestick events without prompt detection and reporting, the health consequences are also based on the assumption that post-exposure prophylaxis (if available), quarantine, and supportive measures are not instituted until the laboratory worker exhibits symptoms and seeks medical attention.

Table 8–3 summarizes the potential health consequences in the laboratory worker and subsequent risk to the public. In the setting of illness caused by needlestick, the presentation and disease course could be different from that of the natural disease, especially for pathogens that are not normally known to cause disease by direct introduction into the host through the skin such as 1918 H1N1V, SARS-CoV and NIPV. However, in the absence of data to suggest otherwise, this RA makes the conservative assumption that the illness caused by needlestick would mimic the natural disease with respect to the course, the organ systems affected, the morbidity and mortality caused and potential for secondary transmission.

The issues of preexisting immunity and potentially available vaccines for each pathogen are further discussed in Appendix J. Full descriptions of the clinical diseases caused by the pathogens are provided in Chapter 3 and Appendix C. The estimates for the case fatality rate (CFR) provided here are summarized in Chapter 3, Appendix C and Mahmoud et al. (2008) and are presumed to apply to heterogeneous

populations containing individuals from medically vulnerable groups. The CFR estimates are assumed to represent the probability that an infected laboratory worker would die from the resulting illness. Applying these CFR estimates to laboratory workers may be conservative, as it is presumed that laboratory workers are healthy adults as discussed further in Appendix I. Potential risk to the public from laboratory workers infected with transmissible pathogens is further discussed in Chapter 9 and Appendix L.

Table 8–3. Summary of health consequences in laboratory worker from needlestick

Pathogen	Pre-existing immunity to pathogen in laboratory worker	Resulting illness via needlestick route	CFR Estimate	Potential for secondary transmission to members of the public if initial infection is undetected and/or unreported
Biosafety Level 3 Pathogens				
B. anthracis	Possible, as vaccine is available	Bacteremia and sepsis with *B. anthracis*	45%	No
F. tularensis	Possible, as vaccine is available	Bacteremia, sepsis and pneumonic form of tularemia	< 2%	No
Y. pestis	No	Bacteremia, primary septicemic plague and pneumonic plague	15%	Yes, if pneumonic plague is present
1918 H1N1V	Possible, due to cross-protection from past influenza vaccines or infections	Influenza	2.5%	Yes, if respiratory symptoms are present
SARS-CoV	No	SARS	10%	Yes, if respiratory symptoms are present
RVFV	Possible, as vaccine is available	Rift Valley fever	0.5–2%	No
ANDV	No	Viremia and Hantavirus pulmonary syndrome	50%	Yes, if respiratory symptoms are present

Pathogen	Pre-existing immunity to pathogen in laboratory worker	Resulting illness via needlestick route	CFR Estimate	Potential for secondary transmission to members of the public if initial infection is undetected and/or unreported
Biosafety Level 4 Pathogens				
EBOV	No	Ebola hemorrhagic fever	40–90%	Yes
MARV	No	Hemorrhagic fever	100%	Yes
LASV	No	Lassa fever (hemorrhagic fever)	1–2%	Yes
JUNV	No	Argentine hemorrhagic fever	< 1%	Yes
TBEV-FE	No	Encephalitis	20–40%	No
NIPV	No	Viremia, encephalitis and/or pneumonia	40–70%	Yes, if respiratory symptoms are present

The CFR estimates in Table 8–3 can be combined with the frequency category for a needlestick incident to estimate the frequency with which deaths would occur among laboratory workers because of infections via needlestick. These CFR estimates are presumably most applicable to needlestick events without prompt detection and reporting, in which treatment of the infection would likely not begin until after symptoms appear, which is likely most often the case during natural disease outbreaks from which the CFR estimates were derived. The frequency of needlestick events without prompt detection and reporting was assigned to the B (0.01 per year to 0.0001 per year) frequency category. This was estimated as the workforce risk for each BSL-3 pathogen as well as each BSL-4 pathogen (see Chapter 4). Frequencies within the category range were multiplied with CFR estimates, with the results shown in Table 8–4.

Table 8–4. Frequency of death (mortality) among laboratory workers for needlestick event without prompt detection and reporting.

BSL-3 Pathogens			
Pathogen	**Assumed CFR**	**Frequency range of laboratory worker mortalities**	**Frequency category**
B. anthracis	0.45	≈ 1 in 200 to 20,000 yrs	**B or C**
F. tularensis	0.02	≈ 1 in 5,000 to 500,000 yrs	**B or C**
Y. pestis	0.15	≈ 1 in 700 to 70,000 yrs	**B or C**
1918 H1N1V	0.025	≈ 1 in 4,000 to 400,000 yrs	**B or C**
SARS-CoV	0.1	≈ 1 in 1,000 to 100,000 yrs	**B or C**
RVFV	0.02	≈ 1 in 5,000 to 500,000 yrs	**B or C**
ANDV	0.5	≈ 1 in 200 to 20,000 yrs	**B or C**
BSL-4 Pathogens			
Pathogen	**Assumed probability of mortality per infection**	**Frequency range of laboratory worker mortalities**	**Frequency category**
EBOV	0.9	≈ 1 in 100 to 10,000 yrs	**B**
MARV	1	≈ 1 in 100 to 10,000 yrs	**B**
LASV	0.02	≈ 1 in 5,000 to 500,000 yrs	**B or C**
JUNV	0.01	≈ 1 in 10,000 to 1 million yrs	**C**
TBEV-FE	0.4	≈ 1 in 200 to 20,000 yrs	**B or C**
NIPV	**0.7**	**≈ 1 in 100 to 10,000 yrs**	**B**

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

8.4 Initial Infections Resulting from Centrifuge Release

8.4.1 Methodology

This section introduces the methodology for generating estimates for initial infections resulting from BSL-3 Centrifuge Infectious Aerosol Release events described in Chapter 4 and Appendix F. A detailed description of the quantitative methodology is presented in Appendix K.

This event involves only those pathogens that can be studied under BSL-3 laboratory conditions (7 of the 13 pathogens). Centrifuge release scenarios involving BSL-4 pathogens are not carried forward to the initial infection analyses because the analysis described in Chapter 4 and Appendix F determined that it would not be credible for an aerosol exposure to go undetected in a BSL-4 laboratory.

All potentially exposed individuals from a centrifuge aerosol release are laboratory workers. The potential route of exposure for laboratory workers during or after a centrifuge release is assumed to be through direct contact, ingestion, or inhalation. Therefore, the dose-response information generated in Appendix J, which focuses primarily on inhalational exposure, can be applied. The centrifuge release information provided in Chapter 4 and the dose-response information provided in Appendix J are synthesized for each of the seven BSL-3 pathogens.

As this RA specifically considers centrifuge release scenarios that are undetected or unreported, the initial infections results also serve as estimates for the frequency of infected laboratory workers leaving the facility after a centrifuge incident, with potential to transmit to the public.

Two types of calculations and results are presented for each pathogen: a *central estimate* and *uncertainty results around that central estimate*. Uncertainty refers to the lack of knowledge of the true value of parameters. Variability refers to the effect of chance and inherent unpredictability of the way events may occur (stochasticity). In the estimation of initial infections, the effect of uncertainty for some key unknown values was assessed by systematically comparing results under different assumptions for their values, and the effect of variability was assessed by performing probabilistic calculations under each tested scenario. The details of these analyses are presented in Appendix K.

8.4.2 Results – Infection frequency

This section summarizes the overall results for the centrifuge aerosol release event. Table 8–5 compiles the central estimate results and Table 8–6 compiles the uncertainty results for all seven BSL-3 pathogens.

Table 8–5. Central estimates for infections from BSL-3 centrifuge infectious aerosol release (with full or partial respiratory protection)

Pathogen	Number of initial infections	Probability of initial infections given release	Average return period for release leading to initial infections	Frequency category
B. anthracis	1 or more	0.0065%	800,000 years	C
F. tularensis	1 or more	12%	400 years	B
	2 or more	0.38%	10,000 years	B/C
Y. pestis	1 or more	0.00091%	5 million years	D
1918 H1N1V	1 or more	0.23%	20,000 years	C
SARS-CoV	1 or more	0.028%	200,000 years	C
RVFV	1 or more	38%	100 years	A/B
	2 or more	6.7%	800 years	B
ANDV	1 or more	0.012%	400,000 years	C

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

For the central estimate example, the results show that *F. tularensis* and RVFV are estimated to result in the highest probability of infections occurring among one or two laboratory workers as a result of a centrifuge aerosol release, with the estimated frequency of those events falling in or near the borders of the B frequency category. For the other pathogens, the frequency of one or more infections falls in the C frequency category, except for *Y. pestis* (D category).

For each pathogen, a single dose-response estimate from Table 8–1 was applied as part of the calculations to arrive at the central estimate results. The literature-based dose-response estimate was applied in all cases except for 1918 H1N1V, for which estimates based on expert opinion were used.

Table 8–6: Summary of uncertainty results: number of 10,000 input combinations that resulted in the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) falling into each frequency category.

Pathogen	Dose-response estimate	Number of initial infections	Frequency category A	Frequency category B	Frequency category C	Frequency category D
B. anthracis	Literature-based	1 or more	0	20 (< 1%)	7606 (76%)	2374 (24%)
		2 or more	0	0	0	10000 (100%)
	Expert-based	1 or more	0	217 (2%)	7484 (75%)	2299 (23%)
		2 or more	0	0	0	10000 (100%)
F. tularensis	Literature-based	1 or more	1513 (15%)	8487 (85%)	0	0
		2 or more	56 (1%)	7036 (70%)	2908 (29%)	0
		3 or more	0	451 (5%)	7854 (79%)	1695 (17%)
		4 or more	0	2 (< 0.1%)	929 (9%)	9069 (91%)
	Expert-based	1 or more	348 (3%)	7557 (76%)	2095 (21%)	0
		2 or more	5 (< 0.1%)	1380 (14%)	5123 (51%)	3492 (35%)
		3 or more	0	23 (< 1%)	1806 (18%)	8171 (82%)
		4 or more	0	0	32 (< 1%)	9968 (> 99%)
Y. pestis	Literature-based	1 or more	0	0	1156 (12%)	8844 (88%)
		2 or more	0	0	0	10000 (100%)
	Expert-based	1 or more	0	24 (< 1%)	3281 (33%)	6695 (67%)
		2 or more	0	0	0	10000 (100%)
1918 H1N1V	Expert-based	1 or more	28 (< 1%)	3916 (39%)	4467 (45%)	1589 (16%)
		2 or more	0	21 (< 1%)	2524 (25%)	7455 (75%)
		3 or more	0	0	7 (0.1%)	9993 (99.9%)
SARS-CoV	Literature-based	1 or more	0	282 (3%)	9694 (97%)	24 (< 1%)
		2 or more	0	0	1 (< 0.1%)	9999 (> 99.9%)
	Expert-based	1 or more	0	28 (< 1%)	2792 (28%)	7180 (72%)
		2 or more	0	0	0	10000 (100%)
RVFV	Literature-based	1 or more	5400 (54%)	4600 (46%)	0	0
		2 or more	1679 (17%)	8313 (83%)	8 (0.1%)	0
		3 or more	310 (3%)	7608 (76%)	2080 (21%)	2 (< 0.1%)
		4 or more	31 (< 1%)	2517 (25%)	4068 (41%)	3384 (34%)
	Expert-based	1 or more	10 (0.1%)	2222 (22%)	7361 (74%)	407 (4%)
		2 or more	0	8 (0.1%)	1091 (11%)	8901 (89%)
		3 or more	0	0	2 (< 0.1%)	9998 (> 99.9%)
		4 or more	0	0	0	10000 (100%)
ANDV	Literature-based	1 or more	0	92 (1%)	9211 (92%)	697 (7%)
		2 or more	0	0	0	10000 (100%)
	Expert-based	1 or more	0	25 (< 1%)	2332 (23%)	7643 (76%)
		2 or more	0	0	0	10000 (100%)

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

The uncertainty results reveal how changing the input values across credible ranges affects changes in the central estimate results. The following summarizes key aspects of results in this table for each pathogen.

B. anthracis infection frequency is placed mostly in the C frequency category, with a significant percentage (> 20 percent) of results placed in D frequency category, and a small number (< 3 percent) in B.

F. tularensis infection frequency is placed mostly in B, with uncertainty shifted mostly toward C. The frequency of release resulting in multiple infections is estimated in B or C, with a wide uncertainty range spanning all four categories.

Y. pestis infection frequency is placed mostly in D frequency category, with 12 percent or 33 percent in C and less than 1 percent in B.

1918 H1N1V infection frequency estimates span all four categories, with most estimates (84 percent) in B or C and < 1 percent in A. Releases resulting in multiple infections are placed in the C category for 25 percent of estimates, in B for < 1 percent, and the remainder in D frequency category.

SARS-CoV infection frequency was placed mostly in C under the literature-based dose-response estimates and mostly in D frequency category under the expert-based estimates. Small percentages (< 3 percent) were placed in B in both cases.

RVFV infection frequency was split more or less evenly between A and B under the literature-based dose-response models and mostly in C under the expert-based models. Frequency of multiple-infection releases displays a wide uncertainty range spanning all four categories across the two sets of estimates.

ANDV infection frequency was placed mostly in C under the literature-based dose-response models and mostly in D frequency category under the expert-based models, with small percentages (< 1 percent) in B in both cases.

8.4.3 Results – Health Consequences

The results described above estimate a non-zero risk of a laboratory worker developing an infection after exposure to any of the BSL-3 pathogens as a result of loss of bio-containment after an event involving a centrifuge. The route of exposure considered is via inhalation. As it is assumed that the exposure from the

centrifuge event is undetected and/or unreported, the health consequences are based on the assumption that post-exposure prophylaxis (if available), quarantine and supportive measures are not instituted unless and until the laboratory worker exhibits symptoms and seeks medical attention. Table 8–7 summarizes the potential health consequences in the laboratory worker and subsequent risk to the public, in the event of an inhalational infection occurring in the laboratory worker.

The issues of pre-existing immunity and potentially available vaccines for each pathogen are further discussed in Appendix J. Full descriptions of the clinical diseases caused by the pathogens are provided in Chapter 3 and Appendix C. The CFR estimates provided here are summarized in Chapter 3, Appendix C and Mahmoud et al. (2008) and are presumed to apply to heterogeneous populations containing individuals from medically vulnerable groups. Applying these CFR estimates to laboratory workers may be conservative, as it is presumed that laboratory workers are healthy adults as discussed further in Appendix I. Potential risk to the public from laboratory workers infected with transmissible pathogens is further discussed in Chapter 9 and Appendix L.

Table 8–7. Summary of Health Consequences in Laboratory Worker from Centrifuge Event

Pathogen	Preexisting immunity to pathogen in laboratory worker	Resulting illness via inhalation route	CFR estimates	Potential for secondary transmission to members of the public if initial infection is undetected and/or unreported
B. anthracis	Possible, as vaccine is available	Inhalational anthrax	45%	No
F. tularensis	Possible, as vaccine is available	Pneumonic form of tularemia	< 2%	No
Y. pestis	No	Pneumonic plague	15%	Yes, if pneumonic plague is present
1918 H1N1V	Possible, due to cross-protection from past influenza vaccines or infections	Influenza	2.5%	Yes
SARS-CoV	No	SARS	10%	Yes
RVFV	Possible, as vaccine is available	Rift Valley fever	0.5%–2%	No
ANDV	No	Hantavirus pulmonary syndrome	50%	Yes, if pulmonary syndrome is present

Finally, the CFR estimates in Table 8–7 were integrated into the initial infections calculations to compute estimates for the frequency of centrifuge releases leading to mortalities among laboratory workers. For *F. tularensis* and RVFV, the conservative value of 2 percent mortality was applied. The results under the central estimate example inputs are shown in Table 8–8.

Table 8–8. Central estimates for BSL-3 centrifuge release fatalities among laboratory workers (with full or partial respiratory protection)

Pathogen	Number of deaths among laboratory workers	Probability of deaths among laboratory workers from a release	Average return period of release leading to deaths among laboratory workers	Frequency category
B. anthracis	1 or more	0.0029%	2 million years	D
F. tularensis	1 or more	0.25%	20,000 years	C
	2 or more	0.00015%	> 10 million years	D
Y. pestis	1 or more	0.00014%	> 10 million years	D
1918 H1N1V	1 or more	0.0058%	900,000 years	C
SARS-CoV	1 or more	0.0028%	2 million years	D
RVFV	1 or more	1.0%	5,000 years	B
	2 or more	0.0027%	2 million years	D
ANDV	1 or more	0.0062%	800,000 years	C

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

Under the central estimate inputs, centrifuge releases leading to one or more deaths among laboratory workers would be placed in the B frequency category for RVFV, the C frequency category for *F. tularensis*, 1918 H1N1V, and ANDV, and the D frequency category for *B. anthracis*, *Y. pestis*, and SARS-CoV. Centrifuge releases leading to two or more deaths among laboratory workers would be placed in the D frequency category for all pathogens. As described under Table 8–5, these results were calculated using literature-based dose-response estimates except for the 1918 H1N1V results, which were calculated using dose-response estimates derived from expert opinion.

Uncertainty results pertaining to these mortality estimates are presented in Appendix K.

8.5 Initial Infections Resulting from Earthquake Event

8.5.1 Methodology

This section describes the methodology for generating estimates for initial infections resulting from earthquake release events described in Chapter 4 and Appendix F.

This event is relevant to all 13 pathogens analyzed in this RA. All potentially exposed individuals are assumed to be members of the public. This section discusses only initial infections, that is, members of the public directly exposed to an aerosol released from NEIDL. Chapter 9 and Appendix L discuss the potential consequences of initially infected individuals interacting with contacts.

The potential route of exposure for members of the public from this event is assumed to be through inhalation. Therefore, the dose-response information generated in Appendix J, which focuses primarily on inhalational exposure, can be applied. The earthquake release information provided in Appendix F and the dose-response information provided in Appendix J are synthesized for each of the 13 pathogens.

The methodology for generating quantitative estimates of the frequency of earthquake release events that lead to one or more initial infections or mortalities from each pathogen, as well as the uncertainty in the estimations and the sensitivity of the estimations to uncertainties in the input values, are described in detail in Appendix K. Two types of calculations and results are presented for each pathogen: a *central estimate* and *uncertainty* results around that central estimate.

8.5.2 Results—Beyond Design Basis Release

The earthquake initial infections calculations were performed using the exposure estimates from the Beyond Design Basis (BDB) Release scenario described in Chapter 4 and Appendix F. The BDB release represents a scenario is which an earthquake causes partial damage to the facility, enough to cause a partial loss of biocontainment but not a total collapse. As the calculations resulted in very low risk estimates for every pathogen under every population scenario, the results are displayed here only in summary form. A more detailed discussion of aspects of each pathogen relevant to potential initial infections in the public is reserved for Section 8.5.3.

Table 8–9. Central estimates for urban site BDB earthquake release

Pathogen	Number of initial infections overall	Probability of initial infections from release	Average return period of release leading to initial infections
B. anthracis	1 or more	1.4×10^{-9}	> 10 million years
F. tularensis	1 or more	5.7×10^{-7}	> 10 million years
Y. pestis	1 or more	3.7×10^{-11}	> 10 million years
1918 H1N1V	1 or more	7.6×10^{-18}	> 10 million years
SARS-CoV	1 or more	5.5×10^{-8}	> 10 million years
RVFV	1 or more	1.6×10^{-4}	> 10 million years
ANDV	1 or more	2.5×10^{-8}	> 10 million years
EBOV	1 or more	4.5×10^{-9}	> 10 million years
MARV	1 or more	9.0×10^{-10}	> 10 million years
LASV	1 or more	2.6×10^{-9}	> 10 million years
JUNV	1 or more	4.7×10^{-10}	> 10 million years
TBEV-FE	1 or more	1.5×10^{-47}	> 10 million years
NIPV	1 or more	1.7×10^{-10}	> 10 million years

For the central estimate example, all pathogens are estimated to produce a low probability that one or more infections occur among members of the public. The highest estimated probability is for RVFV (1.6×10^{-4}), which is about a one-in-6,000 chance. Combined with the central estimate estimated frequency of the occurrence of an earthquake release (one in 100,000 years), the frequency of an earthquake BDB release resulting in one or more infections of any pathogen is estimated to be well into the D frequency category. Many of the probabilities listed in the third column are exceedingly small and are not presumed to precisely represent the actual probability of an infection occurring. The numerical results are used only to the extent that they contribute to the argument that the frequency of infections from this event occur well into frequency category D.

For each pathogen in Table 8–9, a single dose-response estimate from Table 8–1 or 8–2 was applied as part of the calculations to arrive at the central estimate results. The literature-based dose-response estimate was applied in all cases except for 1918 H1N1V, JUNV, and TBEV-FE, for which estimates based on expert opinion were used.

Table 8–10. Summary of uncertainty results: number of 10,000 input combinations that resulted in the frequency of earthquake BDB release (urban site) leading to the given number of initial infections falling into each frequency category.

Pathogen	Number of initial infections	Frequency Category A	Frequency Category B	Frequency Category C	Frequency Category D
B. anthracis	1 or more	0	0	0	10000 (100%)
F. tularensis	1 or more	0	0	0	10000 (100%)
Y. pestis	1 or more	0	0	0	10000 (100%)
1918 H1N1V	1 or more	0	0	0	10000 (100%)
SARS-CoV	1 or more	0	0	0	10000 (100%)
RVFV	1 or more	0	0	0	10000 (100%)
ANDV	1 or more	0	0	0	10000 (100%)
EBOV	1 or more	0	0	0	10000 (100%)
MARV	1 or more	0	0	0	10000 (100%)
LASV	1 or more	0	0	0	10000 (100%)
JUNV	1 or more	0	0	0	10000 (100%)
TBEV-FE	1 or more	0	0	0	10000 (100%)
NIPV	1 or more	0	0	0	10000 (100%)

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

These uncertainty results apply for estimates using both the literature-based and the expert-based range of dose-response estimates. Every combination of dose-response estimates and earthquake release frequency estimates results in an estimated frequency of at least one initial infection in the D frequency category. That means that even the most conservative dose-response assessments combined with the most conservative earthquake frequency (one per 10,000 years) results in an average return period estimate greater than one million years.

Results for urban residents, suburban site, and rural site

The results in Tables 8–9 and 8–10 were calculated using the overall estimated urban population, which includes estimates of area residents and daytime students, workers, hospital patients, and passersby within a 1-km radius. If the population inputs are restricted to residents only, the resulting probabilities in Table 8–9 are even lower, and the same conclusions are drawn regarding the frequency category for one or more infections occurring (D frequency category).

The average, per-person exposure estimates at the suburban and rural sites are slightly higher than at the urban site (see Chapter 4 and Appendix F), but the estimated suburban and rural populations are lower, which results in lower probabilities of at least one infection compared to the urban results in Table 8–9.

Therefore, the same conclusions are drawn regarding the frequency category for one or more infections occurring at the suburban and rural sites (D frequency category).

A more detailed discussion of the effects of site differences on the earthquake initial infections calculations is reserved for Section 8.5.3 in which site and population differences lead to placement of some results in different frequency categories under the larger exposure estimates for the Maximum Reasonably Foreseeable release quantities.

8.5.3 Results—Maximum Reasonably Foreseeable Release

For each pathogen, the results from Chapter 4 and Appendix F detailing the population estimates and exposure amounts from a maximum reasonably foreseeable (MRF) earthquake release are combined with the dose-response information from Appendix J. The MRF release represents an extreme scenario in which an earthquake causes total collapse of the facility. The overall results are tabulated for each pathogen. Step-by-step details for the calculations are shown in Appendix K.

8.5.3.1 Urban site

Table 8–11. Central initial infection and fatality estimates for urban MRF earthquake release, BSL-3 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Frequency of release leading to initial infections or deaths	Frequency category
Initial infections				
B. anthracis	1 or more	0.0053%	> 10 million years	D
F. tularensis	1 or more	2.1%	5 million years	D
Y. pestis	1 or more	0.00014%	> 10 million years	D
1918 H1N1V	1 or more	0.045%	> 10 million years	D
SARS-CoV	1 or more	0.063%	> 10 million years	D
RVFV	1 or more	84%	100,000 years	C
	2 or more	54%	200,000 years	C
	3 or more	27%	400,000 years	C
	4 or more	11%	900,000 years	C
	5 or more	3.8%	3 million years	D
ANDV	1 or more	0.028%	> 10 million years	D

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Frequency of release leading to initial infections or deaths	Frequency category
Deaths among initially infected				
B. anthracis	1 or more	0.0024%	> 10 million years	D
F. tularensis	1 or more	0.043%	> 10 million years	D
Y. pestis	1 or more	0.000021%	> 10 million years	D
1918 H1N1V	1 or more	0.0011%	> 10 million years	D
SARS-CoV	1 or more	0.0063%	> 10 million years	D
RVFV	1 or more	3.6%	3 million years	D
ANDV	1 or more	0.014%	> 10 million years	D

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

The central estimate results for BSL-3 pathogens at the urban site show that, for all pathogens except for RVFV, the estimated probability of any infections occurring is less than 0.1 percent, which, when combined with the estimated low frequency of a release occurring, place the estimated frequency in the D frequency category. The exception, RVFV, produces an estimated 84 percent chance that at least one infection would occur, and up to 4 or more infections would be placed in the C frequency category. The probability of one or more deaths from RVFV infection after a release is estimated to be less than 4 percent under the central estimate assumptions. The calculations leading to these results made use of literature-based dose-response estimates except for 1918 H1N1V, for which dose-response estimates based on expert opinion were used.

Uncertainty results associated with these estimates are presented in Appendix K. They reveal that some parameter combinations for *F. tularensis* (< 20 percent) and 1918 H1N1V (< 10 percent) result in frequency estimates of one or more infections in the C frequency category. For RVFV, the uncertainty estimates reveal that 30 percent of estimates place the frequency of one or more deaths in the C frequency category. On the other hand, most (> 95 percent) of the inputs using the expert-based dose-response curves lead to estimates of one or more RVFV infection in D frequency category.

Table 8–12. Central initial infection and fatality estimates for urban MRF earthquake release, BSL-4 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period for initial infections or deaths	Frequency category
Initial infections				
EBOV	1 or more	1.7%	6 million years	D
MARV	1 or more	0.34%	> 10 million years	D
LASV	1 or more	1.0%	10 million years	D
JUNV	1 or more	0.18%	> 10 million years	D
TBEV-FE	1 or more	0.0025%	> 10 million years	D
NIPV	1 or more	0.065%	> 10 million years	D
Deaths among initially infected				
EBOV	1 or more	1.5%	7 million years	D
MARV	1 or more	0.34%	> 10 million years	D
LASV	1 or more	0.020%	> 10 million years	D
JUNV	1 or more	0.0018%	> 10 million years	D
TBEV-FE	1 or more	0.00098%	> 10 million years	D
NIPV	1 or more	0.045%	> 10 million years	D

Frequency categories: A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years
C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

The central estimate results for BSL-4 pathogens at the urban site show that the highest probabilities of an infection occurring from a release are estimated for EBOV and LASV (1-2 percent chance). However, even those probabilities are low enough that, when combined with the estimated low frequency of a release occurring, the corresponding frequencies are placed in the D frequency category. The calculations leading to these results made use of literature-based dose-response estimates except for JUNV and TBEV-FE, for which dose-response estimates based on expert opinion were used.

Uncertainty results associated with these estimates are presented in Appendix K. They reveal that some parameter combinations for each BSL-4 pathogen result in frequency estimates of one or more infections in the C frequency category, although the percentage of combinations leading to that result is small (< 5 percent, except for EBOV at 13 percent). The uncertainty estimates also reveal that 11 percent of estimates place the frequency of one or more deaths from EBOV in the C frequency category, while all other BSL-4 pathogens are > 99.9 percent D frequency category for the frequency of one or more deaths.

8.5.3.2 Urban residents

The results summarized in the previous section were calculated using the overall estimated urban population, which includes estimates of area residents as well as daytime students, workers, hospital patients, and passersby in a 1-km radius. Another set of results was calculated for population inputs restricted to estimates of the resident population only. Central estimates are shown in tables below.

BSL-3 Pathogens

Table 8–13. Central initial infection and mortality estimates among urban residents for MRF earthquake release, BSL-3 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period of release leading to initial infections or deaths
Initial infections			
B. anthracis	1 or more	8.7×10^{-6}	> 10 million years
F. tularensis	1 or more	3.5×10^{-3}	> 10 million years
Y. pestis	1 or more	2.3×10^{-7}	> 10 million years
1918 H1N1V	1 or more	2.1×10^{-6}	> 10 million years
SARS-CoV	1 or more	1.0×10^{-4}	> 10 million years
RVFV	1 or more	0.26	400,000 years
	2 or more	0.037	3 million years
ANDV	1 or more	4.6×10^{-5}	> 10 million years
Deaths among initially infected			
B. anthracis	1 or more	3.9×10^{-6}	> 10 million years
F. tularensis	1 or more	7.0×10^{-5}	> 10 million years
Y. pestis	1 or more	3.4×10^{-8}	> 10 million years
1918 H1N1V	1 or more	5.2×10^{-8}	> 10 million years
SARS-CoV	1 or more	1.0×10^{-5}	> 10 million years
RVFV	1 or more	6.0×10^{-3}	> 10 million years
ANDV	1 or more	2.3×10^{-5}	> 10 million years

Under the central estimate inputs, an earthquake MRF release resulting in one or more infections among urban residents would be placed in the D frequency category for every BSL-3 pathogen except for RVFV. It is estimated that one or more RVFV infections among residents would occur with about 26 percent probability given an MRF release. An MRF release resulting in multiple RFVF infections among residents would be placed in the D frequency category. The calculations leading to these results made use of literature-based dose-response estimates except for 1918 H1N1V, for which dose-response estimates based on expert opinion were used.

The uncertainty analysis (see Appendix K) reveals that, except for RVFV under the literature-based dose-response estimates, 100 percent of input combinations place the frequency of one or more infections with BSL-3 pathogens among urban residents in the D frequency category. For RVFV, up to three or more infections and one or more death are placed in the C frequency category for a small percentage of inputs (4 percent and 1 percent, respectively). However, the expert-based dose-response models lead to 100 percent D frequency category for even one RVFV infection among urban residents.

BSL-4 Pathogens

Table 8–14. Central initial infection and fatality estimates among urban residents for MRF earthquake release, BSL-4 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period of release leading to initial infections or deaths
Initial infections			
EBOV	1 or more	2.8×10^{-3}	> 10 million years
MARV	1 or more	5.6×10^{-4}	> 10 million years
LASV	1 or more	1.6×10^{-3}	> 10 million years
JUNV	1 or more	2.9×10^{-4}	> 10 million years
TBEV-FE	1 or more	4.8×10^{-9}	> 10 million years
NIPV	1 or more	1.1×10^{-4}	> 10 million years
Deaths among initially infected			
EBOV	1 or more	2.5×10^{-3}	> 10 million years
MARV	1 or more	5.6×10^{-4}	> 10 million years
LASV	1 or more	3.3×10^{-5}	> 10 million years
JUNV	1 or more	2.9×10^{-6}	> 10 million years
TBEV-FE	1 or more	1.9×10^{-9}	> 10 million years
NIPV	1 or more	7.4×10^{-5}	> 10 million years

Under the centrally estimated inputs, an earthquake MRF release resulting in one or more infections among urban residents would be placed in the D frequency category frequency for every BSL-4 pathogen. The highest estimated probabilities are for EBOV and LASV, for which it is estimated that one or more infections among residents would occur with about 0.2 or 0.3 percent probability given an MRF release. Combined with the centrally estimated frequency of the earthquake release (once per 100,000 years), those probabilities result in a projected return period for releases leading to infection among residents greater than ten million years. The calculations leading to these results made use of literature-based dose-

response estimates except for JUNV and TBEV-FE, for which dose-response estimates based on expert opinion were used.

The uncertainty analysis (see Appendix K) reveals that ≥ 99.9 percent of input combinations place the frequency of one or more infections among urban residents in the D frequency category for every BSL-4 pathogen.

8.5.3.3 Suburban site

Another set of results was calculated for exposure and population inputs from estimates on the basis of characteristic of the suburban site (see Chapter 4 and Appendix F). The central estimates are shown in tables below.

BSL-3 Pathogens

Table 8–15. Central initial infection and fatality estimates at suburban site for MRF earthquake release, BSL-3 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period of release leading to initial infections or deaths
Initial infections			
B. anthracis	1 or more	1.1×10^{-6}	> 10 million years
F. tularensis	1 or more	4.6×10^{-4}	> 10 million years
Y. pestis	1 or more	3.0×10^{-8}	> 10 million years
1918 H1N1V	1 or more	1.6×10^{-7}	> 10 million years
SARS-CoV	1 or more	1.3×10^{-5}	> 10 million years
RVFV	1 or more	3.8×10^{-2}	3 million years
ANDV	1 or more	6.0×10^{-6}	> 10 million years
Deaths among initially infected			
B. anthracis	1 or more	5.2×10^{-7}	> 10 million years
F. tularensis	1 or more	9.2×10^{-6}	> 10 million years
Y. pestis	1 or more	4.5×10^{-9}	> 10 million years
1918 H1N1V	1 or more	4.0×10^{-7}	> 10 million years
SARS-CoV	1 or more	1.3×10^{-6}	> 10 million years
RVFV	1 or more	7.8×10^{-4}	> 10 million years
ANDV	1 or more	3.0×10^{-6}	> 10 million years

BSL-4 Pathogens

Table 8–16. Central initial infection and fatality results at suburban site for MRF earthquake release, BSL-4 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period of release leading to initial infections or deaths
Initial infections			
EBOV	1 or more	3.7×10^{-4}	> 10 million years
MARV	1 or more	7.4×10^{-5}	> 10 million years
LASV	1 or more	2.2×10^{-4}	> 10 million years
JUNV	1 or more	3.8×10^{-5}	> 10 million years
TBEV-FE	1 or more	2.6×10^{-6}	> 10 million years
NIPV	1 or more	1.4×10^{-5}	> 10 million years
Deaths among initially infected			
EBOV	1 or more	3.3×10^{-4}	> 10 million years
MARV	1 or more	7.4×10^{-5}	> 10 million years
LASV	1 or more	4.3×10^{-6}	> 10 million years
JUNV	1 or more	3.8×10^{-7}	> 10 million years
TBEV-FE	1 or more	1.0×10^{-6}	> 10 million years
NIPV	1 or more	9.7×10^{-6}	> 10 million years

For the central estimate example, all pathogens are estimated to produce a low probability that one or more infections occur among members of the public at the suburban site. The highest estimated probability is for RVFV (3.8×10^{-0}), which is about a one-in-30 chance. Combined with the central estimate projected frequency of the occurrence of an earthquake release (one in 100,000 years), the frequency of an earthquake MRF release resulting in one or more infections of any pathogen at the suburban site is estimated to be in the D frequency category. The calculations leading to these results made use of literature-based dose-response estimates except for 1918 H1N1V, JUNV, and TBEV-FE for which dose-response estimates based on expert opinion were used.

In the uncertainty analysis (see Appendix K), every combination of dose-response estimates and earthquake release frequency estimates results in an estimated frequency of at least one initial infection at the suburban site in the D category, except for about 32 percent of the estimates using the literature-based dose-response estimates for RVFV.

8.5.3.4 Rural site

Another set of results was calculated for exposure and population inputs from estimates on the basis of characteristic of the rural site (see Chapter 4 and Appendix F). The central estimates are shown in tables below.

BSL-3 Pathogens

Table 8–17. Central initial infection and fatality estimates for rural site, MRF earthquake release, BSL-3 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period of release leading to initial infections or deaths
Initial infections			
B. anthracis	1 or more	5.8×10^{-7}	> 10 million years
F. tularensis	1 or more	2.3×10^{-4}	> 10 million years
Y. pestis	1 or more	1.5×10^{-8}	> 10 million years
1918 H1N1V	1 or more	9.1×10^{-6}	> 10 million years
SARS-CoV	1 or more	6.8×10^{-6}	> 10 million years
RVFV	1 or more	1.9×10^{-2}	5 million years
ANDV	1 or more	3.1×10^{-6}	> 10 million years
Deaths among initially infected			
B. anthracis	1 or more	2.6×10^{-7}	> 10 million years
F. tularensis	1 or more	4.7×10^{-6}	> 10 million years
Y. pestis	1 or more	2.3×10^{-9}	> 10 million years
1918 H1N1V	1 or more	2.3×10^{-7}	> 10 million years
SARS-CoV	1 or more	6.8×10^{-7}	> 10 million years
RVFV	1 or more	3.9×10^{-4}	> 10 million years
ANDV	1 or more	1.5×10^{-6}	> 10 million years

BSL-4 Pathogens

Table 8–18. Central initial infection and fatality estimates for the rural site, MRF earthquake release, BSL-4 pathogens

Pathogen	Number of initial infections/ deaths	Probability of initial infections or deaths from release	Average return period of release leading to initial infections or deaths
Initial infections			
EBOV	1 or more	1.9×10^{-4}	> 10 million years
MARV	1 or more	3.7×10^{-5}	> 10 million years
LASV	1 or more	1.1×10^{-4}	> 10 million years
JUNV	1 or more	1.9×10^{-5}	> 10 million years
TBEV-FE	1 or more	1.3×10^{-6}	> 10 million years
NIPV	1 or more	7.0×10^{-6}	> 10 million years
Deaths among initially infected			
EBOV	1 or more	1.7×10^{-4}	> 10 million years
MARV	1 or more	3.7×10^{-5}	> 10 million years
LASV	1 or more	2.2×10^{-6}	> 10 million years
JUNV	1 or more	1.9×10^{-7}	> 10 million years
TBEV-FE	1 or more	5.4×10^{-7}	> 10 million years
NIPV	1 or more	4.9×10^{-6}	> 10 million years

For the central estimate example, all pathogens are estimated to produce a low probability that one or more infections occur at the rural site. The highest estimated probability is for RVFV (1.9×10^{-2}), which is about a one-in-50 chance. Combined with the central estimate projected frequency of the occurrence of an earthquake release (one in 100,000 years), the frequency of an earthquake MRF release resulting in one or more infections or deaths from any pathogen at the rural site is estimated to be in the D frequency category. The calculations leading to these results made use of literature-based dose-response estimates except for 1918 H1N1V, JUNV, and TBEV-FE, for which dose-response estimates based on expert opinion were used.

In the uncertainty analysis (see Appendix K), every combination of dose-response estimates and earthquake release frequency estimates results in an estimated frequency of at least one initial infection at the rural site in the D category, except for about 17 percent of the estimates using the literature-based dose-response models for RVFV.

8.6 Medically Vulnerable Subpopulations

8.6.1 Methodology

The methodology for the initial infections analyses up to this point uses an assumption that the dose-response and CFR estimates are applicable to all potentially exposed populations. That assumption could be violated for a population that has a different profile of vulnerability to disease or death than did the populations on which the dose-response and mortality estimates were based. For this RA, the issue of population vulnerability is investigated by evaluating populations at the three sites for the presence of five medically vulnerable subpopulations (MVSP), as discussed in Appendix I: children under 5, adults over 65, people with diabetes, people with HIV/AIDS, and pregnant women.

The dose-response models applied in the previous sections of this Chapter are presumed to be applicable to human populations containing MVSP to some extent. The derivation and justification of the literature-based models, discussed in detail in Appendix J, included consideration of the possibility that some potentially exposed individuals could be more susceptible to infection at a given dose than others. The expert-based dose-response models were based on the outcome of a modified Delphi process in which it was not specified to the experts that their estimates should be based on healthy individuals only; presumably, the experts took into consideration the susceptibility profile of a typical human population in arriving at their infectious dose estimates. For CFR estimates, the baseline rates assumed for each pathogen are based on data observed in human outbreaks, which occurred among populations containing many, if not all, of the MVSP considered in this RA.

Despite the fact that the dose-response and CFR estimates are already presumed to encompass MVSP, it is worth considering whether particular potentially exposed populations considered for this RA contain an atypical proportion of individuals belonging to certain MVSP, and then whether that proportion of MVSP is different enough from the norm to warrant adjusting the baseline initial infections and mortality estimates.

The quantitative analysis, described in Appendix K, is applied only to the earthquake release scenarios, in which the potentially exposed group (members of the public surrounding NEIDL) may contain MVSP of higher or lower proportion than a typical human population. As discussed in Appendix I, the population of laboratory workers, who are the potentially exposed group for the needlestick and centrifuge events, might include proportions of some but not all, of the five MVSPs. It is surmised that the population of laboratory workers assigned to work with BSL-3 or BSL-4 pathogens would have a lower proportion of each MVSP, and of unhealthy or immunocompromised individuals in general, than the proportions

occurring in the overall population. Therefore, it is acknowledged that the dose-response models applied in the centrifuge initial infections analysis could overestimate the risk to laboratory workers in this regard, but no attempt is made to quantify the potential degree of overestimation. The needlestick analysis contained the assumption that every exposure would lead to an infection, regardless of whether the exposed worker is a member of a MVSP.

The calculations leading to the central quantitative estimates presented in the next section make use, in part, of MVSP-related values presented in Appendix I. The values for the proportion of MVSP in the populations near the three sites are based on data, whereas the values for relative susceptibility of MVSP members compared to healthy adults are based on expert opinion.

8.6.2 Results

In this section, results from calculations introduced in Appendix K are presented. The results are shown only for the three pathogens that produced the highest estimated probabilities of infection and death under the earthquake MRF release scenario as shown in Section 8.5.3. The three pathogens also represent the highest estimated infection and death probabilities among bacteria (*F. tularensis*), BSL-3 viruses (RVFV), and BSL-4 viruses (EBOV). Results from those three pathogens are sufficient to demonstrate the estimated influence of the MVSP profile at the three sites in comparison to U.S. average rates of MVSP.

8.6.2.1 Earthquake MRF Release Results for Each MVSP

This section contains results for estimated probabilities and frequencies of initial infections and deaths among members of each individual MVSP at each site. Each set of results is compared to what the results would be if the population at each site was the same estimated size but with MVSP in line with overall U.S. proportions. Central estimates are presented here; the accompanying uncertainty results are also presented in Appendix K.

Children under 5

Table 8–19. Central estimates for earthquake MRF release among children under 5

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: Initial Infections				
F. tularensis	1.5×10^{-3}	> 10 million years	1.8×10^{-3}	> 10 million years
RVFV	0.12	≈ 830,000 years	0.14	≈ 720,000 years
EBOV	1.2×10^{-3}	> 10 million years	1.4×10^{-4}	> 10 million years
URBAN SITE: Deaths Among Initially Infected				
F. tularensis	3.3×10^{-5}	> 10 million years	3.9×10^{-5}	> 10 million years
RVFV	2.8×10^{-3}	> 10 million years	3.3×10^{-3}	> 10 million years
EBOV	1.1×10^{-3}	> 10 million years	1.3×10^{-4}	> 10 million years
SUBURBAN SITE: Initial Infections				
F. tularensis	3.0×10^{-5}	> 10 million years	3.8×10^{-5}	> 10 million years
RVFV	2.6×10^{-3}	> 10 million years	3.2×10^{-3}	> 10 million years
EBOV	2.4×10^{-5}	> 10 million years	3.1×10^{-5}	> 10 million years
SUBURBAN SITE: Deaths Among Initially Infected				
F. tularensis	6.7×10^{-7}	> 10 million years	8.4×10^{-7}	> 10 million years
RVFV	5.6×10^{-5}	> 10 million years	7.0×10^{-5}	> 10 million years
EBOV	2.3×10^{-5}	> 10 million years	2.8×10^{-5}	> 10 million years
RURAL SITE: Initial Infections				
F. tularensis	1.6×10^{-5}	> 10 million years	1.9×10^{-5}	> 10 million years
RVFV	1.4×10^{-3}	> 10 million years	1.6×10^{-3}	> 10 million years
EBOV	1.3×10^{-5}	> 10 million years	1.6×10^{-5}	> 10 million years
RURAL SITE: Deaths Among Initially Infected				
F. tularensis	3.6×10^{-7}	> 10 million years	4.2×10^{-7}	> 10 million years
RVFV	3.0×10^{-5}	> 10 million years	3.6×10^{-5}	> 10 million years
EBOV	1.2×10^{-5}	> 10 million years	1.4×10^{-5}	> 10 million years

The results in the left columns of the above table are the central estimate projected probability and return period for initial infection and mortality among children under five at each site after an earthquake MRF release. The calculations of the values incorporated the estimated proportion of children under five present at each site and increased vulnerability of children under five to disease and death. At each site, the estimated return periods would be placed in the D frequency category, except for at least one initial infection with RVFV at the urban site, which would be placed in the C frequency category.

The results in the right columns are displayed for comparison purposes—they are the equivalent estimates for a site with the same population size but with a typical U.S. rate of children under five. There are some small differences across each row, but all the return periods still fall in the D frequency category. The direction of the small differences in each row reflect the fact that all three sites were estimated to have a smaller-than-average proportion of children under five.

Adults over 65

Table 8–20. Central estimates for earthquake MRF release among adults over 65

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: Initial Infections				
F. tularensis	3.6×10^{-3}	> 10 million years	3.6×10^{-3}	> 10 million years
RVFV	0.27	≈ 380,000 years	0.27	≈ 380,000 years
EBOV	2.9×10^{-3}	> 10 million years	2.9×10^{-3}	> 10 million years
URBAN SITE: Deaths Among Initially Infected				
F. tularensis	1.0×10^{-4}	> 10 million years	1.0×10^{-4}	> 10 million years
RVFV	8.4×10^{-3}	> 10 million years	8.4×10^{-3}	> 10 million years
EBOV	2.8×10^{-3}	> 10 million years	2.8×10^{-3}	> 10 million years
SUBURBAN SITE: Initial Infections				
F. tularensis	5.1×10^{-5}	> 10 million years	7.9×10^{-5}	> 10 million years
RVFV	4.3×10^{-3}	> 10 million years	6.6×10^{-3}	> 10 million years
EBOV	4.1×10^{-5}	> 10 million years	6.3×10^{-5}	> 10 million years
SUBURBAN SITE: Deaths Among Initially Infected				
F. tularensis	1.4×10^{-6}	> 10 million years	2.2×10^{-6}	> 10 million years
RVFV	1.2×10^{-4}	> 10 million years	1.8×10^{-4}	> 10 million years
EBOV	4.0×10^{-5}	> 10 million years	6.1×10^{-5}	> 10 million years
RURAL SITE: Initial Infections				
F. tularensis	6.9×10^{-5}	> 10 million years	4.0×10^{-5}	> 10 million years
RVFV	5.7×10^{-3}	> 10 million years	3.3×10^{-3}	> 10 million years
EBOV	5.5×10^{-5}	> 10 million years	3.2×10^{-5}	> 10 million years
RURAL SITE: Deaths Among Initially Infected				
F. tularensis	1.9×10^{-6}	> 10 million years	1.1×10^{-6}	> 10 million years
RVFV	1.6×10^{-4}	> 10 million years	9.1×10^{-5}	> 10 million years
EBOV	5.3×10^{-5}	> 10 million years	3.1×10^{-5}	> 10 million years

The results in the left columns of the table are the central estimate projected probability and return period for initial infection and mortality among adults over 65 at each site after an earthquake MRF release. The calculations of the values incorporated the estimated proportion of adults over 65 present at each site and increased vulnerability of adults over 65 to disease and death. At each site, the estimated return periods would be placed in the D frequency category, except for infections at the urban site, for which the estimated return period is in the C frequency category.

The results in the right columns are displayed for comparison purposes—they are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of adults over 65. There are some small differences across each row, but all the return periods still fall in the D frequency category. The direction of the small differences in each row reflect the fact that the urban and suburban sites were estimated to have a smaller-than-average proportion of adults over 65 and the rural site a higher-than-average proportion.

People with diabetes

Table 8–21. Central estimates for earthquake MRF release among people with diabetes

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: Initial Infections				
F. tularensis	2.1×10^{-3}	> 10 million years	1.4×10^{-3}	> 10 million years
RVFV	0.16	≈ 640,000 years	0.11	≈ 910,000 years
EBOV	1.6×10^{-3}	> 10 million years	1.1×10^{-3}	> 10 million years
URBAN SITE: Deaths Among Initially Infected				
F. tularensis	4.7×10^{-5}	> 10 million years	3.3×10^{-5}	> 10 million years
RVFV	3.9×10^{-3}	> 10 million years	2.7×10^{-3}	> 10 million years
EBOV	1.5×10^{-3}	> 10 million years	1.0×10^{-3}	> 10 million years
SUBURBAN SITE: Initial Infections				
F. tularensis	4.2×10^{-5}	> 10 million years	3.1×10^{-5}	> 10 million years
RVFV	3.4×10^{-3}	> 10 million years	2.5×10^{-3}	> 10 million years
EBOV	3.3×10^{-5}	> 10 million years	2.4×10^{-5}	> 10 million years
SUBURBAN SITE: Deaths Among Initially Infected				
F. tularensis	9.6×10^{-7}	> 10 million years	7.0×10^{-7}	> 10 million years
RVFV	7.8×10^{-5}	> 10 million years	5.7×10^{-5}	> 10 million years
EBOV	3.0×10^{-5}	> 10 million years	2.2×10^{-5}	> 10 million years
RURAL SITE: Initial Infections				
F. tularensis	1.8×10^{-5}	> 10 million years	1.6×10^{-5}	> 10 million years
RVFV	1.5×10^{-3}	> 10 million years	1.3×10^{-3}	> 10 million years
EBOV	1.4×10^{-5}	> 10 million years	1.2×10^{-5}	> 10 million years
RURAL SITE: Deaths Among Initially Infected				
F. tularensis	4.1×10^{-7}	> 10 million years	3.6×10^{-7}	> 10 million years
RVFV	3.3×10^{-5}	> 10 million years	2.9×10^{-5}	> 10 million years
EBOV	1.3×10^{-5}	> 10 million years	1.1×10^{-5}	> 10 million years

The results in the left columns of the table are the central estimate projected probability and return period for initial infection and mortality among people with diabetes at each site after an earthquake MRF release. The calculations of those values incorporated the estimated proportion of people with diabetes present at each site and increased vulnerability of people with diabetes to disease and death. At each site, the estimated return periods would be placed in the D frequency category except for initial infections with RVFV at the urban site, which would be placed in the C frequency category.

The results in the right columns are displayed for comparison purposes—they are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of people with diabetes. There are some small differences across each row, but all the return periods still fall in the D frequency category. The direction of the small differences in each row reflect the fact that all three sites were estimated to have a higher-than-average proportion of people with diabetes.

People with HIV/AIDS

Table 8–22. Central estimates for earthquake MRF release among people with HIV/AIDS

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: Initial Infections				
F. tularensis	1.4×10^{-4}	> 10 million years	1.1×10^{-4}	> 10 million years
RVFV	1.1×10^{-2}	≈ 8.7 million years	9.1×10^{-3}	> 10 million years
EBOV	1.1×10^{-4}	> 10 million years	8.7×10^{-5}	> 10 million years
URBAN SITE: Deaths Among Initially Infected				
F. tularensis	3.2×10^{-6}	> 10 million years	2.6×10^{-6}	> 10 million years
RVFV	2.7×10^{-4}	> 10 million years	2.2×10^{-4}	> 10 million years
EBOV	1.0×10^{-4}	> 10 million years	8.2×10^{-5}	> 10 million years
SUBURBAN SITE: Initial Infections				
F. tularensis	1.2×10^{-6}	> 10 million years	2.3×10^{-6}	> 10 million years
RVFV	9.8×10^{-5}	> 10 million years	2.0×10^{-4}	> 10 million years
EBOV	9.4×10^{-7}	> 10 million years	1.9×10^{-6}	> 10 million years
SUBURBAN SITE: Deaths Among Initially Infected				
F. tularensis	2.8×10^{-8}	> 10 million years	5.5×10^{-8}	> 10 million years
RVFV	2.3×10^{-6}	> 10 million years	4.6×10^{-6}	> 10 million years
EBOV	8.8×10^{-7}	> 10 million years	1.8×10^{-6}	> 10 million years
RURAL SITE: Initial Infections				
F. tularensis	3.2×10^{-7}	> 10 million years	1.2×10^{-6}	> 10 million years
RVFV	2.7×10^{-5}	> 10 million years	9.9×10^{-5}	> 10 million years
EBOV	2.6×10^{-7}	> 10 million years	9.5×10^{-7}	> 10 million years
RURAL SITE: Deaths Among Initially Infected				
F. tularensis	7.7×10^{-9}	> 10 million years	2.8×10^{-8}	> 10 million years
RVFV	6.5×10^{-7}	> 10 million years	2.3×10^{-6}	> 10 million years
EBOV	2.4×10^{-7}	> 10 million years	8.9×10^{-7}	> 10 million years

The results in the left columns of the table are the central estimate projected probability and return period for initial infection and mortality among people with HIV/AIDS at each site after an earthquake MRF release. The calculations of those values incorporated the estimated proportion of people with HIV/AIDS present at each site and increased vulnerability of people with HIV/AIDS to disease and death. At each site, the estimated return periods would be placed in the D frequency category.

The results in the right columns are displayed for comparison purposes—they are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of people with HIV/AIDS. There are some small differences across each row, but all the return periods still fall in the D frequency category. The direction of the small differences in each row reflect the fact that the urban site was estimated to have a higher-than-average proportion of people with HIV/AIDS and the suburban and rural sites a lower-than-average proportion.

Pregnant women

Table 8–23. Central estimates for earthquake MRF release among pregnant women

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: Initial Infections				
F. tularensis	2.5×10^{-4}	> 10 million years	2.5×10^{-4}	> 10 million years
RVFV	2.4×10^{-2}	≈ 4.1 million years	2.4×10^{-2}	≈ 4.1 million years
EBOV	2.3×10^{-4}	> 10 million years	2.3×10^{-4}	> 10 million years
URBAN SITE: Deaths Among Initially Infected				
F. tularensis	5.5×10^{-6}	> 10 million years	5.5×10^{-6}	> 10 million years
RVFV	5.4×10^{-4}	> 10 million years	5.4×10^{-4}	> 10 million years
EBOV	2.2×10^{-4}	> 10 million years	2.2×10^{-4}	> 10 million years
SUBURBAN SITE: Initial Infections				
F. tularensis	5.4×10^{-6}	> 10 million years	5.4×10^{-6}	> 10 million years
RVFV	5.2×10^{-4}	> 10 million years	5.2×10^{-4}	> 10 million years
EBOV	5.0×10^{-6}	> 10 million years	5.0×10^{-6}	> 10 million years
SUBURBAN SITE: Deaths Among Initially Infected				
F. tularensis	1.2×10^{-7}	> 10 million years	1.2×10^{-7}	> 10 million years
RVFV	1.1×10^{-5}	> 10 million years	1.1×10^{-5}	> 10 million years
EBOV	4.6×10^{-6}	> 10 million years	4.6×10^{-6}	> 10 million years
RURAL SITE: Initial Infections				
F. tularensis	2.7×10^{-6}	> 10 million years	2.7×10^{-6}	> 10 million years
RVFV	2.6×10^{-4}	> 10 million years	2.6×10^{-4}	> 10 million years
EBOV	2.5×10^{-6}	> 10 million years	2.5×10^{-6}	> 10 million years
RURAL SITE: Deaths Among Initially Infected				
F. tularensis	6.0×10^{-8}	> 10 million years	6.0×10^{-8}	> 10 million years
RVFV	5.8×10^{-6}	> 10 million years	5.8×10^{-6}	> 10 million years
EBOV	2.3×10^{-6}	> 10 million years	2.3×10^{-6}	> 10 million years

The results in the left columns of the table are the central estimate projected probability and return period for initial infection and mortality among pregnant women at each site after an earthquake MRF release. The calculations of those values incorporated the estimated proportion of pregnant women present at each site and increased vulnerability of pregnant women to disease and death. At each site, the estimated return periods would be placed in the D frequency category.

The results in the right columns are displayed for comparison purposes—they are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of pregnant women. There are no differences across any of the rows, because the proportion of pregnant women at each site and in the United States overall was estimated to be the same (1 percent of the population).

SUMMARY

As noted in the above tables, the frequency of one infection for a member of any MVSP as a result of this type of earthquake and exposure to pathogens is estimated to be in the D frequency category, except for RVFV infection among children under five, adults over 65, and people with diabetes at the urban site. The frequency of one death for a member of any MVSP as a result of such an event is estimated to be well into the D frequency category (less than one in 10 million years) for all pathogens at all three sites. If the local MVSP profiles at each site were in line with average U.S. proportions, the estimates would not change enough to alter these conclusions.

Because the inputs for susceptibility of MVSP relative to healthy adults, described in Appendix I, are based on expert opinion and not on direct data, it is possible that they significantly underestimate the true differences in susceptibility. The numerical results in the above Tables are sensitive to these inputs. The largest change in the results for a particular MVSP would occur if the relative susceptibility of that MVSP is increased and the relative susceptibility of all other MVSP remains the same, in which case the particular MVSP would be disproportionately affected relative to not only healthy adults, but also to the other MVSP. If the relative sensitivities for all MVSP are increased at the same time, then the estimated risk to each MVSP would still increase, but to a much lesser extent. In all cases, however, the results regarding the risk estimates to each local population as compared to what those results would be in a population with typical U.S. proportions of MVSP would not change substantially. Therefore, the relative differences across each row of in the above tables are not sensitive to potential inaccuracies of the estimated susceptibility differences of the MVSP.

8.6.2.2 Earthquake MRF Release Overall Results Adjusted for MVSP

In this section, the earthquake MRF release results presented in Section 8.5.3, for which dose-response and mortality estimates assumed to be applicable to a general U.S. population were used, are compared to adjusted results that apply site-specific MVSP data and estimates for increased susceptibility of MVSP, as described in Appendix K.

Table 8–24. Central estimates for earthquake MRF release

Pathogen	Adjusted results using local site MVSP estimates		Baseline results from Section 8.5.3	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: Initial Infections				
F. tularensis	2.1×10^{-2}	≈ 4.7 million years	2.1×10^{-2}	≈ 4.7 million years
RVFV	0.84	≈ 120,000 years	0.84	≈ 120,000 years
EBOV	1.7×10^{-2}	≈ 5.9 million years	1.7×10^{-2}	≈ 5.9 million years
URBAN SITE: Deaths Among Initially Infected				
F. tularensis	4.3×10^{-4}	> 10 million years	4.3×10^{-4}	> 10 million years
RVFV	3.6×10^{-2}	≈ 2.8 million years	3.6×10^{-2}	≈ 2.8 million years
EBOV	1.5×10^{-2}	≈ 6.5 million years	1.5×10^{-2}	≈ 6.5 million years
SUBURBAN SITE: Initial Infections				
F. tularensis	4.5×10^{-4}	> 10 million years	4.6×10^{-4}	> 10 million years
RVFV	3.7×10^{-2}	≈ 2.7 million years	3.8×10^{-2}	≈ 2.6 million years
EBOV	3.6×10^{-4}	> 10 million years	3.7×10^{-4}	> 10 million years
SUBURBAN SITE: Deaths Among Initially Infected				
F. tularensis	8.9×10^{-6}	> 10 million years	9.3×10^{-6}	> 10 million years
RVFV	7.5×10^{-4}	> 10 million years	7.8×10^{-4}	> 10 million years
EBOV	3.3×10^{-4}	> 10 million years	3.3×10^{-4}	> 10 million years
RURAL SITE: Initial Infections				
F. tularensis	2.4×10^{-4}	> 10 million years	2.3×10^{-4}	> 10 million years
RVFV	2.0×10^{-2}	≈ 5.0 million years	1.9×10^{-2}	≈ 5.1 million years
EBOV	1.9×10^{-4}	> 10 million years	1.9×10^{-4}	> 10 million years
RURAL SITE: Deaths Among Initially Infected				
F. tularensis	5.0×10^{-6}	> 10 million years	4.7×10^{-6}	> 10 million years
RVFV	4.2×10^{-4}	> 10 million years	3.9×10^{-4}	> 10 million years
EBOV	1.8×10^{-4}	> 10 million years	1.7×10^{-4}	> 10 million years

For the urban site, adjusting the overall results according to local proportions of MVSP does not change the estimates (to two significant figures) for probability and frequency of at least one infection or death. That result can be explained by the observation that, while the estimated proportions of people with diabetes and HIV/AIDS are higher at the urban site than the U.S. averages, the estimated proportions of children under five and adults over 65 are lower.

For the suburban site, the estimated probabilities of at least one infection or death are slightly lower after adjusting for the local proportions of MVSP. The largest contributor to that small difference is the estimated suburban site proportion of adults over 65, which is just over half the U.S. average proportion.

The new results here do not change the assigned frequency category for the occurrence of an MRF release resulting in one or more infections or deaths at the suburban site (D frequency category).

For the rural site, the estimated probabilities of at least one infection or death are slightly higher after adjusting for the local proportions of MVSP. The largest contributor to this small difference is the estimated rural site proportion of adults over 65, which is appreciably higher than U.S. average proportion. The new results here do not change the assigned frequency category for the occurrence of an MRF release resulting in one or more infections or deaths at the rural site (D frequency category).

Note that the local site MVSP adjustments for the probability of deaths have a smaller effect on the estimates for EBOV than for *F. tularensis* and RVFV. That can be explained by the fact that the estimated EBOV mortality rate is already quite high, even for healthy adults, so there is little room for increase when the estimates of MVSP-specific mortality rates are calculated.

Uncertainty analyses pertaining to these results are presented in Appendix K, which again show only minor differences from the corresponding results that do not incorporate MVSP. In addition, sensitivity to the inputs for susceptibility of MVSP relative to healthy adults, discussed in Appendix I, is considered. Because those inputs are based on expert opinion and not on direct data, it is possible that they significantly underestimate the true differences in susceptibility. However, the results shown above exhibit very low sensitivity to those inputs. For example, even if the relative susceptibility values are multiplied by a factor of 100, the results do not change appreciably, and all estimated return periods correspond to the same frequency categories.

8.7 References

Bazhutin 1992 — Bazhutin, N.B., Belanov, E.F., Spiridonov, V.A., Voitenko, A.V., Krivenchuk, N.A., Krotov, S.A., Omel'chenko, N.I., Tereshchenko, A.Yu. and Komichev, V.V. [The effect of the methods for producing an experimental Marburg virus infection on the characteristics of the course of the disease in green monkeys]. *Vopr Virusol*, 37(3):153–156, 1992.

Bossart 2009 — Bossart, K.N., Zhu, Z., Middleton, D., Klippel, J., Crameri, G., Bingham, J., McEachern, J.A., Green, D., Hancock, T.J., Chan, Y., Hickey, A.C., Dimitrov, D.S., Wang, L. and Broder, C.C. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLOS Pathogens*, 5(10):e1000642, 2009.

Brachman 1966 — Brachman, P.S., Kaufmann, A.F. and Dalldorf, F.G. Industrial inhalational anthrax. *Bacteriological Reviews*, 30(3):646–657, 1966.

Day 1972 — Day, W.C. and Berendt, R.F. Experimental tularemia in *Macaca mulatta*: relationship of aerosol particle size to the infectivity of airborne *Pasteurella tularensis*. *Infection and Immunity*, 5:77–82, 1972.

Haas 2002 — Haas, C.N. On the risk of mortality to primates exposed to anthrax spores. *Risk Analysis*, 22:189– 193, 2002.

Hooper 2001 — Hooper, J.W., Larsen, T., Custer, D.M. and Schmaljohn, C.S. A lethal disease model for hantavirus pulmonary syndrome. *Virology*, 289:6–14, 2001.

Hooper 2008 — Hooper, J.W., Ferro, A.M. and Wahl-Jensen, V. Immune serum produced by DNA vaccination protects hamsters against lethal respiratory challenge with Andes virus. *Journal of Virology*, 82(3):1332–1338, 2008.

Huang 2010 — Huang, Y., Hong, T., Bartrand, T.A., Gurian, P.L., Haas, C.N., Liu, R. and Tamrakar, S.B. How sensitive is safe? Risk-based targets for ambient monitoring of pathogens. *IEEE Sensors Journal*, 10(3):668–673, 2010.

Kenyon 1992 — Kenyon, R.H., McKee, Jr., K.T., Zack, P.M., Rippy, M.K., Vogel, A.P., York, C., Meegan, J., Crabbs, C. and Peters, C.J. Aerosol infection of rhesus macaques with Junin virus. *Intervirology*, 33:23–31, 1992.

Mahmoud 2008 — Mahmoud, A., D. Burke, S. Eubank, V.S. Freimuth, G Friedman-Jimenez, P. Hamburg, K.A. Holbrook, D.L. Kasper, J. Lewis, W.I. Lipkin, T.H. Murray, M.E. Northridge, J. Patterson, M. Robson, S. Stanley, W. Thomann, S. Bennett, P. Highnam, and R. Khabbaz. (2008). NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratory at Boston University Medical Center, Finding and Recommendations, Part I: Risk Assessment; Briefing of the Advisory Committee to the Director, NIH, June 6, 2008., Department of Health and Human Services.

McCrumb 1961 — McCrumb, Jr, F.R. Aerosol infection of man with *Pasteurella tularensis*. *Bacteriological Review*, 25(3):262–267, 1961.

P'iankov 1995 — P'iankov, O.V., Sergeev, A.N., P'iankova, O.G. and Chepurnov, A.A. [Experimental Ebola fever in *Macaca mulatta*]. *Vopr Virusol*, 40(3):113-115, 1995.

Saslaw 1961 — Saslaw, S., Eigelsbach, H.T., Prior, J.A., Wilson, H.E. and Carhart, S. Tularemia vaccine study. II. Respiratory challenge. *Archives of Internal Medicine*, 107(5):702–714, 1961.

Speck 1957 — Speck, R.S. and Wolochow, H. Studies on the experimental epidemiology of respiratory infections. VIII. Pneumonic plague in macacus rhesus. *Journal of Infectious Diseases*, 100:58–69, 1957.

Stephenson 1984 — Stephenson, E.H., Larson, E.W. and Dominik, J.W. Effect of environmental factors on aerosol-induced Lassa virus infection. *Journal of Medical Virology*, 14:295–303, 1984.

Tamrakar 2008 — Tamrakar, S.B. and Haas, C.N. Dose-response model for Lassa virus. *Human and Ecological Risk Assessment*, 14:742–752, 2008.

Watanabe 2010 — Watanabe, T., Bartrand, T.A., Weir, M.H., Omura, T. and Haas, C.N. Development of a dose-response model for SARS coronavirus. *Risk Analysis*, 30:1129–1138, 2010.

Contents

9. Secondary Transmission

9.1 Introduction

Chapter 8 addressed the questions related to the potential consequences of events leading to loss of biocontainment. The results of those analyses were an estimate of the likelihood of developing initial infections in those who were directly exposed to the pathogens and how often those initial infections would be expected to occur. This chapter follows those analyses by addressing the question: what would happen if an individual with an undetected or unreported initial infection interacts with members of the community? What would be the likelihood of *secondary transmissions* occurring in members of the community the initially infected individual came into contact with? How often would infections and deaths from those secondary transmissions be expected to occur?

Secondary transmission analyses for this RA consist of qualitative and quantitative estimates of potential infection and health effects in those exposed to a pathogen because of contact with an individual already infected as a result of one of the events from the event sequence analyses. In this RA, the term *secondary transmission* refers to the transfer of pathogenic organisms from an initially infected person to another person, causing the establishment of infection in the second person, and any and all subsequent generations of transmission. Secondary transmission is distinguished from initial infection, which describes an infection caused by exposure to pathogens released directly from a NEIDL-associated event.

Under scenarios in which a laboratory worker is infected (e.g., via needlestick or centrifuge aerosol release), the worker could become contagious and transmit infection to other individuals, who could in turn transmit to others, and a chain of transmission could continue through any number of generations. Under such a scenario, the infection of the original laboratory worker is considered an initial infection, while all subsequent infections result from secondary transmission. The potential for secondary transmission increases when the exposure is undetected by the laboratory worker or if protocol in reporting or responding to a known mishap occurring in the laboratory is not followed. In those cases, the exposed worker could develop infection and become contagious while outside the laboratory among members of the public.

A member of the public who becomes infected as a result of an initial infection (e.g., via an earthquake release) or a secondary transmission may also transmit infection to others. Infected members of the public might or might not know or suspect that they have been exposed, and if symptoms develop they might not recognize the source of their disease. In assessing the possibility of extended outbreaks in the public,

lessons can be learned from outbreaks that have occurred in the past regardless of whether they originated from loss of containment at a biological laboratory.

9.2 Methodology

For each pathogen, the potential for secondary transmission is discussed qualitatively. For four of the pathogens that can be transmitted directly from one person to another—*Yersinia pestis* (*Y. pestis*) causing pneumonic plague, 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), and Ebola virus (EBOV)—quantitative analyses were performed through the use of mathematical modeling and statistical procedures. Those analyses involved the following:

- Description of the quantity, quality, and relevance of data from past natural outbreaks involving the pathogen
- Assessment of the likelihood for given numbers of secondary transmissions occurring in the community under the scenarios specific to this RA using branching process modeling methods (Lloyd-Smith 2005)
- Characteristics of the pathogen as derived from published studies of past outbreaks in terms of average reproductive numbers, timing of disease progression and death rates
- Consideration of public health measures that were put into place during past outbreaks of the pathogens to control the outbreaks and the estimated effects of those measures
- For each pathogen, three event sequences were considered for estimating secondary transmission; those were needlestick, centrifuge release, and MRF earthquake release
- Assessment of the uncertainty associated with the likelihood of transmission because of lack of knowledge of the pathogen's characteristics
- Assessment of the variability in transmissions exhibited by observations of past outbreaks
- Discussion of the implications of uncertainty and variability on the assessment of the risk of secondary transmission
- Analyses specific to the three NEIDL sites including differences in populations at the three sites, contact-rate estimates for the local populations, differences in the proportions of medically vulnerable subpopulations in the communities at the three sites and the effect of commuting of workers on the spread of infection in the community

Details of the methods are provided in Appendix L.

9.3 Results

Assessments of the transmission potential for all 13 pathogens are presented here. In addition, the results from quantitative analyses of four of the pathogens (*Y. pestis*, 1918 H1N1V, SARS-CoV, and EBOV) are presented. The quantitative results presented here focus on estimates of how often an undetected or unreported needlestick infection leading to public infections and fatalities would occur. The needlestick event is chosen because it resulted in more frequent initial infections estimates with those four pathogens compared to the centrifuge and earthquake release events.

An initial infection after a needlestick event without prompt detection and reporting was estimated to occur in frequency category B (between once per 100 years and once per 10,000 years) (Appendix K). Values from throughout this range of estimates are multiplied by the estimated likelihood of secondary transmissions occurring in the community (derived from simulations detailed in Appendix L) to arrive at estimates of how often needlestick events leading to outbreaks of different sizes would be expected to occur. Results are displayed for both total numbers of infections and fatalities and for those that occur among Boston city residents. The portion of infections occurring among residents and non-residents are derived from estimates for rates of workers commuting, as detailed in Appendix L.

9.3.1 *Bacillus anthracis*

Bacillus anthracis (*B. anthracis*) causes inhalational, gastrointestinal or cutaneous forms of anthrax. There is no evidence of secondary transmission of inhalational and gastrointestinal anthrax. There are rare reports of person-to-person transmission of cutaneous anthrax. There are reports of humans acting as vectors in physically carrying spores on hands or inanimate items such as clothing to close contacts resulting in infection in the close contact (WHO 2008). The most recent example of this is the cutaneous anthrax that developed in a 7-month old infant who most likely came into contact with *B. anthracis* spores while being held by co-workers of his mother at her workplace in New York City that was contaminated with spores during the 2001 intentional release (Freedman, Afonja et al. 2002). As the person-to-person transmission of cutaneous anthrax is rare, secondary transmission modeling of spread of infection in the community after loss of biocontainment was not performed for *B. anthracis*.

9.3.2 *Francisella tularensis*

Francisella tularensis (*F. tularensis*) is the causative pathogen of tularemia, which is a disease of animals that also affects humans. *F. tularensis* can infect humans through the skin, mucous membranes, gastrointestinal tract, and lungs (Dennis, Inglesby et al. 2001; Ellis, Oyston et al. 2002). There are no reports of direct person-to-person transmission of *F. tularensis*, even from the pneumonic form of

tularemia. There is one published report that suggests that bacteria are aerosolized from patients and in animal models of pneumonic tularemia and this could potentially cause secondary human infections (Jones, Nicas et al. 2005); those conclusions have not been validated by other authors or experts. Because there is no known direct person-to-person transmission of *F. tularensis*, secondary transmission modeling of spread of infection in the community after loss of biocontainment was not performed for this pathogen.

9.3.3 *Yersinia pestis*

Y. pestis causes plague and is transmissible from person to person, particularly in pneumonic form, as described in Chapter 3. Some detailed studies exist in the literature on epidemiological parameters and quantitative transmission modeling of pneumonic plague outbreaks that have occurred. Therefore, *Y. pestis* was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. A detailed review of the published literature on *Y. pestis* with a focus on mathematical models of the spread of pneumonic plague was performed to evaluate available estimates of the reproductive numbers for pneumonic plague, the average generation time between one infection and the next and case fatality rate (CFR), as described in Appendix L.

As shown in Table 9–1, one or more public infections with *Y. pestis* following a needlestick event would be expected to occur between once in 510 years and once in 18,000 years, which places this sequence of events in frequency category B or C. An outbreak with 10 or more total public infections would be expected to occur between once in 1,500 years to once in 740,000 years. An outbreak of greater than 100 total public infections is estimated to occur less than once in 130,000 years (frequency category C or D). Outbreaks are unlikely to grow that large under the assumption that control measures would be highly effective in preventing transmission, a notion that is supported by data from several pneumonic plague outbreaks occurring in the 20th century, in which none exceeded 50 total cases, and standard control measures were usually highly effective once hospitals and communities became aware of an outbreak (Appendix L).

One or more total fatalities in the public following a needlestick would be expected to occur between once in 560 years and once in 38,000 years (category B or C), while 100 or more deaths under this scenario is entirely within frequency category D. Among Boston city residents, frequency estimates are somewhat smaller than the total estimates because some portion of infections during an outbreak would likely occur outside the city. The results show that 10 or more infections might occur with a frequency in categories B, C, or D, while 100 or more infections occurring among residents would be extremely

unlikely, occurring with an average frequency less than once in 1.8 million years, entirely within category D.

Table 9–1. *Y. pestis* results—frequency of public infections and fatalities from an undetected/unreported needlestick (urban site)

Consequence		Frequency range	
		Total	Among Boston city residents
Number of public infections	1 or more	1 in 510 to 18,000 years	1 in 850 to 32,000 years
	10 or more	1 in 1,500 to 740,000 years	1 in 4,600 to 3.5 million years
	100 or more	1 in 130,000 to > 10 million years	1 in 1.8 million to > 10 million years
Number of public fatalities	1 or more	1 in 560 to 38,000 years	1 in 970 to 86,000 years
	10 or more	1 in 6,500 to > 10 million years	1 in 21,000 to > 10 million years
	100 or more	1 in 5.8 million to > 10 million years	1 in > 10 million years

9.3.4 1918 H1N1 Influenza Virus

1918 H1N1V was transmitted person-person worldwide during the 1918 influenza pandemic, as described in Chapter 3. Numerous studies exist in the literature on epidemiological parameters and quantitative transmission modeling of the 1918 pandemic in addition to other influenza pandemics, including the most recent pandemic in 2009, which was also caused by an H1N1 influenza virus (2009 H1N1V). Therefore, 1918 H1N1V was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. A detailed review of the published literature on this pathogen with a focus on mathematical models of the spread of pandemic influenza was performed to evaluate available estimates of the reproductive numbers, the average generation time between one infection and the next, effects of public health control measures, and CFR, as described in Appendix L.

As shown in Table 9–2, one or more transmissions of 1918 H1N1V following a needlestick event would be expected to occur with an average frequency between once in 550 years and once in 16,000 years, which places this sequence of events in frequency category B or C. An outbreak of 1918 H1N1V in the community with 10 or more secondary transmissions following a needlestick would be expected to occur between once in 980 years to once in 43,000 years. The estimated chances of larger outbreaks (100 or more, 1,000 or more, and so on) are subject to wide uncertainty ranges. Some assumptions lead to estimating that an outbreak reaching 100 cases would be extremely infrequent (less than once in 10 million years), while other assumptions lead to estimating that a sustained, widespread outbreak could

occur with average frequency up to once in 23,000 years. Under most assumptions (more than 90 percent), such extreme, unchecked outbreaks starting from a single initially infected case would be considered unlikely (frequency category D, or less than once in 1 million years); however, their possibility is supported by the occurrence of worldwide influenza pandemics in the past.

Table 9–2. 1918 H1N1V results—frequency of public infections and fatalities from an undetected/unreported needlestick (urban site)

Consequence		Frequency range	
		Total	Among Boston city residents
Number of public infections	1 or more	1 in 550 to 16,000 years	1 in 900 to 26,000 years
	10 or more	1 in 980 to 43,000 years	1 in 1,800 to 140,000 years
	100 or more	1 in 1,400 to > 10 million years	1 in 3,900 to > 10 million years
	1,000 or more	1 in 4,300 to > 10 million years	1 in 15,000 to > 10 million years
	10,000 or more	1 in 8,300 to > 10 million years	1 in 350,000 to > 10 million years
	100,000 or more	1 in 23,000 to > 10 million years	1 in > 10 million years
Number of public fatalities	1 or more	1 in 1,100 to 70,000 years	1 in 2,300 to 170,000 years
	10 or more	1 in 2,700 to > 10 million years	1 in 10,000 to > 10 million years
	100 or more	1 in 5,800 to > 10 million years	1 in 50,000 to > 10 million years
	1,000 or more	1 in 23,000 to > 10 million years	1 in > 10 million years

9.3.5 SARS-Associated Coronavirus

SARS-CoV is transmissible person-to-person, as observed in many locations during the 2003 outbreak described in Chapter 3. Numerous studies on epidemiological parameters and quantitative transmission modeling of SARS-CoV exist in the literature. Therefore, SARS-CoV was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. A detailed review of the published literature on this pathogen with a focus on mathematical models of the spread of SARS was performed to evaluate available estimates of the reproductive numbers, the average generation time between one infection and the next, effects of public health control measures, and CFR.

As shown in Table 9–3, one or more transmissions of SARS-CoV following a needlestick event would be expected to occur between once in 760 years and once in 27,000 years, which places this sequence of events in the frequency category B or C. An outbreak of SARS-CoV with 100 or more public infections would be expected to occur between once in 2,500 years and once in 440,000 years. An outbreak of

greater than 1,000 public infections is potentially very unlikely under certain assumptions (less than once in 10 million years), while other under assumptions, a sustained, widespread outbreak could occur in frequency category C, up to once in 260,000 years. Those extreme, higher risk results depend on the assumption that control measures attempting to curb transmission would not be effective at ending an outbreak that becomes large. That scenario is not supported by past experience, given that the entire worldwide outbreak of SARS in 2002–2003 resulted in about 8,000 infections and 800 deaths. For the analysis in this RA, extreme outcomes on par with those numbers or higher were observed only if it was assumed that control measures would remain relatively ineffective for a long period. If control measure effectiveness was on par with what was observed in most locations in 2003, those extreme consequences were found to be much less likely.

Table 9–3. SARS-CoV results—frequency of public infections and fatalities from an undetected/unreported needlestick (urban site)

Consequence		Frequency range	
		Total	Among Boston city residents
Number of public infections	1 or more	1 in 760 to 27,000 years	1 in 1,000 to 37,000 years
	10 or more	1 in 1,100 to 59,000 years	1 in 1,800 to 120,000 years
	100 or more	1 in 2,500 to 440,000 years	1 in 5,600 to 3.2 million years
	1,000 or more	1 in 23,000 to > 10 million years	1 in 160,000 to > 10 million years
	10,000 or more	1 in 67,000 to > 10 million years	1 in > 10 million years
	100,000 or more	1 in 260,000 to > 10 million years	1 in > 10 million years
Number of public fatalities	1 or more	1 in 1,000 to 47,000 years	1 in 1,600 to 80,000 years
	10 or more	1 in 2,500 to 350,000 years	1 in 5,300 to 1.9 million years
	100 or more	1 in 23,000 to > 10 million years	1 in 170,000 to > 10 million years
	1,000 or more	1 in 67,000 to > 10 million years	1 in > 10 million years
	10,000 or more	1 in 260,000 to > 10 million years	1 in > 10 million years

9.3.6 Rift Valley Fever Virus

Rift Valley fever virus (RVFV) is an RNA virus in the larger family of viral hemorrhagic fevers and causes Rift Valley fever. There is no direct person-to-person transmission of RVFV; transmission to humans is via arthropod vectors or by contact with infected animal products. Cattle, sheep, and goats serve as the primary amplifier of the virus. Upon developing adequate viremia (virus in the blood), humans could also serve as a virus reservoir for mosquitoes.

There is a limited literature on disease transmission models of RVFV involving animals, arthropod vectors and human hosts. Several models have addressed other complex variables such as climate conditions and livestock in the prediction and transmission of RVFV in endemic areas (Clements, Pfeiffer et al. 2006; Favier, Chalvet-Monfray et al. 2006; Anyamba, Chretien et al. 2009; Metras, Collins et al. 2011; Mpeshe, Haario et al. 2011). The applicability of those published models and epidemiologic, climate and livestock data to conditions in the United States, specifically to NEIDL sites under study in the RA is unknown. Furthermore, data on ruminants and mosquito vectors are not uniformly available in a format suitable for use in secondary transmission modeling. For these reasons, secondary transmission modeling of spread of infection in the community following a loss of biocontainment was not performed for RVFV.

The needlestick, centrifuge release, and earthquake release scenarios involving RVFV were considered and the subsequent potential for secondary transmission of RVFV in the community via arthropod vectors and infected animal products were qualitatively analyzed.

An initial infection after a needlestick event without prompt detection and reporting was estimated to occur in frequency category B (between once per 100 years and once per 10,000 years). An initial infection with RVFV following a centrifuge release event was estimated, under a higher risk set of dose-response assumptions, to occur in frequency categories A or B (between about once per 10 years and once per 300 years). An initial infection occurring in a member of the public after exposure to RVFV released during an earthquake event was estimated to occur in frequency category C (between about once per 10,000 years and once per 1 million years) (Appendix K).

If a laboratory worker or a member of the public develops Rift Valley fever from any of the above events, there is no possibility of directly transmitting the virus to the workers' close social contacts. However, secondary transmission could occur from an initially infected laboratory worker or member of the public developing sufficiently high viremia and then being bitten by a mosquito vector belonging to a permissive species. The mosquito could act as a biological vector and transmit the virus to another human while taking its next blood meal. That scenario is possible but considered unlikely because (1) the primary amplifiers of RVFV are ruminants; (2) it is rare for humans to serve as a virus reservoir; and (3) very few permissive species of mosquitoes are native to the area.

Similarly, it is possible but unlikely that an initially infected laboratory worker or member of the public would transmit infection via mosquito to ruminants; therefore, it is considered unlikely that secondary

transmission to other humans would occur via infected ruminants. This scenario is distinct from the issue of establishment of RVFV in the environment after a release, which is discussed in Chapter 7.

9.3.7 Andes Virus

Andes virus (ANDV) is a New World hantavirus that causes a severe cardiopulmonary syndrome (HPS). A unique feature of ANDV is that direct person-to-person transmissions of ANDV have been reported. There are reports of HPS in close contacts with genetic evidence of person-to-person transmission, from earlier outbreaks in the 1990s to more recent cases. In such circumstances, transmission generally occurred in close family contacts who exchanged bodily fluids, with evidence of ANDV RNA found in saliva of patients (Enria, Padula et al. 1996; Padula, Edelstein et al. 1998; Martinez, Bellomo et al. 2005; Castillo, Nicklas et al. 2007; Lazaro, Cantoni et al. 2007). A recent study that prospectively studied 476 household contacts of 76 index patients with HPS in Chile found 16 contacts developed confirmed HPS (3.4 percent) (Ferres, Vial et al. 2007). A third of all the cases occurred in family clusters. Person-to-person transmission was definite in only three household contacts and probable in another nine. Sexual contacts were at the highest risk for HPS in this study.

In other reports from Argentina, 16 cases of HPS were suspected to be from person-to-person transmission, though contact or exposure to rodents could not be completely ruled out for a majority of those patients (Wells, Sosa Estani et al. 1997; Cantoni, Padula et al. 2001). There did not appear to be any hospital- or health care-associated person-to-person transmission in one outbreak in Chile (Castillo, Villagra et al. 2004). Other reports have described cases in health care workers (Lopez, Padula et al. 1996; Wells, Sosa Estani et al. 1997; Toro, Vega et al. 1998; Mertz, Hjelle et al. 2006).

In summary, direct person-to-person transmission of ANDV has been documented; although the extent of the transmission is limited to close family and possibly health care contacts and has not resulted in large outbreaks of HPS. There are limited studies available to provide epidemiologic data for detailed secondary-transmission modeling and no published mathematical models for this pathogen. A reproductive number of 0.7 has been estimated for ANDV, which would indicate that a sustained outbreak is unlikely for ANDV (Lloyd-Smith, Schreiber et al. 2005). For those reasons, secondary-transmission modeling of spread of infection in the community following a loss of biocontainment was not performed for this pathogen.

9.3.8 Ebola Virus

EBOV is transmissible person-to-person, as observed during several outbreaks that have occurred in Africa (Legrand 2006). The EBOV transmission estimates are based on information from outbreaks that occurred in Africa. As discussed further in Appendix L, the setting and population in which those outbreaks occurred are in many ways quite different than the United States, and some have suggested that the extent of transmission seen in Africa can be explained by local cultural practices that amplified transmission. However, others have concluded that the locally specific practices accounted for only a small portion of transmission and that many transmissions occurred within families and the community (see Appendix L for references). Given the lack of evidence to suggest otherwise, it was assumed that transmission estimates derived from the Africa outbreaks would be applicable to a potential outbreak in the United States.

Several studies on epidemiological parameters and quantitative transmission modeling of EBOV exist in the literature. Therefore, EBOV was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. A detailed review of the published literature on this pathogen with a focus on mathematical models of the spread of EBOV was performed to evaluate available estimates of the reproductive numbers, the average generation time between one infection and the next, effects of public health control measures, and CFR, as described in Appendix L.

As shown in Table 9–4, one or more transmissions of EBOV following a needlestick event would be expected to occur between once in 550 years and once in 18,000 years, which places this sequence of events in frequency category B or C. An outbreak of EBOV with 10 or more public infections would be expected to occur between once in 1,900 years and once in 76,000 years. An outbreak of 100 or more public infections is considered much less likely than smaller outbreaks, with the estimated frequency falling to the range once in 110,000 years to less than once in 10 million years. Those results are supported by observations from at least 14 outbreaks that occurred in Africa since 1976, which ranged from a total of 12–425 infections per outbreak. Most (9 of 14) of those documented outbreaks involved fewer than 100 cases, and it is presumed that more outbreaks limited to a small number of cases have occurred and gone undocumented. The reason that EBOV outbreaks larger than 100 cases are rarer and more than 1,000 cases have not occurred is that standard, hospital-level control measures were effective once put in place.

Table 9–4. EBOV results—frequency of public infections and fatalities from an undetected/unreported needlestick (urban site)

Consequence		Frequency range	
		Total	Among Boston city residents
Number of public infections	1 or more	1 in 550 to 18,000 years	1 in 920 to 29,000 years
	10 or more	1 in 1,900 to 76,000 years	1 in 5,000 to 250,000 years
	100 or more	1 in 110,000 to > 10 million years	1 in 2.9 million to > 10 million years
Number of public fatalities	1 or more	1 in 610 to 20,000 years	1 in 1,100 to 36,000 years
	10 or more	1 in 3,100 to 240,000 years	1 in 11,000 to 1.0 million years
	100 or more	1 in 420,000 to > 10 million years	1 in 8.9 million to > 10 million years

9.3.9 Marburg Virus

Marburg virus (MARV) is a member of a group of hemorrhagic fever viruses and is closely related to EBOV. Direct person-to-person transmission of MARV from index cases to family and community contacts has been described in the 1967 outbreak and the two large outbreaks in Africa. Secondary transmission is associated with close contact with the ill patient or their bodily fluids, mainly blood (Bausch, Nichol et al. 2006; Feldmann 2006; Towner, Khristova et al. 2006). Other body fluids from infected humans (feces, vomitus, urine, saliva, and respiratory secretions) with high virus concentrations, especially when the fluids contain blood, have also been implicated in transmission.

Secondary transmission modeling of the spread of infection in the community following loss of biocontainment has been performed for the closely related EBOV. The results of the modeling are broadly applicable to MARV; for those reasons, secondary transmission modeling of spread of infection in the community following a loss of biocontainment was not performed for this pathogen.

9.3.10 Lassa Virus

Lassa virus (LASV) is the causative pathogens of Lassa fever, which is a viral hemorrhagic fever. Direct person-to-person transmission of LASV occurs, especially in hospital settings. Person-to-person transmission is associated with direct contact with the blood or other bodily fluids containing virus particles of infected individuals. Airborne transmission has also been postulated to occur. Contact with objects contaminated with virus, such as medical equipment (reused needles), is also associated with transmission in health care settings (Centers for Disease Control 2011). The viruses are generally not known to be spread through casual contact, including skin-to-skin contact without exchange of bodily fluids (Ogbu, Ajuluchukwu et al. 2007). Thus, the risk of direct person-to-person transmission is low and

involves close contact with infected body fluids. Furthermore, there are limited published mathematical models of such transmission. For those reasons, secondary transmission modeling of spread of infection in the community following loss of biocontainment was not performed for this pathogen.

9.3.11 Junín Virus

Junín virus (JUNV) is the causative pathogen of Argentine hemorrhagic fever. As with other hemorrhagic fever viruses in the family Arenaviridae (Lassa fever virus and the New World arenaviruses), there is potential for direct person-to-person transmission of JUNV, postulated to occur via close contact with infectious blood and body fluids (Borio, Inglesby et al. 2002). Note that there have been no reports of person-to-person transmission of JUNV from patients to health care workers, despite the several hundred patients with hemorrhages cared for each year in Argentina (Charrel and de Lamballerie 2003). Thus, the risk of direct person-to-person transmission of JUNV is considered low, and secondary transmission modeling of spread of infection in the community following loss of biocontainment was not performed for this pathogen.

9.3.12 Tick-borne Encephalitis Virus, Far Eastern Subtype

Tick-borne encephalitis virus, Far Eastern subtype (TBEV-FE) was formerly known as Russian spring-summer encephalitis. This virus is one member of the tick-borne encephalitis virus complex. TBEV-FE is one of the causative pathogens of tick-borne encephalitis (TBE) (Lindquist and Vapalahti 2008). The virus is transmitted to humans through the bite of an infected tick. Because there is no direct-to-person transmission of TBEV-FE, secondary transmission modeling of spread of infection in the community following loss of biocontainment was not performed for this pathogen.

9.3.13 Nipah Virus

Nipah virus (NIPV) is an emerging pathogen that was first described in 1998 from an outbreak of encephalitis in Malaysia and Singapore (1999). The mode of transmission of NIPV has changed between the Malaysian/Singapore outbreaks and those in Bangladesh/India. In the Malaysian/Singapore outbreaks, it is postulated that the virus was transmitted from bats (natural reservoir) to pigs, causing an outbreak in pigs, which subsequently led to an outbreak in humans in close contact with the pigs (abattoir workers and pig farmers). In Bangladesh, the transmission from bats to humans appears to be ongoing and via at least three different routes (Luby, Rahman et al. 2006; Luby, Hossain et al. 2009). The most frequent mode is food-borne through ingestion of Nipah-virus-contaminated date palm sap which is a staple food source in that region. A second mode of transmission appears to be via domestic animals that feed on

contaminated fruits or date palm sap that have been licked or partially eaten by fruit bats infected with NIPV. There do not appear to be any arthropod vectors in the transmission of NIPV.

Evidence from epidemiologic investigations of outbreaks in Bangladesh and India indicates that NIPV can be transmitted directly from person to person. That has occurred in patients with respiratory illness. Close physical contact with a known NIPV patient who later died was found to be the strongest risk factor for direct person-to-person transmission (Gurley, Montgomery et al. 2007). NIPV has been found in respiratory secretions of infected patients (Chua, Lam et al. 2001). Though direct transmissions have occurred and are known to be responsible for many of the Bangladeshi outbreaks, the risk of direct transmission appears to be low and requires close contact that might be culture- and region-specific (to Bangladesh) (Luby, Hossain et al. 2009). Given the limitations of conducting field investigations in rural areas of developing countries, the study (Luby, Hossain et al. 2009) estimated that only a few individuals transmitted to others and the generations of transmissions rarely exceeded two. The overall number of secondary cases resulting from an infected person was expected to be less than 0.5, indicating that it was unlikely that any one chain of transmission would result in a large outbreak.

In summary, there is a possibility of person-to-person transmission of NIPV via respiratory secretions; however the risk is low and requires close contact that could be culture- and region-specific (Luby, Hossain et al. 2009). Moreover, there are limited studies available to provide epidemiologic data for detailed secondary transmission modeling and no published mathematical models for this pathogen. For those reasons, secondary transmission modeling of spread of infection in the community following loss of biocontainment was not performed for this pathogen.

9.3.14 Site Differences

This section describes and compares results from simulations based on details specific to the suburban and rural sites. The following differences in assumptions as compared to those for the urban site are applied.

Local population size—The estimates provided in the results sections above used a local population estimate of 1 million individuals, which is representative of the daytime population in the city of Boston. The suburban and rural site simulations were run using local populations of 12,000 (approximate population of Tyngsborough, Massachusetts) and 8,000 (approximate combined population of Hancock and Peterborough, New Hampshire). Those changes have an effect only on larger outbreaks in which the

infected portion of the local population might become substantial enough to appreciably decrease the portion of the local population that remains susceptible.

Effect of commuting—The suburban and rural site simulations incorporate commuting estimates specific to those areas, as described in Appendix L. It is noted that chains of transmission occurring among non-locals (who neither work nor live in the local area) are not subject to the local population constraint noted above. I.e., non-locals have contacts among a wider pool of susceptible individuals than just the local area, so that an extreme outbreak could potentially exceed the local population size of the area in which the outbreak started.

Contact rate differences—A procedure described in Appendix L was applied to adjust estimates for transmission at the suburban and rural sites, on the basis of information that suggests that residents in those areas contact fewer people daily, compared to urban residents (about 15 percent fewer at the suburban site and about 50 percent fewer at the rural site). The adjusted values are applied only to infected individuals in the simulation if they are classified as local residents. Otherwise, the adjusted contact rates are not applicable, and the estimates used for the urban site are applied.

Effectiveness of control measures—It is possible that differences between the sites would lead to different expectations for the timing and effectiveness of control measures. However, no convincing evidence was found to justify concrete assumptions about site differences in this regard, as explained in the following points.

- Any evaluation of the current facilities, resources, personnel, and outbreak preparedness at the suburban and rural sites might not be relevant if the NEIDL was actually located there. Presumably, the presence of NEIDL in the area would bring new medical resources to the area and potentially enhance the preparedness of the local area hospitals.
- Even if it could be concluded that there are differences in overall preparedness of hospitals at different sites, there are important factors contributing to the timing and effectiveness of control measures that are beyond the control of health officials. For example, (1) decisions made by infected individuals in the pre-control phase of an outbreak, such as whether to seek medical attention, could contribute to delays; (2) the human factor, i.e., errors made by individual health care workers could contribute to delays and decrease control effectiveness; and (3) variation in the biology infectious cases, for example, unusually contagious patients, could hinder control efforts. Those examples could occur at any hospital, which supports the notion that the full range of possible delays and control effectiveness could be relevant at any site.

- There is no guarantee that medical facilities closest to the NEIDL would be the ones primarily affected by an outbreak. Given the high rates of commuting observed at the three sites, an outbreak could easily spread outside the local area in the early stages, making it difficult to predict which area or hospital would begin seeing the first cases. For example, in the case of an outbreak started by an infected laboratory worker, the initial cases could be family members or individuals near the worker's home, which might or might not be close to the NEIDL.

Summary of Site Difference Results

Comparisons of results for the suburban and rural sites to the urban site results are summarized here and presented in detail in Appendix L.

For total numbers of infections and fatalities, the estimated likelihoods for a given consequence are generally slightly smaller at the suburban and rural sites as compared to the urban site, but the uncertainty ranges largely overlap, so that no statistically significant difference can be concluded. The reason that the overall results from the suburban and rural sites are so similar to the urban site results is that there is a high estimated rate of commuting to and from the towns at those sites, so that a significant portion of transmissions occur among nonresidents and are not subject to local population constraints or to the estimates for decreased contact rates that were based on residents only.

The effects of the site difference assumptions are more apparent when comparing the results for local residents. There tends to be a lower estimated chance of each consequence among local residents at the suburban and rural sites compared to the urban site because of commuting and contact rate differences, although uncertainty ranges overlap in most cases. The differences suggest that a more substantial portion of the risk from an undetected/unreported laboratory worker infection at the suburban and rural sites would be borne by nonresidents, particularly areas with a strong connection with the local area via commuting.

9.3.15 Medically Vulnerable Subpopulations

A procedure for incorporating information about medically vulnerable subpopulations (MVSP), described in Appendix L, was applied to SARS-CoV outbreak simulations at the three sites. MVSP, as considered in this RA, include children under 5 years of age, adults over 65 years of age, people with diabetes, people with HIV/AIDS, and pregnant women. The following factors contributing to secondary transmission estimates were included.

- Estimates for the proportion of each MVSP within the local population at each site—These estimates were based on data from the three sites (see Appendix I) and affect the likelihood that a secondary transmission would occur to a member of a specific MVSP.
- Estimates of increased susceptibility to infection, given exposure, for each MVSP—These estimates were based on expert opinion and serve to increase the likelihood that transmissions would occur to members of MVSP, compared to what would be estimated using just their proportion in the population.
- Estimates of increased CFR for each MVSP—These estimates were based on expert opinion unless data were available and serve to increase the estimated numbers of fatalities among MVSP, compared to what would be estimated under the assumption of a constant CFR across all groups.

Overall, it was determined that the MVSP profiles at the three sites potentially contribute a small effect on site differences for overall risk to the population, but that the differences are not substantial in light of the overall uncertainty and in comparison to the site differences with respect to other factors discussed above, such as differential contact rates.

In addition, estimates of the risk specific to each individual MVSP at each site were obtained. For each MVSP, the estimated likelihood of infections and fatalities occurring in that group were not substantially different across the sites, and also would not be substantially different if local MVSP profiles were in line with average U.S. proportions. See Appendix L for details.

Some limitations of this part of the analysis are as follows. First, the expert estimates of increased susceptibility to disease and mortality were based on belonging to each MVSP individually; in reality, it is possible that individuals may have multiple concomitant medical vulnerabilities such as being both elderly and diabetic, or both HIV-positive and pregnant. The susceptibility in these situations is potentially increased due to compounding effects of the individual conditions. Published data about the effects of each combination of vulnerabilities on susceptibility are scarce, and as such the simplified approach for this analysis was to assess each medical vulnerability separately. Second, because most of the estimates for susceptibility of MVSP relative to healthy adults are based on expert opinion and not on data, it is possible that they significantly underestimate the true differences in susceptibility. However, as detailed in Appendix L, the conclusions summarized above regarding comparisons across sites and to U.S. averages would not be significantly affected by assuming higher susceptibilities of each MVSP.

9.4 References

(1999). "Update: outbreak of Nipah virus--Malaysia and Singapore, 1999." MMWR Morb Mortal Wkly Rep 48(16): 335-337.

Anyamba, A., J. P. Chretien, et al. (2009). "Prediction of a Rift Valley fever outbreak." Proceedings of the National Academy of Sciences of the United States of America 106(3): 955-959.

Bausch, D. G., S. T. Nichol, et al. (2006). "Marburg hemorrhagic fever associated with multiple genetic lineages of virus." N Engl J Med 355(9): 909-919.

Borio, L., T. Inglesby, et al. (2002). "Hemorrhagic fever viruses as biological weapons: medical and public health management." JAMA 287(18): 2391-2405.

Cantoni, G., P. Padula, et al. (2001). "Seasonal variation in prevalence of antibody to hantaviruses in rodents from southern Argentina." Tropical medicine & international health : TM & IH 6(10): 811-816.

Castillo, C., C. Nicklas, et al. (2007). "Andes Hantavirus as possible cause of disease in travellers to South America." Travel medicine and infectious disease 5(1): 30-34.

Castillo, C., E. Villagra, et al. (2004). "Prevalence of antibodies to hantavirus among family and health care worker contacts of persons with hantavirus cardiopulmonary syndrome: lack of evidence for nosocomial transmission of Andes virus to health care workers in Chile." The American journal of tropical medicine and hygiene 70(3): 302-304.

Centers for Disease Control. (2011). "Special Pathogens Branch: Arenaviruses." Retrieved September 2, 2011, 2011, from http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/arena.htm.

Charrel, R. N. and X. de Lamballerie (2003). "Arenaviruses other than Lassa virus." Antiviral Res 57(1-2): 89-100.

Chua, K. B., S. K. Lam, et al. (2001). "The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia." J Infect 42(1): 40-43.

Clements, A. C., D. U. Pfeiffer, et al. (2006). "Application of knowledge-driven spatial modelling approaches and uncertainty management to a study of Rift Valley fever in Africa." International journal of health geographics 5: 57.

Dennis, D. T., T. V. Inglesby, et al. (2001). "Tularemia as a biological weapon: medical and public health management." JAMA 285(21): 2763-2773.

Ellis, J., P. C. Oyston, et al. (2002). "Tularemia." Clin Microbiol Rev 15(4): 631-646.

Enria, D., P. Padula, et al. (1996). "Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission." Medicina 56(6): 709-711.

Favier, C., K. Chalvet-Monfray, et al. (2006). "Rift Valley fever in West Africa: the role of space in endemicity." Tropical medicine & international health : TM & IH 11(12): 1878-1888.

Feldmann, H. (2006). "Marburg hemorrhagic fever--the forgotten cousin strikes." N Engl J Med 355(9): 866-869.

Ferres, M., P. Vial, et al. (2007). "Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile." The Journal of infectious diseases 195(11): 1563-1571.

Freedman, A., O. Afonja, et al. (2002). "Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old infant." JAMA 287(7): 869-874.

Gurley, E. S., J. M. Montgomery, et al. (2007). "Person-to-person transmission of Nipah virus in a Bangladeshi community." Emerg Infect Dis 13(7): 1031-1037.

Jones, R. M., M. Nicas, et al. (2005). "The Infectious Dose of Francisella tularensis (Tularemia)." Applied Biosafety 10(4): 227-239.

Lazaro, M. E., G. E. Cantoni, et al. (2007). "Clusters of hantavirus infection, southern Argentina." Emerging infectious diseases 13(1): 104-110.

Lindquist, L. and O. Vapalahti (2008). "Tick-borne encephalitis." Lancet 371(9627): 1861-1871.

Lloyd-Smith, J. O., S. J. Schreiber, et al. (2005). "Superspreading and the effect of individual variation on disease emergence." Nature 438(7066): 355-359.

Lopez, N., P. Padula, et al. (1996). "Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina." Virology 220(1): 223-226.

Luby, S. P., M. J. Hossain, et al. (2009). "Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007." Emerg Infect Dis 15(8): 1229-1235.

Luby, S. P., M. Rahman, et al. (2006). "Foodborne transmission of Nipah virus, Bangladesh." Emerg Infect Dis 12(12): 1888-1894.

Martinez, V. P., C. Bellomo, et al. (2005). "Person-to-person transmission of Andes virus." Emerging infectious diseases 11(12): 1848-1853.

Mertz, G. J., B. Hjelle, et al. (2006). "Diagnosis and treatment of new world hantavirus infections." Current opinion in infectious diseases 19(5): 437-442.

Metras, R., L. M. Collins, et al. (2011). "Rift Valley Fever Epidemiology, Surveillance, and Control: What Have Models Contributed?" Vector borne and zoonotic diseases.

Mpeshe, S. C., H. Haario, et al. (2011). "A Mathematical Model of Rift Valley Fever with Human Host." Acta biotheoretica.

Ogbu, O., E. Ajuluchukwu, et al. (2007). "Lassa fever in West African sub-region: an overview." J Vector Borne Dis 44(1): 1-11.

Padula, P. J., A. Edelstein, et al. (1998). "Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus." Virology 241(2): 323-330.

Toro, J., J. D. Vega, et al. (1998). "An outbreak of hantavirus pulmonary syndrome, Chile, 1997." Emerging infectious diseases 4(4): 687-694.

Towner, J. S., M. L. Khristova, et al. (2006). "Marburg virus genomics and association with a large hemorrhagic fever outbreak in Angola." J Virol 80(13): 6497-6516.

Wells, R. M., S. Sosa Estani, et al. (1997). "An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia." Emerg Infect Dis 3(2): 171-174.

World Health Organization. (2008). Anthrax in humans and animals. Geneva, Switzerland, World Health Organization.

Table of Contents

10. Environmental Justice

10.1 Introduction

The objective of the environmental justice analysis for this RA is to addresses the considerations associated with *Executive Order 12898, Federal Actions to Address Environmental Justice in Minority and Low-Income Populations*. CEQ, DOE, and NRC NEPA Guidance, were also used to inform this analysis. Available U.S. Census data was analyzed to determine and identify potential environmental justice areas at the urban, suburban and rural sites. Additionally, this RA examined two types of effects: (1) those that could directly expose environmental justice communities as a result of a release of pathogen from the facility and (2) those that expose people in environmental justice communities indirectly through secondary transmission of pathogens. Each of those types of impacts is addressed separately.

10.2 Environmental Justice—Regulatory Overview

10.1.1 Federal Guidance

In response to public concerns, EPA created the Office of Environmental Justice in 1992. EPA defines *environmental justice* as the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies. *Fair treatment* means that no group of people should bear a disproportionate share of the negative environmental consequences resulting from industrial, governmental, and commercial operations or policies. *Meaningful involvement* means that (1) people have an opportunity to participate in decisions about activities that could affect their environment or health; (2) the public's contribution can influence the regulatory agency's decision; (3) the public's concerns will be considered in the decision-making process; and (4) the decision makers seek out and facilitate the involvement of those potentially affected. EPA established the National Environmental Justice Advisory Council (NEJAC) on September 3, 1993, to provide independent advice, consultation, and recommendations to the EPA Administrator on matters related to environmental justice.

Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations*, directs federal agencies to develop environmental justice strategies to address disproportionately high and adverse human health or environmental effects of their programs on minority and low-income populations, and to focus federal attention on the environmental and human health conditions of minority and low-income populations with the goal of achieving environmental protection for all communities. The order is also intended to promote nondiscrimination in federal programs that affect human health and the environment and provide minority and low-income communities' access to

public information and public participation in matters relating to human health and the environment. In addition to Executive Order 12898, two guidance documents help define how to address environmental justice concerns: The Council on Environmental Quality's (CEQ's) December 1997 document, *Environmental Justice Guidance under the National Environmental Policy Act*, and an April 1998 document produced by an EPA working group titled *Final Guidance for Incorporating Environmental Justice Concerns in EPA's NEPA Compliance Analyses*.

The CEQ has oversight of the federal government's compliance with Executive Order 12898 and NEPA. EPA has lead responsibility for implementation of the Executive Order as Chair of the Interagency Working Group on Environmental Justice (CEQ 1997).

10.2.1.1 Minority Populations

The federal environmental justice criteria identify minority populations as Black or African American; American Indian and Alaska Native; Asian; Native Hawaiian and other Pacific Islander; persons of two or more races; and persons of Hispanic origin. Minority populations should be identified for environmental justice analyses where, either the minority population of the affected area exceeds 50 percent, or the minority population percentage of the affected area is meaningfully greater than the minority population percentage in the general population or other appropriate unit of geographic analysis (CEQ 1997). The latter guidance was used for this analysis to address the federal environmental justice criteria, identifying census tracts with minority population percentages exceeding that of the United States. Census tracts are subdivisions of a county and represent a level at which disproportionate impacts would be more noticeable. As of 2000, 25 percent of the U.S. population was of a minority race or ethnicity (U.S. Census Bureau 2000).

10.2.1.2 Low Income Populations

The federal environmental justice criteria use poverty thresholds established by the U.S. Census Bureau to identify low-income populations (CEQ 1997). Poverty status is reported as the number of persons or families with income below a defined threshold level. The Census defines the 2000 poverty level as $8,791 ($10,956 for 2009) of annual income, or less, for an individual and $17,604($21,954 for 2009) of annual income, or less, for a family of four (U.S. Census Bureau 2010a). The federal environmental justice criteria identify low-income populations for those census tracts with poverty rates exceeding those of the United States. As of the 2000 Census, 12 percent (U.S. Census Bureau 2000) of U.S. residents were classified as living in poverty and in 2008 13.2 percent of the U.S. residents were classified as living in poverty (U.S. Census Bureau 2010b).

10.2.1.3 Disproportionately High and Adverse Human Health Effects

Adverse health effects are measured in risks and rates that could result in fatal or nonfatal adverse impacts on human health. Adverse health effects may include bodily impairment, infirmity, illness, or death. Agencies are required to identify programs, policies or activities which may cause disproportionately high and adverse human health or environmental effects. The agency's determination of disproportionately high and adverse impacts is made in consideration of whether the impacts as summarized in the RA meet the following criteria:

1. The impacts must be significant or above generally accepted norms, such as regulatory limits or state and local statutes and ordinances (NUREG 2003). The significance of impacts is determined in consideration of both the context and the intensity of the impact. The context includes factors such as extent of the impact, (i.e. whether the impact is local, regional, or national) and, therefore, the number of people that might be affected. Intensity refers to the severity of the impact. Direct and indirect impacts are considered as well as immediate and long-term impacts (40 CFR 1508.8).

2. Impacts are disproportionate if the risks to a minority individual, or low-income individual, appreciably exceed the risk to an individual in the general population (CEQ 1997).

10.2.2 State Guidance

The Massachusetts Executive Office of Energy and Environmental Affairs (EEA) designates environmental justice populations are those segments of the population that EEA has determined to be most at risk of being unaware of or unable to participate in environmental decision making or to gain access to state environmental resources. These groups are defined as neighborhoods that meet *one or more* of the following criteria:

- The median annual household income is at or below 65 percent of the statewide median income for Massachusetts; *or*
- 25 percent of the residents are minority; *or*
- 25 percent of the residents are foreign born, *or*
- 25 percent of the residents are lacking English language proficiency.

Neighborhoods, as defined by EEA's Environmental Justice Policy are U.S. Census Bureau census block groups (Massachusetts EEA 2002).

10.3 Environmental Justice Methodology

For purposes of this RA, the analysis for environmental justice is presented to address both the federal and state criteria, separately but sequentially. NIH performed an environmental justice analysis that includes input from the Boston community from January 9, 2004, to the present to identify the disproportionate placement of high and adverse environmental or health impacts from the NEIDL at the urban, suburban, or rural sites on minority or low-income populations. The public input gathered since January 9, 2004, assisted in identifying a geographic scale for which demographic information was obtained on the potential impact area(s). Per CEQ, available demographic data from the U.S. Census Bureau census tracts and block numbers were used to identify the composition of the potentially affected population. Census tracts are small relatively permanent statistical subdivisions of a county. Block Numbering Areas are small statistical subdivisions of a county for grouping and numbering blocks in nonmetropolitan counties where local census statistical committees have not established census tracts. A Block Group is a combination of a census blocks that is a subdivision of a census tract or Block Numbering Area. Census blocks are the smallest geographic area for which the U.S. Census Bureau collects and tabulates decennial census data. Both census tracts and Block Numbering Areas provide the geographic framework for delineating block groups, assigning census block numbers, and tabulating data (U.S. Census Bureau 2000a). It is important to note that the unit of analysis for determining environmental justice communities by the federal criteria is the census tract; whereas for the determination by Massachusetts criteria, the unit of analysis is the census block.

10.4 Considering Environmental Justice in Specific Phases of the NEPA Process

Identification of health and environmental issues under NEPA, CEQ, and the Massachusetts EEA Environmental Justice Policy is accomplished through public involvement and the scoping process. Public involvement and scoping are implemented to ensure fair treatment and meaningful involvement of all people as defined by EPA on matters related to environmental justice.

10.4.1 Scoping

On January 9, 2004, NIH published in the *Federal Register* its Notice of Intent to prepare an EIS on the proposed Boston-NBL. Publication of that notice initiated the NIH scoping activities. On February 9,

2004, NIH published in the *Federal Register* a notice of a public scoping meeting and an extension of the comment period. A public scoping meeting was held at Faneuil Hall in Boston on Tuesday, February 17, 2004, from 7 p.m. to 10 p.m. Comments were provided during the extended public scoping period, which began January 9, 2004, and ended March 2, 2004.

10.4.2 Draft EIS

NIH filed a Draft EIS with EPA on October 15, 2004. On October 22, 2004, EPA published notice that the Draft EIS had been filed, was available for public review and comment, and that a public meeting was scheduled for November 10, 2004. The public meeting was held at Faneuil Hall in Boston on Wednesday, November 10, 2004, from 7 p.m. to 9 p.m. Comments were received during an extended 75-day public comment period, which began October 22, 2004, and ended January 3, 2005.

10.4.3 Supplemental Draft EIS

NIH filed a Supplemental Draft EIS with EPA on March 25, 2005. On April 1, 2005, EPA published notice that the Supplemental Draft EIS had been filed, was available for public review and comment, and that a public meeting was scheduled for April 25, 2005. The public meeting was held at Faneuil Hall in Boston on Monday, April 25, 2005, from 7 p.m. to 9 p.m. Comments were received during an extended 48-day public comment period, which began April 1, 2005, and ended May 18, 2005.

10.4.4 Final EIS

On the basis of comments NIH received from EPA on the Draft EIS, a description of the public outreach efforts to date was provided, and the area of analysis for environmental justice issues in the Final EIS was expanded to include a 1.6-km (one-mile) radius including all of the South End and portions of South Boston, Roxbury, Dorchester, Chinatown, Back Bay, and Kenmore/Fenway (NIH 2005). The Final EIS identifies the NEIDL project area, at the urban location, as an environmental justice area per Massachusetts EEA because its population on average is made up of more than 25 percent minorities (NIH 2005). The environmental justice analysis was performed at just the urban location in the Final EIS and determined that

- The area surrounding NEIDL is an environmental justice community on the basis of the percentage of minorities.
- Within the study area, 52 percent of the residents are minority.

- The median household income in the South End is greater than the median household income of the City of Boston and is close to the statewide average.
- No neighborhoods in the study area have a resident population 25 percent or higher that are foreign born.
- No neighborhoods in the study area have a resident population 25 percent or higher that are lacking in English language proficiency.

The Final EIS also concludes, "It is unlikely that the Proposed Action would have proportionately greater impact on the disadvantaged (e.g. minority) population than any other population in the area"(NIH 2005).

10.5 Additional Considerations for this RA

To address the public and state and federal courts' interest in the demographic and health information regarding the community surrounding the NEIDL and how it relates to environmental justice populations, apart from the demographic data, this RA considers the following.

10.5.1 Health Disparities among Populations Surrounding the Urban and Suburban Sites

Health disparities are differences in health outcomes and their determinants between segments of the population, as defined by social, demographic, environmental, and geographic attributes (Carter-Pokras and Baquet 2002; Truman, Smith et al. 2011). *Health inequalities* (often used interchangeably with disparities) is used in the scientific and economic literature to refer to summary measures of population health associated with individual- or group-specific attributes such as income, education, or race/ethnicity (Asada 2010). *Health inequities* are a subset of health inequalities that are modifiable, associated with social disadvantage, and considered ethically unfair (Braveman and Gruskin 2003).

On the basis of the public health practice, medical research and environmental justice literature, it is noted that there are differences in life expectancy, morbidity, risk factors, and quality of life among segments of the population by race/ethnicity, sex, education, income, geographic location, and disability status (Institute of Medicine (U.S.). Committee on Environmental Justice. and Health Sciences Policy Program (U.S.) 1999; Truman, Smith et al. 2011). For example, the infant mortality rates among black infants are more than double than that of whites (US Department of Health and Human Services 2011). Similarly, minorities experience higher rates of heart disease and diabetes, while they have lower rates of cancer screening and immunizations. Specific examples related to infectious diseases include a higher rate of

HIV/AIDS, syphilis, hepatitis, and tuberculosis among racial and ethnic minorities [reviewed in (US Department of Health and Human Services 2011)]. Those disparities are believed to be the results of the complex interaction among genetic variations, environmental factors, and specific health behaviors. The health disparities are noted in Massachusetts (Office of Health and Human Services (EOHHS) 2011).

10.5.2 Access to Healthcare at Urban and Suburban NEIDL Sites

The Massachusetts Health Care Insurance Reform Law of 2006 mandates that nearly every resident of Massachusetts obtain a state-government-regulated minimum level of healthcare insurance coverage and provides health insurance for residents earning less than 150 percent of the federal poverty level who are not eligible for Mass Health (Medicaid). The law also partially subsidizes health care insurance for those earning up to 300 percent of the federal poverty level. Coverage to almost all residents of Massachusetts is done through a combination of Medicaid expansions, subsidized private insurance programs, and insurance market reforms. Massachusetts residents must demonstrate on their annual tax returns that they have had health insurance meeting *minimum creditable coverage* standards during all months of the previous year, excluding any lapse in coverage of 63 days or less. If unable to do that, filers will face tax penalties as long as an affordable product is available to them. An individual may request an exemption from this requirement if he or she does not obtain coverage because of his or her religious beliefs. The Massachusetts Health Care Insurance Reform Law of 2006 applies to the populations at both the urban and suburban sites in Massachusetts. However, the law does not apply to populations at the rural site because it is in New Hampshire.

With regard to the urban site of NEIDL, a local resource for access to health care is the BMC. This tertiary level care medical facility is a teaching hospital and has nearly one million patient visits per year, more than half of those patients have an annual income at or below $20,420.00 (BMC 2008). About 70 percent of patients come from underserved populations including low-income families, elders, and people with disabilities, minorities and immigrants. The services provided to the patients include comprehensive range of care, inpatient, clinical and diagnostic services, and more than 70 specialties and subspecialties of medicine and surgery. BMC also is part of an integrated health care system that includes 15 community health centers in the Boston area and serves more than 280,000 people annually. Additionally, BMC Health Net Plan coordinates health coverage for Massachusetts residents with low to moderate incomes, serves more than 240,000 members and is the state's largest managed care organization for both MassHealth and Commonwealth care (BMC 2008).

Similarly, the environmental justice communities farther away from the suburban NEIDL site (in the 6- to 10-km radius away from the site) are in the urban community of Lowell, the fourth largest city of Massachusetts. There, access to medical care is available through two large facilities. One is the Lowell Community Health Center (http://www.lchealth.org/Fact-Sheet-2010.pdf), a nonprofit organization whose mission is to "to provide caring, quality and culturally competent health services to the people of Greater Lowell, regardless of their financial status; to reduce health disparities and enhance the health of the Greater Lowell community; and to empower each individual to maximize their overall well-being." That health center served nearly 35,000 local residents in 125,000 visits during 2009; 94 percent of the patients were noted to have incomes below the federal poverty level, and the majority were racial and ethnic minorities with limited English proficiency. The other large provider is Saints Medical Center (http://www.saintsmedicalcenter.com/Community/SaintsMedicalCenterCBreport2010.pdf), which is a nonprofit, full-service, 157-bed acute care community hospital that provided care to nearly 315,000 residents in 25 towns in the greater Lowell area. It had 6,500 inpatients and 281,000 outpatients during the fiscal year 2010.

10.5.3 High Containment Laboratories and Guidelines for Biosafety and Biocontainment

Regardless of location, U.S. high biocontainment laboratories follow guidelines for biosafety and biocontainment. *Biosafety in Microbiological and Biomedical Laboratories*, (BMBL), 5th ed., has become the code of practice for biosafety—the discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials. These principles are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility design and safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. A risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections. The BMBL is periodically updated to refine guidance according to new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health.

Thus, it is expected that NEIDL will be built and operated according to the highest standards of biosafety and biocontainment with administrative and engineering mitigative strategies in place to decrease risk of exposure to the community surrounding NEIDL, which includes environmental justice communities in the urban and suburban sites.

10.5.4 Lack of Occupational Exposure Limits for Biological Agents

An occupational exposure limit is an upper limit of an acceptable concentration of a hazardous substance for a material or class of material in the air at the workplace. Occupational exposure limits are set by legislation and are enforced by a governmental entity to protect the health and safety of the worker and the general public (29 CFR 1910.1000).

The Occupational Safety and Health Administration (OSHA) of the U.S. Department of Labor publishes and sets Permissible Exposure Limits (PELs) to protect workers against the health effects of exposure to hazardous substances. PELs are regulatory limits on the amount of concentration of a substance in the air, and they are enforceable (29 CFR 1910.1000). PELs do not include biological agents. Additionally, other industries such as the nuclear industry, have established occupational and public exposure standards that are set by a regulatory agency. The Nuclear Regulatory Commission, sets and enforces standards of exposure for protection to workers and the general public from ionizing radiation resulting from activities conducted under licenses issued by the Nuclear Regulatory Commission. These regulations are issued under the Atomic Energy Act of 1954, as amended, and the Energy Reorganization Act of 1974 as amended. Nuclear Regulatory Commission regulations under 10 CFR Part 20 subpart C addresses occupational radiation dose exposure limits, and subpart D addresses radiation dose limits for individual members of the public.

Unlike the chemical and nuclear industry, no governmental agency regulates or enforces worker or public exposure to biological agents. Nor is there legislation or a governmental entity that has set occupational or public exposure limit values for biological agents. These limits have not been set because of the essential difference between biological agents and other hazardous substances are their ability to reproduce/replicate. A small amount of a microorganism can grow considerably in a very short time under favorable conditions, therefore the value/level/volume of an organism does not remain constant (Gorny 2007). According to the Department of Biohazards, Institute of Occupational Medicine and Environmental Health, World Health Organization, there is a need for occupational exposure limits for biological agents, however in contrast to chemical or physical hazards for which such criteria already exist, for example the higher the concentration, the longer the time of exposure the more severe the health outcome, biohazards do not show a proportional answer of the human body to the exposure to the risk, which makes it very difficult to determine universally valid evaluation criteria (Gorny 2007).

Thus, without guidelines regarding occupational exposure limits for biological agents, this RA faces the challenge of determining the significance of a release of a pathogen as a result of loss of biocontainment

on the basis of the amount of release of pathogen only. The significance of the release, is thus, based on the consequences of the release of a pathogen, specifically in terms of infections and deaths due to the pathogen.

10.5.5 Public Outreach

Because of human health issues raised in the public, judicial review process, and to respond to findings from the NRC, a component of the RA is to perform an analysis and determine if the NEIDL's location will have a disproportionate impact on low-income and minority populations in the adjacent community, not just at the urban location as was performed in the EIS, but also at the suburban and rural sites. The BRP was established as a Working Group of the Advisory Committee to the Director (ACD) of NIH to guide its response to judicial requests and comments and concerns voiced by the local community, the NRC, and the general public regarding the construction and operation of the NEIDL (BRP 2008). With those objectives in mind, the BRP has engaged the Boston community from the inception of the RA in 2008 for purposes of gathering public input. All public input gathered has been compiled and considered into the environmental justice methodology for the RA.

Table 10-1 lists key public outreach activities, which have occurred throughout the development of this RA. The table includes meetings specifically aimed at informing the public and gaining public input, as well as working meeting that were webcast for public access.

1 **Table 10-1. NEIDL risk assessment: public involvement**

Date	Event	Purpose
March 13, 2008	BRP Meeting, Bethesda	Boston community members were invited and participated in discussions regarding the charge of the BRP, NIH, NEIDL, overview of the principles of environmental protection laws, and RA studies
May 2, 2008	BRP/NRC Meeting Bethesda	BRP recommendations regarding RA work plan
May 16, 2008	BRP Meeting, Boston	Boston community members attended and provided comments on proposed RA work plan recommended by the BRP
June 6, 2008	BRP Presentation to the Advisory Committee to the Director NIH	Briefing to Advisory Committee to the Director NIH regarding the proposed scope and analytic approach for supplementary RA
July 16, 2008	BRP Meeting, Bethesda	Boston community members were invited and participated in roundtable discussions with BRP members on environmental justice and how to effectively engage communities
October 14, 2008	BRP Meeting, Boston	Boston community members attended and provided comments on BRP questions related to community engagement and communication regarding the planning and oversight of biocontainment laboratories
December 5, 2008	BRP Presentation to the Advisory Committee to the Director NIH, Bethesda	Progress update regarding NEIDL RA was presented: Phase I; Interim status of NEIDL; Boston prohibition on use of recombinant DNA at BSL-4;Phase II Tasks; BRP community meetings; Principles and practices for public involvement and communications; Best practices for institutional review
April 7, 2009	BRP Teleconference with the National Research Council	Boston community members attended and provided comments on the RA study design, agents, scenarios and methodology
June 4, 2009	Advisory Committee to the Director (ACD) of NIH, Meeting, Bethesda	BRP Chair presented the community with engagement document(s) developed to address fundamental principles and best practices for public and local community relations and communications regarding a national research resource. Approved by the ACD
March 19, 2010	BRP/NRC Meeting Bethesda	NRC reviewed and provided comments on Tetra Tech's proposed approach to quantitative modeling
April 28, 2010	BRP Meeting, Boston	Boston community members attended and provided comments on proposed approach to quantitative modeling
September 22, 2010	BRP/NRC Meeting Bethesda	NRC reviewed and provided comments on preliminary results on initial and secondary infection rates
October 5, 2010	BRP Meeting, Boston	Boston community members attended and provided comments on initial and secondary infection rates

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All information cited in the above table is also available in greater detail on the BRP website at http://nihblueribbonpanel-bumc-neidl.od.nih.gov/meetings.asp.

10.6 Demographic Data

The source of offsite impacts considered for the NEIDL RA environmental justice analysis is the area within a 10-km (6-mile) radius from the center of the NEIDL at each of the three potential sites (urban, suburban and rural) of a potential release of any or all the 13 selected pathogens. A guideline of 1-km (0.6-mile) radius study area within city limits and a 2.4-km (4-mile) radius outside city limits is provided by the Nuclear Regulatory Commission as generally sufficient for assessing potential environmental justice impacts associated with activities other than nuclear power plants (NUREG 2003). For NEIDL, environmental justice data were collected for a radius of 10 km (6 miles) of each of the three sites, which is 10 times the city recommendation of the Nuclear Regulatory Commission. The larger radius is used to ensure that all potentially affected areas are considered. The population data are taken from the U.S. Census Bureau's 2000 decennial census for each tract within the 10-km (6-mile) area; that is the most recent year for which data are available at the census tract geographic level.

10.6.1 Urban Site Environmental Justice

10.6.1.1 Federal Criteria

Minority populations and poverty rates for each census tract in the Boston site's 10-km (6-mile) boundary area are listed in Table 10-2. Table 10-2 summarizes the totals within each 2-km (1.2 mile) radius boundary within the 10-km (6-mile) area. Of the 297 census tracts identified in the 10-km (6-mile) radius boundary, 152 (51percent) had a higher percentage of minority residents compared to the national average, and 156 of the census tracts (53 percent) had a higher percentage of persons living in poverty compared to the United States. As shown in Table 10-2, the portion of census tracts with a percentage of minority populations or a percentage of persons below poverty that is greater than the national average decreases going away from the interior 2-km (1.2-mile) circle of the Boston site

Table 10-2. Minority and low-income population data for the 10-km (6-mile) boundary area of the urban site

Boundary area radius	Total census tracts	Number of tracts with a minority population above the U.S. level of 31 percent	Percent of tracts with a minority population above the U.S. level of 31 percent	Number of tracts with a poverty level above the U.S. level of 12 percent poverty	Percent of tracts with a poverty level above the U.S. level of 12 percent poverty
Inside 2-km (1.2 mile)	32	24	75%	28	88%
2-4 km (1.2–2.4 mile)	62	40	65%	44	71%
4-6 km (2.4–3.6 mile)	68	42	62%	45	66%
6-8 km (3.6–4.8 mile)	64	28	44%	25	39%
8-10 km (4.8–6.0 mile)	71	18	25%	14	20%
Total for the 10-km (6-mile) area	297	152	51%	156	53%

Source: U.S. Census Bureau 2000

10.6.1.2 State Criteria

To assess environmental justice per the Massachusetts criteria, data were collected on minority populations, foreign-born populations, households lacking English language proficiency, and median annual household income for each neighborhood (defined as a census block group) within a 10-km (6-mile) area of the Boston site. Table 10-3 summarizes the totals within each 2-km (1.2-mile) radius boundary within the 10-km (6-mile) area. Of the 1,112 census block groups identified in the 10-km (6-mile) radius boundary, 622 block groups (56 percent) had a higher percentage of minority residents compared to the state's threshold of 25 percent; 474 census block groups (43 percent) had 25 percent or more residents who are foreign born; 78 block groups (7 percent) had households where 25 percent or more of the residents lacked English language proficiency; and 281 block groups (25 percent) had a median annual household income at or below 65 percent of the statewide median income of $50,500. As shown in Table 10-3, the portion of census block groups meeting one or more of the state environmental justice guidance criteria was, for the most part, higher within the boundaries closest to the Boston NEIDL site and decreases moving away from the site.

Table 10-3. Massachusetts criteria for environmental justice for the 10-km (6-mile) boundary area of the urban site

Boundary area radius	Total census block groups	Percent of block groups with a minority population above the state guidance level of 25 percent	Percent of block groups with a foreign born population above the state guidance level of 25 percent	Percent of block groups lacking English language proficiency above the state guidance level of 25 percent	Percent of block groups with a median annual household income at or below 65 percent of the Massachusetts median income
Inside 2 km (1.2 mile)	100	79%	35%	17%	55%
2-4 km (1.2–2.4 mile)	203	71%	44%	4%	39%
4-6 km (2.4–3.6 mile)	228	66%	57%	11%	27%
6-8 km (3.6–4.8 mile)	252	53%	48%	8%	17%
8-10 km 4.8–6.0 mile	329	35%	30%	2%	12%
Total for the 10-km (6-mile) area	1,112	56%	43%	7%	25%

Source: U.S. Census Bureau 2000

10.6.2 Suburban Site Environmental Justice

10.6.2.1 Federal Criteria

Minority populations and poverty rates for each census tract in the Tyngsborough 10-km (6-mile) boundary area are listed in Table 10-4.

Table 10-4 summarizes the minority and low-income population totals within each 2-km (1.2-mile) radius within the 10-km (6-mile) boundary area. Of the 85 census tracts identified in the 10-km (6-mile) boundary area, 19 (22 percent) had a higher percentage of minority residents compared to the United States, and 20 of the census tracts (24 percent) had a higher percentage of persons living in poverty compared to the United States. As shown in Table 10-4, the portion of census tracts having a percentage of minority populations, or a percentage of persons living below the poverty level, that is greater than the overall U.S. levels, is higher in the exterior circles (6-8 km [3.6–4.8 miles]) and (8–10km [4.8–6.0

miles]), away from the Tyngsborough site. Those tracts with the higher percentages of minority and low-income populations are all in the southeast quadrant of the 10-km (6-mile) circle, near the city of Lowell.

Table 10-4. Minority and low-income population data for the 10-km (6-mile) boundary area of the suburban site

Boundary area radius	Total census tracts	Number of tracts having a percentage of minority persons above the U.S. level of 31 percent	Percent of tracts with a minority population above the U.S. level of 31 percent	Number of tracts having a percentage of persons in poverty above the U.S. level of 12 percent	Percent of tracts with a percentage of persons in poverty above the U.S. level of 12 percent
Inside 2-km (1.2 mile)	4	0	0%	0	0%
2-4 km (1.2–2.4 mile)	6	0	0%	0	0%
4-6 km (2.4–3.6 mile)	16	1	6%	0	0%
3.6–4.8 mile (6–8 km)	29	11	38%	12	41%
4.8–6.0 mile (8–10 km)	30	7	23%	8	27%
Total for the 10-km (6-mile) area	85	19	22%	20	24%

Source: U.S. Census Bureau 2000

10.6.2.2 State Criteria

To assess environmental justice per the Massachusetts criteria, data were collected on minority populations, foreign-born populations, households lacking English language proficiency, and median annual household income for each neighborhood (defined as a census block group) within a 10-km (6-mile) area of the Tyngsborough site. Table 10-5 summarizes the totals within each 2-km (1.2-mile) radius boundary within the 10-km (6-mile) area. Of the 260 census block groups identified in the 10-km (6-mile) radius boundary around Tyngsborough, 72 block groups (28 percent) had a higher percentage of minority residents compared to the state's threshold of 25 percent; 32 block groups (12 percent) had 25 percent or more residents who are foreign born; 9 block groups (3 percent) had households where 25 percent or more of the residents lacked English language proficiency; and 31 block groups (12 percent) had a median annual household income at or below 65 percent of the statewide median income of $50,500. As shown in Table 10-5, the portion of census block groups meeting one or more of the state environmental justice guidance criteria is higher in the exterior circles (6–8 km [3.6–4.8 miles]) out to (8–10 km [4.8–6.0

miles]). Those census block groups are southeast of the proposed Tyngsborough site, near the city of Lowell.

Table 10-5. Massachusetts criteria for environmental justice for the 10-km (6-mile) boundary area of the suburban site

Boundary area radius	Total census block groups	Percent of block groups with a minority population above the state guidance level of 25 percent	Percent of block groups with a foreign born population above the state guidance level of 25 percent	Percent of block groups lacking English language proficiency above the state guidance level of 25 percent	Percent of block groups with a median annual household income at or below 65 percent of the Massachusetts median income
Inside 2km (1.2 mile)	15	0%	0%	0%	0%
2-4 km (1.2–2.4 mile)	21	5%	0%	0%	0%
4-6 km (2.4–3.6 mile)	48	15%	6%	0%	0%
6-8 km (3.6–4.8 mile)	82	46%	22%	5%	20%
8-10 km (4.8–6.0 mile)	94	28%	12%	5%	16%
Total for the 10-km (6 mile) area	260	28%	12%	3%	12%

Source: U.S. Census Bureau 2000

10.6.3 Rural Site Environmental Justice

10.6.3.1 Federal Criteria

Minority populations and poverty rates for each census tract in the Peterborough 10-km (6-mile) boundary area are listed in Table 10-6. Of the 21 census tracts identified in the 10-km (6-mile) area, none had a higher percentage of minority residents compared to the United States, and none of the census tracts had a higher percentage of persons living in poverty compared to the United States.

Table 10-6. Minority and low-income data for the 10-km (6-mile) boundary area of the rural site

Boundary area radius	Total census tracts	Number of tracts with a percentage of minority persons above the U.S. level of 31 percent	Percent of tracts with a minority population above the U.S. level of 31 percent	Number of tracts with a percentage of persons in poverty above the U.S. level of 12 percent	Percent of tracts with persons in poverty above the U.S. level of 12 percent
Inside 2 km (1.2 mile)	3	0	0%	0	0%
2-4 km (1.2–2.4 mile)	3	0	0%	0	0%
4-6 km (2.4–3.6 mile)	4	0	0%	0	0%
6-8 km (3.6–4.8 mile)	4	0	0%	0	0%
8-10 km (4.8–6.0 mile)	7	0	0%	0	0%
Total for the 10 km (6-mile)	21	0	0%	0	0%

Source: U.S. Census Bureau 2000

10.6.3.2 State Criteria

Although the rural site of Peterborough is in New Hampshire, environmental justice analysis per the Massachusetts criteria was conducted for the rural site for consistency and comparison with the urban and suburban sites. Data were collected on minority populations, foreign-born populations, households lacking English language proficiency, and median annual household income for each neighborhood (defined as a census block group) within a 10-km (6-mile) area of the Peterborough site. Table 3-7 summarizes the totals within each 2-km (1.2-mile) radius boundary within the 10-km (6-mile) area. Of the 62 census block groups identified in the 10-km (6-mile) radius boundary around the rural site, none of the block groups exceed any of the Massachusetts environmental justice criteria.

Table 10-7. Massachusetts criteria for environmental justice for the 10-km (6-mile) boundary area of the rural site

Boundary area radius	Total census block groups	Percent of block groups with a minority population above the state guidance level of 25 percent	Percent of block groups with a foreign born population above the state guidance level of 25 percent	Percent of block groups lacking English language proficiency above the state guidance level of 25 percent	Percent of block groups with a median annual household income at or below 65 percent of the Massachusetts median income
Inside 2 km (1.2 mile)	10	0%	0%	0%	0%
2-4km (1.2–2.4 mile)	10	0%	0%	0%	0%
4-6 km (2.4–3.6 mile)	11	0%	0%	0%	0%
6-8 km (3.6–4.8 mile)	11	0%	0%	0%	0%
8-10 km (4.8–6.0 mile)	20	0%	0%	0%	0%
Total for the 10-km (6-mile) area	62	0%	0%	0%	0%

Source: U.S. Census Bureau 2000

10.7 Results

Demographic Data

Environmental justice populations, as defined by the federal environmental justice criteria and the Commonwealth of Massachusetts EEA criteria, were identified at the urban and suburban sites. No environmental justice communities meeting these same criteria were identified at the rural site. At the urban site, populations that met or exceeded the criteria were located throughout the 10-km (6-mile) study area, with the higher percentage of neighborhoods meeting one or more of the criteria located nearer to the center of the study area (see Tables 10-2 and 10-3). At the suburban site, the environmental justice populations are located farther away from the NEIDL location (6–10 km or 3.6–6 miles]) and are associated with the more urbanized, higher-density community of Lowell (the fourth largest city in Massachusetts), southeast of Tyngsborough.

Health Disparities/Chronic Disease

Health disparities are differences in health outcomes and in the determinants between segments of a population, as defined by social, demographic, environmental, and geographic attributes (Carter-Pokras and Baquet 2002; Truman, Smith et al. 2011). The literature shows that health disparities are generally more evident within environmental justice communities; therefore, health disparities are assumed to be present within the identified environmental justice communities at both the urban and suburban sites. If

the health disparities were a result of a health *inequity* factor, such as access to health care, this can somewhat be mitigated at the urban and suburban sites by remedies of the Massachusetts Health Care Insurance Reform Law of 2006. The Law mandates that nearly every resident of Massachusetts obtain a state-government-regulated minimum level of healthcare insurance coverage. The Law also provides health insurance for residents earning less than 150% of the federal poverty level who are not eligible for Mass Health (Medicaid). Additionally, based on available statistics, Boston Medical Center is Boston's largest provider of healthcare accessible to all, regardless of status or ability to pay. The Massachusetts Health Care Insurance Reform Law does not apply to the NEIDL rural site since it is located in the State of New Hampshire. However, there are no environmental justice communities present within 10 km (6 miles) of the rural site..

As noted in section 10.5.1 above, racial and ethnic minorities have increased prevalence and patterns of chronic diseases. These chronic diseases have the potential to contribute to increased susceptibility to pathogens. Preliminary reports from the 2009 H1N1 influenza pandemic have suggested a disproportionate impact on racial and ethnic minorities (Kwan-Gett, Baer et al. 2009; Centers for Disease Control and Prevention 2010; Truelove, Chitnis et al. 2011; Uscher-Pines, Maurer et al. 2011; Wenger, Castrodale et al. 2011), indicating higher rates of hospitalization, morbidity, and mortality among racial/ethnic minorities. No clear reason was noted for the increased hospitalization and mortality, although it was postulated that underlying chronic diseases may have played a role. A detailed study based on survey responses of minorities during the 2009 influenza pandemic has also expressed concern of the disproportionate impact of the pandemic; however, the authors were unable to demonstrate an increased risk of susceptibility when controlled for socioeconomic status and demographics (Quinn, Kumar et al. 2011).

Appendix I provides a discussion on the susceptibility of specific medically vulnerable populations (i.e., the very young and the elderly, diabetics, those with HIV/AIDS, and pregnant women) to the 13 pathogens studied in this RA

Location of NEIDL

With regard to the operations of NEIDL and event sequences that could lead to loss of biocontainment (Chapter 7), this RA assumes no difference with respect to the siting of the laboratory.

Types of Exposure

There are two types of operational impacts to consider: (1) those that may directly expose people in environmental justice communities to a release of pathogen from the facility; and (2) those that may indirectly expose people environmental justice communities by secondary transmission of a pathogen. Each of these types of impacts is addressed separately.

Direct Exposure

Event sequences (e.g., earthquake) could potentially lead to a release of pathogen and directly expose members of the surrounding community at the urban site. This RA has estimated that a person at 1 km (0.6 mi) from the NEIDL facility would be exposed to a very low amount of pathogen (Chapter 7). Environmental justice communities at the urban site are present within a 2-km (1.2-mi) radius of the NEIDL site; therefore, it is assumed that members of these environmental justice communities are potentially at risk for being exposed to pathogens in the event of such a release. The risk of exposure is estimated to be similar for all persons in the area, regardless of their environmental justice status. Based on the results of this RA, in the event of a maximum reasonably foreseeable release earthquake, the public would receive an average exposure that is unlikely to cause infection, with the possible exception of RVFV, for which a release leading to 1–5 infections and 1 fatality at the urban site may occur with an average frequency of once in 10,000 to 1 million years (frequency category C). Infections from other pathogens are mostly estimated to occur with an average frequency less than once in one million years (frequency category D). Initial infections are estimated to be in frequency category D at the suburban and rural sites. The rural and suburban sites do not have environmental justice communities within the 1-km (1.2-mi) radius of exposure and, therefore, impact is not considered significant at either of these two sites.

Secondary Transmission

The risk of exposure from work done at NEIDL is not based on the location of the facility, but on the potential that an infected worker leaves the facility and potentially transmits the pathogen to social contacts. Close social contacts include family members and friends. Other important social contacts include those individuals, regardless of environmental justice status, that are encountered during routine commute to work, including those on mass transit. If there are secondary exposures due to an infected worker leaving the facility, the potential for exposure extends well beyond the 10-km (6-mi) radius used for the demographic study, and those at greatest risk will be the worker's social contacts. If multiple generations of transmission occur, then infections could occur later in the outbreak among people who are not direct contacts of the initially infected laboratory worker. There are no data to indicate that any social

contacts, regardless of race, ethnicity, or economic status are or are not more likely to become infected from an infected worker leaving NEIDL.

This RA considers event sequences that could potentially lead to secondary transmission of the pathogens in the local community. These secondary transmissions are postulated to occur after an *undetected or unreported* loss of biocontainment (i.e., exposure and initial infection from a primary-exposed laboratory worker). For these event sequences, it is to be noted that the potential for secondary transmission exists for pathogens such as *Yersinia pestis* (especially the pneumonic form of disease), 1918 H1N1 influenza virus, SARS-associated coronavirus, Andes virus, Ebola virus, Marburg virus, Lassa virus, Junín virus and Nipah virus.

For four of these pathogens, quantitative estimates were derived for their secondary transmission potential, which provide insight into how frequently infections might be expected to occur in the public. For example, a laboratory worker becoming infected with a transmissible pathogen via needlestick, who subsequently leaves the facility and infects at least one member of the public, is a sequence of events that is estimated to occur with a frequency between once in 500 years to once in 30,000 years. The frequency with which outbreaks resulting in various numbers of infections and fatalities among the public might be expected under this scenario is analyzed in detail in Chapter 9 and Appendix L. The extent to which local individuals (defined as people in the city of Boston, the town of Tyngsborough, and the towns of Peterborough and Hancock) would bear the risk estimated under secondary transmission scenarios is not obvious, due to the unpredictability of where transmissions would occur among people traveling in and out of the local area. Estimates of risk to local and non-local individuals (defined with respect to commuting data, see Appendix L) were generated for this RA, which are relevant in considering potential risk to environmental justice communities.

For the urban site, the estimated risk to local residents is relevant for the environmental justice analysis because environmental justice communities were identified within the city of Boston. Estimates (shown in Appendix L) for the frequency of 10 or more fatalities among Boston residents range from once in 5,300 years to once in more than 10 million years. For the frequency of 100 or more fatalities among Boston residents, estimates range from once in 50,000 years to once in more than 10 million years. It is also noted that outbreaks spreading outside of the city might pose a risk to any environmental justice communities in the surrounding area as well.

For the suburban site, no environmental justice communities were found in the town of Tyngsborough, Massachusetts. Nonetheless, as shown in Appendix L, the estimated effects of commuting into and out of Tyngsborough result in a substantial portion of the risk for large outbreaks being potentially borne by non-locals, a result which suggests that the environmental justice communities identified in nearby Lowell, Massachusetts, could be at risk during a potential outbreak started in Tyngsborough. However, the extent to which there might be a specific risk to residents of Lowell due to observed travel patterns in that area has not been assessed.

For the rural site, no environmental justice communities were found in the towns of Hancock and Peterborough or in towns within a 10-km (6-mi) radius of the site. However, as shown in Appendix L, the estimated effect of commuting into and out of the local area may result in a substantial portion of the risk for large outbreaks being potentially borne by non-locals, and it is possible that large outbreaks would extend beyond the 10-km (6-mi) radius applied in this environmental justice analysis. Therefore, the possibility that a large outbreak starting at the rural site would pose a risk to one or more environmental justice communities in a wider radius cannot be ruled out.

For secondary transmission, the analysis did not determine that people in close proximity to the NEIDL sites were at greater risk than people in the larger vicinity located farther away. Risk of exposure is estimated to be similar for all persons in the area, regardless of their environmental justice status.

Accessibility/Utilization

Addressing environmental justice for purposes of this RA and for the communities at the urban, suburban, and rural site is a very important yet complex topic. As a result, BU and NIH, based on EPA NEJAC guidance initiated a public process going back to January 9, 2004. This process goes beyond that which is regulatory required for public involvement under NEPA for the collection, assimilation, review of public concern, and available data as it pertains to *Executive Order 12898, Federal Actions to Address Environmental Justice in Minority and Low-Income Populations*. In addition, CEQ, DOE and NRC NEPA Guidance were also used to inform this analysis.

The available literature recognizes that large proportions of EJ communities suffer from health disparities(e.g life expectancy, morbidity, risk factors, quality of life). The literature also shows that environmental justice communities most often are comprised of individuals that have lack of access to health services. The analysis for this RA therefore considered factors and available data regarding environmental justice communities and access to services in the state of Massachusetts, keeping in mind

that equal accessibility to health services and medical care is not necessarily the same as equal utilization of health services and medical care. The findings are unique for the State of Massachusetts as this State has a very different health care system then most states in the Union. Massachusetts has a state regulated health care insurance coverage system which was implemented in 2006. Health care insurance coverage for people above 100% poverty level and up to 300% of the poverty level began in February 2007. Additionally, BUMC, a tertiary level care facility, has one million patient visits each year. Annually, one half, or 500,000, of the patients seeking care at BUMC have an annual income below $20,000.00 a year, and 70% of the patients come from underserved populations (BMC, 2008).

Medical Vulnerability

As discussed above in section 10.5.1, there are disparities in life expectancy, morbidity, risk factors, and quality of life among segments of the population by race/ethnicity, sex, education, income, geographic location, and disability status. Those disparities are believed to be the results of the complex interaction among genetic variations, environmental factors, and specific health behaviors. Furthermore, those who are medically compromised among EJ communities are likely to be at higher risk of infection and experience adverse outcomes as a result of those infections. There are reports of higher rates of infectious diseases such as HIV/AIDS, syphilis, hepatitis, and tuberculosis among racial and ethnic minorities. It is postulated in the literature that underlying chronic disease may be a contributing factor of the disproportionate impact of an infectious disease pandemic on racial and ethnic minorities. Published reports based on the experience with the recent 2009 H1N1 influenza pandemic have begun to address these issues. Thus, health disparities along with chronic diseases have the potential to contribute to increased susceptibility to any of the pathogens being studied in this RA. These disparities also have the potential to contribute to differences in how communities respond to exposure and infection with any of the pathogens. There are, however, limited published data on this topic.

Specific sub- populations such as children under 5 years of age, adults over 65 years of age, those with diabetes, those with HIV/AIDS, and pregnant women are likely to exhibit increased susceptibility/vulnerability in terms of increased morbidity and mortality to the pathogens addressed in this RA. With available data, to further address the impact of the effect of pathogens on these sub-populations, a separate analysis was performed to address this concern and is presented in Appendix I and Chapters 8 and 9.

10.8 References

Asada, Y. (2010). "A summary measure of health inequalities for a pay-for-population health performance system." Preventing chronic disease **7**(4): A72.

Braveman, P. and S. Gruskin (2003). "Defining equity in health." Journal of epidemiology and community health **57**(4): 254-258.

Carter-Pokras, O. and C. Baquet (2002). "What is a "health disparity"?" Public health reports **117**(5): 426-434.

Centers for Disease Control and Prevention. (2010). "Information on 2009 H1N1 impact by Race and Ethnicity (Historical Archive) from February 24, 2010." Retrieved September 21, 2011, 2011, from http://www.cdc.gov/h1n1flu/race_ethnicity_qa.htm.

BMC (Boston Medical Center). 2008. *What Makes BMC Special*. Boston Medical Center, Boston, MA.

BRP (Blue Ribbon Panel). 2008. *NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratories at Boston University Medical Center Finding and Recommendations Part I: Risk Assessment Briefing of the Advisory Committee to the Director.* Blue Ribbon Panel.

CEQ (Council on Environmental Quality). 1997. *Environmental Justice Guidance Under the National Environmental Policy Act*. Council on Environmental Quality, Executive Office of the President, Washington, DC.

Institute of Medicine (U.S.). Committee on Environmental Justice. and Health Sciences Policy Program (U.S.) (1999). Toward environmental justice : research, education, and health policy needs. Washington, D.C., National Academy Press.

Kwan-Gett, T. S., A. Baer, et al. (2009). "Spring 2009 H1N1 influenza outbreak in King County, Washington." Disaster medicine and public health preparedness **3 Suppl 2**: S109-116.

Office of Health and Human Services (EOHHS). (2011). "Race and Ethnicity Reports." Retrieved August 26, 2011, 2011, from http://www.mass.gov/Eeohhs2/docs/dph/research_epi/disparity_report.pdf.

Gorny, R. 2007. *Biological agents: Need for Occupational Exposure Limits (OELs) and feasibility of OEL setting.* Institute of Occupational Medicine and Environmental Health, Department of Biohazards, WHO Collaborating Centre, Sosnowiec, Poland.

Massachusetts EEA (Executive Office of Environmental Affairs). 2002. *Environmental Justice Policy of the Executive Office of Environmental Affairs.* Massachusetts Executive Office of Environmental Affairs, MA.

NIH (National Institutes of Health). 2005. *Final Environmental Impact Statement, National Emerging Infectious Diseases Laboratories, Boston, MA*. National Institutes of Health.

NUREG (Nuclear Regulatory Commission). 2003. *Environmental Review Guidance for Licensing Actions Associated with NMSS Programs* (NUREG-1748). <http://www.nrc.gov/reading-rm/doc-collections/nuregs/staff/sr1748/>. Accessed April 2011.

Quinn, S. C., S. Kumar, et al. (2011). "Racial disparities in exposure, susceptibility, and access to health care in the US H1N1 influenza pandemic." American journal of public health **101**(2): 285-293.

Truelove, S. A., A. S. Chitnis, et al. (2011). "Comparison of patients hospitalized with pandemic 2009 influenza A (H1N1) virus infection during the first two pandemic waves in Wisconsin." The Journal of Infectious Diseases **203**(6): 828-837.

Truman, B. I., K. C. Smith, et al. (2011). "Rationale for regular reporting on health disparities and inequalities - United States." MMWR. Surveillance summaries : Morbidity and mortality weekly report. Surveillance summaries / CDC **60 Suppl**: 3-10.

U.S. Census Bureau. 2000. *Census 2000 Summary Files 1 and 3*. <http://factfinder.census.gov/servlet/DatasetMainPageServlet?_program=DEC&_submenuId=&_lang=en&_ts=>.Accessed January 2009.

U.S. Census Bureau. 2000a. Census Tracts and Block Numbering Areas. <http://www.census.gov/geo/www/cen_tract.html>. Accessed July 2011.

U.S. Census Bureau. 2001. *Poverty in the United States: 2000*. <http://www.census.gov/prod/2001pubs/p60-214.pdf>. Accessed April 2011.

U.S. Census Bureau. 2010a. *Historical Poverty Tables – People: Table 1. Weighted Average Poverty Thresholds for Families of Specified Sizes*. <http://www.census.gov/hhes/www/poverty/data/historical/people.html>. Accessed April 2011.

U.S. Census Bureau. 2010b. *USA QuickFacts*. <http://quickfacts.census.gov/qfd/states/00000.html>. Accessed April 2011.

U.S. Census Bureau. 2011. *Overview of Race and Hispanic Origin: 2010*. <http://www.census.gov/prod/cen2010/briefs/c2010br-02.pdf>. Accessed April 2011.

USDHHS (U.S. Department of Health and Human Services). 2011. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>. Accessed August 2011

US Department of Health and Human Services. (2011). "Eliminating Racial & Ethnic Health Disparities." Retrieved August 26, 2011, 2011, from http://www.cdc.gov/omhd/About/disparities.htm.

Uscher-Pines, L., J. Maurer, et al. (2011). "Racial and ethnic disparities in uptake and location of vaccination for 2009-H1N1 and seasonal influenza." American journal of public health **101**(7): 1252-1255.

Wenger, J. D., L. J. Castrodale, et al. (2011). "2009 Pandemic influenza A H1N1 in Alaska: temporal and geographic characteristics of spread and increased risk of hospitalization among Alaska Native and Asian/Pacific Islander people." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **52 Suppl 1**: S189-197.

Contents

Chapter Highlights: This chapter summarizes and integrates the information from the RA, starting with the initiating events, then initial exposures, and finally the potential for secondary transmission of pathogens if loss of biocontainment occurs. This chapter also draws overall conclusions that are supported by these results.

- Potential events were identified that could lead to loss of biocontainment at NEIDL.
- Risks to laboratory workers are from events that result in sufficient exposure to pathogens to cause initial infections in the exposed individual; these are represented by centrifuge release and needlestick injury. The risk varies among pathogens and events.
- Risks to the public are from events that either directly expose the public to pathogens such as earthquakes leading to loss of biocontainment or from undetected and unreported initial infections in laboratory workers from a centrifuge release or needlestick injury event. The risk varies among pathogens.
- For direct exposure events such as an earthquake, there are potential differences (in risk to the public among the urban, suburban, and rural sites, but this risk is almost totally beyond reasonably foreseeable.
- For secondary transmission of pathogens in the public from an undetected and unreported centrifuge release or needlestick event, there are potential differences in risk to the public among different sites, but the estimated differences are small compared to the overall uncertainty.

11. RISK CHARACTERIZATION

11.1 Introduction

The NRC guidance for analyzing risks associated with operating NEIDL suggested that future RA studies be organized around the following three domains (NRC 2008):

1. What could go wrong?
   a. Scenarios of release of an infectious agent (pathogen)
   b. Agents (pathogens) to consider for RA
2. What are the probabilities?
3. What would be the consequences?

In seeking an understanding of the risks of operating NEIDL, federal and Massachusetts courts, the NRC, and the NIH BRP have expressed interest in three related topics:

1. What are the risks to the workers at NEIDL?
2. What are the risks to the public in the communities surrounding NEIDL?
3. Are there differences in risks if NEIDL were located in a less-densely populated site?

This chapter on risk characterization summarizes the RA performed and applies each of the three NRC questions to the three related topics.

What is risk characterization?

"Risk characterization is the summarizing step of risk assessment. The risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative and useful for decision makers." (USEPA 2000)

11.2 Approach

11.2.1 Overview

This RA follows guidelines established by federal agencies for conducting and reporting RAs (USEPA 2000) and has been performed by using available scientific data and established methods of analyses. The RA acknowledges the uncertainty associated with the data and the appropriate role of judgment (expert opinion) in estimating key parameters required for RA.

The four essential principles of RA are applied as follows to this RA and are described in the preceding chapters and associated appendices:

1. Transparency: This is achieved by providing details of the assessment approach applied at each step of the analyses; stating the assumptions used for the analyses and the basis for those assumptions; addressing data gaps and the methods used to overcome the data gaps such as expert judgment; the uncertainties in the available data, qualitative discussions, and quantitative assessments of the impact of the uncertainties in the data and sensitivity analyses to determine impact of variability in key parameters.

2. Clarity: This is achieved by attempting to convey details with brevity; providing lay language summaries and discussions in chapters; providing details in appendices and using tables and graphs where possible to present technical data.

3. Consistency: This is achieved by following established guidance and guidelines, following precedence wherever possible and using established and published methods for all analyses.

4. Reasonableness: The RA is based on best available scientific information, uses generally accepted scientific knowledge and guidance and has been subjected to review by the BRP and NRC. Furthermore this RA strives to include reasonableness and realism in the analyses based on *real-world experience*; however, the limited operational data poses a significant challenge in that regard, and, in several cases, the event sequence assumptions are expected to overestimate the likelihood or consequences of potential events. Such a use of conservative assumptions (i.e., overestimations) to account for uncertainty is consistent with NEPA accident analysis guidance (DOE 2002).

This RA follows the four-step paradigm outlined by EPA for health RAs (USEPA 2000) and includes hazard identification (pathogens and events), dose-response assessment, exposure assessment, and risk characterization.

11.2.2 Selection of Pathogens for Analysis

To address the NRC question of which agents to consider for RA (Question 1b above), 13 pathogens were selected for analysis on the basis of guidance from NIH, the BRP, and discussion of emerging infections (NIH 2009). The pathogens differ in key characteristics such as their ability to be spread from person to person (transmissibility), the method by which they are spread from one person to the next (either directly or via vectors, such as insects), their ability to cause human disease (pathogenicity) and their ability to cause deaths among those infected (case fatality rate). The details of the pathogens are provided in Chapter 3 and Appendix C (Pathogen Characteristics). The pathogens are classified as requiring BSL-3 or BSL-4 biocontainment precautions and are analyzed separately for this RA (Table 11-1).

Table 11-1. Pathogens selected for analysis

Pathogen	Abbreviation
BSL-3	
1. *Bacillus anthracis* (either BSL-2 or BSL-3)	*B. anthracis*
2. *Francisellatularensis*	*F. tularensis*
3. *Yersiniapestis*	*Y. pestis*
4. 1918 H1N1 influenza virus	1918 H1N1V
5. SARS-associated coronavirus	SARS-CoV
6. Rift Valley fever virus	RVFV
7. Andes virus (either BSL-3 or BSL-4[a])	ANDV
BSL-4	
8. Ebola virus	EBOV
9. Marburg virus	MARV
10. Lassa virus	LASV
11. Junín virus	JUNV
12. Tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus)	TBEV-FE
13. Nipah virus	NIPV

a. BSL-4 is required when infecting rodent species permissive for (susceptible to) chronic infection.

11.2.3 Event Sequence Analyses

In addressing possible scenarios for the release of a pathogen from NEIDL as a result of a loss of biocontainment (NRC Question 1a above), the following analyses were performed:

- Selection of events for analysis: This RA considers a broad spectrum of reasonably foreseeable[1] events and generally truncates the analysis at events with a frequency of less than 1 in 10 million years. More than 300 incidents described as occurring at other comparable biocontainment facilities and postulated events with relevance to NEIDL were considered. Many of the 300 incidents were similar, so they were consolidated into about 30 candidate events for further consideration. Many of the 30 candidate events are bounded by (i.e., have a frequency and consequence that is less than) other candidate events. A set of events was selected for analyses that were considered to be the highest risk events, those that provide broad coverage of the routes

[1]Reasonably foreseeable events include events that could have catastrophic consequences, even if their probability of occurrence is low, provided that the analysis of the impacts is supported by credible scientific evidence, is not based on pure conjecture, and is within the rule of reason (DOE 2002).

of exposure and potentially exposed groups. From those candidate events, centrifuge aerosol release, needlestick, earthquake, transportation accident, malevolent acts (as discussed in the threat assessment), and environmental persistence (including escaped animals) were selected for detailed analyses. Those are discussed in Chapter 4 and Appendices D and E (Event Sequence Analysis); Chapter 5 (Transportation Analysis); Chapter 6 (Threat Assessment for malevolent acts); and Chapter 7 (Potential for Released Pathogens to Become Established in the Environment).

- Analysis of event sequences: For each event selected for detailed analysis, scenarios were developed that account for NEIDL-specific operations on the basis of NEIDL equipment and SOPs. The frequency, number of people potentially exposed, and the extent of exposure were estimated for each scenario. Relevant operational experience from similar laboratories was used to the extent possible, and conservative analyses (i.e., use of assumptions that tend to overestimate the frequency or consequences or both) were used to account for variability and uncertainty where data are lacking. Chapter 4 and Appendix F provide details of those analyses.

Operating Experience at BSL-3 and BSL-4 Laboratories: This RA relies on past experience at similar laboratories to the extent data are available and useful. Appendix D summarizes various sources of this operating experience and includes the recent CDC report of 395 potential release events and 7 laboratories associated infections (LAIs) from 2003 to 2009 nationwide at laboratories working with select agents (NRC 2011). The CDC report shows the LAI rate has decreased by more than an order of magnitude over the past decades due to enhanced practices, equipment, and facilities (see Section D.1.1 of Appendix D).

The operating experience was used to identify potential initiating events, develop scenarios, and estimate the scenario frequencies. While helpful for qualitative analyses, the data were not used for quantitative analyses as details of appropriate measures of operating time (e.g., researcher-hours), descriptions of individual incidents leading to loss of biocontainment and biosafety protocols in place at the time of the events are not specified in available reports.

Therefore, past BSL-3 and BSL-4 experience was used to support the RA wherever appropriate, but they are not suitable for quantitative use.

NEIDL safety programs: An overview of NEIDL operations is provided in Chapter 2. Aspects particularly relevant to these analyses include the sharps safety practices (see Section F.7.2.1) and the Culture of Safety Enhancement Program (see Section 2.1 of Chapter 2). The effectiveness of these efforts cannot be fully predicted and incorporated into this analysis, so actual risk may be lower than predicted.

11.2.4 Health Effects Analyses

To address the NRC questions about probabilities and consequences of the loss of biocontainment and the three topics of interest such as risks to workers and the public and differences in risk at an alternate site, the following analyses were performed:

- Qualitative assessments were performed for all 13 pathogens in terms of their characteristics, review of the available scientific literature on their biology, transmission, human disease potential, natural outbreaks, morbidity and mortality (Chapter 3 and Appendix C).

- Quantitative assessments were performed for all 13 pathogens in terms of assessing their dose-response relationships and estimates of initial infections and fatalities in those exposed directly to the pathogens as a result of loss of biocontainment.
    - Estimation of dose-response relationships: On the basis of published evidence from exposures and infections of humans and animals and input from an expert consultation, dose-response relationships were estimated for each of the 13 pathogens. Dose-response relationships describe the likelihood that infection would occur at the levels of exposure estimated for each scenario. Chapter 8 and Appendix J provide details of those analyses.
    - Estimation of initial infections and fatalities: The risk of initial infections and fatalities was estimated for workers and the public under each scenario. Estimates are provided for each event-pathogen pair as appropriate based on BSL status. Chapter 8 and Appendix K provide details of those analyses.

- Estimates of secondary transmission in the community: The potential for secondary transmission was assessed qualitatively for each of the 13 pathogens. To supplement that assessment, secondary transmission estimates were developed for four pathogens for which sufficient epidemiological data and published mathematical models were available (*Y. pestis,* 1918 H1N1V, SARS-CoV and EBOV). Estimates are provided of the risk of secondary infections and fatalities in the community following each relevant event for the four pathogens. Chapter 9 and Appendix L provide details of those analyses.

11.3 Measures of Likelihood

The likelihood of events can be described and calculated in several ways. Table 11-2 compares several equivalent ways of describing (using numbers and measures) the likelihood of events. Because uncertainties are associated with many of the events analyzed, the likelihoods are presented as a range of values (as opposed to a single point estimate).

Table 11-2. Measures of likelihood

Average return period[a] (years)	Average frequency[b] (per year)	Probability/chance of occurrence in facility lifetime[c] (in 50 years)	
1	1	Virtually 100%	Virtually 100-in-100
10	0.1	99%	99-in-100
100	0.01	39%	1-in-2.5
1,000	0.001	4.9%	1-in-21
10,000	0.0001	0.5%	1-in-200
100,000	0.00001	0.05%	1-in-2,000
1,000,000	0.000001	0.005%	1-in-20,000
10,000,000	0.0000001	0.0005%	1-in-200,000

[a] **Average return period in years**: This is the average time, in years, before the event would be expected to occur. If the event was to occur multiple times, this would be the average time between occurrences. This way of describing events is often used in characterizing flood levels; for example, a *1,000-year flood* is a water level that is estimated to occur with a 1,000-year average return period or *1 per 1,000 years*.

[b] **Average frequency per year:** This is the average number of occurrences of the event per year.

[c] **Probability/chance of occurrence in facility lifetime (50 years)**: This is the chance that the event would occur at least once in a 50-year period.

In addition to the numeric values, frequency categories that encompass a range of numeric values are also used to facilitate comparison of results. Table 11-3 identifies the frequency categories (i.e., A, B, C, and D) used in this RA and provides the corresponding average return period.

Table 11-3. Frequency categories used in this RA

Frequency category	Average return period (1 in "this many" years)
A	1 to 100 years
B	100 to 10,000 years
C	10,000 to 1 million years
D	>1 million years

11.4 Summary of Events Analyzed

Two types of potential primary exposure events were analyzed: laboratory associated infections (LAIs) and direct-to-public exposure events. The LAI events analyzed for this RA are centrifuge aerosol releases and needlesticks. As infected workers have the potential for secondary transmission to the public and public risk is a primary focus of this RA, the LAIs analyzed in this RA are assumed to be undetected or

unreported events. Severe earthquake events are direct to public exposure events. Chapters 4, 8, and 9 report the analyses of the LAIs and direct exposure events. Those events are summarized below in this section and the numeric results and conclusions drawn from the results are presented in subsequent sections.

11.4.1 Centrifuge Aerosol Release

NEIDL operations will include use of centrifuges to separate materials. Because of the high centrifugal forces associated with centrifugation, pathogenic aerosols can be generated during centrifugation. A number of preventive and mitigative measures protect the laboratory worker from potential centrifuge aerosol releases, including the following:

- NEIDL protocol requires that both aerosol-tight containers and aerosol-tight rotors/cups be used to contain potential aerosols.
- BSL-3 laboratory workers are required to wear a PAPR at all times, and BSL-4 laboratory workers are required to use one-piece totally encapsulating positive pressure suits at all times.
- Vaccines are offered to all BSL-3 and BSL-4 laboratory workers when such vaccines are available for the pathogens on which they are working.
- If an incident occurs that has the potential to expose a worker to a pathogen, NEIDL procedures and training require that the event be reported and that appropriate medical interventions be implemented. Laboratory workers would be treated and quarantined if such action is deemed appropriate.

Because a primary focus of the RA is risk to the public, the RA focused primarily on scenarios that involve a failure to detect or report the potential exposure because those scenarios have greater potential to expose the public to secondary transmission of the pathogens. Section 4.2.2 of Chapter 4 provides details of the centrifuge release events, which are summarized below.

Exposure frequency—It is conservatively estimated that an undetected or unreported BSL-3 centrifuge aerosol release event is in frequency category A (one in1 to 100 years) with all PAPRs providing full respiratory protection. However, even with PAPRs performing at their full efficiency, laboratory workers can be exposed to low concentrations (i.e., 0.1 percent of the airborne concentration) of the infectious aerosols in the room. The potential that a worker's PAPR provides only partial respiratory protection was also included in the analyses. To account for the possibility that a PAPR does not provide its full respiratory protection, it was assumed that there is a 1 percent chance that one worker's PAPR would operate with only 1 percent of its normal filtering efficiency (i.e., removes only 90 percent of the particles), which means the worker using the PAPR would receive a 100 times greater exposure.(Note:

the results of Appendix K show that this potential reduction in PAPR effectiveness would not occur frequently enough to significantly affect overall worker risk.)

No credible mechanisms were found for a positive pressure suit failure that results in exposure from an undetected or unreported centrifuge aerosol release, so BSL-4 centrifuge scenarios were not analyzed further.

Laboratory worker infections and fatalities—Based on information from the dose-response estimates combined with the known quantities of pathogens that would be present in the laboratory, it was determined that the amount of exposure estimated from a centrifuge release could result in infection of at least one laboratory worker from any of the BSL-3 pathogens. Some pathogen exposures are estimated to be much more likely than others to result in an infection because: (1) some pathogens are present in greater concentrations so the aerosol release results in greater worker exposures, and (2) some pathogens cause infection at lower levels of exposure than other pathogens. Estimates for the risk of laboratory worker infections and fatalities are summarized in Table 11-4a, with further details provided in Chapter 8.

Public infections and fatalities—A laboratory worker who is infected from a centrifuge release and does not detect or report the exposure could leave the laboratory and pose a risk to the public through secondary transmission. Of the BSL-3 pathogens, *Y. pestis* (pneumonic plague), 1918H1N1V, and SARS-CoV pose the highest risk for secondary transmission. Sufficient data from past outbreaks involving those three pathogens were available in the published literature and used to generate quantitative estimates of transmission (secondary transmission modeling), which are summarized in Section 11.5.2. A centrifuge release involving BSL-4 pathogens is not considered to pose a risk to the public because no credible scenario was found in which an aerosol exposure of a laboratory worker would go undetected under BSL-4 laboratory conditions.

11.4.2 Needlestick

NEIDL operations would include use of syringes and other sharp objects that have the potential to puncture the skin of laboratory workers and expose them to pathogens. A needlestick event (i.e., a potential exposure due to a puncture from the needle of a syringe) was analyzed as a surrogate for all other puncture events. A number of preventive and mitigative measures protect the laboratory worker from sharp objects like needles, including the following:

- NEIDL procedures and training on the use of sharp objects reduces the likelihood a skin puncturing event (see Section F.7.2.1 of Appendix F).
- Personal protective equipment (e.g., use of stainless steel mesh gloves when changing knife blades) protects the worker.
- Vaccinations and procedures and training for reporting and medical interventions reduce the likelihood of infection if a potential exposure does occur.
- The NEIDL culture of safety enhancement program (see Section 2.1 of Chapter 2) is intended to reduce the likelihood of mishaps and increase reporting.

As with the centrifuge events, the RA focused primarily on scenarios that involve a failure to detect or report the potential exposure because those scenarios have the potential to expose the public to secondary transmission of the pathogens. Section 4.2.3 of Chapter 4 provides details of the needlestick events, which are summarized below.

Exposure frequency—As with the centrifuge event, the only needlestick events that were analyzed in detail are those that involve a failure to detect or report the potential exposure. An undetected or unreported needlestick would affect only one worker and is estimated to be in frequency category B (one in 100 to 10,000 years). This frequency category assignment is appropriate and even conservative based on: (1) the historic rates indicate that the frequency is on the border of categories A and B, (2) historic values likely overstate the value for current facilities due to enhanced practices, equipment, and facilities (see Section D.1.1 of Appendix D), and (3) the NEIDL is expected to have lower incident rates due to its attention to sharps safety (see Section F.7.2.1 of Appendix F) and the enhancement of safety (see Section 2.1 of Chapter 2). Section F.7.3.2 of Appendix F provides additional details. A needlestick in a BSL-4 laboratory is estimated to be as likely as a needlestick in a BSL-3 laboratory.

Worker infections and fatalities—For each of the13 pathogens, it was conservatively assumed that all needlestick exposures would result in a laboratory worker infection; therefore, an infection is in the frequency category B (one in 100 to 10,000 years). Those results, along with estimates of laboratory worker fatalities, are displayed in Table 11-4b and described in further detail in Chapter 8.

Public infections and fatalities—A laboratory worker who is infected from a needlestick and does not detect or report the exposure may leave the laboratory and pose a risk to the public through secondary transmission. Of the BSL-3 pathogens, *Y. pestis* (pneumonic plague), 1918H1N1V, and SARS-CoV pose

the highest risk for secondary transmission. EBOV represents the highest transmission risk among BSL-4 pathogens. Sufficient data from past outbreaks and mathematical models for those four pathogens were available in the published literature and used to generate quantitative estimates of transmission, which are summarized in Section 11.6.2.

11.4.3 Earthquake

Two severe earthquake events beyond the design basis were analyzed, (1) an earthquake that does not result in structural failure but does partially reduce the filtration of a potential release and (2) the maximum reasonably foreseeable (MRF)event, which is defined as the event with the largest potential pathogen release. The MRF earthquake assumes a loss of all biocontainment features and a ground-level release (effectively a full collapse) that has the potential to expose the public to pathogenic aerosols. The less severe earthquake was analyzed to put the MRF earthquake into perspective, and it shows that public infections or fatalities would be extremely unlikely (occurring with an average frequency much less than 1 in 10 million years); therefore, the more severe, MRF earthquake is the only event addressed further in this section. Section 4.2.4 of Chapter 2 provides details of the earthquake events, which are summarized below.

Exposure frequency—The NEIDL structure was designed to withstand an earthquake with a peak acceleration of 0.12g (g is the acceleration of gravity), per the requirements of the Massachusetts Building Code. The fundamental period of the NEIDL structure is 2 seconds and seismic shaking at the fundamental period has the potential of causing the greatest damage. Based on the U.S. Geological Survey (USGS) seismic hazard maps, the annual exceedance probability for a 2-second 0.12 earthquake is estimated to be 1×10^{-5} (see Attachment E of Appendix F), which corresponds to frequency category C (1 in 10,000 to 1 million years). The MRF earthquake is assigned to frequency category C, but this is considered conservative because a significantly more severe, hence less likely, earthquake would be required to result in a total collapse of the NEIDL structure.

Airborne dispersion calculations for the MRF earthquake show that individual members of the public beyond the NEIDL exclusion fence (i.e., at least 30 m from the facility) would receive an average of less than one unit of any pathogen. Exposures from the release were analyzed for a radius of 1 km from the point of release. The exposure level is pathogen-specific because the maximum working volume and suspension concentration differs for each pathogen.

Public infections and fatalities—Estimates from the dose-response assessment were applied to the exposure and population estimates to calculate the likelihood of infections and fatalities occurring in the public from direct exposure. Of all pathogens, the highest risk was estimated for RVFV, for which a release leading to one to five infections and one fatality at the urban site could occur with average frequency more than 1 in 1 million years, but less than 1 in 10,000 years (category C). Infections could occur from other pathogens as a result of a release, but most of the associated frequency estimates fall in frequency category D (less than 1 in one million years).

11.4.4 Aircraft Crash

The potential for direct public exposure as a result of an aircraft accidentally crashing into the NEIDL was also evaluated (see Section 4.2.5 of Chapter 2). It was concluded that the frequency and consequences of the aircraft crash are no greater than and likely less than the frequency and consequences associated with the MRF earthquake. Therefore, an accidental aircraft crash was not analyzed in detail.

11.4.5 Transportation Analysis

Pathogen samples will be shipped both to and from NEIDL throughout its operating lifetime. Those samples will be packaged and shipped in accordance with the U.S. Department of Transportation (DOT) Hazardous Material Regulations (49 CFR Parts 171-180). The DOT regulations require triple-packaging of infectious substances. NEIDL policy goes beyond the DOT regulations and requires that the DOT-compliant triple packaging be placed in a large, foam-lined plastic case for an added layer of safety. The over-pack will be secured to the bed away from the exterior walls of an exclusive use (i.e., no other cargo) large truck. While some shipments will be mixed mode (i.e., air and ground), all shipments will arrive or depart from NEIDL in trucks (Murphy 2011). The analysis (Chapter 5) considered crash-related injuries, crash-related fatalities, and pathogen releases due to truck and aircraft crashes.

11.4.6 Threat Assessment (Malevolent Acts)

The TA (Chapter 6) evaluates the potential of 11 malevolent scenarios, but it does not include a quantitative estimate of the frequency or consequences of these scenarios. DOE NEPA Guidance (DOE 2002) acknowledges the difficulty of analyzing malevolent acts and suggests that the consequences could be compared to consequences of severe accidents because the forces resulting in releases of hazardous materials could be similar. An evaluation determined that the consequences of the MRF earthquake are equal to or exceed the consequences of malevolent acts that result in pathogen releases from the facility.

11.4.7 Potential For Released Pathogens To Become Established In The Environment

On the basis of the available evidence, it is concluded that five of the pathogens that are likely to be studied at the NEIDL, namely, *F. tularensis*, *Y. pestis*, 1918 H1N1V, RVFV, and TBEV-FE, have potential, at least theoretically, to become established in the environments in the vicinity of the three proposed NEIDL sites (see Tables 8-1 and 8-2 of Chapter 8). The means by which that might occur involve animals (including arthropods) that could be present in those environments. On the basis of the available data, it is concluded that one of those pathogens, *F. tularensis*, might already be present in some areas in the vicinity of proposed NEIDL sites. Also, it is noted that although released *B. anthracis* spores have potential for long term persistence in soil under particular environmental conditions (microenvironments), the evidence indicates that these conditions do not exist in New England. As a result, there is no indication that released spores could cause *B. anthracis* to become established in the local environments (see Section 7.3.1 of Chapter 7 for details).

11.4.8 Environmental Justice

The environmental justice analysis (see Chapter 10) addresses Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations,* and the Massachusetts EEA Environmental Justice Policy. The environmental justice analysis addressed the question of operational impacts of NEIDL resulting in disproportionately high and adverse human health effects on minority and low-income populations at the urban, suburban, or rural sites. This analysis examined two types of impacts: (1) those that could directly expose environmental justice communities as a result of a release of pathogen from the facility, and (2) those that expose people in environmental justice communities indirectly through secondary transmission of pathogens.

11.5 Risk to Workers

This section focuses on the events that pose the greatest risk to workers, i.e., centrifuge release and needlestick events. Those events could also pose risks to the public; the public risk from the events is addressed in Section 11.6.

11.5.1 What Could Go Wrong?

Equipment failures and personnel errors can result in worker exposures to infectious pathogens, but biocontainment features used in NEIDL reduce the likelihood and extent of exposure. The CDC and NIH have published *Biosafety in Microbiological and Biomedical Laboratories* (referred to as BMBL) (CDC

and NIH 2007), which provides biosafety practice and policy recommendations. Protocols identified by BU go beyond the recommendations of BMBL and provide additional protection of workers and the public. A few of the BSL-3 and BSL-4 preventive and mitigative features to be used at NEIDL are:

- BSCs used for all activities involving open pathogen containers
- Aerosol seals on rotors/cups and containers
- Use of non-breakable labware wherever possible
- PPE requirements that include double gloves, gowns, shoe covers, boots, mesh gloves when changing blades, hooded PAPRs for all BSL-3 activities, and positive pressure suits for BSL-4 activities.
- Vaccinations when available
- Procedures and training to report all incidents and medical response in the event of incidents

Even with the implementation of those protocols, there remains risk of worker exposures to BSL-3 or BSL-4 pathogens. Numerous equipment failures and personnel errors have the potential of causing a partial loss of biocontainment, more than 30 of which were considered for analysis in this RA (see Chapter 7).This RA focused on two of the larger risks to the laboratory worker, namely, centrifuge release and needlesticks. Because this RA concentrates primarily on the risk to the public, only centrifuge release and needlestick events that go undetected or unreported were analyzed because they have the potential to expose the public to pathogens via infected laboratory workers.

11.5.2 What are the Frequencies and Consequences?

Experience at other BSL-3 and BSL-4 laboratories has shown that laboratory workers may be exposed to pathogens and that LAIs are a real possibility. The following sections provide a comparison by event and by pathogen.

Centrifuge release and needlestick events were analyzed in this RA because they are two of the more common incidents in laboratories, and they involve different routes of exposure. Tables 11-4a and 11-4b

Operating experience:

- BSL-3 or equivalent laboratories NIH intramural (1982-2003):
  - ~3.2 million worker hours
  - 1 symptomatic infection
  - 4 asymptomatic infections
- BSL-4 laboratories worldwide (1972-2009):
  - ~0.69 million worker hours
  - 0 infections
  - The rate of laboratory-associated infections have decreased by more than an order of magnitude over the past decades.

Details and references are found in Tables D-1 and D-4, and Section D.1.1 of Appendix D.

show the frequency of laboratory worker infections and fatalities resulting from centrifuge release and needlestick events, both of which are assumed to be undetected or unreported. The number of potential worker infections from a needlestick event is limited to one worker, while the potential exists for multiple infections from a centrifuge release occurring in a room with multiple laboratory workers. Tables 11-4a and 11-4b provide a comparison of the two events in a format similar to *Consumer Reports* tables. Each of the 13 pathogens is listed in the left column and the two sets of columns present the frequency category for the number of infections and fatalities specified in the lower header row. Table 11-4a provides the results of the analyses for the centrifuge release event and Table 11-4b provides the results for the analyses for the needlestick event.

(Note: There is a range of potential consequences associated with each event (e.g., 0, 1, 2, 3, 4, or more laboratory worker infections) and Table 11-4 presents the results for each level of consequence. Because there is variability and uncertainty associated with the estimates, there is a range of frequencies for each level of consequence and these estimates may cover more than one frequency category. In order to simplify the presentation of results in Table 11-4 and similar tables, the frequency is assigned on the basis of the highest category that contains more than 25 percent of the range of results.)

Table 11-4a. Risk of laboratory worker infections and fatalities as a result of a centrifuge release event

	Laboratory worker infections					Laboratory worker fatalities				
Pathogen	**1**	**2**	**3**	**4**	**≥ 5**	**1**	**2**	**3**	**4**	**≥ 5**
BSL-3										
B. anthracis	○	·	·	·	·	○	·	·	·	·
F. tularensis	⊙	⊙	○	·	·	⊙	·	·	·	·
Y. pestis	○	·	·	·	·	·	·	·	·	·
1918 H1N1V	⊙	○	·	·	·	○	·	·	·	·
SARS-CoV	○	·	·	·	·	○	·	·	·	·
RVFV	●	⊙	⊙	○	·	⊙	○	·	·	·
ANDV	○	·	·	·	·	○	·	·	·	·
BSL-4										
EBOV	·	·	·	·	·	·	·	·	·	·
MARV	·	·	·	·	·	·	·	·	·	·
LASV	·	·	·	·	·	·	·	·	·	·
JUNV	·	·	·	·	·	·	·	·	·	·
TBEV-FE	·	·	·	·	·	·	·	·	·	·
NIPV	·	·	·	·	·	·	·	·	·	·

Frequency categories: ● = A (1 in 1 to 100 years) ⊙ = B (1 in 100 to10,000 years)
○ = C (1 in 10,000 to 1,000,000 years) · = D (1 in >1 million years)

Table 11-4b. Risk of laboratory worker infections and fatalities as a result of a needlestick event

Pathogen	Infections		Fatalities	
	1	≥ 2	1	≥ 2
BSL-3				
B. anthracis	⦿	×	⦿	×
F. tularensis	⦿	×	O	×
Y. pestis	⦿	×	O	×
1918 H1N1V	⦿	×	O	×
SARS-CoV	⦿	×	O	×
RVFV	⦿	×	O	×
ANDV	⦿	×	⦿	×
BSL-4				
EBOV	⦿	×	⦿	×
MARV	⦿	×	⦿	×
LASV	⦿	×	O	×
JUNV	⦿	×	O	×
TBEV-FE	⦿	×	⦿	×
NIPV	⦿	×	⦿	×

Frequency categories:
● = A (1 in 1 to 100 years)
⦿ = B (1 in 100 to 10,000 years)
O = C (1 in 10,000 to 1,000,000 years)
· = D (1 in >1 million years)
× = not applicable (a needlestick can infect only one worker at a time)

The results in Tables 11-4a and b suggest that the risk of a single infection in a laboratory worker as a result of a needlestick is greater than the risk of a single infection from a centrifuge release for most pathogens; however, this is largely because of a simplifying assumption that all needlestick events result in infections. In reality, it is known that not all needlestick events occurring in laboratories have resulted in infection, but the simplifying assumption was made because (a) accidental needlestick exposure data are not available for the 13 pathogens and (b) the extent of exposure from a needlestick is highly variable and difficult to quantify for a dose-response analysis. Use of a less conservative assumption could result in a reduction in the frequency of needlestick infections and fatalities for NEIDL and alter this comparison.

As shown in Table 11-4a for a centrifuge release, RVFV poses the greatest risk to the laboratory worker because its concentration in the work stock is greater than the concentration of other pathogens, which result in greater exposures, and the dose-response analysis for RVFV suggests that the amount of those exposures could be highly infective.

11.6 Risks to the Public

11.6.1 What Could Go Wrong?

There are two potential ways that NEIDL operations can expose the public to pathogens:

- Indirect public exposure: Undetected or unreported LAIs, such as the centrifuge release and needlestick events analyzed in this RA, have the potential to expose the public indirectly via secondary transmission of pathogen from an infected laboratory worker.

- Direct exposure: Pathogen releases from the facility, such as those that might result from an earthquake, can directly expose the public to pathogens.

Both the indirect and the direct exposures were considered. The needlestick is used as the basis for indirect public risk because it includes both BSL-3 and BSL-4 pathogens, and the estimated likelihood of an infection is higher than for the centrifuge event for the most transmissible pathogens. The MRF earthquake is used as the basis for direct public risk because it is an extremely severe event that includes the loss of all biocontainment features and it results in the maximum credible direct exposure levels to pathogens.

There are multiple layers of biocontainment that limit the likelihood of indirect and direct exposure. The first layer is the standard operating protocols of NEIDL that advocate safe laboratory practices and other mitigative features such as offering FDA-approved vaccinations to laboratory workers. Biocontainment features include use of sealed unbreakable containers; use of biological safety cabinets; seismic design and construction that exceeds requirements; and a ventilation system with high-efficiency air particulate (HEPA) filtration on the discharge (single filtration for BSL-3 and double for BSL-4). In order for either indirect or direct public exposures to result, failure of biocontainment features must occur.

11.6.2 What are the Frequencies and Consequences?

Operating Experience on Secondary Transmission from LAIs:

Operating experience from five facilities shows that LAIs from BSL-4 pathogens have not been a threat to the public. No LAIs involving BSL-4 pathogens have been reported from these facilities. (Johnson 2009)

LAI from BSL-3 pathogens that have resulted in secondary transmission to the public include one LAI that resulted in seven infections with SARS-associated corona virus in China (one of which was fatal) (World Health Organization 2004). Experience at the CDC between 1947 and 1973 showed that, for 109 LAIs diagnosed among its personnel, no secondary infections occurred among family or community members (Centers for Disease Control and Prevention 1999). The National Animal Disease Center (Ames, Iowa) similarly found no secondary infections associated with 18 LAIs at their institution between 1960 and 1975.

Recent published reviews of LAIs linked to BSL-3 or BSL-4 laboratory operations reported no infections in the community at large since those of the 2004 SARS incident in China (Kimman, Smit, and Klein 2008; Harding 2006) (Johnson 2009)

The public risk of infections and fatalities due to the needlestick and MRF earthquake are presented in Tables 11-6a and 11-6b. The risk of public infections and fatalities from a needlestick has been analyzed for four of the pathogens (*Y. pestis*, 1918H1N1V, SARS-CoV, EBOV) for which there are sufficient epidemiological data and published secondary transmission models available, with results summarized in Table 11-6a. In that table, the frequency of one or more public infections or fatalities is placed in category B (1 in 100 to 10,000 years) for all four pathogens. Outbreaks leading to 100 or more fatalities are placed in frequency category C (1 in 10,000 to 1 million years) for 1918 H1N1V and SARS-CoV and in frequency category D (1 in more than 1 million years) for *Y. pestis* and EBOV. 1918 H1N1V and SARS-CoV pose a higher risk of large outbreaks because they are able to spread more quickly and easily from person-to-person and can cause outbreaks that are more difficult to control than do the other pathogens.

For the earthquake event, RVFV is the only pathogen placed in category C (1 in 10,000 to 1 million years) in Table 11-6b for the frequency of one or more infections or fatalities. RVFV poses the greatest risk because its concentration in the work stock is greater than the concentration of other pathogens, which result in greater exposure estimates, and the dose-response analysis for RVFV suggests that those exposure amounts could be infective to a large enough portion of individuals to cause one or more infections.

_Y. pestis_transmission estimates – historical context

Information from outbreaks of pneumonic plague (caused by *Y. pestis*) that have been documented in the 20th century was used as the basis for the quantitative transmission estimates in this RA. The outbreaks occurred in various countries (including the United States) and ranged from 3–42 cases per outbreak. Those observations are reflected in the results for this RA, which show that an outbreak of 100 or more total cases would be much less likely than outbreaks with lower numbers of total infections. The reason that larger outbreaks are less feasible is that, in most cases, standard control measures were observed to be highly effective in ending transmission once hospitals and communities were aware of the outbreak. For more details about the historical outbreaks, see Chapter 9 and Appendix L.

Table 11-6a.Risk of public infections and fatalities as a result of an undetected or unreported initial infection in a laboratory worker following a needlestick event

Pathogen	Public infections					Public fatalities				
	≥ 1	≥ 10	≥ 100	≥ 1,000	≥ 10,000	≥ 1	≥ 10	≥ 100	≥ 1,000	≥ 10,000
BSL-3										
B. anthracis										
F. tularensis										
Y. pestis	⊙	○	·	·	·	⊙	○	·	·	·
1918 H1N1V	⊙	⊙	⊙	○	·	⊙	○	○	·	·
SARS-CoV	⊙	○	○	○	·	⊙	○	○	·	·
RVFV										
ANDV										
BSL-4										
EBOV	⊙	○	·	·	·	⊙	○	·	·	·
MARV										
LASV										
JUNV										
TBEV-FE										
NIPV										

Frequency categories: ● = A (1 in 1 to 100 years) ⊙ = B (1 in 100 to10,000 years)
○ = C (1 in 10,000 to 1,000,000 years) · = D (1 in >1 million years)
Shaded cells indicate that quantitative transmission estimates were not performed

Table 11-6b. Risk of initial infections and fatalities in the public as a result of direct exposure from the MRF earthquake event

Pathogen	Public infections					Public fatalities				
	≥ 1	≥ 10	≥ 100	≥ 1,000	≥ 10,000	≥ 1	≥ 10	≥ 100	≥ 1,000	≥ 10,000
BSL-3										
B. anthracis	·	·	·	·	·	·	·	·	·	·
F. tularensis	·	·	·	·	·	·	·	·	·	·
Y. pestis	·	·	·	·	·	·	·	·	·	·
1918 H1N1V	·	·	·	·	·	·	·	·	·	·
SARS-CoV	·	·	·	·	·	·	·	·	·	·
RVFV	○	·	·	·	·	○	·	·	·	·
ANDV	·	·	·	·	·	·	·	·	·	·
BSL-4										
EBOV	·	·	·	·	·	·	·	·	·	·
MARV	·	·	·	·	·	·	·	·	·	·
LASV	·	·	·	·	·	·	·	·	·	·
JUNV	·	·	·	·	·	·	·	·	·	·
TBEV-FE	·	·	·	·	·	·	·	·	·	·
NIPV	·	·	·	·	·	·	·	·	·	·

Frequency categories: ● = A (1 in 1 to 100 years) ⊙ = B (1 in 100 to10,000 years)
○ = C (1 in 10,000 to 1,000,000 years) · = D (1 in >1 million years)

1918 H1N1V transmission estimates – historical context

Transmission estimates for 1918 H1N1V were generated using information from both the 1918–1919 influenza pandemic, which was caused by the 1918 H1N1V strain, and the 2009 H1N1 influenza pandemic, which provides insights into how influenza pandemics unfold in the modern world. Studies of the pandemics reveal that influenza can spread very rapidly in a population partly because the generation interval, or time between the onset of symptoms of successive cases, is thought to be relatively short (possibly two days or less) compared to other pathogens. That makes transmission difficult to control through strategies such as isolation of infected patients, because it is difficult to isolate patients fast enough before they transmit to others. Those observations explain why the results for this RA show that the frequency for very large outbreaks involving 1918 H1N1V are potentially higher than for the other pathogens. On the other hand, the average number of transmissions from a single infected case that occurred in the 1918 and 2009 pandemics is thought to be relatively low, possibly less than two. That means that there could be a good chance that a small outbreak would die out by random chance, or that control measures that encourage social distancing would have a good chance of bringing a local outbreak under control before it grows large. That possibility is also reflected in the results for this RA, which shows that there could be a very low frequency for outbreaks resulting in 10 fatalities in the public.

Tables 11-6a and 11-6b demonstrate that the public risk is greater from the indirect route of exposure via an undetected or unreported initial infection resulting from a needlestick event when compared to the direct exposure due to an earthquake. The earthquake results in fewer infections because of the small quantities of pathogen in the laboratory, the limited potential for release of that inventory, and the dilution of any release in the atmosphere.

The uncertainty associated with the needlestick results are shown in Table 11-6c and 11-6d, which provide the range of results for each outcome (95 percent of estimates fall within each range). The results demonstrate that the risk of one or more infections among the public resulting from an undetected and unreported initial infection in a laboratory worker following a needlestick event is in the range of 1 in 510 to 1 in 27,000 years for the four pathogens analyzed. The risk decreases further for 10 or more infections. The risk for outbreaks greater than 100 infections among the public are also presented. A similar analysis of fatalities among the public as a result of the pathogens is presented in Table 11-6d.

Table 11-6c. 95% estimate ranges from uncertainty analysis, of the risk of infections among the public resulting from an undetected and unreported initial infection in a laboratory worker following a needlestick event

Number of infections among the public	BSL-3 Pathogens			BSL-4 Pathogens
	Y. pestis	1918 H1N1V	SARS-CoV	EBOV
≥1	1 in 510 to 18,000 years	1 in 550 to 16,000 years	1 in 760 to 27,000 years	1 in 550 to 18,000 years
≥10	1 in 1,500 to 740,000 years	1 in 980 to 43,000 years	1 in 1,100 to 59,000 years	1 in 1,900 to 76,000 years
≥100	1 in 130,000 to >10 million years	1 in 1,400 to >10 million years	1 in 2,500 to 440,000 years	1 in 110,000 to >10 million years
≥1,000	1 in >10 million years	1 in 4,300 to >10 million years	1 in 23,000 to >10 million years	1 in >10 million years
≥10,000	1 in >10 million years	1 in 8,300 to >10 million years	1 in 67,000 to >10 million years	1 in >10 million years
≥100,000	1 in >10 million years	1 in 23,000 to >10 million years	1 in 260,000 to >10 million years	1 in >10 million years

Table 11-6d. 95% estimate ranges from uncertainty analysis, of the risk of fatalities among the public resulting from an undetected and unreported initial infection in a laboratory worker following a needlestick event

Number of Fatalities Among the Public	BSL-3 Pathogens			BSL-4 Pathogens
	Y. pestis	1918 H1N1V	SARS-CoV	EBOV
≥1	1 in 560 to 38,000 years	1 in 1,100 to 70,000 years	1 in 1,100 to 47,000 years	1 in 610 to 20,000 years
≥10	1 in 6,500 to >10 million years	1 in 2,700 to >10 million years	1 in 2,500 to 350,000 years	1 in 3,100 to 240,000 years
≥100	1 in 5.8 million to >10 million years	1 in 5,800 to >10 million years	1 in 23,000 to >10 million years	1 in 420,000 to >10 million years
≥1,000	1 in >10 million years	1 in 23,000 to >10 million years	1 in 67,000 to >10 million years	1 in >10 million years
≥10,000	1 in >10 million years	1 in 23,000 to >10 million years	1 in 260,000 to >10 million years	1 in >10 million years

EBOV transmission estimates—historical context

The EBOV transmission estimates are based on information from outbreaks that have occurred in Africa. As discussed in Chapter 9, the setting and population in which these outbreaks occurred are in many ways quite different than the United States, and some have suggested that the extent of transmission seen in Africa can be explained by local cultural practices that amplified transmission. However, others have concluded that these locally specific practices accounted for only a small portion of transmission and that many transmissions occurred within families and the community. Given the lack of evidence to suggest otherwise, it was assumed that transmission estimates derived from the Africa outbreaks would be applicable to a potential outbreak in the United States.

At least 14 outbreaks have occurred in Africa since 1976, which ranged from a total of 12–425 cases per outbreak. Most (9 of 14) outbreaks involved fewer than 100 cases. That range of outcomes is reflected in the results for this RA, which show that greater than 100 infections are possible following a single laboratory worker infection, although smaller numbers of infections are more likely. The reason that EBOV outbreaks of more than 100 cases are rarer and more than 1,000 cases have not occurred is that standard, hospital-level control measures were effective once put in place.

SARS-CoV transmission estimates: historical context

Information from outbreaks of SARS that have occurred since it first emerged in 2002 was incorporated into the quantitative estimates shown here. Observations of transmission patterns that occurred revealed that infected cases transmitted to about two to four others, on average, during the early stages of outbreaks before public health control measures were implemented. However, the variation in the number of transmissions that occurred from case-to-case was wider than what has been observed for other transmissible pathogens. More than half of people infected with SARS-CoV did not transmit infection to anyone, while a small portion of cases transmitted to large numbers of others in isolated *superspreading* events. That variation can partly explain why some cities (Hong Kong, Singapore, Toronto) experienced explosive outbreaks of SARS in 2003, while other places (such as the United States) did not, even though infected cases traveled there several times. For more details, see Chapter 9 and Appendix L.

Those observations have important implications for considering possible consequences of a single infected laboratory worker entering a community. There has been one observed case of an infected laboratory worker in China causing seven secondary infections and one death. However, given the variation in transmission described above, much different consequences could occur under similar circumstances—from no transmission at all, to a large number of infections and fatalities. The entire worldwide outbreak of SARS in 2002–2003 resulted in about 8,000 infections and 800 deaths. For the analysis in this RA, extreme outcomes on par with those numbers or higher were observed only if it was assumed that control measures would remain relatively ineffective for a long period. If control measure effectiveness was on par with what was observed in most locations in 2003, those extreme consequences were found to be much less likely.

11.7 Site Differences

11.7.1 What Are the Differences in What Could Go Wrong?

There are differences among the urban, suburban and rural sites; those consist of the following:

- Differences in population densities and characteristics.
- Natural phenomena hazards (such as earthquakes) could have different frequencies and severities among the three sites; however, there are no hazards unique to one site.
- Meteorological conditions differ among the three sites; however, that affects only the concentration of potential aerosol from potential pathogen releases.
- The environs surrounding the three sites could affect the potential for environmental persistence of pathogens if there was a release of pathogens to the environment.

Those factors contribute to differences among the three sites; however, those and other factors do not affect the operations of NEIDL. Because it is assumed that the facilities, operations and potential events are independent of the site, there are no discernable differences in what can go wrong among the three sites.

11.7.2 What Are the Differences in Frequencies and Consequences?

The sites were compared on the basis of the risk of infections and fatalities in the general public, medically vulnerable subpopulations (MVSP), and environmental justice communities. In addition, potential differences among the sites were considered for transportation, malevolent acts, and environmental persistence. The potential differences are addressed in the following subsections.

11.7.3 General Public

There are differences among the three sites, including differences in population, meteorological conditions, and commuting patterns. While some of the differences might be large (e.g., differences in population density), they do not necessarily translate to large differences in the risk to the general public. Appendix L provides a comparison of the estimated likelihood of infections and fatalities resulting from secondary transmission among the public at the three sites as a result of an undetected or unreported initial infection in a laboratory worker. The estimated likelihoods for a given total number of infections or fatalities are generally slightly greater at the urban site than at the suburban and rural sites because of the following assumptions.

- It is assumed that residents in the towns adjacent to the suburban and rural sites have about 15 percent and 50 percent fewer contacts on average, respectively, than residents near the urban site, which results in correspondingly fewer numbers of transmissions. Those assumptions are derived from data-based estimates from each local area.

- The populations at the suburban and rural sites are much lower than urban site population. These lower populations are a limit on the maximum size of outbreaks among locals at the suburban and rural sites.

Despite the above differences, the differences in secondary transmission results across the three sites are not substantial, as uncertainty ranges overlap considerably. This is the case because not all infected

individuals are likely to be local residents. Commuting data were used to estimate the portion of transmissions that would occur among individuals who are nonresidents, and for those individuals, the above assumptions are no longer valid. Therefore, a significant portion of infections and fatalities could occur outside the local area, so local population differences have a diminished effect on overall differences among the three sites.

A similar comparison was performed for fatalities among the public as a result of exposure and infections resulting from a direct exposure of pathogen to the public following an MRF earthquake event. The results of the uncertainty analysis indicate that the risk of one or more fatalities from RVFV as a result of the event is higher at the urban site (1 in 340,000 years to 1 in greater than 10 million years) as compared to the suburban and rural sites (1 in greater than 10 million years for all estimates across the uncertainty range). That is because of the higher population density at the urban site. For this event, RVFV is chosen as the representative pathogen because it was shown to pose a greater direct exposure risk to the public than the other 12 pathogens.

11.7.4 Medically Vulnerable Subpopulations

This RA considered five categories of MVSP; those are those under 5 years of age; those over 65 years of age; those with diabetes, those with HIV/AIDS, and pregnant women. Local, regional and national data sources were used to estimate the proportion of each category of MVSP among the populations at the three sites (Appendix I). There are differences among the proportions of the different MVSP among the three sites. Differences in vulnerability among MVSP to the 13 pathogens were estimated from the literature and from expert opinion (Appendix H).

The risk of infections and fatalities from secondary spread of SARS-CoV among members of MVSP at the three sites resulting from an undetected and unreported initial infection in a laboratory worker following a needlestick event was used to assess the potential risk to MVSP. SARS-CoV was chosen as the representative pathogen because that pathogen was considered to pose a relatively high risk to the public as a result of the event. First, the overall MVSP profile across all five categories at each site was assessed to estimate an overall population susceptibility adjustment as compared to a typical U.S. population. It was estimated that the overall population susceptibility to SARS-CoV at each site was not substantially different than a typical U.S. population. In addition, the risk of infections and fatalities to each individual MVSP was assessed, and again it was found that the differences in risk were not substantially different across the sites. For full details, see Appendix L.

11.7.5 Environmental Justice

The goals of the environmental justice (EJ) analysis (see Chapter 10) are to address Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations,* and the Massachusetts EEA Environmental Justice Policy; identify potential environmental justice areas at the urban, suburban and rural sites and assess the effects of exposure to pathogens on environmental justice communities. The exposure scenarios are a direct release of pathogen from the facility resulting in exposure of members of EJ communities to that pathogen and spread of pathogen through EJ communities as a result of secondary person to person transmission.

Demographic Data

The area within a 10-km (6-mile) radius from the center of the NEIDL at each of the three potential sites (urban, suburban and rural) is considered the area of off-site impacts. Using US Census data, EJ communities were identified near the urban site. At the suburban site, EJ communities were identified within 10 km but not within 4 km of the suburban site. No environmental justice communities are present within 10 km of the rural site.

Effects of Exposure to Pathogen on EJ Communities

Pathogens exert an effect at the individual level. This RA recognizes, through available literature that a large proportion of EJ communities suffer from health disparities. Differences in life expectancy, morbidity, risk factors, and quality of life are noted among segments of the population by race/ethnicity, sex, education, income, geographic location, and disability status. Those disparities are believed to be the results of the complex interaction among genetic variations, environmental factors, and specific health behaviors. Furthermore, those who are medically compromised among EJ communities are likely to be at higher risk of infection and experience adverse outcomes as a result of those infections. Health disparities along with chronic diseases have the potential to contribute to increased susceptibility to any of the pathogens being studied in this RA. Published reports based on the experience with the recent 2009 H1N1 influenza pandemic have begun to address these issues. There are, however, limited published data on this topic with respect to the pathogens addressed in this RA. Similarly, there are limited data to enable a detailed quantitative analysis on the spread of infections based on contact networks among individuals in EJ communities.

At the community level, several factors affect the response to pathogen exposure. Health disparities have the potential to contribute to differences in how communities respond to exposure and infection with any

of the pathogens. Environmental justice communities most often are comprised of individuals that have lack of access to services. With regard to access to medical care, the findings are unique for the State of Massachusetts as this State has a very different health care system then most states in the Union. The Massachusetts Health Care Insurance Reform Law of 2006 established a state regulated health care insurance coverage system. The law mandates that nearly every resident of Massachusetts obtain a state-government-regulated minimum level of healthcare insurance coverage. It is important to acknowledge that equal accessibility to health services and medical care is not necessarily the same as equal utilization of health services and medical care.

Direct Exposure to Pathogen and EJ Communities

Event sequences such as earthquakes could lead to release of pathogen from NEIDL and directly expose members of the surrounding community. At the urban site, this would result in exposure of EJ communities that are in the vicinity of NEIDL.

If an MRF earthquake occurred, members of the public adjacent to the facility within a 1 km radius would receive an average exposure that is unlikely to cause infection for 12 of the 13 pathogens. The possible exception is RVFV, for which a release leading to one to five infections and one fatality at the *urban site* is estimated to occur with an average frequency of once in 10,000 to 1 million years (category C). Infections with other pathogens are mostly estimated to occur with an average frequency less than once in one million years (category D). Initial infections from all 13 pathogens are estimated to be in the D frequency category at the *suburban and rural sites*. Since no environmental justice communities are present at the rural and suburban site within the 1-km radius of exposure, the impact is *not* considered *significant* to environmental justice communities at either of those sites.

Secondary Exposure to Pathogen and EJ Communities

The risk of secondary exposure to pathogen from work done at NEIDL is based on the scenario that an infected worker leaves the facility and potentially transmits the pathogen to social contacts. If there are secondary exposures due to an infected worker leaving the facility, initially, those at greatest risk will be the worker's close social contacts. It is to be noted that the workers and their contacts may or may not live in the communities in closest proximity to the NEIDL location. In situations where secondary exposures continue, several persons could be infected one after the other. For example, the lab worker could infect person A, who in turn infects person B, who in turn infects person C and so on; such that after a certain period of time, people getting infected are no longer close social contacts of the original lab worker.

An analysis of the extent to which local individuals at the three NEIDL sites would bear the risk of secondary exposure was estimated using commuting data from the US Census Bureau.

For the urban site, the estimated risk to local residents is relevant for the environmental justice analysis as EJ communities have been identified within the city of Boston. Depending on the pathogen being considered, estimates for the frequency of 10 or more fatalities among Boston residents range from once in 5,300 years to once in more than 10 million years. For the frequency of 100 or more fatalities among Boston residents, estimates range from once in 50,000 years to once in more than 10 million years. It is also noted that outbreaks spreading outside of the city might pose a risk to environmental justice communities in the surrounding area as well.

For the suburban site, no environmental justice communities were found in the town of Tyngsborough, Massachusetts. Nonetheless, the estimated effects of commuting into and out of Tyngsborough result in a substantial portion of the risk for large outbreaks being potentially borne by non-locals, a result which suggests that the environmental justice communities identified in nearby Lowell, Massachusetts, could be at risk during a potential outbreak started in Tyngsborough. However, the extent to which there might be a specific risk to residents of Lowell due to observed travel patterns in that area has not been assessed.

For the rural site, no environmental justice communities were found in the towns of Hancock and Peterborough or in towns within a 10-km (6-mi) radius of the site. The estimated effect of commuting into and out of the local area may result in a substantial portion of the risk for large outbreaks being potentially borne by non-locals, and it is possible that large outbreaks would extend beyond the 10-km (6-mi) radius applied in this environmental justice analysis. Therefore, the possibility that a large outbreak starting at the rural site would pose a risk to one or more environmental justice communities in a wider radius cannot be ruled out.

11.7.6 Transportation

The transportation analysis estimates the likelihood of truck crash-related injuries, truck crash-related fatalities, pathogen releases due to truck crashes, and pathogen releases due to aircraft crashes. The likelihood of pathogen releases due to truck crashes was estimated using two approaches:

1. Because of the robustness of the NEIDL packaging, the NEIDL packages are expected to survive crashes that truck-occupants would not survive; therefore, the occupant-fatal crash rate was used as one estimate of the likelihood for a pathogen release.

2. The NEIDL packages are not expected to fail as a result of impact loads and are most likely to be breached as a result of crushing loads. Occupant-fatal rollovers and train collisions are the types of collisions most likely to produce crushing loads and they were used to estimate the likelihood of a pathogen release.

Table 11-7 presents the frequency categories associated with each event for BSL-3 and BSL-4 shipments. The frequency category for injuries and fatalities from BSL-3 shipments is higher than for BSL-4 shipments because there are expected to be approximately ten times more BSL-3 shipments.

Table 11-7. Frequency category for transportation impacts within 10 km of the laboratory.

Event	Frequency category	
	BSL-3	BSL-4
Crash-related injuries(including the public and the driver of the truck)	⦿	○
Crash-related fatalities(including the public and the driver of the truck)	○	·
Public infections due to the crash of a truck carrying NEIDL pathogens	·	·
Public infections due to the crash of an aircraft carrying NEIDL pathogens	·	·

Frequency categories: ● = A (1 in 1 to 100 years) ⦿ = B (1 in 100 to10,000 years)
○ = C (1 in 10,000 to 1,000,000 years) · = D (1 in >1 million years)

The conclusions of the NEIDL transportation analysis are summarized as follows:

- Crash-related injuries and fatalities from NEIDL shipments are more likely than pathogen-related infections or fatalities in the public. A crash-related injury due to truck or air transport of NEIDL pathogens has an estimated frequency of one in 100 to 10,000 years and a crash-related fatality has an estimated frequency of one in 10,000 to 1 million years. An infectious pathogen release from a truck or aircraft crash resulting in an infection has an estimated frequency of 1 in more than 1 million years, which is considered beyond reasonably foreseeable..

- In the event of an infectious pathogen release from a transportation crash, the exposure levels are expected to be no greater than 5% of the exposures resulting from the MRF earthquake. The probability of one or more infections from a pathogen release due to a transportation crash is also smaller than the probability for the MRF earthquake.

These conclusions are valid for all three sites being evaluated (i.e., urban, suburban, and rural) as it is assumed the protocols followed for pathogen shipments would be similar for all sites.

11.7.7 Malevolent Acts

The security systems (e.g., electronic systems, personnel, policy, procedure, etc.) are assumed to be the same at each of the three sites, so the threat from any malevolent action(s) was the same at all three sites. Therefore, the analysis (Chapter 6) provides no basis for discerning any differences in the frequency among the three sites.

The consequences may be slightly different among the sites for malevolent acts that involve release of pathogens from the facility; however, the analysis determined that any exposures resulting from such releases would be no greater than the exposures from the MRF earthquake.

11.7.8 Environmental Persistence

The means by which the five pathogens (namely, *F.tularensis, Y. pestis*, 1918 H1N1V, RVFV, and TBEV-FE) theoretically could become established in the environments in the vicinity of the three proposed NEIDL sites involve animals (including arthropods) that could be present in those environments. It can be surmised that the intensively urbanized nature of the BioSquare Research Park site supports smaller populations of such animals and, as a result, would be expected to present a less favorable immediate environment for any such potential to be realized. Although a quantitative difference regarding this potential can be surmised, no qualitative difference is noted between the urban, suburban, and rural sites.

11.8 Conclusions

This section provides the major conclusions drawn from this RA.

11.8.1 Conclusions Related to Laboratory Worker Risk

1. NEIDL protocols would reduce and mitigate the risks of laboratory worker exposures and infections. However, the potential for NEIDL laboratory workers to be exposed to and become infected by the pathogens with which they are working remains a risk.

2. An undetected or unreported exposure from a needlestick leading to a laboratory worker infection is estimated to occur with a frequency between 1 in 100 years to 1 in 10,000 years for any of the BSL-3 or BSL-4 pathogens evaluated.

3. An undetected or unreported exposure from a centrifuge release leading to a laboratory worker infection for BSL-3 pathogens is estimated to occur with lower frequency than from a

needlestick, with the possible exception of RVFV. The analysis determined that RVFV poses the greatest risk to the worker, followed by *F. tularensis* and 1918 H1N1V. An undetected exposure from a centrifuge release in a BSL-4 laboratory is not considered credible.

11.8.2 Conclusions Related to Risk to the Public

1. One or more infections or fatalities in the public resulting from an MRF (total collapse) earthquake is expected to occur with a frequency of less than 1 in 1 million years for all pathogens except RVFV. The estimated average frequency of 1 or more infections for RVFV is between 1 in 10,000 years and 1 in million years, while the average frequency of 1 or more fatalities resulting from RVFV infection is between 1 in 340,000 years and 1 in more than 10 million years.

2. The analyses show risk to members of the public from secondary transmission of pathogens from an infected worker leaving the laboratory is significantly greater than the risk of direct exposure to a pathogen from a release due to an earthquake. That is relevant for pathogens that are transmissible directly from one person to another.

3. There does not appear to be any evidence of risk of direct person-to-person secondary transmission of anthrax to the public from a case of inhalational or gastrointestinal anthrax in a laboratory worker. There is a potential for person-to-person secondary transmission of anthrax to the public from a case of cutaneous anthrax in a laboratory worker. There does not appear to be a risk of direct person-to-person secondary transmission to the public from initial infections in laboratory workers of *F. tularensis* or TBEV-FE.. Risk to the public from initial infections in laboratory workers with RVFV is unlikely because that pathogen requires a vector or contact with infected animals for transmission to other humans. There is a potential for secondary transmission of the following pathogens to the public from initial infections in the laboratory worker: *Y. pestis*, 1918 H1N1V, SARS-CoV, ANDV, EBOV, MARV, LASV, JUNV, or NIPV.

4. For all four transmissible pathogens analyzed quantitatively (*Y. pestis*, 1918 H1N1V, SARS-CoV, and EBOV), the estimated average frequency with which one or more secondary transmissions is expected to occur after an undetected or unreported needlestick infection is between about 1 in 500 years and 1 in 30,000 years.

5. Of the four pathogens analyzed quantitatively, SARS-CoV and 1918 H1N1V pose the greatest risk of a large outbreak consisting of 100 or more infections among the public. For example, a SARS-CoV outbreak consisting of 100 or more infections is estimated to occur with an average frequency between 1 in 2,500 years and 1 in 440,000 years.

11.8.3 Conclusions Related to Difference among Sites

1. No site differences were found in risk to laboratory workers because it is assumed that NEIDL operations would be identical at the urban, suburban and rural sites.

2. For 12 of the 13 pathogens, most estimates of the frequency of one or more infections from an earthquake release are estimated to be less than 1 in 1 million years at all three sites. The frequency of one or more fatalities from RVFV as a result of the MRF earthquake event is higher at the urban site (1 in 340,000 years to 1 in greater than 10 million years) as compared to the suburban and rural sites (1 in greater than 10 million years for all estimates). That difference is because of the higher population density at the urban site.

3. Estimates for social contact rates based on data from the urban, suburban, and rural sites suggest that residents at the suburban and rural sites might have about 15 percent and 50 percent fewer average daily contacts, respectively, compared to the urban site, which could result in lower likelihoods of transmissions from those residents. However, on the basis of commuting estimates from each area (including the likelihood that an initially infected laboratory worker is not a local resident), it was determined that a significant portion of infections and fatalities could occur among individuals who are not local residents. As a result, those differences between the local resident populations did not cause the overall estimates of secondary transmissions of pathogens among the public to be substantially different across sites.

11.8.4 Limits of the RA

1. This RA used operational information regarding incidents at other BSL-3 and BSL-4 laboratories to the extent appropriate (see Appendix D). The operational data were an important input to the qualitative analyses, but they were of limited suitability for quantitative analysis of potential frequencies or exposures for NEIDL events. As a result of the data limitations, frequency categories spanning wide ranges were employed and conservative assumptions (i.e., assumptions

that tend to overestimate the frequency or consequences) were made. The results reflect that uncertainty but are generally considered to be conservative (i.e., overstate the true risk).

2. Data regarding the consequences of human exposure (via inhalation or needlestick) were limited for many pathogens and nonexistent for other pathogens. To compensate for incomplete data, expert opinion, information from experimental animal exposures, and conservative assumptions (i.e., assumptions that tend to overestimate the consequences of exposure) were used to inform the analyses. A wide range of initial infection frequency estimates was obtained, and the estimates of overall risk are sensitive to those estimates.

3. This RA focused on scenarios involving potential worker infections from undetected or unreported exposures as those events also pose a risk to the public. As such, the results of the RA are not intended to be an assessment of risk to laboratory workers in general.

4. Changes in the operation of the NEIDL relative to the bases used for this RA will affect the outcomes accordingly and could invalidate the conclusions of this RA if such changes were substantial.

11.9 References

Centers for Disease Control and Prevention, and the National Institutes of Health. 1999.Biosafety in Microbiological and Biomedical Laboratories. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH).

CDC and NIH (Centers for Disease Control and Prevention and National Institutes of Health). 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. U.S. Government Printing Office, Washington, DC. Printing Office, Washington, DC.

DOE (U.S. Department of Energy). 2002. Recommendations For Analyzing Accidents Under The National Environmental Policy Act, U.S. Department of Energy, Office of Environment, Safety and Health, Environment, Safety and Health, Office of NEPA Policy and Compliance July 2002.

EPA 2000 *Science Policy Council Handbook: Risk Characterization*. Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-002. Available on the internet at: http://www.epa.gov/spc/2riskchr.htm,

Harding, A.L., Byers, K.B. 2006. *Epidemiology of Laboratory-Associated Infections*. Edited by D. O. Fleming, Hunt, D.L. 4th ed, *Biological Safety: Principles and Practices*: ASM Press.

Johnson, K.M. 2009. Appendix D - Review of Biocontainment Laboratory Safety Record edited by U. S. Department of Health and Human Services. Washington, D.C.

Kimman, T. G., E. Smit, and M. R. Klein. 2008. Evidence-based biosafety: a review of the principles and effectiveness of microbiological containment measures. *Clin Microbiol Rev* 21 (3):403-25.

Murphy 2011 J. R. Murphy, BUMC, e-mail to N. Boyd, NIH dated Sept. 20, 2011 with attachment.

NIH 2009 *NIH Blue Ribbon Panel to Advise on the Risk Assessment for the BU National Emerging Infectious Diseases Laboratories – Teleconference with the National Research Council on Technical Input,* presentation on April 7, 2009, slide 21. Available on the web at http://nihblueribbonpanel-bumc-neidl.od.nih.gov/docs/2009/April/BRP_NRC_Teleconf_April_7.pdf. Accessed July 27, 2009.

NRC 2008 *Technical Input on Any Additional Studies to Assess Risk Associated with Operation of the National Emerging Infectious Diseases Laboratory, Boston University – Letter Report,* Committee on Technical Input on Any Additional Studies to Assess Risk Associated with Operation of the National Emerging Infectious Diseases Laboratory, Boston University, National Research Council, ISBN: 0-309-12040-3, April 29, 2008. Available on the internet at: http://www.nap.edu/catalog/12208.html . Accessed August 18, 2011.

World Health Organization. 2004. China's recent SARS outbreak: important lessons for global public health. In *proMED; Archive Number 20040704.1792*.

Appendix A.

Facility Design and Operations

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A. Facility Design and Operations

The fundamental objective of any biosafety program is the biocontainment (ie. Safe handling and containment) of hazardous biological material Biocontainment aremicrobiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. . One fundamental principle to eliminate or mitigate hazards is to develop effective controls such as but not limited to:

- Developed training to enhance the understanding and awareness of the hazards;
- Provide clear SOPs for operational safely;
- Provide suitable and appropriate alarms and warnings;
- Restore the system to the safe condition in an off-normal event;
- Establish safety barriers to defeat the hazards; and
- Contain the hazards if the barriers fail.

As described in the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition (CDC and NIH 2007):

> *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) has become the code of practice for biosafety. These principles are [bio]containment and Risk Assessment (RA). The fundamentals of [bio]containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory.

Workers are the first line of defense for protecting themselves, coworkers, and the public from exposure to hazardous pathogens. Protection depends on an awareness of the hazard and a well-established protocol to operate safely. Humans are fallible, and mistakes have the potential to compromise any of the safeguards of the laboratory. For those reasons, it is critical that technical proficiency in using good microbiological practices, safety equipment, and emergency response are continuously trained, tested, emphasized, and enforced. In addition, a program of continuous improvement strengthens the biosafety program by constantly evaluating risk and Standard Operating Procedures (SOPs) and work practices for areas requiring improvement.

This appendix contains an overview of the National Emerging Infectious Diseases Laboratories (NEIDL) facility including laboratory layout, generic floor plans, and equipment and material flow within the facility. It also contains a description of the facility design and construction (secondary barriers), safety equipment (primary barriers), and laboratory practice and technique (administrative controls).

A.1 NEIDL Overview

A.1.1 Laboratory Layout

The NEIDL consists of biosafety level (BSL)-4 laboratories with the associated BSL-4 support space (e.g., access to ventilation system, instrument air compressors); BSL-3 laboratories and animal handling rooms; BSL-2 laboratories and one BSL-3 laboratory on each of those floors; office space, clinical research center space, and building systems support space. Laboratory spaces and air flow systems are segregated as necessary to avoid cross contamination and within the structure to provide optimum workflow. Figures A.5 through A.21 (which are placed at the end of this appendix to avoid breaking-up the textual flow) provide floor plans for the various laboratory rooms.

A.1.2 Generic Floor Plans

The RA team evaluated the floor plans, which indicate that the highest containment BSL laboratories are within the interior spaces of the building. Such a layout provides protection against and lowers the likelihood of an aircraft crash accident penetrating the containment areas. In addition, that location would be anticipated to lower the likelihood of the highest biocontainment area being successfully breached by a malevolent action (i.e., no direct exposure pathway exists from the higher BSL spaces to the exterior of the facility).

The ability to maneuver people, equipment, supplies, and animals throughout the facility efficiently, without compromising the integrity of the research programs or creating an unsafe environment for building occupants is extremely important in the design of the facility. Proper design of such process flow also prevents cross-contamination of vital research and diagnostic

programs and allows for compartmentalization of rooms and suites for decontamination purposes.

A.1.3 Material and Equipment Flow

Materials and equipment entering or leaving the BSL-4 suites do so through one of the following means, depending on the specifics and size of the item:

- Stainless steel chemical dunk tanks;
- Pass-through, double-door autoclaves; or
- Fumigation vestibules (provided for each BSL-4 area).

Smaller samples and other materials enter or exit via any one of the three means as stated above. Large and more sensitive pieces of equipment enter and exit through the fumigation vestibules. When an item exits via the fumigation vestibule, it must be decontaminated inside the vestibule before exiting from the BSL-4 containment.

Waste materials and contaminated instruments exit via a pass-through autoclave.

A.2 Facility Design and Construction (Secondary Barrier)

Facility safeguards are designed to protect the staff and facility and to mitigate the accidental release of a pathogen from the laboratories. This section discusses the secondary safety barriers and how the facility safeguards within the NEIDL were designed to ensure containment and defined to facilitate the evaluation and analysis of consequences associated with operations involving biological pathogens.

Structure features (i.e., secondary barrier) are discussed first, followed by a detailed discussion of the safety equipment (i.e., primary barrier) within the NEIDL.

A.2.1 Structure

The BSL-3 and BSL-4 laboratories within the NIEDL were designed and built to the applicable federal standards, incorporating safety barriers and safeguards to ensure a safe work environment

and mitigate the accidental release of a pathogen. The most fundamental safety barrier for the public at the NEIDL is the high-biocontainment laboratory itself. The BSL-4 core laboratory space incorporates technologically advanced scientific equipment for infectious disease research in a high-biocontainment environment. The BSL-4 laboratory barrier is based on the *box within a box* concept. The BSL-4 facilities were designed and constructed to be easily cleaned and decontaminated. Seams, floors, walls and ceiling surfaces have been sufficiently sealed to facilitate fumigation and be resistant to liquids and chemicals used for cleaning and decontamination. The airtight enclosure allows a negative pressure cascade across functional areas creating directional airflow to help contain pathogens to the laboratory environment. The laboratory consists of multiple functional areas separated by pressure zones including a locker room, showers, suit room, air lock, and laboratory, all of which are surrounded by a second corridor, which serves as a buffer to the balance of the facility. Biological pathogens will be isolated within the laboratory suite from exterior spaces by interlocked doors with the interlocking mechanism allowing only one door to open at a time to ensure proper function of the barrier system (BUMC 2009a).

A.2.2 Codes and Standards

The design team that designed and oversaw construction of the facility is made up of CUH2A, Smith Carter, and Hemisphere Engineering (CUH2A et al. 2005). Those companies have been involved in many of the BSL-4 projects designed and constructed in North America, including the following (BUMC 2009b):

- National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Rocky Mountain Laboratories, Hamilton, Montana
- Centers for Disease Control and Prevention (CDC), Emerging Infectious Diseases Laboratory
- NIH, NIAID, Integrated Research Facility, Fort Detrick, Maryland
- United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland

The overall facility design and construction is in conformance with the following applicable codes (BUMC 2009b):

- The NIH *Design Policy and Guidelines*, November 2003 ed.
- The U.S. Department of Health and Human Services, CDC/NIH, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th ed.
- The Massachusetts State Building Code (780 CMR), 6th ed.
- Boston University Medical Center (BUMC) *Biosafety Manual*

A.2.3 HVAC Systems

The integrated heating, ventilation, and air conditioning (HVAC) system provides a controlled indoor environment. The system allows the ability to adjust temperature, pressurization, directional airflow and humidity (within selected laboratories) to parameters required by individual research activities. The NEIDL facility has incorporated a redundant design for the HVAC system. Each air handling system and corresponding exhaust system in the high-biocontainment labs (i.e., the BSL-3 and BSL-4 spaces) incorporate redundant air handlers and exhaust fans to ensure continuity of proper directional airflow (Kajunski, Joe, Assistant Director Engineering and NEIDL, Boston University, MED National Bio Lab. Telephone Meeting Notes, HVAC. Conversation with Tetra Tech, Inc., June 19, 2009).

A.2.4 Alarm Modes

This section describes the alarms for both BSL-3 and BSL-4. Note that no local alarms are planned for BSL-2.

A.2.4.1 BSL-3 Alarms

BSL-3 laboratories will have a local alarm, currently being planned, based on the operational state of directional airflow within the BSL-3 space (a wall-mounted device that captures attention with strobe lights and sound).

A.2.4.2 BSL-4 Alarms

Laboratory Internal Alarm Lights. A strobe light annunciating in the NEIDL indicates an emergency alarm condition in the laboratories. In addition, a local light tree will identify the type of alarm. Two lights relate to the following alarms:

- Red – Critical Evacuation; and
- Amber – Critical Seek Information.

Fire Alarm Mode. The fire signal for all Control Cells is an alert signal only, and it does not have any effect on the cell operation. If the room occupant decides to evacuate the Control Cell, the exit is through prescribed egress sequences. Room isolation is then achieved remotely through a building automation system (BAS) command of the bioseal dampers.

Power Interruption Alarm Mode. When a power interruption to the air distribution system in each cell occurs, the space goes into the system shutdown mode. Restart is by the BAS.

Emergency Mode. Alarm strobe lights and enunciator panels inform the occupants of any alarm that affects building safety.

Occupant Emergency Alarm Mode. When one of the panic buttons in each of the laboratories is activated, all lights for that suite are switched on, and a critical alarm goes to the BAS. The ventilation system remains operating in steady-state mode.

Breathing Air Alarm Mode. Failure of the breathing air system sends a critical alarm to the BAS. Failure of the backup breathing air system sends a critical alarm to the BAS.

Chemical Shower Alarm Mode. Low levels of chemical shower solution prompts a critical alarm to the BAS.

Door Override Alarm Mode. When either of the door override panic buttons is activated, the hard-wired magnetic door lock is released, and a critical alarm is sent to the BAS.

Biohazard Alarm Mode. The system has two levels of alarm function. The first occurs when a Control Cell pressure is ±0.06-inch water gage (wg) from a set point (operator adjustable). An alarm is initiated as described in Emergency Mode (above). The second level of alarm function occurs when the Control Cell static pressure is ±0.1-inch wg from a set point (operator adjustable), thereby approaching a positive pressure when referenced to adjacent Control Cells.

All bioseal dampers associated with the room or space close, and a critical alarm is sent to the BAS.

Decon Mode. BAS originated with a dedicated sequence during a room decontamination process.

A.2.5 Backup Systems

Redundant, critical systems are incorporated in the utility and building infrastructure to facilitate safe operations. As a backup to the electrical utility, the facility is equipped with an on-site diesel generator that is capable of providing 48 hours of uninterruptable power to prioritized loads. In addition, an uninterruptible power supply is available to selected prioritized loads. Additionally, three emergency power generators are connected in parallel to supply emergency power (BUMC 2009). Two independent heating mediums are available—district steam service and a natural-gas-fired heating plant. In addition, the water service has two independent utility connections A networked electrical service provides four separate incoming feeds installed such that if any one feed is disrupted, the remaining two will provide the necessary service (BUMC 2009).

A.3 Safety Equipment (Primary Barriers)

A.3.1 BSL-2

The BSL-2 areas of the NEIDL will be suitable for work involving pathogens of moderate potential hazard to personnel and the environment. Laboratory personnel have specific training in handling pathogenic agents and are supervised by competent researchers. In addition, access to the laboratory is limited when work is being conducted, and personnel take extreme precautions with contaminated sharp items. Processes in which infectious aerosols or splashes can be created are conducted only in biological safety cabinets (BSCs) or other physical biocontainment equipment.

Guidance provided in *Safety Equipment* (Primary Barriers and personnel protective equipment [PPE]) specifies the following (BUMC 2009b, Appendix E):

- Properly maintained BSCs (preferably Class II), appropriate PPE, or other physical biocontainment devices must be used when dealing with high concentrations or large

volumes of infectious pathogens or when conducting procedures with the potential of creating infectious aerosols or splashes.

- Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn when working with hazardous materials (HazMat).
- Eye and face protection is used for anticipated splashes, or sprays of infectious or other HazMat when the microorganisms must be handled outside the BSC or biocontainment device.
- Gloves must be worn to protect hands from exposure to HazMat.

A.3.2 BSL-3

The areas of the NEIDL that have been designed and designated as BSL-3 are those areas where work will be conducted with dangerous and exotic pathogens that pose a potential risk for respiratory transmission and that can cause serious and potentially lethal infection.

At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all activities will be confined to Class III BSCs or Class II BSCs, and the use of positive air-purifying respirators (PAPRs) will also be required. Secondary barriers for this level will include strictly controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

Guidance provided in *Safety Equipment* (Primary Barriers and PPE) specifies the following (BUMC 2009b, Appendix F):

- All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III) or other physical biocontainment device.
- Protective laboratory clothing with a solid front, such as tie-back or wrap-around gowns, scrub suits, coveralls, and PAPRs are worn in the laboratory.
- Gloves must be worn to protect hands from exposure to HazMat.

A.3.3 BSL-4

BSL-4 is required for work with dangerous and exotic pathogens that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Pathogens with a close

or identical antigenic relationship to BSL-4 pathogens are also handled at this level until sufficient data are obtained either to confirm continued work at this level or to verify working with them at a lower level can be conducted safely without undue risk. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious pathogens, and they understand the primary and secondary biocontainment functions of the standard and special practices, the biocontainment equipment, and the laboratory design characteristics. They are supervised by competent researchers who are trained and experienced in working with those pathogens. Access to the BSL-4 laboratories is strictly controlled by the laboratory director. The BSL-4 facilities are in a controlled area, which is completely isolated from all other areas of the building. A specific facility operations manual will govern the operations of the spaces.

Within BSL-4 work areas, all activities are confined to Class III BSCs or Class II BSCs and all activities are performed with one-piece, positive-pressure, personnel suits equipped with a life support system (e.g., an escape bottle air apparatus). Air is supplied to the suits from an exterior source and is not recirculated. The BSL-4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

Guidance provided in *Safety Equipment* (Primary Barriers and PPE)—Suit Laboratory specifies the following (BUMC 2009b):

- All procedures must be conducted by personnel wearing a one-piece, positive-pressure suit ventilated with a life support system.
- All manipulations of infectious pathogens must be performed within a Class III BSC or other primary barrier system.
- Equipment that can produce aerosols must be contained in devices that exhaust air through a HEPA filtration system before being discharged into the environment.
- Protective laboratory clothing such as a scrub suit must be worn by workers before entering the room used for donning positive pressure suits. All protective clothing must be removed in the *dirty* side change room before entering the personal shower. Reusable laboratory clothing must be processed in an autoclave before being laundered.
- Inner gloves must be worn to protect against break or tears in the outer suit gloves.

A.3.4 Biosafety Cabinets

Primary barriers include BSCs (or other biocontainment systems used for open handling of pathogens) and full-body, air-supplied, positive-pressure suits. The type of BSC used depends on the pathogens being handled. In general, the BMBL (CDC and NIH 2007) recommends Class I or II BSCs for BSL-2 and BSL-3 areas, and Class III BSCs (or Class I or II BSCs with positive-pressure suits) for BSL-4 areas. The NEIDL Final Environmental Impact Statement (FEIS) (NIH and DHHS 2005) reports anticipated use of Class III BSCs for the BSL-4 laboratory. Class III BSC within processing rooms for diagnostic samples; the FEIS (NIH and DHHS 2005) also reports anticipated use of Class II Type A2 BSC and/or Class II Type B1 BSCs in the BSL-4 laboratories and necropsy room in conjunction with required use of positive-pressure suits. Diagrams and brief descriptions of the above described BSCs follow in Figures A-1, A-2, and A-3 (CDC and NIH 2007).

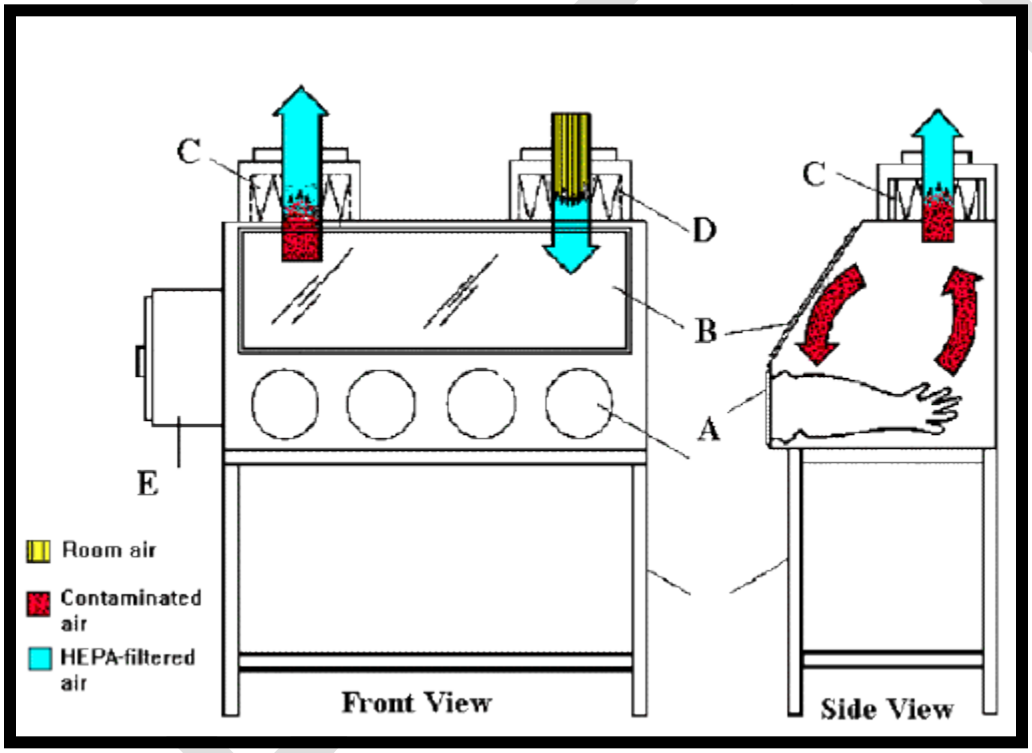

Figure A-1. Class III BSC.

Cabinet air exits through an exhaust HEPA filter (C) into a facility airtight ducted negative pressure exhaust. Exhaust air from the cabinet is drawn through a final set of inline HEPA filters before discharge to the outdoors. A redundant (parallel) exhaust air system is available to accommodate servicing the units. An additional chemical dunk tank can also be installed, which would be beneath the work surface of the BSC with access from above.

Notes:

A. glove ports with O-ring for attaching arm-length gloves to cabinet

B. sash

C. exhaust HEPA filter

D. supply HEPA filter

E. double-ended autoclave or pass-through box

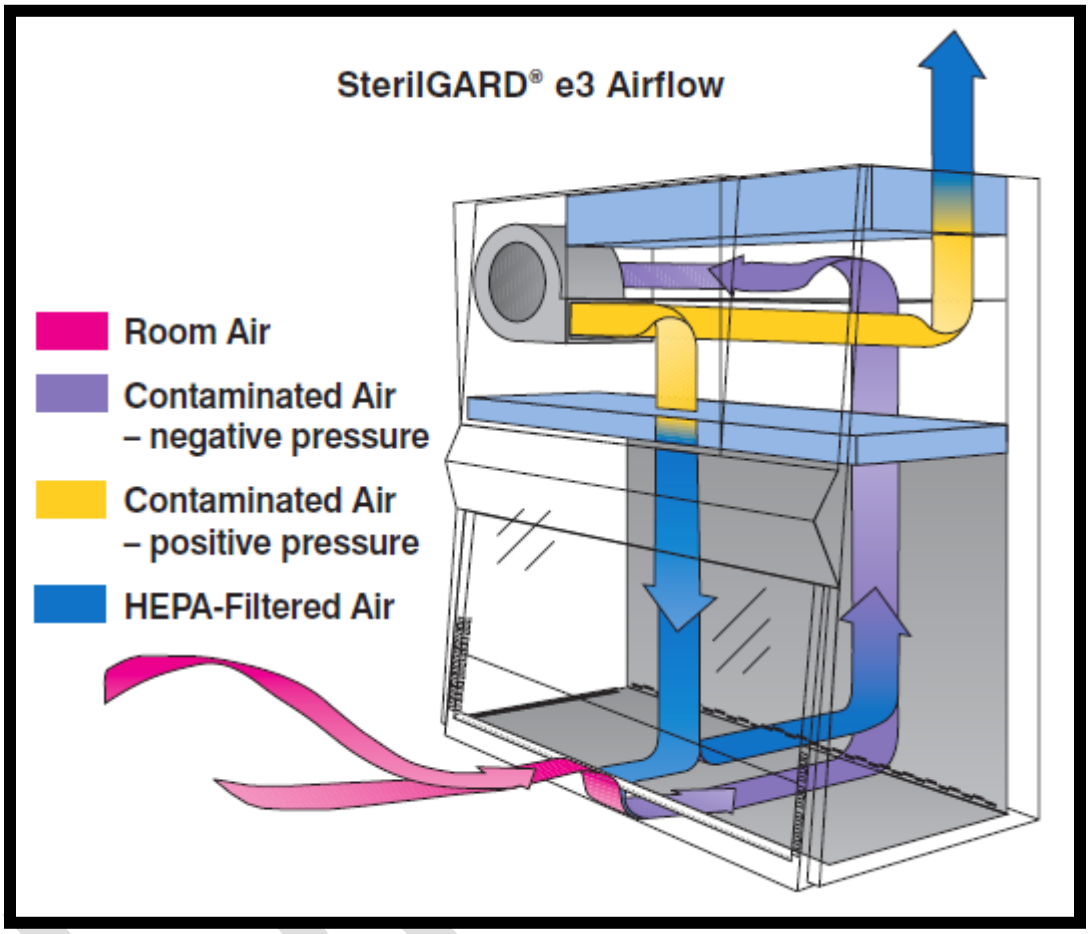

Figure A-2. Class II, type B2 BSC airflow patterns.

As shown in Figure A-2, HEPA-filtered exhaust air flows from the top to the work surface. A portion exits from the rear grill. A portion of the down-flow air is pulled into the front grill along with room air to prevent room contaminants from entering the work space. The air from the rear and front grills passes through the supply and exhaust filters preventing airborne contaminants from entering the room or the cabinet work area.

Figure A-3. Class II BSCs do not provide total barrier containment.

Note: An RA could require additional PPE to prevent aerosol exposure. Examples in BSL-3 labs include pathogens with low inhalation infectious doses (*Francisella tularensis* and *Coxiella burnetii*). One option is the PAPR.

A.4 Laboratory Practice and Technique

A.4.1 BUMC Biosafety Manual

The purpose of the BUMC *Biosafety Manual* (BUMC 2008) is to define and communicate the biological safety policies and procedures pertaining to research operations at BU and Boston Medical Center. The manual provides policies and procedures that are designed to safeguard personnel and the environment from biologically hazardous materials and to comply with federal, state, and local regulatory requirements. All BU and BUMC developed policies for principal investigators (PIs) and laboratory workers must adhere to the biological safety policies and procedures as they conduct their research and manage laboratories. For programs or operations not covered in the manual, the manual requires that PIs contact the Institutional Biosafety Committee (IBC) office or the biosafety officer to begin developing suitable programs before initiating their projects.

Descriptions of BSLs and assigned BSLs for specific organisms are in the CDC/NIH document, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th ed. The BMBL outlines four BSLs, summarized in Table A-1 below.

Table A-1. Biocontainment elements for each BSL

BSL	Pathogens	Practices	Safety equipment	Facilities
1	Not known to cause disease in healthy adults; RG1	Standard microbiological practices	None required	Open bench-top, sink required
2	Associated with human disease, which is rarely serious and for which preventive or therapeutic interventions are often available; RG2	BSL-1 practice plus: - Limited access - Biohazard warning signs - Sharps precautions - Biosafety manual	Primary barriers: Class I or II BSCs or other containment used for manipulating pathogens that cause splashes or aerosols of infectious materials. PPE: lab coats; gloves; eye/face protection as needed.	BSL-1 plus: Autoclave available
3	Associated with human disease for which preventive or therapeutic interventions might be available; RG3	BSL-2 practice plus: - Controlled access - Decontamination of all waste - Decontamination of lab clothing before laundering - Baseline serum	Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulations of pathogens. PPE: protective lab clothing; gloves; respiratory protection as needed.	BSL-2 plus: - Physical separation from access corridors - Self-closing, double-door access - Exhausted air not recirculated - Negative airflow into laboratory
4	Pathogens are likely to cause serious or lethal human diseases for which preventive or therapeutic interventions are not usually available; RG4	BSL-3 practices plus: - Clothing change before entering - Shower on exit - All material decontaminated on exit from facility	Primary barriers: All procedures conducted in Class III BSCs or Class I or Class II BSCs in combination with full-body, air-supplied, positive-pressure personnel protective suit.	SL-3 plus: - Separate building or isolated zone - Dedicated supply/exhaust, vacuum, and decontamination systems - Other requirements outlined in BMBL

Note: For a more complete description of the four BSLs and for recommended specific organisms, see the BMBL.

A.4.2 Procedures (SOPs)

BUMC has a system in place to evaluate risk and determine which types of research projects will be performed at the various BSLs. BUMC safeguards personnel and the environment from

biologically hazardous materials through the use of an internal biological RA process and IBC. As required by NIH when conducting biological research with recombinant DNA (rDNA) and receiving federal grant money to comply with the rDNA guidelines, BU has maintained an active IBC for several decades. NIH guidelines are quite prescriptive for IBCs and require that they ensure that all proposed research projects are in compliance with NIH and CDC guidelines (and other guidelines). After the projects are initiated, the IBC must also ensure that the work is being done safely so that the laboratory worker and the public are safe.

During and following approval from the IBC, NEIDL personnel develop SOPs outlining the appropriate safety equipment that will be required during the research, as well as the appropriate research equipment, the appropriate safety processes and procedures, and the appropriate BSL. During the internal biological RA process, the reviewers (including biosafety and the IBC) can modify the level of containment required for a pathogen. During the biological RA, many aspects of the experiment protocols are evaluated to determine which (if any) of the procedures could cause an aerosol, the mode of entry, transmission and exposure of the required experimental pathogen, PPE requirements, and required equipment (e.g., type and use of BSC). When the IBC is presented with a request for a change in use of a new pathogen, the IBC reviews the project again. Additional requirements are followed depending on containment and the type of work being performed.

A.4.3 Training

The purpose of the Laboratory Training Program for the NEIDL is to promote excellence in conducting safe practices; to keep the laboratory and equipment in a safe operating order; to maintain compliance with institutional policies and local, state, and federal regulations and guidelines; and to reinforce awareness that laboratory safety protects the research, support personnel, and the environment from potential hazards associated with level 4 research.

The NEIDL Biosafety Training Plan presents a strategy for developing a comprehensive training program for the scientists, technicians, and guest scientists at BU who are authorized to conduct research in the NEIDL; the operations and maintenance employees of BU who manage the NEIDL's containment and engineering systems; and other groups of BU such as employees,

visitors, vendors, and service personnel who work in support of the NEIDL. In addition, the plan includes training for members of the Boston community whose roles and interests support the NEIDL mission.

The plan recommends six training tracks: (1) Orientation; (2) Operations and Maintenance; (3) IBC and Institutional Animal Care and Use Committees (IACUC); (4) Science Program; (5) NEIDL First Responders, and (6) Public Sector First Responders. For each track, the plan includes recommended courses, learning objectives, and suggested content. The plan requires that participants demonstrate that they have met the learning objectives of each course within their training track. In addition, participants in the Science Program track a required to pass a hands-on protocol proficiency examination (BUMC 2009b).

Table A-2 provides the NEIDL training matrix.

1 **Table A-2. BUMC NEIDL training matrix**

Course no.	Course title	Intended participants
1	General Orientation	Track 1: Administrative; visitors; vendors; service personnel; community members Track 2: Operations and Maintenance Track 3: IBC and IACUC Track 5: NEIDL Emergency Response Team Track 6: Public Safety Emergency Responders
2	Introduction to Microbiology and the Control of Infectious Diseases	Track 2: Operations and Maintenance
3	Fundamentals of Secondary Barrier Containment	Track 2: Operations and Maintenance Track 5: NEIDL Emergency Response Team Track 6: Public Safety Emergency Responders
4	Verification and Certification of Secondary Barriers	Track 2: Operations and Maintenance
5	Incident Response for Non-laboratory NEIDL Personnel	Track 2: Operations and Maintenance
6	NEIDL Emergency Response Team Incident Response Procedures	Track 2: Operations and Maintenance Track 4: Science Program Track 5: NEIDL Emergency Response Team Track 6: Public Safety Emergency Responders
7	Emergency Response Preparedness	Track 2: Operations and Maintenance Track 4: Science Program Track 5: NEIDL Emergency Response Team Track 6: Public Safety Emergency Responders
8	Comprehensive Review of BSL-4 Containment	Track 3: IBC and IACUC
9	Compliance Issues - Select Agent Rule, NIH Recombinant DNA Guidelines and BPHC Biological Laboratory Regulation	Track 3: IBC and IACUC
10	Independent Assessment of Risks	Track 3: IBC and IACUC
11	Biosafety and Biosecurity in the Conduct of Exemplary Research	Track 4: Science Program
12	Assessing Risks of NEIDL Research Protocols	Track 4: Science Program
13	Preparation of Research Protocols to Obtain Approval to Conduct Research Project in the NEIDL	Track 4: Science Program
14	Maintaining Compliance with the Select Agent Rule, NIH Guidelines, and the BPHC Biological Laboratory Regulation	Track 4: Science Program
15	Comprehensive Review of BSL-4 Safeguards and Research Practicum	Track 4: Science Program
16	First Aid, CPR and Automated External Defibrillator (AED)	Track 4: Science Program
17	Planning Emergency Response Drills	Track 5: NEIDL Emergency Response Team

2 Note: From BUMC 2009b, program under development..

A.4.4 Access Control

A combination of security systems and staffing reinforce the layers of access control at the NEIDL. Access to the BSL-3 and BSL-4 labs is restricted to workers who have received appropriate immunization and security clearances for the pathogens in use at the labs. Access to different areas or layers within the facility can require positive identification and signing in with a security officer (i.e., a public safety officer), using access control systems such as biometrics or proximity card technologies, requesting access via escort by an authorized colleague, or a combination of those approaches. Work being performed within high-level biocontainment areas will be monitored by systems to ensure that at least two authorized persons are in each area at all times to ensure safety and minimize risk of an individual initiating a malevolent or unauthorized act. Figure A-4 shows the secure perimeter, access-control points, and a few representative locations of security cameras for the NEIDL. Similar provisions would be used for the other candidate locations (NIH and DHHS 2005). Note that the specifics of the NEIDL security systems are considered restricted distribution security information, the disclosure of which could present an increased risk to the facility and its occupants and thereby potentially endanger the operations of the facility. Therefore, in accordance with guidance provided in the Bioterrorism Act of 2002, the details of the NEIDL security systems and the specifics of the Threat Assessment (TA) performed to evaluate the systems are not included in this document, nor will they be disclosed during public meetings or briefings for non-cleared individuals. However, detailed findings of that evaluation are included in the TA.

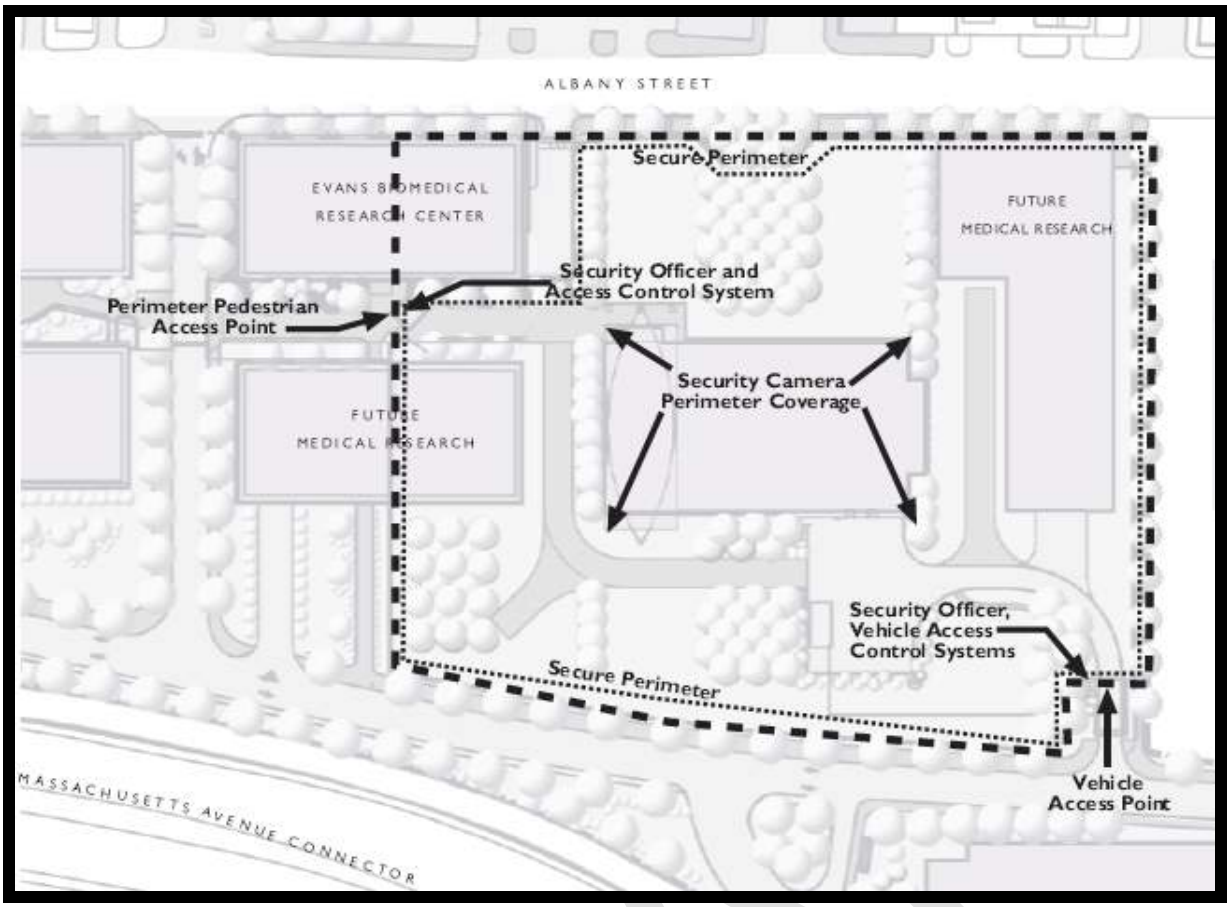

Figure A-4. Representative NEIDL security feature locations.

A.4.5 Disease Surveillance Plan

The Boston Public Health Commission's (BPHC's) *Guidelines for the Implementation and Enforcement of Boston Public Health Commission's Disease Surveillance and Reporting Regulation* requires laboratory registration and the implementation and maintenance of a medical surveillance program for research laboratories working with select agents and other high-risk pathogens that require containment in BSL3 and BSL-4 facilities. The BPHC compliance guidelines specify practices for ensuring that the BPHC receives timely access to information regarding incidence of disease syndromes, any outbreak or cluster of a disease, and potential exposures to reportable diseases deemed harmful to the public health. The BUMC Disease Surveillance Plan sets forth the roles and responsibilities of researchers and compliance staff at BU and Boston Medical Center (BMC), as mandated by the BPHC Disease Surveillance and Reporting Regulation.

The Disease Surveillance Plan is an integral component of the BU and BMC's select agent research and occupational health and safety programs. The purpose of the plan is to prevent laboratory-acquired infections associated with the receipt, storage, handling, and disposal of select agents and high-risk pathogens in BSL-4 laboratory environments and to protect workers outside the BSL-4 laboratory containment, the environment, and the public health from exposure to those agents. The NEIDL plan includes policies and provisions for identifying at-risk individuals, maintaining vigilance for the recognition of potential exposures, identifying potentially exposed individuals and providing a quick and appropriate medical response to an accidental exposure or to symptoms suggestive of a laboratory-acquired infection. The plan also provides the direction, approaches, and responsibilities for maintaining compliance with the BPHC's *Disease Surveillance and Reporting Regulation*; the federal government's *Final Rule on the Possession, Use, and Transfer of Select Agents and Toxins* (Select Agent Rule); and the NIH's *Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines).

The NEIDL has implemented a written biosafety plan that is commensurate with the risk of possessing, using and transferring a select agent. In developing the plan, NEIDL considered (1) the principles of biosafety and guidance described in the CDC, and NIH publication BMBL, 5th ed., including all appendices; and (2) the NIH Guidelines. The BMBL guidance on occupational health (BMBL Section VII) serves as the foundation for the comprehensive health and safety management program that will enable the NEIDL to operate as a safe and healthy workplace. The BMBL emphasizes that prevention is the most effective strategy for maintaining occupational health and that prevention is achievable in a biomedical research setting where the occupational health and safety programs are broadly shared responsibilities involving every group and individual associated with the conduct and support of the research program.

At BU and BMC, those groups include the Office of the Associate Vice President Research Compliance; the Office of Environmental Health and Safety (OEHS); the Research Occupational Health Program; the BMC Emergency Care programs; Public Safety; IBC; and the IACUC. The PIs, researchers and support staffs who work in the NEIDL all have primary roles in implementing the Disease Surveillance and Biosafety Plans.

The NEIDL will also establish and maintain a health surveillance program for personnel engaged in animal research involving viable rDNA-containing microorganisms that require BSL-4 containment in the laboratory. The overall program includes a system for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. The NEIDL requires researchers to immediately report spills and accidents that result in overt exposures to organisms containing rDNA molecules to the Biological Safety Officer, IBC, and to the NIH Office of Biotechnology Assessment.

The disease surveillance program also takes into consideration the potential hazards to which employees can be exposed and that have the potential to cause adverse health consequences. The surveillance program evaluates the work processes, tasks performed by individuals, the hazardous pathogens in use, and the potential exposure that can occur in handling the pathogens. The outcome of such risk and hazard assessments will determine the overall needs of the individuals with potential for exposure.

A.5 Waste Management

Waste management practices at the NEIDL are laboratory-specific and dependent on the wastestreams generated by individual BSL-2, BSL-3, and BSL-4 laboratory activities. In general, disposal of waste is particular to the organisms or SOPs of the research group, but, in general, the following principles apply:

- Rigid containers labeled with the universal biohazard symbol and lined with red biohazard bags are provided in every clinical and research laboratory at NEIDL for all biohazard waste.
- Sharp containers are also provided for the safe disposal of needles, syringes, and scalpel blades.

Solid waste includes all nonhazardous waste generated from offices and maintenance areas, including recyclable materials. In addition to normal solid waste, it is anticipated that the NEIDL will generate three types of special waste: biological and non-biological waste, radioactive waste, and hazardous-chemical waste. NEIDL operations will not generate mixed waste (a U.S. Environmental Protection Agency-defined term denoting wastes that are both radioactive and

hazardous). The use, storage, and disposal of all solid and special waste will be performed in accordance with state and local regulations.

A.5.1 Biologic Waste

Biological waste will be disposed of in strict compliance with the Massachusetts's Department of Public Health State Sanitary Code Title VIII (105 CMR 480.00), the Massachusetts Solid Waste regulations (310 CMR 19.000) and Section 2.01 of the BPHC Regulation titled *Waste Container Lot, Junk Yard, and Recycling Facilities*.

Solids Handling. No waste materials will be removed from the BSL-3 or BSL-4 laboratories without first being processed in an autoclave or decontaminated by a method approved and managed by the BU's OEHS. Several materials require special decontamination methods to assure safe removal from the BSL-3 and BSL-4 laboratories. BSL-3 and BSL-4 laboratories use a disinfectant that is particular to each pathogen used as outlined in the research-specific SOPs and an autoclave to further decontaminate solid biological and non-biological waste. The BSL-2 laboratories will use the conventional system of bagging biohazardous waste and shipping the material off-site for incineration using a licensed, third-party contractor.

Liquid Effluents. The proposed system will include a multi-sterilization system for BSL-3 and BSL-4 facilities, tissue digesters outside containment for animal carcasses after sterilization, and a dedicated liquid effluent decontamination system for BSL-4. All liquid waste from the BSL-4 laboratories will first be decontaminated with a chemical disinfectant and then be piped to a biowaste processor and heated under pressure until the temperature reaches 121º C (249.8 ºF) for at least 60 minutes to ensure that two decontamination processes are completed. Decontamination will be verified by using biological indicators and electronic monitoring and charting of the process verified to meet acceptable discharge limits levels. The liquid waste treated liquid waste effluent will be discharged to the Boston Water and Sewer Commission sanitary sewer system. Ventilation from plumbing systems will pass through a HEPA filter before discharge to the atmosphere. The filters will be decontaminated and disposed of as appropriate (NIH and DHHS 2005).

The NEIDL operates in accordance with all plumbing codes and Massachusetts Water Resources Authority regulations requiring that sinks in laboratories drain to a pH adjustment system, where pH adjustment and verification, flow monitoring, and water sampling take place. The NEIDL has a plumbing system that will carry laboratory wastewater from every non-BSL-4 area to mixing tanks in the basement where pH adjustment and compliance sampling occur (NIH and DHHS 2005).

The sterilization system for the BSL-3 and BSL-4 laboratories will include 5 large autoclaves and 11 medium autoclaves. Animal carcass materials will be placed on rack sterilizers for easy introduction or removal of large materials, while smaller autoclave models will be used for general laboratory waste. Once waste material has been processed in the autoclave in biodegradable bags and removed from the BSL-3 and BSL-4 contained space, all animal carcass waste will be placed in the tissue digestion system and undergo alkaline hydrolysis for final processing and disposal (mineral oil could be added to the tissue digester to aid in removal of the autoclave bags following processing. Following completion of laboratory work in the BSL-3 facilities, workspace areas would be disinfected using a newly prepared 1:10 bleach solution or other appropriate pathogen-specific disinfectant.

A.5.2 Radioactive Waste

Radioactive waste generated at the NEIDL will consist primarily of solid waste such as paper, plastic and glass contaminated with trace amounts of radioactive isotopes (radioisotopes). Such wastes will be limited to those materials that meet the definition of low-level radioactive waste (LLRW) as defined by the Nuclear Regulatory Commission and codified in Title 10 of the *Code of Federal Regulations* (CFR) Part 20. BUMC's Radioisotope Committee oversees the disposal and management of LLRW. Researchers typically place LLRW in labeled, special containers at the point of generation, and contact the BUMC's Radiation Protection Office (RPO) when the special container is filled. An RPO representative then removes the waste, obtains an inventory of the materials placed in the container (and their suspected level of activity and contamination, both surface and volumetric), and manifests and transports the container to a licensed radioactive waste storage facility for storage and handling. The RPO maintains all records associated with LLRW, beginning from waste collection to final disposition. The radioisotopes that are

anticipated to be used at the NEIDL consist of both long-lived and short-lived radioisotopes. Long-lived radioisotopes require disposal off-site at a licensed LLRW-disposal facility. Waste contaminated with short-lived radioisotopes would be held on-site in BUMC's decay-in-storage facility for periods ranging anywhere from one week to not more than 2 years and 9 months, depending on the radioisotope's half-life, to allow sufficient decay and subsequently disposed of as nonradioactive sanitary waste.

A.5.3 Hazardous Waste

The generation of hazardous-chemical waste (as defined by the 40 CFR Part 261) at the entire NEIDL has been estimated on the basis of biological laboratories in comparable-sized facilities at BUMC. EPA has delegated Massachusetts to implement most aspects of hazardous waste regulation in Massachusetts and regulations regarding the generation of hazardous waste apply in lieu of federal regulations. Typical wastestreams anticipated at the NEIDL include the following:

- Flammable liquids
- Flammable, toxic liquids
- Corrosive liquids
- Oxidizing liquids
- Ethidium bromide solids
- Liquid effluents

A.6 Facility Support for BSL-2, -3, and -4 Areas

Decontamination (Equipment Wash Room). An equipment wash room will be provided for various pieces of equipment including socialization penning, animal caging systems and other reusable animal supplies. Typically, such equipment is capable of washing material in 82 °C (180 °F) water as recommended in the National Research Council guide and supported with redundant, instantaneous water heaters and will include the following:

- Bulk pass-through type equipment and cage washing systems;
- Gross wash for initial spray-down and acid soak of equipment, animal racks, caging, and penning;
- Staging for dirty and clean cages;

- Equipment zones and dedicated space for placing washing equipment (equipped with floor pits and overhead clearance for utility services); and
- Detergent storage, directly adjacent to the equipment zones to stage and pump chemical detergents to the washing equipment.

Dunk tanks. A barrier-designed dunk tank allows for the passage of materials that are heat sensitive or capable of being decontaminated using a liquid disinfectant or virucide across the biocontainment barrier. The types of disinfectants (e.g., phenolics, glutaraldehydes, quaternary ammonium compounds, hydrogen peroxide, alcohols, proteinated iodines, sodium hypochlorite) vary according to the types of infectious pathogens and their characteristics, such as corrosiveness, viability over time, and concentrations in use. Biosafety protocols (and SOPs) determine which disinfectant is used, when it is replenished, and what concentrations are required. Dunk tanks will be provided at all necropsy rooms in the NEIDL.

Autoclaves. Materials taken from primary biocontainment zones must be decontaminated. Those include waste (e.g., disposable PPE, paper goods, medical supplies); mobile and shared equipment; isolators, cages, racks, and penning; clinical waste; and samples (e.g., pathologic waste). For most materials, decontamination can be achieved via a steam autoclave. For certain sensitive items, however, alternate methods of decontamination (e.g., vaporized hydrogen peroxide [VHP], chlorine dioxide, paraformaldehyde burn) are available. Double-door autoclaves at the biocontainment barrier envelope allow material to be passed from the contaminated (i.e., *dirty*) side to the uncontaminated (i.e., *clean*) side with a full sterilization cycle. Interlocking doors also prevent both doors from being opened simultaneously. The double-door barrier autoclave has two types of barrier seals. It is recommended that the tight seal be pressure capable to at least 500 Pascal (Pa) (2-inch wg) of static pressure, have a flange that is bolted with a flexible neoprene (or silicone) seal, and have a receiving flange that is cast into the wall cavity. The location of the flange seal with respect to the autoclave body is important to consider in terms of maintenance requirements. Some autoclaves have close to 30 valves, numerous filters, and high-maintenance door seals. Such a combination requires that maintenance be both preventative and corrective. The autoclave body should be on the clean side of the biocontainment barrier, with the chamber condensate hard-piped directly to the waste

treatment system. Autoclaves are required inside the biocontainment zones of the necropsy rooms (i.e., within primary containment areas) and along the secondary biocontainment corridor leading to the xones outside the biocontainment area. For each zone within the facility, NEIDL is considering two autoclaves for redundancy and is considering three sets of redundant bulk autoclaves serving the BSL-3 animal facility including three medium-sized autoclaves to serve smaller loads, when needed.

Gas Decontamination. Gas decontamination will be considered for large pieces of equipment (e.g., penning, BSCs, carts) because gases pass between barriers of biocontainment. Both VHP and paraformaldehyde gases are effective for such an application, which is dependent on room size. A paraformaldehyde gas-generating machine capable of decontaminating large room spaces might also be used. Such equipment also incorporates gas neutralization (using ammonium carbonate) as part of the sterilization cycle. The humidity level and temperature are important for effective sterilization (70 percent relative humidity and 20 °C [68 °F]). The decontamination rooms are designed to be airtight via the use of bioseal dampers on the ventilation ducts, leak-tight barrier doors with specialized seals, and leak-tight service penetrations. Fans, which are used to circulate the gas within the room, can either be permanently mounted or portable. The rooms can also be used for transferring materials and animals into the facility and will require interlocking doors, penning, and door windows. Animal air locks are designed to accommodate gaseous decontamination of equipment. The VHP process is an alternative for room decontamination in the facility. Infrastructure will be required to be in place to support VHP equipment, including supply and exhaust ports into the room, circulating fans, vaporizer equipment, and dehumidification equipment. Because the infrastructure isprovided in the NEIDL facility, it is intended to use certain rooms or airlocks as a means to decontaminate large pieces of equipment periodically.

Decontamination Waste Treatment. Solid waste and liquid effluent decontamination is required of all materials infected with pathogens, including animal carcasses. For any waste disposal technology, NEIDL must consider the following design criteria (according to the biological RA and level of biocontainment):

- Ease of transport and loading into treatment equipment;

- Worker protection and reduction of biohazard aerosol generation;
- Decontamination effectiveness of given technologies;
- Consistent, repeatable, and verifiable performance;
- Volume reduction for final disposal;
- Compliance with local, state, and federal environmental requirements;
- Cost-effectiveness (capital and operating);
- Technical maturity and degree of automation to achieve effective labor savings; and
- Reliability and maintainability.

Several decontamination and sterilization technologies were initially reviewed and will be studied further including chemical, incineration, rendering, autoclave, and alkaline digestion.

A.7 References

BUMC (Boston University Medical Center). 2008. *Boston University Medical Center Biosafety Manual*. Boston University Medical Center, Boston, MA.

BUMC (Boston University Medical Center). 2009a. Tetra Tech Site Visit. December 4, 2008, and June 1–4, 2009.

BUMC (Boston University Medical Center). 2009b. Boston Public Health Commission Biological Laboratory Safety Permit Application, March 3, 2009.

CDC and NIH (Centers for Disease Control and Prevention and National Institutes of Health). 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. U.S. Government Printing Office, Washington, DC.

CUH2A, Smith Carter, and Hemisphere Engineering. 2005. National Emerging Infectious Disease Laboratory, Basis of Design, 100 percent Construction Drawings.

NIH (National Institutes of Health). 2008. *National Institutes of Health (NIH) Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities (DRM)*. <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/DesignRequirementsManualPDF.htm>. Accessed September 28, 2009.

NIH and DHHS (National Institutes of Health and U.S. Department of Health and Human Services). 2005. *Final Environmental Impact Statement, National Emerging Infectious Disease Laboratory, Boston, Massachusetts*. National Institutes of Health, Bethesda, MD, and U.S. Department of Health and Human Services, Washington, DC.

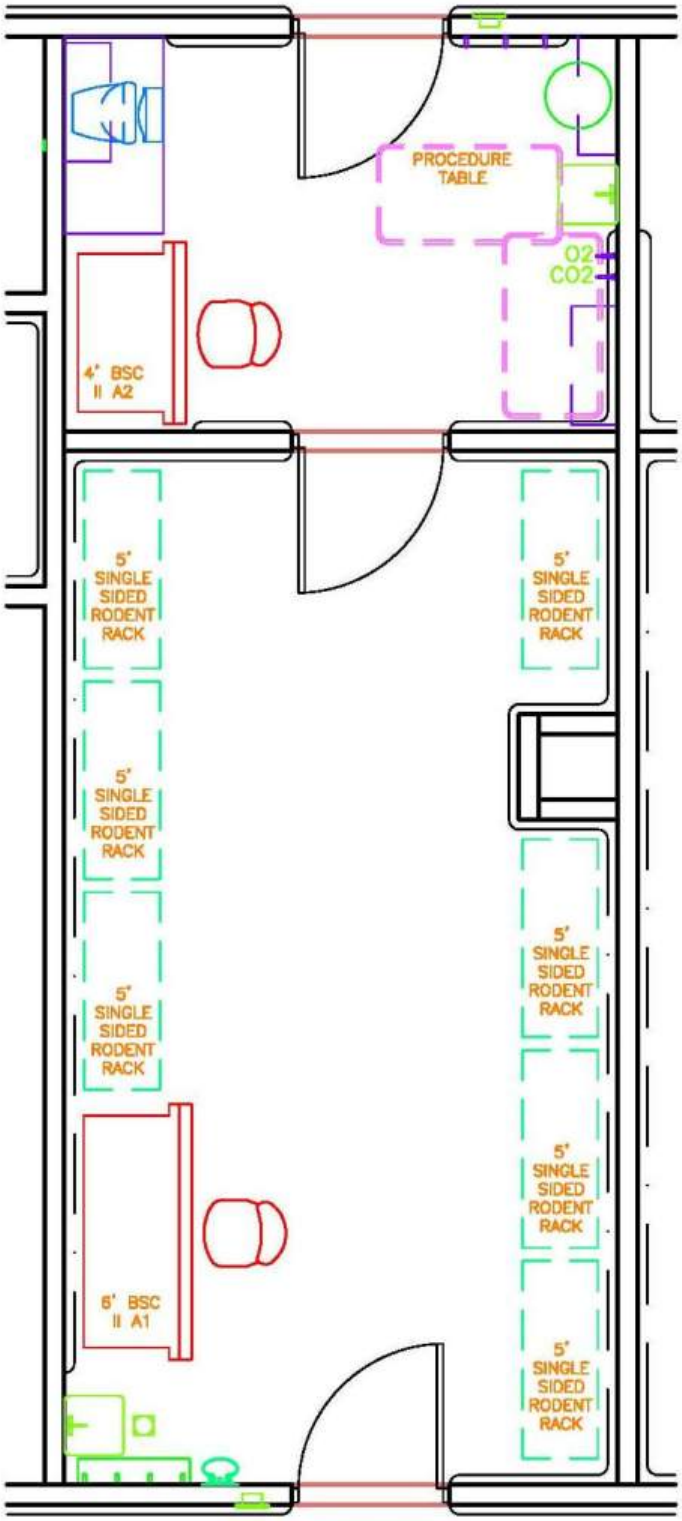

Figure A-5. Typical BSL-3 animal holding.

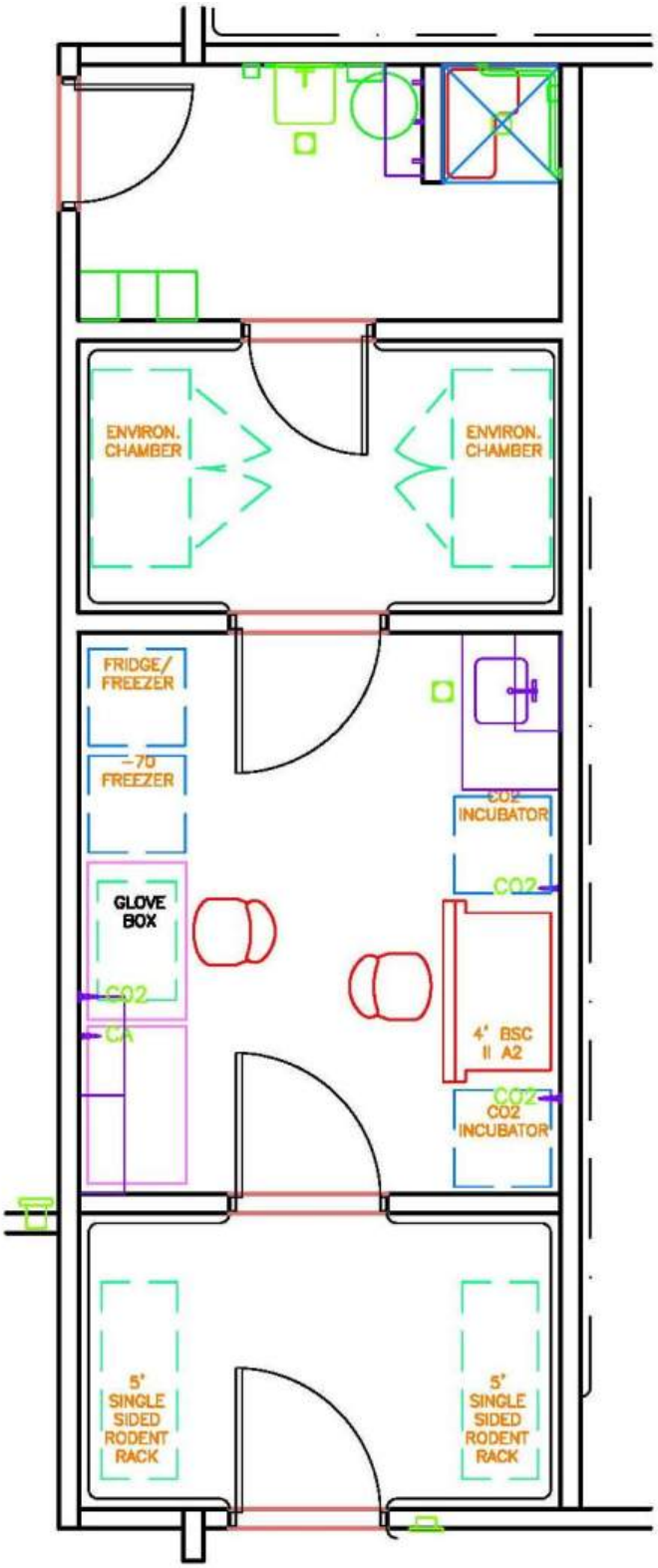

Figure A-6. BSL-3 insectary.

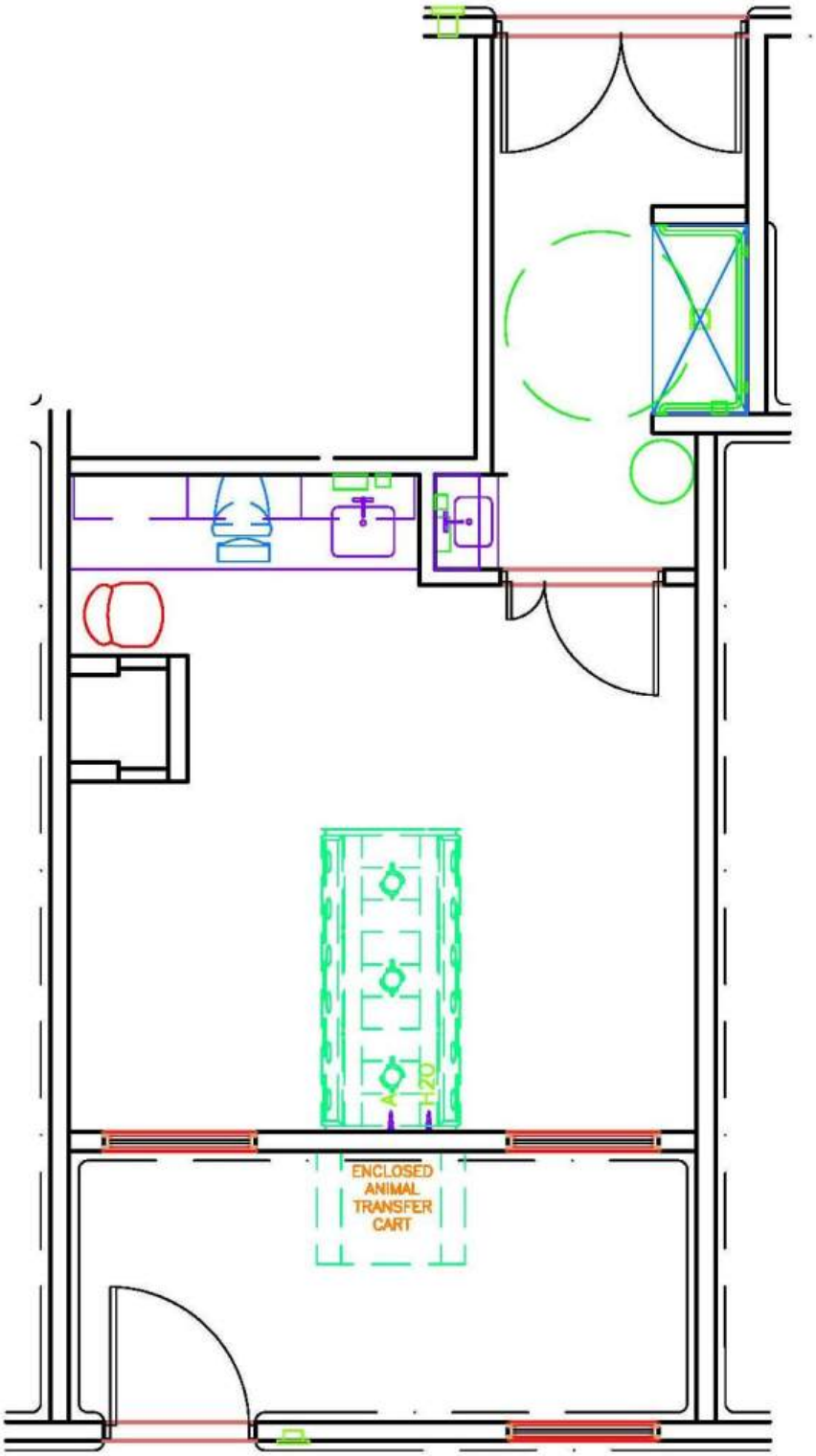

Figure A-7. BSL-3 aerobiology suite.

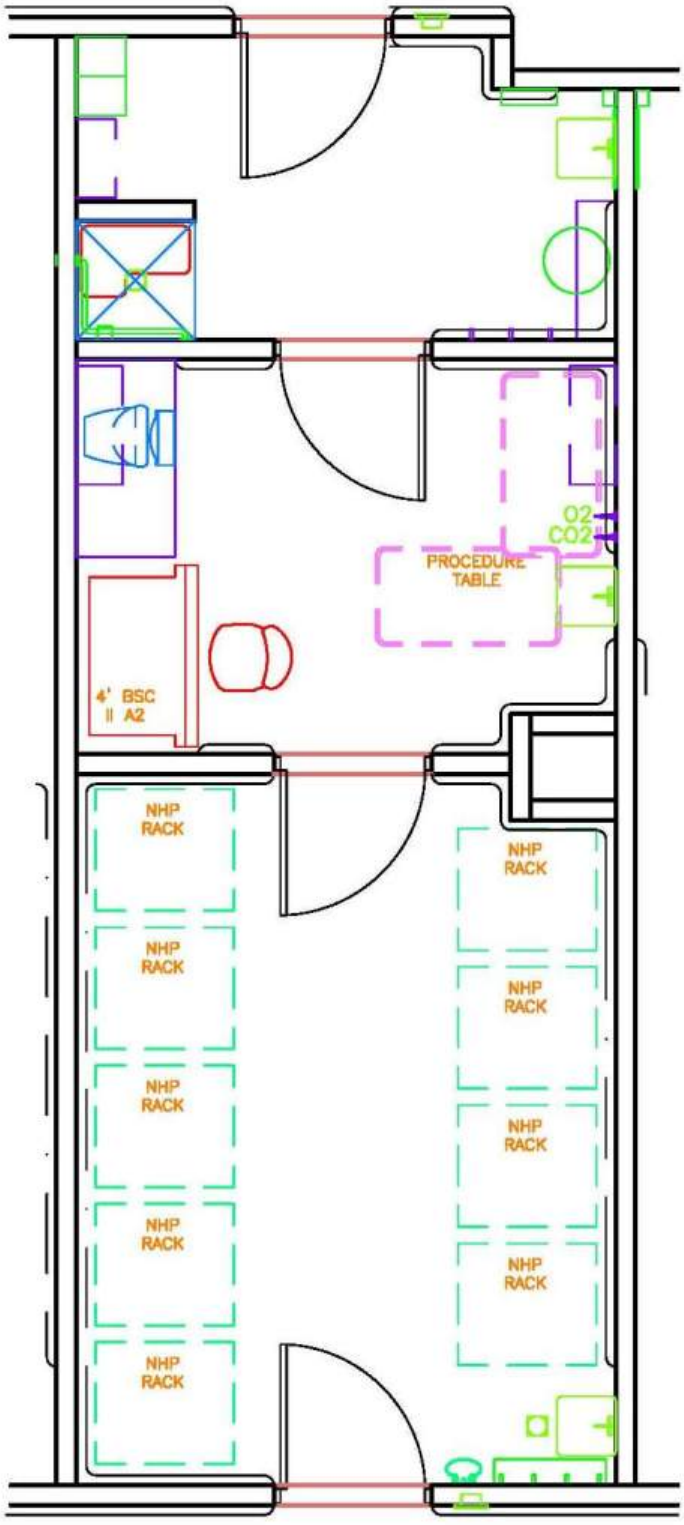

Figure A-8. BSL-3 animal holding with ante room.

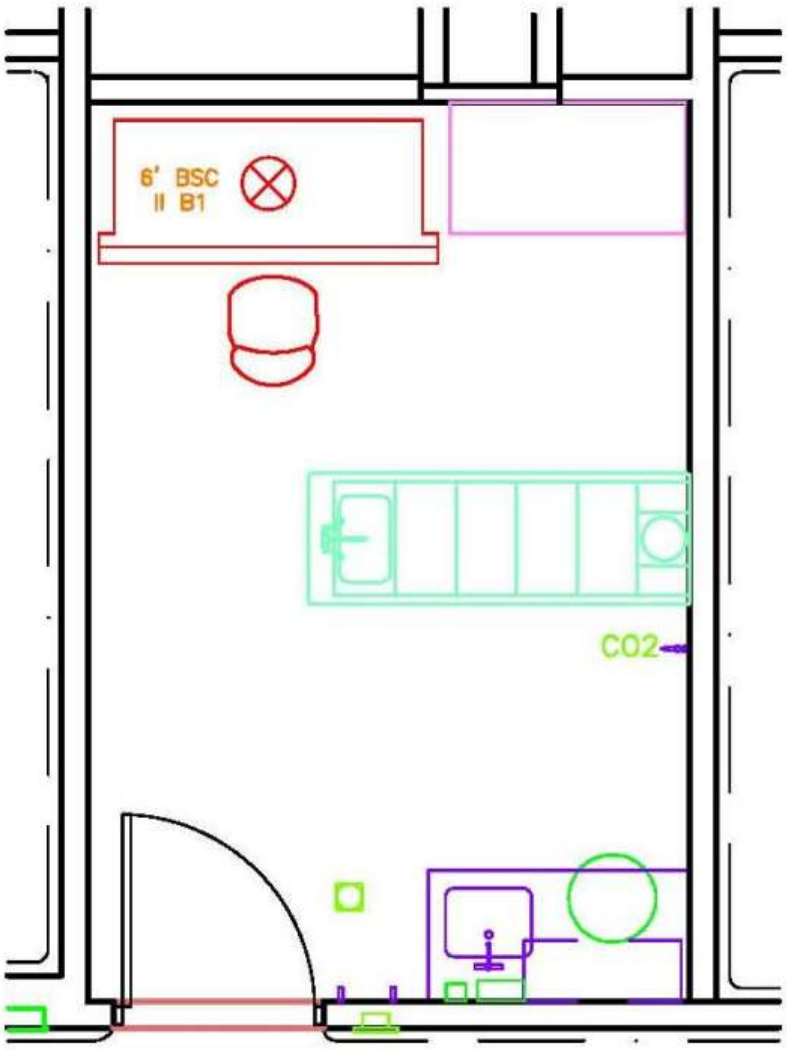

Figure A-9. BSL-3 necropsy.

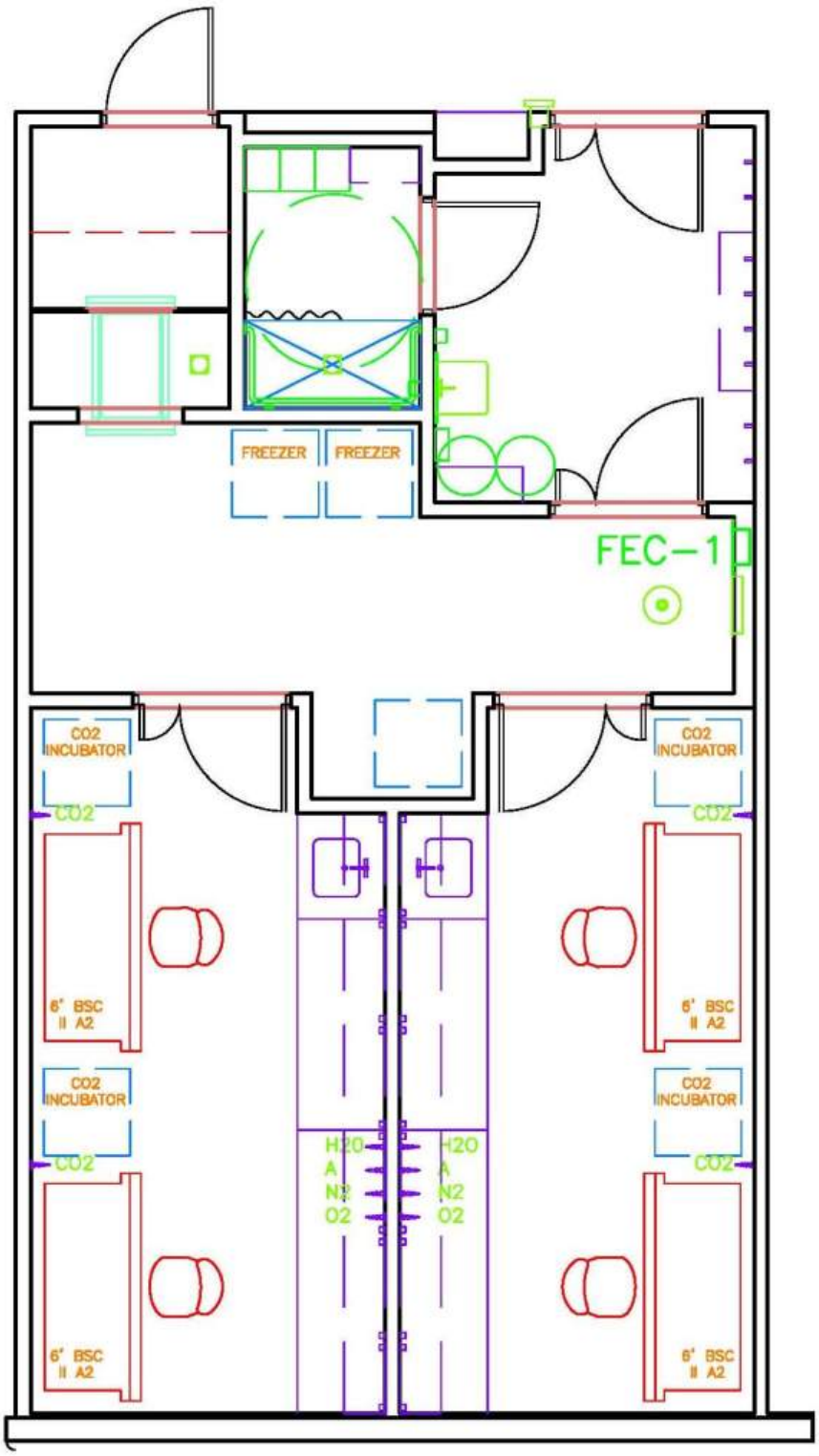

Figure A-10. Typical BSL-3 laboratory.

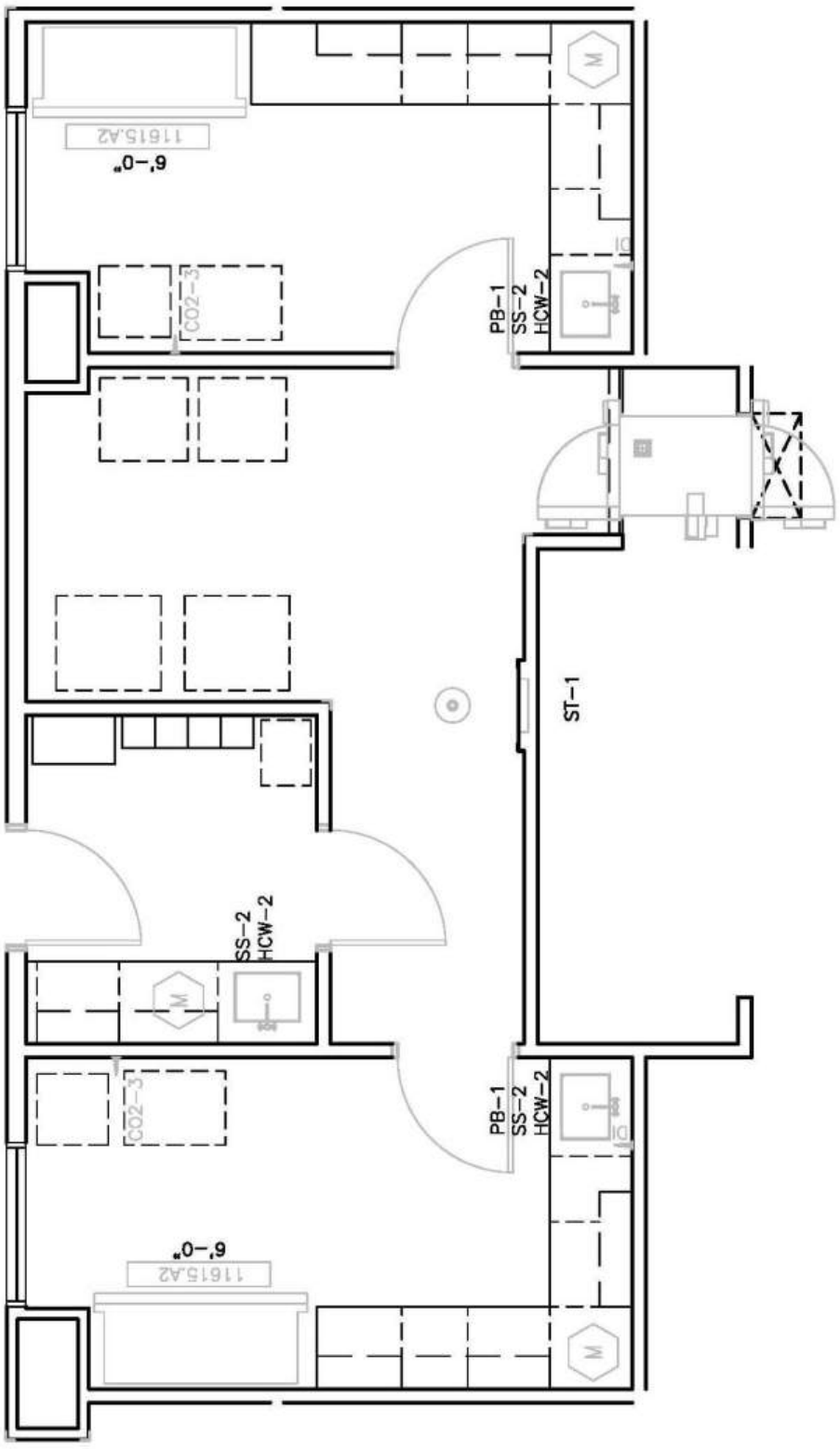

Figure A-11. BSL-3 specimen suite.

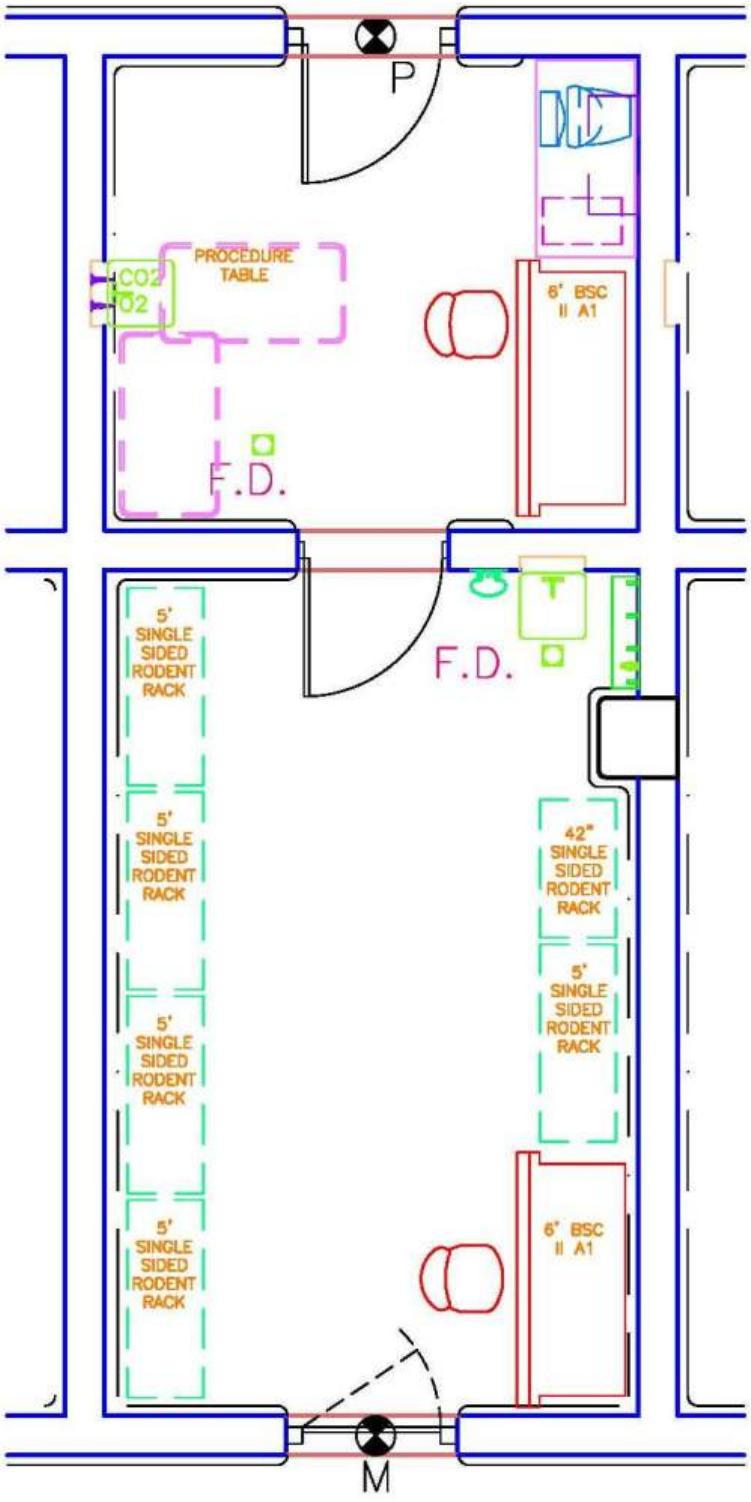

Figure A-12. Typical BSL-4 animal holding.

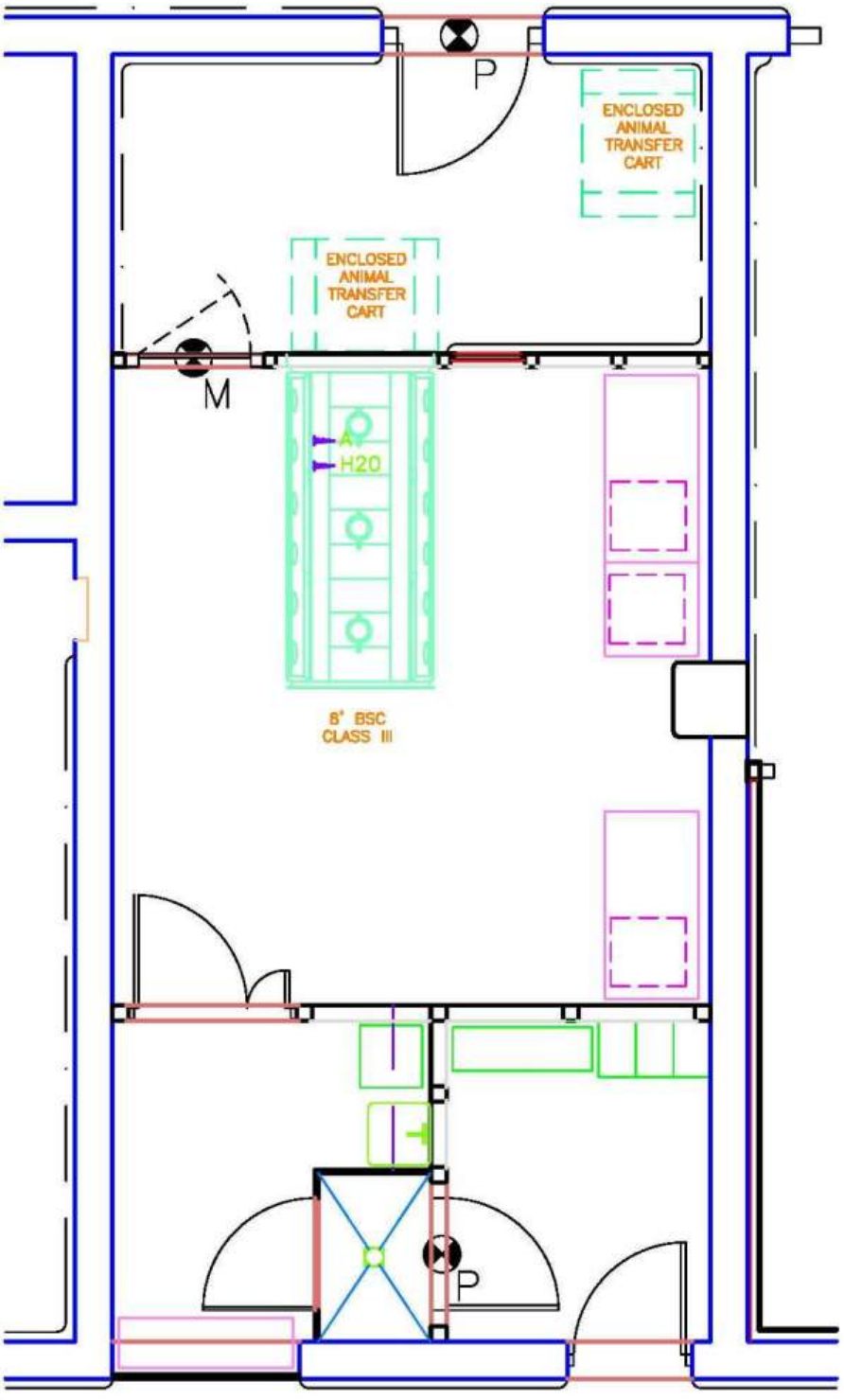

Figure A-13. BSL-4 aerobiology suite.

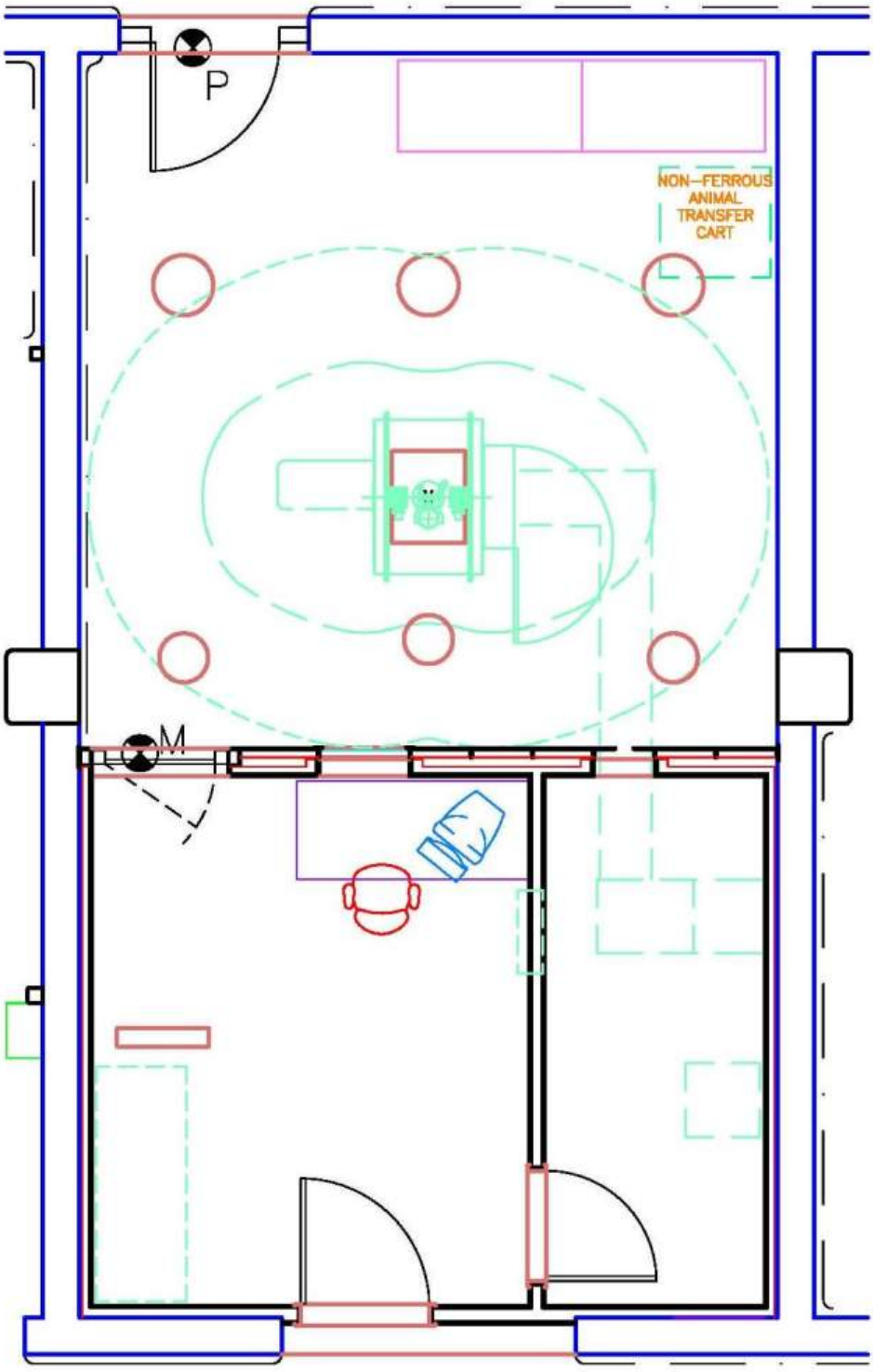

Figure A-14. BSL-4 MRI suite.

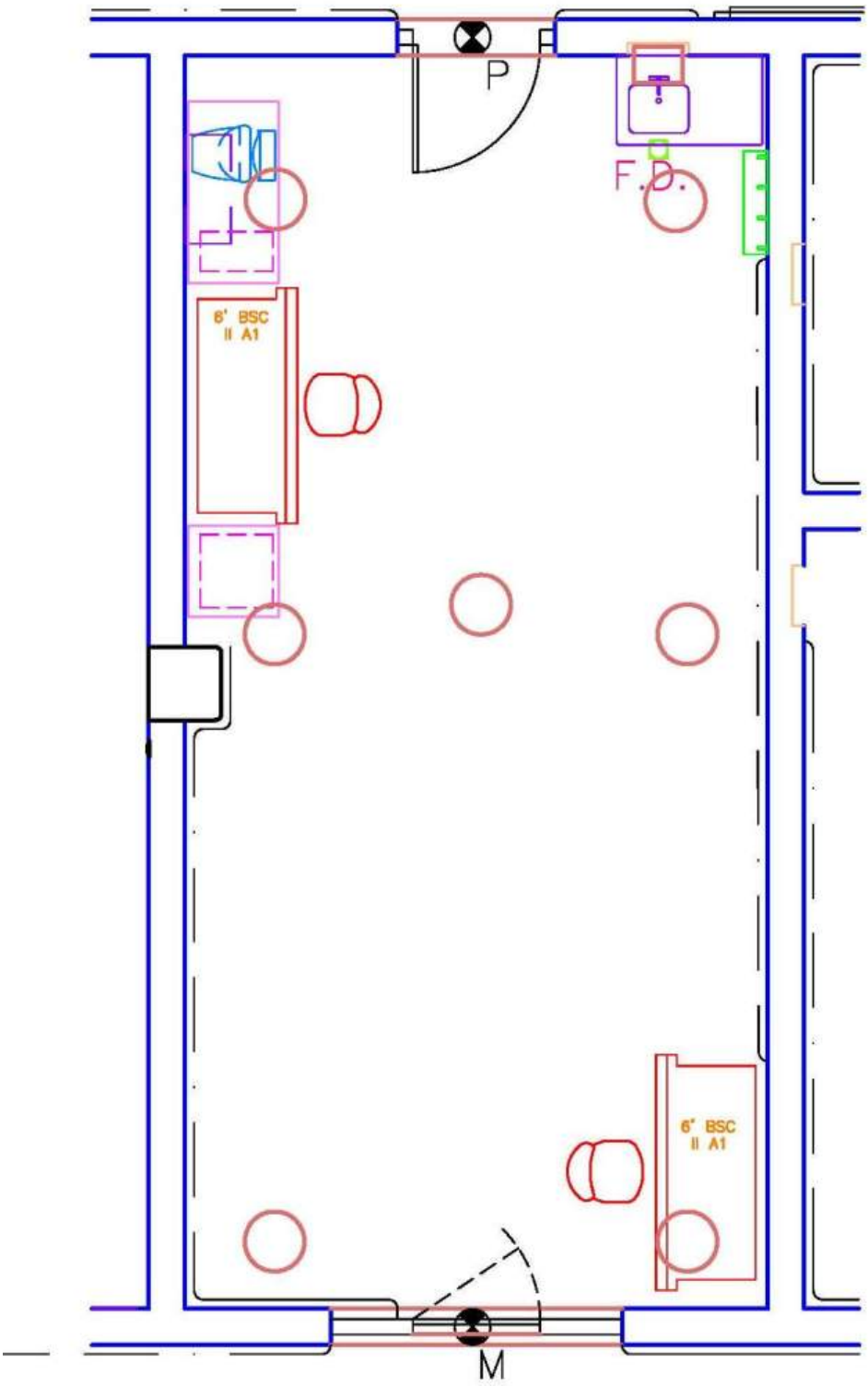

Figure A-15. BSL-4 imaging suite.

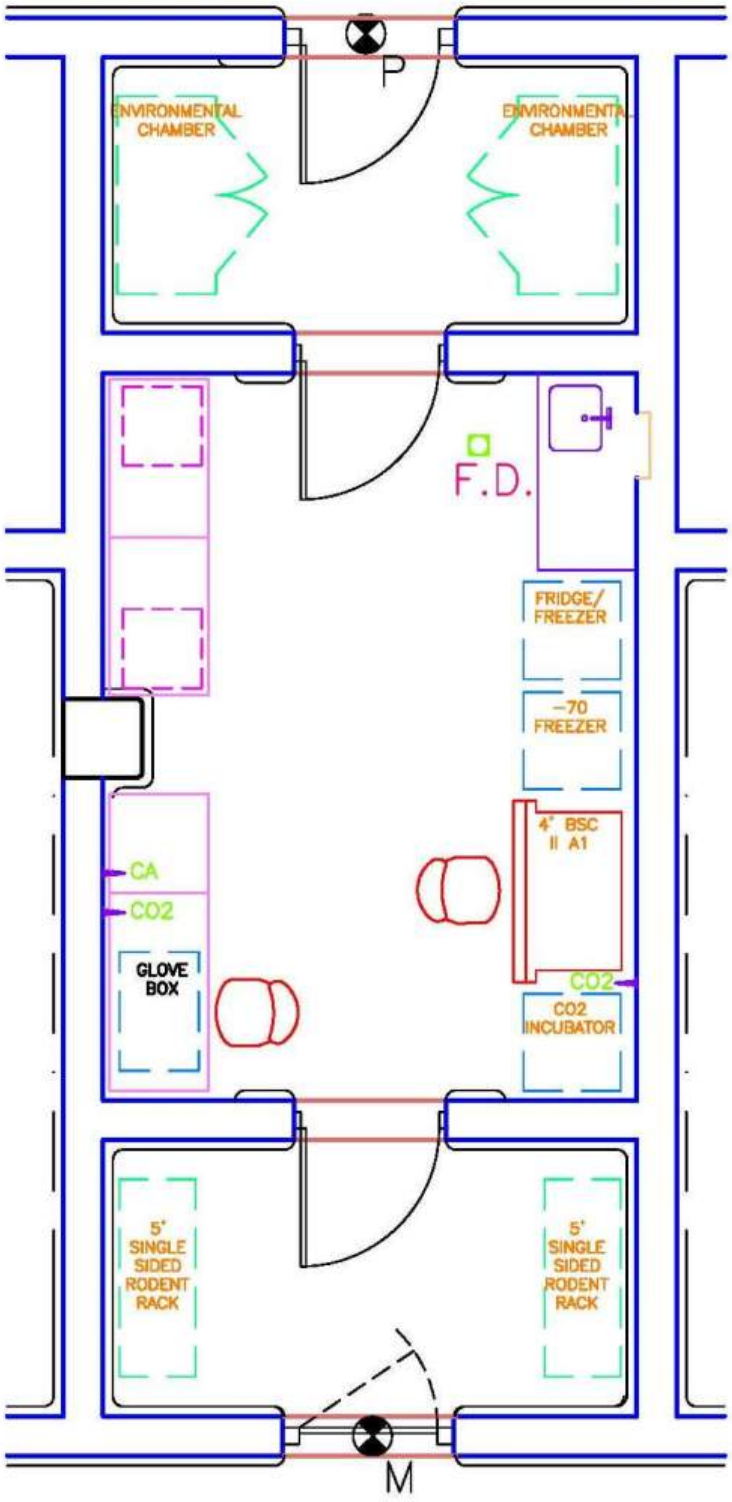

Figure A-16. BSL-4 insectary.

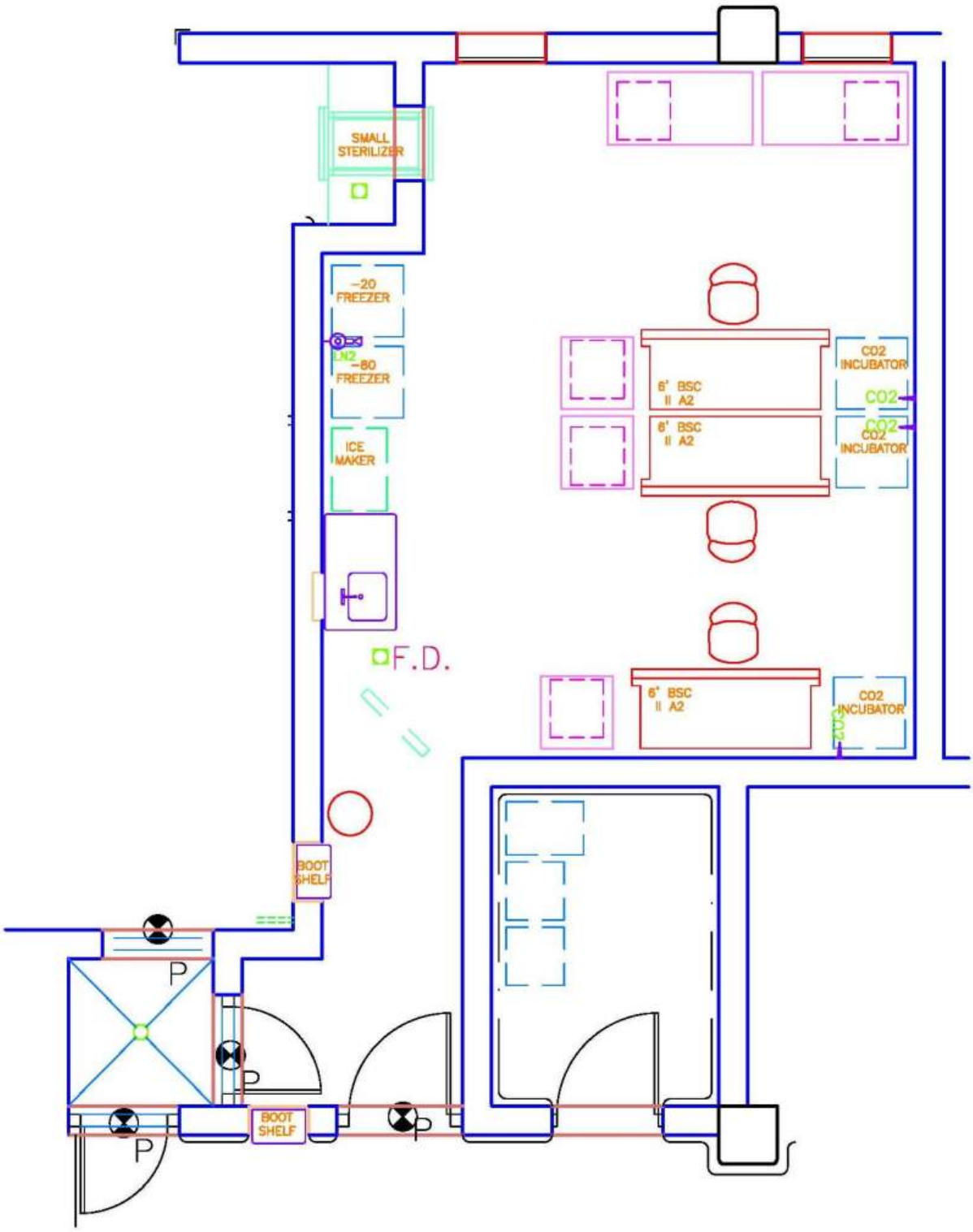

Figure A-17. Typical BSL-4 laboratory.

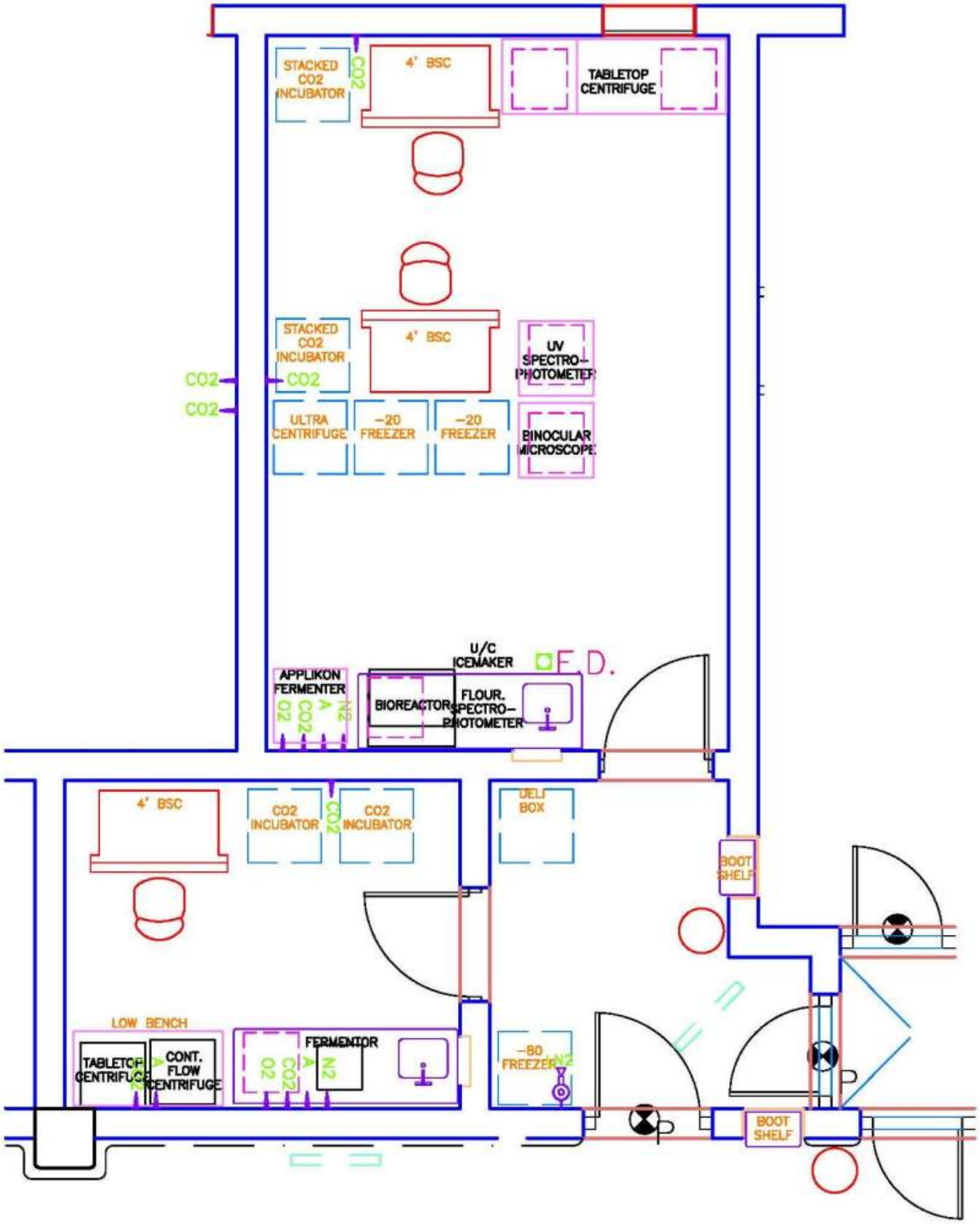

Figure A-18. BSL-4 biomolecular production.

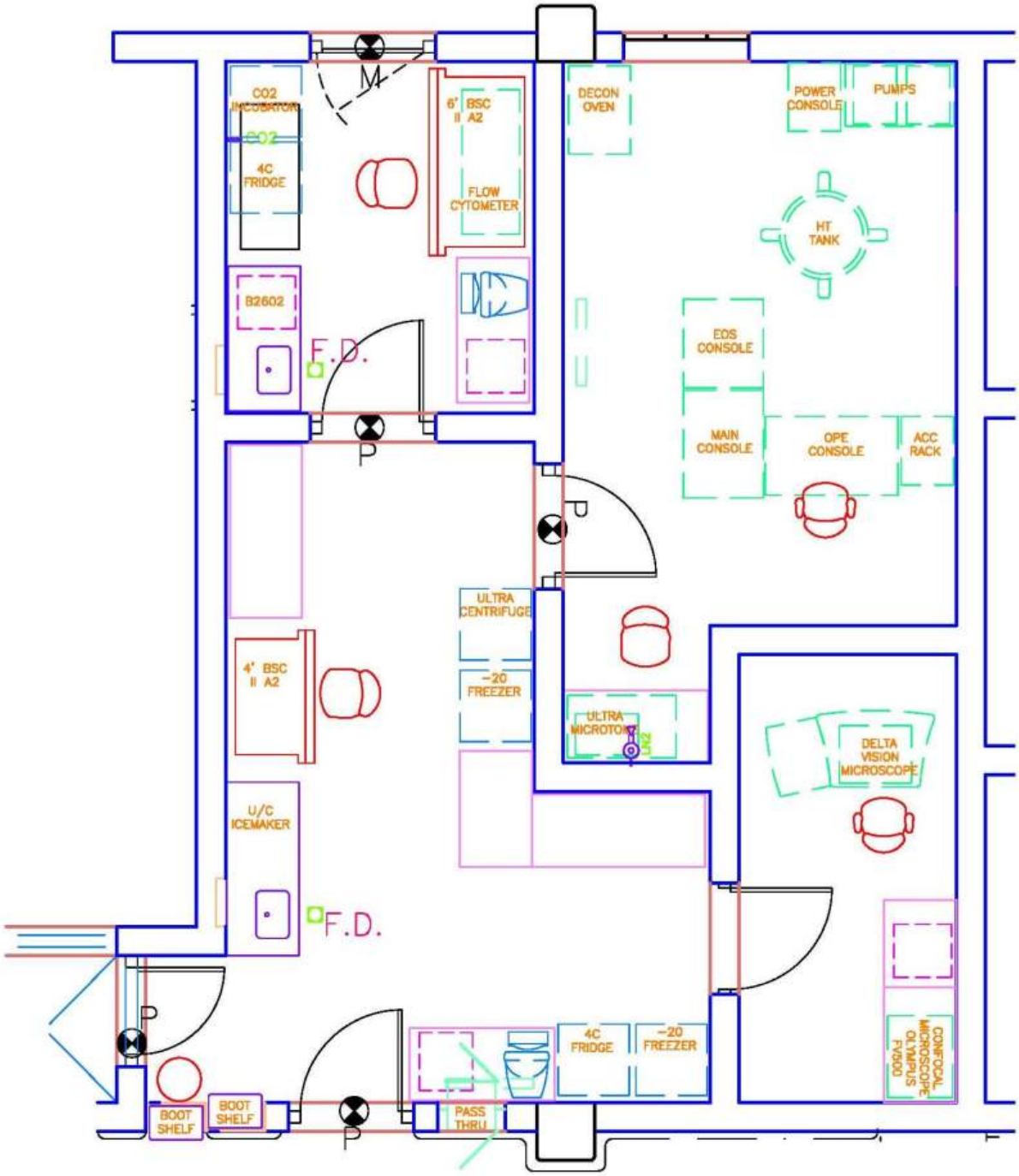

Figure 4-19. BSL-4 microscopy/cryo EM.

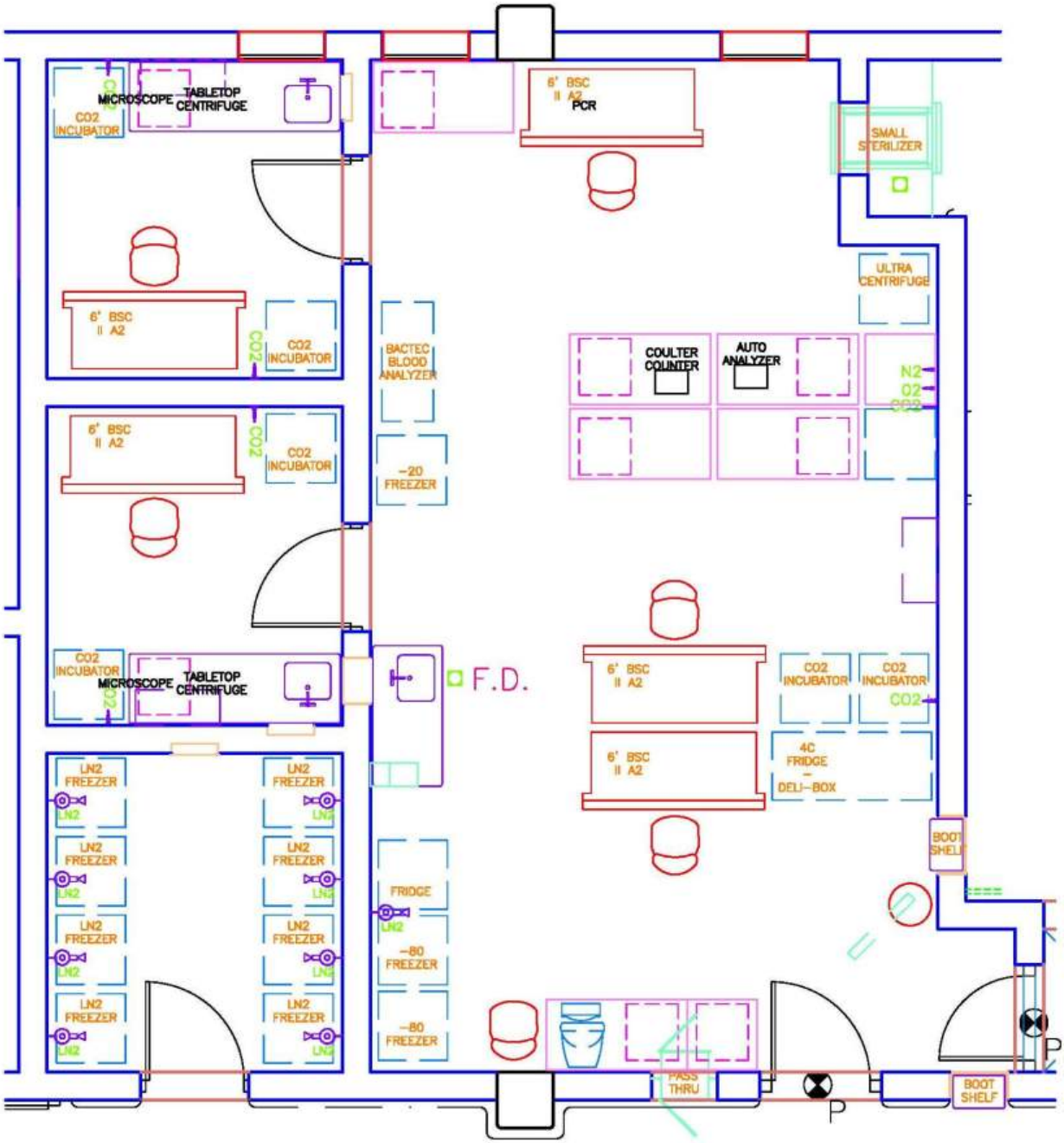

Figure 4-20. BSL-4 specimen processing.

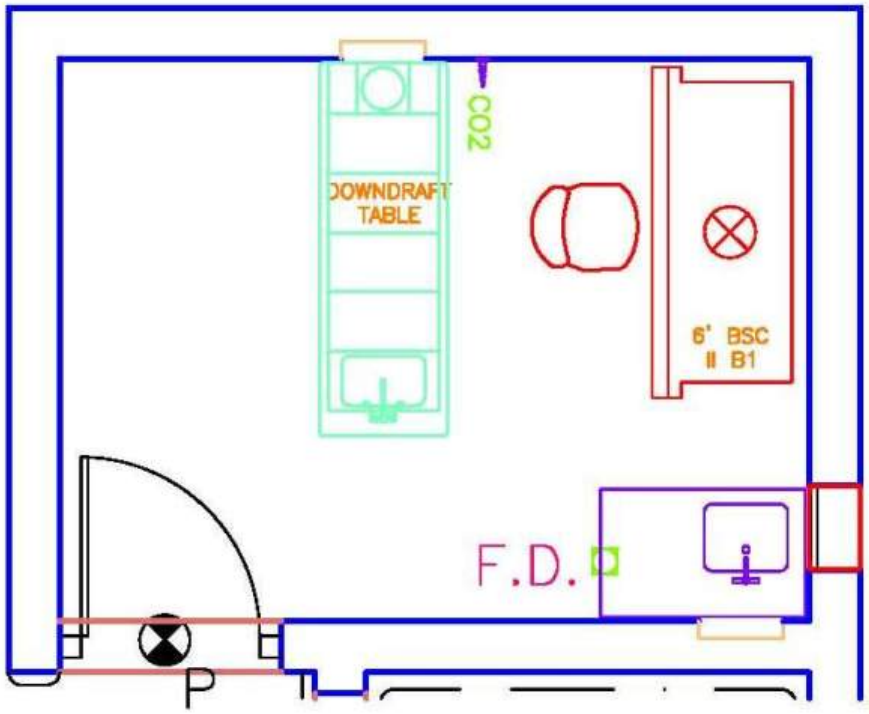

Figure A-21. BSL-4 necropsy.

Appendix B.
Site Characteristics

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B. Site Characteristics

Much of the site characterization information presented in this appendix, such as site location information, utilities descriptions, transportation access, and health care infrastructure was taken from the *Draft Supplementary Risk Assessment and Site Suitability Analyses* (DSRASSA) prepared by National Institutes of Health's (NIH's) Division of Occupational Health and Safety in July 2007 (NIH 2007). The data were augmented by site visits and additional research and data-gathering activities.

B.1 BUMC BioSquare Research Park—Urban Setting

B.1.1 Location

The National Emerging Infectious Diseases Laboratories (NEIDL) site is in the southeast portion of the South End in Boston, within Suffolk County. The South End is a densely developed residential area bordered by institutional and industrial areas south of Harrison Avenue. Commercial activity in the South End is concentrated along Columbus Avenue, Tremont Street, and Washington Street and includes numerous restaurants, while the medical and research uses are concentrated along Albany Street and Harrison Avenue. The institutional and industrial uses south of Harrison Avenue include the Boston University Medical Center (BUMC), the BioSquare, the Boston Flower Exchange facility on Albany Street, and the Suffolk County House of Correction.

B.1.2 Livestock and Animals

According to the Massachusetts State and County Data-2002 Census of Agriculture Suffolk County, in which Boston is located, has no livestock animals in the county inventory. Wildlife in the area would be typical of those adapted to city environments (i.e., mice, rats, feral cats and dogs, and various avian species).

B.1.3 Utilities

Natural gas is provided and purchased from KeySpan Energy. The NEIDL is designed to use either district steam or natural gas as the primary heating medium. Gas service is provided by a natural gas service connection from Albany Street. The NEIDL is capable of using district steam as its heating medium. NStar Electric provides electric service. An existing Boston Water and

Sewer Commission (BWSC) water main, in Albany Street, provides water service, and a BWSC sanitary sewer line in Albany Street provides sanitary sewer service to the NEIDL. Stormwater runoff from the site discharges into the existing BWSC system entering the Roxbury Canal Conduit, which runs through the site and flows easterly toward an outfall in the Fort Point Channel, a coastal waterbody approximately 0.9 mile from the site.

B.1.4 Transportation and Access

Boston is New England's leading port; a regional rail, bus, and truck terminal center; and an important air transport center. Boston is a hub from which many highways extend to serve the cities to the north, west, and south. Route 128 is a highway that circles Boston . The Central Artery provides access to the downtown area, and the Southeast Expressway extends to the South Shore area. The Massachusetts Turnpike (i.e., Interstate [I]-90) crosses Route 128 in Weston and terminates in West Stockbridge, with an extension to the New York Thruway. Amtrak provides passenger service to New York City and Washington, D.C. Massachusetts Bay Transportation Authority (MBTA) subway service is available. Conrail and the Springfield Terminal Railway offer freight service to Boston. Conrail has an intermodal facility in Allston and a Flexi-Flo terminal in Boston. Boston is a member of the MBTA, which provides fixed route bus service in the city and to surrounding towns. The MBTA also provides THE RIDE, a paratransit service for the elderly and disabled. Logan International Airport, easily accessible from downtown Boston, is the busiest Primary Commercial Service facility in New England. Also, Hanscom Field in Bedford, Massachusetts, and Norwood Memorial Airport provide commercial service. The Nashua Street Heliport is near North Station. MBTA commuter boat service is available to Charlestown and Hingham.

B.1.5 Healthcare and Emergency Response

Within Boston's city limits are 22 hospitals and 35 colleges and universities. Many are in the immediate area surrounding the NEIDL site. Boston Metropolitan Medical Response System (MMRS) has a sophisticated, cooperative, multiagency emergency response system. The Boston MMRS develops and exercises plans to mitigate the medical consequences of a weapon of mass destruction (WMD) event by creating a highly trained, readily deployable, fully equipped response system of medical, law enforcement, fire service, and other professionals to support

local resources. Those resources and plans will be used to respond to any major medical incident affecting large numbers of people. Boston University works with the Boston Public Health Commission (BPHC) on emergency planning related to the NEIDL. Those efforts include training responders as well as drills and exercises. Additionally many activities are underway as part of the Boston MMRS and include the following:

- An interagency management team has been established, including officials from the BPHC, the BPHC Communicable Disease Control Bureau (CDCB), Boston Police and Fire Departments, Boston EMS, Boston Emergency Management Agency, U.S. Department of Veterans Affairs, Massachusetts Port Police and Fire Departments, the MBTA Police, the Conference of Boston Teaching Hospitals, representatives of metro-Boston's community and neighborhood health centers, and numerous academic institutions.
- Cooperative partnerships are in place with many of Boston University's schools of pharmacy, public health, and medicine.
- Advanced personnel protective equipment is in place for response personnel. The personnel protective equipment allows responders to remain safe during a response to a WMD event.
- Boston's local pharmaceutical cache is in place, ready for deployment in response to an act of terrorism involving chemical or biological weapons.
- Syndromic surveillance program in operation through BPHC CDCB. The Web-based surveillance system monitors daily volume at 10 acute care hospitals, one community health care center, and call-code volume data from Boston EMS and the Massachusetts Poison Control Center, ensuring that, subsequent to a release of a biological or chemical agent, BPHC can rapidly detect changes in healthcare seeking patterns and mount an effective and coordinated investigation and response.

Expanded citywide personnel training includes the following:

- All EMS personnel are trained to Hazardous Materials (HazMat) Operations level;
- All EMS personnel are trained to Department of Justice WMD Technician requirements;
- Approximately 40 EMS personnel are also trained as HazMat Technicians;

- A pilot HazMat training course is underway for Hospital Providers that specifically addresses issues of hospital-based care for patients involved in HazMat and WMD incidents; and
- A pilot MMRS Volunteer Responder Course is underway, preparing volunteers (i.e., BU students) from Boston's pharmacy, public health, and medical schools to participate in response to a WMD event or other large-scale emergency.

Boston Medical Center (BMC)

Boston Medical Center (BMC) is a private, not-for-profit, 581-bed licensed, academic medical center and is the primary teaching affiliate for the BU School of Medicine. The BMC is the largest safety net hospital in New England providing a full spectrum of pediatric and adult care services, from primary to family medicine to advanced specialty care. BMC is the largest 24-hour Level I trauma center in New England. A full complement of support services is available. BMC is fully accredited by the Joint Commission on Accreditation of Healthcare Organizations (Joint Commission). The Joint Commission is an independent, not-for-profit organization that is the nation's leading accreditor of hospitals. Obtaining Joint Commission accreditation is important for hospitals, as the Medicare Act of 1965 decreed that accredited hospitals were deemed to have satisfied federal health and safety requirements necessary to participate in Medicare. Hospitals also have considerable incentive to become accredited for marketing purposes, often using Joint Commission accreditation as a *third-party endorsement of quality*. As a result, approximately 80 percent of the 6,000 U.S. hospitals have sought Joint Commission accreditation.

The Infectious Disease Section of the Adult Department of Medicine provides Fellow and Attending physician coverage for expert consultation 24 hours a day. Those specialists are available to respond if a NEIDL worker becomes ill. Likewise, pediatric infectious disease attending physicians from the Pediatric Infectious Disease Section of the Department of Pediatrics are available 24 hours a day.

BMC has 60 negative-flow rooms throughout the institution. Five of those rooms are in the Emergency Department (ED). Air to the patient rooms is supplied by general purpose air

handlers equipped with high efficiency HEPA filters. BMC's radiology department is open and staffed by technicians to complete the studies, and radiologists are available to interpret the results 24 hours a day. Radiology suites are on both inpatient campuses and have mobile capabilities to accommodate portable X-rays for patients requiring continuous isolation.

BMC is a BSL-2 lab with service availability 24 hours a day. In addition, BMC is a designated Level A Support lab for the Massachusetts State Laboratory. BMC participates in multidisciplinary, interdepartmental planning for emergencies. BMC's planning activities include active participation in, and plan integration with, regional and state entities, such as the following:

- Conference of Boston Teaching Hospitals Disaster Committee;
- Region 4 C Surge Committee;
- Urban Area Security Initiative Region Homeland Security Committee;
- Metropolitan Medical Response System Committee;
- Local Emergency Planning Committee; and
- Massachusetts State Surge Committee.

The following Mutual Aide Agreements are in place with the following organizations:

- MMRS—Boston Hospitals: Share services, staffing, and supplies;
- Lemuel Shattuck Hospital—Capacity – Isolation specialty;
- Quincy Medical Center—Capacity;
- Boston Fire Department—Decontamination;
- Massachusetts Department of Public Health—Regional benchmarks for preparedness, laboratory support, National Disaster Medical System (NDMS) participating hospital;
- Centers for Disease Control and Prevention Quarantine Center Boston Logan Airport—Designated Receiving Hospital; and
- Vendor contracts for critical supplies.

BMC routinely participates in emergency response drills and exercises. The BMC also has experience in responding to mass casualty events and disasters. The BUMC emergency response

activities are supported by the Boston Police Department. The new headquarters is equipped with perhaps the most advanced imaging and ballistics identification technology in the country; a DNA laboratory (one of only 18 departments in the country with in-house DNA testing capacity; and an enhanced 9-1-1 call center, and a Computer-aided Dispatch system linked to Mobile Data Terminals.

B.2 Tyngsborough, Massachusetts (Suburban)

B.2.1 Location

The town of Tyngsborough is a small residential community in the northwest section of Middlesex County, in northeastern Massachusetts. Composed of 17.86 mi^2 (46.3 km^2) of land and surface water, bordering towns include Dunstable and Groton on the west and northwest; Westford and Chelmsford on the south; Dracut and Lowell on the east; and Nashua and Hudson, New Hampshire on the north. The town is bisected by the Merrimack River. Tyngsborough is about 7 miles (11.27 km) west of Lowell, 31 miles (49.89 km) northwest of Boston, 26 miles (41.84 km) northeast of Fitchburg, and 235 miles (378.20 km) from New York City. Tyngsborough is dotted with numerous streams, lakes, great ponds, and wetlands.

The suburban site is on a 210-acre (0.85 km^2) forested site overlooking a private pond. The site contains a mix of pine and mixed hardwood forests. Several old, abandoned quarries are found within the borders of the property. The site is bordered by residential areas on all sides except for the southeast property line, which is bordered by fields. The property is in the Merrimack River watershed, and all stormwater and groundwater is carried to the river. Stormwater from the site runs into a small brook on-site that flows into the Merrimack River.

B.2.2 Livestock and Animals

According to the Massachusetts State and County Data-2002 Census of Agriculture, Middlesex County, in which Tyngsborough is located, had

- 2,827 cattle and calves;
- 2,214 hogs and pigs;
- 1,964 horses and ponies;
- 816 sheep and lambs;

- negligible populations of goats; and
- 16,691 poultry species (i.e., chickens and turkeys).

Livestock populations in the vicinity of the site are low or negligible. The numbers of livestock have been decreasing since the 1997 census. Wildlife present are typical of those found in Massachusetts and composed of amphibians, birds, fish, mammals, and reptile species, such as deer mice, shrew, voles, rats, chipmunks, bats, squirrels, raccoons, bobcats, black bear, rabbits, fox, coyotes, white tailed deer, and moose. The varied habitats in the Merrimack River watershed support almost any bird species found in Massachusetts.

B.2.3 Utilities

If the NEIDL were constructed at the suburban site, Electric power would be supplied by the Massachusetts Electric Power Company. Electric power for chillers is on the grid that supplies the town. Heating most likely would be provided by natural-gas-fired boilers supplied by the town utility. The site is supplied by the municipal water system and sewage is disposed of through an on-site sanitary disposal system with a leaching field that was installed 10 years ago. Municipal water service is available at the site. Municipal sewer service is not available from Tyngsborough. It might be possible to tie into municipal sewer service from the adjacent town of Chelmsford approximately 600 ft (182.88 m) away. Water for fire suppression is obtained from a fire pond on the property. The NEIDL would probably tie into existing electrical and natural gas lines.

B.2.4 Transportation and Access

The development of transportation resources in the Merrimack River Valley, in which Tyngsborough is located, was shaped by the history of the region as a major site of American industrial development in the nineteenth century. The area has highway and rail facilities linking major cities and towns to each other and to the port, airport, and intermodal facilities of Boston. Principal highways are U.S. Route 3 running north-south between Nashua, New Hampshire, and the Boston region, and State Route 113. Tyng Road, the main access road to the property, is a collector street/quiet residential road with limited traffic. It is a country road with no lines and no

shoulder. The existing road infrastructure might not be ideal for the types and volume of construction, operational, and service-related NEIDL traffic.

Commuter rail service to North Station, Boston, is available from neighboring Lowell. Travel time is 45–49 min, and 680 MBTA parking spaces are available. Freight rail service is available from the Springfield Terminal Railway. Tyngsborough is a member of the Lowell Regional Transit Authority, which provides fixed bus service between Lowell and Tyngsborough. Paratransit services for the elderly and disabled are available through the Tyngsborough Council on Aging.

The Tew-Mac Airport, a General Aviation facility has two asphalt runways 600 ft x 60 ft and 2,830 ft x 26 ft (182.88 m x 18.29 m and 862.58 m x 7.92 m). Non-precision instrument approaches are permitted. Other nearby airports are the Lawrence Municipal Airport in North Andover and L.G. Hanscom Field in Bedford. The region is served by Logan International Airport for most commercial and general aviation needs, and the primary airport that would service the NEIDL if it were sited at Tyngsborough. There are no direct mass transportation opportunities directly to the site.

B.2.5 Healthcare and Emergency Response

Tyngsborough has professional police and fire departments and a professional staff assisting the Board of Health. The police department employs 25 full-time officers. The fire department employs 38 on-call firefighters, rotated through four full-time slots. The nearest fire station is 1.7 mi (2.74 km) north of the site. The small, local department could be challenged to respond to the safety and security requirements of a major research facility.

Tyngsborough does not have a hospital within the town limits but is served by Lowell General Hospital (LGH) approximately 8 miles away across the Merrimack River. LGH is a 200-bed community hospital with a 12-bed intensive care unit (ICU); an 18-bed intermediate care unit; 2 medical/surgical units with 40 beds each; and pediatrics and maternity services and an outpatient cancer center. LGH has a daily patient census of 130–135 with between 11,000 and 12,000 discharges per year. LGH also operates an off-site outpatient surgery center. The ED has a

negative-pressure isolation room with no anteroom. Eight standard isolation rooms are in other areas of the hospital. One of those rooms has an anteroom. LGH has a Health Resources and Services Administration grant to renovate the isolation room in the ED to provide exhaust from that room directly to the exterior of the facility.

LGH has both an infection control practitioner and an emergency response coordinator. Two flexible patient transporters and two mass decontamination units are available on-site, as well as a portable chemical decontamination shower for use at the ambulance entrance. Emergency drills are performed once a year. Ambulance services are provided by a local company under contract to LGH with an established patient transport agreement and requisite protocols. An employee parking lot is cleared in the event that patient transport by helicopter is necessary. Members of the medical staff are board certified specialists; however LGH does not have an infectious disease specialist on staff though there is an infectious disease physician who provides consultations as necessary. A full-service clinical laboratory is on the premises, augmented by a reference laboratory as needed. LGH is fully accredited by the Joint Commission. If a serious incident, exposure, or infection occurred in an individual associated with the NEIDL, LGH would stabilize and then transport the patient to a more comprehensive healthcare facility by an appropriate method (e.g., ambulance, helicopter) according to the patient's stability and other factors such as weather conditions. In addition to LGH, the Tyngsborough area has a small number of ambulatory healthcare services (e.g., doctor's offices, clinics) and convalescent/nursing residential care facilities.

B.3 Boston University (BU) Sargent Center for Outdoor Education (Rural)

B.3.1 Location

The ruralsite is in Hillsborough County within the town limits of Peterborough, zoned as a Rural District in the scenic Monadnock Region of southwestern New Hampshire 85 miles (136.79 lm) from Boston and 125 miles (201.17 km) from Hartford, Connecticut. The site, composed of 700 acres (2.83 km^2) of open fields, forested land, streams, wetlands, and a river, is bordered on the north by Hancock, New Hampshire; to the northeast by Hunt's Pond and Nubanusit Lake; to the south by West Peterborough and the McDowell Artist Colony; to the southeast by Peterborough,

Sheiling Forest and the Wapack Nature and Wildlife Preserve. A 60-acre (0.24 km^2) pond is on the site (Halfmoon Pond), a 20-acre (0.08 km^2) open meadow, and 22 miles (35.41 km) of walking and hiking trails.

B.3.2 Livestock and Animals

According to the New Hampshire State and County Data-2002 Census of Agriculture Hillsborough County, in which Peterborough is located, has a significant number of livestock animals. According to the 2002 agricultural census, 2,325 cattle and calves, 3,774 goats, and 461 bison were in the county. Wildlife present are typical of those found in southern New Hampshire and composed of amphibians, birds, fish, mammals, and reptile species such as deer mice, shrew, voles, rats, chipmunks, bats, squirrels, raccoons, bobcats, black bear, rabbits, fox, coyotes, white tailed deer, and moose. The varied habitats along the Contoocook and North Branch rivers support almost any bird species found in southern New Hampshire. Many species of waterfowl use the river as a migratory stop in the spring and fall.

B.3.3 Utilities

Electric power is provided by Northeastern Utilities; two diesel-fired back-up generators (150 kilowatts [kW] each)—one for the north circle and for the south circle are provided for back-up power because power failure is fairly routine in the area. Heat at the rural site is provided by a combination of liquid propane gas and fuel oil, both stored underground. On-site water is provided via wells from an aquifer running under the property, and septic fields and a sewage lagoon complete the utilities. Peterborough has a municipal sewage collection and treatment system; however, the rural siteis not currently served by the sewerage system.

Fueling NEIDL operations at the other location would be a challenge. The site does not tie into natural gas lines. Therefore, either large, aboveground storage tanks would be required to fuel all operations or natural gas tie in lines would need to be installed to support the facilities needs.

B.3.4 Transportation and Access

Road access to the area is somewhat limited to U.S. Route 202 and State Routes 101, 123, and 136. The nearest interstate routes are Everett Turnpike and I-89, 27 and 33 miles (43.45 and

53.11 km) away, respectively. Access from the west is via a dirt road. The university encourages traffic to arrive from the east; however, the road is an unlined country road with no shoulder. Access to the site is by a rural, unpaved road that would not support the needs of a large biomedical research facility. The existing road infrastructure would not support the types and volume of construction, operational, and service-related NEIDL traffic. No railroad or public transportation is available near the site. The region is served by Logan International Airport for most commercial and general aviation needs, and the primary airport that would service the NEIDL if it were sited at the rural site.

B.3.5 Healthcare and Emergency Response

Peterborough has three full-time administrative employees; a town administrator, assistant administrator, and town clerk. The town has a volunteer fire and rescue service department with one full-time employee and 50 volunteer and on-call firefighters. The Peterborough fire station 4.8 mile southeast of the site (7.72 km). The Hancock fire station is 3.9 miles (6.28 km) from the site, which is approximately a 9-min drive north of the site. The town has 12 full-time police officers. The local Board of Health is made up of volunteers who might not necessarily be trained to address health and safety issues associated with a research facility. The nearest medical facility is Monadnock Community Hospital (MCH) in Peterborough, a 7.4 mi (11.91 km) from the proposed site. MCH at 452 Old Street Road, is a 25-bed acute care facility (licensed for 62 beds) including 4 ICU beds, 7 obstetrical beds, and 14 medical surgical beds. MCH is the primary care facility for 13 surrounding towns and serves a total population of approximately 36,000. The large majority of patients are Medicare patients; many people in the surrounding area are either self-employed or employed by small businesses and do not have other health insurance.

The MCH ED offers health services 24 hours a day, 7 days a week to patients of all ages with all presenting complaints. The ED is responsible for the immediate treatment of any medical or surgical emergency; for initiating life saving procedures in all types of emergency situations; and for providing emergency and initial evaluations and treatment for other conditions including minor illnesses and injuries, and sub-acute medical problems. After initial assessment and stabilization, patients are transported to other medical institutions if necessary. The MCH ED

services about 12,000 patients per year, most of which are primary care visits. It has two trauma rooms with two beds each, and one of the other rooms is maintained under slight negative pressure to have inward airflow. Decontamination showers are available at the MCH ED, and the MCH has a decontamination trailer and a heliport with room for a second. The hospital has an Emergency Response Plan that was developed in conjunction with town administrators and operates under the Hospital Incident Command System.

In the event of the need to transport a patient to a tertiary care facility, either the Monadnock Emergency Medical Services or the Dartmouth-Hitchcock Advanced Response Team (DHART) would be used. The DHART is based in Lebanon, at Dartmouth-Hitchcock Medical Center, New Hampshire's only verified Level 1 Trauma Center. DHART crews provide both ground and air medical transportation services to the medical communities of Northern New England. In addition, DHART flight crews respond to public safety agency requests for medical evacuation of trauma patients from scenes of injury and will transport to the closest trauma center in the region's five states. University of Massachusetts-Worcester helicopters can also be used. BUMC is one and a half hours away by ground transport or 35–40 min by helicopter. Transport decisions are based on the case urgency and prevailing weather patterns. Under severe weather conditions, transportation of an ill NEIDL worker would be problematic. The MCH medical staff includes more than 125 primary and specialty care physicians, 15 dentists, and 23 health professional affiliates. Medical staff offices are in the Medical Arts Building on MCH's campus and in the communities of Peterborough, Jaffrey, Antrim, and New Ipswich. One hundred percent of the medical staff are board certified in their specialty area. However no infectious disease specialists are at MCH. An infectious diseases specialist consults as necessary by telephone. A pathologist is available two days a week in the clinical laboratory. MCH is not accredited by the Joint Commission. The hospital is accredited as *Critical Access* through the Centers for Medicare and Medicaid Services (CMS, formerly the Health Care Financing Administration).

Peterborough is one of the most flood-prone areas in the state and has been included in three disaster declarations since 1987. It is subject to a variety of natural hazards including riverine flooding, wildfires, ice storms, and river ice jams. The town has more than 40 dams, 2 of which

have been classified as high-hazard dams. Specifically, the rural site property is encompassed by a Special Flood Hazard Area designated as Zone A, a 100-year floodplain. The site is adjacent to U.S. Army Corps of Engineers flood control easements and the spillway for the MacDowell Lake. In an emergency, the spillway can be opened to prevent Peterborough from flooding. Peterborough has joined the disaster resistant efforts of the Federal Emergency Management Agency's Project Impact, a national effort to change the way disasters are handled. That effort shifts the focus of emergency management from responding to disasters to taking actions before disasters that reduce potential damage. Peterborough has adopted a strict floodplain management program and introduced intensive community growth management efforts, including planning for open space and conservation areas as a Project Impact disaster-resistant community.

B.4 References

NIH and DHHS (National Institutes of Health and Department of Health and Human Services), 2005. Final Environmental Impact Statement, National Emerging Infectious Disease Laboratory, Boston, Massachusetts. December.

CUH2A, Smith Carter, and Hemisphere Engineering 2005 National Emerging Infectious Disease Laboratory, Basis of Design, 100 percent Construction Drawings. November.

CDC and NIH (Centers for Disease Control and Prevention and National Institutes of Health). 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. U.S. Government Printing Office, Washington, DC.

BUMC 2008. Boston University Medical Center Biosafety Manual. Revised. October 2008.

BUMC 2009a. Telephone Meeting Notes, Uninterruptible Power Supply (UPS), Emergency On-Site Power, and Spill Response. Conversation with Tetra Tech, Inc. June 19, 2009.

BUMC 2009b. Tetra Tech Site Visit. December 4, 2008 and June 1-4, 2009.

BUMC 2009c. Boston Public Health Commission Biological Laboratory Safety Permit Application, March 3, 2009

BUMC 2009d Telephone Meeting Notes, HVAC/Ventilation System Overview, Conversation with Tetra Tech, Inc. June 16, 2009.

Kajunski, Joe 2009. Telephone Meeting Notes, HVAC. Conversation with Tetra Tech, Inc. June 19, 2009.

NIH 2008. *National Institutes of Health (NIH) Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities (DRM)*, 2008. Available on the web at http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/DesignRequirementsManualPDF.htm

Appendix C.

Pathogen Characteristics

Table of Contents

C. PATHOGEN CHARACTERISTICS

C.1 INTRODUCTION

The following data are selected findings from literature searches that were conducted by TetraTech scientists and subject matter experts on the Health and Human Ecology team, using methods and materials as described in Chapter 3. Results were sorted by pathogen and by biosafety level (BSL) designation, with references provided such that questions or needs for additional information by the Tetra Tech team could be efficiently addressed. These carefully documented data were organized and updated as a set of working notes for internal use by TetraTech in preparing the Risk Assessment. The data are presented here in the form used by the scientists and subject matter experts, and are presented solely for purposes of contributing to transparency of the Risk Assessment process. These data have not been substantially edited or reorganized for the lay reader. Instead, for that purpose, Chapter 3 has been constructed as a summation of key points about pathogen characteristics, and interested readers are directed there for representative information that has been selected and presented in a format that, in general, is more easily understood by the lay reader. Data are representative of the open literature as of January 2009. A cursory systematic update from the open literature was performed in April, 2010. Other information subsequently was added as needed. Any additional relevant data that might exist under classified status were not available to TetraTech.

C.1.1 BSL-3 Pathogens

C.1.1.1 Bacillus anthracis (B. anthracis)

Host range

a. Field

Natural reservoir is soil (typically pH >6) (Turnbull et al. 1998) (Hugh-Jones and Blackburn 2009). No animal reservoirs are known (Turnbull et al. 1998).

Grazing herbivores, especially cattle, sheep, goats, and horses are particularly susceptible; pigs are more resistant (Beyer and Turnbull 2009).

Not typically communicable between animals except through consumption (i.e. consumption by predatorsor scavenger, and those species are relatively resistant to infection) (Beyer and Turnbull 2009).

Birds are resistant with some exceptions such as the ostrich (Beyer and Turnbull 2009).

Outbreaks recently in Canada (Kumor, Bates, and Stephens; Center for Infectious Disease Research & Policy 2006).

Outbreaks in chimpanzees in Ivory Coast and gorilla in Cameroon (Leendertz et al. 2006; Leendertz et al. 2004).

b. Experimental

Mice, rats, guinea pig, and NHPs have been used for experimental models of disease (Welkos, Keener, and Gibbs 1986; Goossens 2009)

Prevalence/incidence/ attack rate

26,954 human cases in Turkey between 1960 and 2005 (Doganay and Metan 2009). Incidence per 100,000 ranged from 0.38–1.08 yearly (yearly data available 1990–2005) (Doganay and Metan 2009). 426 of those were reviewed: 96.9 percent were cutaneous, 1.9 percent were GI (1 case intestinal, 7 cases OP), 1.2 percent were meningitis (Doganay and Metan 2009).

2,000 to 20,000 human infections annually in Africa, the Middle East, and Southeast Asia. Most are cutaneous in settings of ruminant disease.

Largest human epidemic, 10,000 cases, occurred in Zimbabwe (1979–1985) (Mwenye, Siziya, and Peterson 1996).

71 naturally occurring inhalational human infections documented in the last century (Holty et al. 2006).

In the United States, from 1996 to 2001, 21 outbreaks in animals resulting in 1,862 deaths (Johnson 2008)

Anthrax is enzootic in several counties in southwestern Texas near the border of Mexico (Johnson 2006) and in pockets in South Dakota, Nebraska, and Oklahoma (McBride et al. 1998).

Endemic in the Middle East, equatorial Africa, Mexico and Central America, Argentina, Cambodia, Chile, China , Myanmar, Papua New Guinea, Peru, Thailand, Vietnam .

One million sheep deaths in Iran in 1945 (Amidi et al. 1974).

Attack rate among 170 postal mail personnel for letter sorting incident was 1.2 percent (Greene et al. 2002; Inglesby et al. 2002).

Historical circumstantial evidence from at-risk industrial occupations suggests that humans are relatively resistant to infection from naturally occurring exposures (Turnbull 1998).

R_0/incubation period/infectious period/infectious dose

No human-to-human spread ($R_0 = 0$) with GI or inhalational disease (Inglesby et al. 2002; Meselson et al. 1994; Turnbull 1998).

Very rare spread to caregiver (cutaneous infection from changing dressings) (Weber and Rutala 2001).

Incubation period for inhalational anthrax is suggested by modeling to be dose dependent and that the relatively long period of the Sverdlovsk outbreak was related to exposure level (Wilkening 2006). That contrasts with evaluations of natural environment in goat hair mills where workers inhaled > 500 *B. anthracis* particles in an 8-hour shift (Dahlgren et al. 1960).

Cutaneous disease develops 1–12 days after infection and is characterized by ulcer and ultimately eschar with significant swelling (Smyth 1941; Kunanusont, Limpakarnjanarat, and Foy 1990; Gold 1955; Bravata et al. 2007; Fair et al. 2007; Amidi et al. 1974).

Incubation period for cutaneous anthrax in humans ranges from 9 hours to 2 weeks, but it mostly is 2 to 6 or 7 days (Turnbull 1998).

GI/OP symptoms appear 2–5 days after ingestion (Ndyabahinduka et al. 1984; Sirisanthana and Brown 2002; Sirisanthana et al. 1984).

Incubation period 1–13 days (Doganay and Metan 2009).

Cynomolgus monkeys, incubation periods are 7–18 days (Brachman, Kaufman, and Dalldorf 1966).

Incubation period in experimental animals is 36–72 hours regardless of the route of infection (Beyer and Turnbull 2009).

Infectious doses for humans have not been established (Turnbull 1998).

ID_{50} for pulmonary infection in humans is estimated at 8,000 to 50,000 spores (Franz et al. 1997).

Extrapolations suggest: LD_{50} is 2,500 to 55,000 spores (Defense Intelligence Agency 1986);

LD_{10} as low as 100 spores; LD_1 of as low as 1–3 spores (Peters and Hartley 2002).

Mathematical modeling of airborne anthrax infection (based on the USPS 2001 experience) suggests exposures ranging from 18 to 863 spores and perhaps as low as 2–9 spores (Fennelly et al. 2004).

One model of the Sverdlovsk outbreak suggests 50 percent of victims were infected by doses of ~2 spores (Wilkening 2006).

One estimate of the human ID_{50} is 8,600 spores (Wilkening 2006).

Infectious dose for cutaneous disease is unknown. Spores will germinate 1–3 hours after inoculation (Bischof, Hahn, and Sohnle 2007).

Infectious dose for GI anthrax is unknown. In animal models (guinea pigs, rabbits and monkeys) researchers were unable to induce infection using an inoculum of 10^8 spores (Beatty et al. 2003).

As few as 100 spores can be sufficient to cause infection in monkeys (Brachman, Kaufman, and Dalldorf 1966), but the amount needed to cause infection and death in 50 percent of monkeys was 4,130 (1,980–8,630) spores (Glassman 1966).

Morbidity/case fatality ratio

a. Humans

In the 2001 U.S. outbreak, no evidence of mild form of the disease was detected through follow up serologic testing of exposed persons (Baggett 2005).

Untreated patients can infrequently progress to sepsis and, ultimately, death.

CFR for untreated infection varies by cutaneous (5–20 percent) (Smyth 1941), GI/OP (25–60 percent), and inhalational (85–100 percent).

Zimbabwe, 19 cases were reported with a case fatality rate of 26 percent (95 percent CI 5–47 percent) (Mwenye, Siziya, and Peterson 1996).

Turkey, CFRs were 0.96 percent for cutaneous infection, 37.5 percent for GI infection, 100 percent for meningitis, and 2.8 percent overall (Doganay and Metan 2009).

Appropriate antimicrobial therapy reduced reported CFR to < 1 percent (Gold 1955), 12.5 percent (Beatty et al. 2003; Sirisanthana and Brown 2002), and 45–75 percent (Jernigan et al. 2001; Holty, Kim, and Bravata 2006), in the most recent outbreaks. Patients with later symptom onset after exposure (e.g., >30 days) had better survival.

CFR was lower among patients in the United States 2001 outbreak who received antibiotics during the prodromal phase (< 4.7 days) compared to patients who received antibiotics later (40 percent versus 75 percent) (Holty, Kim, and Bravata 2006). Available data from a pediatric review is limited but showed a similar CFR (Bravata et al. 2007).

b. Animals

Species-specific mortality during a 1971 epizootic in Louisiana was 4.3–9.1 percent (cow) and 12.5–22.5 percent (horse) (Johnson 2006).

1974 epizootic in Texas, species-specific mortality of 10.5 percent (cattle), 40 percent (horses), 50 percent (mules), and 0 percent (pigs) (Johnson 2006).

Cynomolgus monkeys incurred mortality rates (percent) of 7.1, 10.0. 11.8, 22.5, and 43.8, respectively for five experiments (overall mortality rate of 23.5 percent) (Brachman, Kaufman, and Dalldorf 1966).

Aerosol infection/routes of transmission

Infection following laboratory exposure has been described, including a recent cutaneous case in a laboratory worker (Brachman 1980)(Centers for Disease Control and Prevention 2002).

Human disease is acquired by three routes: cutaneous, inhalation, and GI. More recently, injection infections have been reported following illegal drug use (heroin) (ProMED mail 2010). Cutaneous anthrax results from introducing the spore through the skin; inhaling anthrax via the respiratory tract; and GI anthrax (abdominal or OP) through ingestion. Cutaneous disease (~95 percent) characterized by ulcer and ultimately eschar with significant swelling (Smyth 1941; Kunanusont, Limpakarnjanarat, and Foy 1990; Gold 1955; Bravata et al. 2007; Fair et al. 2007; Amidi et al. 1974).

GI/OP infection follows ingestion and accounts for less than 5 percent of cases (Ndyabahinduka et al. 1984; Sirisanthana and Brown 2002; Sirisanthana et al. 1984).

Inhalation/pulmonary infection historically occurs rarely in manufacturing settings or, more recently, after accidental or deliberate release of weaponized spores (Meselson et al. 1994). Germination of spores is followed by rapid hemorrhage, edema and necrosis of surrounding tissue from release of bacterial toxins . Meningitis is a frequent complication of inhalational disease (Rangel and Gonzalez 1975; Sejvar, Tenover, and Stephens 2005).

Naturally occurring disease in the United States is rare. No GI/OP cases were recorded in the last century, and 18 inhalational cases were reported between 1900 and 1976 (Brachman 1980). A single inhalational case with exposure to dried animal hides in 2006 was the first naturally occurring in United States since 1976 (CDC 2006; Walsh et al. 2007). Only 225 cutaneous cases occurred between 1944 and 2000.

Two naturally occurring cases of cutaneous infection since 2006 were related to Djembe drums (Centers for Disease Control and Prevention 2008).

A 2009 case of GI infection in the United States was linked to animal hide drums (Centers for Disease Control and Prevention 2010; Goodnough 2009; Brooks 2010).

Infection can occur from contact with infected tissues (butchering, contaminated meat) (Woods et al. 2004), consumption of undercooked meat (Sirisanthana et al. 1984), and contact with contaminated hair, wool, hides (Wattiau et al. 2008).

Bacterial concentrations/pathogenesis

Source cultures for animal experimentation are usually 10^9 CFU/mL (Abalakin and Cherkasskii 1978; Keppie, Smith, and Harris-Smith 1955; Welkos, Keener, and Gibbs 1986).

Culture concentration of 10^9 CFU/mL comprise of 90% spores (Welkos, Keener, and Gibbs 1986).

In naturally infected animals and in the laboratory, bacteremic animals achieve 10^7 to 10^8 CFU/mL (Turnbull 1998).

Certain organs of infected animals can have even higher concentration (e.g., 10^9 CFU/g in spleen) (Welkos, Keener, and Gibbs 1986).

10 mL of $5x10^9$ as inoculum for mouse challenges created by filtration (Osorio et al. 2009; Pickering et al. 2004).

Pathogen stability

Spores seeded into a plant/soil model had survival ratio at day 2 and day 4 of approx. 60% and 53%, respectively, and the percentage of cells that were vegetative was 47% and 43% , respectively (Saile and Koehler 2006).

Vegetative bacteria have poor survival outside the host (Johnson 2006)(Titball, Turnbull, and Hutson 1991).

Spores resist drying, heat, ultraviolet (UV) light, and some disinfectants (Turnbull 1998).

Anthrax spores can remain viable after standard DNA purification procedures (Rantakokko-Jalava and Viljanen 2003).

Heating to 121 °C for 45 minutes and gamma irradiation appear to eliminate viability (Fasanella et al. 2003; Dauphin et al. 2008).

Anthrax spores can survive in soil for months or even decades depending on pH, temperature, and nutrients in the soil (Montville et al. 2005; Manchee et al. 1990).

Animal data suggest that anthrax spores can survive in lungs for 60 days, or even more than 100 days in the lymph nodes of a monkey (Henderson, Peacock, and Belton 1956).

The spore surface is highly hydrophobic, and spores in soil can be transported in clumps of organic matter by water runoff. Evaporation of water redistributes spores onto vegetation and are consumed by susceptible herbivores (Johnson 2006).

Seawater and formaldehyde used on contaminated soil (Inglesby et al. 2002). Chlorine dioxide fumigation used after 2001 attack in buildings (Wein, Craft, and Group 2005).

Vectors

Tabanid flies have been associated with rare cases of cutaneous disease (Turell and Knudson 1987).

Other epidemiological/ecological data

Spore germination occurs at temperatures of 8–45 °C, pH 5–9, when relative humidity (RH) is greater than 95 percent, and adequate nutrients are present (Turnbull et al. 1998).

Therapeutics/vaccines

B. anthracis typically is susceptible to penicillin, doxycycline, fluoroquinolones. Penicillin is not given as monotherapy. Initial IV therapy with multiple agents for inhalational disease with total course of 60 days. Oral FQ or doxycycline for cutaneous disease (CDC 2001).

A human monoclonal antibody drug is available (raxibacumab) and was developed for the U.S. government (Human Genome Sciences 2009).

Anthrax immune globulin is available (HHS 2006).

Prophylaxis: 60 day course of either doxycycline or ciprofloxacin recommended and effective in the recent bioterrorism event with vaccination if available (CDC 2001).

Anthrax vaccine adsorbed (AVA), a precipitated preparation of protective antigen from attenuated, non-encapsulated Sterne strain bacteria, affords protection for rhesus monkeys for inhalational and cutaneous disease and a clinical trial for cutaneous disease among mill workers (Gladstone 1946; Brachman et al. 1962; Mahlandt et al. 1966; Turnbull 1986).

A licensed vaccine for anthrax is available for at-risk personnel for preexposure or in post-exposure situations (to be taken with antibiotic therapy) (CDC 2002; CDC and NIH 2007; Wright et al.).

Other remarks

Although they may have limited applicability to animals, the findings as summarized by the Working Group on Civilian Biodefense showed no significant threat to personnel from aerosolization of settled spores (Inglesby et al. 1999).

Re-suspension of weapons grade anthrax can occur, but risk to exposed persons is unknown (Weis et al. 2002).

In 1996, The United States Department of the Army reported its findings from field tests to study the potential for reaerosolization of settled spores. Although they may have limited applicability to animals, the findings as summarized by the Working Group on Civilian Biodefense showed no significant threat to personnel from reaerosolization of settled spores (Inglesby et al. 1999). BSL-3 biocontainment precautions are recommended for activities with a high potential for creating aerosols (CDC and NIH 2007).

Taxonomy/antigenic relationships/synonyms

B. anthracis is a large (1–8 μm x 1–1.5 μm), gram-positive, non-motile, spore forming (1 μm), bacterial rod that is responsible for both epizootics and enzootic disease and occasional human disease (Johnson 2006).

B. anthracis has three primary virulence factors, which are all plasmid-mediated: edema toxin, lethal toxin, and a poly-D-glutamic acid capsule (Turnbull 1998).

C.1.1.2 Francisella tularensis (F. tularensis)

Host range

a. Field

Infections have been reported in more than 200 species of terrestrial and aquatic mammals (esp. *Sylvilagus*-cottontail in United States) such as ground squirrels, rabbits, hares, voles, muskrats, water rats and other rodents, as well as reptiles, birds, and fish (Gelman 1961; Hopla 1974; Morner 1992; Dennis 1998; Sjostedt 2007).

Natural reservoirs are poorly understood (Keim, Johansson, and Wagner 2007).

b. Experimental

Primates (Lyons and Wu 2007; Sawyer et al. 1966; Day and Berendt 1972), mice, rats (Lyons and Wu 2007), and rabbits (Lyons and Wu 2007) have been used in experimental animal models.

Prevalence/incidence/attack rate

120 cases per year in the United States are recorded; Arkansas has the highest rate; Martha's Vineyard has periodic outbreaks (Sjostedt 2007)(Centers for Disease Control and Prevention 2002; Matyas, Nieder, and Telford 2007). Endemic foci exist in Russia, Finland, and Sweden.

R_0/incubation period/infectious period/infectious dose

No, or minimal, human-to-human spread is reported ($R_0 = 0$) (Dennis et al. 2001).

Ulceroglandular form after a bite from a vector or handling infected meat (with a 3- to 5-day incubation, and the range is 1–21 day) (Dennis 1998; Cross 2000; Dennis et al. 2001; Sanders and Hahn 1968; Penn 2005; Evans et al. 1985)Incubation period averages 3–5 days (and the range is 1–21 days) (Penn 2010).

Incubation period is 3–5 (1–14) days (Dennis et al. 2001).

Extremely low infective dose. ID_{50} of 10-50 cells (SchuS4). As few as 10 cells skin inoculation or 15 cells by aerosol as determined in human challenges (Franz et al. 1997; Saslaw et al. 1961). 200 aerosolized cells infected 2/2 volunteers (McCrumb 1961).

Morbidity/case fatality ratio

Human disease has three major clinical forms: Ulceroglandular is the most common (45–85 percent), usually after a bite of vector that fed on infected animal or handling infected meat (Dennis 1998; Cross 2000; Dennis et al. 2001; Sanders and Hahn 1968; Penn 2005; Evans et al. 1985).

F. tularensis subsp. *tularensis*, (type A), is found almost exclusively in North America and is the most virulent species (Clades 1 and 2) (Ellis et al. 2002; Petersen 2006; Penn 2005). *F. tularensis* subspecies *novicida* is of low virulence (Penn 2005). Depending on strain, illness can be relatively benign or even asymptomatic (Penn 2005).

Untreated CFR varies by strain/clinical presentation. CFR before antibiotics ~7 percent, but ~4 percent for ulceroglandular (Pullen 1945).

CFR can be as high as 50 percent for pneumonia (Dennis et al. 2001; Pullen 1945).

CFR for Type A-East (Clade1) is 14 percent, Type B is 7 percent, Type A-West (Clade 2) is 0 percent (Staples et al. 2006).

Antibiotics reduce CFR from severe disease in pre-antibiotic era to ~2 percent (Evans et al. 1985).

Poor outcome is associated with comorbid conditions, late presentation, delayed antibiotics (Penn and Kinasewitz 1987; Rohrbach, Westerman, and Istre 1991).

Extremely low infective dose is reported. ID_{50} of 10–50 cells (SchuS4). As few as 10 cells through skin inoculation or 15 cells by aerosol as determined in human challenges (Franz et al. 1997; Saslaw et al. 1961).

Aerosol infection/routes of transmission

Tularemia is highly infectious for lab workers (1 infection/1,000 at-risk employee years in *vaccinated* employees) (Pike 1976; Shapiro and Schwartz 2002; Burke 1977; Overholt et al. 1961).

Can be transmitted through apparently intact skin. Oculoglandular is a rare form (< 5 percent) contracted through the conjunctiva (Lillie and Francis 1937). OP or enteral tularemia (< 5 percent) can result from ingestion of infected food or water. Pneumonic tularemia (< 5 percent)

can be primary (inhalation) or secondary (spread from other forms)(Stuart and Pullen 1945; Syrjala et al. 1986; Lillie and Francis 1937). Typhoidal tularemia (< 5 percent) is a sepsis presentation in the absence of a known point of entry.

Infected arthropods (Klock, Olsen, and Fukushima 1973; Markowitz 1985).

Handling infectious tissues (Young 1969).

Ingestion (Greco et al. 1987; Mignani et al. 1988; Reintjes et al. 2002; KuoLee et al. 2007).

Inhalation of aerosols (Dahlstrand, Ringertz, and Zetterberg 1971; Feldman et al. 2001; Feldman et al. 2003).

Possibly direct contact with soil or water, or ingestion of water (Willke et al. 2009) }(Greco et al. 1987; Meric et al. 2008; Keim, Johansson, and Wagner 2007).

Bacterial concentrations/pathogenesis

Heavy bacterial load occur in blood and tissues (Lyons and Wu 2007).

Most models use SCHU S4. Human challenge models from the 1950s and 1960s cutaneous and aerosol used a challenge of 10^4-10^8 (Lyons and Wu 2007).

Concentrations used in primate models varied from 10^4 to greater than10^8 (Lyons and Wu 2007; Sawyer et al. 1966; Day and Berendt 1972).

Mouse/rat animal models used challenge doses of 10^2 to10^4 CFU; tissue burdens of $10^{7\text{-}8}$ are reported (Lyons and Wu 2007).

Rabbit models have used aerosol challenge ranging from 10^5 to 4 x 10^8 cells (Lyons and Wu 2007).

Pathogen stability

Viability of *F. tularensis* aerosols in a chamber ventilated with outdoor air ranged from 7% (45 minutes at 79-82% RH) [HL=11.7 min] to 25% (30 minutes at 73% relative humidity) [HL=15min] (Hood 2009).

F. tularensis can persist in water, mud, and animal carcasses for months (Morner 1992).

Water mammals or protozoa might play role in persistence in water but are likely not required. Chlorination protects municipal systems (Mitchell and Penn 2005; Greco et al. 1987).

Under natural conditions, *F tularensis* can survive for extended periods in a cold, moist environment. The working group lacks information on survival of intentionally dispersed particles but would expect a short half-life because of desiccation, solar radiation, oxidation and other environmental factors (Dennis et al. 2001).

Vectors

A wide range of arthropod vectors is implicated in transmission between mammalian hosts (Dennis 1998; Hopla 1974; Sjostedt 2007).

Tabanid flies (e.g., *Chrysops discalis)* are most important for *F. tularensis tularensis* Clade 2 in Utah, Nevada, and California ; ticks [*D. variabilis, A. americanum* (Clade 1); *D. andersoni* (Clade 2)] most important east of the Rocky Mountains (Farlow et al. 2005; Jellison 1950; Olsuf'ev and Emel'ianova 1966).

In Russia, Central Asia, and Sweden, it is spread by mosquitoes (*Aedes*, *Culex*, and *Anopheles)* (Sjostedt 2007) and *Ixodes* sp of ticks. Mosquitoes and tabinids are likely infected by a water source.

Preservation in the fly gut for at least 56 hrs. is reported (Sjostedt 2007).

Other epidemiological/ecological data

Tularemia is primarily a rural disease, but natural infections can occur in suburban and even urban areas as well (Dvorak 2005; Martone et al. 1979; Halsted and Kulasinghe 1978; Dennis et al. 2001).

Therapeutics/vaccines

Doxycycline or ciprofloxacin are used for prophylaxis (Sawyer et al. 1966; Dennis et al. 2001). Aminoglycosides are the treatment of choice for severe disease (Pediatrics 2006; Enderlin et al. 1994; Hassoun, Spera, and Dunkel 2006).

Fluoroquinolones are as effective as aminoglycosides in animal models and limited human studies (Johansson et al. 2000; Perez-Castrillon et al. 2001; Limaye and Hooper 1999).

Tetracyclines are acceptable, but failures occur (Brouillard et al. 2006; Tarnvik and Chu 2007; Overholt et al. 1961; Evans et al. 1985). Beta-lactams should not be used.

Live attenuated vaccine strain (LVS), not licensed in western countries, had been in use since 1959 for immunizing personnel at risk of lab infection, but it is use in the U.S. has been suspended (Penn 2005) .

Partial protection against aerosol challenge (Griffin, Oyston, and Titball 2007; Burke 1977). mproved LVS is in clinical evaluation (Pasetti 2008) Experimental formalin-inactivated, attenuated, and recombinant vaccines might be efficacious or available for at-risk personnel, but no licensed vaccines are available (Sjostedt 2003; Jia et al. 2009).

Other remarks

Fully virulent *F. tularensis* is a BSL-3 pathogen (Dennis et al. 2001; CDC and NIH 2007). Conditionally virulent or attenuated *F. tularensis* is BSL-2.

Taxonomy/antigenic relationships/synonyms

Francisella species are small (0.2–0.5 µm x 0.7–1.0 µm), aerobic, catalase-positive, pleomorphic, gram-negative coccobacilli with four recognized subspecies (*tularensis*, *holarctica*, *mediasciatica*, and *novicida*) (Ellis et al. 2002; Wong 1999).

C.1.1.3 Yersinia pestis (Y. pestis)

Host range

a. Field

Approximately 215 mammalian species from 73 genera are naturally infected; rodents are the most important host (Butler 1991; Gabastou et al. 2000; Dennis and Meier 1997; Gage 1998).

Occasional spread to amplifying hosts (prairie dogs, ground squirrels, chipmunks) causes epizootics (Christie 1980; Gage et al. 2000; Dennis and Meier 1997; Reed et al. 1970; von Reyn et al. 1976; Wild, Shenk, and Spraker 2006; Gage and Kosoy 2005).

Occurs in rats, tarabagans (Pharaoh's rat), susliks (ground squirrel),ground squirrels, prairie dogs, field mice, bobcats, chipmunks, and camels (Dennis and Mead 2010).

Cats, when orally infected, develop buboes and bacteremia and transmit infection via scratches, bites, and close contact (Gage et al. 2000).

Occurs in great gerbils, Kazakhstan (Stenseth et al. 2006; Enscore et al. 2002).
Partially resistant mammals (*Peromyscus* and *Microtus*) and their fleas responsible for enzootic maintenance (Christie 1980; Gage et al. 2000; Dennis and Meier 1997; Reed et al. 1970; von Reyn et al. 1976; Wild, Shenk, and Spraker 2006; Gage and Kosoy 2005).

Many mammals have high susceptibility and high CFR; others are more resistant (Christie 1980; Gage et al. 2000; Dennis and Meier 1997; Reed et al. 1970; von Reyn et al. 1976; Wild, Shenk, and Spraker 2006; Gage and Kosoy 2005).

Carnivores appear to be highly resistant (Salkeld et al. 2007; Boone, Kraft, and Stapp 2009).

b. Experimental

Guinea pigs are the most common historical experimental models for epizootic plague because of their high susceptibility.

Aerosol transmission in mice and guinea pigs is difficult to establish, but there is a rat model (Agar et al. 2009).

Mice are used for experimental study (Agar et al. 2008).

Prevalence/incidence/attack rate

In the United States, 415 cases were reported from 1970 to 2007 (Dennis and Mead 2010). About seven cases are recorded annually, most (~80 percent) bubonic (Centers for Disease Control and Prevention 1994, 2006, 1991).

Occurs in 17 of the contiguous western U.S. states (Dennis and Mead 2010).
80 percent of U.S. cases occur in New Mexico, Arizona, and Colorado; approximately 10 percent are in California (Dennis and Mead 2010).

Globally, 38 countries reported more than 80,000 cases over past 50 years. Annual reporters include Brazil, Democratic Republic of Congo (DRC), Madagascar, Myanmar, Peru, United States, Vietnam (Dennis and Mead 2010).

Outbreaks have occurred in Vietnam, India, Tanzania, and Madagascar (Dennis and Mead 2010). Recently, pneumonic outbreaks have occurred in Madagascar 1997, DRC 2006, and adjacent Uganda in 2007.Attack rate is 8 percent in untreated close contacts in Uganda (Begier et al. 2006). That is similar to the attack rate experienced in Madagascar (Ratsitorahina et al. 2000).

R_0/incubation period/infectious period/infectious dose

R_0 is estimated at 1.3 from model of 20th century pneumonic outbreaks (Gani and Leach 2004).

R_0 of 1.32 (90% confidence interval: (1.01-1.61)) estimated from six historical outbreaks (Lloyd-Smith et al. 2005).

In rhesus and *C. philippinensis* primates, 120–270 cells caused infection (Meyer 1961). Monkeys with pneumonic plague exhaling 20 or more CFU were able to infect other monkeys (Meyer 1961).

Bubonic plague develops 2–6 days after infection (Butler 1972, 1991; Hull, Montes, and Mann 1987; McGovern and Friedlander 1997).

Primary pneumonic plague develops 1–3 days after droplet exposure (2-5 ft)(Kool 2005; Craven et al. 1993; Meyer 1961; Doll et al. 1994; Gasper et al. 1993).

Plague's latent period has a mean and standard deviation of 4.3 and 1.8 days, respectively (Gani and Leach 2004).

Incubation period is stated by others to be 1–3 days for primary pneumonic plague (Perry and Fetherston 1997).

Incubation period is 2–6 days for the bubonic form (Winters et al. 2009).

Infectious period has a mean and standard deviation of 2.5 and 1.2 days, respectively (Gani and Leach 2004).

Estimated dose is 100–500 CFU in humans (Perry and Fetherston 1997; Franz et al. 1997). LD_{50} in mice has been determined to be 2.1×10^3 CFU (Agar et al. 2008; Agar et al. 2009).

Infectious dose is low—approximately120–270 organisms in *Macaca rhesus* and *Cynomolgus philippinensis* (*M. cynomolgus philippinensis*) (Meyer 1961).

LD_{50} is ~20,000 inhaled cells in *M. rhesus*.

LD_{50} data in rats is 1.6 x 10^3 (Agar et al. 2009).

LD_{50} determined for mice (Agar et al. 2008).

Morbidity/case fatality ratio

In the United States, 415 cases were reported from 1970–2007 with 59 deaths (Dennis and Mead 2010).

Three major clinical forms of human diseases exist: bubonic, septicemic, and pneumonic. Asymptomatic or minimally symptomatic infections have not been reported (Dennis and Mead 2010). Secondary septicemia, pneumonia, and meningitis are the most common complications. Sub-clinical infections can occur in endemic areas (Ratsitorahina et al. 2000).

Bubonic plague (~85 percent of U.S. cases) are characterized by fever, headache, chills, swollen, tender lymph nodes (mainly inguinal and femoral in adults, mainly cervical or axillary in children) (Butler 1972, 1991; Hull, Montes, and Mann 1987; McGovern and Friedlander 1997).

Bacteremia or secondary sepsis is frequent with higher CFR (Butler et al. 1976; Dennis and Mead 2010).

Primary septicemic plague occurs without adenopathy (approximately 10–25 percent of U.S. cases) (Butler 1991; Hull, Montes, and Mann 1987; Sebbane et al. 2006; Dennis and Meier 1997).

Primary pneumonic plague is a rare (about 2 percent in the United States but higher in other areas) but deadly form of the disease (Kool 2005; Craven et al. 1993; Meyer 1961; Doll et al. 1994; Gasper et al. 1993).

Secondary pneumonia occurs in about12 percent of U.S. cases (Doll et al. 1994).

CFR in untreated bubonic plague ranges from 40 to 60 percent (Dennis 1997).

Untreated septicemic form is uniformly fatal (Perry and Fetherston 1997; Crook and Tempest 1992).

Untreated pneumonic form is uniformly fatal (Dennis 1997).

In the United States, treated bubonic CFR is less than 5 percent (Butler 1991).

In the United States, overall CFR for plague is about 14 percent (Craven et al. 1993). However, antibiotics used empirically for undifferentiated sepsis are not effective against *Y. pestis*, so the CFR remains high.

Overall CFR for children in recent U.S. outbreaks is 15.8 percent (Mann, Shandler, and Cushing 1982).

Aerosol infection/routes of transmission

For the pneumonic form, inhalation of droplets (greater than 5 microns) from infected animals such as cats can occur (Burmeister, Tigertt, and Overholt 1962).

Cats are a recent source, including 5/23 cases primary pneumonic (Eidson et al. 1988; Gage et al. 2000).

Inhalation of respiratory droplets from person with primary or secondary pneumonic plague occurs (Burmeister, Tigertt, and Overholt 1962).

At least five laboratory-acquired infections have occurred in the U.S. (Inglesby et al. 2000) Four of these occurred prior to availability of now-standard biocontainment technologies. The fifth case occurred from an attenuated strain in an unsuspected immune-compromised host, during work at biosafety level 2. In addition to being immune-compromised, the researcher apparently did not always follow BSL-2 biocontainment requirements regarding the use of personal protective equipment (gloves) (Centers for Disease Control and Prevention 2011)(Burmeister, Tigertt, and Overholt 1962).

Bubonic form of infection can occur from bites by flea vectors, or from bites or scratches by infected animals such as cats (Gage et al. 2000).

Bubonic form can occur from direct contact with animal carcasses (Christie 1980; von Reyn et al. 1976; Reed et al. 1970).

Pharyngeal infection can occur via ingestion of organisms (Bin Saeed, Al-Hamdan, and Fontaine 2005; Arbaji et al. 2005).

Bacterial concentrations/pathogenesis

Inocula for aerosol challenges in mice were in tenfold steps from 10^8 to 10^{10} CFU/mL (Agar et al. 2008)(Torosian et al. 2009; Feodorova and Golova 2005).

In lab animals, bacterial concentrations reach $10^8$to 10^9 CFU/mL blood(Agar et al. 2008) (Torosian et al. 2009; Feodorova and Golova 2005).

A rat model used 10 mL of 10^{10} CFU/mL as aerosol inoculum (Agar et al. 2009).
Bacteremic load of 10^4 to 10^7 CFU/mL reported from humans (Butler et al. 1976).

Routes of *Y. pestis* infection other than the aerosol route (i.e. i.p., s.c., i.v.) in mice do not significantly alter 50% lethal doses (LD_{50}s) (Perry and Fetherston 1997).

Pathogen stability

Y. pestis can survive approximately 3 hours on flea mouthparts (Bibikova 1977).

Can survive days to weeks in flea feces, tissues of dead animals, and up to 40 weeks in some soils (Ayyadurai et al. 2008; Eisen et al. 2008; Drancourt, Houhamdi, and Raoult 2006).

Survival up to 3 weeks has been noted in blood-contaminated soil (Mollaret 1963).

Stability on laboratory surfaces varies [steel (6 hours), glass (7 hours), polyethylene (24 hours), and paper (5 days)] (Rose et al. 2003).

World Health Organization (WHO) estimates that aerosolized *Y. pestis* would remain viable for 1 hour (Borio and Hynes 2010).

An approximately 3-log decay over 90 minutes using an avirulent strain in aerosolized heart infusion broth at conditions of 26 °C and RH varying from 20 to 50 percent is reported (Won and Ross 1966).

Vectors

Of the 1,500 species of fleas, more than 200 are naturally infected, about 30 are proven vectors of the plague (Perry and Fetherston 1997).

Squirrel fleas (*O. montanus*) are the most common source of human plague in the United States. *O montanus* and *X. cheopis* likely transmit early without complete blocking (Eisen et al. 2006).

Cat, dog, and so-called human fleas are very poor vectors (Eisen et al. 2006; Kartman, Quan, and Stark 1962).

An accepted paradigm has been that *Y. pestis* proliferation in the flea causes gut blockage with subsequent starvation, aggressive biting and regurgitation of *Y. pestis* into the bite site. In contrast, O *montanus* and *X. cheopis* likely transmit early without complete blocking of midgut (Eisen et al. 2006).

Unblocked and occasional blocked fleas can live for many months or over a year in the wild (Eisen et al. 2006; Kartman, Quan, and Stark 1962).

Y. pestis has been isolated from lice and ticks (Houhamdi et al. 2006).

Experimentally infected ticks can maintain plague bacilli for up to a year but cannot transmit (Thomas, Karstens, and Schwan 1990).

Other epidemiological/ecological data

For infection control, respiratory droplet isolation is recommended by using a surgical mask and eye protection when caring for symptomatic patient until 48 hours after initiating effective treatment (Weber and Rutala 2001). Quarantine of asymptomatic persons is not recommended.

Soil is suggested as a possible site for interepizootic maintenance, but it is not well supported (Baltazard 1964).

U.S. males and females are equally affected (Dennis and Mead 2010).

The last case of human-to-human in the United States occurred in the 1924 Los Angeles outbreak (Meyer 1961).

Three pandemics originating in Egypt 542; Italy 1347; China 1894. United States-via China in 1900 was originally urban in southern California and became endemic in wild animal populations (Kaufmann, Boyce, and Martone 1980). Since then, it occurs seasonally in warm months among persons in the southwestern United States with close contact to animals (about 7 cases annually, about 80 percent bubonic) (Centers for Disease Control and Prevention 1994, 2006, 1991).

Warm springs and wet summers increase its prevalence in great gerbils in Kazakhstan, which can also affect human disease frequency (Stenseth et al. 2006; Enscore et al. 2002).

Blocking in fleas was described in 1914. Many factors influence blocking and transmission including strain differences and transmission factors, flea species, proventricular morphology, and temperature (Gage and Kosoy 2005). Vector efficiency is defined as the product for infection potential, vector or infective potential, and transmission potential. Flea blood meal = 0.1–0.3 μL, therefore, must have at least 10^8 CFU/mL (Lorange et al. 2005; Hinnebusch 2003).

25,000–100,000 *Y. pestis* inoculated with flea bite (Reed et al. 1970). Vector index from multiplying blocking-survival potential and vector efficiency. *X. cheopis* is a classic and very effective vector (becomes blocked in as few as 5 days versus 2–3 weeks).

Therapeutics/vaccines

Streptomycin has been the treatment of choice. It can be efficacious for treatment, but gentamicin is as efficacious and more available (Inglesby et al. 2000; Heine 2007; Mwengee et al. 2006; Boulanger et al. 2004).

Doxycycline is also FDA approved and used, particularly when aminoglycosides are not available or cannot be used. Fluoroquinolones are as effective in animal models (Russell et al. 1996; Steward et al. 2004).

Resistance to imipenem, rifampin, and macrolides (Frean et al. 2003; Wong et al. 2000).

A multidrug resistant isolate was reported from Madagascar, but it was susceptible to fluoroquinolones, cephalosporins (Galimand et al. 1997; Welch et al. 2007).

Prophylactic tetracycline, doxycycline, sulfonamides, chloramphenicol, and FQ can be used for exposed asymptomatic individuals (Inglesby et al. 2000). In a 2,000-person review, no cases occurred among those receiving prophylaxis (Centers for Disease Control 1984).

In the United States, a licensed, formaldehyde–killed, whole bacilli vaccine was discontinued in 1999 (Titball and Williamson 2001).

The vaccine demonstrated efficacy in prevention and attenuation of bubonic disease, but was not helpful for primary pneumonic. Eight cases of plague occurred among vaccinated U.S. servicemen in Vietnam (equating to one case/10^6 person years of exposure), versus (333 cases/10^6 person years of exposure in non-vaccinated civilians(Titball and Williamson 2001).

Heat-killed vaccine was used by British in the Gulf War (Allen et al. 2006). F1/LcrV combination vaccine worked well in macaques, but showed limited usefulness in African green monkeys.

Other remarks

Fully virulent *Y. pestis* is a BSL 3 pathogen (CDC and NIH 2007). Conditionally virulent or attenuated *Y. pestis* is BSL-2 (CDC and NIH 2007; Sewell 2003).

Taxonomy/antigenic relationships/synonyms

Y. pestis is a gram-negative, non-motile, non-spore-forming coccobacillus (0.5–0.8µm x 1–3µm) responsible for epizootics and enzootic disease and intermittent human disease (Perry and Fetherston 1997). Three classic biovars are recognized: *antique*, *medievalis*, and *orientalis* (Dennis and Meier 1997).

C.1.1.4 1918 H1N1 influenza virus (1918 H1N1V)

NOTE: Information on the 1918 H1N1 virus is limited. Accordingly, information for other influenza strains is shown as well. Data points referring specifically to 1918 H1N1 are clearly marked.

Host range

a. Field

1918 H1N1 virus is known to be infectious humans, birds, and swine (Taubenberger and Morens 2006; Babiuk et al. 2010) .

Research indicates that descendants of the 1918 virus still persists enzootically in pigs (Taubenberger and Morens 2006).

The origin of the 1918 H1N1 virus remains unknown (Taubenberger and Morens 2006). Influenza A viruses cause chronic, asymptomatic infection in the GI tracts of wild birds but are also able to infect and cause disease in a variety of mammals. On rare occasions, an influenza A virus is introduced into human populations and spreads rapidly to cause a global pandemic. That can occur either when an avian virus with a novel hemagglutinin

(HA) protein adapts to human-to-human transmission, or when an avian virus undergoes genomic reassortment during co-infection of an influenza virus-infected mammal such as a pig. As a pandemic virus circulates, it undergoes progressive antigenic drift in its HA and neuraminidase (NA) proteins, permitting it to reinfect the same populations in regular outbreaks of *seasonal* influenza (Barnard 2009).

"Influenza A viruses have infected many different animals, including ducks, chickens, pigs, whales, horses, and seals. However, certain subtypes of influenza A virus are specific to certain species, except for birds, which are hosts to all known subtypes of influenza A. Subtypes that have caused widespread illness in people either in the past or currently are H3N2, H2N2, H1N1, and H1N2. H1N1 and H3N2 subtypes also have caused outbreaks in pigs, and H7N7 and H3N8 viruses have caused outbreaks in horses. Influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species. For example, until 1998, only H1N1 viruses circulated widely in the U.S. pig population. However, in 1998, H3N2 viruses from humans were introduced into the pig population and caused widespread disease among pigs. Most recently, H3N8 viruses from horses have crossed over and caused outbreaks in dogs."(Centers for Disease Control and Prevention 2005).

Wild, aquatic birds, predominantly dabbling ducks, appear to be the reservoir of influenza A viruses (Weber and Stilianakis 2008). Birds are hosts to all known subtypes of influenza A virus (Centers for Disease Control and Prevention 2005).

b. Experimental

The virus is able to infect and replicate in experimentally infected pigs, and the available data suggest that natural infections occurred widely in swine during the 1918 pandemic (Weingartl et al. 2009).

1918 H1N1V has low pathogenicity in birds (Babiuk et al. 2010). Data indicated that 1918 H1N1V does not replicate efficiently in experimentally infected chickens and, although the virus does replicate in ducks (as shown by serological testing), the level of

replication in most ducks was below the limit of detection (for nucleic acid-based detection tests) (Babiuk et al. 2010).

1918 H1N1 virus does not spread from inoculated (10^6 PFU intransal instillation) mice to uninfected cagemates (Lowen et al. 2006; Tumpey 2008).

Mice are a poor model for 1918 H1N1 transmission, and ferrets are used instead (Tumpey 2008)

10^6 PFU 1918 H1N1 is the highest concentration that can be given to mice in a 50 uL volume; interferon-deficient mice exhibit 100 percent mortality. Ferrets exhibit 50 percent mortality (Tumpey 2008).

10^5 PFU 1918 H1N1 in outbread mice is not lethal (Tumpey 2008).

1918 H1N1 lethality in mice (LD_{50}) is 3.5 $\log_{10}$ PFU or EID_{50}; LD_{50} in ferrets is 10^6 PFU; the same dose in a susceptible mouse strain (Mx1 gene deficient) give an LD_{100} (intranasal inoculation) (Tumpey 2008).

Guinea pigs have been used as an animal model for 1918 H1N1 (Van Hoeven et al. 2009).

ID_{50} of H3N2 for guinea pigs was determined to be 5 PFU (Lowen et al. 2006).

ID_{50} of H0N1 for mice was determined to be 0.079 to 5 EID_{50} (Yetter et al. 1980).

H3N2 found to spread between guinea pigs by droplets; cages up to 91 cm apart (Lowen et al. 2006).

Mice, ferrets, rats, pigs, and NHPs have been used as an animal model (regarding influenza A) (Barnard 2009).

Prevalence/incidence/attack rate

1918 H1N1 virus is not circulating in humans, and is not known to be circulating in animals (Tumpey, Basler, et al. 2005).

At least 3 laboratory-acquired infections with non-1918 H1N1V have been reported, and 2 of these occurred in the ABSL-3 setting (Wentworth et al. 1997; Harding 2006). Figure C-1, below, shows 1918 H1N1 influenza plus pneumonia (P&I) (combined) age-specific incidence rates per 1,000 persons per age group (panel A), death rates per 1,000 persons, ill and well combined (panel B), and case-fatality rates (panel C, solid line), U.S. Public Health Service house-to-house surveys, 8 states, 1918 (36). A more typical curve of age-specific influenza case-fatality (panel C, dotted line) is taken from the U.S. Public Health Service surveys during 1928–1929 (37) (Taubenberger and Morens 2006).

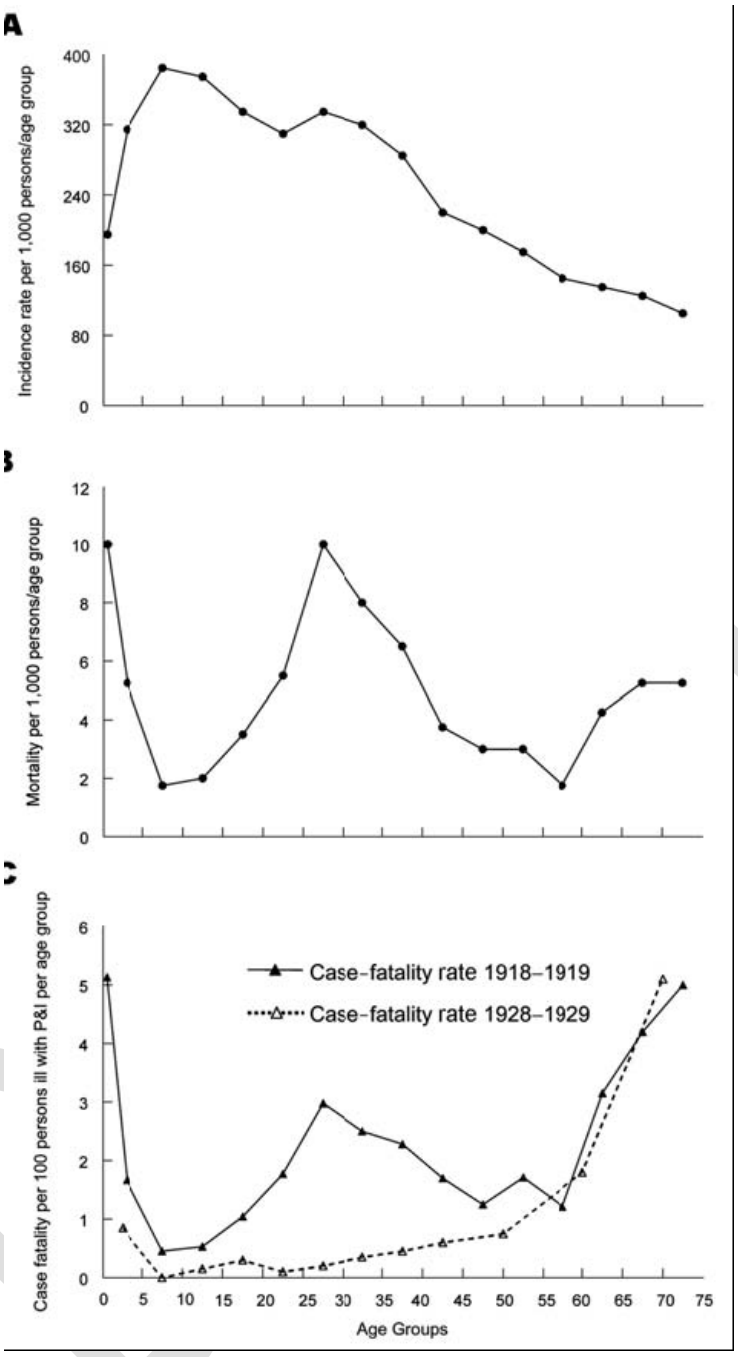


Figure C-1. 1918 attack rates by age group.

The major epidemiological differences between 1918 and 2008 human populations include immunity to H1, immunity to N1, and co-circulation of H1N1 and H3N2; all humans older than 2–3 years have immunity to both H1N1 and H3N2 viruses (Murphy 2008).

Current vaccines can boost antibody to 1918 virus (Tumpey et al. 2004; Murphy 2008).

A 53 percent attack rate (9/17) among human volunteers for influenza A/Wisconsin 67/2005 H3N2 (Zaas et al. 2009).

For pandemic influenza A virus, not otherwise specified, using R_0 values of 1.6, 1.9, 2.1 and 2.4 percent, model-determined attack rates of 32.6, 43.5, 48.5, and 53.7 percent, respectively, were found without interventions. School closures alone were projected to limit the attack rate to 1.0 but only if the R_0 did not exceed 1.6. The "model suggests that the combination of targeted antiviral prophylaxis (TAP), school closure, and social distancing can be successful up to R_0 = 2.4, without any vaccination." Success is defined as limiting the attack rate to that of annual influenza epidemics (about 10 percent of the U.S. population). Various combinations of interventions (including unlimited TAP, and excluding vaccination) resulted in projected attack rates of 0.02, 0.07, 0.14, and 2.8 percent, respectively. However, it is noted that a supply of 20 million courses of targeted antiviral therapy courses would be exceeded if the R_0 were 1.8 or greater (as of the publication date—April 2006—5 million courses were stockpiled nationally). See table C-3 below by German et al "Simulated mean number of ill people (cumulative incidence per 100) and for TAP, the number of antiviral courses required for various interventions and R_0" (Germann et al. 2006).

Attack rate for H2N2 was predicted (by modeling) to be 33 percent without intervention and 2 percent with the use of targeted antiviral prophylaxis (Longini et al. 2004).

Attack rate for influenza A/Taiwan/1/86 (H1N1) during an outbreak at a naval base was 39 percent in those under 35 years old and 25 percent for all other ages (Klontz et al. 1989).

Attack rate for H1N1 in Seattle (December 15, 1978, through March 31, 1979) was 0.290 overall. For the age groups (years) 0–4, 5–19, and 20+, the attack rates were 0.346, 0.524, and 0.023, respectively (Longini et al. 1982).

Clinical attack rate of 34 percent for 1977-1978 H1N1 virus was reported among 200 males (98 percent were 20 years of age or younger) aboard a navy ship (Ksiazek et al. 1980). For the same virus and period, clinical attack rates of 2.36 percent and 0.62 percent were reported for age groups ≤23 years and ≥23 years, respectively, at a 38,000 student campus (Pons, Canter, and Dolin 1980).

R_0/incubation period/infectious period/infectious dose

Literature analysis (1965–2005) indicates influenza generation time (cf incubation period) can be as short as 2.5 days on average (range 1.5–4.0 days) (Carrat et al. 2008). That value is substantially shorter than that assumed by Longini et al. 2004 (Longini et al. 2004; Germann et al. 2006).

The incubation period for the 2009 novel swine-origin influenza A H1N1 virus (S-OIV) infection appears to be 2–7 days; however, additional information is needed (Novel Swine-Origin Influenza et al. 2009).

"The incubation period for seasonal influenza is reported to average 2 days (range: 1–4 days) [but that is] not supported by high-quality evidence" (Carrat et al. 2008).

The infectious period for seasonal influenza is 1 day before the onset of symptoms through 5–7 days after onset of symptoms or until symptoms have resolved (Novel Swine-Origin Influenza et al. 2009).

The actual R_0 for 1918 H1N1 in Iceland was 2.2 (Dowell and Bresee 2008).

The estimated R_0 for 1918 H1N1 is as follows:

- Estimated to be approximately 2–3; median R_0 based on data from 45 U.S. cities is 2; "Initial R" = 2.0 (Interquartile range 1.7-2.3); "Extreme R" = 2.7 (Interquartile range 2.3-3.4); Maximum R = 6.5 (upper bound); estimate over 45 U.S. cities (Mills, Robins, and Lipsitch 2004);

- Estimated R_0 for England and Wales, assuming a 3 day generation interval, for Autumn wave: 1.39 (1.36–1.43); for winter wave: 1.39 (1.29-1.49). Assuming a 6 day generation interval the numbers are 1.84 (1.75-1.92) and 1.82 (1.61-2.05) respectively (Chowell et al. 2008);
- Estimated R_0 for Copenhagen and three other Scandinavian cities was substantially higher in summer (2.0–5.4) than in fall (1.2–1.6) (Andreasen, Viboud, and Simonsen 2008);
- Estimated R_0 for H2N2 (for modeling purposes) was 1.68 (Longini et al. 2004);
- R_0 estimated at 2.2 (95% C.I. 1.7-2.7) extreme at 3.5, Iceland (Gottfredsson et al. 2008);
- R_0 estimated at 2.1, England and Wales (Viboud et al. 2006);
- R_0 estimated at 1.49 (95% CI 1.45-1.53) spring wave; 3.75 (95% CI 3.57-3.93) fall wave, Geneva (Chowell et al. 2006);
- R_0 of 1.79 – 2.1, estimates from eight model variants over 16 U.S. cities; largest confidence interval 1.3-3.2 (Bootsma and Ferguson 2007);
- R_0 of 1.58 – 3.41 estimated for Prussia, Germany (Nishiura 2007);
- R_0 estimated at 1.3 – 3.1, New Zealand (Sertsou et al. 2006);
- R_0 of 2.68 estimated for Sao Paulo, Brazil (Massad et al. 2007);
- R_0 estimated at 1.7-2.0 for 83 cities in the U.K. (Ferguson et al. 2006);
- R_0 of 1.70 estimated for the UK and Wales (Gani et al. 2005);
- R_0 estimated at 2.4, 3.5 for San Francisco (Chowell, Nishiura, and Bettencourt 2007) .

Tables C-2 and C-3 below pertain to a pandemic influenza A virus, not otherwise specified. R_0 estimates of 1.6, 1.9, 2.1, and 2.4 have been used to model the spread of influenza and efficacy of intervention strategies (Germann et al. 2006).

Table C-1. Characteristics of simulated pandemic influenza in the United States without intervention

Basic reproductive number, R_0	1.6	1.9	2.1	2.4
Rate of spread: 1,000th ill person*	14	13	12	11
10,000th ill person*	29	24	22	19
100,000th ill person*	48	37	34	29
1,000,000th ill person*	70	52	46	39
Peak of epidemic*	117	85	75	64
Daily number of new cases at peak activity	2.3 M	4.5 M	6.0 M	7.9 M
Number of days with >100,000 new cases	86	68	60	52
Cumulative number of ill persons	92 M	122 M	136 M	151 M

M, million.
*Days after initial introduction.

Source: (Germann et al. 2006)

Table C-2. Simulated mean number of ill people (cumulative incidence per 100) and for TAP, the number of antiviral courses required for various interventions and R_0

Intervention	R_0 = 1.6	R_0 = 1.9	R_0 = 2.1	R_0 = 2.4
Baseline (no intervention)	32.6	43.5	48.5	53.7
Unlimited TAP (no. of courses)*	0.06 (2.8 M)	4.3 (182 M)	12.2 (418 M)	19.3 (530 M)
Dynamic vaccination (one-dose regimen)†‡	0.7	17.7	30.1	41.1
Dynamic child-first vaccination†‡	0.04	2.8	16.3	35.3
Dynamic vaccination (two-dose regimen)‡§	3.2	33.8	41.1	48.5
Dynamic child-first vaccination‡§	0.9	25.1	37.2	47.3
School closure¶	1.0	29.3	37.9	46.4
Local social distancing¶	25.1	39.2	44.6	50.3
Travel restrictions during entire simulation‖	32.8	44.0	48.9	54.1
Local social distancing and travel restictions¶‖	19.6	39.3	44.7	50.5
TAP,* school closure,** and social distancing**	0.02 (0.6 M)	0.07 (1.6 M)	0.14 (3.3 M)	2.8†† (20 M)
Dynamic vaccination,†‡ social distancing,¶ travel restrictions,¶‖ and school closure**	0.04	0.2	0.6	4.5
TAP,* dynamic vaccination,†‡ social distancing,¶ travel restrictions,¶‖ and school closure**	0.02 (0.3 M)	0.03 (0.7 M)	0.06 (1.4 M)	0.1 (3.0 M)
Dynamic child-first vaccination,†‡ social distancing,¶ travel restrictions,¶‖ and school closure**	0.02	0.2	0.9	7.7

M, million.
*60% TAP, 7 days after pandemic alert, antiviral supply of 20 M courses unless stated.
†10 million doses of a low-efficacy vaccine (single-dose regimen) per week.
‡Intervention continues for 25 weeks, beginning such that the first individuals treated develop an immune response on the date of the first U.S. introduction.
§10 million doses of a high-efficacy vaccine (two-dose regimen) per week.
¶Intervention starting 7 days after pandemic alert.
‖Reduction in long-distance travel, to 10% of normal frequency.
**Intervention starting 14 days after pandemic alert.
††Exhausted the available supply of 20 M antiviral courses.

Source: (Germann et al. 2006)

The typical incubation period is 2 days, with a range of 1–4 days (cites internal reference #28) (Bridges, Kuehnert, and Hall 2003).

Compiled data from H1N1 volunteer challenge studies in the literature (1965–2005) show an inoculum range of 10^3–$10^{7.2}$ $TCID_{50}$, and an infection rate of 362 per 532 volunteers (68 percent), but aerosol challenge was used in only one of those studies (Carrat et al. 2008).

A human infectious dose (HID_{30}) of 3 x TID_{50} for influenza A2/Bethesda/10/63 was determined for 23 male volunteers, 21–40 years of age (7/23 infected) (Alford et al. 1966).

Eleven of fifteen (73 percent) human volunteers were infected using an H1N1 inoculum of 10^4 TCID50 instilled intranasally (Clements et al. 1986).

Nine of seventeen (53 percent) human volunteers were infected using H3N2 influenza A (Wisconsin 67/2005) using inocula of either 10^2 , 10^3 , 10^4 , or 10^5 $TCID_{50}$ instilled intranasally (Zaas et al. 2009).

Intranasal instillation of influenza virus in volunteers might not mimic real exposure/infection (Carrat et al. 2008).

The HID_{50} for 1918 H1N1 in humans is unknown but is estimated to be 1–10 virions (Subbarao 2008).

Data on HID_{50} for other influenza viruses range from 3 TID_{50} to $10^{7.2}$ TID_{50} (Carrat et al. 2008; Alford et al. 1966; Zaas et al. 2009).

Morbidity/case fatality ratio

Asymptomatic infection with 1918 influenza virus possibly could occur and would not be detected by illness monitoring of lab personnel (Uyeki 2008; Monto 2008).

As many as one in three infections with influenza (non-1918 H1N1) are asymptomatic (Carrat et al. 2008).

Although primary infection in young children is usually symptomatic, overall, approximately 50 percent of influenza infections can be asymptomatic. Nevertheless, infected persons with few or no signs of illness can shed virus and, therefore, be infectious to others (cites ref #29). Infected persons can become contagious (i.e., they can shed detectable amounts of influenza virus) the day before symptoms begin. Adults usually shed virus for about 3–5 days (author cites refs #28, 30), whereas young children can shed the virus for up to 3 weeks (author cites refs #31–33) (Bridges, Kuehnert, and Hall 2003).

Symptoms of seasonal influenza typically include high fever, chills, headache, sore throat, dry cough, myalgia, anorexia, and malaise. Complications include primary viral pneumonia, secondary bacterial pneumonia, or combined bacterial and viral pneumonia. Severe infections are caused by the recently emerged avian influenza A H5N1 virus are characterized by rapid development of diffuse interstitial pneumonia, viremia, and shock leading to death (Barnard 2009).

Morbidity data are pending from the 2009–2010 H1N1 pandemic [Update: morbidity & mortality data are available from Lemaitre and Carrat 2009 (Lemaitre and Carrat 2010)]. Mortality attributed to 1918 H1N1 in Iceland was 2.8 percent (Dowell and Bresee 2008). Case fatality rate in 1918 was about 2.5 percent (Taubenberger 2008).

"Age-specific death rates in the 1918 pandemic exhibited a distinct pattern that has not been documented before or since: a W-shaped curve, similar to the familiar U-shaped curve but with the addition of a third (middle) distinct peak of deaths in young adults about 20–40 years of age. Influenza and pneumonia death rates for those 15–34 years of age in 1918–1919, for example, were more than 20 times higher than in previous years" (Taubenberger and Morens 2006).

"Overall, nearly half of the influenza-related deaths in the 1918 pandemic were in young adults 20–40 years of age, a phenomenon unique to that pandemic year" (Taubenberger and Morens 2006).

"The 1918 pandemic is also unique among influenza pandemics in that absolute risk of influenza death was higher in those < 65 years of age than in those > 65; persons < 65 years of age accounted for > 99 percent of all excess influenza-related deaths in 1918–1919. In comparison, the < 65-year age group accounted for 36 percent of all excess influenza-related deaths in the 1957 H2N2 pandemic and 48 percent in the 1968 H3N2 pandemic" (author cites a primary ref.) (Taubenberger and Morens 2006).

In the 1918 pandemic, "[t]hose 5 to 14 years of age accounted for a disproportionate number of influenza cases but had a much lower death rate from influenza and pneumonia than other age groups" (Taubenberger and Morens 2006).

Histological and bacteriological evidence suggests that the vast majority of influenza deaths during the 1918 pandemic resulted from secondary bacterial pneumonia (Morens, Taubenberger, and Fauci 2008) (Klugman, Astley, and Lipsitch 2009). Antibiotics were not available in 1918, and medical support services have improved dramatically in the past 90 years (Murphy 2008).

A model-predicted case fatality rate for H2N2 infections was 0.58/1,000 persons in the absence of intervention and 0.04/1,000 persons if targeted antiviral prophylaxis is used (Longini et al. 2004).

Case fatality rates for adults (ages 14 years and older) in a large prepaid group practice (Portland, Oregon) for the 1968-1969 and 1972-1973 H3N2 viruses were 13 percent and 12 percent, respectively (Barker and Mullooly 1980).

Aerosol infection/routes of transmission

As of 2004, the CDC/NIH Influenza Agent Summary Statement Committee estimated the likely infectious dose for 1918 H1N1 virus to be 1 to 10 [particles] (Subbarao 2008).

Droplet transmission of influenza viruses occurs when contagious droplets produced by the infected host via coughing or sneezing are propelled a short distance and come into contact with another person's conjunctiva, mouth, or nasal mucosa. Because the droplets generally are large (110 μm) and do not stay suspended in the air, this mode of transmission is not affected by special air handling or control of room pressures. Airborne transmission entails the production of infectious droplet nuclei, generally < 5 μm in diameter, which, in contrast to droplets, can remain suspended in the air and be disseminated by air currents (Bridges, Kuehnert, and Hall 2003).

Evidence exists to support the transmission of influenza viruses by direct and indirect contact and by droplet and droplet nuclei (i.e., airborne) transmission. However, experimental studies involving humans are limited, and the relative contribution of each mode of transmission remains unclear (Bridges, Kuehnert, and Hall 2003).

Transmission of H1N1 (strain Pan 99) by fomites was found to be inefficient using a guinea pig model (Mubareka et al. 2009).

"Flu viruses are thought to spread mainly from person to person through coughing or sneezing of people with influenza. Sometimes people [can] become infected by touching something with flu viruses on it and then touching their mouth or nose" (Centers for Disease Control and Prevention 2010).

"The pathogenesis of H5N1 in mammals raises some new concerns about the waterborne route; in cats" (Weber and Stilianakis 2008).

"The waterborne route of transmission is traditionally not considered to be relevant for respiratory viruses. The emergence of highly pathogenic avian influenza virus H5N1 as a perceived pandemic threat has changed this situation" (Weber and Stilianakis 2008).

Virus titers/concentrations/pathogenesis

The CDC in 2008 estimated the range of concentrations of 1918 H1N1 virus a laboratory might work with to be 10^2 to 10^8 [virions] (Subbarao 2008).

Table C-3, Properties of recombinant influenza viruses used in this study

Virus[a]	Titer[b]		% Weight loss[c]	Lung titer (log_{10} EID_{50}/ml ± SE)[d]	LD_{50}[e]
	log_{10} EID_{50}/ml	PFU/ml			
Wild-type Tx/36/91	8.2	2.0×10^7	0.7	3.3 ± 0.2	>6
Parental rTx/36/91	8.7	4.4×10^7	0.1	3.6 ± 0.3	>6
Parental rWSN	8.2	2.2×10^7	21.8	7.2 ± 0.2	2.75
1918 HA/NA:Tx/36/91	8.7	2.5×10^7	15.9	6.0 ± 0.2	4.75
1918 HA/NA:WSN	8.5	2.1×10^7	21.7	7.0 ± 0.3	2.25

[a] All viral genomic segments derived from the WSN or Tx/91 virus unless otherwise indicated.
[b] Calculated by the method of Reed and Muench (47) from titrations in eggs and MDCK cells.
[c] Average percent weight loss on day 4 postinfection (five mice per group).
[d] Average lung titers of four mice on day 4 postinfection.
[e] Expressed as the log_{10} PFU required to give 1 LD_{50}.

Source: (Tumpey, Garcia-Sastre, et al. 2005):

1918 virus challenge doses used in guinea pig model: 10^6 EID_{50} (i.e., the dilution that causes infection in 50 percent of eggs is the egg infectious dose – EID_{50}; the upstream dilution tube that is 10^6 higher than the EDI_{50} is the 10^6 EID_{50}). Other challenge doses of 1918 H1N1 varied between 10^3 and 10^6 PFU of EID_{50} (Van Hoeven et al. 2009).

The 1918 H1N1 peak titer in guinea pig nasal wash was greater than 10^5 PFU/mL or EID_{50}/mL(Van Hoeven et al. 2009).

A concentration of 10^8 PFU/ml for a recombinant virus containing the 1918 NS1 sequence achieved in Madin-Darby canine kidney (MDCK) cell culture within 36 hours (Jackson et al. 2008).

In experimental infections in healthy volunteers, influenza A viral replication peaks approximately 48 hours after inoculation into the nasopharynx, declining thereafter, with usually little or no virus shed after 6 days (Barnard 2009).

Human volunteers intranasally infected with H3N2 influenza A (Wisconsin 67/2005) had a median time to peak symptoms of 80 hours (range 50–110 hours) (Zaas et al. 2009).

Unpublished data show no transmission of 1918 H1N1 among caged mice over a 14-day period (Tumpey 2008).

The 1918 H1N1 virus replicates effectively in both the upper and lower respiratory tract, in contrast to other H1N1 viruses, and that is suspected to be a virulence factor for the strain (Barnard 2009)(Watanabe et al. 2009).

Using a mouse model, it was shown that viruses engineered to contain four C-terminal residues of the nonstructural, nonessential, NS1 gene from the 1918 (or HPAI as well) virus were more virulent than wild type virus (Jackson et al. 2008).

The 1918 H1N1 virus is less lethal in mice than contemporary H5N1 isolates, requiring 63–500 times more virus to cause death (Tumpey 2008).

Pathogen stability

Human influenza viruses can survive on a variety of surfaces at 35–49 percent humidity and a temperature of 28 °C. Both influenza A and B viruses were cultured from experimentally contaminated, nonporous surfaces, such as steel and plastic, up to 24–48 hours after inoculation, and from cloth, paper, and tissues up to 8–12 hours after inoculation (Bean et al. 1982).

Influenza viruses could be recovered from hands for only 5 minutes and only if the hands were contaminated with a high viral titer. Viable virus could be transferred from nonporous surfaces to hands for 24 hours and from tissues to hands for 15 minutes. Those data support the feasibility of spread of influenza by indirect contact (Bean et al. 1982). However, the importance of that mode of transmission probably depends on the type of surface and the amount of virus present (Bridges, Kuehnert, and Hall 2003).

Effects of humidity on the ability of influenza viruses to infect mice in a non-ventilated room with constantly agitated air have been studied (Loosli et al. 1943). At an RH of 17–24 percent, animals became infected with influenza as late as 24 hours after the virus was first aerosolized into the room, although the proportion of animals infected decreased over time. Infectivity was

enhanced at 22 hours after influenza virus was introduced, when the floor was vigorously swept, suggesting that desiccation of the virus does not eliminate infectivity. Whether sufficient numbers of virus-laden particles can remain viable to infect humans in a similar setting is unknown (Bridges, Kuehnert, and Hall 2003).

The D_{37} value for influenza A virus was calculated to be 7.5 [HL=18 min at maximum solar conditions] (Lytle and Sagripanti 2005).

Solar radiation-induced infectivity reduction for influenza A virus has been reported to range from 0.1 to 7.5 $\log_{10}$ /day in U.S. locations depending on latitude and season (Sagripanti and Lytle 2007).

"Study of the inactivation of influenza virus as a function of RH and temperature has produced contradictory results." "Maximum survival times vary between 1 hour (80 percent RH) and 24 hours (20 percent RH)" (Weber and Stilianakis 2008).

Transmission of H3N2Pan/99 among guinea pigs in separate cages at 20 °C was 75–100 percent, 100 percent, 25 percent, 75 percent, and 0 percent, for RHs of 20 percent, 35 percent, 50 percent, 65 percent, and 80 percent, respectively (Lowen et al. 2007).

Absolute humidity (AH) was reported in 2009 to be more important than RH in determining survival and transmission (the conclusion stands that low humidity, whether measured by RH or AH, favors viral survival). Data derived by calculating AH from a previous study (Harper 1961) that examined RH and temperature showed an inverse linear correlation between survival and AH, with survival being greatest (about 80 percent, 63 percent, 62 percent, at 0,6 and 23 hours, respectively) as AH nears zero (i.e., 2 mb). 50 percent of influenza virus transmission variability and 90 percent of influenza virus survival variability were explained by AH, whereas, respectively, only 12 percent and 36 percent were explained by RH (Shaman and Kohn 2009).

"Avian influenza viruses can be isolated from natural, open fresh water" (Weber and Stilianakis 2008).

Avian strains of H1N1 can be isolated from bodies of fresh water (Deboosere et al. 2011).

"The influenza-related risk posed by water resources, water supplies and sanitation has received some limited attention. There is apparently no quantitative information on the inactivation of human influenza A viruses in open, liquid water; H1 sequences have been isolated from Siberian lake water, but no further information on inactivation rates is provided (authors cites reference number 123). The most recent work on low- and high pathogenic avian influenza virus inactivation in water investigates 8 subtypes of low-pathogenic avian influenza (LPAI) viruses and two strains of high-pathogenic H5N1 (Anyang/01 and Mongolia/05) (author cites reference number 124). Virus inactivation depends on pathogenicity, salinity and temperature (see Table [C-5]): survival decreases with salinity and temperature and LPAI survive longer than HPAI. These results imply that avian influenza viruses can under circumstances of low salinity and low temperatures persist many weeks in water" (Weber and Stilianakis 2008).

Table C-4. Daily inactivation rates for LPAI and HPAI avian influenza viruses in water

T = 17 ^{0}C		T = 28 ^{0}C
Salinity Z 0 ppt	0.023 (LPAI)	0.116 (LPAI)
	0.051 (HPAI)	0.215 (HPAI)
Salinity Z 15 ppt	0.038 (LPAI)	0.184 (LPAI)
	0.053 (HPAI)	0.216 (HPAI)
Salinity Z 30 ppt	0.067 (LPAI)	0.220 (LPAI)
	0.063 (HPAI)	0.281 (HPAI)

The values for LPAI are the means of the values of 8 subtypes, the values for HPAI the mean of 2 strains of H5N1.

Vectors

No vectors exist for the 1918 H1N1V.

Other epidemiological/ecological data

A credible scenario leads to the conclusion that a 1918 influenza virus-infected (LAI) researcher could lead to an uncontained outbreak among the public (Osterholm 2008).

A bite from an infected ferret is considered the most likely exposure event for 1918 H1N1 (Osterholm 2008).

The pandemic potential of 1918 influenza virus is thought to be significant (Henkel 2008). Clinical impact of the 1918 H1N1 virus in 2008 would be much less than in 1918—even less than the H2N2 epidemic of 1957 (Murphy 2008).

1918 H1N1 virus should not be treated differently than other human influenza A viruses with pandemic potential (Murphy 2008).

"Findings [on] …data on the timing of 19 classes of NPI in 17 U.S. cities during the 1918 pandemic… support the hypothesis that rapid implementation of multiple NPIs [non-pharmaceutical interventions] can significantly reduce influenza transmission, but that viral spread will be renewed on relaxation of such measures [6 weeks or less]" (Dowell and Bresee 2008; Hatchett, Mecher, and Lipsitch 2007; Markel et al. 2007; Bootsma and Ferguson 2007).

"An important component of the current pandemic planning strategies in the United States and many other countries is to keep ill persons out of the hospital and have large numbers of them cared for at home, with the idea of avoiding the amplification of infections in hospitals seen with SARS in 2003 and with a range of other modern epidemics (author here citing CDC 2007 - Community Strategy for Pandemic Influenza Mitigation)" (Dowell and Bresee 2008).

"Regardless of R_0, unless drastic travel restrictions are imposed, the extent or duration of the pandemic [referring to influenza A, not otherwise specified] is insensitive to details of the amount and locations(s) of introductions of pandemic influenza virus in our simulations" (Germann et al. 2006).

Studies generally have shown that susceptibility to influenza infection, preexisting antibody titer, viral shedding, and symptomatic illness are related in the following ways (Bridges, Kuehnert, and Hall 2003):

- The higher a person's existing antibody titer against the same or a related influenza virus strains, the larger the inoculum of virus needed for infection and the less likely that clinical illness will develop (author cites refs 28, 36);
- The amount of viral shedding correlates with the severity of illness and temperature elevation (author cites ref 31);
- The amount of virus required to induce infection is inversely related to the size of infectious particles administered, with particles smaller than 10 mm in diameter more likely to cause infection in the lower respiratory tract (author cites ref 36).

In February 2007, CDC published a planning guidance document for non-pharmaceutical interventions (NPI). The rationale for NPI is, "[i]t is highly unlikely that the most effective tool for mitigating a pandemic (i.e., a well-matched pandemic strain vaccine) will be available when a pandemic begins." Furthermore, the pandemic could find populations "potentially without sufficient quantities of influenza antiviral medications. In addition, it is not known if influenza antiviral medications will be effective against a future pandemic strain." "The use of NPIs for mitigating a community-wide epidemic has three major goals: (1) delay the exponential growth in incident cases and shift the epidemic curve to the right in order to *buy time* for production and distribution of a well-matched pandemic strain vaccine, (2) decrease the epidemic peak, and (3) reduce the total number of incident cases, thus reducing community morbidity and mortality." "Communities, individuals and families, employers, schools, and other organizations will be asked to plan for the use of these interventions to help limit the spread of a pandemic, prevent disease and death, lessen the impact on the economy, and keep society functioning. This interim guidance introduces a Pandemic Severity Index to characterize the severity of a pandemic, provides planning recommendations for specific interventions that communities might use for a given level of pandemic severity, and suggest sic when these measures should be started and how long they should be used" (CDC 2007).

"The pandemic mitigation interventions described in this document include:

Isolation and treatment (as appropriate) with influenza antiviral medications of all persons with confirmed or probable pandemic influenza. Isolation may occur in the home or healthcare setting, depending on the severity of an individual's illness and/or the current capacity of the healthcare infrastructure.

Voluntary home quarantine of members of households with confirmed or probable influenza case(s) and consideration of combining this intervention with the prophylactic use of antiviral medications, providing sufficient quantities of effective medications exist and that a feasible means of distributing them is in place. Dismissal of students from school (including public and private schools as well as colleges and universities) and school-based activities and closure of childcare programs, coupled with protecting children and teenagers through social distancing in the community to achieve reductions of out-of-school social contacts and community mixing.

Use of social distancing measures to reduce contact between adults in the community and workplace, including, for example, cancellation of large public gatherings and alteration of workplace environments and schedules to decrease social density and preserve a healthy workplace to the greatest extent possible without disrupting essential services. Enable institution of workplace leave policies that align incentives and facilitate adherence with the non-pharmaceutical interventions (NPIs) outlined above.
All such community-based strategies should be used in combination with individual infection control measures, such as hand washing and cough etiquette" (CDC 2007).

Therapeutics/vaccines

Immunity induced by current influenza viruses or vaccines will restrict the replication of a 1918 H1N1 virus in current human populations (Murphy 2008, 2008).

Current vaccines can boost antibody to 1918 virus (Tumpey et al. 2004; Murphy 2008). Immunization of mice with 1999 H1N1 vaccine reduced replication of 1918 virus by 50-fold (Tumpey et al. 2004; Murphy 2008), and reduction would be expected to be greater if the mice, like current 2008 humans, had been repeatedly infected with wild type H1N1 (Murphy 2008).

Oseltamivir (Tamiflu®) protects mice from a lethal challenge of 1918 influenza virus (Katz 2008).

"Recombinant viruses possessing the 1918 NA or both the 1918 HA and 1918 NA were inhibited effectively in both tissue culture and mice by the NA inhibitors, zanamivir and oseltamivir (Tumpey et al. 2002). A recombinant virus possessing the 1918 M segment was inhibited effectively both in tissue culture and in vivo by the M2 ion-channel inhibitors amantadine and rimantadine. These data suggest that current antiviral strategies would be effective in curbing the dangers of a re-emergent 1918 or 1918-like virus" (Tumpey et al. 2002).

"Mice that received an intramuscular immunization of the homologous or Sw-Iowa-30-inactivated vaccine developed HI and VN antibodies to the 1918 recombinant virus and were completely protected against lethal challenge. Mice that received A-PR-8-34, A-Texas-36-91, or A-New Caledonia-20-99 H1N1 vaccines displayed partial protection from lethal challenge" (Tumpey et al. 2004).

Intranasal or intramuscular vaccination with 1918 influenza virus-like particles protect mice from a lethal 8-gene 1918 influenza virus challenge (Tumpey 2008).

"Mice vaccinated with 1918 HA plasmid DNAs showed complete protection to a lethal challenge from the 1918 virus" (Kong et al. 2006).

Four measures of influenza vaccine efficacy have been estimated in detail on the basis of challenge studies in human volunteers from 1980 to 2008 (Basta et al. 2008).

Vaccines for influenza A : inactivated vaccines (Fluzone®, Fluvirin™) obtained from infected chicken embryos are most commonly used. Attenuated vaccines include FluMist (Barnard 2009).

Approved therapeutics for seasonal influenza A virus infections include the neuraminidase inhibitors oseltamivir phosphate (Tamiflu) and zanamivir (Relenza®) and the M2 ion channel blockers amantadine (Symmetrel®) and rimantadine (Flumadine®) (Barnard 2009).

"Influenza-associated pneumonia patterns may now be influenced by the administration of pneumococcus, *Hemophilus influenzae* b, and meningococcus vaccine, and cases have tended to occur in elderly individuals" (Morens, Taubenberger, and Fauci 2008).

Modeling (based on the assumption that antiviral therapy would reduce the period of illness by 1 day) has predicted targeted antiviral prophylaxis to be nearly as effective as vaccinating 80 percent of the population and has potential as an effective measure for containing influenza until adequate quantities of vaccine become available (Longini et al. 2004).

Modeling has projected that stockpiles sufficient to cover 20–25 percent of the population would be sufficient to treat most of the clinical cases and could lead to 50–77 percent reductions in hospitalizations (Gani et al. 2005).

Other remarks

"The 1918 H1N1 virus appears to be an avian-like influenza virus derived *in toto* from an unknown source" [author cites two primary sources] (Taubenberger and Morens 2006).

Recommendations for work with 1918 influenza virus include enhanced BSL-3 and ABSL-3 practices, procedures and facilities; large laboratory animals such as NHPs should be housed in primary barrier systems in ABSL-3 facilities (Henkel 2008).

Seasonal influenza vaccination for researcher working with 1918 influenza virus is recommended by CDC (Uyeki 2008).

The current policy of the Division of Select Agents and Toxins regarding the 1918 influenza virus requires oseltamivir (Tamiflu) preexposure prophylaxis in enhanced BSL-3 biocontainment; it is not required for work in BSL-4 biocontainment. That requirement was based on a risk assessment conducted by Intragovernmental Select Agents and Toxins Technical Advisory Committee (which advises the Division of Select Agents and Toxins) (Henkel 2008). The NIH-RAC is considering whether to recommend this policy to the NIH (Corrigan-Curay 2008).

The RAC opinion, by the end of the December 2, 2008, meeting, was strongly against recommending preexposure prophylactic use of antiviral agents for scientists working with 1918 H1N1 virus.

One view is that a series of incorrect assumptions led to the existing recommendation of mandatory antiviral prophylaxis for work with 1918 H1N1 virus, and new data on restricted replication of 1918 virus in H1N1 immune mice makes continuation of the policy unnecessary and unwise (Murphy 2008, 2008).

The RAC is considering, "whether the public health objective should be to prevent [by requiring preexposure prophylaxis] any researcher from developing an active case of 1918 H1N1 [infection] that could be spread to the public, or is it acceptable to be prepared to contain the infection once in the community" (Shapiro 2008). Further, the RAC is considering whether "the ethical obligation to protect the public [is] higher because this virus was recreated in the laboratory" (Shapiro 2008). The CDC's perspective is that, without a vaccine for the 1918 virus, the public health objective is to prevent a case of illness in a lab worker by use of biocontainment, training, seasonal influenza vaccination and oseltamivir preexposure prophylaxis, and that there is a higher obligation to the public with regard to a recreated virus (Uyeki 2008).

Pre-exposure prophylaxis with antiviral agents would not necessarily be 100 percent effective in preventing infection with 1918 H1N1 virus (Uyeki 2008).

The Occupational Medicine response plan for NIH Division of Intramural Research Laboratory of Infectious Diseases, Subbarao lab (SARS-CoV, HPAI, H2N2 viruses) (Subbarao 2008) is as follows:

- If an employee reports a spill but had intact respiratory protection, the employee returns to work, monitors symptoms and temperature and reports to Occupational Medicine physician daily;
- If an employee reports a spill but had questionable respiratory protection or a percutaneous exposure, the employee is sent home and is asked to stay home, started on

post-exposure prophylaxis, avoids contact with others and wears a surgical mask, monitors temperature and symptoms, and reports to Occupational Medicine physician twice a day;

- If an employee reports a fever (temp of more than 100.4 °F), the employee stays in place, dons a surgical mask, and notifies an Occupational Medicine physician. The Occupational Medicine physician obtains a medical history and a work and social history for the prior 14 days, consults with PI and infectious disease specialists. If indicated, an Occupational Medicine physician coordinates safe transport and appropriate hospital isolation and diagnostic laboratory testing.

The Select Agent Program requires work with 1918 virus be predicated on approval by the Division of Select Agents and Toxins of an [application] amendment, and this also requires a detailed inspection from the Division of Select Agents and Toxins group (Henkel 2008).

Antigenic relationships/synonyms

Influenza viruses are spherical or pleomorphic, single-stranded, negative-sense RNA enveloped viruses of the genus *Influenzavirus A* belonging to family *Orthomyxoviridae*. Influenza A and B viruses contain eight separate ribonucleoprotein (RNP) segments, while influenza C virus contains seven, each of which encodes 1 or 2 proteins. The internal antigens (M1 and NP proteins) are the type-specific antigens used to determine if a virus is A, B, or C, while the external hemagglutinin (HA) and neuraminidase (NA) are the subtype- and strain-specific antigens (Barnard 2009).

C.1.1.5 SARS-associated coronavirus (SARS-CoV)

Host range

a. Field

Coronaviruses are important pathogens of mammals and birds causing enteric or respiratory tract infections in a variety of animals including humans, livestock, and pets (Wang and Eaton 2007; Shi and Hu 2008).

Samples from wild animals sold as food in the local market in Guangdong, China, yielded SARS-associated coronavirus from palm civets (*Paguma* sp.), but the animals did not always show clinical signs (Wang and Eaton 2007; Shi and Hu 2008).

Virus crossed the animal-human barrier from palm civets to humans. More than 10,000 masked palm civets were destroyed in Guangdong Province (Wang and Eaton 2007).

Virus has been isolated from raccoon dogs (*Nyctereutes* sp.), Chinese ferret badgers (*Melogale moschata*), and domestic cats (Wang and Eaton 2007; Shi and Hu 2008). 2005 two studies identified a number of SARS-like coronaviruses in Chinese bats (Wang and Eaton 2007) (Shi and Hu 2008).

Phylogenetic analysis of bat viruses indicated a high probability that SARS-associated coronavirus originated in bats and spread to humans either directly or through animals held in Chinese markets. The bats did not show any visible signs of disease but are the likely natural reservoirs (Wang and Eaton 2007; Shi and Hu 2008).

Bats are natural reservoirs of a very similar virus [bat SARS-CoV] and civet cats might be involved in the virus's emergence (Feng and Gao 2007).

In China, original infected humans had a typical history of contacting animals in the food industry (Feng and Gao 2007; Shi and Hu 2008).

In live-animal markets in Guangdong Province 13–40 percent of wild animal traders and slaughterers were revealed to be seropositive (Feng and Gao 2007; Shi and Hu 2008).

Suspicion focused on palm civets because more than 70 percent of SARS seropositives were among the traders who were primarily trading masked palm civets; SARS-CoV-like viruses were isolated from Himalayan palm civets (*Paguma larvata*) [nasal samples], and nasal and fecal samples were RT-PCR positive; and a raccoon dog (*Nyctereutes procyonoides*) [virus isolates and RT-PCR from nasal and fecal samples] at an animal

market; neutralizing antibody in serums from *P. larvata*, *N. procyonoides*, and *Melogale moschata* [Chinese ferret-badger] (Guan et al. 2003; Feng and Gao 2007).

A subsequent survey failed to support widespread infection in wild and/or farmed civets (Feng and Gao 2007).

Civets may be the carrier source of SARS-CoV-like virus (Feng and Gao 2007).

Virus exists in civets and other common animals within wet-market systems. It might reflect an *artificial* market cycle in native species rather than an indication of a natural reservoir (Feng and Gao 2007).

Bats function as the natural reservoirs and rarely display clinical signs despite persistent infections with multiple viruses (Feng and Gao 2007).

Bats or bat products in food and traditional medicine markets make it possible that SARS-CoV-like viruses infect humans from bats, subsequently adapt to humans, and trigger human-to-human transmission (Feng and Gao 2007).

Closely related coronaviruses isolated from horseshoe bat species of the genus *Rhinolophus* within the family *Rhinolophidae* (Shi and Hu 2008).

High prevalence of antibodies observed in the lesser horseshoe bat *Rhinolophus pusillus* ((Daddario-DiCaprio) 2/6), the great-eared horseshoe bat *Rhinolophus macrotis* (5/7) and the Pearson's horseshoe bat *Rhinolophus pearsoni* (13/46) by a sandwich ELISA method (Shi and Hu 2008).

Chinese horseshoe bat *Rhinolophus sinicus* (12/18 by Western blots, 31/37 positive by enzyme immunoassay (EIA) with titer ≥1:400, and 8/19 by a neutralization assay for human SARS-CoV with titer ≥1:20); very low percentage of positive samples were detected in the fruit bat *R. leschenaultia* (1.2 percent) (Shi and Hu 2008).

Using RT-PCR, a low prevalence of viruses was detected in fecal swabs of *Rhinolophus ferrumequinum* (1/8), *R. macrotis* (1/8) and *R. pearsoni* (3/30), as compared with a high prevalence in *R. sinicus* (23/59); 39 percent of fecal swabs from wild Chinese horseshoe bats, *R sinicus*, contained genetic material similar to SARS-CoV (Shi and Hu 2008; Wang et al. 2006).

In contaminated markets, red foxes, domestic cats, Lesser rice field rats, and others might harbor the virus (Shi and Hu 2008).

The prime suspect as an animal reservoir host remains the civet cat (*Paguma larvata*), and the foci for spread from this host to humans seems to be the animal markets in China (Shi and Hu 2008).

Domestic cats living in the Amoy Gardens apartment block in Hong Kong, where more than 100 residents contracted SARS (2003), were found to be infected with SARS-CoV (Martina et al. 2003).

Investigations of SARS-CoV in animals are summarized in Table C-7 (Shi and Hu 2008).

Table C-7. Summary of investigations of SARS-CoV in other animal species (does not include masked palm civets and bats.

Species	Common name	Methods	Prevalence	Reference (as cited by Shi & Hu {Shi, 2008 #13541}
Macaca mulatta	Rhesus macaque	RT-PCR	0/20	Lau et al. (2005)
Canis familiaris	Dog	Antibody	0/20	Chen et al. (2005)
		Real-time RT-PCR	0/5	(Wang et al., 2005a) and (Wang et al., 2005b)
Nyctereutes procyonoides	Raccoon dog	RT-PCR, virus isolation	1/1	Guan et al. (2003)
		RT-PCR	15/15	Kan et al. (2005)
Vulpes vulpes	Red fox	Real-time RT-PCR	3/5	(Wang et al., 2005a) and (Wang et al., 2005b)
Melogale moschata	Chinese ferret-badger	Antibody	1/2	Guan et al. (2003)
Arctonyx collaris	Hog-badger	RT-PCT, antibody	0/3	Guan et al. (2003)
Mustela vison	Mink	Real-time RT-PCR	0/1	(Wang et al., 2005a) and (Wang et al., 2005b)
Felis catus	Domestic cat	RT-PCT, antibody	0/4	Guan et al. (2003)
		Real-time RT-PCR	4/20	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/13	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/3	(Wang et al., 2005a) and (Wang et al., 2005b)
		Antibody	0/11	Chen et al. (2005)
Sus scrofa domestica	Pig	Antibody, RT-PCR	2/108	Chen et al. (2005)
S. scrofa	Wild boar	Real-time RT-PCR	1/19	(Wang et al., 2005a) and (Wang et al., 2005b)
Muntiacus reevesi	Chinese muntjac	RT-PCT, antibody	0/2	Guan et al. (2003)

		Antibody	0/9	(Wang et al., 2005a) and (Wang et al., 2005b)
Bos tarurs	Cattle	Real-time RT-PCR	0/60	Chen et al. (2005)
Capra hircas	Goat	Real-time RT-PCR	0/3	(Wang et al., 2005a) and (Wang et al., 2005b)
Oryctolagus cuniculus	Rabbit	Real-time RT-PCR	0/6	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/5	(Wang et al., 2005a) and (Wang et al., 2005b)
Lepus sinensis	Chinese hare	RT-PCT, antibody	0/3	Guan et al. (2003)
Castor fiber	Beaver	RT-PCT, antibody	0/3	Guan et al. (2003)
Niviventer fulvescens	Chestnut spiny rat	RT-PCR	0/12	Lau et al. (2005)
Rattus rattus flavipectus	Buff-bellied rat	RT-PCR	0/4	Lau et al. (2005)
Rattus sikkimensis	Sikkim rat	RT-PCR	0/44	Lau et al. (2005)
Rattus losea	Lesser rice field rat	Real-time RT-PCR	1/6	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/16	(Wang et al., 2005a) and (Wang et al., 2005b)
Anas domestica	Duck	Antibody	0/30	Chen et al. (2005)
Anas platyhynchos	Spotbill duck	Real-time RT-PCR	0/13	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/9	(Wang et al., 2005a) and (Wang et al., 2005b)
Anser anser	Greylag goose	Real-time RT-PCR	1/10	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/14	(Wang et al., 2005a) and (Wang et al., 2005b)

1

Gallus domestiaus	Chicken	Antibody	0/11	Chen et al. (2005)
Gallus gallus	Red jungle fowl	Real-time RT-PCR	0/46	(Wang et al., 2005a) and (Wang et al., 2005b)
Francolinus pintadeanus	Chinese francolin	Real-time RT-PCR	0/31	(Wang et al., 2005a) and (Wang et al., 2005b)
		Real-time RT-PCR	0/2	(Wang et al., 2005a) and (Wang et al., 2005b)
Phasianus colchicus	Common pheasant	Real-time RT-PCR	0/8	(Wang et al., 2005a) and (Wang et al., 2005b)
Pavo muticus	Green peafowl	Real-time RT-PCR	0/2	(Wang et al., 2005a) and (Wang et al., 2005b)
Columba livia	Pigeons	Real-time RT-PCR	0/6	(Wang et al., 2005a) and (Wang et al., 2005b)

b. Experimental

A mouse-adapted strain has been used in BALB/c mice (Roberts et al. 2007; Day et al. 2009).

Replicates in cynomolgus macaques (Cinatl et al. 2005).

Replicates in the lungs of mice, hamsters and domestic cats; these animals remain asymptomatic (Perlman and Dandekar 2005).

Initial reports indicated that cynomolgus macaques (*M. fascicularis*) and ferrets develop clinically evident respiratory disease and would be useful animal models for studying SARS. But the results are not reproducible (Perlman and Dandekar 2005).

Experimental infection of civets with human isolates reproduced overt clinical signs similar to that of SARS patients. It is unlikely that civets play a role as natural reservoir hosts but are susceptible hosts (Feng and Gao 2007).

SARS-CoV infects a wide-range of hosts, including masked palm civets, monkeys [common marmoset, *Callithrix jacchus*], cats, ferrets, mice, pigs, chickens, guinea pigs, and golden Syrian hamsters (Shi and Hu 2008) (Wang and Eaton 2007).

Virus replicates in the respiratory tracts of African green monkeys (*Chlorocebus aethiops sabaeus*) and rhesus (*M. mulatta*) and cynomolgus macaques (*M. fascicularis*) (titer of infecting virus was $10^{6.3}$ $CCID_{50}$) but minimal to no clinical disease was observed. Clinical disease was observed in cynomolgus macaques, but observations were not uniformly reproducible (McAuliffe et al. 2004; Gillim-Ross and Subbarao 2006).

Virus replicates to high titers in the lungs and nasal turbinates of 6- to 8-week-old mice. Viral nucleic acid is detected in the lungs and intestines (Gillim-Ross and Subbarao 2006).

Failure to isolate the virus and the lack of virus shedding indicate that neither pigs nor chickens are likely to play a role as an amplifying host (Weingartl, Copps, et al. 2004).

Most reproducible data on virus replication obtained from inbred mice and from golden Syrian hamsters that are not inbred but are of limited genetic heterogeneity (Subbarao and Roberts 2006).

In golden Syrian hamsters, the virus replicates to a high titer in the respiratory tract, 10^8 $CCID_{50}$/gram of lung tissue following intranasal administration (Subbarao and Roberts 2006).

Ferrets (*Mustela furo*) and domestic cats (*Felis catus*) are susceptible to infection by SARS-CoV and can efficiently transmit the virus to previously uninfected animals housed with them (Wang et al. 2004).

In domestic cats and ferrets inoculated intratracheally with 10^6 $CCID_{50}$ obtained from a patient who died from SARS and passaged 4 times on Vero cells: no clinical signs in

cats; 3/6 ferrets exhibited clinical signs and one died; all cats and ferrets shed virus from pharynx, trachea, and lungs from 2–10 (cats)/–14 (ferrets) days after infection. SARS-CoV was isolated from the trachea and lungs: cats 10^3+/– 0.51$CCID_{50}$/mL; ferrets 10^6 +/– 0.70 $CCID_{50}$/mL. In the GI and urinary tracts, SARS-CoV was detected by RT–PCR. All animals seroconverted. All attempts to infect suckling mice through intracerebral inoculation failed. Infection via non-direct contact was shown when uninoculated cats and ferrets housed with inoculated cats and ferrets became infected with SARS-CoV (Wang et al. 2004).

SARS-CoV injected intratracheally into chickens, turkeys, geese, ducks, and quail, or into the allantoic sac of their embryonating eggs, failed to cause disease or replicate. That suggests that domestic poultry are unlikely to have been the reservoir, or associated with dissemination, of SARS-CoV in the animal markets of southern China (Swayne et al. 2004).

SARS animal model was established by inoculating SARS-CoV into rhesus macaques (*M. mulatta*) through the nasal cavity; RNA was detected by nested RT-PCR in the pharyngeal swab and nasal swab samples on the first day after infection and in all monkeys from the 5th to 16th day post-infection (Qin et al. 2005).

Animal studies provide proof from experimental infection of cynomolgus macaques (*M. fascicularis*) that SARS-CoV is the etiological agent of SARS (Fouchier et al. 2003).

Prevalence/incidence/attack rate

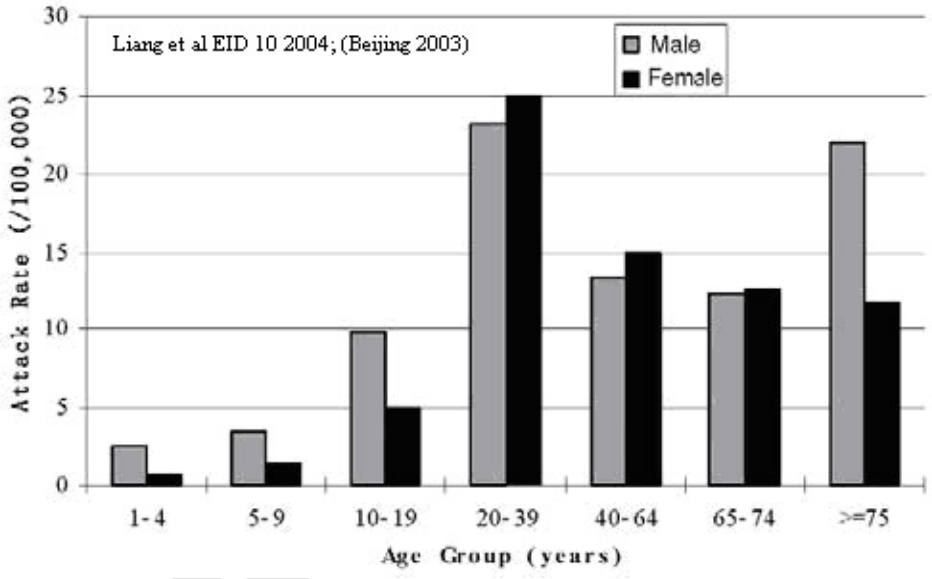
Seroprevalence rate was 88.9 percent (80/90) for healthcare workers with SARS and 1.4 percent (15/1,057) for healthcare workers who were apparently healthy with seroprevalence in a reference group at 0.4 percent (3/709); findings suggest that inapparent infection is uncommon in humans (Chu et al. 2004).

Attack rate for probable SARS among healthcare workers in Beijing is estimated as 465 per 100,000 (Liang et al. 2004).

Highest attack rate in Beijing 2003 as 25 percent among males 20–39 y.o. (Liang et al. 2004).

Demographic characteristics of case-patients: children <10 years of age accounted for 0.9 percent of probable cases, median age of those who became ill was 33 years; age-specific attack rates were highest in those 20–39 years of age (relative risk [RR] 1.7, 95 percent confidence interval[CI] 1.53 to 1.89], as compared with those 40–64 years, and significantly lower in children (1–4 years of age, RR 0.12 [CI 0.05 to 0.28], 5–9 years, RR 0.17 [CI 0.09 to 0.31] and 10–19 years, RR 0.53 [CI 0.44 to 0.64]), as compared with those aged 40–64 years; overall, male patients had similar rates as female patients, but the risk differed significantly in certain age groups: among those 10–19 years of age, the RR for SARS in male patients was 1.96, 95 percent CI 1.36 to 1.83, as compared with that of females; and in those >75 years, RR for male patients was 1.88 (95 percent CI 1.08 to 3.29) (Liang et al. 2004).

Figure C-2. Attack rates (cases per 100,000 population) by age and sex, Beijing 2003.



Source: (Liang et al. 2004).

Attack rates were >50 percent in hospitals in China, Hong Kong, and Singapore (Goh et al. 2004).

Secondary household attack rates were 6.2 percent in Singapore and 4.6 percent in China (Goh et al. 2004).

Secondary attack rate in households was 8 percent in Hong Kong (Lau et al. 2004).

Attack rates by percentage: male, 46.6, females, 53.4; 18–30 years, 46.6, 31–40 years, 15.3, 41–50, 16.2, 51–60 years, 10.9, >60 years, 11.1; overall, 14.9 percent (Lau et al. 2004).

R_0/incubation period/infectious period/infectious dose

R_0 estimated between 2 and 3; because the transmission route for SARS-CoV superspreaders were atypical of the disease in most cases estimates of R_0 have not included superspreaders to not skew results; therefore, some R_0 values reported might not be a true indication of transmissibility during the epidemic (Chan, Tang, and Hui 2006).

R_0 values/modeling reported by several investigators (Anderson et al. 2004; Chan, Tang, and Hui 2006; Riley et al. 2003; Lipsitch et al. 2003; Galvani, Lei, and Jewell 2003; Cauchemez et al. 2006; Fraser et al. 2004; Hufnagel, Brockmann, and Geisel 2004; Lloyd-Smith, Galvani, and Getz 2003).

1.63 (90% ₑonfidence interval: 0.54-2.65) for first three generations of transmission in Singapore 2003; 2.55 (90% confidence interval: 0.50-4.50) for first two generations of transmission in Singapore 2003. Third generation occurred before centralized control measures were in place, but after WHO's global alert on SARS, so drop in R0 estimate could be explained by informal behavior changes or informal increased isolation of patients. These are direct estimates of R in Singapore from contact tracing data (Lloyd-Smith et al. 2005).

1.88 (90% confidence interval 0.41-3.32) for first two generations of transmission in Beijing 2003; 0.94 (90% confidence interval 0.27-1.51) for only second generation of transmission in Beijing 2003. The first generation consisted of a single infected individual who directly transmitted to 33 others. These are direct estimates of R in Beijing from contact tracing data (Lloyd-Smith et al. 2005).

3.6 (95% confidence interval 3.1-4.2) for Hong Kong; 2.4 (95% confidence interval 1.8-3.1) for Vietnam; 3.1 (95% confidence interval 2.3-4.0) for Singapore; 2.7 (95% confidence interval 1.8-3.6) for Canada: these are estimates inferred from epidemic curves and generation interval data from each city, using a likelihood-based estimation procedure (Wallinga and Teunis 2004).

Estimated 0.86 (0.24-1.18) for Toronto, 1.70 (0.44-2.29) for Hong Kong, 1.83 (0.47-2.47) for Singapore (Chowell, Castillo-Chavez, et al. 2004). Ranges are the inter-quartile range. These are model-based estimates assuming a portion of population is less susceptible, which explains why these are lower than other estimates. For example, with uniform susceptibility, estimate for Hong Kong increases to 2.6 (Bauch et al. 2005).

2.7 (95% confidence interval 2.2-3.7) for Hong Kong (Riley et al. 2003). This excluded super-spreading events. Including them may increase the estimate to about 3.2 (Bauch et al. 2005). Estimate was based on fitting stochastic spatial simulation to case-incidence data.

3.5 (90% confidence interval 1.5-7.7) for Singapore using Bayesian procedure. Range of 2.2-3.6 using deterministic model estimate (Lipsitch et al. 2003).

4.80 for Toronto; 3.60 for Hong Kong; 5.04 for Singapore; 4.91 for Beijing (Gumel et al. 2004): these are deterministic dynamic model-based estimates. The reason they are higher than other estimates may be because of unrealistic assumptions about the efficacy of control measures (Bauch et al. 2005).

4.2 for Taiwan (Hsieh, Chen, and Hsu 2004). May be higher than other estimates because of uncertainties in model infectiousness parameters and/or unusually high rate of hospital transmission in Taiwan.

2.1 for Hong Kong (Zhou and Yan 2003). Lower because estimate includes data from after implementation of control measures (Bauch et al. 2005).

1.5 for Toronto, 2.0 for Hong Kong (Choi 2003). Estimates are too low because of authors' interpretation of generation time parameter. After adjusting for this, results are 2.5 for Toronto and 3.4 for Hong Kong (Bauch et al. 2005).

1.1-3.3 for Beijing (Wang and Ruan 2004). Lower end of range assumes no transmission after entering hospital, highly unrealistic (Bauch et al. 2005).

2.23 for Taiwan (Bombardt 2006) using approach similar to Wallinga and Teunis (Wallinga and Teunis 2004).

2.87 for mainland China (Zhang 2007).

3 (range 1.5-5)) (Lloyd-Smith, Galvani, and Getz 2003).

2-3 (Anderson et al. 2004).

2.5-2.9 Hong Kong (Fang, Chen, and Hu 2005).

Hong Kong R_0 = 2–3, excluding superspreading events (Riley et al. 2003).

Main conclusion to draw from these estimates of R_0 is that SARS-CoV is of low transmissibility by comparison with other directly transmitted viruses such as influenza A (R_0 of *ca* 7 or more) and the measles virus (R_0 of *ca* 15–18 before widescale immunization) (Anderson et al. 2004).

Estimated that a single infectious case of SARS will infect about three secondary cases in a population that has not yet instituted control measures (Lipsitch et al. 2003).

In Toronto, mean incubation period was 5 days (median 4 day; range 2–10 days)(Varia et al. 2003).

Incubation period: estimates of 2–12 days; range 4–5.3 days, mean 4.5–4.6 days from China/Hong Kong data (Anderson et al. 2004).

Incubation period estimates of mean and median for patients in China, Hong Kong, Singapore and Canada consistently ranged from 4 to 6 days (Donnelly et al. 2004).

Infectious period (Amoy Gardens, Hong Kong): range 5–15 days; peak viral load (naso-pharyngeal wash) at day 10 (Peiris, Chu, Cheng, Chan, Hung, Poon, Law, Tang, Hon, Chan, Chan, Ng, Zheng, Ng, Lai, Guan, Yuen, et al. 2003).

Infectious dose for humans is unknown.

Infectious dose used in *M. mulatta* monkeys was 10^3, 10^5, and 10^7 $CCID_{50}$; optimal dose was 10^5 $CCID_{50}$ which produced serologic conversion in 5 of 8 animals (HID63).(Qin et al. 2005).

Morbidity/Case fatality ratio

Primary symptoms include fever, headache, myalgias and malaise, followed by a dry cough, and shortness of breath in more severe cases. About 25 percent of patients developed severe disease that progressed to adult respiratory distress syndrome (McIntosh and Perlman 2010).

Asymptomatic or minimally symptomatic infections apparently are exceedingly rare (~0.1 percent of people tested) (Leung et al. 2006; Yang et al. 2009).

One near pandemic between November 2002 and July 2003, with 8,096 known infected cases and 774 deaths (a case-fatality rate of 9.6 percent) worldwide (Feng and Gao 2007; Lam, Zhong, and Tan 2003).

Mortality by age group is less than 1 percent for people aged younger than 25, 6 percent for those 25 to 44, 15 percent for 45 to 64 and more than 50 percent for 65 and older (Lam, Zhong, and Tan 2003; Zhong and Wong 2004; Feng and Gao 2007).

Rapid transmission and high mortality rate (Feng and Gao 2007; Lam, Zhong, and Tan 2003). Overall mortality rate is about 10 percent but the mortality varies with age; few children and generally appeared to be milder in pediatric age group, mortality rate in the elderly was as high as 50 percent (Cinatl et al. 2005).

By 2007, 8,400 cases with more than 812 fatalities in more than 30 countries (Feng and Gao 2007).

Case-fatality rate during early outbreaks was nearly 10 percent (range 0–40 percent) (Feng and Gao 2007).

Case fatality rates, Hong Kong, age related, younger than 30 years, less than 1 percent; 30–44 years, males = 10–12 percent, females= 5 percent; 45–59 years, males = 20–22 percent, females = 10 percent; 60–74 years, males = females = 40–45 percent; 75+ years, males = 65 percent, females = 70 percent (Anderson et al. 2004).

Case fatality rate for SARS was less than 1 percent for patients aged 24 years or younger, 6 percent for 25–44 years, 15 percent for 45–64 years, and more than 50 percent for patients aged 65 years or older (Chan, Tang, and Hui 2006).

Mortality rates of mechanically ventilated patients ranged from 44.8 to 48 percent at 28 days after ICU admission and 51.7 percent at 8 weeks; through discharge from the hospital, mortality rate was 64 percent; global case fatality rate was 9.6 percent (Tai 2006).

As of May 20, 2003, case-fatality rate was 6.4 percent for probable SARS case-patients; case-fatality rates increased with age (0.5 percent in younger than 20 year olds; 4.8 percent for those 20–64 years; and 27.7 percent for 65 years or older, $p < 0.001$); case fatality rate among probable case-patients, excluding those still hospitalized, was 8.4 percent (Liang et al. 2004).

Overall complication rate of 23.7 percent and case-fatality rate of 19.7 percent (Wang et al. 2004).

Amoy Gardens mortality rate was 6.7 percent (Peiris, Chu, Cheng, Chan, Hung, Poon, Law, Tang, Hon, Chan, Chan, Ng, Zheng, Ng, Lai, Guan, and Yuen 2003).

Toronto, 2003, case-fatality rate was 2.9 percent among the 102 cases less than 60 years of age and 53.8 percent among the 26 cases 60 years or older; overall, 13.3 percent (Varia et al. 2003).

In Toronto nosocomial cases, death rate, 41.2 in females, 58.8 in males (Varia et al. 2003).

CFR in Hong Kong increased with age as in other parts of the world: 14.7 percent in persons under 44 years of age, 21.4 percent between 45 and 64 years and 63.9 percent over 64 years; experiences suggested that deaths were associated with pre-existing illnesses in the older age group (> 64 years)(Chan-Yeung and Xu 2003).

Case fatality rate for SARS-CoV infection in 2003 was estimated to be 13.2 percent for individuals younger than 60 years and close to 50 percent for individuals 60 years and older (Gillim-Ross and Subbarao 2006).

Average overall CFR for all countries increased from 10.4 percent on April 21, 2003, to 14.7 percent on May 12 (largely attributable to the sudden rise in CFRs in China and Taiwan) (Galvani, Lei, and Jewell 2003).

Table C-5. Summary of probable SARS cases with onset of illness from November 1, 2002 to July 31, 2003

	Cumulative number of cases					
Areas	**Female**	**Male**	**Total**	**Median age (range)**	**Number of deaths[a]**	**Case fatality ratio (%)**
Australia	4	2	6	15 (1-45)	0	0
Canada	151	100	251	49 (1-98)	43	17
China	2674	2607	5327[b]	Not available	349	7
China, Hong Kong Special Administrative Region	977	778	1755	40 (0-100)	299	17
China, Macao Special Administrative Region	0	1	1	28	0	0
China, Taiwan	218	128	346[c]	42 (0-93)	37	11
France	1	6	7	49 (26 - 61)	1	14
Germany	4	5	9	44 (4-73)	0	0
India	0	3	3	25 (25-30)	0	0
Indonesia	0	2	2	56 (47-65)	0	0
Italy	1	3	4	30.5 (25-54)	0	0
Kuwait	1	0	1	50	0	0
Malaysia	1	4	5	30 (26-84)	2	40
Mongolia	8	1	9	32 (17-63)	0	0
New Zealand	1	0	1	67	0	0
Philippines	8	6	14	41 (29-73)	2	14
Republic of Ireland	0	1	1	56	0	0
Republic of Korea	0	3	3	40 (20-80)	0	0
Romania	0	1	1	52	0	0
Russian Federation	0	1	1	25	0	0
Singapore	161	77	238	35 (1-90)	33	14
South Africa	0	1	1	62	1	100

1

Republic of Ireland	0	1	1	56	0	0
Republic of Korea	0	3	3	40 (20-80)	0	0
Romania	0	1	1	52	0	0
Russian Federation	0	1	1	25	0	0
Singapore	161	77	238	35 (1-90)	33	14
South Africa	0	1	1	62	1	100
Spain	0	1	1	33	0	0
Sweden	3	2	5	43 (33-55)	0	0
Switzerland	0	1	1	35	0	0
Thailand	5	4	9	42 (2-79)	2	22
United Kingdom	2	2	4	59 (28-74)	0	0
United States	13	14	27	36 (0-83)	0	0
Viet Nam	39	24	63	43 (20-76)	5	8
Total			**8096**		**774**	**9.6**

a. Includes only cases whose death is attributed to SARS.
b. Case classification by sex is unknown for 46 cases.
c. Since 11 July 2003, 325 cases have been discarded in Taiwan, China. Laboratory information was insufficient or incomplete for 135 discarded cases, of which 101 died.
d. Includes HCWs who acquired illness in other areas.
e. Due to differences in case definitions, the United States has reported probable cases of SARS with onsets of illness after 5 July 2003.

Source: WHO (World Health Organization 2010)

Table C-6. Case fatality rate for SARS in Asian-Pacific Region, November 2002—August 7, 2003

	Cumulative # of cases	# deaths	Case fatality rate %	
Australia	5	0	—	
Canada		251	41	17
China		5,327	349	7
Hong Kong,	1,755	300	17	
Taiwan		346	37	11
Indonesia	2	0	—	
Malaysia	5	2	—	
New Zealand	1	0	—	
Philippines	14	2	—	
Korea		3	0	—
Singapore	238	33	14	
Thailand	9	2	—	
Vietnam	63	5	8	
Global		8,098	774	9.6

Source: (Chan-Yeung and Xu 2003)

Table C-7. Case fatality ratio for SARS in affected countries

Area	Cases	Median age	Number of deaths	Case fatality ratio (%)
Australia	6	15	0	0
Brazil	1	4	0	0
Canada	251	49	38	15
China (Manland)	5327	-	349	7
Hong Kong (Chlna)	1755	40	300	17
Macau (China)	1	28	0	0

Area	Cases	Median age	Number of deaths	Case fatality ratio (%)
Taiwan (China)	665	46	180	27
Colombia	1	28	0	0
Finland	1	24	0	0
France	7	49	1	14
Germany	9	44	0	0
India	3	25	0	0
Indonesia	2	56	0	0
Italy	4	30.5	0	0
Kuwait	1	50	0	0
Malaysia	5	30	2	40
Mongolia	9	32	0	0
New Zealand	1	67	0	0
Philippines	14	41	2	14
Ireland	1	56	0	0
Korea	3	40	0	0
Romania	1	52	0	0
Russian Federation	1	25	0	0
Singapore	238	35	33	14
South Africa	1	62	1	100
Spain	1	33	0	0
Sweden	3	33	0	0
Switzerland	1	35	0	0
Thailand	9	42	2	22
United Kingdom	4	59	0	0
USA	33	36	0	0
Vietnam	63	43	5	8
Total	**8,422**	**-**	**916**	**11**

Source: (Zhong and Wong 2004)

Aerosol infection/routes of transmission

Suspected SARS definition = sexual or casual contact with someone with a diagnosis of SARS within the last 10 days and clinical symptoms (Anonymous 2007).

Diagnostic laboratory staff members most at risk handled respiratory samples and were exposed to aerosols from leaking samples (Barkham 2004).

Transmission rate among health care workers high, 63 percent of cases in Hanoi hospitals, 46 percent in Hong Kong, 76 percent in Singapore, equally high rates in Toronto (Low and Wilder-Smith 2005).

Transmission of most respiratory viruses is a combination of direct contact (touch), short-range (large droplet; within 1 m) and long-range (droplet nuclei; beyond 1 m and farther) (Chan, Tang, and Hui 2006).

High infectivity of the viral illness is supported by the fact that 138 patients (many of whom were healthcare workers) were infected with SARS-CoV within 2 weeks after the admission of this single index case from Hotel M; superspreading event is thought to be related to the administration of a nebulized bronchodilator to the index case, together with overcrowding and poor ventilation in the hospital ward (Chan, Tang, and Hui 2006).

Possible spread by long-range airborne transmission in a major community outbreak in a private residential complex in Hong Kong; possible passive carriage of viruses by pests, drying up of U-shaped bathroom floor drain, which allowed the backflow of contaminated sewage or its aerosolized particles and creation of infectious aerosol current by the use of residential exhaust fans in the toilet (Chan, Tang, and Hui 2006) (Verreault, Moineau, and Duchaine 2008). Possible airborne transmission shown by air samples obtained from a room occupied by a SARS patient, and swabs taken from frequently touched surfaces in rooms and at a nurse station were positive for SARS-CoV by PCR (Chan, Tang, and Hui 2006).

* nosocomial transmission was the primary accelerator of SARS infections, accounting for 72 percent of cases in Toronto and 55 percent of probable cases in Taiwan (McDonald et al. 2004). Transmission appears to be primarily through exposure to respiratory droplets and direct contact with patients and their contaminated environment, especially if exposed during aerosol-generating procedures (McDonald et al. 2004).

Airborne droplets from infected patients might be the main route of transmission, with a secondary transmission route via blood and fecal-oral transmission (Weiss and Navas-Martin 2005).

Transmission other than by droplets, bodily fluids, and fomites is unknown (Lam, Zhong, and Tan 2003; Lim, Ng, and Tsang 2006).

Most respiratory pathogens transmitted by direct contact and short-range routes, with occasional instances where long-range transmission can be the only explanation, e.g., influenza; source for such transmission events is normally the infected patient's upper respiratory tract, and not via the hematogenous or fecal–oral routes (Chan, Tang, and Hui 2006).

In United States, under certain circumstances, SARS-CoV was not readily transmitted to close contacts, despite ample unprotected exposures (Peck et al. 2004).

Superspreading events, in which a single person spread the infection to many other people, were an important component of SARS-CoV transmission globally; in Singapore and Taiwan single case-patients might have transmitted the virus to >60 persons; for most SARS case-patients, transmission was limited—after the institution of intensive infection-control measures in Singapore, 81 percent of probable SARS patients had no evidence of transmission to other persons (Peck et al. 2004).

In United States, lack of virus transmission among 110 healthcare workers with exposure within droplet range (i.e., 3 feet) to six SARS-CoV–positive patients; 45 healthcare workers had exposure without any mask use, 72 had exposure without eye protection, and 40 reported direct

skin-to-skin contact; potential droplet- and aerosol-generating procedures were infrequent: 5 percent of healthcare workers manipulated a patient's airway, and 4 percent administered aerosolized medication; despite numerous unprotected exposures, no serologic evidence of healthcare-related SARS-CoV transmission; could be related to the relative absence of high-risk procedures or patients (Park et al. 2004).

Transmitted primarily through direct mucous membrane contact with infectious respiratory droplets and through exposure to fomites (Lo et al. 2005).

Amoy Gardens, 2003, high virus concentration aerosol spread from building plumbing through floor drains; initial exposures in bathrooms from feces and urine, then spread via prevailing winds to adjacent buildings, exposing additional humans; virus travelled hundreds of feet between buildings/apartments (McKinney, Gong, and Lewis 2006; Verreault, Moineau, and Duchaine 2008).

In Amoy Gardens, spread of the airborne, virus-laden aerosols generated by the index patient was modeled with the use of airflow-dynamics studies, including studies performed with the use of computational fluid-dynamics and multizone modeling (Yu et al. 2004).
Transmission mechanical, including airborne particles, contaminated equipment, and contact (Anonymous 2007).

Transmission of SARS-CoV is similar to other respiratory viruses but with several important differences: first international dissemination of SARS-CoV was related to a superspreader, a 64-year-old physician from southern China who visited Hong Kong on February 21, 2003, and stayed in Hotel M; died 10 days later with severe pneumonia; spread the virus to at least 16 hotel guests or visitors who had visited the floor where he stayed and spread subsequently in Hong Kong and to Vietnam, Singapore, and Canada (Chan, Tang, and Hui 2006; Verreault, Moineau, and Duchaine 2008).

Hong Kong transmission study showed that the probability of transmission from index cases whose date of symptom onset occurred before public health interventions imposed on March 26,

2003, was slightly higher during the first 2 days after symptom onset and peaked again on day 9 after symptom onset; probability of transmission from index patients whose LDH level was above the expected level was markedly higher than that from those whose LDH level was below the predicted level, peaking on days 8–9 of illness; index cases aged 60 years or older had a high probability of transmission as compared with younger index patients, approximately 3 times higher than in younger index patients during the first week of illness and remained high throughout the first 9 days of illness(Pitzer, Leung, and Lipsitch 2007).

Virus titers/concentrations/pathogenesis

In Vero cells, 10^6–10^7 $CCID_{50}$/mL produced in roller bottles/flasks (Johnson 2008).

In BHK-21 (baby hamster kidney) cells, titers of 10^8 PFU used as animal inoculum but no details are provided about whether this was artificially concentrated from culture (Weingartl, Czub, et al. 2004).

In A72 canine cells, canine coronavirus titers attain $3.14 \pm 0.58 \times 10^{6.0}$ $CCID_{50}$/mL (Amici et al. 2006).

In human lung epithelial cells [A549 cell line], SARS-CoV titers attain $4.39 \pm 1.2 \times 10^{3.0}$ $CCID_{50}$/mL (Amici et al. 2006).

In VERO cells, SARS-CoV titers attained 1.74×10^6 $CCID_{50}$/mL [titers in some tests attained $>10^7$ $CCID_{50}$/mL] (Amici et al. 2006).

In canine coronavirus-infected dogs, by RT-PCR, virus was excreted in feces to $10^{4.0}$ to $10^{5.0}$ RNA copies/µL, peaking at 2.11×10^5 copies/µL (Amici et al. 2006).

SARS-CoV from trachea and lungs of infected domestic cats, titers 1×10^3 $CCID_{50}$/mL (Weiss and Navas-Martin 2005).

In VERO cells and chicken embryo kidney epithelial cells, virus titers $10^{5.8}$–$10^{6.0}$ PFU/µL (Weingartl, Copps, et al. 2004).

* Virus isolated retrospectively from stored clinical specimens that were RT-PCR positive for viral RNA; virus more readily isolated from the respiratory tract than from stool specimens, was most successful during the first 2 weeks of the illness, and was generally negative after day 22 of illness, even though virus [=signal=RNA] was detectable in the specimens by RT-PCR (Chan et al. 2004).

Macaques infected with 1 x 10^6 $CCID_{50}$ of SARS-CoV suspended in 5 mL phosphate-buffered saline, 4 mL was applied intratracheally, 0.5 mL intranasally, and 0.25 mL on each conjunctiva; shed SARS-CoV from sputum, nose, and pharynx from 2 days after infection through >/= 6 days; virus isolated from the lung (1 x 10^5 $CCID_{50}$/g tissue) and kidney (1x10^3 $CCID_{50}$/g tissue) of 1 macaque, and from the lung of a second (1x10^4 $CCID_{50}$/g tissue), other samples RT-PCR positive (Kuiken et al. 2003).

In ferret (*Mustela furo*) females, infected intratracheally with 10^3 to 10^4 $CCID_{50}$ of SARS-CoV, produced $10^{2·6–4·0}$ $CCID_{50}$ and were RT-PCR positive (ter Meulen et al. 2004).

In African green monkeys, mean peak virus titers were $10^{3.1}$ and 10^3 $CCID_{50}$/mL in the upper and lower respiratory tract; no virus, but viral genome detected by RT-PCR in fecal samples (McAuliffe et al. 2004).

In African green monkeys, virus titered $10^{1.5–3.0}$ $CCID_{50}$/mL on nasal/throat swabs; $10^{3.7–5.5}$ $CCID_{50}$/g of turbinate tissue; $10^{0.5–3.2}$ $CCID_{50}$/mL in tracheal lavage fluid; $10^{2.5–4.2}$ CCID50/g in tracheal tissue; $10^{1.5–7.2}$ $CCID_{50}$/g of lung tissue (McAuliffe et al. 2004).

In Rhesus monkeys, titers $10^{1.0–1.5}$ $CCID_{50}$/mL and in cynomolgus monkeys, $10^{1.0–2.2}$ $CCID_{50}$/mL [with both species, lower and upper respiratory tracts, respectively] (McAuliffe et al. 2004).
In several strains of laboratory mice, infection produces $10^{7.0}$ $CCID_{50}$/g lung tissue after intranasal infection (Glass et al. 2004).

In cynomolgus monkeys, infection produced up to 170 copies of RNA/ng of respiratory tissues; In Rhesus, up to 7120 copies RNA/ng in respiratory tissues (Rowe et al. 2004).

In ferrets, from early in the infection and up to 5 days post-infection, titers ranged from 4 x 10^3 to 1.4 x 10^4 PFU per pharyngeal swab; sensitivity of real-time RT-PCR titrated to be 0.1 PFU per ml, while the classical RT-PCR showed sensitivity as low as 10^{-4} PFU per mL (Weingartl, Czub, et al. 2004).

In *C. jacchus*, virus isolation and titration attempted, but no viral growth was detected in samples that included oral swabs, rectal swabs, and tissue homogenates from the first group of six monkeys; despite sample-processing limitations, viral RNA was detected with RT-PCR in tissue homogenates from two of six animals (Greenough et al. 2005).

In a second group of *C. jacchus*, used RT-PCR only, because of the absence of titratable virus in the first group; threshold values ranged from 2,500 to 17,000 copies/g of respiratory tissues to > 70 x 10^6 copies/g of tissue; average values from $10^{4.3–5.0}$ copies/g tissue to peak concentrations of $10^{5.6–7.9}$/g tissue (Greenough et al. 2005).

In BALB/c mice, intranasal inoculation, virus titers ranged from $10^{6.0}$ $CCID_{50}$/g turbinate tissue to $10^{7.0}$ $CCID_{50}$/g lung tissue at 1–5 days post-infection (Subbarao et al. 2004). In BALB/c mice, intranasal inoculation, $10^{2.8–3.0}$ $CCID_{50}$/g turbinate, $10^{6.0–6.3}$ $CCID_{50}$/g lung (Stadler et al. 2005). In infected humans, using quantitative PCR, at median 3.9 days after onset of symptoms, 10^0 to $10^{8.8}$ RNA copies/mL of nasopharyngeal specimens (Chu et al. 2004). With RT-PCR, presence of the RNA of SARS-CoV in nasopharyngeal aspirate (NPA) in 31 percent of infected humans on day 2, 43 percent on days 3–5, and 60 percent of patients from 6 to 8 days to 2 weeks after onset of the illness; in stool samples in almost 100 percent of patients at the end of 2 weeks; 32/316 (10 percent) exposed asymptomatic subjects had positive RT-PCRin their NPA (Chan-Yeung and Xu 2003). Virus detectable in the blood, feces, urine and respiratory secretions of SARS patients (Chen et al. 2004).

RT-PCR and ELISA tests performed in infected humans to evaluate viral RNA load (as an indicator of viremia) and immune responses: both IgG and IgM antibodies present 1 week after diagnosis in > 50 percent of patients, positivity rate for IgM increased for a month and remained constant at approx 70 percent, positivity rate for IgG reached and remained at 100 percent; RT-

PCR was more sensitive in detecting blood-borne viral RNA in the early phase of the disease than the ELISA method, but positivity rate was lower than that of antibody assays; viral RNA was detectable in the blood samples 1 week after diagnosis and peaked at around 2 weeks, reaching 75 percent (Chen et al. 2004).

Median nasopharyngeal viral load of patients living near the index case in a Hong Kong community ($10^{5.09}$ RNA copies/mL) was much higher than in patients (10^{0} copies/mL) not living near the index case (Chen et al. 2004).

Amoy Gardens, at 5, 10, 15 days after first symptoms in patients, RT-PCR titers of NPAs were 10^{3} to 10^{7} RNA copies/mL, $10^{5.5}$ to $10^{8.5}$ RNA copies/mL, 10^{3} to $10^{6.5}$ RNA copies/mL, respectively, with mean geometric virus loads of $2.3x10^{5}$ RNA copies/mL, $1.9x10^{7}$ RNA copies/mL, $9.8x10^{4}$ RNA copies/mL, respectively (Peiris, Chu, Cheng, Chan, Hung, Poon, Law, Tang, Hon, Chan, Chan, Ng, Zheng, Ng, Lai, Guan, and Yuen 2003).

From chicken, turkey, geese, ducks, quail in Vero cells, detected virus to titers of 10^{7} $CCID_{50}$/mL; with real-time RT-PCR test detected SARS-CoV to $10^{5–6}$ copies RNA; with RT-PCR, detected virus in OP swab specimens from two chickens on day 1 PI; no infectious virus was isolated from any of the birds at any time from OP or cloacal swab specimens, plasma, or tissues (Swayne et al. 2004).

Viral loads in humans: serum, $10^{2.7}$ RNA copies/mL; urine, $10^{4.4}$ RNA copies/mL; feces, $10^{7.0}$ RNA copies/mL (Hung et al. 2004).

In Vero cells, titers of $10^{5.8}$ to $10^{6.0}$ $CCID_{50}$/mL (Darnell et al. 2004).

Infectious dose used in *M. mulatta* monkeys was 10^{3}, 10^{5}, and 10^{7} $CCID_{50}$; optimal dose was 10^{5} $CCID_{50}$ which produced serologic conversion in 5 of 8 animals (HID63).(Qin et al. 2005).

Infectious dose studies in NHPs have used between $10^{3.0}$ and $10^{7.0}$ $CCID_{50}$/mL to infect (Cheng et al. 2007).

Pathogen stability

At Amoy Gardens, virus is believed to have spread between apartment block buildings via aerosols from sewage system carried by ambient wind (Yip et al. 2007).

Virus was inactivated by UV light at 254 nm (UVC wavelength). UV-A had no significant effect. Details as follows: virus suspension 1 cm deep in 24-well plates, UV source at height of 3 cm from the bottom of the wells. UVC (254 nm) at 4016 $\mu W/cm^2$ (where $\mu W = 10^{-6}$ J/s) and UV-A (365 nm) at 2133 $\mu W/cm^2$. UV-A had no significant effect. UVC resulted in a 1 log reduction at 1 minute, a 2 log reduction at 2 minutes, a 3 log reduction at 3 minutes, a 3.5 log reduction at 4 minutes, a 4 log reduction at 6 minutes, complete inactivation at 15 minutes (Darnell et al. 2004) Note that UVC radiation is absorbed by the ozone layer; a negligible amount reaches earth's surface (World Health Organization 2002).

Titer decreased from $10^{7.5}$ $CCID_{50}$ to $10^{3.2}$ $CCID_{50}$ within 5 days @ room temperature (details not available) (from Bao, et al, 2003 [Chinese]) (Wang et al. 2005).

Viral shedding studies that are available to date suggest that transmission could occur via close contact involving respiratory tract excretions, and via fecal or urine contamination of surfaces (Anderson et al. 2004; Lim, Ng, and Tsang 2006).

Virus can survive in respiratory samples for 5 days at room temperature and 3 weeks at 4 °C (Lim, Ng, and Tsang 2006).

In diarrheal feces, can survive for a few days at room temperature; fecal droplets with a high titer of virus, 10^6 $CCID_{50}$/mL, can remain infectious for 4–5 days (Lim, Ng, and Tsang 2006).

Airborne infections from virus aerosols (Lim, Ng, and Tsang 2006).

Virus will survive on paper and absorbent paper gowns for about 5 minutes and on non-absorbent paper gowns and on non-absorbent gloves for more than 5 minutes (Lim, Ng, and Tsang 2006).

Virus persists in the environment—1+ day on surfaces and 4 days in feces (McKinney, Gong, and Lewis 2006).

On dry surfaces at room temperature, virus survives for 2–3 days; in fecal samples, survival for 2–4 days; resistance to heat and chemical disinfectants similar to other enveloped viruses [heat, organic solvent, and detergent sensitive](Wong and Yuen 2005).

Coronaviruses are resistant to trypsin [addition appears to facilitate isolation in cell cultures]; rather acid labile; sensitive to lipid solvents, detergents, UV radiation, disinfectants, and heat (Murphy et al. 1994; Anonymous 2007).

Virus was inactivated by UV light at 254 nm, heat treatment of >/= 65 °C, alkaline (pH > 12) or acidic (pH < 3) conditions, formalin and glutaraldehyde treatments (Darnell et al. 2004). SARS-CoV detected in the blood of infected individuals might have potential to contaminate donated blood and plasma-derived products; viral inactivation by heat treatment at 60 °C required 15'-30', UV light-C inactivated virus in 40', UV-A required addition of psoralen to enhance inactivation of the virus; presence of bovine serum albumin limited the ability of UV-C and UV-A to inactivate virus; octanoic acid treatment does not reduce the infectivity of virus in protein solutions; solvent/detergent treatment required 2, 4, and up to 24 hours for Triton X-100, between 80, and sodium cholate inactivation, respectively (Darnell and Taylor 2006).

In vitro, virus persists for 2 days in hospital wastewater, domestic sewage and dechlorinated tap water; 3 days in feces, 14 days in PBS, 17 days in urine at 20 °C; at 4 °C, virus persists for 14 days in wastewater and more than 17 days in feces or urine: free chlorine inactivates virus better than chlorine dioxide; free residue chlorine over 0.5 mg/L for chlorine or 2.19 mg/L for chlorine dioxide in wastewater ensures complete inactivation (Wang et al. 2005).

At Amoy Gardens, optimum environmental temperature range associated with SARS cases was 16–28 °C (Yip et al. 2007).

Indication that viruses with structural lipids survive best in aerosols at low RH (Benbough 1971).

MHV coronavirus [surrogate for SARS-CoV] highly susceptible to inactivation by aerosolization and sampling; an enveloped virus is more likely to be inactivated by mechanical stress; MHV coronavirus easily inactivated in PBS with 0.01 percent between but relatively stable when suspended in MEM with 10 percent FBS, a protein concentration similar to that of saliva; inactivated by a relatively low dose of 254 nm UV- high UV susceptibility of coronavirus aerosols suggests that UV air disinfection might be an effective tool for preventing important respiratory viral diseases such as SARS (Walker and Ko 2007).

Infectivity maintained at least 10 days at 4 °C; titer decreased from $10^{7.5}$ $CCID_{50}$ to $10^{3.2}$ $CCID_{50}$ within 5 days at room temperature; virus inactivated by heat at 56 °C for 30' or at 70 °C for 5' (from Bao, et al, 2003 [Chinese]) (Wang et al. 2005).

The virus survived at RT for 9 days in cell culture suspension and 6 days in dried state on plastic (Rabenau et al. 2005).Common laboratory and hospital disinfectants will inactivate the virus, e.g., detergents, hypochlorite solution, and peroxygen compounds (Lim, Ng, and Tsang 2006).

In a Pennsylvania patient, serial stool specimens collected on days 14, 18, 21, and 26 after the onset of illness were positive by RT-PCR [cannot necessarily correlate with isolation of virus] (Peck et al. 2004).

In United States, using RT-PCR, virus detected in a day-14 sputum specimen from one case-patient and in five stool specimens from two case-patients; in one case-patient, SARS-CoV persisted in stool for at least 26 days after symptom onset (Isakbaeva et al. 2004).

Virus loses its infectivity after exposure to several commonly used disinfectants such as Clorox (sodium hypochlorite), 75 percent ethanol, and fixatives such as formaldehyde and paraformaldehyde (Chan-Yeung and Xu 2003).

At 6–8 weeks of illness all specimens from throat, conjunctiva, and urine were negative for SARS-CoV by RT-PCR; stool specimens from five patients were positive by three RT-PCR assays (Leong et al. 2004).

The D_{37} value for BEV (Berne) was calculated to be 3.1 [HL=7 min at maximum solar radiation] (Lytle and Sagripanti 2005). BEV was used a surrogate for SARS-CoV; both are classified in the family *Coronaviridae.*

Human coronavirus 229E (not SARS-associated) at 20°C survived best at 50 percent RH, having a half-life of 67.33 ± 8.24 hrs (Ijaz et al. 1985).

Vectors

No arthropod vectors.

Other epidemiological/ecological data

Control through the use of quarantine (Cinatl et al. 2005; Low and Wilder-Smith 2005).

Made public spotlight in 2/2003, businessman traveling from China became afflicted with pneumonia-like symptoms while on a flight to Singapore, which stopped in Vietnam, where the victim died; several medical staff members who treated him soon developed the same disease despite basic hospital procedures; an Italian doctor identified the threat and communicated it to WHO and the Vietnamese government and later succumbed to the disease (Anderson et al. 2004; Feng and Gao 2007).

Hong Kong, a mainland doctor who arrived in 2/2003 stayed on the 9th floor of a hotel and infected 16 other hotel visitors who spread SARS-CoV to others in Canada, Singapore, Taiwan, and Vietnam (Chan, Tang, and Hui 2006) (Low and Wilder-Smith 2005).

On a flight from Hong Kong to Beijing, 2003, symptomatic passenger infected no less than nine tourists from Hong Kong, three Taiwanese businessmen, one Singaporean, and four Chinese (Low and Wilder-Smith 2005).

Another larger cluster of cases in Hong Kong centered on Amoy Gardens and spread is suspected to have been facilitated by defects in the sewage system (Chan, Tang, and Hui 2006).

Relatively rare disease with 8,096 cases as of 2003 with at least 774 deaths (Cinatl et al. 2005).

Netherlands, 2003, scientists demonstrated that SARS-CoV fulfilled Koch's postulates thereby confirming it as the causative agent; macaques, *M. fascicularis*, infected with the virus developed the same signs as human SARS victims (Kuiken et al. 2003).

Improper handling of SARS-CoV in laboratories caused the infection of two researchers in Singapore and one in Taiwan (Barkham 2004).

20 percent of patients can progress to acute respiratory distress syndrome, requiring mechanical ventilatory support (Cinatl et al. 2005).

Rapid spread of the infection to involve clusters of health care workers attending patients was noted (Lam, Zhong, and Tan 2003).

Temporal–spatial spread of SARS-CoV among patients in a hospital medical ward consistent with airborne transmission; easily spread between healthcare workers and patients via direct or short-range transmission and existence of *superspreaders* who generate a far greater than average number of secondary cases (Chan, Tang, and Hui 2006; Verreault, Moineau, and Duchaine 2008).

Modeling of transmission dynamics for this virus has been addressed (Lipsitch et al. 2003; Riley et al. 2003; Hsieh, Chen, and Hsu 2004; Meltzer 2004).

In contrast to Canada, China, and Taiwan, the United States and other countries did not experience any superspreaders or superspreading events, and the reason is unknown (McDonald et al. 2004).

Coronaviruses cause acute and chronic respiratory, enteric, and central nervous system diseases in humans and animals (Weiss and Navas-Martin 2005).

Since the large global outbreak of 2003, a limited number of cases of SARS have occurred that included four community-acquired infections associated with mild disease the following winter, and at least four laboratory-acquired infections, one of which resulted in secondary spread and severe illness and mortality in contacts (Subbarao and Roberts 2006).

In Toronto, transmission from the index case resulted in at least six generations of transmission, four of which were a result of nosocomial spread (Varia et al. 2003).

Superspreading events pivotal in the global spread of SARS-CoV; investigated superspreading in one transmission chain early in Beijing's epidemic; *superspreading* is defined as transmission of SARS-CoV to at least eight contacts; an index patient with onset of SARS 2 months after hospital admission was the source of four generations of transmission to 76 case-patients, including 12 healthcare workers and several hospital visitors; 4 (5 percent) case circumstances met the superspreading definition; appeared to be associated with older age (mean 56 versus 44 years), case fatality (75 percent vs. 16 percent, $p = 0.02$, Fisher exact test), number of close contacts (36 versus 0.37) and attack rate among close contacts (43 percent versus 18.5 percent, $p < 0.025$) (Shen et al. 2004).

Largest community SARS outbreak at Amoy Gardens, Hong Kong, 2003 affected 329 residents, 1.7 percent of population, 42 deaths; airborne pathway hypothesized with possible meteorologic factors contributing: virus-laden aerosols transported between apartment blocks via ambient wind, low mixing might have prevented efficient dispersal of aerosols, and drop in temperature might have fostered survival of virus or increased susceptibility of the exposed population (Yip et al. 2007).

Nucleocapsid protein (NP) is the most predominant virus-derived structural protein shed in high amounts in serum and NPA during the first week of infection; using immunoswab method with a panel of monoclonal antibodies (MAbs)against the NP, detected an NP concentration of 20 pg/mL in saline, 20–200 pg/mL in pig NPA, and 500 pg/mL in rabbit serum (Kammila et al. 2008).

Therapeutics/vaccines

About 10–20 percent of cases require mechanical ventilation (Cinatl et al. 2005; Low and Wilder-Smith 2005).

Treatment largely supportive with antipyretics, supplemental oxygen, and ventilatory support as needed (Lam, Zhong, and Tan 2003).

Suspected cases must be isolated preferably in negative pressure rooms with full barrier nursing precautions taken for contact with these patients (Cinatl et al. 2005; Low and Wilder-Smith 2005).

Initial anecdotal support for sterroids and ribavirin but no published evidence; many clinicians suspect that ribavirin is detrimental (Lam, Zhong, and Tan 2003).

Indomethacin, a cyclopentanone cyclooxygenase metabolite shown to be a potent antiviral against canine and human SARS-CoV, *in vitro* and *in vivo* (Amici et al. 2006).
Ribavirin (1-_-d-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a purine nucleoside analogue and has limited clinical efficacy (Cinatl et al. 2005).

Intranasal recombinant interferon might be an effective prophylactic treatment; limits duration and severity of clinical disease in humans; effective in cynomolgus macaques (Cinatl et al. 2005).

HIV-1 protease inhibitors [lopinavir and ritonavir] show lower mortality rates (2.3 percent) in treated humans (Cinatl et al. 2005).

Human convalescent plasma apparently had a beneficial effect if used relatively early in the course of illness (Cinatl et al. 2005).

No antiviral is proven to be of beneficial value (Cinatl et al. 2005).

12/2004, report that Chinese researchers had produced vaccine; tested on a group of 36 volunteers, 24 developed antibodies against the virus (Anderson et al. 2004).

Inactivated vaccine elicits potent spike protein-specific neutralizing antibodies that block receptor binding and virus entry (Feng and Gao 2007).

DNA vaccine encoding virus S GP can induce T cell and neutralizing antibody responses and protective immunity in a mouse model (Feng and Gao 2007).

Vaccines targeting several animal CoVs have been developed and are efficacious, but a phenomenon of enhanced disease following vaccination has been observed in cats when infected with feline infectious peritonitis virus (a related coronavirus) [could be indicative of a potential problem with vaccines] (Gillim-Ross and Subbarao 2006).

Extensive ongoing research on vaccines in animals, but no imminent candidates for human use; inactivated, attenuated, subunit/expressed protein, vectored, and DNA vaccines (Gillim-Ross and Subbarao 2006).

Administration of a human monoclonal antibody might offer prophylaxis (ter Meulen et al. 2004).

Other remarks

Viral cell culture is insensitive (Wong and Yuen 2005).

Within a matter of weeks in early 2003, SARS-CoV spread rapidly from the Guangdong province of China (11/2002) to infect individuals in some 37 countries around the world (Feng and Gao 2007; Lam, Zhong, and Tan 2003).

SARS-CoV causes severe disease, is transmissible in the community, and there is no effective prophylaxis or treatment, perhaps fulfilling the criteria for biohazard group 3 or 4 (Barkham 2004).

Recommended to use BSL-3 procedures in a BSL-2 diagnostic laboratory environment with strict controls in administration, engineering (especially airflow and air filters), and PPE (especially BSCs and disposable gloves, gowns, and surgical masks/respirators/full face shields/powered air-purifying respirators [PAPRs]) (Barkham 2004; Gillim-Ross and Subbarao 2006; Lim, Ng, and Tsang 2006).

Research with replication or growth of virus and with animals should be done in a BSL-3 laboratory (Lim, Ng, and Tsang 2006).

Virus escapes reported in from BSL-3 and BSL-4 laboratories in China, Singapore, and Taiwan were due to lapses in good microbiologic practices, not equipment or laboratory failures (Lim, Ng, and Tsang 2006).

Mainland China and Hong Kong, SARS, accounted for 87 percent of all cases and 84 percent of all deaths (Lam, Zhong, and Tan 2003).

Excellent recommendations for handling, storing, and transporting samples/virus, facilities/equipment, training, health and medical surveillance, test exercises, and emergency responses (Lim, Ng, and Tsang 2006).

Coronaviruses (general)—tropism for respiratory and intestinal tracts, liver, and brain; frequently seen by electron microscopy in human feces (White 1994).

Non-SARS human coronaviruses—frequent causes of *common cold* (White 1994).

Coronaviruses have the largest genomes of RNA viruses (White 1994).

In general, coronaviruses replicate poorly in cell cultures, necessitating use of molecular techniques (White 1994).

WHO recommends that manipulation of active viral cultures of SARS-CoV be performed in containment laboratories at BSL3 (Darnell et al. 2004).

Antigenic relationships/synonyms

Order: *Nidovirales*, Family: *Coronaviridae*, Genus: Coronavirus (Weiss and Navas-Martin 2005) (Murphy et al. 1994; Weiss and Navas-Martin 2005).

Also called atypical pneumonia in China (Feng and Gao 2007).

Related animal coronaviruses: porcine transmissible gastroenteritis virus, bovine coronavirus [enteritis, winter dysentery], avian infectious bronchitis viruses, mouse hepatitis virus [lethal intestinal virus disease of infant mice], feline infectious peritonitis virus, feline enteric coronavirus, turkey coronavirus [blue comb], hemagglutinating encephalomyelitis virus of pigs, porcine epidemic diarrhea virus, pheasant coronavirus, canine coronavirus [diarrhea], rabbit coronavirus, sialodacryadenitis coronavirus of rats (Murphy et al. 1994; Weiss and Navas-Martin 2005)

C.1.1.6 Rift Valley fever virus (RVFV)

Host range

a. Field

1912–1913 epizootics of abortion occurred with high mortality in domestic animals during which humans were infected (Meegan and Bailey 1989).

1930–1931, viral transmission to lambs/sheep, lab animals, and humans occurred (Meegan and Bailey 1989).

Severe losses in sheep, cattle, goats, water buffalo, and camel herds have been recorded (Meegan and Bailey 1989).

Severe, usually fatal disease occurs in lamb, kid, mouse, and vole (Meegan and Bailey 1989).

RVF is often a fatal clinical disease in sheep and cattle (Meegan and Bailey 1989).

RVF can be a nonfatal clinical disease in human, goat, buffalo, grey squirrel, and rodent [multiple spp] (Meegan and Bailey 1989).

Subclinical infection can occur in horse, pig, camel, rodent [multiple spp], mongoose, hedgehog, tortoise, frog, most avians, hippopotamus, and monkey [certain spp] (Meegan and Bailey 1989).

Abortions in camel and buffalo were reported (Meegan and Bailey 1989).

From 1950 to 1976, at least 16 major epizootics in sub-Saharan Africa (Murphy et al.). 1951, Johannesburg, 100,000 sheep died and 500,000 ewes aborted (Swanepoel and Coetzer 1994).

1974 to 1976, southwest Africa, losses in Angora goats resulted in a reduction of 230,000 Karakul pelts annually during the 2-year period (Swanepoel and Coetzer 1994).

1978, Zimbabwe, nearly 100 percent of pregnant cows aborted and 3 percent died; serologic evidence showed 30 percent infection rates; estimates of 60,000 abortions and 10,000 deaths (Swanepoel and Coetzer 1994).

Outbreaks characterized by economically disastrous *abortion storms* (newborn fatality rates in excess of 10 percent) and a newborn animal mortality approaching 100 percent is reported among livestock (Bird et al. 2008).

1977 to 1978, Egypt, wild rodents do not serve as RVFV reservoirs; domestic sheep, cattle, buffaloes, camels, goats, donkeys, and dogs act as amplifying hosts; more than 30 percent of camels sampled at the southern border of Egypt were serologically positive for antibodies to RVFV (Hoogstraal et al. 1979).

Guinea, bats (*Micropteropus* spp and *Hipposideros* spp), one isolate each (Weinbren 2008).

Disease is most severe in sheep, goats, and cattle, in which it produces abortions in pregnant animals and a high mortality rate in the newborn; older, nonpregnant animals, while susceptible to infection, are more resistant to clinical disease; considerable variation in susceptibility of animals of different genotypes, breeds or strains exotic to Africa or are from areas where RVF is not endemic, tend to be more susceptible; camels suffer an inapparent infection with RVF, but abortion rates can be as high as in cattle (Anonymous 2004; Bird et al. 2009).

Indigenous African animals might have only inapparent infections (Anonymous 2004; Bird et al. 2009).

Hippopotamus, donkeys, weaverbirds might be susceptible (Shimshony and Barzilai 1983).

Susceptibility of vertebrates has been reported (Brown and Torres 2008)as follows: extremely susceptible are hamsters, kids, kittens, lambs, mice, puppies; highly susceptible are calves, sheep; moderately susceptible are South American/Asian monkeys, buffalo, cattle, goats, humans; less susceptible are camel, cats, dogs, equines, guinea pigs, pigs, rabbits; refractory are amphibians, birds, reptiles.

The host range is broad(Shimshony and Barzilai 1983; Brown and Torres 2008).

Table C-8. Host range of Rift Valley fever virus reported by Shimshony and Barzilai

100% Fatal	High mortality	Severe, mostly nonfatal	Subclinical	Refractory to experimental infection
Lamb (1)[a]	Sheep (1)	Human (1)	Monkey (African) (1)	Mongoose (1)
Kid (1)	Calf (1)	Monkey (Indian and South American) (1)	Wild rodents (6)	Hedgehog (1)
White mouse (1)	Rat[b] (1)	Cattle (1)	Rabbit (1)	Spider monkey (1)
Hamster (1)	Gerbil (3)	Goat (1)	Pig (1)	Tortoise (1)
Field mouse (1)	Laucha (3)	Sheep[b] (4)	Dog (2)	Frog (1)
Dormouse (1)	Puppy (2)	Buffalo (African) (1)	Cat (2)	Hedgehog (1)
Field vole (1)	Kitten (2)	Buffalo (Asian) (5)	Horse (7)	Geckos (8)
Rat[b] (3)		Gray squirrel (1)	Rat[b] (3)	Hen (1)(9)
		Wild rodents (6)	Guinea pig (3)	Canary (1)
		Camel (5)		Pigeon (1)
				Parakeet (1)

Source: (Shimshony and Barzilai 1983)

b. Experimental

A severe, usually fatal, disease in mice, hamsters, and rats [certain spp] (Meegan and Bailey 1989).

Often fatal in puppies, kittens, and gerbils (Meegan and Bailey 1989).

Nonfatal clinical disease in monkeys [multiple spp] and rats [certain spp] (Meegan and Bailey 1989).

Subclinical infection in rabbits, guinea pigs, cats, dogs, chickens, rats [certain spp], monkeys [multiple spp] has been reported (Meegan and Bailey 1989).

Pigs resistant, but develop transient viremia with high infecting doses (Swanepoel and Coetzer 1994).

4/5 African buffalo were viremic, one-half of the pregnant cows aborted (Swanepoel and Coetzer 1994).

Rhesus monkeys and *Cercopithecus* monkeys, mild or asymptomatic infection, develop antibodies (Weinbren 2008).

Death reported in newborn Merino lambs (Weinbren 2008).

Among pregnant ewes, abortions and occasional deaths reported (Weinbren 2008).

Field voles, dormice, wood mice are susceptible (Weinbren 2008).

Death in kittens and puppies reported(Weinbren 2008).

One-day-old puppies died from a dose of $10^{0.2}$ $MICLD_{50}$ (Shimshony and Barzilai 1983). In kittens, 81 percent died from doses of $10^{2.2}$ to $10^{8.2}$ $MICLD_{50}$ (Shimshony and Barzilai 1983).

In a gerbil (*Meriones unguiculatus*) model for the encephalitic form of RVF, resistance to necrotizing encephalitis was age-dependent, 100 percent mortality at 3 weeks, decreasing to approx. 20 percent by 10 weeks, inoculated subcutaneously (SQ) (Anderson, Slone, and Peters 1988).

In rhesus monkeys, *M. mulatta*, three experiments: SQ inoc with$10^{5.3}$ PFU/mL, no viremia in one, $10^{3.6}$ PFU/mL in second monkey lasting 1 day, $10^{6.8}$ viremia on day 2 and $10^{4.9}$ PFU/mL viremia on day 3 in the third monkey; IV inoculation of $10^{4.7}$ PFU/mL, three developed transient viremia without clinical signs, but the fourth developed hemorrhagic diathesis and was euthanatized; IV inoculation of $10^{4.1}$ PFU/mL had transient viremia and one was ill with hemorrhagic signs, but recovered; inoculation (route not specified; IV is implied) of $10^{4.8}$ PFU/mL, one became ill and was killed in a moribund state; viremias to $10^{5.5\text{-}6.5}$ PFU/mL (Peters et al. 1988).

Prevalence/incidence/attack rate

1977, Egypt, 20,000 human clinical cases were reported (Meegan and Bailey 1989).

Kenya, systematic multistage cluster sampling across Garissa District in 1997–1998 indicated a 14 percent prevalence of acute (IgM-positive) cases, with an estimated 20–26 percent of the population having either recent or past infection with RVFV; some populations had RVF IgG seropositivity as high as 32 percent; estimated 27,500 infections occurred in Garissa District, making it the largest recorded outbreak of RVFV in East Africa (LaBeaud et al. 2007).

1977–1978 Egyptian epizootic, acute fever was reported in humans with encephalitic, ocular, or fatal hemorrhagic disease; estimates of human cases ranged from 18,000 to 200,000 (Swanepoel and Coetzer 1994); purportedly millions were infected, more than were 200,000 infected (White 1994).

1987, Senegal and Mauritania, 400 human isolates were reported (Meegan and Bailey 1989; Swanepoel and Coetzer 1994; Flick and Bouloy 2005).

Nigeria, humans, 146/2,223 with CF antibody (Weinbren 2008).

Uganda, humans, seven isolates were reported (Weinbren 2008).

Egypt, 1978, humans, 10 isolates were reported (Weinbren 2008).

Egypt, 1979, humans, 53 isolates were reported (Weinbren 2008).

1997–1998, from Somalia, through Kenya to Tanzania, 90,000 human cases were reported (Murphy et al.).

2000, outbreaks occurred in Saudi Arabia and Yemen (Sidwell and Smee 2003; Flick and Bouloy 2005).

Largest reported human outbreak occurred in Kenya during 1997–1998, in which an estimated 89,000 persons (according to a systematic serosurvey) were infected (CDC 2007).

2000, Arabian Peninsula, simultaneous outbreaks; total number of human cases unknown, but if the hospitalized patients represent a small fraction (usually less than 1 percent) of the total infections, the 884 and 1087 patients hospitalized, respectively, in Yemen and Saudi Arabia, provide a strong indication of the magnitude of this epidemic (Flick and Bouloy 2005).

1977–1978, widespread outbreaks in Nile Valley and Delta region of Egypt occurred with extensive human involvement; morbidity was 200,000 (Meegan, Niklasson, and Bengtsson 1979).

R_0/incubation period/infectious period/infectious dose

R_0 is predicted by one modeling effort to have a mean of 1.193 (95 percent CI 1.177–1.209), (median 1.113, max. 3.743, min. 0.037) (Gaff, Hartley, and Leahy 2007). Note this is an estimate for R_0 for spread through a mosquito population via mosquito-mosquito and mosquito-livestock-mosquito transmission. It is not a prediction for R_0 for human transmission.

Incubation period in livestock is 1–3 days; in humans, 2–6 days (Bird et al. 2009).

Infectious period in ruminants, including camels, is considered to be 30 days (Anonymous 2007).

Infectious dose in humans is unknown (no reports found).

Aerosol infection in lambs, rhesus monkeys, cynomolgus monkeys, hamsters, puppies, kittens, mice used doses varying from $10^{<1.0 \text{ to } 9.0}$ $MIPLD_{50}$ or $10^{1.9\text{-}2.6}$ PFU (Shimshony and Barzilai 1983).

Morbidity/case fatality ratio

a. Human

Human infection results in self-limiting febrile disease that in 1 to 2 percent of patients progresses to more serious complications including hepatitis, encephalitis, and retinitis or a hemorrhagic syndrome with high fatality (Bird et al. 2008).

Disease can be severe, occasionally fatal (Meegan and Bailey 1989).

Disease can be hemorrhagic (Meegan and Bailey 1989).

Kenya, 1998, 400 deaths reported (Sidwell and Smee 2003; Flick and Bouloy 2005).

1977, Egypt, 20,000 human clinical cases with 598 deaths (mortality rate of 3 percent); morbidity of 20,000 to 200,000; military estimated 0.2 percent mortality rate; 14percent hospitalized patients died (Meegan and Bailey 1989).

1977–1978 Egyptian epizootic, acute fever in humans with encephalitic, ocular, or fatal hemorrhagic disease; estimates of human cases ranged from 18,000 to 200,000, with 598 deaths (Swanepoel and Coetzer 1994); purportedly millions infected with thousands of deaths, more than 200,000 were infected, with more than 600 deaths (White 1994).

1977–1978, Egypt, widespread outbreaks occurred in the Nile Valley and Delta region, with extensive human involvement, morbidity 200,000 with 600 deaths (Meegan, Niklasson, and Bengtsson 1979).

1987, Senegal AND Mauritania, high human mortality rate with 400 human isolates and 224 deaths (Meegan and Bailey 1989; Swanepoel and Coetzer 1994; Flick and Bouloy 2005).

Largest reported human outbreak occurred in Kenya during 1997–1998, in which an estimated 89,000 persons (from a systematic serosurvey) were infected and 478 died (CDC 2007).

1987, Senegal and Mauritania, high human mortality rate with 400 human isolates and 224 deaths (Meegan and Bailey 1989; Swanepoel and Coetzer 1994; Flick and Bouloy 2005).

1997–1998, from Somalia, through Kenya to Tanzania, 90,000 human cases and more than 500 deaths (Murphy et al.).

2006–2007, Kenya, about 75 people died and another 183 were infected (Bird et al. 2009; Gerdes 2002, 2004).

2006–2007, Kenya, the human case fatality rate was 29 percent (CDC 2007).

2007, Somalia reported 14 human deaths (Bird et al. 2009; Gerdes 2002, 2004).

2007, Sudan reported 125 human cases including 60 deaths (Bird et al. 2009; Gerdes 2002, 2004).

1975 South African epizootic, four human deaths from hemorrhagic syndrome and three deaths from encephalitis were reported (Swanepoel and Coetzer 1994; Meegan and Bailey 1989).

A mortality ratio of 5–10 percent in humans has been reported (White 1994).

Human case fatality rate of 1–2 percent, unless hemorrhagic symptoms occur, where it can reach 10 percent (Murphy et al.).

Approximately 1 percent of infected humans die; in livestock, the fatality level is significantly higher; and in pregnant livestock, abortion of virtually 100 percent of fetuses is reported (Anonymous 2004; Bird et al. 2009; Gerdes 2004, 2002).

Overall human mortality rate from RVF has been estimated at 0.5–1.0 percent of those infected, but the rate is much higher among those with severe disease (CDC 2007). Human morbidity, 1977, estimated at 20,000–200,000 and higher, with at least 598 fatalities (Shimshony and Barzilai 1983).

b. Animal

Depending on the species, up to 30 percent of infected adult animals die (Meegan and Bailey 1989).

80–100 percent pregnant animals abort (Meegan and Bailey 1989).

90–100 percent of infected 7-day-old or younger lambs die (Meegan and Bailey 1989; Gerdes 2005).

20–60 percent of older lambs and adult sheep die (Meegan and Bailey 1989; Gerdes 2005).

90–100 percent of pregnant ewes abort (Meegan and Bailey 1989).

10–70 percent of calves die (Meegan and Bailey 1989; Gerdes 2005).

10–20 percent of adult cattle die (Meegan and Bailey 1989; Gerdes 2005).

80–100 percent of pregnant cattle abort (Meegan and Bailey 1989).

Morbidity and mortality are associated with differences in strain virulence and susceptibility of hosts (Swanepoel and Coetzer 1994).

Lambs suffer more severe disease than monkeys, yet monkeys can be infected by slightly lower doses (Swanepoel and Coetzer 1994).

Exotic livestock breeds are less resistant that indigenous breeds (Swanepoel and Coetzer 1994).

Sub-Saharan Africa, lambs are extremely susceptible and can be fatally infected with 0.1 $MIPLD_{50}$ (Swanepoel and Coetzer 1994).

Mortality in kids, abortions in pregnant nannies, adults goats resistant (Swanepoel and Coetzer 1994).

Egypt, horses develop low level viremia; 4 abortions in donkeys; low antibody prevalence in both spp (Swanepoel and Coetzer 1994).

Kenya and Egypt, antibody in camels, abortions, low level viremia; in Egypt, 1 abortion, 56 deaths, but circumstantial evidence only (Swanepoel and Coetzer 1994).

Egypt: high antibody prevalence in Asian water buffalo, 12.1 percent abortion rates; mortality rates of 7.2 percent, but might be circumstantial evidence (Swanepoel and Coetzer 1994).

Mortality rate of 90 percent in lambs, 20-60 percent in adult sheep, and 10-30 percent in calves and adult cattle (Murphy et al.).

Mortality of newborn lambs and kids is reported as 70–100 percent, older lambs, kids and adult sheep, and goats is 10–70 percent; cattle 10 percent; calves 20 percent (Brown and Torres 2008).

Aerosol infection/routes of transmission

Humans infected from mosquito bites and contact with infected tissues and blood probably via abraded skin, mucous membranes, aerosol, and intranasally (Swanepoel 1994; White 1994).

Human disease is most frequently seen only during epizootics and can result from either feeding by an infected mosquito vector or aerosol transmission, usually associated with slaughtering sick animals (LeDuc 1989).

Humans infected from mosquito bites and contact with infected tissues and blood probably via abraded skin, mucous membranes, aerosol, and intranasally (Swanepoel and Coetzer 1994).

Humans are susceptible to infection via contact with infected material or mosquito bites. Human infections via vectors is a striking feature in countries with a relatively small population of animal hosts. In such areas, RVF can be recognized first in humans. It causes serious disease in laboratory workers and must be handled with high level biosecurity, laboratory workers should be vaccinated (Anonymous 2004; Bird et al. 2009).

Human infections typically occur either from infected mosquito bites or as the result of percutaneous/aerosol exposure while slaughtering infected animals or via contact with aborted fetal materials (Bird et al. 2008).

Several field researchers with no physical contact with an infected sheep slaughtered via traditional Islamic method in Egypt became viremic with RVFV 3 days after aerosol exposure; sheep blood titered 10^{10} $MICLD_{50}$/mL (Hoogstraal et al. 1979).

Mechanical and biological transmission via arthropods, fomites, direct contact with animal blood, and maternal and fetal tissues and fluids (White 1994).

Frequency of airborne transmission under natural conditions is unknown. But it is known that laboratory workers acquired RVFV after inhaling infectious aerosols generated by careless handling of infected tissues (Brown, Dominik, and Morrissey 1981).

RVFV causes serious human infection in laboratory workers in the absence of appropriate biocontainment precautions; staff should either be vaccinated and work under BSL-3, work under BSL- 4 conditions, or wear respiratory protection. Workers must take care when working with infected animals or when performing necropsy examinations (Anonymous 2004).

RVFV has caused frequent laboratory infections [in the absence of appropriate biocontainment precautions] (Meadors, Gibbs, and Peters 1986).

A 1967 publication reported 29 laboratory infections and 1 death [in the absence of appropriate biocontainment precautions] (Hanson et al. 1967; Pike 1979).

A 1980 publication reported an additional 18 infections [in the absence of appropriate biocontainment precautions] and no deaths; the source of infection in these 47 cases was unknown (Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses).

Potential for infection via ingestion of raw milk (Flick and Bouloy 2005).

A nonfatal, dengue-like illness most frequently occurs in lab workers, field investigators, and animal handlers (Meegan and Bailey 1989).

1951, Johannesburg, humans assisting in a necropsy of a bull became ill (Swanepoel and Coetzer 1994).

Aerosol infection in lambs, rhesus monkeys, cynomolgus monkeys, hamsters, puppies, kittens, mice, doses varied from $10^{<1.0 \text{ to } 9.0}$ $MIPLD_{50}$ or $10^{1.9\text{-}2.6}$ PFU (Shimshony and Barzilai 1983).

ICR-strain mice infected by exposure to infectious aerosols ($10^{0.5 \text{ to } 4.5}$ PFU) composed of particles with a mass median diameter of 0.96 ,µm; respiratory median lethal doses for different RVFV strains were $10^{2.2}$, $10^{1.9}$, $10^{2.6}$, and $10^{1.9}$ PFU; single group of mice infected with $10^{3.1}$ PFU assayed sequentially through 96 hours post infection—between 6 and 30 hours, demonstration of an increasing virus concentration only in the lungs indicated that initial replication occurred there, without evidence of pneumonia; virus isolated from the liver by 48 hours, with fulminating and fatal hepatic necrosis (Brown, Dominik, and Morrissey 1981).

Puppies infected via aerosol of 17–36 $MICLD_{50}$ had inapparent infection, viremias to $10^{3.2}$ $MICLD_{50}$; the infectious dose is estimated at 25 $MICLD_{50}$ (Keefer, Zebarth, and Allen 1972).

Kittens infected via aerosol of 5–7 $MICLD_{50}$ had inapparent infection, viremias $10^{2.2\text{-}3.8}$ $MICLD_{50}$ (Keefer, Zebarth, and Allen 1972).

Puppies and kittens exposed via ingestion at $10^{5.9\text{-}6.4}$ $MICLD_{50}$ were not infected [virus probably inactivated by stomach acid] (Keefer, Zebarth, and Allen 1972).

Virus titers/concentrations/pathogenesis

RVFV is one of most prolific viruses known, replicating to extremely high titers after a 30–72 hour incubation period (Murphy et al.).

In humans, maximum titers to $10^{8.6}$ $MICLD_{50}$ (Swanepoel and Coetzer 1994).
Virus titers in infected humans were $10^{4.2 \text{ to } 8.6}$ (Shimshony and Barzilai 1983).

1977–1978, Egypt, virus titers in infected humans were $10^{4.1\text{-}8.6}$ $SMICLD_{50}$ (Meegan 1979).

In Vero cells, 10^{7}-10^{9} $CCID_{50}$/mL produced in roller bottles/flasks, concentrated 10–100 times via ultracentrifuging to produce concentrations of 10^{8}–10^{11} $CCID_{50}$/mL, roller bottles/flasks (Johnson 2008).

In Vero cells, 10^{8} PPU/mL after a 48-hour incubation (Brown, Dominik, and Larson 1982).

Maximal titers in hamster kidney cell line attained $10^{8.2}$ $MIPLD_{50}$/mL (Shimshony and Barzilai 1983).

$10^{9.0}$ $MIPLD_{50}$/mL in suspended L cell (mouse L-cells) cultures (Shimshony and Barzilai 1983).

In diploid rhesus monkey cell line, virus titers attained 4.0–10x10^{6} PFU/mL and 0.19–3.89x10^{6} MIP [mouse, intraperitoneal] LD_{50}/mL (Meadors, Gibbs, and Peters 1986).

Multiplicities of infecting virus from 1 to 10 SMICLD$_{50}$ produced $10^{6.0}$ to $10^{7.99}$ SMICLD$_{50}$/mL in L cell monolayer s from 0 and 72 hour post-inoculation, respectively, and $10^{7.5}$ to $10^{5.34}$ in L cell suspension cultures from 0 through 96 hours post-inoculation, respectively [virus infectious doses very low] (Orlando, Delauter, and Riley 1967).

In 50 L virus fermentors with L cells, $10^{8.2 \text{ to } 9.0}$ MICLD$_{50}$/mL produced (Klein, Jones Jr, et al. 1971).

Before/after ultrafiltration, cell culture origin virus was $10^{5.52 \text{ to } 7.19}$/$10^{6.52 \text{ to } 8.85}$ MICLD$_{50}$/mL with total virus infectivity/final volume = $10^{8.28 \text{ to } 10.67}$ MICLD50 (Klein, Mahlandt, et al. 1971).

Virus titers high in susceptible animals (Meegan and Bailey 1989).

Maximum lamb viremic load reach $10^{10.1}$ MIPLD$_{50}$/mL; $10^{7.6}$ in adult sheep; $10^{7.5}$ in calves; $10^{8.2}$ in kids; $10^{5.6}$ in goats (Swanepoel and Coetzer 1994).

Rhesus monkeys, *M. mulatta*, three experiments: SQ were inoculated with$10^{5.3}$ PFU/mL, no viremia in one, $10^{3.6}$ PFU/mL in second monkey lasting 1 day, $10^{6.8}$ viremia on day 2 and $10^{4.9}$ PFU/mL viremia on day 3 in the third monkey; IV inoculation of $10^{4.7}$ PFU/mL, three developed transient viremia without clinical signs, but the fourth developed hemorrhagic diathesis and was euthanatized; IV inoculation of $10^{4.1}$ PFU/mL had transient viremia and one was ill with hemorrhagic signs, but recovered; inoculation (route not specified; IV is implied) of $10^{4.8}$ PFU/mL, one became ill and was killed in a moribund state; viremias to $10^{5.5\text{-}6.5}$ PFU/mL (Peters et al. 1988).

In African buffalo, maximum titers to $10^{5.4}$ TCID$_{50}$; to $10^{4.9}$ MICLD$_{50}$ in dogs; to $10^{2.5}$ MIPLD$_{50}$ in ponies (Swanepoel and Coetzer 1994).

In newborn and weanling mice, IP and IC inoculation, causes death, virus titers from $10^{6.6}$ to $10^{9.0}$ log$_{10}$/mL [test systems not reported] (Weinbren 2008; Shimshony and Barzilai 1983).

Hamsters with viremias $\geq 10^{7.4 \text{ to } 10.6}$ PFU/mL of blood; mosquitoes/biting gnats ingested $10^{4.9 \text{ to } 8.1}$ PFU/mosquito/biting gnat for transmission studies (Turell et al. 2008).

Viremias $>10^8$ PFU/mL of blood (Bird et al. 2009).

Cattle and sheep maintain very high level viremias for 3–5 days (White 1994).

Vaccinated rats challenged, SQ, with 1.0×10^3 PFU of virulent wild-type RVFV, lethal to non-vaccinates; induced challenge virus titers of 3.4×10^7 PFU/mL blood in non-vaccinated rats (Bird et al. 2008).

Several domestic animal species can act as amplifying hosts with viremias of 10^{10} mouse LD_{50}/mL blood (Hoogstraal et al. 1979).

Several field researchers with no physical contact with an infected sheep slaughtered via traditional Islamic method in Egypt became viremic with RVFV 3 days after aerosol exposure; sheep blood titered 10^{10} $MICLD_{50}$/mL (Hoogstraal et al. 1979).

Susceptible rat spp develop fulminating liver necrosis within 3–5 days when inoculated with less than 5 PFU dose; great variation is reported across strains of virus in virulence (Shimshony and Barzilai 1983).

Clinical responses of cattle inoculated with doses from $10^{4.3}$ to $10^{7.3}$ $MIPLD_{50}$ were similar; produced viremias to $10^{7.5}$ MIPLD50 (Shimshony and Barzilai 1983).
In pregnant cattle, dose 10^5 PFU, aborted, severe clinical disease, death; virus in tissues to $10^{4.5}$ $MIPLD_{50}$; live borne fetuses were viremic at 10 days post-infection (Shimshony and Barzilai 1983).

Virus titers in sheep, $10^{8.9\text{-}10.0}$ (Shimshony and Barzilai 1983).

In puppies, virus in saliva; in sheep, $10^{5.3}$ PFU/mL in saliva; isolated from sheep and cow milk (Shimshony and Barzilai 1983).

High titers in fetuses and fetal membranes; virus in human throat washings (Shimshony and Barzilai 1983).

Fatal encephalitis in 10-week-old adult gerbils was dose-independent [$10^{1.0\text{-}7.0}$ PFU, SQ]; viral replication evident in the brains of young from day 4 ($10^{3.0}$ PFU/g) through day 7 ($10^{6.0}$ PFU/g); virus detected in the brain of a single adult gerbil (at day 7, $10^{4.0}$ PFU/g); in two moribund adult gerbils, $10^{7.0}$ PFU/g of virus in the brain tissue on days 8 and 11; when young and adult gerbils were inoculated with a low dose (50 PFU) of virus, IC, no detectable differences in the course of infection with all animals succumbing to fatal necrotizing encephalitis at approx 7 days post-inoculation (Anderson, Slone, and Peters 1988).

1977–1978, Egypt, virus titers in aborting sheep, $10^{8.9–10.0}$ SMICLD50 (Meegan 1979).

The virus can be isolated from blood, preferably collected in an anticoagulant, during the febrile stage of the disease, or from liver, spleen and brain tissues of animals that have died and from the organs of aborted fetuses; primary isolations made on cell cultures of various types, such as African green monkey kidney (Vero) cells, baby hamster kidney cells, chicken embryo reticulum, or primary cells of sheep or cattle origin; hamsters, adult or suckling mice, embryonating chicken eggs or 2-day-old lambs can be used for primary virus isolation (Anonymous 2004).

Pathogen stability

RVFV survives in aedine mosquito ova that are subject to an obligatory drying period (Swanepoel and Coetzer 1994).

During interepidemic periods, the virus is maintained in nature via transovarial transmission in mosquitoes, as was shown in *Aedes lineatopennis* in Kenya and in *Aedes vexans* in Senegal (Flick and Bouloy 2005).

Virus can be maintained by mosquitoes through transovarial transmission. The mosquito species most frequently associated with virus maintenance often breed in natural depressions (dambos) on the African savanna. Heavy rainfall floods the breeding sites creating conditions resulting in hatching of vertically infected mosquitoes and in subsequent transmission of the virus to vertebrates that use the flooded dambos as watering holes. If sufficient vectors and susceptible vertebrate hosts are available, epizootic transmission can follow (LeDuc 1989).

Virus persists in sheep liver and spleen for 20–21 days (Meegan and Bailey 1989).

Virus is very stable in serum; survives for several months at 4 °C and > 3 hours at 56 °C (Swanepoel and Coetzer 1994).

Virus from blood collected in oxalate-phenol-glycerin solution remains viable for more than 8 years after storage under variable refrigeration temperatures (Swanepoel and Coetzer 1994).

Virus is very stable at less than 60 °C or after lyophilization (Swanepoel and Coetzer 1994).

Virus is very stable in aerosols at 23 °C and 50–85 percent RH (Swanepoel and Coetzer 1994; Shimshony and Barzilai 1983).

Half life of the virus is 77, 15.8, and 6.9 minutes for 30, 55, and 80 percent RH, respectively (Brown, Dominik, and Larson 1982).

Geometric mean of the biological decay rate is 2.3 percent per minute over range of 50 and 80 percent RH. Aerosol stability is not influenced by RH (Miller et al. 1963).

Virus is inactivated by lipid solvents, formalin, and pH less than 6.8 (Swanepoel and Coetzer 1994).

Virus is sensitive to heat and acid. It is inactivated readily by detergents, lipid solvents, and common disinfectants (Murphy et al.).

Inactivated rapidly at pH 6.2 (Gerdes 2005).

Virus lyophilizes well; blood preserved in oxalate-phenol-glycerin solution retained virulence for more than 8 months 4 °C; sheep plasma retained infectivity for more than 8 years under a variety of refrigeration conditions; resistant to temperatures less than 60 °C, recovered from serum after several mo storage at 4 °C or after 3 hours at 56 °C; inactivated by acetone at –30 °C overnight; by 0.25 percent solution of 10 percent formalin for 3 days, by methylene blue with light; lipid solvents, i.e., ether and sodium deoxycholate inactivate; susceptible to trypsin digestion; in cell monolayers, incomplete inactivation by acetone at –60 °C for 48 hours; inactivated rapidly at pH of less than 6.8 (Shimshony and Barzilai 1983).

Highly stable in aerosol form at 23 °C and RH of 50–85 percent (Shimshony and Barzilai 1983). Indication that viruses with structural lipids survive best in aerosols at low RH (Benbough 1971).

UV radiation inactivates viruses by chemically changing the RNA and DNA; most effective UV is 250 nm; filoviruses [MARV and EBOV] most sensitive to solar UV_{254}, requiring 20–100 minutes at mid-day exposure to inactivate one $\log_{10}$ of virus: bunyaviruses (hantaviruses [AND virus] and RVFV), arenaviruses (Lassa, JUN), and flaviviruses (TBE complex viruses) more resistant (Lytle and Sagripanti 2005).

Vectors

a. Field

Transmission occurs by large number of mosquito spp.—23 spp in 5 genera [table p. 62] (Meegan and Bailey 1989).

More than 30 species of genera (*Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Eretmapodites*, *Mansonia*) (Bird et al. 2009).

Biologic vectors are *Aedes* spp, *Culex* spp, *Anopheles* spp, *Eretmapodites* spp, *Mansonia* spp; mechanical vectors= *Culicoides* spp, phlebotomids, stomoxids, simulids (Brown and Torres 2008).

RVFV has been isolated from *Culicodes* (biting gnats or midges), *Simulium* (black flies), *Rhipicephalus* ticks (Meegan and Bailey 1989).

Biological transmission occurs via mosquitoes, and mechanical transmission is via biting flies (White 1994).

During the 1977–1978 epizootic in Egypt, *Culex pipiens* was the most ubiquitous and prevalent mosquito species in the Nile Valley and Delta; isolation of RVFV from engorged and unengorged *C pipiens*, and demonstration of laboratory transmission of the virus by this species, strongly implicate it as the chief vector (Hoogstraal et al. 1979).

RVFV has been isolated from *Aedes africanus, A caballus, A cinereus, A circumluteolus, A dentatus, A dendrophilus, A coustani, A juppi, A lineatopennis, A tarsalis, Anopheles cinereus, An coustani, Coquillettidia fuscopennata, Culex antennatus, Cx pipiens, Cx theileri, Eretmapodites* spp, *E quinquevittatus, Mansonia africana, Culicoides* spp, *Simulium* spp from varied ecosystems: inland plateaus, coastal lowlands, highlands, bushed grasslands, cities, deltas, forests, and rivers (Shimshony and Barzilai 1983).

Occasional isolations occur from *Rhipicephalus* spp ticks (Flick and Bouloy 2005).

During interepidemic periods, the virus is maintained in nature via transovarial transmission in mosquitoes, as was shown in *Aedes lineatopennis* in Kenya and in *Aedes vexans* in Senegal (Flick and Bouloy 2005).

Virus can be maintained by mosquitoes through transovarial transmission. The mosquito species most frequently associated with virus maintenance often breed in natural depressions (dambos) on the African savanna. Heavy rainfall floods the breeding sites

creating conditions resulting in hatching of vertically infected mosquitoes and in subsequent transmission of the virus to vertebrates that use the flooded dambos as watering holes. If sufficient vectors and susceptible vertebrate hosts are available, epizootic transmission can follow (LeDuc 1989).

RVFV adapted quickly to variety of ecosystems outside traditional areas of Africa. From Egypt's arid to semiarid climate, the virus spread into the Middle East, with transmission via *Culex pipiens* (Meegan, Niklasson, and Bengtsson 1979).

b. Experimental

Viral load in viremic domestic ruminants, humans, and rodents is sufficient to infect mosquitoes; viremias of $10^{5.7}$ to $10^{9.7}$ $MICLD_{50}$/mL will infect 50 percent of mosquitoes (Swanepoel and Coetzer 1994).

Biologic transmission occurs by 7 spp of *Aedes*, 6 spp of *Culex*, and 2 spp of *Eretmapodites* (Meegan and Bailey 1989).

Mechanical transmission is by *Culicoides, Simulium* and *Lutzomyia* (sand flies) (Meegan and Bailey 1989).

Transovarial transmission to mosquito ova of *Aedes* floodwater spp with isolates from male and female mosquitoes raised from field collected ova (Meegan and Bailey 1989).

Virus is transmitted by bites of *Erchrysogaster*, *Ae caballus*, and *Ae aegypti* (Weinbren 2008).

Culex pipiens from the Nile Delta transmitted the virus after infection from infected hamsters; infection rates were 87 percent, and transmission rates were 40 percent (Weinbren 2008).

Arthropod transmission studies have implicated 18 mosquito species of the genera *Aedes*, *Mansonia*, *Culex*, *Anopheles,* and *Eretmapodites* as possible RVFV vectors (Hoogstraal et al. 1979).

Most effective vector, *C theileri*, was infected with doses of $5x10^{4.0}$ $MICLD_{50}$/mL (Shimshony and Barzilai 1983).
Experimental transmission, *Aedes aegypti, A caballus, A coustani, A juppi, A lineatopennis, A triseriatus, Culex neavei, Cx pipiens, Cx quinquefasciatus, Cx theileri, Cx univittatus, Cx zambaensis, Eretmapodites chrysogaster, E quinquevittatus* (Shimshony and Barzilai 1983).

In one study, *Culicoides variipennis* [a North American spp] was not susceptible (Shimshony and Barzilai 1983).

Virus isolated from *Culicoides* spp (Shimshony and Barzilai 1983).
Phlebotomus duboscqi, Phlebotomus papatasi, Phlebotomus sergenti, and *Sergentomyia schwetzi* (Diptera: *Psychodidae*) fed on infected hamsters (10^4 PFU/mL inoculum, at 24 hours); with virus titers in hamsters of $10^{8.0–8.6}$ PFU/mL, *P papatasi* were not infected and did not transmit the virus, 18 percent of *P. sergenti* were infected, but did not transmit the virus, 50 percent of *P. duboscqi* were infected and 6 percent transmitted, 12 percent of *S. schwetzi* were infected and 7 percent transmitted; with hamster viremias of $10^{10.6}$ PFU/mL, 32–45 percent of *P. papatasi* were infected and 2–6 percent transmitted, 57 percent of *P. sergenti* were infected, 14 percent transmitted, 22 percent of *S. schwetzi* were infected and 14 percent transmitted; via intrathoracic inoculation of 10^2 PFU, 100 percent of *P. papatasi* were infected and 14 percent transmitted, 100 percent of *P. duboscqi* were infected and 50 percent transmitted, 41 percent of *S. schwetzi* were infected and 0 percent transmitted (Dohm et al. 2000).

In North America, potential vector species from Colorado and the Midwest, *Culex tarsalis* transmitted efficiently (infection rate = 93 percent, dissemination rate = 56 percent, and estimated transmission rate = 56 percent), but using the same virus dose,

none of other species tested transmitted under laboratory conditions; *Aedes dorsalis* was susceptible to infection (78 percent) and had a moderate dissemination rate (33 percent), but had a salivary gland barrier and rarely transmitted RVFV by bite; only 30 percent of *Aedes vexans* became infected and 3 percent developed a disseminated infection, but 32 percent of those with disseminated infection transmitted virus by bite, so about 1 percent of orally exposed *Ae vexans* would be expected to transmit RVFV by bite; none of the *Culicoides sonorensis* became infected, even after intrathoracic inoculation; none of the *Anopheles quadrimaculatus* tested transmitted RVFV by bite, even after intrathoracic inoculation, indicating that *C. sonorensis* and *An quadrimaculatus* would not be competent vectors of RVFV [terminology: infection rate = percentage of orally exposed mosquitoes/biting gnats that contain virus: dissemination rate = percentage of orally exposed mosquitoes/biting gnats that contained virus in legs; transmission rate = percentage of orally exposed mosquitoes/biting gnats that re-fed on an uninfected host and transmitted virus] (Turell 2008).

Other epidemiological/ecological data

1987–1988, Egypt, the virus was recognized in more than 18 African countries; spread over 4,200 miles (north to south), circulating in a number of different geographic and climatic settings, with penetration into Egypt showing amplification in a totally new ecosystem (Shimshony and Barzilai 1983).

Before Egypt, the virus was in 20 countries: Angola, Botswana, Cameroun, Chad, Egypt, Ethiopia, Gabon, Kenya, Lesotho, Malawi, Mali, Mozambique, Namibia, Nigeria, Somalia, S Africa, Sudan, Tanzania, Uganda, Zaire, Zambia, Zimbabwe (Shimshony and Barzilai 1983).

Recent outbreaks in Kenya, Madagascar, Mauritania, Somalia**,** Tanzania, and Yemen indicate the potential for RVFV to cause severe disease in both humans and domestic animals and its potential to be introduced into new areas, including North America (Turell et al. 2008).

RVFV is found in a wide range of ecological zones (Meegan and Bailey 1989).

Outbreaks historically are associated with indigenous forests and coastal bush areas (Swanepoel and Coetzer 1994).

Outbreaks occur in central and eastern Africa when particularly heavy rains favor mosquito breeding by flooding breeding habitats, *dambos* (small to large shallow depressions in the earth that are normally dry, but which flood during rainy seasons/years) [*oshanas* in southwestern Africa] (Meegan and Bailey 1989; Bird et al. 2009).

Outbreaks in South Africa during the 1950s centered on the *panveld* where surface waters gather in undrained shallow depressions (pans) after heavy rains or in ponds formed by farm dams that are subjected to periodic drying (Swanepoel and Coetzer 1994).

A correlation in Zimbabwe between endemic cycle and broad vleis (low-lying grassy area serving as a water seepage channel for rain water from higher ground) (Swanepoel and Coetzer 1994).

In eastern Africa, epidemics are associated with above average rainfall at irregular intervals of 5–15 years (Swanepoel and Coetzer 1994).

In many areas of Africa, the climatic cycle lasts 18 years with 9 years of rainfall followed by 9 years of drought; interepizootic periods can last 8–24 years (Swanepoel and Coetzer 1994).

Outbreaks in northeastern Africa (Egypt, Sudan) associated with movement of infected mosquitoes and transportation of infected sheep and cattle. Virus activity is associated with *Culex pipiens* rather than aedine species (Swanepoel and Coetzer 1994).

Historic distribution of RVF is the sub-Saharan African continent [including Egypt and Sudan], Madagascar, and the Arabian Peninsula (Anonymous 2007).

Epidemics occur in infected areas after flooding; separated by inter-epidemic periods that can last for several decades in arid areas. During those periods, the prevalence of infection in humans, animals, and mosquitoes can be difficult to detect (Anonymous 2004).

Disease occurs in climatic conditions favoring breeding of mosquito vectors and is characterized by liver damage (Anonymous 2004).

Excessively heavy rainfall in semiarid regions often precedes large, periodic outbreaks of RVFV activity, allowing for the abundant emergence of transovarially infected *Aedes* spp. mosquitoes and the subsequent initiation of an outbreak by transmission of virus to livestock and humans via infected mosquito feeding (Bird et al. 2008).

The most important epidemics/epizootics occur after periods of unusually heavy rains or in association with construction of dams (Flick and Bouloy 2005).

The virus is found in many areas in sub-Saharan Africa, although it reached beyond the normal distribution in 1978 when a major epidemic occurred in Egypt. In Africa, it is normally associated with excessively heavy rainfall (LeDuc 1989).

Epidemics in Kenya are associated with the El Niño phase of the Southern Oscillation (ENSO) (Hay et al. 2000; Davies, Linthicum, and James 1985; Bird et al. 2009).

As a group, the other viral hemorrhagic fever (VHF) agents are linked to the ecology of their vectors or reservoirs, whether rodents or arthropods (Geisbert and Jahrling 2004).

Epizootic RVF occurred 4 times over a 30-year period in Kenya and was associated with widespread, frequent, persistent rainfall; raises water levels in grassland depressions (dambos), habitats of immature forms of ground-pool-breeding aedine mosquitoes in which RVFV is probably transmitted transovarially; outbreaks last 1–3 years and recur at 5–15 year intervals (Davies, Linthicum, and James 1985).

In humans, most infections result in undifferentiated febrile disease, but about 1 percent of infections result in hemorrhagic complications, which are often fatal and ocular sequellae occur, which can cause retinal damage, including blindness (Turell et al. 2008).

All known RVF outbreaks in East Africa from 1950 to 1998, and probably earlier, followed periods of abnormally high rainfall. An analysis of the record and Pacific and Indian Ocean sea surface temperature anomalies, coupled with satellite normalized difference vegetation index data, shows that prediction of outbreaks can be made up to 5 months in advance of outbreaks in East Africa. Concurrent, near-real-time monitoring with satellite normalized difference vegetation data could identify actual affected areas (Linthicum et al. 1999).

Therapeutics/vaccines

Formalinized vaccine is used in cattle and sheep (Meegan and Bailey 1989).

Attenuated vaccine is used in sheep and cattle, but is abortigenic in pregnant ewes (Meegan and Bailey 1989).

Smithburn vaccine strain was derived by serial intracerebral passage of wild-type Uganda virus until it lost its liver tropism and became neuro-adapted. After that, it is passed in mice and embryonating hen's eggs, grown in cell culture, and lyophilized (Weinbren 2008).

Since 1967, a formalin-inactivated vaccine produced in infected primary monkey kidney cells has been used to protect laboratory workers (Meadors, Gibbs, and Peters 1986).

Formalin-inactivated product that uses a more acceptable seed virus was cloned and propagated in cell culture and a well-characterized diploid fetal rhesus monkey cell substrate. It causes mild vaccine reactions in humans with neutralizing antibody, but no viremia, are produced (Meadors, Gibbs, and Peters 1986).

Formalinized cell culture origin human vaccine (USAMRIID) exists (Pittman et al. 1999; Meegan and Bailey 1989).

Attenuated human vaccine exists (Meegan and Bailey 1989).

Vaccine attenuation has been approached by selection of small [minute] plaque variant (Meegan and Bailey 1989; Swanepoel and Coetzer 1994); chemical mutagenesis with 5-fluorouracil (Swanepoel and Coetzer 1994; Meegan and Bailey 1989); and serial passage in permissive systems, e.g., cell culture, suckling mice (Swanepoel and Coetzer 1994).
Treatment of exposed humans with immune plasma and ribavirin has been recommended (Swanepoel and Coetzer 1994).

No preventive RVF medications or licensed vaccines for humans exist (CDC 2007).

In vivo studies of a reverse genetics-generated recombinant RVFV vaccine candidate containing precise deletions of complete virus genes with known roles in virulence show promise for humans (Bird et al. 2008).

Inactivated RVFV vaccine TSI-GSD-200 is safe and provides good, long- term immunity in humans when the primary series and one boost are administered. It is used for at-risk laboratory personnel and military personnel (LaBeaud et al. 2007).

Vaccine created from the twelfth mutagenesis passage (RVFV ZH-548-P12) of a virus originally isolated from a human with a nonfatal case of RVF. The final vaccine product markedly attenuated for rhesus macaques inoculated intravenously. The vaccine produced transient, low-titer viremias and minimal serum enzyme elevations; potential for use in humans (Morrill and Peters 2003).

DNA vaccines expressing Gn and Gc genes were tested in mice alone or in various combinations. The DNA vaccine elicited antibodies and protected mice from challenge with 100 LD50 when delivered alone or in combination with other DNA (Spik et al. 2006).

No approved vaccine is available for livestock or humans (Bird et al. 2009).

No specific treatment exists; give supportive care (Bird et al. 2009).

The Working Group for Civilian Biodefense has recommended intravenous ribavirin be administered in the case of a contained casualty situation with RVF infections, and in the case of mass casualties, an oral regimen of ribavirin is recommended (Sidwell and Smee 2003).
A major focus for preventing and controlling RVF outbreaks is reducing numbers of amplifying hosts by immunizing livestock (Morrill, Mebus, and Peters 1997).

In Rhesus macaques, passive administration of 0.025 mL/kg of immune serum (PRN titer of 1:2,560) failed to induce detectable serum-neutralizing antibody in recipients, but it prevented viremia and illness [suggests therapy for humans] (Peters et al. 1988).

Administration of antibodies, interferon, interferon inducer, or the nucleoside analog ribavirin in experimentally RVFV-infected mice, rats, or monkeys was efficient in protecting against the disease; however, those treatments have never been tested in RVFV-infected patients (Flick and Bouloy 2005).

Other remarks

First described in 1912–1913 from an outbreak of abortion in ewes, with accompanying human disease in the Rift Valley of Kenya. The virus was isolated in 1931 (Meegan and Bailey 1989; Swanepoel and Coetzer 1994) and reported in 1930 in another publication (Sidwell and Smee 2003).

Virus isolated from a newborn lamb in 1930 in an area of upland tropical savannah (Weinbren 2008).

Detailed clinical signs and pathologic changes with extensive photos have been published (Swanepoel and Coetzer 1994).

RVFV is assigned to SALS (American Committee on Arthropod-Borne Viruses (ACAV) Subcommittee on Arbovirus Laboratory Safety) level 3 (Weinbren 2008).

RVFV possession in the United States requires a USDA permit, and is a USDA restricted, USDA high consequence agent. A Department of Commerce permit is required, and vaccination is recommended (Weinbren 2008).

Peracute (excessively acute) or acute zoonotic disease occurs among domestic ruminants in Africa (Anonymous 2004).

Considered a BSL-4 pathogen unless humans are immune from prior infection or vaccinated (Murphy et al.).

RVFV is a high-priority concern from a bioterrorism perspective, Category A from NIH, Category B from CDC (Sidwell and Smee 2003).

RVF is a mosquito-transmitted [*Aedes* or *Culex*] zoonotic disease of livestock. (Anonymous 2007; Murphy et al. 1999).

A common pathogenic feature of the hemorrhagic fever viruses is the ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response (Geisbert and Jahrling 2004).

Outbreaks occur across sub-Saharan Africa and into Egypt, Sudan, Mauretania, and the Middle East (Meegan, Niklasson, and Bengtsson 1979; Geisbert and Jahrling 2004; Bird et al. 2009).

Taxonomy/antigenic relationships/synonyms

Genus *Phlebovirus*, family *Bunyaviridae* (Meegan and Bailey 1989; Anonymous 2004; White 1994; Murphy et al.; Shimshony and Barzilai 1983; Bird et al. 2009).

Before the 1987 Mauritanian outbreak, RVFV, previously identified as Zinga virus, had been isolated in West Africa from mosquitoes (Saluzzo et al. 1989).

Biological and antigenic traits distinguish the strains isolated during the 1977 Egyptian epidemic from the sub-Saharan strains (Saluzzo et al. 1989).

Twelve distinct viruses associated with hemorrhagic fever in humans are classified among four families: *Bunyaviridae*, which includes Rift Valley fever, Crimean-Congo hemorrhagic fever, and Hantaan viruses (LeDuc 1989).

C.1.2 BSL-4 Pathogens

C.1.2.1 Andes virus (ANDV)

Note: BSL-4 biocontainment precautions are recommended for ANDV when infecting rodent species permissive for (susceptible to) chronic infection. Otherwise, BSL-3 biocontaiment precautions are recommended.

Host range

a. Field

Long-tailed pygmy rice rat, *O. longicaudatus*, and other species of genus *Oligoryzomys* are the reservoir (Centers for Disease Control and Prevention 2008; Wells et al. 1997; McCaughey and Hart 2000; Padula et al. 2004).

Rodent reservoirs are in the sub-family *Sigmodontinae* within the family *Muridae*, order Rodentia with which the hantaviruses have evolved for thousands of years (Toro et al. 1998; McCaughey and Hart 2000; Mertz et al. 2006).

Occasional evidence of infection (antibody) is found in numerous other species of rodents and their predators (e.g., dogs, cats, and coyotes), indicating that many (perhaps any) mammal species coming into contact with an infected host might become infected; no evidence supports the transmission of infection to other animals or to humans from those *dead-end* hosts, but domestic cats and dogs might bring infected rodents into contact with humans (Centers for Disease Control and Prevention 2008).

In Chile, most frequently captured rodent species in outbreak area, *O. longicaudatus*, 12.7 percent hantavirus-antibody–positive; *Akodon olivaceus*, 7.5 percent antibody-positive; *A. longipilis*, 2.7 percent antibody-positive (Toro et al. 1998).

In areas of Chile and Argentina, natural reservoir of a milder pathogen appears to be *Callomys laucha* (Ferres and Vial 2004).

As far as known, all *Hantaviruses* are maintained in nature through chronic infection of rodent and other small mammalian hosts, with transmission between rodents and to humans primarily via aerosolized, infectious excreta (LeDuc 1989).

Isolates from *O. chacoensis* and *O. flavescens* captured where hantavirus pulmonary syndrome (HPS) patient lived and worked (Gonzalez Della Valle et al. 2002).

Argentina, serologic evidence of infection in *O. flavescens* (rice rat), *A. azarae* (grass field mouse), *Bolomys obscurus* (dark field mouse) (Levis et al. 1997).

b. Experimental

Causes death in Syrian hamster with course of disease that closely models human infection (Wahl-Jensen et al. 2007)(Centers for Disease Control and Prevention 2008).

Cynomolgus macaques, *M. fascicularis*, via IV or aerosol, 4.4 x 10^5 PFU; no clinical disease, hematologic changes (↓lymphocytes), both IgM and IgG antibodies against the viral nucleocapsid protein, neutralizing antibody response; via plaque assay, serum samples negative for infectious virus, but by non-nested reverse transcriptase–polymerase chain reaction, viral S-segment genomes were detected in whole blood (McElroy et al. 2002).

Prevalence/incidence/attack rate

Paraguay, hantavirus seroprevalence of 45 percent in Indian populations of Gran Chaco and 35 percent seropositives in non-Indian populations (Ferres and Vial 2004).

Temuco, Chile, Andes virus (AndesV) seroprevalence in mountainous areas of 2.15 percent and seronegative at lower altitudes (Ferres and Vial 2004).

Jujuy, northern Argentina, 6.5 percent seropositivity (Ferres and Vial 2004).

In Jardinopolis, Brazil, seroprevalence 14.3 percent (Ferres and Vial 2004).
In Argentina and Chile, 16 percent of cases occurred in children < 16 years old (Ferres and Vial 2004).

In Chile, seroprevalence varies from 1 to 40 percent depending on geographic area and ethnic differences (Pini 2004).

In Chile from 1995-2006, a total of 492 cases of hantavirus cardiopulmonary syndrome were reported (Torres-Perez et al. 2009).

Seroprevalence in southern Chile, 2.15 percent (Tager Frey et al. 2003).

Seroprevalence in northern Argentina study, 1.5 percent of 135 persons (Levis 1995).

Northern Argentina, prevalence of hantavirus antibodies in the general human population was 6.5 percent, one of the highest reported in the literature; high prevalence of hantavirus antibody seemed to be associated with high infestation of rodents detected in domestic and peridomestic habitats (Pini et al. 2003).

Rodent seroprevalence in northern Chile, *O. longicaudatus*, 5.9 percent, *Abrothrix longipilis*, 1.9 percent (Medina et al. 2009).

Rio Negro Province, Argentina, seroprevalence:

- In subantarctic forests without presence of HPS cases: *O. longicaudatus* , 4.4 percent; in *A. longipilis,* 0.2 percent; in *Loxodontomus microtus*, 8.3 percent;
- in subantarctic forests with related HPS: *O longicaudatus*, 7.4 percent;

- in steppes without HPS: in *A. olivaceus,* 2.4 percent (Larrieu et al. 2008).

Chile, 10th Region, seropositive rodent species distributed from the Pacific coast to the Andes mountains, *O. longicaudatus*, 2.7 percent, *A. longipilis*, 0.6 percent; seasonal movement to open habitats close to human outdoor activity during dry season (Murua et al. 2003).
Diego Gaynor, northwest of Buenos Aires, seroprevalence of 9.3 percent in total sample of 291 *A azarae* and 13.5 percent for 37 *O. flavescens* using ELISA; higher rates of seroprevalence in older males than in females or juveniles (Suarez et al. 2003).

South-central Chile, correlation of seropositive rodent species to confirmed HPS cases; *O. longicaudatus*, 10.4 percent seropositive using immunoassay (Torres-Perez et al. 2004).

Argentina study, Nt and IFAT antibodies detected in 22.5 percent of laboratory *Rattus norvegicus* and 23.5 percent of wild-caught *Callomys musculinus* (Weissenbacher et al. 1990) [uncertain significance as study used Old World hantaviruses—Hantaan, Seoul, Puumala, Prospect Hill viruses—and was done before the identification of Andes virus].

Seroprevalence in small mammals in Patagonian Andes mountain range, Rio Negro province, Argentina; rodent blood samples collected in natural and peri-urban habitats and at the home of HPS case patient analyzed by ELISA and organ tissue samples tested by PCR and nucleotide sequence analysis; AND virus antibody was detected in 5.4 percent of 555 *O. longicaudatus*, 0.7 percent of 411 *A. longipilis*, and 10 percent of 10 *Loxodontomys microtus*; seroprevalence in *O. longicaudatus* were 13.7 percent in spring 1996, 59.3 percent in summer 1996, 2.1 percent in autumn 1997, 12.4 percent in winter 1997, and 3.1 percent in spring 1997; much higher seroprevalence (33 percent) found during trapping around the residence of an HPS case patient; higher seroprevalence found in older male *O. longicaudatus*; no apparent correlation of seroprevalence with rodent population density, or of rodent population density or seroprevalence with numbers of human cases (Cantoni et al. 2001).

Northern Argentina, HPS-endemic area composes Salta and Jujuy provinces; 1997–2000, 30 HPS cases diagnosed in Jujuy province (population 512,329) (Pini et al. 2003).

R_0/incubation period/infectious period/infectious dose

Incubation period estimated at 14–32 (median 18) days from studies of 19 hantavirus cardiopulmonary syndrome (HCPS) patients with history of high risk activities and a biologist with HCPS who was bitten by an infected *O. longicaudatus* (Vial et al. 2006).

Incubation period estimated at 8–43 days (Castillo et al. 2007).

Incubation period 15–24 days (Martinez et al. 2005).

Infectious dose in humans is unknown.

Lethal dose required to kill 50 percent of Syrian hamsters (LD_{50}) calculated to be 8 PFU; virus titers reach $10^{8.0–9.0}$ PFU/mL in liver (Hooper et al. 2001).

A 50 percent lethal dose (LD_{50}) of 1.54 FFU (focus-forming units) for i.p. injections in hamsters was determined by inoculating a ten-fold serial dilution of 0.8 to 80,000 FFU (Safronetz et al. 2009).

Hantaan virus ("related" to Andes hantavirus) has a reported ID_{50} = O.5 PFU (95% C.I. 0.3-1.1) via aerosol in rats (Nuzum et al. 1988).

Morbidity/case fatality ratio

HPS in humans typically presents in a very nonspecific way with a relatively short febrile prodrome lasting 3-5 days. In addition to fever and myalgias, early symptoms include headache, chills, dizziness, non-productive cough, nausea, vomiting, and other gastrointestinal symptoms. Malaise, diarrhea, and lightheadedness are reported by approximately half of all patients, with less frequent reports of arthralgias, back pain, and abdominal pain (Centers for Disease Control and Prevention 2008).

Results from serologic studies to determine the prevalence of exposure to the virus among populations in endemic areas suggest that clinically asymptomatic infections occur (Ferres and Vial 2004; Pini et al. 2003).

Case fatality rate of 25–35 percent in Argentina and 37 percent in Chile (Centers for Disease Control and Prevention 2008).

In Chile, 477 cases reported through 4/2006 with CFR of 37 percent; numbers of cases range from 56 in 2004 to 81 cases in 2001 (Mertz et al. 2006).

Argentina, CFR = 50 percent, 55 percent of patients were male (Lopez et al. 1996).

Chile, CFR in children 43.8 percent (Toro et al. 1998; Ferres and Vial 2004).

Chile, 82 cases reported in children as of 2010, CFR 36.6% (Ferres et al.).

Death rate in northern Argentina 13.3 percent in children with *mild* form of disease (Ferres and Vial 2004).

46.7 percent lethality rate in children (Ferres and Vial 2004).

1993–1997, Andean Region, Argentina, 38 cases, 60 percent males, 50 percent fatal (Cantoni et al. 2001).

Temuco, Chile, 1997–1999, 82 percent male, 88 percent farm workers, mortality rate = 43.8 percent of 15 patients with HPS (Castillo et al. 2001).

Rapidly progressing human disease with 30–50 percent case fatality rate (Custer et al. 2003).

1995–1997, 108 cases of HPS with mortality rate of 48 percent reported in Argentina; approx 1/3 cases in and about El Bolson, a locality in the province of Rio Negro in southwestern Argentina, with remaining cases in clusters in other parts of the country, including the northern province of Salta, the islands of the Parana River and Patagonia (Ferrer et al. 1998).

Cases of HPS in Salta, Argentina increased annually since 1995 reaching 35 in 1997, 29 in 1998, 22 in 1999, 15 in 2000, with mortality rates of 23, 7, 24, and 27 percent, respectively (Gonzalez Della Valle et al. 2002).

1995–96, Argentina, 77 HPS cases with 48 percent mortality (Enria et al. 1996).
CFR = 55.6 percent in southern Argentina associated with clusters of cases (Lázaro 2007).

Northern Argentina, most patients had a mild clinical course, and the death rate (13.3 percent) was low (Pini et al. 2003).

Aerosol infection/routes of transmission

Hantaviruses are transmitted by persistently infected rodents via aerosolization of feces, saliva, and urine [and contaminated fomites] for many weeks, if not for life (Centers for Disease Control and Prevention 2008).

In rodent reservoirs, causes generalized, chronic, asymptomatic infection with virus shedding in urine, feces, saliva; transmission to humans via inhalation of aerosols of urine or feces, or from rodent bites (Ferrer et al. 1998).

Infections after rodent bites (Centers for Disease Control and Prevention 2008).
Transmission via bite of *O. longicaudatus* in mammalogist; developed HCPS 14 days later (Merino 2002).

1995–1996, Argentina, person-to-person transmission documented from HPS patient to heath care professionals 27–28 days after first contact (Enria et al. 1996).

Direct genetic evidence of person-to-person transmission (Padula et al. 1998).

1996, Andean Region, Argentina, 85 percent (16 cases) person-to-person transmission were suspected (Cantoni et al. 2001; Wells et al. 1997). However, in all those cases except one,

infection from rodents could not be ruled out; the exception was a physician who had never visited an endemic area but had treated an infected patient (Enria et al. 2001).

Viral antigen demonstrated in saliva and might be a route of transmission in humans (Gonzalez Della Valle et al. 2002).

No evidence of nosocomial transmission in southern Chile (Castillo et al. 2004).
7 of 64 (11 percent) cases in Chile occurred in family clusters, affecting principally the secula partner (Castillo et al. 2007).

Person-to-person transmission was described for an outbreak of HPS in southwest Argentina with four clusters in two endemic areas during, or shortly after, the prodromal phase, with an incubation period of 15–24 days (Martinez et al. 2005).

1993–2005, southern Argentina, retrospective review, six of eight clusters of apparent person-to-person transmission showed no evidence of exposure to rodents (Lázaro et al. 2007).

Person-to-person transmission rates of 4 percent for surviving patients and 41 percent for those who died (Lázaro et al. 2007).

It appears that close and prolonged contact is necessary for person-to-person transmission (Lázaro et al. 2007; Castillo et al. 2007).

HPS in Argentina and Chile, unique predilection for limited person-to-person transmission; prospectively followed 421 household contacts of patients with laboratory-confirmed infection to test the hypothesis that the virus retains the ability to be transmitted from person to person; sex partners of patients with laboratory-confirmed HPS at the greatest risk of infection, with an estimated secondary attack rate of 2.5 percent and detectable viremia 5–15 days before the onset of symptoms (Ferres et al. 2007; Montgomery, Ksiazek, and Khan 2007).

Occurs in household clusters and transmitted person-to-person (Ferres et al. 2007).

Sleeping in the same bed or room; exposure to saliva (deep kissing), urine, and semen; and demolition of a shed were all associated with an increased risk (Ferres et al. 2007).

Andes virus is only hantavirus with (albeit uncommonly) person-to-person transmission (Centers for Disease Control and Prevention 2008).
Evidence for person-to-person transmission, including nosocomial transmission (Lopez et al. 1996; Wells et al. 1997; Toro et al. 1998; Mertz et al. 2006)(Centers for Disease Control and Prevention 2008).

Using Puumala virus, Old World hantavirus, virus RNA could be detected in human saliva several days after onset of disease symptoms; raises the question whether interhuman transmission of hantavirus can occur through saliva; might apply to virus person-to-person transmission (Pettersson et al. 2008).

While person-to-person transmission does not appear to be a significant problem with the other viruses related to Andes virus, a unique situation has been identified for some of these viruses in which several laboratory workers and animal handlers were infected after exposure to infected laboratory rat colonies (LeDuc 1989).

Virus injected intramuscularly causes disease in Syrian hamsters closely resembling HPS in humans; lethal in hamsters when administered by routes that model most common routes of human infection, i.e., the subcutaneous, intranasal, and intragastric routes even at very low doses, i.e., highly pathogenic when introduced by the mucosal routes (LD_{50} = 100 PFU) (Hooper, Ferro, and Wahl-Jensen 2008).

Transmission of virus among *O. longicaudatus* reservoir populations occurs with face-to-face and excrement exposure, from 130 attempts with direct contact, 12.3 percent resulted in virus transmission, and with infectious animals from 93 attempts,16 animal infections (17.2 percent) occurred; transmission from *O. longicaudatus* to *A. olivaceus*, as well (Padula et al. 2004).

Bats and birds might be involved in transmission cycle of some hantaviruses (Centers for Disease Control and Prevention 2008).

Virus titers/concentrations/pathogenesis

In Vero cells, 10^6 $CCID_{50}$/mL produced in roller bottles (Johnson 2008).

Lethal dose required to kill 50 percent of Syrian hamsters (LD_{50}) calculated to be 8 PFU; virus titers reach $10^{8.0–9.0}$ PFU/mL in liver (Hooper et al. 2001).

A 50 percent lethal dose (LD_{50}) of 1.54 FFU (focus-forming units) for i.p. injections in hamsters was determined by inoculating a ten-fold serial dilution of 0.8 to 80,000 FFU (Safronetz et al. 2009).

Titers to $10^{6–7}$ PFU/mL in serum, $10^{7–8}$ PFU/mL in whole blood of infected hamsters (Wahl-Jensen et al. 2007).

Pathogen stability

The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for hanta virus (a related virus, but not Andes virus) was calculated to be 12 [HL=28 min at maximum solar radiation] (Lytle and Sagripanti 2005).

Inactivated by heat, detergents, organic solvents, and hypochlorite solutions (Pini 2004).

Indication that viruses with structural lipids survive best in aerosols at low RH (Benbough 1971).

Virus not completely inactivated by secretions in the airways or gut (e.g., saliva, mucus, and gastric juices) possibly because of inherent stability of the virions, rapid adherence and uptake of virions by cells lining the luminal space, or a combination of inherent stability and rapid uptake (Hooper, Ferro, and Wahl-Jensen 2008).

Vectors

ANDV is not known to be vector-borne.

Other epidemiological/ecological data

Spring-summer seasonality of human infections (Centers for Disease Control and Prevention 2008).

In contrast to old world hantaviruses, which cause hemorrhagic fever with renal syndrome (HFRS), new world hantaviruses cause hantavirus cardio-pulmonary [or pulmonary] syndrome; death from cardiogenic shock rather than from respiratory failure (Mertz et al. 2006).

Each hantavirus species associated with a rodent reservoir, which is persistently and asymptomatically infected (Mertz et al. 2006).

Field data suggest that transmission in host populations occurs horizontally and more frequently among male than female rodents; transmission from rodent to rodent believed to occur primarily after weaning and through physical contact, perhaps through aggressive behavior, such as fighting (Centers for Disease Control and Prevention 2008; Schonrich et al. 2008).

1995, a novel hantavirus (Andes virus) identified in samples from patients in southern Argentina (Lopez et al. 1996; Wells et al. 1997; Toro et al. 1998).

Most hemorrhagic fever viruses are zoonoses; found in both temperate and tropical habitats; generally infect both genders and all ages, although there might be an influence of occupational exposure; transmission to humans is frequently by bite of an infected tick or mosquito or via aerosol from infected rodent hosts; aerosol and nosocomial transmission are especially important with Junin [and, probably Andes] viruses; seasonality of hemorrhagic fever among humans is influenced by the dynamics of infected arthropod or vertebrate hosts; mammals, especially rodents, appear to be important natural hosts for many hemorrhagic fever viruses; transmission cycle for each hemorrhagic fever virus is distinct and dependent on the characteristics of the primary vector species and the possibility for its contact with humans (LeDuc 1989).

Most viruses in the genus are capable of causing severe human disease and death; most of the hantaviral diseases are highly seasonal, with peak incidence in the late fall and early winter for

the Asian and Scandinavian viruses, although a recently recognized member of the group, found in Greece, appears to have a different seasonality, with most human disease seen in the warmer months of the year, probably because of differences in the behavior of the natural rodent host of the virus in that area; populations at greatest risk for infection are those with significant rural exposure, such as farmers, shepherds, and persons involved with harvesting grains (LeDuc 1989).

As a group, the other VHF agents are linked to the ecology of their vectors or reservoirs, whether rodents or arthropods (Geisbert and Jahrling 2004).

Risk of HCPS was greatest among sex partners; among household contacts who developed HCPS, viremia preceded onset of symptoms, and appearance of anti-hantavirus antibodies by up to 2 weeks (Ferres et al. 2007).

First isolated from asymptomatic 10-year-old male 6 days before death from HPS; virus isolated in Vero cells and identified via rt-PCR, ELISA, and IFAT (Galeno et al. 2002).
Rio Negro Province, Argentina, human cases of HPS in subantarctic forests and virus in *O. longicaudatus* (Larrieu et al. 2008).

Northern Argentina, HPS-endemic area composes Salta and Jujuy provinces; 1997–2000, 30 HPS cases diagnosed in Jujuy province (population 512,329) (Pini et al. 2003).

As documented with other hantaviruses, population booms of *Oryzomys longicaudatus* and *A. olivaceus* associated with availability of feed/seeds; flowering and seeding of Chilean shrub provides abundant food for cyclic population expansion of AND virus reservoirs (Murúa 1996).

Therapeutics/vaccines

Ribavirin of questionable efficacy (Mertz et al. 2006).

DNA vaccine for Hantaan virus (HTNV, an AndesV relative) tested in mice alone or in various combinations; vaccines delivered by gene gun; in general, the HTNV DNA vaccine not very immunogenic in mice (Spik et al. 2006).

Constructed expression plasmid, pWRG/AND-M, containing full-length M genome segment of Andes virus tested in Rhesus monkeys via gene gun; elicits high-titer NAbs in NHPs that protects hamsters from lethal HPS, even when administered 5 days after challenge (Custer et al. 2003).

In passive transfer experiments, neutralizing antibodies produced in rabbits vaccinated by electroporation with ANDV M gene-based DNA vaccine, pWRG/AND-M protected hamsters against intranasal challenge (21 LD_{50}) (Hooper, Ferro, and Wahl-Jensen 2008).

DNA vaccine plasmid (pWRG/HA-M) that contains both the HTNV and ANDV M gene segments tested in Rhesus macaques, produced antibodies that bound the M gene products (i.e., G1 and G2 GPs), and neutralized both HTNV and ANDV; neutralizing antibody titers elicited by the dual-immunogen pWRG/HA-M, or single-immunogen plasmids expressing only the HTNV or ANDV GPs, increased rapidly to high levels after a booster vaccination administered 1–2 years after the initial vaccination series (Hooper et al. 2006).

Replication-deficient adenovirus vectors constructed to deliver either nucleocapsid protein AdN, GP AdG_N, or GP AdG_c were able both alone and in combination to prevent illness in the Syrian hamster model (Safronetz et al. 2009).

Other remarks

Hantavirus cardiopulmonary syndrome in humans, or HPS (renal variant) (McCaughey and Hart 2000) (Centers for Disease Control and Prevention 2008).

Zoonotic disease transmitted from rodents (Centers for Disease Control and Prevention 2008). Hantaviruses are not arboviruses (no arthropod vectors) (Centers for Disease Control and Prevention 2008).

Recommend laboratory work at BSL-3 (Mertz et al. 2006) except BSL-4 biocontainment is required when infecting rodent species permissive for chronic infection (CDC and NIH 2007).

Common pathogenic feature of the hemorrhagic fever viruses is ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response (Geisbert and Jahrling 2004).

Taxonomy/antigenic relationships/synonyms

Genus *Hantavirus*, family Bunyaviridae (Centers for Disease Control and Prevention 2008). More than 20 recognized sero/genotypes of hantaviruses; each type appears to be specific to a different rodent host (McCaughey and Hart 2000).

12 distinct viruses associated with hemorrhagic fever in humans are classified among four families: family Bunyaviridae, which includes Rift Valley fever, Crimean-Congo hemorrhagic fever, and the genus *Hantavirus* (Hantaan viruses) (LeDuc 1989).
Known that at least four (and possibly several more) distinct viruses make up the genus *Hantavirus* (LeDuc 1989).

Almost 30 different hantaviruses have been identified, more than half of which are known to cause disease (McElroy et al. 2002; Schonrich et al. 2008).

C.1.2.2 Ebola virus (EBOV)

Host range

a. Field

Virus first isolated from an adult female human with a severe, prostrating, febrile illness with hemorrhagic signs and rash, located in undulating tropical rain forest at 400 m altitude in Zaire (Pattyn, Bowen, and Webb 2008).

Isolated in intraperitoneally inoculated guinea pigs and in cell cultures (Pattyn, Bowen, and Webb 2008).

Hemorrhagic fever with rash, death in humans; 651 cases with 452 deaths reported in Sudan and Zaire [Democratic Republic of Congo], 1976 (Pattyn, Bowen, and Webb 2008).Cases listed above include several nosocomial hospital personnel infections in all three outbreaks and, in addition, there were about 90 cases by syringe needle transmission in Zaire; 1976 (Pattyn, Bowen, and Webb 2008).Numerous epidemics in sub-Saharan Africa (Swanepoel 1994; Warfield, Deal, and Bavari 2009).

Virus believed to be transmitted to humans via contact with infected animal host (Swanepoel et al. 1996; Pourrut et al. 2005).

Zoonotic virus with devastating effect on western lowland gorillas of Central Africa (Leroy, Rouquet, et al. 2004).

Fruit bats/Old world fruit bats/flying foxes in three genera of *Pteropus*, family *Pteropodidae* [*Hyspignathus monstrosus*, *Epomops franqueti,* and *Myonycteris torquata*] are considered the reservoir (Leroy et al. 2005; Warfield, Deal, and Bavari 2009).

No virus isolates from more than 30,000 mammals, birds, reptiles, amphibians, and arthropods captured in epidemic areas (Pourrut et al. 2005; Groseth, Feldmann, and Strong 2007; LeDuc 1989).

Virus isolated from gorilla, chimpanzee, and duiker carcasses in 2001 and 2003 (Leroy, Rouquet, et al. 2004).

Natural reservoir remains unknown, but unlikely to be NHPs, because they are especially sensitive to filovirus infection as evidenced by both experimental studies and outbreaks among gorillas and chimpanzees in Africa; surveys of wild populations as well as experimental inoculations of animals, arthropods, and even plants have failed to identify a potential reservoir (Leffel and Reed 2004) 2006, massive gorilla and chimpanzee die-offs in the Democratic Republic of Congo due to an EBOV outbreak serves as an

unfortunate testament to the deadly nature of filovirus infections in primates (Swenson et al. 2008).

Filoviruses circulate widely in the central African rain forests and can infect humans and many NHPs, including previously undocumented members, e.g., mandrills and baboons as well as chimps and gorillas, which has created substantial additional concern for these already endangered species (McCormick 2004).

In central Africa rain forests where outbreaks occurred during 2001–2005, fruit bats of the suborder *Megachiroptera* were naturally infected with EBOV without clinical signs: *Hypsignathus monstrosus*, hammer-headed fruit bat; *Epomops franqueti*, singing fruit bat; *Myonycteris torquata*, little-collared fruit bat (Gonzalez, Pourrut, and Leroy 2007).

Test of more than 1,000 small vertebrates during 2001–2003 outbreaks in humans and great apes in Gabon and DRC; evidence of asymptomatic infection in 3 species of fruit bat indicating possible reservoir for EBOV (Leroy et al. 2005).

IgG for EBOV detected in serum from *Hypsignathus monstrosus, Epomops franqueti, Myonycteris torquata*; no virus isolated, but nucleotide sequences demonstrated (Leroy et al. 2005).

EBO-Reston, Philippines, 2007–2008, first known outbreak in pigs; previously found only in sick monkeys and few contacts; no known incidents of serious illness or death in humans; concern "because this is new, because it is unexpected, because virus is slightly different from previous EBO-Reston isolates, and because it is in pigs," which live in close proximity to humans; farms quarantined and pigs being depopulated (Normile 2009).

b. Experimental

Causes sickness and death in newborn and suckling mice inoculated IC, SC, and IP (Pattyn, Bowen, and Webb 2008).

Death in guinea pigs inoculated IP, titers in liver to $10^{6.5}CCID_{50}$/mL (Pattyn, Bowen, and Webb 2008).
Fever and death in IP inoculated vervet, cynomolgous, and rhesus monkeys with virus titers to $10^{6.0}$ to $10^{7.5}CCID_{50}$/mL in blood, serum, liver, and spleen (Pattyn, Bowen, and Webb 2008).

Of 24 plant species and 19 vertebrate species experimentally inoculated, only bats became infected (Swanepoel et al. 1996).

Clinical signs absent in bats.

Several NHP species have been used to model EBOV (Zaire) HF including African green monkeys (*Chlorocebus aethiops*, formerly *Cercopithecus aethiops*), cynomolgus macaques (*M. fascicularis*), rhesus macaques (*M. mulatta*), and hamadryad baboons (*Papio hamadryas*; similar pathologic features of EBOV infection have been documented among these species; however, African green monkeys do not present with a macular cutaneous rash, which is a characteristic feature of disease in macaques and baboons and a prominent feature of human disease (Geisbert et al. 2003).

In cynomolgus monkeys inoculated i.m. with 10^3 PFU, onset of plasma viremia was rapid, within 3 days, ranged from $10^{1.4}$ to $10^{4.2}$ PFU/ml (mean, peak viremia of $10^{6.9}$ occurred at 6 dpi (Geisbert et al. 2003).

NHP models for human disease comparative; rodent (mouse, guinea pigs) models have significant gaps and used for evaluating antivirals (Paragas and Geisbert 2006).

Laboratory studies show that fruit and insectivorous bats support replication and circulation of high titers of EBOV without showing overt illness (Hensley et al. 2005).

Several primate species used to model human filoviral HF, including African green monkeys, cynomolgus macaques, rhesus macaques (*M. mulatta*), and hamadryad baboons (*Papio hamadryas*) (Hensley et al. 2005).

Hypsignathus monstrosus, hammer-headed fruit bat; *Epomops franqueti*, singing fruit bat; *Myonycteris torquata*, little-collared fruit bat confirmed as reservoirs (Gonzalez, Pourrut, and Leroy 2007).

33 varieties of 24 species of plants and 19 species of vertebrates and invertebrates were inoculated with Zaire EBOV; fruit and insectivorous bats supported replication and circulation of high titers of virus without necessarily becoming ill; deaths occurred only among bats that had not adapted to the diet fed in the laboratory (i.e., death might not have been due to EBOV infection) (Swanepoel et al. 1996).

Rhesus monkeys exposed to 1 x $10^{5.2}$, by i.m., oral, or conjunctival routes experienced lethal infection (Jaax et al. 1996).

Prevalence/incidence/attack rate

There have been more outbreaks of *Zaire ebolavirus* than any other strain (Mahanty and Bray 2004).

Using EBOV-Zaire as antigen in an ELISA test, an overall seroprevalence of 5.3 percent was found among populations in the rain forest of the Central African Republic (Gonzalez et al. 2000).

Using ELISA for Zaire subtype, 1985–2000, Cameroun, Central African Republic, Gabon—in 638 primates seroprevalence of wild-born chimpanzees (*Pan troglodytes*), 12.9 percent; antibodies in *Papio annulus* [baboon (4.0 percent)], *Gorilla gorilla* (6.75 percent), *Mandrillus* spp (2.8 percent), *Cercopithecus neglectus* (0.9 percent) (Leroy, Telfer, et al. 2004).

Dogs in Ebola outbreak areas had Ebola-specific IgG rates ranging from 8.9 percent to 25.2 percent; in areas with active cases, seroprevalence was 31.8 percent; infections subclinical (Allela et al. 2005).

Sudan, 1976, secondary attack rate of 12 percent (Feldmann, Slenczka, and Klenk 1996).
Zaire, 1976, 5 percent secondary attack rate, 20 percent in close relatives of patients (Feldmann, Slenczka, and Klenk 1996).

EBO-Reston, isolated in 1989 from cynomolgus macaques imported from the Philippines for medical research in the United States; unusual numbers of the monkeys started dying in quarantine, about 1000 monkeys died or were euthanized; subsequently, 21 animal handlers at the Philippine exporter and four employees of the quarantine facility were found to have antibodies to the virus, indicating that they had been infected, but just one reported flulike symptoms (Normile 2009).

R_0/incubation period/infectious period/infectious dose

R_0 is estimated to be 1.83 (SD 0.06) and 1.34 (SD 0.03) for the 1995 Congo and 2000 Uganda outbreaks, respectively (Chowell, Hengartner, et al. 2004). Alternate analysis gives median 1.89 (Interquartile range 1.66-2.28) based on empirical data from Zaire 1976 outbreak (Chowell, Hengartner, et al. 2004).

R_0 of 1.36 (SD 0.13) estimated from 1995 Congo outbreak (Lekone and Finkenstadt 2006).
1.50 (90% confidence interval: (0.85-2.08)) estimated from 2000 Uganda outbreak (Lloyd-Smith et al. 2005).

Secondary transmission of EBOV in humans caused by close contact with infected patients, direct contact with infected blood, tissue, or body fluids, or improper needle hygiene (Jaax et al. 1995).

Human-to-human transmission is possible for all VHF viruses; majority of the person-to-person transmission for the arenaviruses and filoviruses attributed to direct contact with infected blood

and body fluids; potential for airborne transmission of the VHF agents appears to be an infrequent event but cannot be categorically excluded as a mode of transmission (Geisbert and Jahrling 2004).

Infectious doses are unknown (Said et al. 2007; Johnson 2008).
"Incubation period for African-derived strains 3–8 days in primary cases, slightly longer in secondary cases; however cases with incubation periods of 19 and 21 days have occurred (King 2008).

High mortality rate, low infective dose indicated de facto of Category A agent (Leffel and Reed 2004).

Morbidity/case fatality ratio

Results from serologic studies to determine the prevalence of exposure to the virus among populations in endemic areas suggest that clinically asymptomatic, or minimally symptomatic, infections occur (Gonzalez et al. 2000).

Mortality of 23 to 90 percent, depending on the virus strain (Leffel and Reed 2004).
CFR = 20–90 percent (Said et al. 2007).

CFR ranges from 41 to 90 percent (Warfield et al. 2004).

Mortality ranges from 22 to 88 percent (Feldmann, Slenczka, and Klenk 1996).

Zaire and Sudan strains appear to be the most virulent, with the mortality rate approaching 90 percent for the Zaire strain and 50 to 60 percent for the Sudan strain (Leffel and Reed 2004; Groseth, Feldmann, and Strong 2007).

Reston and Ivory Coast strains virulent for NHPs, but the few reported cases in humans have not resulted in any fatalities, although the Ivory Coast strain appears to be highly pathogenic in humans (Leffel and Reed 2004).

Case fatality rate is 85–90 percent for the Zaire strains and 25 percent for Uganda strains (Warfield, Deal, and Bavari 2009; King 2008).

The strain of Ebola in Zaire has one of the highest case fatality rate of any human pathogenic virus, roughly 90 percent (Warfield, Deal, and Bavari 2009; King 2008).

Zaire ebolavirus has the highest case fatality rate, up to 90 percent in some epidemics, with an average case fatality rate of approximately 83 percent in patients over 27 years; case fatality rates were 88 percent in 1976, 100 percent in 1977, 59 percent in 1994, 81 percent in 1995, 73 percent in 1996, 80 percent in 2001–2002, and 90 percent in 2003 (King 2008).

Sudan EBOV isolated in 1976; average fatality rates were 54 percent in 1976, 68 percent in 1979, and 53 percent in 2000/2001; average case-fatality rate is 54 percent (McCormick and Fisher-Hoch 1999; King 2008).

Strain isolated later in Sudan has a case fatality rate of around 50 percent (King 2008).

Reston ebolavirus is suspected as either another subtype or a new filovirus or Asian origin discovered in crab-eating macaques in 1989; despite its status as a BSL-4 pathogen, it is non-pathogenic to humans and mildly fatal to monkeys (McCormick and Fisher-Hoch 1999; King 2008).

During the incident in which *Reston ebolavirus* was discovered, six animal handlers seroconverted [one of whom had cut himself while performing a necropsy on an infected monkey; and failed to become ill]; concluded that the virus had a very low pathogenicity for humans (McCormick and Fisher-Hoch 1999; King 2008).

Tai ebolavirus isolated in 1994, Côte d'Ivoire from chimpanzees, possibly infected from eating Western Red *Colobus* monkeys; one scientist performing necropsies was infected with febrile illness, but recovered 6 weeks after infection (McCormick and Fisher-Hoch 1999; King 2008).

Bundybugyo ebolavirus isolated in Uganda in 2007; total of 149 cases with 37 deaths and a case fatality rate of 24.83 percent(King 2008).

DRC, 11 deaths/35 infected humans in 2008; in 2007, 217 illnesses; in 1995, 245 deaths (King 2008).

Uganda, 2007, 37 dead of 149 cases (King 2008).

Emerging pathogens, Marburg and EBOV cause very severe hemorrhagic fevers and mortality as high as 90 percent (Huggins, Zhang, and Bray 1999).

High rates of morbidity and mortality (Huggins, Zhang, and Bray 1999).

Acute mortality caused by Zaire species of EBOV is approximately 80 percent in human outbreaks and > 90 percent in monkey models of the genus *Macaca* (Geisbert et al. 2003).
Sudan, 1976, 1/3 hospital staff infected with 41 dead; approx 15 generations of person-to-person transmission due to close contact; total of 284 probable/confirmed cases with 151 (53 percent) dead (Feldmann, Slenczka, and Klenk 1996).

Sudan, 1979, 33 cases with 22 dead (65 percent mortality); 7 generations of transmission, with 89 percent mortality in first 4 generations and 38 percent in last 3 generations (Feldmann, Slenczka, and Klenk 1996).

2001–2003, central African Republic, Gabon: 5 outbreaks, 313 cases, 264 dead; 78 percent patients died within 5–7 days; coincided with outbreaks in gorillas, chimpanzees, and duikers (Leroy, Telfer, et al. 2004).

1976, Ebola Zaire and Ebola Sudan outbreaks associated with high mortality, especially among healthcare providers; secondary transmission propagated by reuse of needles and syringes; outbreak in the DRC involved 318 cases with 88 percent mortality; in Sudan, mortality was 53 percent among 284 total cases (Salvaggio and Baddley 2004).

Mortality 100 percent when infection source was contaminated syringes and needles (Salvaggio and Baddley 2004).

* Outbreaks of filovirus infection in human beings or captive NHPs (Mahanty and Bray 2004).

Table C-9. EBOV infections

Date	Location	Source of infection	Number of cases	Case-fatality rate (%)
Zaire ebolavirus				
1976	Zaire (now DRC)	Unknown	318	88
1977	Zaire (now DRC)	Unknown	1	100
1994	Gabon	Unknown	49	65
1995	DRC	Unknown	317	77
1996	Gabon	Dead chimp	37	57
1996	Gabon	Unknown	60	75
2001	Gabon	Contact with NHP	123	79
2003	Republic of Congo	Contact with NHP	143	90
Sudan ebolavirus				
1976	Sudan	Unknown	284	53
1979	Sudan	Unknown	34	65
2000	Uganda	Unknown	425	53
Ivory Coast ebolavirus				
1994	Cote d'Ivoire	Dead chimp	1 0	21
Reston ebolavirus				
1989	Virginia, USA	Imported macaques	--	--

Date	Location	Source of infection	Number of cases	Case-fatality rate (%)
1990	Pennsylvania, USA Imported macaques	--		--
1992	Italy		Imported macaques	--
1996	Texas, USA	Imported macaques	--	

DRC = Democratic Republic of Congo; NHP = nonhuman primate.

* In 1976 more than 550 cases of severe hemorrhagic fever with more than 430 fatalities occurred simultaneously in [DRC] and Sudan (Beer, Kurth, and Bukreyev 1999).

Aerosol infection/routes of transmission

Not naturally transmitted by aerosol, but highly infectious as respirable particles under laboratory conditions (Leffel and Reed 2004).

"Epidemiological data from natural outbreaks would suggest … that the survivability of filoviruses as a respirable particle is very short outside of controlled laboratory conditions, or that infected patients do no expire infectious virus particles" (Leffel and Reed 2004).

Large outbreaks usually driven by person-to-person transmission, with caregivers both at home and in hospitals at particular risk (Bausch et al. 2007).

LAI can occur by needle stick (Emond et al. 1977).

Human-to-human transmission via contact with blood and body fluids and via contaminated medical equipment; might infect via skin and mucous membranes (Leffel and Reed 2004).

Transmission primarily associated with close contact with infected persons and perhaps by contaminated needles (LeDuc 1989).

Communicable primarily through direct contact with infected blood or tissues or both; some evidence of infectivity via the respiratory, oral, and conjunctival routes (Leffel and Reed 2004).

Most documented cases have been either secondary or nosocomial infections; institution of basic isolation procedures generally sufficient to stop outbreaks (Leffel and Reed 2004).

During the 2000 EBOV outbreak in Uganda, however, 14 healthcare workers were exposed after isolation procedures were instituted; while possibility of aerosol exposure cannot be ruled out in some cases, it is clear that direct contact is the primary means of transmission (Leffel and Reed 2004).

Although transmission during naturally occurring outbreaks is believed to occur from close personal contact with blood or other body fluids, or the failure to practice proper medical hygiene as relates to blood-borne pathogens, in the past 10 years several publications have indicated that filoviruses possess a number of properties that would make them suitable as biological weapons (Leffel and Reed 2004).

Airborne transmission between monkeys demonstrated by an accidental outbreak in a laboratory, but very limited evidence for human-to-human airborne transmission in any reported epidemics (Peterson, Bauer, and Mills 2004).

After the original outbreak of MARV in 1967, concern increased about transmission of filoviruses, especially aerosol transmission, even with few secondary cases; epidemiological analysis of the outbreak suggested aerosol transmission between shipments of primates had occurred (Leffel and Reed 2004).

1995, lethal experimental infection of rhesus monkeys by aerosol exposure to Ebola Zaire; exposed to doses of 400 pfu or 50,000 pfu; all monkeys died or were euthanized after becoming moribund between days 7 and 9 postexposure; within the same time frame that rhesus monkeys die from parenteral exposure to Ebola Zaire (Leffel and Reed 2004).

Filoviruses can infect via aerosol, and extraordinarily low doses are lethal for both guinea pigs and NHPs; epidemiological data from natural outbreaks suggests that the aerosol infectious dose for humans is considerably higher, that survivability of filoviruses as a respirable particle is very short outside controlled laboratory conditions or that infected patients do not expire infectious virus particles (Leffel and Reed 2004).

Reported transmission of Zaire strain to Rhesus monkey (*M. mulatta*) controls under BSL-4 biocontainment without direct contact with infected monkeys in same room; most likely route of infection by aerosol, oral, or conjunctival exposure to virus-laden droplets secreted or excreted from infected monkeys (Jaax et al. 1995). The study did not exclude the possibility that exposure had occurred from excreted virus that was aerosolized during routine cleaning of the cages rather than primate-to-primate transmission (Leffel and Reed 2004).

Might not be highly contagious early in infection and contact with a patient might not transmit the virus; as the illness progresses, bodily fluids from diarrhea, vomiting, and bleeding represent an extreme biohazard (Bausch et al. 2007; Leffel and Reed 2004).

Secondary transmission of EBOV in humans caused by close contact with infected patients, direct contact with infected blood, tissue, or body fluids, or improper needle hygiene (Jaax et al. 1995).

Secondary cases from direct physical contact with ill persons, with additional risk from exposure to body fluids (Dowell et al. 1999).

Transmitted from infected patients via blood, secretions (saliva, respiratory, urine, feces, vomitus), organs, semen; via handling and eating ill or dead animals; via contaminated syringes and needles; associated with customary burial practices (Curtis 2006).

Transmission via breaks in skin or contact with mucous membranes (Jaax et al. 1996).

EBOV transmitted via unprotected physical contact with bodily fluids from infected persons, Uganda studies with clinical specimens from 26 laboratory-confirmed cases and from environmental specimens collected from an isolation ward using culture or rtPCR; positives in 16 of 54 clinical specimens (including saliva, stool, semen, breast milk, tears, nasal blood, and a skin swab) and in 2 of 33 environmental specimens; concluded that EBOV is shed in a wide variety of bodily fluids during the acute period of illness, but risk of transmission from fomites in an isolation ward and from convalescent patients is low when recommended infection control guidelines for the viral hemorrhagic fevers are followed (Bausch et al. 2007).

Kikwit, 1995, person-to-person transmission by intimate contact, possible droplet and small aerosol transmission; reservoir unknown (Feldmann, Slenczka, and Klenk 1996).

Transmission via inhalation, inoculation, ingestion, person-to-person, direct contact, and by doing autopsies (Said et al. 2007).

Transmission via respiratory droplets less than 2 m, but aerosolized virions have greater range (Said et al. 2007).

Little data from animal studies with aerosolized filoviruses; animal models of filovirus exposure are not well characterized, and there are discrepancies between these models and what has been observed in human outbreaks (Leffel and Reed 2004).
Some recent outbreaks attributed to the consumption or handling of bush meat (Leffel and Reed 2004).

Human infections documented via handling of infected dead and living chimpanzees, gorillas, and forest antelopes (Peterson, Bauer, and Mills 2004).

No documented sexual transmission, but PCR signal documented in semen of partner of woman who seroconverted (Salvaggio and Baddley 2004).

Possible infections from breast milk (Salvaggio and Baddley 2004).

Risk of transmission to infants from breast milk and of sexual transmission from contaminated semen (Bausch et al. 2007).

Isolation of EBOV from only one saliva specimen, in contrast to the 8 that were RT-PCR positive, suggests that virus is rapidly inactivated by salivary enzymes or other factors in the oral cavity that are unfavorable to virus persistence and replication (Bausch et al. 2007).

Fruit bats eaten by local populations in outbreak areas and could be the source of human infections (Leroy et al. 2005) (Leroy et al. 2005).

While feeding, chronically, asymptomatic infected bats shed the virus in saliva; drop partially eaten fruit and masticated fruit pulp to the ground where ground-dwelling mammals [great apes and forest duikers (*Cephalophus* spp) particularly sensitive to the virus] eat the fruit/pulp and are infected; during reproductive periods, high titers of virus are found in birthing fluids, blood, and placental tissues (Gonzalez, Pourrut, and Leroy 2007).

Experimental transmission in monkeys via aerosols and direct inoculation (Jaax et al. 1996).

Rhesus monkeys infected via aerosol (droplet size 0.8–1.2 µm) with doses as low as 400 PFU caused rapidly fatal disease in 4–5 days (Johnson et al. 1995).

Virus titers/concentrations/pathogenesis

Vero cells, 2.5 x10^{7}–10^{8} $CCID_{50}$/mL; max 3 L quantities produced in roller bottles (Johnson 2008).

Vero cells, titer $10^{7.3}$ (Halfmann et al. 2008).

Vero cells, titer $10^{5.5}$ $CCID_{50}$/mL from human serum (Pattyn, Bowen, and Webb 2008).

Vero cells, titer $10^{6.4}$ PFU/mL (Pattyn, Bowen, and Webb 2008).

Virus titer tissue burdens via plaque formation in VERO cells = 2.5 x 10^6 pfu; via IFA= 3.0 x 10^7 $TCID_{50}$; Intracerebral suckling mouse inoculation = 9.0 x 10^6 $MICLD_{50}$ (Moe, Lambert, and Lupton 1981).

Rhesus monkeys: in interferon-treated; $10^{5.7}$ PFU/g lung, $10^{5.2}$ PFU/g lymph node, $10^{4.2}$ /g heart, $10^{4.1}$ /g gonad; in untreated, $10^{4.5}$ PFU/g lung, $10^{4.0}$ PFU/g lymph node, $10^{3.9}$ PFU/g heart, $10^{3.5}$ PFU/g gonad (Jaax et al. 1995).

Cynomolgus monkeys infected with EBOZ virus- viremias more than $10^{7.0}$ PFU/mL (Jahrling et al. 1996).

Viremia titers in Rhesus macaques attain $10^{7–8}$ PFU/mL (Jahrling et al. 2007; Martin et al. 2006).

Mice inoculated with lethal dose: on day 2, liver had an average virus titer of 3 x 10^5 PFU/g liver and spleen had 2 x 10^6 PFU/g; on day 3, liver had an average virus titer of 2 x 10^7 PFU/g and spleen had 2 x 10^8 PFU/g (Huggins, Zhang, and Bray 1999).

EBOV replicated in bats (*Tadarida condylura*, Angola free-tailed bat; *T pumila*, little free-tailed bat; *Epomophorus wahlbergi*, Wahlberg's epauletted fruit bat): titers of $10^{4.6}$–$10^{7.0}$ fluorescent focus-forming units (FFU)/mL recorded in sera and titers of $10^{2.0}$–$10^{6.5}$ FFU/g in pooled viscera of fruit bats; virus recovered from feces of a fruit bat on day 21 postinoculation (Swanepoel et al. 1996).

Pathogen stability

Based on experimental data, aerosol decay rates for EBOV were calculated to be 15 and 24 minute half-lives for the Zaire and Reston strains, respectively (Piercy et al. 2010).

Filoviruses are relatively stable in aerosols, retain virulence after lyophilization, and can persist for long periods on contaminated surfaces (Leffel and Reed 2004).

Predicted inactivation of a filovirus was calculated for six geographic points for as many as five times of year. The time for a 1 log decrease varied from 20 to 100 minutes[HL range of 6 to 30 min] (Lytle and Sagripanti 2005). The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for filovirus was calculated to be 7.3 [HL=17 min at maximum solar radiation] (Lytle and Sagripanti 2005).

Indication that viruses with structural lipids survive best in aerosols at low RH (Benbough 1971). UV radiation inactivates viruses by chemically changing the RNA and DNA; most effective UV is 250 nm; filoviruses [MARV and EBOV] most sensitive to solar UV_{254}, requiring 20'–100' at mid-day exposure to inactivate 1 $\log_{10}$ of virus: bunyaviruses (hantaviruses [AND virus] and RVFV), arenaviruses (Lassa, Junin), and flaviviruses (TBE complex viruses) more resistant (Lytle and Sagripanti 2005).

Filoviruses retain infectivity at room temperature on environmental surfaces; thus, fomites could be sources of transmission, e.g., blankets and sleeping mats identified (Salvaggio and Baddley 2004).

Animal carcasses (NHP) left in the forest are not infectious after 3–4 days (Leroy, Rouquet, et al. 2004).

EBOV and MARV survive for weeks; rapidly inactivated in environment, being sensitive to heat, sunlight, or drying [Note: Some data reported in this section by others might challenge that position]; susceptible to chlorine disinfectants, heating to 60 °C for 1 hour, UV light; survives in carcasses/body fluids and on fomites (Said et al. 2007).

Sensitive to lipid solvent (50 percent ether) at 4 °C, 1 hour, titer $10^{6.3}CCID_{50}$/mL reduced to less than $10^{1.7}CCID_{50}$/mL (Pattyn, Bowen, and Webb 2008).

Negative-stranded RNA virus with lipid envelope; stable at a neutral pH, as a result of which the virus can survive for long periods in blood, and viral isolation is possible weeks after exposure, even during convalescence (Leffel and Reed 2004).

Vectors

No known vectors.

Maintenance and transmission unknown; recent evidence suggests that fruit bats might have a reservoir role; it is unclear as to whether other species are involved or how transmission to humans or apes occurs (Groseth, Feldmann, and Strong 2007).

EBO Reston virus does not replicate in *Culex* or *Aedes* mosquitoes or *Ornithodoros* ticks (Monath 1999).

Other epidemiological/ecological data

Most hemorrhagic fever viruses are zoonoses; found in both temperate and tropical habitats; generally infect both sexes and all ages, although there might be an influence of occupational exposure; transmission to humans is frequently by bite of an infected tick or mosquito or via aerosol from infected rodent hosts; aerosol and nosocomial transmission are especially important with EBOV; seasonality of hemorrhagic fever among humans is influenced by the dynamics of infected arthropod or vertebrate hosts; mammals, especially rodents, appear to be important natural hosts for many hemorrhagic fever viruses; transmission cycle for each hemorrhagic fever virus is distinct and dependent on the characteristics of the primary vector species and the possibility for its contact with humans (LeDuc 1989).

EBOV first recognized during an outbreak of human disease, 1976; two major epidemics of what in retrospect appears to have been sustained nosocomial transmission started within a few weeks of one another, one in DRC and the other a few hundred miles away in southern Sudan; subsequent to these outbreaks, isolated cases have occurred, and serologic surveys have found an antibody to EBOV in other African countries, but, as yet, little is known as to how the viruses are maintained in nature (LeDuc 1989; Feldmann, Slenczka, and Klenk 1996).

Near-simultaneous outbreaks of the Ebola Zaire and Sudan occurred in 1976; Reston and Ivory Coast are virulent for NHPs, but the few reported cases in humans have not resulted in any fatalities (Feldmann, Slenczka, and Klenk 1996; Leffel and Reed 2004).

Although a lot of epidemiological evidence of human transmission of disease is not available, what is known suggests that transmission of EBOV does not occur before the appearance of symptoms; experiments in NHPs support this assumption (Leffel and Reed 2004).

Reemergence in Kikwit, 1995 (Feldmann, Slenczka, and Klenk 1996).

For surveillance, failed to detect antibodies against EBOV in oral fluid specimens obtained from patients with seropositive serum samples; patients with positive serum RT-PCR results had positive results for their oral fluid specimens; oral fluid samples useful for investigation of Ebola outbreaks (Formenty et al. 2006).

Ecological niche modeling of potential habitats (Peterson et al. 2006; Peterson, Bauer, and Mills 2004).

Persistence in body fluids from 12 convalescent patients studied via virus isolation and RT-PCR during 1995 Ebola hemorrhagic fever outbreak in Kikwit, DRC; RNA detected for up to 33 days in vaginal, rectal, and conjunctival swabs of one patient and up to 101 days in the seminal fluid of four patients; infectious virus detected in one seminal fluid sample obtained 82 days after disease onset; patient samples selected to include some from a suspected line of transmission with at least three human-to-human passages, some from 5 survivors and 4 deceased patients, and 2 from patients who provided multiple samples through convalescence(Rodriguez et al. 1999).

Geographic range of fruit bats (*Epomops franqueti, Hypsignathus monstrosus, Myonycteris torquata*) overlaps the distribution of cases (Warfield, Deal, and Bavari 2009).

Late 1980s, an outbreak of EBOV occurred in an NHP-holding facility in Reston, Virginia; appeared to jump from animal to animal and room to room in a manner that suggested aerosol transmission; animal handlers in the facility seroconverted, indicating they had been infected; identified as new subtype originating in the Philippines; suggestion that primates were infected with Ebola Reston by aerosol exposure; no evidence to indicate that primate-to-primate

transmission by aerosol actually occurred; in an outbreak of Ebola Reston in the Philippines it was concluded that transmission between cages and buildings was due to poor sanitation and hygiene (Warfield, Deal, and Bavari 2009; Leffel and Reed 2004).

Therapeutics/vaccines

No approved vaccine or treatment is available (Feldmann, Slenczka, and Klenk 1996; Groseth, Feldmann, and Strong 2007; Warfield, Deal, and Bavari 2009).

No vaccine or therapy for EBOV or MBGV hemorrhagic fever is approved for human use (Geisbert et al. 2003).

Experimental vaccines have been produced for both Ebola and Marburg with 99 percent efficacy to protect monkeys from the disease; vaccine is based on either a recombinant vesicular stomatitis virus or adenovirus carrying the Ebola spike protein on its surface; vaccine trial demonstrated an immune response in humans, but the vaccine must be given within 1–4 days after the symptoms begin (Paragas and Geisbert 2006; Geisbert et al. 2008).

No standard treatment, but primarily supportive, including minimizing invasive procedures, balancing electrolytes, replacing coagulation factors lost due to dehydration to help stop bleeding, maintaining oxygen and blood levels, and treating any complicating infections; convalescent plasma shows promise, Ribavirin and interferon are ineffective, and administration of an inhibitor of coagulation (rNAPc2) has shown some benefit in monkeys [protecting 33 percent of infected monkeys from a usually 100 percent lethal infection- does not work on humans] (Swenson et al. 2008).

LAI (needle stick) treated with interferon and convalescent serum resulted in complete recovery (Emond et al. 1977).

No treatments for Marburg and EBO viral hemorrhagic fevers; ribavirin, an antiviral drug used to treat several other hemorrhagic fevers, has no *in vitro* effect on Marburg and EBOV, failed to

protect in multiple primate studies, and is unlikely to have any clinical value to human patients (Huggins, Zhang, and Bray 1999).

Human convalescent plasma containing antibodies has been used for treatment in the past, despite the lack of coherent clinical or experimental data regarding its use. Equine IgG with high titer neutralizing antibodies to EBOV protected guinea pigs and baboons but failed to protect rhesus monkeys (Huggins, Zhang, and Bray 1999).

In 2006, USAMRIID scientists announced a 75 percent recovery rate after infecting four Rhesus monkeys and administering antisense drugs (Martin et al. 2006).

α-interferon ineffective to treat Rhesus monkeys infected with Zaire strain (Jaax et al. 1995).

S-Adenosylhomocysteine hydrolase inhibitors show promise as antivirals; significant protection (90 percent) when treatment began on day 2, at which time, the liver had an average virus titer of 3×10^5 pfu/g and the spleen had 2×10^6 pfu/g; treatment on day 3, when the liver had an average virus titer of 2×10^7 pfu/g and the spleen had 2×10^8 pfu/g, resulted in 40 percent survival (Huggins, Zhang, and Bray 1999).

Blended vesicular stomatitis virus vector expressing glycoprotein from multiple EBOV strains and 1 MARV strain protected *Macaca fascicularis* primates from lethal challenges of MARV and EBOV virus strains (Geisbert et al. 2009).

A multivalent adenovirus-based vector vaccine candidate (EBO7) expressing glycoproteins of *Zaire ebolavirus* and *Sudan ebolavirus* protected nonhuman primates against the parenteral and aerosol routes of lethal challenge (Pratt et al.).

Tested panfilovirus vaccine based on a complex adenovirus (CAdVax) technology that expresses multiple antigens from five different filoviruses *de novo*; vaccination of NHPs [IM with 10^{10} PFU, challenged with 10^3 PFU] demonstrated 100 percent protection against infection by two

species of EBOV and three MARV subtypes, each administered at 1,000 times the lethal dose (Swenson et al. 2008).

Candidate vaccine based on recombinant replication-defective adenovirus protects NHP from EBOV infection (Paragas and Geisbert 2006).

Candidate vaccine based on recombinant replication-competent vesicular stomatitis virus protected monkeys against EBOV (Paragas and Geisbert 2006).

Immunization with DNA or replication-defective adenoviral vectors (rAd) encoding the Ebola glycoprotein (GP) and nucleoprotein (NP) shown to confer specific protective immunity in NHPs, cynomolgus macaques (*M. fascicularis*); might work as human vaccine (Sullivan et al. 2006).

Vaccine candidate using human parainfluenza virus type 3 (HPIV3) as vector with HPIV3 recombinants expressing the EBOV (*Zaire* species) surface GP alone or in combination with the nucleocapsid protein NP or with the cytokine adjuvant granulocyte-macrophage colony stimulating factor; administered by the respiratory route to rhesus monkeys; single immunization with any construct expressing GP moderately immunogenic against EBOV and protected 88 percent of the monkeys against severe hemorrhagic fever and death caused by EBOV; two doses highly immunogenic, and all the animals survived the virus challenge without clinical signs or viremia (Bukreyev et al. 2007).

Single injection of attenuated recombinant vesicular stomatitis virus vector expressing the EBOV GP completely protected rodents and NHPs from lethal EBOV challenge; in guinea pig and mouse models possible to protect 50 percent and 100 percent of the animals, respectively, following treatment as late as 24 h after lethal challenge; in rhesus macaques protection if treated 20'–30'after an otherwise uniformly lethal infection (Feldmann et al. 2007).

Recombinant VSV-based *Zaire ebolavirus* (ZEBOV) and *Marburg virus* (MARV) vaccines used against aerosol challenge in cynomolgus macaques; all monkeys vaccinated with a VSV vector

expressing the GP of ZEBOV protected against an aerosol exposure of ZEBOV; all monkeys vaccinated with a VSV vector expressing GP of MARV protected against an aerosol exposure of MARV (Geisbert et al. 2008).

Cynomolgus monkeys immunized with hyperimmune IgG horse serum somewhat protected from EBOV challenge, viremias reduced, clinical signs delayed, but death still occurred (Jahrling et al. 1996).

Expression of viral structural proteins in cells leads to assembly and release of particles that resemble virions in size and morphology: virus-like particle vaccines highly effective to protect small lab animals and NHP against lethal doses of virus (Yang et al. 2008).

Passive immunotherapy of monkeys with convalescent-phase blood from immune monkeys not protective (Jahrling et al. 2007).

Candidate EBOV DNA vaccine; plasmids expressing EBOV GP (Zaire), GP (Sudan/Gulu), and NP (Zaire) are safe and well-tolerated and induce EBOV-specific antibody and T-cell responses in healthy adults (Martin et al. 2006).

Neutralizing human monoclonal antibody, KZ52, fails to protect against EBOV in rhesus macaques (Oswald et al. 2007).

Despite restriction of replication of human paramyxovirus-vectored vaccine by preexisting HPIV3-specific immunity, the expressed EBOV GP was highly immunogenic in guinea pigs (Yang et al. 2008).

DNA vaccines offered protective immunity via gene gun to NHP and laboratory animals (Riemenschneider et al. 2003).

Other remarks

SALS BSL-4 virus (Pattyn, Bowen, and Webb 2008).

Department of Commerce permit required (Pattyn, Bowen, and Webb 2008).

Zaire is now known as Democratic Republic of Congo.

Considerable media attention and fear generated by outbreaks of filoviruses because they can cause a severe VHF syndrome that has a rapid onset and high mortality (Leffel and Reed 2004).

Great bioterrorism concern because of their high mortality rate; low infective dose; ease of dissemination; potential for major public health impact, public panic, or social disruption; and requirement for major public health preparedness measures , i.e., a Category A pathogen (Leffel and Reed 2004).

Progress in understanding origins of pathophysiologic changes that make EBOV infections of humans so devastating have been slow; a primary reason is the status of filoviruses as BSL-4 pathogens necessitating study in high biocontainment settings (Geisbert et al. 2003).

Common pathogenic feature of the hemorrhagic fever viruses is the ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response (Geisbert and Jahrling 2004).

Natural reservoir for *Filoviridae* remains unknown (Geisbert and Jahrling 2004).

Taxonomy/antigenic relationships/synonyms

EBOV and MARV are the sole members of the genus *Filovirus* in the family *Filoviridae* (Leffel and Reed 2004; Feldmann, Slenczka, and Klenk 1996).

Family *Flaviviridae* is divided into two genera: *Ebolavirus* and *Marburgvirus*; genus *Ebolavirus* further divided into four species, *Zaire ebolavirus* (ZEBOV), *Ivory Coast ebolavirus*, *Sudan ebolavirus* (SEBOV), and *Reston ebolavirus*); 2007 Uganda outbreak virus might be a 5th species; *Marburgvirus* genus, is represented by a single species (*Lake Victoria marburgvirus*) (Swenson et al. 2008).

Order Mononegavirales, family Filoviridae, divided into two genera: *Marburgvirus* and *Ebolavirus*. *Lake Victoria marburgvirus* is the lone species in the genus *Marburgvirus* while the genus *Ebolavirus* contains four distinct species: *Ivory Coast ebolavirus* (ICEBOV), *Sudan ebolavirus* (SEBOV), *Zaire ebolavirus* (ZEBOV) and *Reston ebolavirus* (REBOV) (Hensley et al. 2005).

MARV group [weak cross reaction via indirect immunofluorescence] (Pattyn, Bowen, and Webb 2008).

12 distinct viruses associated with hemorrhagic fever in humans are classified among four families: *Filoviridae*, which includes Marburg and EBOV (LeDuc 1989).

4 known subtypes of EBOV: Zaire, Sudan, Reston, Ivory Coast (Leffel and Reed 2004).

4 subtypes of ebolavirus (McCormick and Fisher-Hoch 1999; King 2008).

C.1.2.3 Marburg virus (MARV)

Host range

a. Field

First isolated from human blood in 1967 via IP inoculation of guinea pigs with blood, salivary gland, nasopharyngeal swabs, liver, spleen, and CNS tissue in newborn mice, suckling hamsters, and WI-26 human diploid embryo fibroblasts (Siegert and Simpson 2008; Warfield, Deal, and Bavari 2009).

First isolated and Marburg hemorrhagic fever first described in 1967 during outbreaks in Germany and Yugoslavia; outbreaks linked to infected monkeys imported from Uganda (Warfield, Deal, and Bavari 2009; Bausch et al. 2006).

First filovirus described, identified after cluster of hemorrhagic fever cases occurred in laboratory workers, Marburg, Germany, 1967; all infected workers handled blood and tissue or cell cultures from African green monkeys originating from Uganda; 32 cases, 26 primary and 6

secondary (contacts of primary patients) documented, with overall mortality 23 percent (Salvaggio and Baddley 2004).

Disease, isolates from humans in West Germany, Yugoslavia laboratories, Southern Africa (Siegert and Simpson 2008).

No specific CF antibodies or virus isolations from *Cercopithecus aethiops* monkeys ; positive CF tests reported with sera from primate species is possible evidence of natural infection of primates in Uganda, but specificity of CF antibodies employing infected guinea pig crude liver antigens still questionable (Siegert and Simpson 2008).

No firm evidence of infection of monkeys in Uganda (Swanepoel 1994).

Two vervet monkeys and three baboons IFA positive in Kenya (Siegert and Simpson 2008).

Studies for reservoir hosts, examined the fauna of a mine in northeastern DRC associated with protracted outbreak of hemorrhagic fever, 1998–2000; MARV nucleic acid detected in 12 bats, composing 3.0 percent–3.6 percent of two species of insectivorous bat, *Miniopterus inflatus* (1/33, 3.0 percent), *Rhinolophus eloquens* (7/197, 3.6 percent) and one species of fruit bat, *R. aegyptiacus* (4/127, 3.1 percent); antibody to the virus in the serum of 9.7 percent of one of the insectivorous species (*Rh eloquens*)and in 20.5 percent of the fruit bat species, but attempts to isolate virus were unsuccessful (Swanepoel et al. 2007).

Fruit bat (*R. aegyptiacus*), Gabon, virus detected via virus-specific RNA; IgG antibody detected in individual bats, captured in area outside of known range of MAR virus indicating potential for expanding infected areas (Towner et al. 2007).

MARV RNA detected is tissue of *Rousettus aegyptiacus* bat in Kenya (Kuzmin et al.). Investigation of July and September, 2007, infections among miners in Kitaka Cave, Uganda, found the likely source of infection to be Egyptian fruit bats (Rousettus aegyptiacus) based on

detection of Marburg virus RNA in 31/611 (5.1%) bats, and virus-specific antibody in bat sera (Towner et al. 2009).

Experimental

Several primate species used to model human filoviral hemorrhagic fevers, including African green monkeys, cynomolgus macaques, rhesus macaques (*M. mulatta*), and hamadryad baboons (*Papio hamadryas*) (Hensley et al. 2005).

One study failed to demonstrate transmission of MARV from infected rhesus macaques to uninfected macaques (Leffel and Reed 2004).

Aerosol exposure of African green monkeys with lyophilized virus; 60 percent monkeys died within 13–22 days; lyophilization reduced virulence by nearly 10^3 $\log_{10;}$ time to death appeared extended as compared to parenteral exposure of cynomolgus macaques to 1,000 pfu of MARV; however, the doses mentioned were extremely low (0.1 to 0.003 guinea pig LD_{50} (Leffel and Reed 2004).

NHP models for human disease comparative; rodent (mouse, guinea pigs) models have significant gaps; used for evaluating antivirals (Paragas and Geisbert 2006).

Inapparent infections in IC, IP, SC inoculated newborn and weanling mice (Siegert and Simpson 2008).

Only the guinea pig has been successfully adapted as a rodent model of the human disease; time course and pathogenesis via parenteral exposure are similar to reports for NHPs and humans (Leffel and Reed 2004).

From original human tissue, febrile illness in IC, IP, SC inoculated guinea pigs (Siegert and Simpson 2008).

First guinea pig passage material produced sickness and death in IC, IP, SC inoculated guinea pigs (Siegert and Simpson 2008).

Original and first guinea pig-passaged materials produced sickness and death in IC, IP, SC inoculated vervet, squirrel, and rhesus monkeys (Siegert and Simpson 2008).

Death in newborn hamsters from IP, IC inoculated 9th gp and 9th hamster passage material (Siegert and Simpson 2008).

Laboratory studies show that fruit and insectivorous bats support replication and circulation of high titers of EBOV without showing overt illness (Hensley et al. 2005).

Prevalence/incidence/attack rate

1 percent of more than 400 human serums IFA positive in Liberia (Siegert and Simpson 2008). Serosurveys in general population of eastern and southern Africa showed prevalence rates of <2 percent (Bausch et al. 2003).

Using MARV (Musoke strain) as antigen in an ELISA test, an overall seroprevalence of 2.4 percent was found among populations in the rain forest of the Central African Republic (Gonzalez et al. 2000).

Serologic studies using ELISA for risk factors in area with confirmed MAR virus transmission in DRC; 2 percent of 912 in general village cross-sectional survey positive for IgG, 87 percent of 15 seropositives were males who worked in local gold mines; work in mines and receiving injections associated with seropositivity; all of 103 health care workers were seronegative in study; primary transmission from unknown reservoir in mines, secondary transmission less common than with EBOV (Bausch et al. 2003).

31, 3, 4 isolates from humans in W Germany, South Africa, Yugoslavia, respectively (Siegert and Simpson 2008).

Virus prevalence in presumed reservoir, fruit bat, might be as low as 1 percent (Fisher-Hoch 2005).

First documented in 1967, when 31 people became ill in the Germany and Yugoslavia; 25 primary infections, with 7 deaths in laboratory staff working with *Cercopithecus aethiops* from Uganda, and 6 secondary cases in medical staff, with no deaths from blood exposure to primary cases or needle sticks (CDC 2009).

R_0/incubation period/infectious period/infectious dose

"Epidemiological data from natural outbreaks would suggest … that the survivability of filoviruses as a respirable particle is very short outside of controlled laboratory conditions, or that infected patients do no expire infectious virus particles" (Leffel and Reed 2004).

Morbidity/case fatality ratio

Results from serologic studies to determine the prevalence of exposure to the virus among populations in endemic areas suggest that clinically asymptomatic, or minimally symptomatic, infections occur (Siegert and Simpson 2008; Bausch et al. 2003; Gonzalez et al. 2000).

2004–2005, Angola, more than 328 deaths in children; 80 percent of the deaths in the early stages of the outbreak were children under the age of 15, but that dropped to 30–40 percent in later stages, 14 nurses and 2 doctors infected (Fisher-Hoch 2005; CDC 2009).

Recent outbreaks of MARV in DRC and Angola, where mortality of over 80 percent has been reported, comparable with that in outbreaks of Zaire EBOV in Yambuku (88 percent) and Kikwit (81 percent), higher than that reported in outbreaks of Zaire EBOV (59–78 percent) in Gabon and DRC, and more than 3 times that (23 percent) in the European MARV outbreak in Europe (Ascenzi et al. 2008).

Mortality rate in Angola very high, 88 percent (329 of 374), compared with only 23 percent in the original 1967 outbreak in Germany; in the outbreak in Durba, DRC, in 1998–2000, mortality was also high (83 percent) (Fisher-Hoch 2005).

2007–2008, Uganda, 2/3 cases dead (Warfield, Deal, and Bavari 2009; CDC 2009).

Seven human deaths from 31 cases (Siegert and Simpson 2008).

Case fatality rate is from 23 percent to over 90 percent (Leffel and Reed 2004; Siegert and Simpson 2008).

Mortality of 23 to 90 percent, depending on the virus (Leffel and Reed 2004).

First outbreak, 1967, 32 cases with 23 percent mortality rate (Leffel and Reed 2004).

1998–2000, Durba, DRC, 103 cases and a fatality rate of 67 percent (Leffel and Reed 2004).

In 1975, three people in South Africa infected from a human from Zimbabwe, resulting in one death (Warfield, Deal, and Bavari 2009; CDC 2009).

1980, 1987, 2 similar cases with 2 deaths in Kenyan European visitors to a cave (Warfield, Deal, and Bavari 2009; CDC 2009).

Table C-10. Outbreaks of filovirus infection in human beings or captive NHPs

Date	Location	Source of infection	Number of cases	Case-fatality rate (%)
Marburgvirus				
1967	Europe	Imported monkeys	31	23
1975	South Africa	Unknown	3	33
1980	Kenya	Unknown	2	50
1987	Kenya	Unknown	1	100
1998	DRC	Unknown	141	82

Source: (Mahanty and Bray 2004)

DRC = Democratic Republic of Congo; NHP = non-human primate.

Emerging pathogens, MARV and EBOV cause very severe hemorrhagic fevers and mortality as high as 90 percent (Swenson et al. 2008).

High rates of morbidity and mortality (Swenson et al. 2008).

1998–1999, DRC, total of 154 cases (48 laboratory-confirmed and 106 suspected) identified (case fatality rate, 83 percent); 52 percent of cases in young male miners; only 27 percent men reported contact with other affected persons, whereas 67 percent of patients who were not miners reported such contact ($P < 0.001$); most affected miners (94 percent) worked in an underground mine (Bausch et al. 2003; Bausch et al. 2006) (Fisher-Hoch 2005; Warfield, Deal, and Bavari 2009; CDC 2009).

Mortality ranges from 22 to 88 percent (Feldmann, Slenczka, and Klenk 1996).

Mortality 30–35 percent (Kiley 1988).

Aerosol infection/routes of transmission

Seven lab deaths in Europe (Pike 1979).

Spread through bodily fluids, including blood, excrement, saliva, and vomit (CDC 2005).

Nosocomial transmission occurs, especially after close or intimate contact (LeDuc 1989).

Two major MAR outbreaks, in eastern DRC and in 2004–2005) in Angola; most, but not all, were single-point source epidemics, with index cases often unidentified and with subsequent spread in hospitals and in rural villages because of inadequate facilities and poor practices in caring for the sick (Fisher-Hoch 2005).

Although transmission during naturally occurring outbreaks is believed to occur from close personal contact with blood or other body fluids, or the failure to practice proper medical hygiene as relates to blood-borne pathogens, in the past 10 years several publications have indicated that filoviruses possess a number of properties that would make them suitable as biological weapons (Leffel and Reed 2004).

1967 outbreak, majority of cases were primary infections as a result of handling tissues from infected African green monkeys; 9 cases considered secondary cases, attributed to inadvertent needle sticks and unprotected contact ; in one case MARV was transmitted via semen 3 months after the patient had recovered from the disease (Leffel and Reed 2004; Martini and Schmidt 1968).

Communicable primarily through direct contact with infected blood and/or tissues; some evidence of infectivity via the respiratory, oral, and conjunctival routes (Leffel and Reed 2004).

Suggestion that human-to-human transmission needs relatively close contact although aerosol transmission might be increased in cases of hemorrhagic syndrome with high level viremia (Belanov et al. 1996).

Sexual transmission documented (Salvaggio and Baddley 2004).

Possible infection from breast milk (Salvaggio and Baddley 2004).

Not naturally transmitted by aerosol, but highly infectious as respirable particles under laboratory conditions (Leffel and Reed 2004).

1998-99, Democratic Republic of Congo, multiple short, apparently independent chains of transmission noted, with 7 being the largest number of cases noted in any single chain; incidences of secondary spread from at least 20 patients, tertiary spread from at least 3 patients, and quaternary spread from at least 2 patients documented, most often related to exposure of family members caring for a sick miner; nosocomial infection did not play an important role in virus transmission (Bausch et al. 2006).

Human-to-human transmission possible for all VHF viruses; majority of the person-to-person transmission for the arenaviruses and filoviruses attributed to direct contact with infected blood and body fluids; potential for airborne transmission of the VHF agents appears to be an infrequent event, but cannot be categorically excluded as a mode of transmission (Geisbert and Jahrling 2004).

Virus has been isolated from semen (Ascenzi et al. 2008).

Transmitted from infected patients via blood, secretions(saliva, respiratory, urine, feces, vomitus), organs, semen; via handling and eating ill or dead animals; via contaminated syringes and needles; associated with customary burial practices (Curtis 2006).

Transmission via inhalation, inoculation, ingestion, person-to-person, direct contact, and by doing autopsies (Said et al. 2007).

Some recent outbreaks attributed to the consumption or handling of bush meat (Huggins, Zhang, and Bray 1999).

Little data from animal studies with aerosolized filoviruses; animal models of filovirus exposure are not well characterized, and there are discrepancies between these models and what has been observed in human outbreaks (Leffel and Reed 2004).

1995, guinea pigs exposed via aerosol; homogenates of guinea pig liver containing 3 x 10^7 LD_{50} were aerosolized with 10 percent glycerol in a biological aerosol generator; dose was reported in the range of 2 to 6 aerosol LD_{50} (Leffel and Reed 2004).

Filoviruses can infect via aerosol and extraordinarily low doses are lethal for both guinea pigs and NHPs (0.1 to 0.003 guinea pig LD_{50}); epidemiological data from natural outbreaks suggests that the aerosol infectious dose for humans is considerably higher, that survivability of filoviruses as a respirable particle is very short outside of controlled laboratory conditions, or that infected patients do not expire infectious virus particles (Leffel and Reed 2004).

In guinea pigs and Rhesus monkeys (*M. mulatta*), contact and aerosol infection demonstrated (Pokhodiaev, Gonchar, and Pshenichnov 1991).

Aerosolized infectious doses ranging from 0.1 - 5 conditional units were uniformly fatal for 17 green monkeys (a conditional unit was defined as the amount of virus equivalent to guinea pig intraperitoneal LD50). Lower concentrations of virus (0.003-0.1 CU per animal delivered by aerosol) caused lethal infection of 6 of 10 animals (Bazhutin et al. 1992).

Transmission via respiratory droplets is less than 2 meters, but aerosolized virions have a greater range (Said et al. 2007).

Virus titers/concentrations/pathogenesis

In Vero cells, 2.5 x 10^6- 5 x 10^7 $CCID_{50}$/mL produced in flasks and roller bottles (Johnson 2008). Infects and replicates in a variety of mammalian cell cultures, including primary and continuous cell cultures, primary and continuous primate cell cultures, primary and continuous guinea pig cell cultures and BHK-21 cell cultures with variable or absent CPE; one primary and one continuous primate cell culture, a continuous human cell culture, and BHK-21 exhibited distinct

CPE after infection; variable CPE was produced in Vero cell cultures, and CPE endpoints generally were 10-100 times lower than infectious virus titrations (Siegert and Simpson 2008).

High mortality rate; low infective dose (0.1 to 0.003 guinea pig LD_{50} in African green monkeys) (Leffel and Reed 2004).

Rhesus monkey (*M. mulatta*) studies, virus titers to $10^{8.3}$ LD_{50}/mL or /g tissue at 9-10 post infection (Lub et al. 1995).

Pathogen stability

Based on experimental data, the aerosol decay rate for MARV was calculated to be 14 minutes (half-life) (Piercy et al. 2010).

Filoviruses are relatively stable in aerosols, retain virulence after lyophilization, and can persist for long periods on contaminated surfaces (Leffel and Reed 2004).

Survives up to 5 days on contaminated surfaces; unstable in aerosols with specific rate of inactivation= 0.05/min (Belanov et al. 1996).

Filoviruses retain infectivity at room temperature on environmental surfaces; thus, fomites might be sources of transmission, e.g., blankets and sleeping mats identified (Salvaggio and Baddley 2004).

Predicted inactivation of a filovirus was calculated for six geographic points for as many as five times of year. The time for a 1 log decrease varied from 20 to 100 minutes [HL range of 6 to 30 min] (Lytle and Sagripanti 2005). The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for filovirus was calculated to be 7.3 [HL=17 min at maximum solar radiation] (Lytle and Sagripanti 2005).

EBO and MAR viruses survive for weeks; rapidly inactivated in environment, being sensitive to heat, sunlight, or drying [NOTE-- some data reported in this section by others might challenge

that position]; susceptible to chlorine disinfectants, heating to 60 °C for 1 hour, UV light; survives in carcasses/body fluids and on fomites (Said et al. 2007).

Negative-stranded RNA virus with lipid envelope; stable at a neutral pH, as a result of which the virus can survive for long periods in blood, and viral isolation is possible weeks after exposure, even during convalescence (Leffel and Reed 2004).

Indication that viruses with structural lipids survive best in aerosols at low RH (Benbough 1971).

UV radiation inactivates viruses by chemically changing the RNA and DNA; most effective UV is 250 nm; filoviruses [MAR and EBOV] most sensitive to solar UV_{254}, requiring 20'-100' at mid-day exposure to inactivate 1 $\log_{10}$ of virus: bunyaviruses (hantaviruses [AND virus] and RVFV), arenaviruses (Lassa, JUN), and flaviviruses (TBE complex viruses) more resistant (Lytle and Sagripanti 2005).

Lipid solvent (ether, chloroform) & deoxycholate (detergent) sensitive; titer reduced from $10^{6.0}CCID_{50}$/mL to $<10^{1.7}CCID_{50}$/mL (Siegert and Simpson 2008).

Vectors

a. Field

1978, Rhodesia (Zimbabwe), possible infection via spider or horsefly bite (Conrad et al. 1978; Feldmann, Slenczka, and Klenk 1996).

b. Experimental

Replicates in *Aedes aegypti* but not in *Anopheles maculipennis* mosquitoes or *Ixodes ricinus* ticks after intrathoracic inoculation (Siegert and Simpson 2008).

Virus persists in *Aedes* mosquitoes for 3+ weeks (Monath 1999).

Other epidemiological/ecological data

22/22 infected humans were CF antibody positive in Germany (Siegert and Simpson 2008; CDC 2009).

Epidemiology of this virus sketchy, with the exception of two major outbreaks that led to discovery; MARV first recognized during an outbreak of a severe hemorrhagic disease associated with importation of African green monkeys (*Cercopithicus aethiops*) from East Africa to Germany; subsequent, isolated human cases have been reported, primarily from sub-Saharan Africa (LeDuc 1989).

Natural reservoir for *Filoviridae* remains unknown (Geisbert and Jahrling 2004).

Ecological niche modeling of potential MAR virus habitats (Towner et al. 2007).
Sporadic cases occurred from 1975 to 1987, with total of 6 cases and 3 deaths; from 1998 to 2000, series of cases occurred near Durba in the Democratic Republic of Congo; all the cases have been associated with miners working in gold mines, with 103 cases and a fatality rate of 67 percent (Leffel and Reed 2004).

1998–1999, Democratic Republic of Congo, evidence of multiple introductions of infection into the population substantiated by detection of at least nine genetically distinct lineages of virus in circulation during the outbreak; implication that reservoir hosts of MARV inhabit caves, mines, or similar habitats (Bausch et al. 2006).

1975, Rhodesia (Zimbabwe), index case and 2 secondary cases, probably via fomites, aerosol, or close contact; possible insect/arachnid transmission (Conrad et al. 1978; Feldmann, Slenczka, and Klenk 1996).

Northeastern Democratic Republic of Congo, October 1998, in gold-mining village; sporadic cases and short chains of human-to-human transmission continued until September 2000; suspected cases identified on the basis of a case definition and confirmed by the detection of virus antigen and nucleic acid in blood, cell culture, antibody responses, and

immunohistochemical analysis; outbreak ceased with flooding of the mine (CDC 2009; Bausch et al. 2006).

Most hemorrhagic fever viruses are zoonoses; found in both temperate and tropical habitats; generally infect both genders and all ages, although there might be an influence of occupational exposure; transmission to humans is frequently by bite of an infected tick or mosquito or via aerosol from infected rodent hosts; aerosol and nosocomial transmission are especially important with MARV; seasonality of hemorrhagic fever among humans is influenced by the dynamics of infected arthropod or vertebrate hosts; mammals, especially rodents, appear to be important natural hosts for many hemorrhagic fever viruses; transmission cycle for each hemorrhagic fever virus is distinct and dependent on the characteristics of the primary vector species and the possibility for its contact with humans (LeDuc 1989).

First filovirus identified was MARV, 1967, after a severe outbreak of VHF that began in Marburg, Germany, with subsequent cases appearing in Frankfurt and Belgrade; 32 cases with 23 percent mortality rate; majority of cases were primary infections as a result of handling tissues from infected African green monkeys, *Cercopithecus aethiops*; 9 cases considered secondary cases, attributed to inadvertent needle sticks and unprotected contact ; in one case MARV was transmitted via semen 3 months after the patient had recovered from the disease (Feldmann, Slenczka, and Klenk 1996; Leffel and Reed 2004).

Angola, 2004–2005 outbreak shows again devastating and rapid spread of viral hemorrhagic fevers in medical settings where hygiene practices are poorly applied or ignored; legacy of years of war and poverty in Angola resulted in very poor medical education and services; initial high rate of infection among infants in Angola might be related to poor hospital practices, possibly administration of vaccines; though the outbreak in Angola was in a part of Africa not previously known to have filovirus infection, prior ecological modeling predicted this location (Fisher-Hoch 2005).

Greater than 75 percent of the Marburg cases in Angola were in children, mostly infants (Fisher-Hoch 2005).

Geographical distribution of fruit bats, *R. aegyptiacus*, overlaps distribution of cases (Warfield, Deal, and Bavari 2009).

Therapeutics/vaccines

No treatments for Marburg and Ebola viral hemorrhagic fevers; ribavirin, an antiviral drug used to treat several other hemorrhagic fevers, has no in vitro effect on MARV and EBOV, failed to protect in multiple primate studies, and is unlikely to have any clinical value to human patients (Huggins, Zhang, and Bray 1999).

No specific antiviral therapy; hospital care is supportive in nature, e.g., early administration of vasopressors and hemodynamic monitoring with attention to fluid and electrolyte balance, circulatory volume, and blood pressure, but fluid infusions might cause pulmonary edema (Swenson et al. 2008).

Blended vesicular stomatitis virus vector expressing glycoprotein from multiple EBOV strains and 1 MARV strain protected *Macaca fascicularis* primates from lethal challenges of MARV and EBOV virus strains (Geisbert et al. 2009).

First experimental vaccine from USAMRIID completely protects animals from lethal infection; now being tested in NHP (Swenson et al. 2008).

MARV surface protein inserted on vesicular stomatitis vector developed in Canada shows promise in mice and NHP; effective in rhesus monkey even after infection (Geisbert et al. 2008). Tested panfilovirus vaccine based on a complex adenovirus (CAdVax) technology that expresses multiple antigens from five different filoviruses *de novo*; vaccination of NHPs [IM with 10^{10} PFU, challenged with 10^{3} PFU] demonstrated 100 percent protection against infection by two species of EBOV and three MARV subtypes, each administered at 1,000 times the lethal dose (Swenson et al. 2008).

Candidate vaccine based on recombinant replication-competent vesicular stomatitis virus completely protected monkeys against MARV (Paragas and Geisbert 2006).

Recombinant VSV-based *Zaire ebolavirus* (ZEBOV) and *Marburg virus* (MARV) vaccines used against aerosol challenge in cynomolgus macaques; all monkeys vaccinated with a VSV vector expressing the GP of ZEBOV protected against an aerosol exposure of ZEBOV; all monkeys vaccinated with a VSV vector expressing GP of MARV protected against an aerosol exposure of MARV (Geisbert et al. 2008).

Expression of viral structural proteins in cells leads to assembly and release of particles that resemble virions in size and morphology: virus-like particle vaccines highly effective to protect Small lab animals and NHP against lethal doses of virus (Yang, Ye, and Compans 2008; Warfield et al. 2004).

Attenuated recombinant vesicular stomatitis virus vaccine shown to have preventive and post-exposure efficacy in NHP (Bausch and Geisbert 2007).

DNA vaccines offered protective immunity via gene gun to NHP and laboratory animals (Riemenschneider et al. 2003).

No medical interventions or vaccines are approved for humans (Warfield, Deal, and Bavari 2009).

Other remarks

BSL-4 pathogen as determined by SALS (Siegert and Simpson 2008).

Department of Commerce permit required (Siegert and Simpson 2008).

Green monkey disease or Marburg hemorrhagic fever (Swanepoel 1994).

Zoonotic pathogen related to Ebola -virus genes isolated from Egyptian fruit bats, *R. aegyptiacus*; Uganda, Angola, Congo and African fruit bats in Gabon; antibodies found in healthy bats (Warfield, Deal, and Bavari 2009; Peterson et al. 2006; Peterson, Bauer, and Mills 2004; Towner et al. 2007; Mackenzie 2007).

Considerable media attention and fear generated by outbreaks of filoviruses because they can cause a severe VHF syndrome that has a rapid onset and high mortality (Leffel and Reed 2004).

Great bioterrorism concern because of their high mortality rate; low infective dose; ease of dissemination; potential for major public health impact, public panic, or social disruption; and requirement for major public health preparedness measures (Leffel and Reed 2004).

Common pathogenic feature of the hemorrhagic fever viruses is ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response (Geisbert and Jahrling 2004).

Taxonomy/antigenic relationships/synonyms

Distantly related via indirect immunofluorescence to Ebola (Siegert and Simpson 2008).

Twelve distinct viruses associated with hemorrhagic fever in humans are classified among four families: Filoviridae, which includes Marburg and EBOV is 1 of the 4 families (LeDuc 1989).

MARV poorly understood virus; family Filoviridae (Kiley 1988; LeDuc 1989).

EBOV and MARV are the sole members of the genus *Filovirus* in the family Filoviridae (Feldmann, Slenczka, and Klenk 1996; Leffel and Reed 2004).

Family Flaviviridae is divided into two genera: *Ebolavirus* and *Marburgvirus*; genus *Ebolavirus* further divided into four species, *Zaire ebolavirus* (ZEBOV), *Ivory Coast ebolavirus*, *Sudan ebolavirus* (SEBOV), and *Reston ebolavirus*; *Marburgvirus* genus, is represented by a single species (*Lake Victoria marburgvirus*) (Swenson et al. 2008).

Order Mononegavirales, family Filoviridae, divided into two genera: *Marburgvirus* and *Ebolavirus*. *Lake Victoria marburgvirus* is the lone species in the genus *Marburgvirus* while the genus *Ebolavirus* contains four distinct species: *Ivory Coast ebolavirus* (ICEBOV), *Sudan*

ebolavirus (SEBOV), *Zaire ebolavirus* (ZEBOV) and *Reston ebolavirus* (REBOV) (Mahanty and Bray 2004).

C.1.2.4 Lassa virus (LASV)

Host range

a. Field

Reservoir host: *Mastomys natalensis* where infection is life-long (Fisher-Hoch 2005).
Zoonotic in rodents particularly the *Mastomys* species complex (Curtis 2006).
M. natalensis is very likely the only reservoir host of LASV in Guinea and Sierra Leone
Evidence of vertical as well as horizontal transmission of the virus in *M. natalensis* (Lecompte et al. 2006).

"Rodents are infected *in utero*" (McCormick and Fisher-Hoch 2002).
Infection in the rodent is "life-long, persistent, and mostly silent" (Fisher-Hoch 2005).
Ag or Ab were found in very few *Mus musculus* and *Rattus rattus* and none were culture positive; multiple explanations given including non-specificity of the reagents. Cites a sole prior study claiming positive culture outside *Mastomys* but the possibility of misidentification was raised (Demby et al. 2001).

b. Experimental

Marmoset *C. jacchus* model due to macaques shortage; infected SQ with 10^3 and 10^6 PFU (Carrion, Brasky, et al. 2007).

An interference phenomenon in which lower doses (11 PFU) were more infective than higher doses (PFU $10^{6.1}$) has been reported for rhesus monkeys (Peters et al. 1987).
Strain 13 guinea pigs (Carrion, Patterson, et al. 2007), uniform lethal infection by 2 or more PFU (Jahrling et al. 1982).

Mice, guinea pigs, Rhesus, African green, and capuchin monkeys (Peters et al. 1987).
Pathogenicity of LASV for guinea pigs depends both on the virus and the host strain. For example, the Josiah strain of LASV has an LD_{50} of 0.3 PFU for strain 13 guinea pigs, but

kills only about 30 percent of outbred Hartley animals receiving between 2 and 200,000 PFU (Peters et al. 1987).

Outbred Hartley strain guinea pigs and cynomolgus monkeys: monkeys exposed to inhaled doses greater than or equal to 465 PFU were infected and died. The median infectious dose (ID_{50}) for guinea pigs was 15 PFU (Stephenson, Larson, and Dominik 1984).

Prevalence/incidence/attack rates

Prevalence of antibodies to LASV in the general population varies greatly (from 1.9 percent to 55 percent) among different regions or even villages within the endemic countries (authors cite internal references 139,141–143,145,152,161,171). Longitudinal studies in selected villages in Sierra Leone revealed a high incidence of LASV-specific seroconversion (5–20 percent per year) in susceptible (i.e., antibody negative) individuals (authors cite internal reference 139). However, the majority of these subjects did not report a febrile illness temporally associated with seroconversion. This is in agreement with the high seroprevalence of LASV specific antibodies in the general population, and indicates that most LASV infections are mild or even asymptomatic (Gunther and Lenz 2004).

Endemic in Guinea, Sierra Leone, Liberia, Nigeria, and also in Ghana, Ivory Coast or Burkina Faso (Drosten et al. 2003).

Mano River basin is considered epicenter of Lassa fever (Fisher-Hoch 2005).

Incidence of Lassa fever is highest in Sierra Leone (Khan et al. 2008).

As high as 52 percent seroconversion in Sierra Leone (Jeffs 2006).

Antibody prevalence in Sierra Leone ranges from 5 percent in coastal villages to 40 percent in forests and savannahs (average prevalence is 18 percent) (McCormick and Fisher-Hoch 2002).

Serostudies in Liberia and Sierra Leone—up to 50 percent seropositivity w/ 6 percent of seronegative converting per year (ter Meulen 2000).

In Sierra Leone infection rates of up to 20 percent of the population each year have been documented (Curtis 2006).

Guinea seroprevalence found by cross-sectional study to be 12.9% (10.8-15%) and 10.0% (8.1-11.9%) in rural and urban areas, respectively (Kerneis et al. 2009).

Estimated antibody prevalence: 4–6 percent Guinea, 15–20 percent Nigeria; in some villages in Sierra Leone as many as 60 percent "have evidence of exposure" (presumably antibodies, but this is not stated) (Fisher-Hoch and McCormick 2004).

In endemic areas accounts for 10–16 percent of adult medical admissions and 30 percent of adult deaths (Fisher-Hoch and McCormick 2004).

Prevalence of antibody is 1–6 percent in Liberia & Guinea, and 5–25 percent (average 21 percent) in Nigeria (McCormick and Fisher-Hoch 2002).

Some studies indicate that 300,000 to 500,000 infections and 5,000 deaths occur annually across West Africa (Ogbu, Ajuluchukwu, and Uneke 2007; Gunther and Lenz 2004).
100,000 to 300,000 infections annually in endemic areas are estimated (McCormick et al. 1987; Gunther and Lenz 2004).

The at risk seronegative population (in Sierra Leone, Guinea, and Nigeria) estimated to be as high as 59 million, with an annual incidence of illness of three million, fatalities up to 67 000, and up to three million reinfections (Richmond and Baglole 2003).

Seropositivity has also been found in the Central African Republic, DRC, Mali, and Senegal (Richmond and Baglole 2003).

Highest case rates occur in the dry season from February through May (McCormick and Fisher-Hoch 2002).

5–20 percent of susceptible persons are infected each year (McCormick and Fisher-Hoch 2002).

"About 25 cases of imported Lassa fever have been reported worldwide (cites 243–249). Four cases of Lassa fever have been imported into Europe in the year 2000 (cites 178,180,186,204) and a further case was imported in 2003 (cites 179). At least two of these patients died from typical Lassa fever (cites 204, 234)…" So far, no clinically apparent secondary cases were reported after import of Lassa fever, despite high and medium risk contacts" (Gunther and Lenz 2004).

A survey was conducted of small mammals in selected regions of Guinea to assess the degree to which LV poses a public health risk in that country; the proportion of LV-infected *Mastomys* per region ranged from 0 to 9 percent and was highest in the savannah and forest zones. The proportion of infected animals per village varied considerably, even between villages in close proximity. Infected animals tended to cluster in relatively few houses, suggesting the existence of focal *hot spots* of LV-infected *Mastomys* that might account for the observed heterogeneous distribution of Lassa fever (Demby et al. 2001).

R_0/incubation period/infectious period/infectious dose

R_0 N/A. Person-to-person transmission is associated with direct contact with the blood or other excretions, containing virus particles, of infected individuals (CDC 2008; Gunther and Lenz 2004).

Incubation period reported to range from 1–24 days (Mertens et al. 1973) and 7–18 days (McCormick and Fisher-Hoch 2002).

Incubation up to 3 weeks, flu-like, gastrointestinal symptoms—subset with hemorrhage & organ failure associated with high mortality, neurological complication of tremor, convulsions and coma, sensorineural deafness complication of convalescence phase (Drosten et al. 2003).

7–18 day incubation period (Carrion, Brasky, et al. 2007; Fisher-Hoch 2005).

3–21 day incubation (Ogbu, Ajuluchukwu, and Uneke 2007).

Interference phenomenon in which lower doses are more infective that higher doses might have implications for modeling in the Risk Analysis (Peters et al. 1987).

Morbidity/case fatality ratio

Results from serologic studies to determine the prevalence of exposure to the virus among populations in endemic areas suggest that clinically asymptomatic, or minimally symptomatic, infections occur, and that that most LASV infections are mild or even asymptomatic (Gunther and Lenz 2004).

The onset of illness is typically indolent, might result in a spectrum of clinical effects ranging from asymptomatic to multi-organ system failure and death (Khan et al. 2008).

Lassa fever is mild in about 80 percent of people infected with the virus—20 percent have a severe multisystem disease. Lassa fever is also associated with occasional epidemics, during which the case-fatality rate can reach 50 percent (CDC 2008).

Symptoms include progressive fever, malaise, myalgia, abdominal pain, vomiting, diarrhea, chest pain, headache, cough, sore throat, weakness, dizziness, tinnitus, deafness, hypotension, shock, facial or cervical swelling, bleeding , conjunctivitis, petechiae, rales, and wheezing. Case fatality ratios have been reported from 9–52 percent. Reports of ribavirin use relate a 2–3 fold decrease in mortality (Peters 1991) but must be used within the first week for optimal efficacy (Fisher-Hoch and McCormick 2004).

Symptoms also include pulmonary edema (Fisher-Hoch and McCormick 2004).

Fever, weakness malaise severe headache. Up to one-third of hospitalized patients progress to prostrating illness with persistent vomiting and diarrhea. Bleeding in 15–20 percent of patients.

Severe pulmonary edema and adult respiratory distress is common in fatal cases (Fisher-Hoch 2005).

Sensorineural deafness is the major chronic sequelae of Lassa fever (Khan et al. 2008).

Inflammation of the throat with white tonsillar patches helps in differentiating Lassa Fever from other tropical diseases (Roberts and Kemp 2002).

Data from human observation shows that a single infection with LASV provides long term protection (Fisher-Hoch and McCormick 2004).

"Pathology triggered by arenaviruses has a more insidious onset. For Lassa fever patients, hemorrhagic manifestations are not pronounced; and neurological complications are infrequent, develop late and manifest only in the most severely ill group. Deafness is a frequent long-term consequence of severe Lassa fever" (Geisbert and Jahrling 2004).

Viremia $\geq 10^3$ is associated with increased fatality; high titer viremia and elevated AST levels together indicate a risk of death of 80 percent (McCormick and Fisher-Hoch 2002).

4 concurrent symptoms- fever, pharyngitis, retrosternal pain, & proteinuria- correctly predicated 70 percent of laboratory confirmed Lassa cases (Roberts and Kemp 2002).

Overall fatality rate estimated at 1–2 percent (McCormick and Fisher-Hoch 2002).
Estimated overall fatality rate of 1–2 percent among the estimated 100,000–300,000 annual cases (much lower than the rate among hospital admissions (Gunther and Lenz 2004).

"Mortality is about 16 percent in untreated hospitalized cases, perhaps only about 2 percent in the community, but the number of people exposed in West Africa is very large; therefore, the actual number of cases is considerable" (Fisher-Hoch 2005).

Mortality is reported as 16 percent in hospitalized untreated points. In Nigeria some outbreaks with 50 percent mortality in community and as high as 70 percent in hospitalized patients (McCormick and Fisher-Hoch 2002).

Hospitalized fatality rate is 16 percent in Nigeria, and in 3rd trimester pregnant women as high as 30–70 percent (Fisher-Hoch 2005).

Case fatality ratio of 28% (18/64) was found from a retrospective review of medical records at one teaching hospital in Nigeria (Inegbenebor, Okosun, and Inegbenebor).

Mortality 17 percent among hospitalized patients if untreated (Fisher-Hoch 2005).

Some studies indicate that 300,000 to 500,000 infections and 5,000 deaths occur annually across West Africa (Ogbu, Ajuluchukwu, and Uneke 2007; Gunther and Lenz 2004).

Lower case mortality than other VHF, but more common, therefore ↑morbidity and mortality overall. Nosocomial infection mortality reported as high as 65 percent (Jeffs 2006).

Approximately 15–20 percent of patients hospitalized for Lassa fever die from the illness. However, overall only about 1 percent of infections with LASV result in death. The death rates are particularly high for women in the third trimester of pregnancy, and for fetuses, about 95 percent of which die in the uterus of infected pregnant mothers (CDC 2008).

Aerosol infection/routes of transmission

Aerosol infections in laboratory animals—anecdotal and experimental infections by aerosols. Monkeys housed in room that had been previously used to infect guinea pigs with the LASV (Josiah strain) contracted the virus and died. Macaques exposed to inhaled doses ranging from 2.7–4.5 $\log_{10}$ PFU all died, mean time to death 14 days (Peters et al. 1987).

Probability of aerosol dissemination function of 3 variables (1) aerosols occur primarily from infected host or secondarily from animal bedding or other source (2) stability of the virus once aerosolized (3) infectivity of small particle aerosol for animal at risk (Peters et al. 1987).

"Data from this study, combined with epidemiological evidence and the occurrence of nosocomial hospital infections, strongly suggested that airborne droplet nuclei represent an additional means of indirect virus dissemination. The biological half-lives at both 24 and 32 °C were sufficient for aerosol transmission of LASV to considerable distances in natural situations" (Stephenson, Larson, and Dominik 1984).

Primary infection from aerosolized rodent sources and ingestion of food contaminated by rodent feces, and by consumption of the rodent host. "Secondary human to human transmission" by blood and body fluids (Curtis 2006).

Presence of virus in pharyngeal secretions and urine for 3 to 4 weeks after onset of clinical signs provides an excellent source of airborne, infectious virions (Stephenson, Larson, and Dominik 1984).

Primary route is via cuts and abrasions of the skin, or by mucosal contact; does not mention pulmonary route ; "stirring of dust contaminated with urine might cause some cases but epidemiological data (secondary attack rates in households) do not support respiratory infection as a frequent event;" rodents roaming in the houses deposit urine on utensils, etc., and virus gets in through skin breaks and perhaps by ingestion in some cases (McCormick and Fisher-Hoch 2002).

"Needle stick injuries estimated to deliver approximately 10^2 to 10^4 infectious particles from patient blood that contains 10^5 to 10^7 infectious particles per milliliter have been described previously (author cites internal reference 62)" (Djavani et al. 2007).

Human infection with arenaviruses is incidental to the natural cycle of the viruses and occurs when an individual comes into contact with the excretions or materials contaminated with the

excretions of an infected rodent, such as ingestion of contaminated food, or by direct contact of abraded or broken skin with rodent excrement [and urine]. Infection can also occur by inhalation of tiny particles soiled with rodent urine or saliva (aerosol transmission). The types of incidental contact depend on the habits of both humans and rodents. For example, where the infected rodent species prefers a field habitat, human infection is associated with agricultural work. In areas where the rodent species' habitat includes human homes or other buildings, infection occurs in domestic settings (CDC 2008).

LASV and Machupo virus are transmissible from human to human. For those reasons, the highly pathogenic arenaviruses must be handled in laboratories of BSL-4 (Gunther and Lenz 2004). Some arenaviruses, such as Lassa and Machupo viruses, are associated with secondary person-to-person and nosocomial (healthcare setting) transmission. That occurs when a person infected by exposure to the virus from the rodent host spreads the virus to other humans. That could occur in a variety of ways. Person-to-person transmission is associated with direct contact with the blood or other excretions, containing virus particles, of infected individuals. Airborne transmission has also been reported in connection with certain viruses. Contact with objects contaminated with these materials, such as medical equipment, is also associated with transmission (CDC 2008).

By *M. natalensis* feces or by close personal contact (Carrion, Brasky, et al. 2007). Nosocomial human to human transmission in Africa because of poor medical practices (Drosten et al. 2003).

Humans presumably become infected through contact with infected rodent excreta, urine, tissues, or blood (3,27). Transmission to man is through fecal-oral route, or respiratory tract by inhaling contaminated air containing the virus, or when infected blood touches bruised skin or by sexual intercourse (Ogbu, Ajuluchukwu, and Uneke 2007).

Person-to-person transmission of Lassa fever can also occur through contaminated medical equipment, such as reused needles or when a person comes into contact with virus in blood, tissue, secretions, or excretions of an infected individual, the but virus cannot be spread through

casual contact (including skin-to-skin contact without exchange of body fluids) (Ogbu, Ajuluchukwu, and Uneke 2007).

Virus titers/concentrations/pathogenesis

"Nearly all patients with fatal Lassa fever remain viremic until death, with terminal viremia ranging from 10^3–10^8 50 percent -tissue culture infectious doses ($TCID_{50}$/mL.)" (Gunther and Lenz 2004).

In humans, a viremia level of $x10^{3.6}$ $TCID_{50}$/mL in Lassa fever patient admission was associated with case fatality rate of 76 percent (Carrion, Brasky, et al. 2007).

Samples of human serum were adjusted to contain 10^6–10^7 $TCID_{50}$/mL of Lassa fever virus (Lloyd, Bowen, and Slade 1982).

2 x 10^7 FFU in vitro (in supernatant) (Cosset et al. 2009).

Infectious titers of 5 x 10^6 PFU/mL. grown in Vero E6 cells (Carrion, Brasky, et al. 2007).
Chronic viruria in infected *Mastomys* ranges from 10^4–10^6 PFU/ml urine (Stephenson, Larson, and Dominik 1984).

In lab-infected marmosets viremia levels of 5 to 7 $\log_{10}$ on day 14 and day of necropsy (Carrion, Brasky, et al. 2007).

"Needle stick injuries estimated to deliver approximately 10^2 to 10^4 infectious particles from patient blood that contains 10^5 to 10^7 infectious particles per milliliter have been described previously (author cites reference 62)" (Djavani et al. 2007).

Pathogenesis is poorly understood. The virus can be isolated from almost every organ, but histopathology reveals lesions not sufficient to cause organ failure and death (Drosten et al. 2003).

"…the currently available data are not sufficient to reconstruct the chain of pathophysiological events in Lassa fever. Some mechanisms are likely to play a role: i) early during infection LASV may target immune cells and interfere with their activation; ii) the high affinity of LASV for the receptor cy-DG may allow virus replication in several organs: iii) the relatively low susceptibility of the virus to IFN may facilitate virus spread; iv) a continuous and uncontrolled rise in virus load in several organs may eventually trigger a fatal inflammatory syndrome in the terminal stage of the disease" (Gunther and Lenz 2004).

"Pathogenesis of LF appears to be related to unchecked viremia … Cell mediated immunity appears to be the most important arm in recovery... LASV infection probably results in lifelong immunity, at least against severe disease" (Khan et al. 2008).

Microvascular instability and impaired hemostasis are the pathophysiologic hallmarks (Khan et al. 2008).

"Severe disease appears to result from the interaction of LASV with macrophages and dendritic cells, either directly or indirectly via soluble mediators, resulting in a process akin to septic shock, with activation of a host of inflammatory and vasoactive mediators leading to cellular dysfunction, insufficient effective circulating intravascular volume, and multi-organ system failure" (Khan et al. 2008).

Hemorrhage appears to be primarily attributable to LASV-induced release of a soluble mediator impairing platelet aggregation (Khan et al. 2008).

Pathogen stability

Biological half-lives of the virus in aerosols at both 24 °C and 32 °C ranged from 10.1 to 54.6 minutes and were sufficient for aerosol dispersion of virus to considerable distances in natural situations; ability of the virus to readily infect two mammalian hosts via the respiratory route—monkeys exposed to inhaled doses 5,465 PFU were infected and died. The median infectious dose (ID_{50}) for guinea pigs was 15 PFU (Stephenson, Larson, and Dominik 1984).

The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for Lassa was calculated to be 13 [HL = 30 min at maximum solar radiation] (Lytle and Sagripanti 2005).

Stability of LASV at 32 °C indicates that airborne transmission of the virus in the natural environment most likely could occur during the dry season when the RH is minimal (Lloyd, Bowen, and Slade 1982).

In acute-phase serum containing 10^5 $TCID_{50}$/mL of LASV, 0.2 percent beta-propiolactone (BPL) reduced infectivity to undetectable levels within 30 min at 37 °C (Lloyd, Bowen, and Slade 1982).

Complete inactivation of Lassa fever virus in human sera containing 10^6 $TCID_{50}$/mL of the virus occurs in 60 min at 60 °C (Lloyd, Bowen, and Slade 1982).

Virus is inactivated by heating to 56 °C, pH less than 5.5 or more than 8.5, UV or gamma radiation, or detergents (Curtis 2006).

"…prolonged presence of high virus titers in the lungs and upper respiratory tract tissues, pharyngeal secretions, and urine of infected humans or rodents would provide exemplary seed sources for infectious aerosols" (Stephenson, Larson, and Dominik 1984).

Virus is excreted in urine 3 to 9 weeks, semen 3 months from infection (McCormick and Fisher-Hoch 2002).

Vectors

LASV is not known to be vector-borne (CDC 2008).

Other epidemiological/ecological data

In 2000, at least four cases were imported to Europe (Fisher-Hoch 2005).

Human contact with rodent excreta, tissues, urine or blood (Ogbu, Ajuluchukwu, and Uneke 2007).

Endemic in parts of West Africa with outbreaks of the disease occurring typically in the dry season between January and April. Potential for large nosocomial outbreaks due to secondary human to human transmission (Curtis 2006).

Nosocomial transmission can be avoided by taking preventive precautions against contact with patient secretions by instituting strict barrier nursing—using PPE, complete sterilization of medical equipment, patient isolation and properly disposing of all contaminated supplies (Ogbu, Ajuluchukwu, and Uneke 2007).

Four strains of virus: *Josiah*— Sierra Leone, *Nigeria* and *LP*-Nigeria, and *AV* imported to Germany by a traveler who had visited Ghana, Côte D'Ivoire, and Burkina Faso (Ogbu, Ajuluchukwu, and Uneke 2007).

The main feature of fatal illness is impaired or delayed cellular immunity leading to fulminant viremia (Richmond and Baglole 2003).

Therapeutics/vaccines

Ribavirin effective (Drosten et al. 2003).

Rx with Ribavirin reduces case fatality of pts with ↑ AST from 55 to 5 percent if given within the first 6 days (Jeffs 2006).

Because of its expense, need for intravenous administration, potential toxicity, and teratogenicity, empiric therapy with ribavirin is undesirable (Ogbu, Ajuluchukwu, and Uneke 2007).

Supportive treatment is often necessary and includes fluid replacement, blood transfusion, administration of paracetamol, phylometadione, ringer lactate, hemocele quinine and broad spectrum antibiotics (Ogbu, Ajuluchukwu, and Uneke 2007).

No vaccine success as of 2004 (Fisher-Hoch and McCormick 2004).

"Guinea pigs vaccinated with a ML29 reassortant vaccine experienced sterilizing immunity and complete protection when challenged on day 30 either with homologous virus or with the distantly related Nigerian isolate. Simultaneous vaccination–challenge or challenge on day 2 after vaccination also protected 60–100 percent of the animals against both strains, but without sterilizing immunity. These results indicate that simultaneous replication of ML29 and LASV attenuates the virulence of LASV infection" (Carrion, Patterson, et al. 2007).

"A killed vaccine tested in NHPs elicited antibodies to major structural proteins of LASV but did not protect animals against challenge [authors cite reference 5]. Controlled clinical trials failed to show protective efficacy of human convalescent plasma [authors cite reference 6]. In addition, there is a solid body of evidence that the clearance of LASV and recovery of Lassa fever patients is not dependent on antibody responses but is associated with cell-mediated immunity" (Carrion, Patterson, et al. 2007).

Other remarks

Increased prevalence of LASV in rainy season in Guinea (Fichet-Calvet et al. 2008).

Taxonomy/antigenic relationships/synonyms

Taxonomically, LASV is classified in the genus *Arenavirus*, family *Arenaviridae* and is an enveloped RNA virus (ICTVdB - The Universal Virus Database 2006; Ogbu, Ajuluchukwu, and Uneke 2007).

"Arenaviruses are classified as segmented negative-strand RNA viruses. The genes are oriented in both negative and positive senses on the two RNA segments, a coding strategy which is called

ambisense.'" LASV is serologically, phylogenetically, and geographically part of the Old World complex of Arenaviruses (Africa, Europe, and Asia) (Gunther and Lenz 2004).

C.1.2.5 Junín virus (JUNV)

Host range

a. Field

The disease was first reported in 1950s with virus isolation in 1958.
Junín, Argentina, 1958; virus isolated from humans in IP-inoculated guinea pigs, then passed in suckling mice (Parodi 1958).

Mus musculus, 10/33 pools (411 rodents) were virus positive according the Arbovirus Catalogue (Parodi 2008). Examination of the papers cited in the catalog document culture of 40 *M. musculus* mice. The 40 mice were divided into three pools and two of the three were positive by culture in guinea pig followed by culture in suckling mice (Parodi et al. 1959; Parodi et al. 1961).

C. laucha, 1/28 pools (229 rodents) and 4/36 were virus positive (Parodi 2008).
A. arenicola, 1/13 pools (78 rodents) were virus positive (Parodi 2008).
Primary hosts are the rodents *C. laucha* and *C. musculinus* (LeDuc 1989; Carballal, Videla, and Merani 1988).

Based on combined tests for antibody and antigen detection, the most recent field data showed a prevalence of JUNV in *C. musculinus* of 11% (Mills et al. 1994).

Field mouse *C. musculinus* is the main natural reservoir; other rodent species like *C. laucha, Mus musculus*, and *A. azarae* reported as less important natural hosts (Carballal, Videla, and Merani 1988; Ambrosio et al. 2006). However, examination of Carballal et al (Carballal, Videla, and Merani 1988) shows no mention of *Mus musculus,* and attribution by Ambrosio et al (Ambrosio et al. 2006) is not entirely clear. Translation of the original Parodi articles (Parodi, A.S., et al. 1959. Prensa Medica 46:554; and Parodi, A.S., et al. 1961. Prensa Medica 48:2321) clarifies that viral antigen was detected via a complement-

fixation test, and that each of the papers reports one *Mus musculus* pool positive for JUNV antigen [this is at odds with the information listed in the Arbovirus Catalogue that 10 of 33 pools, consisting of 411 rodents, were antigen positive].

One of 217 *Mus musculus* was antigen-positive (Mills, Ellis, et al. 1991).

One of 286 *Mus musculus* was antigen-positive (Mills, Calderon, et al. 1991).

Virus isolations from *C. musculinus* (family *Muridae*, subfamily *Sigmodontinae*), *C. laucha*, *A. azarae*, *Bolomys obscurus*, *Oxymycterus rufus*, *O. flavescens*, *Necromys benefactus*; viral antigen positive via ELISA, *Mus musculus* (Mills, Ellis, et al. 1991; Enria and Barrera Oro 2002).

Argentina, strains of Junin virus isolated from *C. musculinus* captured at same sites as *Mus musculus* from which LCM virus was isolated(Sabbatini 1974).

Echinolaelaps spp (mites), > 40 isolates (Parodi 2008).

Other isolates from *C. laucha*, *A. azarae*, *A. obscurus*, *C. musculinus*, *Oryzomys flavescens*, *Cavia pamparum* (wild cavy), and *Lepus europaeus* (hare) (Parodi 2008). First isolated by IP-inoculated guinea pig, 1958, Argentina, infected human with hemorrhagic syndrome (Parodi 2008).

Argentina, > 60 isolates from human blood; 20–60 percent of collected human paired serum samples had CF antibody (Parodi 2008).

Córdoba Province, Argentina, field and experimental studies indicate predominant role of cricetid rodents in JUNV maintenance over an 11 year period (Sabbatini 1977).

b. Experimental

Rhesus macaques (*M. mulatta*), disease most closely simulates human disease with clinical course/symptoms reflecting disease caused by respective strains isolated from infected humans, i.e., strains causing serious disease in humans caused serious disease in macaques, while less virulent human pathogens produced less severe disease in macaques (Peters et al. 1987).

Infection of rhesus monkeys and marmosets produced lesions similar to those reported in human cases; include hemorrhage, bone marrow necrosis, mild hepatocellular necrosis, poliencephalomyelitis and autonomic glanglioneuritis; *C. jacchus* infected with Junin virus developed acute hematologic and neurologic manifestations with anemia, leucopenia and thrombocytopenia and death within 17–24 days (Enria, Briggiler, and Sanchez 2008).

Rhesus monkeys were killed by 1MICLD$_{50}$ (mouse intracranial LD), whereas cynomolgus monkeys were more resistant (Peters et al. 1987).

Aerosol infection of Rhesus macaques (*M. mullata*) with $10^{1.6–1.9}$ or $10^{3.9–4.3}$ PFU produced illness after 3 weeks' incubation, and death similar to effects of parenteral inoculation and mimicking human disease; virus isolated from blood ($10^{1–3}$ PFU/mL), OP swabs ($10^{3–>6}$ PFU/mL), and visceral organs and central nervous system (up to $10^{7.3}$ PFU/g) (Kenyon, McKee, et al. 1992).

NHPs in *M. mulatta* model mimics the human clinical syndrome via parenteral infection of adults with low-passage isolates; distinct hemorrhagic or neurologic disease that correlated with clinical illness patterns present in the humans from whom the viral strains were obtained; however, patterns of viremia, OP viral shedding, and antibody response were different; with postmortem virologic and histopathologic findings, suggests that viral-strain-specific factors are important determinants of clinical disease patterns (Peters et al. 1987).

Intramuscular inoculation of *Aotus trivirgatus* with $3x10^1$ or $3x10^5$ $TCID_{50}$; subclinical infection, no clinical response, minimal viremia (10^{1-2} SMICLD50/mL) detectable at 2 and 3 weeks post-infection, and NT antibodies produced (Samoilovich et al. 1983). Inoculation: *Alouatta caraya*, *Saimiri sciureus* responded like *A trivirgatus* (subclinical infection); *Callithrix jacchus* (Samoilovich et al. 1983).

Virus non virulent for *Alouatta caraya* ; no signs, viremia, or lesions, NT antibody produced to high titers (Weissenbacher et al. 1978; Weissenbacher et al. 1979).

Cebus apella inoculated IM develop inapparent infection with transient viremia, virus in saliva, and leukothrombocytopenia followed by high antibody titers, similar to the mild AHF forms in humans (Carballal, Videla, and Merani 1988).

Infection with pathogenic strain of JUNV causes 100 percent mortality in guinea pigs and *Callithrix jacchus* marmoset (Samoilovich et al. 1988).

Marmoset, *Callithrix jacchus,* develops a fatal disease that mimics the natural infection in humans in clinical course, 100 percent lethal around the third week of infection, characterized by hemorrhagic or neurologic signs, pronounced and long-lasting viremia, wide virus spread, leukopenia, anemia, and thrombocytopenia (Weissenbacher et al. 1986).

Susceptibility of the marmoset, *Callithrix jacchus* to TAC virus infection investigated to perform cross-protection studies between JUN and TAC viruses; no signs of disease or hematologic changes, no viremia, anti-TAC neutralizing antibodies produced 3 weeks post-infection; TAC virus-infected marmosets challenged with JUNV showed no signs of disease, no viremia, and no challenge virus replication, but anti-JUN NT antibodies were produced (Weissenbacher et al. 1982).

Callithrix jacchus is useful model of human disease; *Cebus* spp produced mild or inapparent responses, while *Alouatta caraya*, *Saimiri sciureus*, *Aotus trivirgatus* seroconverted without disease (Peters et al. 1987).

Callithrix jacchus useful model for human disease; clinical syndrome, death, virus in urine, blood, and all tissues (Weissenbacher et al. 1979).

Calomys callidus, *Akodon. molinae* develop persistent infections with virus shedding (Carballal, Videla, and Merani 1988).

Newborn mice, IC inoculation, death, $10^{5.25}$ ID_{50}/mL [test system not reported](Parodi 2008).

Guinea pig, IC inoculation, death, $10^{6.0}$ ID_{50}/mL [test system not reported](Parodi 2008).

Depending on strain, lethal to guinea pgs at less than 1 PFU dose (Peters et al. 1987).

In guinea pig, some JUNV strains required less than 1 PFU to produce an LD_{50} while other strains killed 20 percent regardless of dose (Peters et al. 1987).

Median infectious dose (stated by authors to be the same as an LD_{50}) in outbred guinea pigs 15 PFU (Peters et al. 1987).

ID_{66} (66 percent infection with no mortality) for a dose of $10^{3.5}$ $TCID_{50}$ in guinea pigs (Samoilovich et al. 1988; Peters et al. 1987).

Hamsters, IP; *Saguinus geoffreyii* (marmoset); *Callitrix jacchus* (marmoset)—death (Parodi 2008).

Establishes persistently infected carrier cultures in a variety of cell lines and produces chronic infection in a natural rodent host (Candurra et al. 1990).

7-day-old chick embryo, CAM inoculation, $10^{4.0}$ ID_{50}/mL [test system not reported](Parodi 2008).

Prevalence/incidence/attack rate

Affects a 150,000 square mile area in Argentina with an at risk population of approx 5 million (Vainrub and Salas 1994; Jay, Glaser, and Fulhorst 2005).

Incidence rates 140/100,000 in general population; 335/100,000 in adult males in rural areas (Enria and Barrera Oro 2002).

Cases reported from 1958–1987, 21,000, annual from 100 to 4,000 cases; initially estimated to affect an area of 16,000 km^2 with 250,000 persons at risk, now considered 150,000 km^2 with more than 2 million at risk (Vainrub and Salas 1994).

Estimated 200 to 2,000 cases of AHF reported annually in the north-central Argentine Pampas; distinct seasonal peak in the fall (February to May) during the agricultural harvest (Jay, Glaser, and Fulhorst 2005).

Since the 1950s, JUNV is estimated to have caused about 30,000 cases of symptomatic disease (Charrel and de Lamballerie 2003).

Since 1991 vaccination has reduced disease to 94 suspected and 19 confirmed cases in 2005 (Vainrub and Salas 1994).

Prevalence in rodents assessed by ELISA on samples of body fluids or organs or both, variable (0–3.7 percent); prevalence 1.4 percent in AHF epidemic area, 0.6 percent in the historic low incidence area, 0.4 percent beyond the defined endemic area (Mills, Ellis, et al. 1991).

Using indirect fluorescence antibody test, the cricetid rodent *C. musculinus* had a 30-month prevalence of 7.9 percent; with data on antigen detection, provided an estimated total prevalence of infection of 10.9 percent for this species; other infected species included two cricetids, *C.*

laucha (seroprevalence 0.2 percent) and *Bolomys obscurus*, and a predatory carnivore, *Galictis cuja* (a felid); approximately half of infected animals simultaneously carried serum antibody and antigen in blood and saliva; *C laucha* associated with crop habitats, other seropositive animals strongly associated with the relatively rare roadside and fence-line habitats; seropositive *C. musculinus* were predominantly males in the oldest age and heaviest body mass classes and seropositive males were twice as likely to have body scars as seronegative males; these observations suggest that most infections were acquired through horizontal transmission and that aggressive encounters among adult, male *C musculinus* in relatively densely populated roadside and fence-line habitats are an important mechanism of transmission within reservoir populations. Of the 437 rodents captured only 2% were *Mus musculus* and all were negative for antibodies and antigen (Mills et al. 1994).

Virus prevalence via ELISA in rodent spp in 3 distinct zones of Argentina, 1990–1991: the central Argentine pampas area, the historic AHF zone in Buenos Aires province, and the current AHF zone in Santa Fe province; in high incidence zone, 1.7 percent ELISA positive; in historic zone, 4 percent positive; in disease-free zone, 0.2 percent positive; *C. musculinus* in high incidence zone, 4.0 percent positive; *Bolomys obscuris*, 1.2 percent positive; *C. laucha, A. azarae, Mus musculus*, 0.3–0.5 percent positive; virus isolated from all spp except *M musculus*; although *O. flavescens* were ELISA negative, virus was isolated (Mills, Calderon, et al. 1991).

R_0/incubation period/infectious period/infectious dose

R_0 is <1 (Enria and Barrera Oro 2002).

5 days post-inoculation in guinea pigs (Weissenbacher, de Guerrero, and Boxaca 1975).

7- to12-day post-inoculation in mice (Weissenbacher, de Guerrero, and Boxaca 1975).

Morbidity/case fatality ratio

80 percent of infections result in clinical disease; viremia occurs throughout the acute febrile period. Subclinical infections occur (Enria, Briggiler, and Sanchez 2008).

Severe hemorrhagico-neurologic disease occurs in 20–30 percent of cases in the second week; late neurologic disease occurs later in 10 percent of cases as a truncal cerebellar ataxia with fever in immune serum-treated patients (Enria and Barrera Oro 2002).

In humans, primates, and rodents, JV disease is characterized by viral replication in lymphoid, hemopoietic, or neural tissue, by a cellular or humoral immune response, or by long-term viral persistence (Samoilovich et al. 1988).

Chronic infection of the host (accompanied by a chronic viremia or viruria) appears to be crucial for the long-term persistence of arenaviruses in nature (Charrel and de Lamballerie 2003).

Fatal outcome more frequently occurs in pregnant women in last trimester and with high fetal mortality—associated with both JUN and Machupo virus infections (Charrel and de Lamballerie 2003).

Mortality rate in untreated humans is 15–30 percent (Jay, Glaser, and Fulhorst 2005).

In early years, about 1,000 cases per year recorded, with a high mortality rate (more than 30 percent) in untreated humans (Jay, Glaser, and Fulhorst 2005).

Mortality 20–30 percent (Bushar and Sagripanti 1990).

Case fatality rates up to 30 percent without treatment (Enria and Barrera Oro 2002).

Mortality of 10 percent registered among clinical cases of AHF (Weissenbacher et al. 1980).

Significant morbidity, case-fatality rate without treatment between 15–30 percent (Enria, Briggiler, and Sanchez 2008).

Up to 17 percent mortality among hospitalized patients (Mills et al. 1996).

Aerosol infection/routes of transmission

Lab infections from rodents [in the absence of appropriate biocontainment precautions] are documented (Carballal, Videla, and Merani 1988).

In virology labs [in the absence of appropriate biocontainment precautions], arenaviruses have been infectious to humans under circumstances implicating aerosol spread (Charrel and de Lamballerie 2003).

Risk of laboratory infection for those who handle JUNV is high [in the absence of appropriate biocontainment precautions] (Weissenbacher et al. 1980).

1967, laboratory infections [in the absence of appropriate biocontainment precautions] consisted of 5 cases and one death (Hanson et al. 1967; Pike 1979).

Previous report [in the absence of appropriate biocontainment precautions]—5 laboratory infections, 1 death; 11 clinical infections, 5 subclinical infections, 0 deaths; 1 aerosol infection, other 20 infections source unknown or other non-aerosol route (Scherer 1980).

Transmission of JUNV to humans is thought to be by aerosol (LeDuc 1989; Kenyon, McKee, et al. 1992; Enria and Barrera Oro 2002).

Prenatal infection, acquired through placenta (Ambrosio et al. 2006).

Human-to-human transmission is possible for all VHF viruses; majority of the person-to-person transmission for the arenaviruses and filoviruses has been attributed to direct contact with infected blood and body fluids; potential for airborne transmission of the VHF agents appears to be an infrequent event, but cannot be categorically excluded as a mode of transmission (Geisbert and Jahrling 2004).

Human-to-human transmission occurs (Enria and Barrera Oro 2002).

In rodents, transmission generally horizontal through close contact with; congenital infection not seen (Mills, Ellis, et al. 1991).

Mouse suffers chronic subclinical infection and virus is excreted in saliva and urine (Jay, Glaser, and Fulhorst 2005).

Annual epidemics of AHF occur at the time of the fall harvest, and primarily affect rural males between the ages of 15 and 55; transmission to humans believed to occur by inhaling aerosolized viral particles from contaminated soil and plant litter, which are disturbed during the mechanized harvesting process (Mills et al. 1994).

In nosocomial arenavirus infections, most dangerous route is parenteral exposure through improperly sterilized needles, autopsy accidents, or other failings in techniques (Charrel and de Lamballerie 2003).

Fetal infection and death common in pregnant women infected with JUNV, with an associated increase in maternal mortality; virus isolated from breast milk (Khan 1997).

Aerosol infection of guinea pigs (IN inoculation)- at $10^{4.5}$ $TCID_{50}$ = 100 percent infection, 100 percent mortality; at $10^{3.5}$ $TCID_{50}$ = 100 percent infection, 83 percent mortality; attenuated strain at $10^{6.5}$ $TCID_{50}$ = 100 percent infection, 25 percent mortality, at $10^{3.5}$ $TCID_{50}$ = 66 percent infection, no mortality (Parodi 1958).

Viremic pregnant *C. musculinus* produced negative offspring; became infected within 2 weeks from dam's infected oral secretions and urine; breast milk not tested; horizontal transmission demonstrated between adults and juveniles (Sabbatini 1977).

Virus titers/concentrations/pathogenesis

Rhesus macaques, no to low viremias from human low virulence strains; viremias to $10^{5–7}$ PFU/mL, and persisting at greater than 10^{3} PFU/mL until death with highly virulent humans strains (Peters et al. 1987).

Alouatta caraya, titers in blood and tissues from $10^{1.5}$ to $10^{6.0}$ $TCID_{50}$ (Weissenbacher et al. 1978; Weissenbacher et al. 1979).

In 1965–1966, Córdoba Province, Argentina, JUNV first recovered from *C. musculinus*, *C laucha*, and *A.* azarae; high virus titers in blood, brain, spleen to titers of $10^{6 \text{ to } 7}$ $SMICLD_{50}$/mL (Sabbatini 1977).

Rodent body fluids, i.e., urine, blood, and saliva carry substantial amounts of infectious virus (Kenyon, McKee, et al. 1992).

In suckling mice, 10^6 $SMICLD_{50}$ produced (Weissenbacher et al. 1986).

In marmosets, JUNV inoculation replicated to 10^6 $SMICLD_{50}$/mL (Weissenbacher et al. 1982).

In guinea pigs, different strains of JUN produced wide variety of clinical syndromes and viremia titers; from viscerotropic to neurologic disease with paralysis; viremias from 10^2 to 10^6PFU/mL and virus in tissues from 10^3 to $10^{>7}$ PFU/g and death rates from 0 to 100 percent (Kenyon et al. 1988).

In tissues of guinea pigs, titers to $10^{5–6}$ PFU/g (Peters et al. 1987).

HeLa cell line, $10^{5.0}$ to $10^{7.0}$ $TCID_{50}$/mL (Parodi 2008).

LLC-MK2 cell line, $10^{7.2}$ PFU/mL (Parodi 2008).

Vero cell line, $10^{7.0}$ PFU/mL (Parodi 2008).

In Vero cells, 10^6 $CCID_{50}$/mL produced in roller bottles (Johnson 2008).

In Vero cells, titers of $10^{6.3}$ to $10^{7.3}$ PFU/mL (Videla et al. 1989).

In Vero cells, produces 10^6 to 10^7 PFU/mL (Candurra et al. 1990).

Pathogen stability

Virus in guinea pig plasma, diluted in isotonic phosphate buffer with 2% rabbit plasma was placed into glass tubes and monitored for inactivation of virus at various time points. Inactivation at 37^0C was 47% [66 min], 59% [280 min], and 99.9% [157 min] at time points of 1, 6, and 26 hours, respectively. At 25^0C, inactivation was 75% [780 min] and 99% [650 min] at 26 and 72 hours, respectively (Parodi et al. 1966). Bracketed information is half-life as derived by Ken Bulmahn (TetraTech) from the Parodi et al data.

The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for Junín was calculated to be 13 [HL = 30 min at maximum solar radiation] (Lytle and Sagripanti 2005),

Resistant to pH 6.5–9.5 (Parodi 2008).

Indication that viruses with structural lipids survive best in aerosols at low RH (Ambrosio et al. 2006).

UV radiation inactivates viruses by chemically changing the RNA and DNA; most effective UV is 250 nm; filoviruses [MARV and EBOV] most sensitive to solar UV_{254}, requiring 20'–100' at mid-day exposure to inactivate 1 $\log_{10}$ of virus: bunyaviruses (hantaviruses [AND virus] and RVFV), arenaviruses (Lassa, JUNV), and flaviviruses (TBE complex viruses) more resistant (Lytle and Sagripanti 2005).

Rapidly inactivated at 56 °C, at pH less than 5.5 or greater than 8.5, or by exposure to UV or gamma irradiation or both (Charrel and de Lamballerie 2003).

Inactivated by lipid solvent (ether, chloroform), formalin, trypsin, phenol, and deoxycholate (Parodi 2008; Videla et al. 1989).

Vectors

a. Field

No evidence of arthropod vectors (Jay, Glaser, and Fulhorst 2005).

b. Experimental

Mesostigmata spp mites able to transmit JUNV infection (Parodi 2008).

Other epidemiological/ecological data

80 percent of the affected humans are males 15–60 years of age; reside or work in rural areas (Vainrub and Salas 1994).

Most hemorrhagic fever viruses are zoonoses; found in both temperate and tropical habitats; generally infect both genders and all ages, although there might be an influence of occupational exposure; transmission to humans is frequently by bite of an infected tick or mosquito or via aerosol from infected rodent hosts; aerosol and nosocomial transmission are especially important with JUN virus; seasonality of hemorrhagic fever among humans is influenced by the dynamics of infected arthropod or vertebrate hosts; mammals, especially rodents, appear to be important natural hosts for many hemorrhagic fever viruses; transmission cycle for each hemorrhagic fever virus is distinct and dependent on the characteristics of the primary vector species and the possibility for its contact with humans (LeDuc 1989).

Disease localized in a relatively well-defined area of Argentina, where it causes annual epidemics from about January to August of each year; persons most commonly infected are farmers and others with exposure to rural areas (LeDuc 1989).

Argentina labs, over 20 years, nearly 280 persons worked with JUNV; of 150 people considered as high risk personnel, 35 developed clinical disease with recovery but 3 died; figures indicate that nearly 25 percent of the personnel exposed to virulent virus strains acquired an overt disease while the rest of infected personnel experienced subclinical infections (Weissenbacher et al. 1980).

As a group, the other VHF agents are linked to the ecology of their vectors or reservoirs, whether rodents or arthropods (Geisbert and Jahrling 2004).

Ability to establish chronic infections in their respective principal rodent hosts is the hallmark of the arenaviruses (Jay, Glaser, and Fulhorst 2005).

Therapeutics/vaccines

Specific treatment hyperimmune human plasma from recovered patients; if started early, is extremely effective and reduces mortality to 1 percent (Bushar and Sagripanti 1990).

Immunoglobulin is effective if used within first 8 days of clinical illness, but late neurologic symptoms occur in ~10 percent recipients (Peters et al. 1987).

Attenuated strain of JUNV is the basis of the only arenaviral vaccine (Candid #1) that has been evaluated in humans. Its status is that of an investigational new drug. The vaccine appears to cross-protect against Machupo virus challenge in guinea pigs and NHPs (Jay, Glaser, and Fulhorst 2005).

Immune therapy effective, though a late neurologic syndrome occasionally associated with treatment; attempted to determine in the infected marmoset *Callithrix jacchus* whether immune therapy leads to protection and/or CNS damage; reduced mortality from 100 percent to 25 percent, lowered viremia and viral titers in organs; late neurologic signs in 30 percent of treated marmosets (Avila et al. 1987).

Immune plasma reduces mortality to less than 3 percent (Bushar and Sagripanti 1990).
Treatment with immune plasma during first week; approx 10 percent of patients treated develop a late neurologic disease (Enria and Barrera Oro 2002).

Ribavirin has shown some promise for treatment (Enria, Briggiler, and Sanchez 2008).

Ribavirin limited efficacy in Rhesus macaques (Peters et al. 1987).

Immune serum treatment of JUN virus-infected marmosets reduced mortality from 100 percent to 25 percent (Enria, Briggiler, and Sanchez 2008).

Early use of immune plasma in patients with a clinical diagnosis of AHF is standard specific treatment in Argentina (Enria, Briggiler, and Sanchez 2008).

Callothrix jacchus, immune serum significantly reduces lethality, although late neurologic syndrome observed similar to that in humans; Ribavirin, limited efficacy (Weissenbacher et al. 1986).

Some attenuated strains show neurovirulence on intracerebral inoculation in *Cebus* sp. primates; related Tacaribe (TAC) virus seems nonpathogenic for humans and causes no overt illness in guinea pigs and marmosets (nasal inoculation), which remain protected against later challenge with JUNV–TAC virus conferred protection against AHF and the intrathalamic inoculation showed lack of neurovirulence (Samoilovich et al. 1988).

NT antibody persists in 86 percent of Candid #1 vaccinates for more than 42 months (Levis 1993).

Marmoset *C. jacchus* can be considered an experimental model for protection studies with arenaviruses; TAC virus could be considered as a potential vaccine against JUNV (Weissenbacher et al. 1982).

Efficacy trial of Candid 1, attenuated JUNV vaccine; no serious adverse events were attributed to vaccination; Candid 1, the first vaccine for the prevention of illness caused by an arenavirus, is safe and highly efficacious; produced from original isolate made in guinea pigs from a fatal case of AHF; following 44 newborn mouse brain passages, candidate vaccine strain was cloned by single-burst selection and passed in certified fetal rhesus lung cells (Maiztegui et al. 1998).

Candid #1, attenuated JUNV vaccine, s/q in rhesus macaques; no significant effect on physical, hematologic, or biochemical parameter measured; virus recovered from peripheral blood

mononuclear cells (PBMC); no reversion to virulence detected; all NHP developed a detectable neutralizing antibody response; Candid #1 is safe and immunogenic for NHP (McKee et al. 1993).

Many vaccine initiatives [reviewed] (Enria and Barrera Oro 2002).

Attenuated vaccine markedly reduced incidence of AHF; specific therapy involves transfusion of immune plasma in defined doses of neutralizing antibodies during the prodromal phase of illness; Ribavirin might be effective; immune immunoglobulin or monoclonal antibodies might be useful (Enria, Briggiler, and Sanchez 2008).
1985, *Candid #1* vaccine applied to adult high-risk population; 95.5 percent effective (Maiztegui et al. 1998).

Attenuated vaccine, Candid #1, used since 1991 is immunogenic and protective (Ambrosio et al. 2006).

Formalin inactivated virus produced high neutralizing and low IFA antibody in mice and guinea pigs; guinea pigs were not protected from challenge (Videla et al. 1989).

Other remarks

SALS level 4 (Parodi 2008).

Zoonotic hemorrhagic disease of humans, transmitted from the corn mouse, *C* musculinus (Mills, Ellis, et al. 1991).

First described 1958, causes an arenaviral disease known as AHF (LeDuc 1989; Ambrosio et al. 2006).

Case definitions of AHF (Maiztegui et al. 1998; Harrison et al. 1999).

Common pathogenic feature of the hemorrhagic fever viruses is ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response (Geisbert and Jahrling 2004).

Taxonomy/antigenic relationships/synonyms

Tacaribe group, family *Arenaviridae*, genus *Arenavirus*, caused by JUN virus which is closely related to Machupo virus (Parodi 2008).

JUNV is in Tacaribe (New World) complex of *Arenaviridae* associated with subfamily Sigmodontinae (New World rats and mice) (Jay, Glaser, and Fulhorst 2005).

Mal de los rastropos (Mills, Ellis, et al. 1991).

12 distinct viruses associated with hemorrhagic fever in humans are classified among four families including *Arenaviridae*, which includes Lassa, JUN, and Machupo viruses (LeDuc 1989).

At least 20 related enveloped viruses (23) divided into 2 groups: Old World (5 viruses: Lassa, lymphocytic choreomeningitis (LCM), Mobala, Mopeia, Ippy); New World (Tacaribe complex); 3+ antigenic subgroups within the Tacaribe group- Allpahuayo, Amapari, Bear Canyon, Cupixi, Flexal, Guanarito, Junin, Latino, Machupo, Oliveras, Pampa, Parana, Pichinde, Pirital, Sabia, Tacaribe, Tamiami, Whitewater Arroyo viruses (Enria and Barrera Oro 2002; Carballal, Videla, and Merani 1988).

Family *Arenaviridae* includes 23 viral species, 5 can cause viral hemorrhagic fevers with a case fatality rate of about 20 percent, JUN, Machupo, Guanarito, Sabia and Lassa virus; manipulation requires BSL-4 facilities; other viruses with known human significance- LCM, Flexal, Tacaribe, Whitewater Arroyo (Charrel and de Lamballerie 2003).

Using IFAT, cross reactions between JUNV and LCM viruses; differentiate antibodies between the two viruses using NT (Ambrosio 2001).

C.1.2.6 Tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE)

Host range

a. Field

More than 10 spp of forest rodents positive for virus; 17–20 percent tested had antibodies (Chumakov 2008).

37 percent *Irinaceus roumanicus* (hedgehog) tested had antibodies (Chumakov 2008). Since 1995, TBE virus has been isolated from blood samples of sentinel dogs, ticks, and rodents in Japan (Kunze 2008).

TBE isolates, Japan, 1993, Hokkaido strain, from blood of sentinel dogs, tick pools, and rodent spleens via suckling mouse inoculations (Takashima et al. 2001).

Western European (CEE) TBE virus isolated in Austria from a mouflon (*Ovis ammon musimon*), a ruminant species, infested with *Ix ricinus* (Bago et al. 2002).

In Finland, TBE virus [probably CEE virus or TBEV-W] isolated from red squirrels (*Sciurus vulgaris*), snow hare (*Lepus timidus*), field vole (*Microtus agrestis*), blackbirds (*Turdus merula*), song thrush (*T. philomelos*), yellow hammer (*Emberiza citronella*) (Brummer-Korvenkontio et al. 1973).

In endemic forest area of Russia, seroprevalence of 27 percent HI, 25 percent CF, 25 percent NT antibodies in goats; of 17 percent HI, 29 percent CF antibodies in cattle (Korenberg, Pchelkina, and Spitsina 1984).

Vertical transmission of TBE virus [probably Siberian TBEV] between generations of the small rodents—red voles *M. rutilus* Pallas (previously known as *Clethrionomys rutilus* Pallas) shown for naturally infected reservoir hosts (Bakhvalova et al. 2009). The red

vole is endemic to Alaska but to no other U.S. States (U.S. Department of Agriculture Forest Service).

RSSE/FE-TBE first isolate via IC inoculated mice, 1937, Far East USSR, human, residing in Taiga forest camp (Chumakov 2008).

W-TBE first isolated from humans in Europe in 1948 (Gunther and Haglund 2005).

2008 marked the first time that ticks infected with TBE virus were detected at more than 1,500 meters above sea level; medical authorities recommending vaccination (Leitner 2009).

Ticks act as both the vector and reservoir for TBEV (Leitner 2009).

b. Experimental

Monkeys are not uniformly susceptible to TBEV; no known satisfactory disease model for TBE in monkeys; in bonnet monkeys (*M. radiata*) disease demonstrated with one member of the TBE virus complex, Kyasanur Forest disease virus, but the monkeys did not develop disease consistently when infected with RSSEV or CEEV; subclinical infections of rhesus macaques (*M. mulatta*) were observed after intravenous inoculation of a Turkish strain of a TBE complex virus (Schmaljohn et al. 1999; Kenyon, Rippy, et al. 1992) intranasal infection of adult rhesus monkeys with a member of the TBE complex of flaviviruses mimics the human disease but does not cause pyrexia (Hambleton et al. 1983).

M. radiata infected with RSSE virus developed clinical signs in the central nervous system; RSSE virus was isolated from the brain of one monkey and viral antigen was localized in neurons (Kenyon, Rippy, et al. 1992).

One serious constraint for studying TBE pathogenesis and for vaccine development has been the absence of realistic animal models that mimic the human disease. Mice have

been the animal species most often used for studies of flavivirus behavior in mammals, but the resulting illness bears little resemblance to human infection. Both guinea pigs and several macaque species become viremic after peripheral inoculation of TBE complex viruses, however, clinical illness is mild to absent. Although clinical, virologic, and pathologic evidence of encephalitis has been produced in rhesus macaques by intranasal inoculation of CEE virus, it is likely that infection by this route subverts normal disease pathogenesis by allowing virus to pass directly across the cribriform plate and into the CNS (Kenyon, Rippy, et al. 1992).

TBEV-W, sq, in Rhesus monkeys, at 10^3 $SMICLD_{50}$ or 10^9 $SCICLD_{50}$—inapparent infection—no clinical signs, histopathologic lesions, or viremia, but antibodies produced (Slonim and Zavadova 1977).

Vertical transmission of TBE virus [probably Siberian TBEV] between generations of the small rodents—red voles *M. rutilus* Pallas (previously known as *Clethrionomys rutilus* Pallas) shown experimentally with different sublethal doses of viral strains. In wild red voles and progeny born 240–280 days after experimental infection of their parents, the TBEV was detected in up to 90 percent of samples by RT-PCR, ELISA and bioassays. Small amounts of viral RNA found in embryos, placenta, and blood cells could serve as evidence of prenatal transmission. Postnatal transfer of virus might occur through rodent milk (Calisher 1988).

Prevalence/incidence/attack rate

Prevalence of ticks infected with TBEV in endemic areas in Europe usually varies from 0.5 percent to 5 percent, but in some regions of Russia, a prevalence of 40 percent has been reported (Dumpis, Crook, and Oksi 1999).

51% of humans living in the taiga have Nt antibodies to RSSE virus (Gresikova 1989).

Since 1990, more than 157,500 cases of TBE [caused by the CEE virus] were recorded in Europe, corresponding to 8,755 cases annually. Closely related virus in Far Eastern Eurasia,

RSSE virus, is responsible for a similar disease with a more severe clinical course (Leitner 2009).

TBE is one of the most dangerous human infections occurring in Europe and many parts of Asia, and is believed to cause at least 11,000 human cases of encephalitis in Russia and about 3,000 cases in the rest of Europe annually (Gritsun, Lashkevich, and Gould 2003).

Total number of annual cases tabulated in Western European countries has averaged 3000 for the past 5 years (Gritsun, Lashkevich, and Gould 2003).

In 1990s most of 6,000–10,000 annual TBE case numbers from Russia and increasing annually (1990 = 5,486, 1993 = 7,893, 1996 = 9,548) (Takashima et al. 2001).

TBE is the most important flavivirus infection of the CNS in Europe and Russia, with 10,000–12,000 people diagnosed annually (Gunther and Haglund 2005).

W-TBE endemic in scattered areas within central, eastern and northern Europe (Gunther and Haglund 2005).

TBE incidence increases from West [Europe] to East [Russia, Siberia]; in some areas of European Russia and Scandinavia, up to 184 cases/100,000 inhabitants; seroprevalence ranges from 22 percent to 83 percent (Alciati et al. 2001).

In the past 10 years, incidence of TBE has increased in virtually all European countries where TBE is endemic. Between 1974 and 2003, the number of reported cases increased by 400 percent with further increases between 2004 and 2006 to the highest incidences ever reported. Between 1993 and 2006, the number of reported clinical TBE cases in Germany and Switzerland increased by 200 percent, in Poland by more than 60 percent, Czech Republic, Slovenia, and the Baltic States are most affected [probably CEE virus] (Kunze 2008).

Incidence of TBE varies from year to year in different geographic regions; Pre-Ural and Ural region and Siberia have the highest records of hospitalized cases—during the 1950s and 1960s, TBE was recorded primarily in forest workers, reaching 700–1,200 cases annually; thereafter, up to 11,000 recorded cases per year among urban dwellers who became infected when they visited the local forests. 35–45 percent of all infected persons are children from 2.5 to 12 years old (Gritsun, Lashkevich, and Gould 2003).

Average incidence: Latvia, 30 cases/100,000, Estonia, 16.5/100,000, Slovenia, 14/100,000, Lithuania, 11.2/100,000, Kemerovo Russia, 20.5/100,000, Tomsk Russia, 72.5/100,000, southern Germany, 2/100,000 (Kaiser 2008).

Russia, 1990s, incidence 6.8 cases/100,000 and ranges up to 72.5/100,000 in Tomsk (Kaiser 2008).

Highest incidence is registered in Latvia and in the Urals and the Western Siberian regions of Russia, where attack rates might reach 115–199 reported cases per 100,000 inhabitants per year (Dumpis, Crook, and Oksi 1999).

Highest incidence recorded in Austria, Czech Republic, Hungary, Slovakia, Slovenia with several hundred cases annually in each country; dramatic decrease in Austria because of its vaccination program (Avsic-Zupanc et al. 1995).

Table C-11. Change in TBE incidence in selected European countries, 1979-1999

Country	Number of TBE cases		% Change
	1979	1999	
Austria	677	41	−94
Sweden	23	53	+130
Switzerland	41	108	+163
Lithuania	41	171	+317
Estonia	35	185	+429
Germany	11	115	+945

Data from the World Health Organization. [WHO[16]].

Source: (Banzhoff, Broker, and Zent 2008)

Note: Austria has an active vaccination policy

Currently, 10,000–12,000 cases of encephalitis caused by all subtypes of TBEV (W-TBEV, FEKFDV, TBEV and S-TBEV) are reported annually (Gunther and Haglund 2005).

Between 1990 and 2007 a total of 157,584 TBE cases were documented. In Europe without Russia, a total of 50,486 cases. Average of 8,755 cases per year in Europe within this 18-year period, or 2,805 cases in Europe excluding Russia (Süss 2008).

R_0/incubation period/infectious period/infectious dose

Person-to-person transmission has not been reported (Leitner 2009).

Vertical transmission from mother to fetus has occurred (Seligman and Morozova 2009; Leitner 2009).

Incubation period is 7–14 days (Takashima et al. 2001; Alciati et al. 2001).

Incubation period is 4-28 days (Banzhoff, Broker, and Zent 2008) (Lindquist and Vapalahti 2008) with a median of 8 days (Lindquist and Vapalahti 2008; Kaiser 2008).

The infectious dose in humans unknown.

Morbidity/case fatality ratio

Serosurveys concerning TBEV (RSSE) suggest that between 70 and 95 percent of human infections in endemic regions are subclinical (asymptomatic) (Gritsun, Lashkevich, and Gould 2003).

RSSE is a severe human disease with a case-fatality rate of 8–54 percent (Gresikova 1989).

CEE is a relatively mild disease with a case-fatality rate of 1–5 percent (Gresikova 1989).

Three subtypes have mortality rates of 1–2 percent [European—CEE], 6–8 percent [Siberian], and 20–60 percent [Far Eastern], respectively, indicating that there are important [virulence] differences among them (Seligman and Morozova 2009).

Death rate from Western virus is up to 4 percent and patients with encephalitis, 5 percent; from Eastern virus, average of 20 percent and patients with encephalitis, 60 percent; death rate from CCHF virus might reach 50 percent (Alciati et al. 2001).

Case fatality rate for Far Eastern subtype (RSSE virus) = 5–20 percent, and 5–30 percent survivors with permanent paresis; for Western European subtype (CEE virus), CFR = 0.5–2.0 percent with 2–10 percent permanent paresis (Schmaljohn et al. 1999) (Takashima et al. 2001). Lethality of TBE in Europe is 0.5 percent and a post-encephalitic syndrome is seen in more than 40 percent of affected patients (Gunther and Haglund 2005).

Mortality rate with FE-TBEV = 15–20 percent, with W-TBEV, 1–4 percent (Kaiser 2008). Western Siberia, high morbidity and frequency of tick bites—2.6–5.7 percent of bitten persons hospitalized with TBE; children younger than 14 years = 20–30 percent cases, working adults at highest risk, disease becomes chronic in 1–1.7 percent, mortality varies from 1.8–3 percent (Poponnikova 2006).

With TBEV-FE, CFR = 20–40 percent with seroprevalence of 1–20 percent in Europe and 20–40 percent in Russia; with TBEV-S, CFR= 2–3 percent (Lindquist and Vapalahti 2008).

In Russia, CFR of 30–60 percent (Gould and Solomon 2008).

CFR in Siberia of TBEV-S is 6–8 percent (Gritsun, Lashkevich, and Gould 2003).

Fatality rate of Western type TBEV is low (approx 1 percent), morbidity rate high, and long-term neurologic sequelae common, but 2/3 of infections inapparent (Avsic-Zupanc et al. 1995).

CFR (RSSE) = 30–60 percent (Gould and Solomon 2008).

Aerosol infection/routes of transmission

Person-to-person transmission has not been reported (Leitner 2009).

Infection can follow consumption of raw milk from infected goats, sheep, or cows (Leitner 2009).

Laboratory infections common before the use of vaccines and availability of biosafety precautions to prevent exposure to infectious aerosols (Leitner 2009).

1967, lab infections reported [in the absence of appropriate biocontainment precautions] 18 cases, 2 deaths (Hanson et al. 1967; Pike 1979).

Eight laboratory infections [in the absence of appropriate biocontainment precautions] reported to SALS, zero deaths; infections via aerosol (Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses 1980).

Vertical transmission from an infected mother to fetus has occurred (Leitner 2009).

Humans are infected in rural areas through tick bites (Leitner 2009).

Infection can persist, particularly with the Siberian subtype (Seligman and Morozova 2009).

Vertical transmission in laboratory animals demonstrated to be widespread; virus detected for five generations in small rodents without involvement of arthropod vectors (Seligman and Morozova 2009).

Aerosols either as water droplets or in the form of powder present an infectious hazard in the laboratory and should be avoided by appropriate [bio]containment (Gritsun, Lashkevich, and Gould 2003).

Humans walking through dense vegetation in forests are most likely to become infected following the bite of an infected tick (Gritsun, Lashkevich, and Gould 2003).

Consumption of goat milk; experimentally demonstrated that TBEV can be isolated from the milk of goats for 5–25 days following infection and the infectivity survives in various milk products such as yoghurt, cheese and butter; drinking infected milk might cause biphasic milk fever (Gritsun, Lashkevich, and Gould 2003).

TBEV complex viruses are secreted into the feces and urine of infected mice; possible route of infection (Gritsun, Lashkevich, and Gould 2003).

Laboratory infections are associated with accidental needle-stick injuries during injections of animals. Aerosol infections among laboratory personnel were reported in laboratories when glass bottles containing high virus concentrations were accidentally broken in walk-in incubators (Gritsun, Lashkevich, and Gould 2003).

France, 2003, cases linked to consumption of imported goat cheese; via breast milk reported (Randolph 2008).

Slovenia, handling of TBE viruses potentially hazardous, as indicated by number of LAIs in the pre-vaccination era. Laboratory-acquired, full-blown TBE was reported in a microbiologist who isolated the virus from blood sample of tick-bite patient, probably acquired by aerosol. The

causative virus strain was isolated in Slovenia similar to European prototype strain [CEE virus= Western TBEV= TBE (Western type) = TBEV Western European] (Avsic-Zupanc et al. 1995). Transmission to humans via ixodid ticks in moist, lush, organic under burden of forests; 3,500–10,000 cases/yr in Russia with estimated 5 percent infection rate in ticks (Gould and Solomon 2008).

Virus titers/concentrations/pathogenesis

In Vero, BHK-21, pig kidney cells, 10^6–$3x10^7$ $CCID_{50}$/mL in roller bottles/flasks (Johnson 2008).

In tick cell lines from *Ix ricinus* and *Ix scapularis*, TBE, European subtype replicates to titers of $10^{7–8}$ PFU/mL; in *Boophilus microplus*, *Ornithodorus moubata*, and *Rhipicephalus appendiculatus* cell lines, $10^{4–6}$ 10^6 PFU/mL (Ruzek et al. 2008).

Chick embryo or pig embryo cell cultures produce virus titers to 10^7–10^8/MID_{50}/mL (Chumakov 2008).

Newborn, IC and IP, death, titers 10^7–10^9 ID_{50}/mL [test system not reported]; weanling mice, IC, death, titers 10^7–10^8 ID_{50}/mL [test system not reported], IP, 10^6–10^7 ID_{50}/mL [test system not reported] (Chumakov 2008).

3–4 wk Syrian hamster, IP and IC, death, 10^5–10^8 ID_{50}/mL [test system not reported] (Chumakov 2008).

1–2 d white rats, IC, death, $10^{7.2}$ ID_{50}/mL [test system not reported] (Chumakov 2008).

3–4 wk pigs, death, 10^7 ID_{50}/mL [test system not reported] (Chumakov 2008).

Guinea pigs, IC, death, 10^4–10^6 ID_{50}/mL [test system not reported] (Chumakov 2008).

Chick embryos, 10^6–10^8 ID_{50}/mL [test system not reported] (Chumakov 2008).

Virus replicates in many cell culture systems (Gresikova 1989).

Chick embryo and pig embryo cell cultures, 10^7 to 10^8 MID_{50}/mL (Chumakov 2008).

Mice inoculated SQ or IC with 10,000 focus forming units (FFU) of Hokkaido isolate produced more than 10^9 FFU/gm of brain (Takashima et al. 2001).

Sheep, death; rhesus monkey, paralysis; no virus titers reported (Chumakov 2008).

In *M. radiata*, no viremia and no RSSE virus detected by plaque assay in organs; blind passage of brain tissue, IC, in suckling mice caused death and high viral titers isolated from their brains; virus identified as RSSE virus by neutralization with specific antiserum (Kenyon, Rippy, et al. 1992).

Siberian strain of TBEV, passage in mice = $10^{7.5}$ $MICLD_{50}$/mL (Bakhvalova et al. 2009).

Pathogen stability

Stable for at least 6 hours in liquid aerosol suspension at room temperature and 23–80 percent humidity (Gritsun, Lashkevich, and Gould 2003).

The D_{37} value for West Nile virus was calculated to be 24 [HL=56 min at maximum solar UV conditions](Lytle and Sagripanti 2005). RSSE virus and West Nile virus are classified in the genus *Flavivirus*.

Virions stable for up to 2 hours in normal gastric juice at pH 1.49–1.80 and in gastric juice with reduced acidity (pH 1.87–2.21); in gastric juice taken from humans after a meal (pH 2–7) the virus is stable for 2 hours. Milk foods move out of the stomach quickly (the first milk consumed reaches the duodenum within minutes, and after 1.5–2 hours, there is no milk in the stomach). Hydrochloric acid is secreted in the stomach between 45 minutes and 60 minutes after consumption of milk. The human digestive tract is an efficient route of infection (Gritsun, Lashkevich, and Gould 2003).

Survives in milk for prolonged periods, even when passing through the acidic stomach environment (Kaiser 2008).

Heat labile and inactivated by pasteurization (Kaiser 2008).

Ether and deoxycholate reduce virus titer by 50 percent in 24 hours (Chumakov 2008).

I M $MgCl_2$ at 25 °C inactivates virus within 6 days (Chumakov 2008).

Readily inactivated by organic solvents and detergents; nonionic detergents such as Triton X solubilize the entire envelope releasing M and E proteins. Sodium deoxycholate appears to remove only E, leaving M associated with the nucleocapsid (Gritsun, Lashkevich, and Gould 2003).

Envelope protects the genome from cellular nucleases. The naked nucleocapsids released by detergent treatment are degraded by ribonuclease. Purified naked RNA infectious following direct intracerebral injection of mice (Gritsun, Lashkevich, and Gould 2003).

Infectivity optimally stable at pH 8.4–8.8; residual infectivity over the broader pH range 1.42–9.19 being roughly comparable with the stability of enteroviruses; at acidic pH the E protein undergoes specific conformational changes that reduce virus infectivity, virions remain infectious in curdled milk and gastric juice, which could explain why the virus can infect via the alimentary route (Gritsun, Lashkevich, and Gould 2003).

Rapidly inactivated at 50 °C, 50 percent of infectivity lost in 10 minutes. The total inactivation of the virus suspended in blood or other protein solutions occurs within 30 minutes at 56 °C (Gritsun, Lashkevich, and Gould 2003).

Ultra-low temperatures preserve infectivity almost indefinitely (Gritsun, Lashkevich, and Gould 2003).

In freeze-dried form, they survive almost indefinitely at room temperature (Gritsun, Lashkevich, and Gould 2003).

Inactivated by UV light, gamma-irradiation, and disinfectants, including 3–8 percent formaldehyde, 2 percent glutaraldehyde, 2–3 percent hydrogen peroxide, 500–5,000 ppm available chlorine, alcohol, 1 percent iodine, and phenol iodophors (Gritsun, Lashkevich, and Gould 2003).

Indication that viruses with structural lipids survive best in aerosols at low RH (Benbough 1971). UV radiation inactivates viruses by chemically changing the RNA and DNA; most effective UV is 250 nm; filoviruses [MAR and EBOV] most sensitive to solar UV_{254}, requiring 20–100 minutes at mid-day exposure to inactivate 1 $\log_{10}$ of virus: bunyaviruses (hantaviruses [AND virus] and RVFV), arenaviruses (Lassa, JUN), and flaviviruses (TBE complex viruses) more resistant (Lytle and Sagripanti 2005).

Vectors

The virus can chronically infect ticks and is transmitted both transtadially (from larva to nymph to adult ticks) and transovarially (from adult female tick through eggs (Leitner 2009).

Relatively ancient divergence separates the western populations of the North American *Ixodes* deer ticks from the eastern array of populations, and another apparently more recent divergence separates the northeastern from the southeastern arrays of ticks. Indeed, those in the western clade, designated *Ix pacificus*, are more closely related to Eurasian *Ix persulcatus* than to the more *Ix ricinus*-like ticks of eastern North America [phylogenetic relationships do not necessarily reflect potential ability of the North American tick species to transmit TBE viruses; ticks in MA are genetically more closely related to *Ix ricinus*, the primary vector of CEE virus] (Rich et al. 1995).

Canadian and northern United States, *Ixodes scapularis,* among other *Ixodes* species, are reservoirs and vectors of Powassan virus, which is closely related to viruses of the TBE-complex (Turell 2008).

It is likely that indigenous mice would be competent hosts for amplification of viruses of the TBE complex (Turell 2008).

Ticks flourish best at a humidity above 85 percent, in temperatures of 6–7 °C, and in the presence of large numbers of blood-delivering hosts; in many areas in Europe, those three basic requirements have been changing in favor of the tick population allowing tick movement northward and upward, now inhabiting mountainous areas higher than 1,000 meters above sea level. The number of tick life cycles will increase, tick habitats will expand, and the density of the tick population will continue to rise (Kunze 2008).

In Russia, the range of taiga ticks has been extending southward during the 1990s (Poponnikova 2006).

a. Field

Ixodes persulcatus ticks transmit RSSE virus (Gresikova 1989).

Ix ricinus ticks transmit CEE virus (Gresikova 1989).

Many isolates from *Ixodes* spp, *Dermacentor* spp, and *Hyalomma* spp ticks(Chumakov 2008).

Isolates from Laelapid (Gamasoid) mites (Chumakov 2008).

Most important tick spp. in Europe: *Ixodes persulcatus* and *Haemaphysalis spinigera*, responsible for transmission of RSSE virus; *Ix ricinus*, responsible for transmission of middle European TBE virus; and *Hyalomma* spp, responsible for transmission of CCHF virus (Alciati et al. 2001).

Middle European or Western subtype of TBE virus transmitted by *Ix ricinus* and RSSE (Eastern or Russian TBE virus) transmitted by *Ix persulcatus* (Alciati et al. 2001).

Ticks remain infected throughout their life cycle and transmit the virus to uninfected ticks when co-feeding on small wild rodents (Gritsun, Lashkevich, and Gould 2003).

Persistence in ticks occurs via viremic, transstadial, or transovarial transmission. Transmission of virus from infected to noninfected ticks occurs when they co-feed on the same host (virus replicates at a local skin site), and it is not necessary for the host to develop a detectable viremia (Gritsun, Lashkevich, and Gould 2003).

Isolated sporadically from at least 15 other ticks species and from flies, fleas, lice (Gritsun, Lashkevich, and Gould 2003).There is a report of virus having been isolated from mosquitoes (Gritsun, Lashkevich, and Gould 2003). However, Dr. M. Turell (USAMRIID) states that he knows of no reproducible experimental work showing any mosquitoes species capable of being infected with TBE viruses. He states there is one discredited report from the former Soviet Union in older literature (Turell 2008).

High prevalence of *Ix persulcatus* correlated more frequently with severe and fatal cases; milder cases were recorded in years when *Hy concinna* was the dominant tick species. When these tick species were at equivalent levels in any one focus, there was a relatively similar proportion of severe and mild forms of TBE. Ticks can select high and low-virulence TBEV strains [also demonstrated experimentally] (Gritsun, Lashkevich, and Gould 2003).

Main vector in Japan is *Ix ovatus* (Kunze 2008).

Principal vector of the European TBEV subtype is the tick *Ix ricinus* ; virus infects ticks chronically for the duration of their life and circulates between ticks and their vertebrate hosts. The virus is also transmitted transovarially and during co-feeding of ticks on the same host (Ruzek et al. 2008; Labuda and Nuttall 2004).

Ix trianguliceps, *Ix hexagonus*, and *Ix arboricola* are considered amplifying vectors. Secondary vectors, *Haemaphysalis inermis* and *Dermacentor reticulatus* exhibit lower

transmission rates. The virus was isolated from *Hae inermis* in the Czech Republic (Ruzek et al. 2008).

TBE virus was isolated from *Ix ricinus* nymphs (Danielova et al. 2002).

The virus spread in ticks includes transovarial, transtadial, sexual, and nonviremic transmission (Bakhvalova et al. 2009).

b. Experimental

There is no known experimental work (as of October 2008) assessing whether the viruses of TBE complex can survive in tick species indigenous to North America, and there is no reproducible work showing mosquitoes capable of being infected (Turell 2008).

In *Ix persulcatus* ticks that fed on viremic mice, titers reach 10^6 MLD_{50} (Gresikova 1989).

Transstadial and transovarial infection demonstrated in ticks (Gresikova 1989). Sexual transmission demonstrated in ticks from infected *Ix persulcatus* males to noninfected females (Gresikova 1989).

Haemaphysalis spinigera and *Hae turturis* transmit TBEV under laboratory conditions, *D marginatus* and *D reticulatus* are possible vectors, though infection and transmission rates were lower than in vector species of the genera *Ixodes* and *Haemaphysalis* (Ruzek et al. 2008).

Saliva-activated viremia demonstrated when ticks feed on infected hosts; non-viremic transmission from infected to noninfected *Ix ricinus* during co-feeding (Danielova et al. 2002; Labuda and Nuttall 2004).

Other epidemiological/ecological data

Cases occur during the highest period of tick activity (between April and November) [seasonal activity](Leitner 2009).

Despite their names [European, Siberian, Far Eastern] , all three subtypes co-circulate throughout most of the TBEV endemic areas. The Siberian subtype dominates in many endemic regions from Eastern Europe to Eastern Siberia (Seligman and Morozova 2009).

Maintained in a natural cycle involving ticks and wild vertebrate hosts in forests of Europe and Asia where ticks find high humidity in dense undergrowth (Gritsun, Lashkevich, and Gould 2003).

The TBE virus is endemic in several non-European countries (Mongolia, China, Japan, and South Korea); Heilongjiang province in the northeast of the People's Republic of China, 2,202 cases between 1980 and 1998, 420 of them between 1995 and 1998; cases in Japan (Kunze 2008).

Ticks transmit a greater variety of pathogenic microorganisms than any other arthropod vector group; TBE is the most important tick-borne viral disease of humans in Europe and Asia; distribution of TBEV extends from Western Europe to China and Japan, Hokkaido strain (Ruzek et al. 2008).

TBE virus isolates from Japan, 1993, Hokkaido strain, are the same as Russian subtype (Takashima et al. 2001).

Overview of tick-borne encephalopathies in humans (Gunther and Haglund 2005).
FE-TBE is endemic in Latvia, Russia, Asia, Japan; FE-TBE in eastern Russia more severe than other TBE types (Gunther and Haglund 2005).

Satellite imagery used for prediction of tick seasonal dynamics and presence of TBE (Gunther and Haglund 2005).

Table C-12. TBE subtype locations, vectors, and conditions

Pathogen	Region	Vector	Condition
Western TBEV	Central, eastern/northern Europe, Russia, Baltic States	*Ix ricinus, Ix persulcatus*	Meningo-encephalitis, spinal nerve paralysis
Siberian TBEV	Russia, eastern Europe	*Ix persulcatus*	Meningo-encephalitis, spinal nerve paralysis
Far-eastern TBEV	Eurasia, Asia	*Ix persulcatus*	Meningo-encephalitis, spinal nerve paralysis

W-TBE isolates homogeneous across all European endemic areas; S-TBEV and FE-TBEV activity overlap in Asian Russia and China; 96 percent identity in E-protein amino acid sequences between the S- and FE-TBEV and the W-TBEV (Kaiser 2008).

Once considered a local health issue in certain regions of Russia and Central/Eastern Europe, TBE is now an international health problem with expansion of endemic areas and rapidly increasing numbers of cases and due to travel to and pursuit of leisure activities into high-risk areas (Banzhoff, Broker, and Zent 2008).

W-TBEV, endemic in scattered areas in central, eastern, and northern Europe from the Alsace-Lorraine area in the west to Scandinavia to the north to Italy, Greece, and Crimea in the south; FE-TBEV found in Russia, northeastern China, parts of northern Japan, and as far west as the Baltic states; S-TBEV (Siberian) found westward to Finland; all three subtypes co-circulate in Estonia (Banzhoff, Broker, and Zent 2008).

Reservoirs are wild living vertebrates capable of transmitting infection (reservoir competence). In the natural foci, these reservoirs need to be available in high numbers coupled with a high-reproduction rate and a rapid generation change. In case of TBEV, they need to be receptive to the virus and enable virus multiplication, including viremia, for a long period of time with high-virus titer without becoming clinically ill at the same time. The significance of antiviral immunity of the vertebrate host for virus transmission has changed during the last years. In

contrast to a vector, Spielman et al. defined a reservoir host as the passive member in the vector–reservoir relationship. *Indicator hosts* cannot transmit the virus to other vectors as they can endure only brief viremia with low-virus titers. Indirectly, they support virus circulation by enabling the ticks to multiply in masses (roe, deer). In addition, they are highly valuable sentinels for antibody detection in epidemiological studies. *Accidental hosts* can be infected by the pathogen and can develop viremia, in case of TBEV. In general, they do not participate in virus circulation (Suss 2003).

More than 70–95 percent of human infections are subclinical (Gritsun, Lashkevich, and Gould 2003).

Through vertical transmission among generations of its adapted mammal reservoir hosts, virus transfer might occur before, during, or after birth of the small rodents with high frequencies. In the wild it could provide the TBEV long-term persistence in mammal hosts without involvement of arthropod vectors thus selecting dangerous mammal-adapted variants (Bakhvalova et al. 2009).

TBEV circulate harmlessly in forests, on moorlands, in steppe regions of Europe and Asia until humans enter the ecosystem; normal transmission between ticks and rodents/other animals; eastern strains now found as far west as Latvia possibly carried via ticks on migratory birds (Gould and Solomon 2008).

Therapeutics/vaccines

Formalin-inactivated mouse brain origin vaccine used in the USSR (Gresikova 1989).
Formalin-inactivated embryonating hen's egg origin vaccine used in the USSR (Gresikova 1989).

Formalin-inactivated chick embryo cell culture origin vaccine used in the USSR (Gresikova 1989).

Only successful way of prevention [western Europe] is active immunization (Kunze 2008).

European TBE virus vaccine effective in Japan in humans against Hokkaido strain (Takashima et al. 2001).

Specific immunoglobulin used in Russia; several vaccines used in Europe and Russia. Purified, formalin-inactivated virus vaccine (FSME Immune Inject™) is used effectively in Europe (Takashima et al. 2001).

Two inactivated vaccines (FSME - IMMUN™, Baxter Vaccine AG and Encepur™, Chiron) licensed in Europe for active immunization against TBEV: (Gunther and Haglund 2005).

- Austrian vaccine (predecessor related to FSME-IMMUN) active against both W- and FE-TBEV was introduced in 1976; FSME-IMMUN vaccine introduced in 2001—product based on a formalin-inactivated Austrian W-TBEV, propagated on primary chick embryo cells with several modifications, most importantly a switch from propagation on mouse brain cells to chick embryo cells, highly purified using continuous-flow zonal ultracentrifugation; contains 2–3.5µg of viral antigen; protection rate of from 94 to greater than 98 percent reported (Gunther and Haglund 2005).
- German vaccine (Encepur), 1991, W-TBE strain, contains 0.1µg of viral antigen; protection rate not reported; chick embryo cell culture-derived (Gunther and Haglund 2005; Schmaljohn et al. 1999).
- Both the German and Austrian vaccines induce neutralizing antibodies against both the W-TBE and FE-TBE viruses (Gunther and Haglund 2005).

Rhesus macaques were vaccinated by gene gun inoculation of DNA vaccines expressing the *prM* and *E* genes of RSSEV, CEEV, or both DNA vaccines. The antibody titers similar in monkeys vaccinated with both DNA vaccines or with inactivated virus vaccine (Austria), protective immunity, as measured by passive transfer of serum from monkeys to mice, also appeared very similar. All mice that received sera from monkeys vaccinated with both DNA vaccines or in groups that received sera from monkeys vaccinated with the inactivated-virus vaccine remained healthy after RSSEV challenge [monkeys do not respond predictably to infection with TBEV, so vaccine efficacy was measured by cross-protection of mice via serum] (Schmaljohn et al. 1999).

From vaccine studies, presence of virus-neutralizing antibodies in serum provides an excellent correlate for protection against virus challenge. Protection by TBE vaccination in Austria ranges to 99 percent with no significant differences between age groups (Heinz et al. 2007).

Inactivated vaccine prepared after continuous-flow zonal ultracentrifugation produce fewer problems that other inactivated vaccines (Gresikova 1989).

Attenuated vaccines have been used for CEEV in cattle to prevent human infection from consumption of raw milk (Gresikova 1989).

Human immune serum used for prophylaxis (Gresikova 1989).

Noninfectious, self-replicating RNAs generated by deletion mutagenesis of the capsid protein C represent a promising new future for TBE virus vaccines. The study used a Western subtype of TBE virus [probably a strain of CEE virus]. No indication that the vaccine-induced antibody will protect against RSSE virus (Kofler et al. 2004).

New genetic vaccine based on self-replicating, noninfectious RNA, which contains the necessary genetic information to establish its replication machinery in the host cell, thus mimicking a natural infection. Genetic modifications in the region encoding the capsid protein simultaneously prevents the assembly of infectious virus particles and promotes the secretion of noninfectious subviral particles that elicit neutralizing antibodies. Mice challenged IP with more than 1,000 LD_{50} of TBEV-W (Kofler et al. 2004).

DNA vaccine TBEV tested in mice alone or in various combinations; TBEV expressed preM and E genes; vaccine delivered by gene gun; TBEV DNA vaccine elicited antibodies and protected mice from challenge when delivered alone or in combination with other DNAs (Spik et al. 2006).

Other remarks

RSSE first described in 1932 as a severe encephalitis in humans residing in the far eastern regions of the Russia [USSR] (Gresikova 1989).

CEE first described in 1949 as a mild form of RSSE in the European USSR and eastern Europe (Gresikova 1989).

Tick transmitted encephalitis was first described in Austria in 1927, [CEE] virus isolated in 1937 (Alciati et al. 2001).

SALS level 4 (Chumakov 2008).

Caused by TBEV, previously known as RSSE virus; discovered in 1937 in far-east Russia (Gritsun, Lashkevich, and Gould 2003).

Flaviviruses transmitted by *Ixodes* spp ticks cause neurologic or hemorrhagic disease (Gould and Solomon 2008).

Viruses in the TBE virus complex can produce mild febrile illness, biphasic fever, encephalitis, or hemorrhagic fever in humans (Gould and Solomon 2008).

Note: While the RSSE virus is the NEIDL/BU/NIH preferred pathogen, frequently in the literature no distinction is made between RSSE virus, CEE virus, and the other viruses in the TBE virus complex. While the literature is replete with references to *TBE virus*, there is no single TBE virus, and there is no official registration of or recognition of a virus by that name in the Arbovirus Catalog, which stands as the authoritative publication on arthropod-borne viruses. The point is not academic because there are substantial differences in virulence and pathogenicity among the viruses in this complex (Calisher 1988).

Taxonomy/antigenic relationships/synonyms

Family Flaviviridae, genus *Flavivirus* (Gresikova 1989).

TBE virus complex [close antigenic relationship between RSSE and CEE viruses with different primary tick vectors] (Gresikova 1989):

- RSSE = Spring-summer TBE; Far East encephalitis; forest spring encephalitis; Far Eastern TBE; Taiga encephalitis; Far Eastern Russian encephalitis; TBE (Eastern type) (Gresikova 1989);
- CEE = TBE; Central European TBE; Czechoslovak TBE; diphasic milk fever; biphasic meningoencephalitis; TBE (Western type) (Gresikova 1989).

Two antigenic complexes (Gresikova 1989):

- Tyuleniy complex;
- RSSE virus complex: RSSE virus; Powassan virus; Karshi virus; Royal Farm virus;

Seven subtypes of RSSE virus are recognized (Gresikova 1989).

- RSSE virus in Far Eastern Russia;
- CEE virus in central and western Europe;
- Kumlinge virus in Finland;
- Louping ill virus in the British isles (Scotland, England, Ireland);
- Omsk hemorrhagic fever in western Siberia;
- Kyasanur Forest disease in India;
- Langat virus in Asia.

Although a new subdivision of the virus species into four subtypes has been suggested, the currently accepted classification (ICTVdB - The Universal Virus Database, version 3. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/ does not separate TBEV into two species but lists three subtypes: European, Siberian, and Far Eastern (Seligman and Morozova 2009). TBEV species includes three subtypes, Far Eastern (previously RSSE), Siberian (previously west-Siberian) and Western European (previously Central European Encephalitis, CEE) virus (Gritsun, Lashkevich, and Gould 2003).

In recent literature [~2000 and on], TBE virus = RSSE virus (Gritsun, Lashkevich, and Gould 2003).

TBE virus taxonomically classified into European, Siberian, and Far Eastern subtypes (Ruzek et al. 2008).

Far Eastern subtype = RSSE virus, CEE virus = Western European subtype (Takashima et al. 2001).

Antigenically related TBE viruses (Gunther and Haglund 2005; Kaiser 2008):

- Central European encephalitis W-TBEV or Western TBEV (W-TBEV);
- Siberian subtype TBEV (S-TBEV);
- Russian spring-summer encephalitis virus or Far Eastern TBEV (FE-TBEV);
- Louping-ill virus;
- Powassan virus (POWV);
- Omsk hemorrhagic fever virus (OHFV);
- Kyasanur forest virus (KFDV);
- Langat virus.

Nipah virus (NIPV)

Host range

a. Field

Broad species tropism and highly pathogenic (McEachern et al. 2008).

Flying fox (fruit bats)—*Pteropus* sp natural reservoir (Chua et al. 2002).

Grey-headed fruit bats (*P. poliocephalus*) a reservoir species for NIPV (Weingartl, Berhane, and Czub 2009).

Flying foxes (*Pteropus* sp.), the natural reservoir for the NiV (Blum et al. 2009).

Pigs (Aljofan et al. 2009).

Dogs and cats are susceptible to NIPV infection. In Malaysia, 46–55 percent of the domestic dogs living in the regions of the epidemics were seropositive (Wild 2009). Cats (Torres-Velez et al. 2008).

Dogs, cats, horses, and goats (pigs being the original source for these animals) as dead end hosts (Chua 2003).

Dead-end hosts: dogs, cats, ferrets, pigs, horses (Lo and Rota 2008).

Serological evidence of infection with NIPV in the field in humans, pigs (amplifying host), dogs, cats, horses, and goats (Weingartl, Berhane, and Czub 2009) (Chua 2003).

Bats (flying foxes) serve as natural host but also naturally infect humans, cats, dogs, and pigs (Eaton, Broder, and Wang 2005).

Evidence of NIPV found in four fruit bat species and one insectivorous bat in Malaysia—also evidence of Cambodian bats being infected as well as pigs, dogs, and cats (Bellini et al. 2005).

Natural reservoirs: *Pteropus* sp as well as non-*Pteropus* sp from Cambodia, Thailand, Indonesia, Bangladesh, and Madagascar (Lo and Rota 2008).

In Malaysia, 46–55 percent of dogs and cats in the epidemic regions were seropositive for NIPV while in other areas they were sero-negative (Wild 2009).

In some regions (India) 54 percent of bats tested had ab's to NIPV. Laboratory studies with infected bats show they manifest a subclinical infection (Wild 2009).

b. Experimental

8 African green monkeys, oral and/or intratracheal inoc ranging from $2.5 \times 10^3 – 1.3 \times 10^6$. All symptomatic, one survived (Geisbert et al.)

Squirrel monkeys (Marianneau et al.)

Pigs (Berhane et al. 2008).

Female piglets (Weingartl et al. 2005).

Guinea pig, cats, hamsters, mice (did not appear to be susceptible), grey headed fruit bats (seroconversion) (Torres-Velez et al. 2008).

Mice, guinea pigs, hamsters infected with 10^7 PFU IP. Mice and guinea pigs were not susceptible (Wong et al. 2003).

Cat and golden hamster are considered suitable small animal models of NIPV infection (Weingartl et al. 2009).

Feline vaccine model, 5 x 10^3 $TCID_{50}$ given oronasally (McEachern et al. 2008).

Hamster vaccine model (Guillaume et al. 2004).

Golden hamster model became infected with as little as 100 pfu NIPV inoculated intraperitoneally or 1,000 pfu NIPV inoculated intranasally and appeared to reproduce the pathology and pathogenesis of acute NIPV infection in humans, with lesions most severe in the brain (Halpin and Mungall 2007) (Wong et al. 2003).

Golden hamster model mimics human infection (Bellini et al. 2005).

Chicken embryos (Tanimura et al. 2006).

Prevalence/incidence/attack rate

Study of the 24 months after initial outbreak (1998–1999) showed a prevalence of relapsed and late-onset encephalitis (thought to be the same disease entity) of 7.5 percent and 3.4 percent, respectively (Tan et al. 2002).

Survey of NIPV infection among various risk groups in Singapore (after 1999 outbreak): 1.5 percent of people tested had antibodies to NIPV. Of those, 54.6 percent were symptomatic, while 45.4 percent were asymptomatic, historically. All 1.5 percent were abattoir workers (responsible for the slaughter of pigs) (Chan et al. 2002).

Survey of (pig) abattoir workers in Malaysia after the 1998 outbreak showed 1.6 percent prevalence of NIPV antibodies in those tested (Sahani et al. 2001).

Seroprevalence study of 2004 outbreak in Bangladesh shows nosocomial transmission risk small (Gurley, Montgomery, Hossain, Islam, et al. 2007).

R_0/incubation period/infectious period/infectious dose

R_0 was estimated to be 0.48 for outbreaks in rural Bangladesh. Four limitations of the estimate were noted: (1) It is likely that not all human infections were identified; (2) The estimated rate assumed all persons infected by a primary case-patient were identified, which might not have been true; (3) Only 9 persons were identified as having transmitted the virus, so statistical power of the calculations is limited; (4) It was assumed that persons who developed Nipah illness within 5–15 days after exposure to a Nipah patient were infected from that patient rather than from an unrelated environmental exposure; (Luby et al. 2009).

Among the 10 clusters, 5 involved person-to-person transmission ranging from 2 to 5 generations of transmission. The 60 primary Nipah case-patients infected 29 subsequent persons (Luby et al. 2009).

Incubation period ranged from 4 days to 2 months with more than 90 percent of patients giving a history of 2 weeks or less (Chua 2003).

In Sigiluri West Bengal, India 2001 outbreak clear evidence of person–to-person transmission pointing to an incubation period of 5 to 10 days (Harit et al. 2006).

Incubation 2 days to 2 months with most 2 weeks or less (Bellini et al. 2005).

Incubation period in original outbreaks was 4 days–2 months (average 2 weeks) while in additional outbreaks incubation was 6–11 days with an increased frequency of respiratory symptoms, altered mental status, and vomiting (Lo and Rota 2008).

Duration of illness ranged from 2-34 days (Wong, Shieh, Kumar, et al. 2002).

An LD_{50} was determined for guinea pigs. Death required infection by two routes simultaneously, and the LD_{50} for this approach was 270 PFU given by intraperitoneal injection in conjunction with 47,000 PFU given intranasally (Wong et al. 2003).

10^4 $TCID_{50}$ was a lethal dose (as intended) for golden hamsters (control group N=2) (Freiberg et al.).

Morbidity/case fatality ratio

Results from serologic study following the 1999 Singapore outbreak found 22 people with anti-NIPV antibodies indicative of infection with NIPV. Ten of the 22 were asymptomatic, which suggests that clinically asymptomatic infections do occur (Chan et al. 2002).

Asymptomatic or mild infection might occur in 8–15 percent of infected persons, with some suggesting subclinical infection rates up to 60 percent (Sejvar et al. 2007).

Rate of subclinical infection ranged from 8 to 15 percent (Chua 2003).

Estimated symptomatic versus asymptomatic 3 to 1 (Wong, Shieh, Kumar, et al. 2002).

Symptoms consist of fever, headache, myalgia, altered sensorium (confusion to coma), respiratory distress, convulsions, and vomiting (Chadha et al. 2006).

Main presenting clinical features are fever, headache, dizziness, and vomiting with more than 50 percent of the patients having a reduced level of consciousness and prominent brainstem dysfunction (Chua 2003).

Direct cause of death thought to be mainly due to the direct consequence of encephalitis (Chua 2003).

In humans, widespread multisystemic vasculitis with clinical and pathologic manifestations in the brain, lung, and spleen (Torres-Velez et al. 2008).

Fever, headache, dizziness, and vomiting, reduced level of consciousness and prominent brainstem dysfunction nonproductive cough, myalgia, focal neurologic signs (Goh et al. 2000).

NIPV causes a severe, rapidly progressive encephalitis with a high mortality rate and features that suggest involvement of the brainstem. Neurological relapse can occur in mild or asymptomatic cases (Goh et al. 2000).

Infection with NIPV can also take a more chronic course, with serious neurological disease occurring late (in excess of 4 years) following a non-encephalitic or asymptomatic infection (Eaton et al. 2006).

Sudden onset—fever, headache, dizziness, vomiting, and reduced level of consciousness (Bellini et al. 2005).

Poor prognostic factors: brainstem involvement, virus in CSF, diabetes mellitus (Wong, Shieh, Zaki, et al. 2002).

Persistent neurological deficits in 15 percent of survivors (Sejvar et al. 2007).

Relapse or late onset encephalitis appears to be the same disease and is distinct from acute NiV encephalitis and can manifest up to 53 months after initial exposure (Tan and Chua 2008).

Patients infected with NiV excreted the virus in their respiratory secretions and urine (Chua et al. 2001).

The brain was the most severely affected organ, but other organs including the lungs, heart, and kidneys were also involved (Chua 2003).

Widespread vasculitis → thrombosis, vascular occlusion, ischemia and microinfarction in many organs but most severely in the CNS (Wong et al. 2003).

Endothelial cells are a particular target of the viruses (henipaviruses) in all species (Weingartl, Berhane, and Czub 2009).

In the original Malaysian outbreak, the primary pathology was multiorgan vasculitis associated with infection of endothelial cells. Infection was most prominent in the CNS (Chua et al. 2000).

Pathology found is similar to other viral encephalitis but presence of syncytial multinucleated endothelial cells is characteristic of Nipah and Hendra virus infection (Epstein et al. 2006).

Multiorgan vasculitis with infection of endothelial cells most pronounced in CNS (Bellini et al. 2005).

Two distinct clinicopathological forms of NIPV encephalitis: (1) Acute encephalitis occurring 1–2 weeks post exposure; (2) late-onset (relapsed) encephalitis several weeks after acute infection has subsided (Wong et al. 2009).

Main necropsy finding in initial outbreak was disseminated micro-infarction associated with vasculitis and direct neuronal involvement (Tan and Chua 2008).

Original outbreak in Malaysia, 1998, 265 cases of encephalitis resulting in 105 deaths (Aljofan et al. 2009).

In subsequent outbreaks (India and Bangladesh), case mortality rate was approximately 70 percent (Aljofan et al. 2009; Luby et al. 2009).

Eight outbreaks between 2001–2008 avg fatality 70 percent (Blum et al. 2009).

Original outbreak case-fatality rate 38.5 percent (Chua 2003).

Mortality rates as high as 92 percent in later outbreaks, which no longer involved contact with swine (Torres-Velez et al. 2008).

Rapidly progressive encephalitis that carried a high mortality rate (Chua 2003).

Outbreak in Siliguri, India 2001 case-fatality rate 74 percent (Chadha et al. 2006).

Bangladesh, 8 outbreaks between 2001–2008 with average case fatality ratio greater than 70 percent (Blum et al. 2009).

Nearly a 10 percent incidence rate of late encephalitic manifestation, with a mortality rate of 18 percent (Eaton et al. 2006).

Initial outbreaks in Malaysia and Singapore transmission was through close contact with infected pigs and CFR was 38.5 percent. Subsequent outbreaks in Bangladesh and India transmission was food-borne, person-to-person as well as nosocomial with CFR as high as 92 percent in some outbreaks (Lo and Rota 2008).

Aerosol infection/routes of transmission

Bangladesh, eight outbreaks between 2001–2008 with average case/fatality ratio greater than 70 percent; current hypothesis is virus spillover from bats (date palm sap and partially bat eaten fruit

consumption) to humans, then person-to-person spread (requiring close contact, caregiver) (Blum et al. 2009).

"NIPV is highly infectious and transmitted via the respiratory tract, it can be amplified and spread in livestock serving as a source for transmission to humans, and recently it has been shown to be transmitted directly from person to person and via contaminated food" (Aljofan et al. 2009).

"NIPV has continued to re-emerge causing fatal encephalitis in humans and person-to-person transmission has been documented" (Aljofan et al. 2009).

Epidemiological evidence suggests that person-to person transmission occurs, related to the degree of close contact with a highly infectious case (Blum et al. 2009; Luby et al. 2009). Appeared to be direct transmission of the virus from its natural host, the flying fox, to humans(Aljofan et al. 2009).

NiV spills over from bats to people when a person ingests bat secretions that contain virus, for example, by eating a piece of partially eaten fruit dropped by a bat or drinking date palm sap contaminated by a bat (Blum et al. 2009).

In original outbreak transmission of the virus to humans, was through close contact with infected pigs (Chua 2003).

Transmission of the virus from infected human to human and from infected dogs to human has been documented (Chua 2003).

Transmission thought to be of the oro-nasal route (Torres-Velez et al. 2008).

Oral ingestion and/or aerosol inhalation of infected secretions is thought to be responsible for pig-to-human viral transmission (Wong et al. 2003).

"Human-to-human transmission of NIPV was not shown in the Malaysia and Singapore outbreaks, but several findings from the Bangladesh outbreaks suggest that close contact might have resulted in transmission. In Meherpur and in Naogaon Bangladesh), clusters of cases occurred within family households" (Hsu et al. 2004).

In Sigiluri West Bengal, India 2001 outbreak, clear evidence of person-to-person transmission (Harit et al. 2006).

Nipah case patients with respiratory difficulty were much more likely to be Nipah spreaders (Luby et al. 2009).

In 62 (51 percent) case-patients illness developed from apparent person-to-person transmission (Luby et al. 2009).

Transmission from patients to healthcare workers is uncommon (Hsu et al. 2004) (Gurley, Montgomery, Hossain, Bell, et al. 2007).

Malaysia and Singapore outbreaks—swine intermediate host, Bangladesh outbreak case control study supports person-to-person transmission, Siliguri, India, outbreak supports nosocomial transmission (Gurley, Montgomery, Hossain, Bell, et al. 2007).

Food-borne (date palm sap) transmission of the NiV infections in the Tangail district of Bangladesh outbreak of December 2004 (Luby et al. 2006).

Virus titers/concentrations/pathogenesis

Squirrel monkeys (*Saimiri sciureus*), IV or intranasal inoc of 10^3 or 10^7 PFU (3 animals per group); 10^3 PFU intranasal failed to infect (Marianneau et al.),

1 x $10^8 TCID_{50}$/mL cell culture for Hendra (not Nipah) virus (Aljofan et al. 2009; Crameri et al. 2002). Authors state, "Nipah virus replicated and formed syncytia in Vero cells with the same kinetics as that observed with Hendra virus (data not shown)" (Crameri et al. 2002).

In MRC-5 (human lung fibroblast cells) cells Viral RNA peaked at 7.7 log PFU/uL – in PS (porcine stable kidney cells) cell 8.3 log PFU/uL (Chang et al. 2006).

$10^{7.1}$ PFU/mL Vero cells, 10^{7} PFU/mL PT-K75 (porcine turbinate) cells.

50,000 $TCID_{50}$ of a low passage NIPV isolate (EUKK 19817; stock virus titer 4.3 × 106 $TCID_{50}$/mL (McEachern et al. 2008).

Virus titers reached $2x10^{7}$ pfu/mL (Guillaume et al. 2004).

Virus titer of the stock was 3.2 x 10^{7} $TCID_{50}$/ml (Yoneda et al. 2006).

NIPV grows in cultured cells to titers as high as 10^{8} $TCID_{50}$ or PFU/mL (Daniels, Ksiazek, and Eaton 2001).

Virus titer of $2x10^{6}$ (Field et al. 2001).

NIPV stock titers were adjusted to 1 x 10^{6} $TCID_{50}$/mL (Mungall et al. 2008).

$10^{5.2}$ PFU at dose volume 0.1 mL and $10^{3.9}$ at dose volume 0.1 mL (Tanimura et al. 2006).

Gray headed fruit bats, subcutaneous inoculation with 50,000 $TCID_{50}$ of NIPV, guinea pigs inoculated with same dose IP (Middleton et al. 2007).

500 $TCID_{50}$ for SQ inoculation of cats from stock titer 1.1 x 10^{7} $TCID_{50}$/mL (Mungall et al. 2007).

Viral concentrations in tissue of exp. Piglets: Respiratory tract $10^{5.3}$ PFU/g; spleen 10^{6} PFU/g; nervous system $10^{7.7}$ PFU/g of tissue (Weingartl et al. 2005).

In experimentally infected piglets NIPV enters CNS via blood-brain barrier and possibly infects the CNS directly via peripheral nerves (Weingartl et al. 2005).

Pathogen stability

"Survival of henipaviruses in the environment is highly sensitive to temperature and desiccation. Under most conditions survival time was brief, with half-lives limited to a few hours, indicating that transmission to a new host requires close contact with an infected animal or exposure to contaminated material shortly after excretion. However, under optimal conditions henipaviruses can persist for a number of days and under these circumstances, vehicle-borne transmission may be possible" (Fogarty et al. 2008).

Henipavirus viable for more than4 days in bat urine and viable 1–4 days in various fruit juices at 22 °C, tolerates a wide range of pH. It is inactivated in 1 day at 37 °C and is highly susceptible to dessication (Fogarty et al. 2008).

Irradiation of sera or pretreatment by heat inactivation at 56 °C for 30 minutes following a 1:5 dilution in PBS buffer containing 0.5 percent between 20 and 0.5 percent Triton-X100. These treatments had been shown previously at AAHL and CDC to inactivate HeV (Selleck P., Kzaisak T., unpublished results) (Daniels, Ksiazek, and Eaton 2001).

Evidence of virus persisting in humans up to 4 years before causing a recurrence of often fatal disease—no evidence of late onset or recurrence in the outbreaks in Bangladesh or India (Halpin and Mungall 2007).

Virus isolation rate in challenged grey headed fruit bats reflects extensive field surveillance, suggesting that there are very narrow windows of opportunity for Henipavirus transmission among and from bats (Middleton et al. 2007).

Vectors

a. Field

To date (2004) there is no evidence of vector or vehicle transmission of NIPV (Hyatt et al. 2004).

Other epidemiological/ecological data

Initial outbreak in Malaysia route of transmission was bat (*Pteropus* sp) to pig (amplifying host) to human, additional. Five subsequent outbreaks in Bangladesh (2001–2005) shows no evidence of amplifying host, and there is evidence of human-to-human transmission. All five outbreaks between Jan. and May (Epstein et al. 2006).

Deforestation, planting of fruit orchards in close proximity to pig farms and the natural reservoir of the NIPV (*Pteropus* sp) all contributed to the initial 1998 Malaysian outbreak (Epstein et al. 2006).

Deforestation is probably responsible for fruit bats leaving their ecological niches and approaching farms and villages (Wild 2009).

"A scenario emerges of fruit bat populations under stress, of altered foraging and behavioral patterns, of niche expansion, and of closer proximity to man" (Field et al. 2001).
"Henipaviruses likely exist across the entire global distribution of pteropid bats" (Epstein et al. 2006).

"Our recent studies have identified a high prevalence of antibodies to NIPV in colonies of both *P. vampyrus* and *P. hypomelanus* throughout peninsular Malaysia, suggesting that these bats are true reservoirs and that the virus is endemic to the region (Rahman et al., unpublished observations)" (Epstein et al. 2006).

The distribution of *Pteropus* ranges from Madagascar eastward across the Indian Ocean islands, South Asia, Southeast Asia, Australia, and much of the Pacific islands and includes some of the most densely populated regions on earth (Epstein et al. 2006).

Results indicate that henipavirus is present within West Africa (henipavirus seropositive fruit bats in Ghana) (Hayman et al. 2008).

Horizontal and vertical transmission of NIPV is possible in cats and virus has been previously shown to be shed via urine and nasopharynx in cats (Mungall et al. 2007).
Although no NIPV outbreaks to date in Thailand bat species (*P. lylei)* have been infected with the NIPV with highest recovery of Nipah RNA occurring in May (Wacharapluesadee et al. 2009).

A total of 12 NIPV outbreaks since 1998—Malaysia, Singapore, India, and Bangladesh (Wacharapluesadee et al. 2005).

No evidence of Australian pteroid bats being infected with NIPV in the wild, although they have been infected experimentally (Daniels et al. 2007).

Study showed evidence of NIPV infection in bats of Thailand by demonstrating IgG ab's in serum and RNA in urine and saliva (Wacharapluesadee et al. 2005).

Therapeutics/vaccines

Combination choroquine/ribavirin treatment was not effective in a hamster model, in contrast to results in vitro (Freiberg et al.).

Ribavirin treatment was associated with a 36 percent reduction in mortality in initial outbreak (Epstein et al. 2006).

No commercially available vaccines or approved therapeutics for NIPV; however, in the initial outbreak, Ribivirin treatment was associated with a 36 percent reduction in mortality in patients with acute encephalitis (Epstein et al. 2006).

There are no vaccines or post-exposure therapeutics specifically indicated for henipavirus infection (Halpin and Mungall 2007).

As of 2008, no active or passive therapeutic procedures exist for preventing or treating NIPV infection (McEachern et al. 2008).

Ribavirin useful in treatment with one limited study indicating 36 percent↓ in mortality (Tan and Chua 2008).

No vaccine available, but Ribavirin has some efficacy (Aljofan et al. 2009).

Other remarks

In the initial outbreak in Malaysia in 1998, almost all cases involved contact with swine that were also clinically affected though with a lower mortality rate (Torres-Velez et al. 2008).

Unique genetic makeup, wide host range and high virulence set the henipaviruses apart from other paramyxoviruses (Eaton et al. 2006; Eaton, Broder, and Wang 2005).

No serological evidence for NIPV infection of rodents in Malaysia (Eaton, Broder, and Wang 2005).

NIPV IgM present shortly after onset, all pts. + in 3 days, all IgG+ in 17–18 days (Bellini et al. 2005).

Taxonomy/antigenic relationships/synonyms

NIPV is a member of the *Henipavirus* genus (along with Hendra virus) in the *Paramyxoviridae* family. It is a single stranded negative sense RNA virus with a large genome and a high mutation rate (Lo and Rota 2008).

"A new genus was created, named Henipavirus (Hendra/Nipah) to accommodate these two phylogenetically closely related viruses with HeV as the type species and NIPV as the second member of the genus" (Chua 2003).

NIPV is a large enveloped virus (Weingartl, Berhane, and Czub 2009).

C.2 REFERENCES

Abalakin, V. A., and B. L. Cherkasskii. 1978. [Use of inbred mice as a model for the indication and differentiation of Bacillus-anthracis strains]. *Zh Mikrobiol Epidemiol Immunobiol* (2):146-7.

Agar, S. L., J. Sha, S. M. Foltz, T. E. Erova, K. G. Walberg, W. B. Baze, G. Suarez, J. W. Peterson, and A. K. Chopra. 2009. Characterization of the rat pneumonic plague model: infection kinetics following aerosolization of Yersinia pestis CO92. *Microbes Infect* 11 (2):205-14.

Agar, S. L., J. Sha, S. M. Foltz, T. E. Erova, K. G. Walberg, T. E. Parham, W. B. Baze, G. Suarez, J. W. Peterson, and A. K. Chopra. 2008. Characterization of a mouse model of plague after aerosolization of Yersinia pestis CO92. *Microbiology* 154 (Pt 7):1939-48.

Alciati, S., E. Belligni, S. Del Colle, and A. Pugliese. 2001. Human infections tick-transmitted. *Panminerva Med* 43 (4):295-304.

Alford, R. H., J. A. Kasel, P. J. Gerone, and V. Knight. 1966. Human influenza resulting from aerosol inhalation. *Proc Soc Exp Biol Med* 122 (3):800-4.

Aljofan, M., S. Saubern, A. G. Meyer, G. Marsh, J. Meers, and B. A. Mungall. 2009. Characteristics of Nipah virus and Hendra virus replication in different cell lines and their suitability for antiviral screening. *Virus Res* 142 (1-2):92-9.

Allela, L., O. Boury, R. Pouillot, A. Delicat, P. Yaba, B. Kumulungui, P. Rouquet, J. P. Gonzalez, and E. M. Leroy. 2005. Ebola virus antibody prevalence in dogs and human risk. *Emerg Infect Dis* 11 (3):385-90.

Allen, J. S., A. Skowera, G. J. Rubin, S. Wessely, and M. Peakman. 2006. Long-lasting T cell responses to biological warfare vaccines in human vaccinees. *Clin Infect Dis* 43 (1):1-7.

Ambrosio, A. M., L. M. Riera, C. Saavedra Mdel, and M. S. Sabattini. 2006. Immune response to vaccination against Argentine hemorrhagic Fever in an area where different arenaviruses coexist. *Viral Immunol* 19 (2):196-201.

Ambrosio, A.M. Riera, L., Saavedra,M.C., Sottosanti, J.J. . 2001. Prueba de neutralización del virus de la coriomeningitis linfocítica para diferenciar infeccciones con dos arenavirus en Argentina. . *Rev Argent Microbiol* 33:235-240.

Amici, C., A. Di Coro, A. Ciucci, L. Chiappa, C. Castilletti, V. Martella, N. Decaro, C. Buonavoglia, M. R. Capobianchi, and M. G. Santoro. 2006. Indomethacin has a potent antiviral activity against SARS coronavirus. *Antivir Ther* 11 (8):1021-30.

Amidi, S., W. Dutz, E. Kohout, and A. Ronaghy. 1974. Human anthrax in Iran. Report of 300 cases and review of literature. *Tropenmed Parasitol* 25 (1):96-104.

Anderson, G. W., Jr., T. W. Slone, Jr., and C. J. Peters. 1988. The gerbil, Meriones unguiculatus, a model for Rift Valley fever viral encephalitis. *Arch Virol* 102 (3-4):187-96.

Anderson, R. M., C. Fraser, A. C. Ghani, C. A. Donnelly, S. Riley, N. M. Ferguson, G. M. Leung, T. H. Lam, and A. J. Hedley. 2004. Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic. *Philos Trans R Soc Lond B Biol Sci* 359 (1447):1091-105.

Andreasen, V., C. Viboud, and L. Simonsen. 2008. Epidemiologic characterization of the 1918 influenza pandemic summer wave in Copenhagen: implications for pandemic control strategies. *J Infect Dis* 197 (2):270-8.

Anonymous. 2007. In *Dorland's Illustrated Medical Dictionary*. Philadelphia: Saunders Elsevier.

Repeated Author, ed. 2004. *Rift Valley Fever*. Edited by W. O. f. A. Health. 8 ed. Vol. 2, *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* . Paris: OIE.

Repeated Author, ed. 2007. *Rift Valley Fever*. 2 ed. Vol. 2, *Terrestrial Animal Health Code, World Organisation for Animal Health,* . Paris: OIE.

Arbaji, A., S. Kharabsheh, S. Al-Azab, M. Al-Kayed, Z. S. Amr, M. Abu Baker, and M. C. Chu. 2005. A 12-case outbreak of pharyngeal plague following the consumption of camel meat, in north-eastern Jordan. *Ann Trop Med Parasitol* 99 (8):789-93.

Ascenzi, P., A. Bocedi, J. Heptonstall, M. R. Capobianchi, A. Di Caro, E. Mastrangelo, M. Bolognesi, and G. Ippolito. 2008. Ebolavirus and Marburgvirus: insight the Filoviridae family. *Mol Aspects Med* 29 (3):151-85.

Avila, M. M., S. R. Samoilovich, R. P. Laguens, M. S. Merani, and M. C. Weissenbacher. 1987. Protection of Junin virus-infected marmosets by passive administration of immune serum: association with late neurologic signs. *J Med Virol* 21 (1):67-74.

Avsic-Zupanc, T., M. Poljak, M. Maticic, A. Radsel-Medvescek, J. W. LeDuc, K. Stiasny, C. Kunz, and F. X. Heinz. 1995. Laboratory acquired tick-borne meningoencephalitis: characterisation of virus strains. *Clin Diagn Virol* 4 (1):51-9.

Ayyadurai, S., L. Houhamdi, H. Lepidi, C. Nappez, D. Raoult, and M. Drancourt. 2008. Long-term persistence of virulent *Yersinia pestis* in soil. *Microbiology* 154 (Pt 9):2865-71.

Babiuk, S., R. Albrecht, Y. Berhane, P. Marszal, J. A. Richt, A. Garcia-Sastre, J. Pasick, and H. Weingartl. 2010. 1918 and 2009 H1N1 influenza viruses are not pathogenic in birds. *J Gen Virol* 91 (Pt 2):339-42.

Baggett, H.C., Rhodes, J.C., Fridkin, S.K., et al. . 2005. No evidence of a mild form of inhalational *Bacillus anthracis* infection during a bioterrorism-related inhalational anthrax outbreak in Washington, DC, in 2001. . *Clinical Infectious Diseases* 41:991–997.

Bago, Z., B. Bauder, J. Kolodziejek, N. Nowotny, and H. Weissenbock. 2002. Tickborne encephalitis in a mouflon (Ovis ammon musimon). *Vet Rec* 150 (7):218-20.

Bakhvalova, V. N., O. F. Potapova, V. V. Panov, and O. V. Morozova. 2009. Vertical transmission of tick-borne encephalitis virus between generations of adapted reservoir small rodents. *Virus Res*.

Baltazard, M. 1964. THE CONSERVATION OF PLAGUE IN INVETERATE FOCI. *J Hyg Epidemiol Microbiol Immunol* 120:409-21.

Banzhoff, A., M. Broker, and O. Zent. 2008. Protection against tick-borne encephalitis (TBE) for people living in and travelling to TBE-endemic areas. *Travel Med Infect Dis* 6 (6):331-41.

Barker, W. H., and J. P. Mullooly. 1980. Impact of epidemic type A influenza in a defined adult population. *Am J Epidemiol* 112 (6):798-811.

Barkham, T. M. 2004. Laboratory safety aspects of SARS at Biosafety Level 2. *Ann Acad Med Singapore* 33 (2):252-6.

Barnard, D. L. 2009. Animal models for the study of influenza pathogenesis and therapy. *Antiviral Res* 82 (2):A110-22.

Basta, N. E., M. E. Halloran, L. Matrajt, and I. M. Longini, Jr. 2008. Estimating influenza vaccine efficacy from challenge and community-based study data. *Am J Epidemiol* 168 (12):1343-52.

Bauch, C. T., J. O. Lloyd-Smith, M. P. Coffee, and A. P. Galvani. 2005. Dynamically modeling SARS and other newly emerging respiratory illnesses: past, present, and future. *Epidemiology* 16 (6):791-801.

Bausch, D. G., M. Borchert, T. Grein, C. Roth, R. Swanepoel, M. L. Libande, A. Talarmin, E. Bertherat, J. J. Muyembe-Tamfum, B. Tugume, R. Colebunders, K. M. Konde, P. Pirad, L. L. Olinda, G. R. Rodier, P. Campbell, O. Tomori, T. G. Ksiazek, and P. E. Rollin. 2003. Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. *Emerg Infect Dis* 9 (12):1531-7.

Bausch, D. G., and T. W. Geisbert. 2007. Development of vaccines for Marburg hemorrhagic fever. *Expert Rev Vaccines* 6 (1):57-74.

Bausch, D. G., S. T. Nichol, J. J. Muyembe-Tamfum, M. Borchert, P. E. Rollin, H. Sleurs, P. Campbell, F. K. Tshioko, C. Roth, R. Colebunders, P. Pirard, S. Mardel, L. A. Olinda, H. Zeller, A. Tshomba, A. Kulidri, M. L. Libande, S. Mulangu, P. Formenty, T. Grein, H. Leirs, L. Braack, T. Ksiazek, S. Zaki, M. D. Bowen, S. B. Smit, P. A. Leman, F. J. Burt, A. Kemp, R. Swanepoel, Scientific International, and Congo Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the. 2006. Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med* 355 (9):909-19.

Bausch, D. G., J. S. Towner, S. F. Dowell, F. Kaducu, M. Lukwiya, A. Sanchez, S. T. Nichol, T. G. Ksiazek, and P. E. Rollin. 2007. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis* 196 Suppl 2:S142-7.

Bazhutin, N. B., E. F. Belanov, V. A. Spiridonov, A. V. Voitenko, N. A. Krivenchuk, S. A. Krotov, N. I. Omel'chenko, AIu Tereshchenko, and V. V. Khomichev. 1992. [The effect of the methods for producing an experimental Marburg virus infection on the characteristics of the course of the disease in green monkeys]. *Vopr Virusol* 37 (3):153-6.

Bean, B., B. M. Moore, B. Sterner, L. R. Peterson, D. N. Gerding, and H. H. Balfour, Jr. 1982. Survival of influenza viruses on environmental surfaces. *J Infect Dis* 146 (1):47-51.

Beatty, M. E., D. A. Ashford, P. M. Griffin, R. V. Tauxe, and J. Sobel. 2003. Gastrointestinal anthrax: review of the literature. *Arch Intern Med* 163 (20):2527-31.

Beer, B., R. Kurth, and A. Bukreyev. 1999. Characteristics of Filoviridae: Marburg and Ebola viruses. *Naturwissenschaften* 86 (1):8-17.

Begier, E. M., G. Asiki, Z. Anywaine, B. Yockey, M. E. Schriefer, P. Aleti, A. Ogden-Odoi, J. E. Staples, C. Sexton, S. W. Bearden, and J. L. Kool. 2006. Pneumonic plague cluster, Uganda, 2004. *Emerg Infect Dis* 12 (3):460-7.

Belanov, E. F., V. P. Muntianov, V. D. Kriuk, A. V. Sokolov, N. I. Bormotov, V. P'Iankov O, and A. N. Sergeev. 1996. [Survival of Marburg virus infectivity on contaminated surfaces and in aerosols]. *Vopr Virusol* 41 (1):32-4.

Bellini, W. J., B. H. Harcourt, N. Bowden, and P. A. Rota. 2005. Nipah virus: an emergent paramyxovirus causing severe encephalitis in humans. *J Neurovirol* 11 (5):481-7.

Benbough, J. E. 1971. Some factors affecting the survival of airborne viruses. *J Gen Virol* 10 (3):209-20.

Berhane, Y., H. M. Weingartl, J. Lopez, J. Neufeld, S. Czub, C. Embury-Hyatt, M. Goolia, J. Copps, and M. Czub. 2008. Bacterial infections in pigs experimentally infected with Nipah virus. *Transbound Emerg Dis* 55 (3-4):165-74.

Beyer, W., and P. C. Turnbull. 2009. Anthrax in animals. *Mol Aspects Med* 30 (6):481-9.

Bibikova, V. A. 1977. Contemporary views on the interrelationships between fleas and the pathogens of human and animal diseases. *Annu Rev Entomol* 22:23-32.

Bin Saeed, A. A., N. A. Al-Hamdan, and R. E. Fontaine. 2005. Plague from eating raw camel liver. *Emerg Infect Dis* 11 (9):1456-7.

Bird, B. H., C.G. Albarino, A.L. Hartman, B.R. Erickson, T.G. Ksiazek, and S.T. Nichol. 2008. Rift Valley Fever Virus Lacking the NSs and NSm Genes Is Highly Attenuated, Confers Protective Immunity from Virulent Virus Challenge, and Allows for Differential Identification of Infected and Vaccinated Animals. *Journal of Virology* 82 (6):2681–2691.

Bird, B. H., T. G. Ksiazek, S. T. Nichol, and N. J. Maclachlan. 2009. Rift Valley fever virus. *J Am Vet Med Assoc* 234 (7):883-93.

Bischof, T. S., B. L. Hahn, and P. G. Sohnle. 2007. Characteristics of spore germination in a mouse model of cutaneous anthrax. *J Infect Dis* 195 (6):888-94.

Blum, L. S., R. Khan, N. Nahar, and R. F. Breiman. 2009. In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. *Am J Trop Med Hyg* 80 (1):96-102.

Bombardt, J. N. 2006. Congruent epidemic models for unstructured and structured populations: analytical reconstruction of a 2003 SARS outbreak. *Math Biosci* 203 (2):171-203.

Boone, A., J. P. Kraft, and P. Stapp. 2009. Scavenging by mammalian carnivores on prairie dog colonies: implications for the spread of plague. *Vector Borne Zoonotic Dis* 9 (2):185-90.

Bootsma, M. C., and N. M. Ferguson. 2007. The effect of public health measures on the 1918 influenza pandemic in U.S. cities. *Proc Natl Acad Sci U S A* 104 (18):7588-93.

Borio, L., and N. A. Hynes. 2010. Plague as a Bioterrorism Weapon. In *Principles and Practice of Infectious Diseases*, edited by G. L. Mandell, J. E. Bennett and R. Dolan. Philadelphia: Churchill Livingston Elsevier.

Boulanger, L. L., P. Ettestad, J. D. Fogarty, D. T. Dennis, D. Romig, and G. Mertz. 2004. Gentamicin and tetracyclines for the treatment of human plague: review of 75 cases in new Mexico, 1985-1999. *Clin Infect Dis* 38 (5):663-9.

Brachman, P. S. 1980. Inhalation anthrax. *Ann N Y Acad Sci* 353:83-93.

Brachman, P. S., H. Gold, S. A. Plotkin, F. R. Fekety, M. Werrin, and N. R. Ingraham. 1962. Field Evaluation of a Human Anthrax Vaccine. *Am J Public Health Nations Health* 52 (4):632-45.

Brachman, P. S., A. F. Kaufman, and F. G. Dalldorf. 1966. Industrial inhalation Anthrax. *Bacteriol Rev* 30 (3):646-59.

Bravata, D. M., J. E. Holty, E. Wang, R. Lewis, P. H. Wise, K. M. McDonald, and D. K. Owens. 2007. Inhalational, gastrointestinal, and cutaneous anthrax in children: a systematic review of cases: 1900 to 2005. *Arch Pediatr Adolesc Med* 161 (9):896-905.

Bridges, C. B., M. J. Kuehnert, and C. B. Hall. 2003. Transmission of influenza: implications for control in health care settings. *Clin Infect Dis* 37 (8):1094-101.

Brooks, D. 2010. Anthrax, Human - USA (08): (New Hampshire). *ProMED-mail*, http://promedmail.oracle.com/pls/otn/f?p=2400:1001:1471340831993185::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000,82333.

Brouillard, J. E., C. M. Terriff, A. Tofan, and M. W. Garrison. 2006. Antibiotic selection and resistance issues with fluoroquinolones and doxycycline against bioterrorism agents. *Pharmacotherapy* 26 (1):3-14.

Brown, C., and A. Torres, eds. 2008. *Rift Valley Fever*. 7th ed, *Foreign Animal Diseases*: U.S. Animal Health Association

Brown, J. L., J. W. Dominik, and R. L. Morrissey. 1981. Respiratory infectivity of a recently isolated Egyptian strain of Rift Valley fever virus. *Infect Immun* 33 (3):848-53.

Brown, J.L., J. W. Dominik, and E. W. Larson. 1982. Airborne survival of Rift Valley fever virus. edited by U.S. Army Medical Research Institute of Infectious Diseases Aerobiology Division. Frederick: U.S. Department of Defense.

Brummer-Korvenkontio, M., P. Saikku, P. Korhonen, and N. Oker-Blom. 1973. Arboviruses in Finland. I. Isolation of tick-borne encephalitis (TBE) virus from arthropods, vertebrates, and patients. *Am J Trop Med Hyg* 22 (3):382-9.

Bukreyev, A., P. E. Rollin, M. K. Tate, L. Yang, S. R. Zaki, W. J. Shieh, B. R. Murphy, P. L. Collins, and A. Sanchez. 2007. Successful topical respiratory tract immunization of primates against Ebola virus. *J Virol* 81 (12):6379-88.

Burke, D. S. 1977. Immunization against tularemia: analysis of the effectiveness of live Francisella tularensis vaccine in prevention of laboratory-acquired tularemia. *J Infect Dis* 135 (1):55-60.

Burmeister, R. W., W. D. Tigertt, and E. L. Overholt. 1962. Laboratory-acquired pneumonic plague. Report of a case and review of previous cases. *Ann Intern Med* 56:789-800.

Bushar, G., and J. L. Sagripanti. 1990. Method for improving accuracy of virus titration: standardization of plaque assay for Junin virus. *J Virol Methods* 30 (1):99-107.

Butler, T. 1972. A clinical study of bubonic plague. Observations of the 1970 Vietnam epidemic with emphasis on coagulation studies, skin histology and electrocardiograms. *Am J Med* 53 (3):268-76.

Repeated Author, ed. 1991. *Plague*. Edited by G. T. Strickland, *Tropical medicine*. Philadelphia: WB Saunders.

Butler, T., J. Levin, N. N. Linh, D. M. Chau, M. Adickman, and K. Arnold. 1976. Yersinia pestis infection in Vietnam. II. Quantiative blood cultures and detection of endotoxin in the cerebrospinal fluid of patients with meningitis. *J Infect Dis* 133 (5):493-9.

Calisher, C. H. 1988. Antigenic classification and taxonomy of flaviviruses (family Flaviviridae) emphasizing a universal system for the taxonomy of viruses causing tick-borne encephalitis. *Acta Virol* 32 (5):469-78.

Candurra, N. A., L. A. Scolaro, S. E. Mersich, E. B. Damonte, and C. E. Coto. 1990. A comparison of Junin virus strains: growth characteristics, cytopathogenicity and viral polypeptides. *Res Virol* 141 (5):505-15.

Cantoni, G., P. Padula, G. Calderon, J. Mills, E. Herrero, P. Sandoval, V. Martinez, N. Pini, and E. Larrieu. 2001. Seasonal variation in prevalence of antibody to hantaviruses in rodents from southern Argentina. *Trop Med Int Health* 6 (10):811-6.

Carballal, G., C. M. Videla, and M. S. Merani. 1988. Epidemiology of Argentine hemorrhagic fever. *Eur J Epidemiol* 4 (2):259-74.

Carrat, F., E. Vergu, N. M. Ferguson, M. Lemaitre, S. Cauchemez, S. Leach, and A. J. Valleron. 2008. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *Am J Epidemiol* 167 (7):775-85.

Carrion, R., Jr., K. Brasky, K. Mansfield, C. Johnson, M. Gonzales, A. Ticer, I. Lukashevich, S. Tardif, and J. Patterson. 2007. Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues. *J Virol* 81 (12):6482-90.

Carrion, R., Jr., J. L. Patterson, C. Johnson, M. Gonzales, C. R. Moreira, A. Ticer, K. Brasky, G. B. Hubbard, D. Moshkoff, J. Zapata, M. S. Salvato, and I. S. Lukashevich. 2007. A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity. *Vaccine* 25 (20):4093-102.

Castillo, C., J. Naranjo, A. Sepulveda, G. Ossa, and H. Levy. 2001. Hantavirus pulmonary syndrome due to Andes virus in Temuco, Chile: clinical experience with 16 adults. *Chest* 120 (2):548-54.

Castillo, C., C. Nicklas, J. Mardones, and G. Ossa. 2007. Andes Hantavirus as possible cause of disease in travellers to South America. *Travel Med Infect Dis* 5 (1):30-4.

Castillo, C., E. Villagra, L. Sanhueza, M. Ferres, J. Mardones, and G. J. Mertz. 2004. Prevalence of antibodies to hantavirus among family and health care worker contacts of persons with hantavirus cardiopulmonary syndrome: lack of evidence for nosocomial transmission of Andes virus to health care workers in Chile. *Am J Trop Med Hyg* 70 (3):302-4.

Cauchemez, S., P. Y. Boelle, C. A. Donnelly, N. M. Ferguson, G. Thomas, G. M. Leung, A. J. Hedley, R. M. Anderson, and A. J. Valleron. 2006. Real-time estimates in early detection of SARS. *Emerg Infect Dis* 12 (1):110-3.

CDC. 2001. Notice to Readers: Updated Recommendations for Antimicrobial Prophylaxis Among Asymptomatic Pregnant Women After Exposure to *Bacillus anthracis*. ***Morbidity and Mortality Weekly Report*** 50 (43):960.

Repeated Author. 2001. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. *MMWR - Morbidity & Mortality Weekly Report* 50 (42):909-919.

Repeated Author. 2002. Use of Anthrax Vaccine in Response to Terrorism: Supplemental Recommendations of the Advisory Committee on Immunization Practices *Morbidity and Mortality Weekly Report* 51 (45):1024-1026.

Repeated Author. 2005. Interim guidance for managing patients with suspected viral hemorrhagic fever in US hospitals. http://www.cdc.gov/ncidod/dhqp/pdf/bbp/VHFinterimGuidance05_19_05.pdf.

Repeated Author. 2006. Inhalation anthrax associated with dried animal hides--Pennsylvania and New York City, 2006. *MMWR Morb Mortal Wkly Rep* 55 (10):280-2.

Repeated Author. 2007. Community strategy for pandemic influenza mitigation. edited by HHS. Washington, D.C,.

Repeated Author. 2007. Rift Valley fever outbreak--Kenya, November 2006-January 2007. *MMWR Morb Mortal Wkly Rep* 56 (4):73-6.

Repeated Author. 2008. Lassa Fever Fact Sheet.

Repeated Author. 2009. Marburg hemorrhagic fever: Known cases and outbreaks of Marburg hemorrhagic fever, in chronological order. CDC National Center for Infectious Diseases, Special Pathogens Branch.

CDC, and NIH. 2007. Biosafety in Microbiological and Biomedical Laboratories. edited by H. H. Services.

Center for Infectious Disease Research & Policy. *Anthrax in Canada kills 149 animals, infects man*. University of Minnesota 2006. Available from http://www.cidrap.umn.edu/cidrap/content/bt/anthrax/news/jul1706anthrax.html.

Centers for Disease Control. 1984. Plague pneumonia--California. *MMWR Morb Mortal Wkly Rep* 33 (34):481-3.

Centers for Disease Control and Prevention. 1991. Summary of notifiable diseases. *MMWR - Morbidity & Mortality Weekly Report* 48 (53).

Repeated Author. 1994. Human plague--United States, 1993-1994. *MMWR Morb Mortal Wkly Rep* 43 (13):242-6.

Repeated Author. 2002. Suspected cutaneous anthrax in a laboratory worker--Texas, 2002. *MMWR Morbidity and Mortality Weekly Report* 51 (13):279-81.

Repeated Author. 2002. Tularemia--United States, 1990-2000. *MMWR Morb Mortal Wkly Rep* 51 (9):181-184.

Repeated Author. 2005. Transmission of Influenza A Viruses Between Animals and People. http://www.cdc.gov/flu/avian/gen-info/transmission.htm.

Repeated Author. 2006. Human plague--four states, 2006. *MMWR Morb Mortal Wkly Rep* 55 (34):940-3.

Repeated Author. 2009. *All about hantaviruses*. Centers for Disease Control and Prevention, National Center for Infectious Diseases, Special Pathogens Branch 2008 [cited 11, Oct. 2009].

Repeated Author. 2008. Cutaneous Anthrax Associated with Drum Making Using Goat Hides from West Africa --- Connecticut, 2007. *MMWR - Morbidity & Mortality Weekly Report* 57 (23):628-631.

Repeated Author. 2010. Gastrointestinal anthrax after an animal-hide drumming event -- New Hampshire and Massachusetts, 2009. *MMWR - Morbidity & Mortality Weekly Report* 59 (28):872-877.

Repeated Author. *Preventing the spread of influenza (the flu) in child care settings: guidance for administrators, care providers, and other staff*. Centers for Disease Control and Prevention 2010 [cited March 24 2010. Available from http://www.cdc.gov/flu/professionals/infectioncontrol/childcaresettings.htm.

Repeated Author. 2011. Fatal Laboratory-Acquired Infection with an Attenuated Yersinia pestis Strain --- Chicago, Illinois, 2009. *Morbidity and Mortality Weekly Report* 60 (07):201-205.

Chadha, M. S., J. A. Comer, L. Lowe, P. A. Rota, P. E. Rollin, W. J. Bellini, T. G. Ksiazek, and A. Mishra. 2006. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis* 12 (2):235-40.

Chan-Yeung, M., and R. H. Xu. 2003. SARS: epidemiology. *Respirology* 8 Suppl:S9-14.

Chan, K. H., L. L. Poon, V. C. Cheng, Y. Guan, I. F. Hung, J. Kong, L. Y. Yam, W. H. Seto, K. Y. Yuen, and J. S. Peiris. 2004. Detection of SARS coronavirus in patients with suspected SARS. *Emerg Infect Dis* 10 (2):294-9.

Chan, K. P., P. E. Rollin, T. G. Ksiazek, Y. S. Leo, K. T. Goh, N. I. Paton, E. H. Sng, and A. E. Ling. 2002. A survey of Nipah virus infection among various risk groups in Singapore. *Epidemiol Infect* 128 (1):93-8.

Chan, P. K., J. W. Tang, and D. S. Hui. 2006. SARS: clinical presentation, transmission, pathogenesis and treatment options. *Clin Sci (Lond)* 110 (2):193-204.

Chang, L. Y., A. R. Ali, S. S. Hassan, and S. AbuBakar. 2006. Nipah virus RNA synthesis in cultured pig and human cells. *J Med Virol* 78 (8):1105-12.

Charrel, R.N., and X. de Lamballerie. 2003. Arenaviruses other than Lassa virus. *Antiviral Res* 57:89-100.

Chen, W., Z. Xu, J. Mu, L. Yang, H. Gan, F. Mu, B. Fan, B. He, S. Huang, B. You, Y. Yang, X. Tang, L. Qiu, Y. Qiu, J. Wen, J. Fang, and J. Wang. 2004. Antibody response and viraemia during the course of severe acute respiratory syndrome (SARS)-associated coronavirus infection. *J Med Microbiol* 53 (Pt 5):435-8.

Cheng, V. C., S. K. Lau, P. C. Woo, and K. Y. Yuen. 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin Microbiol Rev* 20 (4):660-94.

Choi, B. C. K., Pak, A. W. P. 2003. A simple approximate mathematical model to predict the number of severe acute respiratory syndrome cases and deaths

J Epidemiol Community Health 57:831-835.

Chowell, G., C. E. Ammon, N. W. Hengartner, and J. M. Hyman. 2006. Transmission dynamics of the great influenza pandemic of 1918 in Geneva, Switzerland: Assessing the effects of hypothetical interventions. *J Theor Biol* 241 (2):193-204.

Chowell, G., L. M. Bettencourt, N. Johnson, W. J. Alonso, and C. Viboud. 2008. The 1918-1919 influenza pandemic in England and Wales: spatial patterns in transmissibility and mortality impact. *Proc Biol Sci* 275 (1634):501-9.

Chowell, G., C. Castillo-Chavez, P. W. Fenimore, C. M. Kribs-Zaleta, L. Arriola, and J. M. Hyman. 2004. Model parameters and outbreak control for SARS. *Emerg Infect Dis* 10 (7):1258-63.

Chowell, G., N. W. Hengartner, C. Castillo-Chavez, P. W. Fenimore, and J. M. Hyman. 2004. The basic reproductive number of Ebola and the effects of public health measures: the cases of Congo and Uganda. *J Theor Biol* 229 (1):119-26.

Chowell, G., H. Nishiura, and L. M. Bettencourt. 2007. Comparative estimation of the reproduction number for pandemic influenza from daily case notification data. *J R Soc Interface* 4 (12):155-66.

Christie, A.B., Chen, T.H., Elberg, S.S. 1980. Plague in camels and goats: their role in human epidemics. *J Infect Dis* 141 (6):724-726

Chu, C. M., L. L. Poon, V. C. Cheng, K. S. Chan, I. F. Hung, M. M. Wong, K. H. Chan, W. S. Leung, B. S. Tang, V. L. Chan, W. L. Ng, T. C. Sim, P. W. Ng, K. I. Law, D. M. Tse, J. S. Peiris, and K. Y. Yuen. 2004. Initial viral load and the outcomes of SARS. *CMAJ* 171 (11):1349-52.

Chua, K. B. 2003. Nipah virus outbreak in Malaysia. *J Clin Virol* 26 (3):265-75.

Chua, K. B., W. J. Bellini, P. A. Rota, B. H. Harcourt, A. Tamin, S. K. Lam, T. G. Ksiazek, P. E. Rollin, S. R. Zaki, W. Shieh, C. S. Goldsmith, D. J. Gubler, J. T. Roehrig, B. Eaton, A. R. Gould, J. Olson, H. Field, P. Daniels, A. E. Ling, C. J. Peters, L. J. Anderson, and B. W. Mahy. 2000. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 288 (5470):1432-5.

Chua, K. B., C. L. Koh, P. S. Hooi, K. F. Wee, J. H. Khong, B. H. Chua, Y. P. Chan, M. E. Lim, and S. K. Lam. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4 (2):145-51.

Chua, K. B., S. K. Lam, K. J. Goh, P. S. Hooi, T. G. Ksiazek, A. Kamarulzaman, J. Olson, and C. T. Tan. 2001. The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. *J Infect* 42 (1):40-3.

Chumakov, M.P., Levkovich, E.N., . *Russian Spring Summer Encephalitis*. CDC 2008. Available from <http://www.ncid.cdc.gov/arbocat/catalog-listing.asp?VirusID=404&SI=1>.

Cinatl, J., Jr., M. Michaelis, G. Hoever, W. Preiser, and H. W. Doerr. 2005. Development of antiviral therapy for severe acute respiratory syndrome. *Antiviral Res* 66 (2-3):81-97.

Clements, M. L., R. F. Betts, E. L. Tierney, and B. R. Murphy. 1986. Resistance of adults to challenge with influenza A wild-type virus after receiving live or inactivated virus vaccine. *J Clin Microbiol* 23 (1):73-6.

Conrad, J. L., M. Isaacson, E. B. Smith, H. Wulff, M. Crees, P. Geldenhuys, and J. Johnston. 1978. Epidemiologic investigation of Marburg virus disease, Southern Africa, 1975. *Am J Trop Med Hyg* 27 (6):1210-5.

Corrigan-Curay, J. 2008. *Development of Recommendations under the NIH Guidelines for Research with Recombinant DNA Molecules for Research with 1918 H1N1 Influenza Virus*. Bethesda: NIH-RAC.

Cosset, F. L., P. Marianneau, G. Verney, F. Gallais, N. Tordo, E. I. Pecheur, J. ter Meulen, V. Deubel, and B. Bartosch. 2009. Characterization of Lassa virus cell entry and neutralization with Lassa virus pseudoparticles. *J Virol* 83 (7):3228-37.

Crameri, G., L. F. Wang, C. Morrissy, J. White, and B. T. Eaton. 2002. A rapid immune plaque assay for the detection of Hendra and Nipah viruses and anti-virus antibodies. *J Virol Methods* 99 (1-2):41-51.

Craven, R. B., G. O. Maupin, M. L. Beard, T. J. Quan, and A. M. Barnes. 1993. Reported cases of human plague infections in the United States, 1970-1991. *J Med Entomol* 30 (4):758-61.

Crook, L. D., and B. Tempest. 1992. Plague. A clinical review of 27 cases. *Arch Intern Med* 152 (6):1253-6.

Cross, J.T., Penn, R.L. 2000. *Francisella tularensis*. In *Principles and practice of infectious diseases*, edited by G. L. Mandell, Bennett, J.E., Dolin, R. . New York: Churchill Livingstone.

Curtis, N. 2006. Viral haemorrhagic fevers caused by Lassa, Ebola and Marburg viruses. *Adv Exp Med Biol* 582:35-44.

Custer, D. M., E. Thompson, C. S. Schmaljohn, T. G. Ksiazek, and J. W. Hooper. 2003. Active and passive vaccination against hantavirus pulmonary syndrome with Andes virus M genome segment-based DNA vaccine. *J Virol* 77 (18):9894-905.

Daddario-DiCaprio, Kathleen M. , Thomas W. Geisbert, Ute Stroher, Joan B. Geisbert, Allen Grolla, Elizabeth A. Fritz, Lisa Fernando, Elliott Kagan, Peter B. Jahrling, Lisa E. Hensley, Steven M. Jones and Heinz Feldman. Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. *The Lancet* 367:1399-404.

Dahlgren, C. M., L. M. Buchanan, H. M. Decker, S. W. Freed, C. R. Phillips, and P. S. Brachman. 1960. Bacillus anthracis aerosols in goat hair processing mills. *Am J Hyg* 72:24-31.

Dahlstrand, S., O. Ringertz, and B. Zetterberg. 1971. Airborne tularemia in Sweden. *Scand J Infect Dis* 3 (1):7-16.

Danielova, V., J. Holubova, M. Pejcoch, and M. Daniel. 2002. Potential significance of transovarial transmission in the circulation of tick-borne encephalitis virus. *Folia Parasitol (Praha)* 49 (4):323-5.

Daniels, P., T. Ksiazek, and B. T. Eaton. 2001. Laboratory diagnosis of Nipah and Hendra virus infections. *Microbes Infect* 3 (4):289-95.

Daniels, P. W., K. Halpin, A. Hyatt, and D. Middleton. 2007. Infection and disease in reservoir and spillover hosts: determinants of pathogen emergence. *Curr Top Microbiol Immunol* 315:113-31.

Darnell, M. E., K. Subbarao, S. M. Feinstone, and D. R. Taylor. 2004. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. *J Virol Methods* 121 (1):85-91.

Darnell, M. E., and D. R. Taylor. 2006. Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products. *Transfusion* 46 (10):1770-7.

Dauphin, L.A., B.R. Newton, M.V. Rasmussen, and et al. 2008. Gamma irradiation can be used to inactivate Bacillus anthracis spores without compromising the sensitivity of diagnostic assays. *Applied and Environmental Microbiology* 74 (14):4427-4433.

Davies, F. G., K. J. Linthicum, and A. D. James. 1985. Rainfall and epizootic Rift Valley fever. *Bull World Health Organ* 63 (5):941-3.

Day, C. W., R. Baric, S. X. Cai, M. Frieman, Y. Kumaki, J. D. Morrey, D. F. Smee, and D. L. Barnard. 2009. A new mouse-adapted strain of SARS-CoV as a lethal model for evaluating antiviral agents in vitro and in vivo. *Virology* 395 (2):210-22.

Day, W. C., and R. F. Berendt. 1972. Experimental tularemia in Macaca mulatta: relationship of aerosol particle size to the infectivity of airborne Pasteurella tularensis. *Infect Immun* 5 (1):77-82.

Deboosere, N., S. V. Horm, A. Pinon, J. Gachet, C. Coldefy, P. Buchy, and M. Vialette. 2011. Development and Validation of a Concentration Method for the Detection of Influenza A Viruses from Large Volumes of Surface Water. *Appl Environ Microbiol*.

Defense Intelligence Agency. 1986. Soviet biological warfare threat. Washington, D.C.

Demby, A. H., A. Inapogui, K. Kargbo, J. Koninga, K. Kourouma, J. Kanu, M. Coulibaly, K. D. Wagoner, T. G. Ksiazek, C. J. Peters, P. E. Rollin, and D. G. Bausch. 2001. Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne Zoonotic Dis* 1 (4):283-97.

Dennis, D. , and F. Meier. 1997. Plague. In *Pathology of emerging infections*, edited by C. R. Horsburgh and A. M. Nelson. Washington, D.C.: ASM Press.

Dennis, D. T., T. V. Inglesby, D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. D. Fine, A. M. Friedlander, J. Hauer, M. Layton, S. R. Lillibridge, J. E. McDade, M. T. Osterholm, T. O'Toole, G. Parker, T. M. Perl, P. K. Russell, and K. Tonat. 2001. Tularemia as a biological weapon: medical and public health management. *JAMA* 285 (21):2763-73.

Dennis, D.T. 1998. Tularemia. In *Public Health and Preventive Medicine*, edited by R. B. Wallace. Stamford: Appleton & Lange.

Dennis, D.T., T.V. Inglesby, D.A. Henderson, J.G. Bartlett, M. S. Ascher, E. Eitzen, A. D. Fine, A.M. Friedlander, J. Hauer, M. Layton, S.R. Lillibridge, J.E. McDade, M.T. Osterholm, T. O'Toole, G. Parker, T.M. Perl, P.K. Russell, and K. Tonat. 2001. Health Management Tularemia as a Biological Weapon: Medical and Public Health Management. *JAMA* 285 (21):2763-2773.

Dennis, D.T., and P.S. Mead. 2010. *Yersinia* species, Including Plague. In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, edited by G. L. Mandell, J. E. Bennett and R. Dolin. Philadelphia: Churchill Livingstone.

Djavani, M. M., O. R. Crasta, J. C. Zapata, Z. Fei, O. Folkerts, B. Sobral, M. Swindells, J. Bryant, H. Davis, C. D. Pauza, I. S. Lukashevich, R. Hammamieh, M. Jett, and M. S. Salvato. 2007. Early blood profiles of virus infection in a monkey model for Lassa fever. *J Virol* 81 (15):7960-73.

Doganay, M., and G. Metan. 2009. Human anthrax in Turkey from 1990 to 2007. *Vector Borne Zoonotic Dis* 9 (2):131-40.

Dohm, D. J., E. D. Rowton, P. G. Lawyer, M. O'Guinn, and M. J. Turell. 2000. Laboratory transmission of Rift Valley fever virus by Phlebotomus duboscqi, Phlebotomus papatasi, Phlebotomus sergenti, and Sergentomyia schwetzi (Diptera: Psychodidae). *J Med Entomol* 37 (3):435-8.

Doll, J. M., P. S. Zeitz, P. Ettestad, A. L. Bucholtz, T. Davis, and K. Gage. 1994. Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. *Am J Trop Med Hyg* 51 (1):109-14.

Donnelly, C. A., M. C. Fisher, C. Fraser, A. C. Ghani, S. Riley, N. M. Ferguson, and R. M. Anderson. 2004. Epidemiological and genetic analysis of severe acute respiratory syndrome. *Lancet Infect Dis* 4 (11):672-83.

Dowell, S. F., and J. S. Bresee. 2008. Pandemic lessons from Iceland. *Proc Natl Acad Sci U S A* 105 (4):1109-10.

Dowell, S. F., R. Mukunu, T. G. Ksiazek, A. S. Khan, P. E. Rollin, and C. J. Peters. 1999. Transmission of Ebola hemorrhagic fever: a study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis* 179 Suppl 1:S87-91.

Drancourt, M., L. Houhamdi, and D. Raoult. 2006. *Yersinia pestis* as a telluric, human ectoparasite-borne organism. *Lancet Infect Dis* 6 (4):234-41.

Drosten, C., B. M. Kummerer, H. Schmitz, and S. Gunther. 2003. Molecular diagnostics of viral hemorrhagic fevers. *Antiviral Res* 57 (1-2):61-87.

Dumpis, U., D. Crook, and J. Oksi. 1999. Tick-borne encephalitis. *Clin Infect Dis* 28 (4):882-90.

Dvorak, P. 2005. Health Officials Vigilant for Illness After Sensors Detect Bacteria on Mall. *The Washington Post*, October 2, 2005.

Eaton, B. T., C. C. Broder, D. Middleton, and L. F. Wang. 2006. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* 4 (1):23-35.

Eaton, B. T., C. C. Broder, and L. F. Wang. 2005. Hendra and Nipah viruses: pathogenesis and therapeutics. *Curr Mol Med* 5 (8):805-16.

Eidson, M., L. A. Tierney, O. J. Rollag, T. Becker, T. Brown, and H. F. Hull. 1988. Feline plague in New Mexico: risk factors and transmission to humans. *Am J Public Health* 78 (10):1333-5.

Eisen, R. J., S. W. Bearden, A. P. Wilder, J. A. Montenieri, M. F. Antolin, and K. L. Gage. 2006. Early-phase transmission of Yersinia pestis by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics. *Proc Natl Acad Sci U S A* 103 (42):15380-5.

Eisen, R. J., J. M. Petersen, C. L. Higgins, D. Wong, C. E. Levy, P. S. Mead, M. E. Schriefer, K. S. Griffith, K. L. Gage, and C. B. Beard. 2008. Persistence of Yersinia pestis in soil under natural conditions. *Emerg Infect Dis* 14 (6):941-3.

Ellis, J., P. C. Oyston, M. Green, and R. W. Titball. 2002. Tularemia. *Clin Microbiol Rev* 15 (4):631-46.

Emond, R. T., B. Evans, E. T. Bowen, and G. Lloyd. 1977. A case of Ebola virus infection. *Br Med J* 2 (6086):541-4.

Enderlin, G., L. Morales, R. F. Jacobs, and J. T. Cross. 1994. Streptomycin and alternative agents for the treatment of tularemia: review of the literature. *Clin Infect Dis* 19 (1):42-7.

Enria, D. A., and J. G. Barrera Oro. 2002. Junin virus vaccines. *Curr Top Microbiol Immunol* 263:239-61.

Enria, D. A., A. M. Briggiler, N. Pini, and S. Levis. 2001. Clinical manifestations of New World hantaviruses. *Curr Top Microbiol Immunol* 256:117-34.

Enria, D. A., A. M. Briggiler, and Z. Sanchez. 2008. Treatment of Argentine hemorrhagic fever. *Antiviral Res* 78 (1):132-9.

Enria, D., P. Padula, E. L. Segura, N. Pini, A. Edelstein, C. R. Posse, and M. C. Weissenbacher. 1996. Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. *Medicina (B Aires)* 56 (6):709-11.

Enscore, R. E., B. J. Biggerstaff, T. L. Brown, R. E. Fulgham, P. J. Reynolds, D. M. Engelthaler, C. E. Levy, R. R. Parmenter, J. A. Montenieri, J. E. Cheek, R. K. Grinnell, P. J. Ettestad, and K. L. Gage. 2002. Modeling relationships between climate and the frequency of human plague cases in the southwestern United States, 1960-1997. *Am J Trop Med Hyg* 66 (2):186-96.

Epstein, J. H., H. E. Field, S. Luby, J. R. Pulliam, and P. Daszak. 2006. Nipah virus: impact, origins, and causes of emergence. *Curr Infect Dis Rep* 8 (1):59-65.

Evans, M. E., D. W. Gregory, W. Schaffner, and Z. A. McGee. 1985. Tularemia: a 30-year experience with 88 cases. *Medicine (Baltimore)* 64 (4):251-69.

Fair, J., E. Jentes, A. Inapogui, K. Kourouma, A. Goba, A. Bah, M. Tounkara, M. Coulibaly, R. F. Garry, and D. G. Bausch. 2007. Lassa virus-infected rodents in refugee camps in Guinea: a looming threat to public health in a politically unstable region. *Vector Borne Zoonotic Dis* 7 (2):167-71.

Fang, H., J. Chen, and J. Hu. 2005. Modelling the SARS epidemic by a lattice-based Monte-Carlo simulation. *Conf Proc IEEE Eng Med Biol Soc* 7:7470-3.

Farlow, J., D. M. Wagner, M. Dukerich, M. Stanley, M. Chu, K. Kubota, J. Petersen, and P. Keim. 2005. Francisella tularensis in the United States. *Emerg Infect Dis* 11 (12):1835-41.

Fasanella, A., S. Losito, R. Adone, F. Ciuchini, T. Trotta, S. A. Altamura, D. Chiocco, and G. Ippolito. 2003. PCR assay to detect Bacillus anthracis spores in heat-treated specimens. *J Clin Microbiol* 41 (2):896-9.

Feldman, K. A., R. E. Enscore, S. L. Lathrop, B. T. Matyas, M. McGuill, M. E. Schriefer, D. Stiles-Enos, D. T. Dennis, L. R. Petersen, and E. B. Hayes. 2001. An outbreak of primary pneumonic tularemia on Martha's Vineyard. *N Engl J Med* 345 (22):1601-6.

Feldman, K. A., D. Stiles-Enos, K. Julian, B. T. Matyas, S. R. Telford, 3rd, M. C. Chu, L. R. Petersen, and E. B. Hayes. 2003. Tularemia on Martha's Vineyard: seroprevalence and occupational risk. *Emerg Infect Dis* 9 (3):350-4.

Feldmann, H., S. M. Jones, K. M. Daddario-DiCaprio, J. B. Geisbert, U. Stroher, A. Grolla, M. Bray, E. A. Fritz, L. Fernando, F. Feldmann, L. E. Hensley, and T. W. Geisbert. 2007. Effective post-exposure treatment of Ebola infection. *PLoS Pathog* 3 (1):e2.

Feldmann, H., W. Slenczka, and H. D. Klenk. 1996. Emerging and reemerging of filoviruses. *Arch Virol Suppl* 11:77-100.

Feng, Y., and G.F. Gao. 2007. Towards our understanding of SARS-Co-V, an emerging and devastating but quickly conquered virus. *Comp Immunol Microbiol & Infect Dis* 30:309-327.

Fennelly, K. P., A. L. Davidow, S. L. Miller, N. Connell, and J. J. Ellner. 2004. Airborne infection with Bacillus anthracis--from mills to mail. *Emerg Infect Dis* 10 (6):996-1002.

Feodorova, V. A., and A. B. Golova. 2005. Antigenic and phenotypic modifications of Yersinia pestis under calcium and glucose concentrations simulating the mammalian bloodstream environment. *J Med Microbiol* 54 (Pt 5):435-41.

Ferguson, N. M., D. A. Cummings, C. Fraser, J. C. Cajka, P. C. Cooley, and D. S. Burke. 2006. Strategies for mitigating an influenza pandemic. *Nature* 442 (7101):448-52.

Ferrer, J. F., C. B. Jonsson, E. Esteban, D. Galligan, M. A. Basombrio, M. Peralta-Ramos, M. Bharadwaj, N. Torrez-Martinez, J. Callahan, A. Segovia, and B. Hjelle. 1998. High prevalence of hantavirus infection in Indian communities of the Paraguayan and Argentinean Gran Chaco. *Am J Trop Med Hyg* 59 (3):438-44.

Ferres, G. M., C. C. Sandoval, B. I. Delgado, P. V. Sotomayor, N. A. Olea, and C. Pa Vial. 2010. [Hantavirosis: clinical and epidemiological characteristics of pediatric patients in Chile]. *Rev Chilena Infectol* 27 (1):52-9.

Ferres, M., and P. Vial. 2004. Hantavirus infection in children. *Curr Opin Pediatr* 16 (1):70-5.

Ferres, M., P. Vial, C. Marco, L. Yanez, P. Godoy, C. Castillo, B. Hjelle, I. Delgado, S. J. Lee, and G. J. Mertz. 2007. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in chile. *J Infect Dis* 195 (11):1563-71.

Fichet-Calvet, E., E. Lecompte, L. Koivogui, S. Daffis, and J. ter Meulen. 2008. Reproductive characteristics of Mastomys natalensis and Lassa virus prevalence in Guinea, West Africa. *Vector Borne Zoonotic Dis* 8 (1):41-8.

Field, H., P. Young, J. M. Yob, J. Mills, L. Hall, and J. Mackenzie. 2001. The natural history of Hendra and Nipah viruses. *Microbes Infect* 3 (4):307-14.

Fisher-Hoch, S. P. 2005. Lessons from nosocomial viral haemorrhagic fever outbreaks. *Br Med Bull* 73-74:123-37.

Fisher-Hoch, S. P., and J. B. McCormick. 2004. Lassa fever vaccine. *Expert Rev Vaccines* 3 (2):189-97.

Flick, R., and M. Bouloy. 2005. Rift Valley fever virus. *Curr Mol Med* 5 (8):827-34.

Fogarty, R., K. Halpin, A. D. Hyatt, P. Daszak, and B. A. Mungall. 2008. Henipavirus susceptibility to environmental variables. *Virus Res* 132 (1-2):140-4.

Formenty, P., E.M. Leroy, A. Epelboin, F. Libama, M. Lenzi, H. Sudeck, P. Yaba, Y. Allarangar, P. Boumandouki, V.B. Nkounkou, C. Drosten, A. Grolla, H. Feldmann, and C. Roth. 2006. Detection of Ebola virus in oral fluid specimens during outbreaks of Ebola virus hemorrhagic fever in the Republic of Congo. *Clinical Infectious Diseases* 42:1521–6.

Fouchier, R. A., T. Kuiken, M. Schutten, G. van Amerongen, G. J. van Doornum, B. G. van den Hoogen, M. Peiris, W. Lim, K. Stohr, and A. D. Osterhaus. 2003. Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* 423 (6937):240.

Franz, D. R., P. B. Jahrling, A. M. Friedlander, D. J. McClain, D. L. Hoover, W. R. Bryne, J. A. Pavlin, G. W. Christopher, and E. M. Eitzen, Jr. 1997. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA* 278 (5):399-411.

Fraser, C., S. Riley, R. M. Anderson, and N. M. Ferguson. 2004. Factors that make an infectious disease outbreak controllable. *Proc Natl Acad Sci U S A* 101 (16):6146-51.

Frean, J., K. P. Klugman, L. Arntzen, and S. Bukofzer. 2003. Susceptibility of Yersinia pestis to novel and conventional antimicrobial agents. *J Antimicrob Chemother* 52 (2):294-6.

Freiberg, A. N., M. N. Worthy, B. Lee, and M. R. Holbrook. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. *J Gen Virol* 91 (Pt 3):765-72.

Gabastou, J. M., J. Proano, A. Vimos, G. Jaramillo, E. Hayes, K. Gage, M. Chu, J. Guarner, S. Zaki, J. Bowers, C. Guillemard, H. Tamayo, and A. Ruiz. 2000. An outbreak of plague including cases with probable pneumonic infection, Ecuador, 1998. *Trans R Soc Trop Med Hyg* 94 (4):387-91.

Gaff, H.D., D. M. Hartley, and N.P. Leahy. 2007. An epidemiological model of Rfit Valley fever. *Electronic Journal of Differential Equations* 115:1-12.

Gage, K. L., D. T. Dennis, K. A. Orloski, P. Ettestad, T. L. Brown, P. J. Reynolds, W. J. Pape, C. L. Fritz, L. G. Carter, and J. D. Stein. 2000. Cases of cat-associated human plague in the Western US, 1977-1998. *Clin Infect Dis* 30 (6):893-900.

Gage, K. L., and M. Y. Kosoy. 2005. Natural history of plague: perspectives from more than a century of research. *Annu Rev Entomol* 50:505-28.

Gage, K.L. 1998. Plague. In *Topley and Wilson's Microbiology and Microbial Infections*, edited by L. Collier, A. Balows, M. Sussman and W. J. Hausler. London: Arnold.

Galeno, H., J. Mora, E. Villagra, J. Fernandez, J. Hernandez, G. J. Mertz, and E. Ramirez. 2002. First human isolate of Hantavirus (Andes virus) in the Americas. *Emerg Infect Dis* 8 (7):657-61.

Galimand, M., A. Guiyoule, G. Gerbaud, B. Rasoamanana, S. Chanteau, E. Carniel, and P. Courvalin. 1997. Multidrug resistance in Yersinia pestis mediated by a transferable plasmid. *N Engl J Med* 337 (10):677-80.

Galvani, A. P., X. Lei, and N. P. Jewell. 2003. Severe acute respiratory syndrome: temporal stability and geographic variation in case-fatality rates and doubling times. *Emerg Infect Dis* 9 (8):991-4.

Gani, R., H. Hughes, D. Fleming, T. Griffin, Jolyon Medlock, and S. Leach. 2005. Potential Impact of Antiviral Drug Use during Influenza Pandemic. *Emerging Infectious Diseases* 11 (9):1355-1362.

Gani, R., and S. Leach. 2004. Epidemiologic determinants for modeling pneumonic plague outbreaks. *Emerg Infect Dis* 10 (4):608-14.

Gasper, P. W., A. M. Barnes, T. J. Quan, J. P. Benziger, L. G. Carter, M. L. Beard, and G. O. Maupin. 1993. Plague (Yersinia pestis) in cats: description of experimentally induced disease. *J Med Entomol* 30 (1):20-6.

Geisbert, T. W., K. M. Daddario-Dicaprio, J. B. Geisbert, D. S. Reed, F. Feldmann, A. Grolla, U. Stroher, E. A. Fritz, L. E. Hensley, S. M. Jones, and H. Feldmann. 2008. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine* 26 (52):6894-900.

Geisbert, T. W., K. M. Daddario-DiCaprio, A. C. Hickey, M. A. Smith, Y. P. Chan, L. F. Wang, J. J. Mattapallil, J. B. Geisbert, K. N. Bossart, and C. C. Broder. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS ONE* 5 (5):e10690.

Geisbert, T. W., J. B. Geisbert, A. Leung, K. M. Daddario-DiCaprio, L. E. Hensley, A. Grolla, and H. Feldmann. 2009. Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of ebola virus. *J Virol* 83 (14):7296-304.

Geisbert, T. W., L. E. Hensley, T. Larsen, H. A. Young, D. S. Reed, J. B. Geisbert, D. P. Scott, E. Kagan, P. B. Jahrling, and K. J. Davis. 2003. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *American Journal of Pathology* 163 (6):2347-70.

Geisbert, T. W., and P. B. Jahrling. 2004. Exotic emerging viral diseases: progress and challenges. *Nat Med* 10 (12 Suppl):S110-21.

Gelman, A.G. 1961. The ecology of tularemia. In *Studies in disease ecology*, edited by J. M. May. New York: Hafner Publishing Co.

Gerdes, G. H. 2002. Rift valley fever. *Vet Clin North Am Food Anim Pract* 18 (3):549-55.

Repeated Author. 2004. Rift Valley fever. *Rev Sci Tech* 23 (2):613-23.

Repeated Author. 2005. Rift Valley Fever. In *The Merck Veterinary Manual*, edited by C. M. Kahn and S. Line. Whitehouse Station, N.J.: Merck & Co. Inc.

Germann, T. C., K. Kadau, I. M. Longini, Jr., and C. A. Macken. 2006. Mitigation strategies for pandemic influenza in the United States. *Proc Natl Acad Sci U S A* 103 (15):5935-40.

Gillim-Ross, L., and K. Subbarao. 2006. Emerging respiratory viruses: challenges and vaccine strategies. *Clin Microbiol Rev* 19 (4):614-36.

Gladstone, G.P. 1946. Immunity to anthrax: protective antigen present in cell-free culture filtrates. *Br J Exp Pathol* 27:394-418

Glass, W. G., K. Subbarao, B. Murphy, and P. M. Murphy. 2004. Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. *J Immunol* 173 (6):4030-9.

Glassman, H.N. 1966. Discussion: Industrial inhalation anthrax. *Bacteriological Reviews* 30 (3):657-659.

Goh, D. L., B. W. Lee, K. S. Chia, B. H. Heng, M. Chen, S. Ma, and C. C. Tan. 2004. Secondary household transmission of SARS, Singapore. *Emerg Infect Dis* 10 (2):232-4.

Goh, K. J., C. T. Tan, N. K. Chew, P. S. Tan, A. Kamarulzaman, S. A. Sarji, K. T. Wong, B. J. Abdullah, K. B. Chua, and S. K. Lam. 2000. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 342 (17):1229-35.

Gold, H. 1955. Anthrax; a report of one hundred seventeen cases. *AMA Arch Intern Med* 96 (3):387-96.

Gonzalez Della Valle, M., A. Edelstein, S. Miguel, V. Martinez, J. Cortez, M. L. Cacace, G. Jurgelenas, S. Sosa Estani, and P. Padula. 2002. Andes virus associated with hantavirus pulmonary syndrome in northern Argentina and determination of the precise site of infection. *Am J Trop Med Hyg* 66 (6):713-20.

Gonzalez, J. P., E. Nakoune, W. Slenczka, P. Vidal, and J. M. Morvan. 2000. Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. *Microbes Infect* 2 (1):39-44.

Gonzalez, J. P., X. Pourrut, and E. Leroy. 2007. Ebolavirus and other filoviruses. *Curr Top Microbiol Immunol* 315:363-87.

Goodnough, A. 2009. Anthrax case linked to drumming circle, New Hampshire officials say. *New York Times*, December 29, 2009.

Goossens, P. L. 2009. Animal models of human anthrax: the Quest for the Holy Grail. *Mol Aspects Med* 30 (6):467-80.

Gottfredsson, M., B. V. Halldorsson, S. Jonsson, M. Kristjansson, K. Kristjansson, K. G. Kristinsson, A. Love, T. Blondal, C. Viboud, S. Thorvaldsson, A. Helgason, J. R. Gulcher, K. Stefansson, and I. Jonsdottir. 2008. Lessons from the past: familial aggregation analysis of fatal pandemic influenza (Spanish flu) in Iceland in 1918. *Proc Natl Acad Sci U S A* 105 (4):1303-8.

Gould, E. A., and T. Solomon. 2008. Pathogenic flaviviruses. *Lancet* 371 (9611):500-9.

Greco, D., G. Allegrini, T. Tizzi, E. Ninu, A. Lamanna, and S. Luzi. 1987. A waterborne tularemia outbreak. *Eur J Epidemiol* 3 (1):35-8.

Greene, C. M., J. Reefhuis, C. Tan, A. E. Fiore, S. Goldstein, M. J. Beach, S. C. Redd, D. Valiante, G. Burr, J. Buehler, R. W. Pinner, E. Bresnitz, and B. P. Bell. 2002. Epidemiologic investigations of bioterrorism-related anthrax, New Jersey, 2001. *Emerg Infect Dis* 8 (10):1048-55.

Greenough, T. C., A. Carville, J. Coderre, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, and K. Mansfield. 2005. Pneumonitis and multi-organ system disease in common marmosets (Callithrix jacchus) infected with the severe acute respiratory syndrome-associated coronavirus. *Am J Pathol* 167 (2):455-63.

Gresikova, M., Calisher,C.H. . 1989. Tick-borne Encephalitis. In *Epidemiology and Ecology*, edited by T. P. Monath. Boca Raton: CRC Press Inc.

Griffin, K. F., P. C. Oyston, and R. W. Titball. 2007. Francisella tularensis vaccines. *FEMS Immunol Med Microbiol* 49 (3):315-23.

Gritsun, T. S., V. A. Lashkevich, and E. A. Gould. 2003. Tick-borne encephalitis. *Antiviral Res* 57 (1-2):129-46.

Groseth, A., H. Feldmann, and J. E. Strong. 2007. The ecology of Ebola virus. *Trends Microbiol* 15 (9):408-16.

Guan, Y., B. J. Zheng, Y. Q. He, X. L. Liu, Z. X. Zhuang, C. L. Cheung, S. W. Luo, P. H. Li, L. J. Zhang, Y. J. Guan, K. M. Butt, K. L. Wong, K. W. Chan, W. Lim, K. F. Shortridge, K. Y. Yuen, J. S. Peiris, and L. L. Poon. 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 302 (5643):276-8.

Guillaume, V., H. Contamin, P. Loth, M. C. Georges-Courbot, A. Lefeuvre, P. Marianneau, K. B. Chua, S. K. Lam, R. Buckland, V. Deubel, and T. F. Wild. 2004. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol* 78 (2):834-40.

Gumel, A. B., S. Ruan, T. Day, J. Watmough, F. Brauer, P. van den Driessche, D. Gabrielson, C. Bowman, M. E. Alexander, S. Ardal, J. Wu, and B. M. Sahai. 2004. Modelling strategies for controlling SARS outbreaks. *Proc Biol Sci* 271 (1554):2223-32.

Gunther, G., and M. Haglund. 2005. Tick-borne encephalopathies : epidemiology, diagnosis, treatment and prevention. *CNS Drugs* 19 (12):1009-32.

Gunther, S., and O. Lenz. 2004. Lassa virus. *Crit Rev Clin Lab Sci* 41 (4):339-90.

Gurley, E. S., J. M. Montgomery, M. J. Hossain, M. Bell, A. K. Azad, M. R. Islam, M. A. Molla, D. S. Carroll, T. G. Ksiazek, P. A. Rota, L. Lowe, J. A. Comer, P. Rollin, M. Czub, A. Grolla, H. Feldmann, S. P. Luby, J. L. Woodward, and R. F. Breiman. 2007. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg Infect Dis* 13 (7):1031-7.

Gurley, E. S., J. M. Montgomery, M. J. Hossain, M. R. Islam, M. A. Molla, S. M. Shamsuzzaman, K. Akram, K. Zaman, N. Asgari, J. A. Comer, A. K. Azad, P. E. Rollin, T. G. Ksiazek, and R. F. Breiman. 2007. Risk of nosocomial transmission of Nipah virus in a Bangladesh hospital. *Infect Control Hosp Epidemiol* 28 (6):740-2.

Halfmann, P., J. H. Kim, H. Ebihara, T. Noda, G. Neumann, H. Feldmann, and Y. Kawaoka. 2008. Generation of biologically contained Ebola viruses. *Proc Natl Acad Sci U S A* 105 (4):1129-33.

Halpin, K., and B. A. Mungall. 2007. Recent progress in henipavirus research. *Comp Immunol Microbiol Infect Dis* 30 (5-6):287-307.

Halsted, C. C., and H. P. Kulasinghe. 1978. Tularemia pneumonia in urban children. *Pediatrics* 61 (4):660-2.

Hambleton, P., J. R. Stephenson, A. Baskerville, and C. N. Wiblin. 1983. Pathogenesis and immune response of vaccinated and unvaccinated rhesus monkeys to tick-borne encephalitis virus. *Infect Immun* 40 (3):995-1003.

Hanson, R. P., S. E. Sulkin, E. L. Beuscher, W. M. Hammon, R. W. McKinney, and T. H. Work. 1967. Arbovirus infections of laboratory workers. Extent of problem emphasizes the need for more effective measures to reduce hazards. *Science* 158 (806):1283-6.

Harding, A.L., Byers, K.B. 2006. *Epidemiology of Laboratory-Associated Infections*. Edited by D. O. Fleming, Hunt, D.L. 4th ed, *Biological Safety: Principles and Practices*: ASM Press.

Harit, A. K., R. L. Ichhpujani, S. Gupta, K. S. Gill, S. Lal, N. K. Ganguly, and S. P. Agarwal. 2006. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. *Indian J Med Res* 123 (4):553-60.

Harper, G.J. 1961. Airborne micro-organisms: survival tests with four viruses. *J Hyg* 59:479-486.

Harrison, L. H., N. A. Halsey, K. T. McKee, Jr., C. J. Peters, J. G. Barrera Oro, A. M. Briggiler, M. R. Feuillade, and J. I. Maiztegui. 1999. Clinical case definitions for Argentine hemorrhagic fever. *Clin Infect Dis* 28 (5):1091-4.

Hassoun, A., R. Spera, and J. Dunkel. 2006. Tularemia and Once-Daily Gentamicin. *Antimicrob Agents Chemother* 50 (2):824.

Hatchett, R. J., C. E. Mecher, and M. Lipsitch. 2007. Public health interventions and epidemic intensity during the 1918 influenza pandemic. *Proc Natl Acad Sci U S A* 104 (18):7582-7.

Hay, S. I., M. F. Myers, D. S. Burke, D. W. Vaughn, T. Endy, N. Ananda, G. D. Shanks, R. W. Snow, and D. J. Rogers. 2000. Etiology of interepidemic periods of mosquito-borne disease. *Proc Natl Acad Sci U S A* 97 (16):9335-9.

Hayman, D. T., R. Suu-Ire, A. C. Breed, J. A. McEachern, L. Wang, J. L. Wood, and A. A. Cunningham. 2008. Evidence of henipavirus infection in West African fruit bats. *PLoS ONE* 3 (7):e2739.

Heine, H.S., Louie, A., Sorgel, F., et al. 2007. Comparison of 2 antibiotics that inhibit protein synthesis for the treatment of infection with *Yersinia pestis* delivered by aerosol in a mouse model of pneumonic plague. *J Infect Dis* 1 (196 (5)):782-787.

Heinz, F. X., H. Holzmann, A. Essl, and M. Kundi. 2007. Field effectiveness of vaccination against tick-borne encephalitis. *Vaccine* 25 (43):7559-67.

Henderson, D. W., S. Peacock, and F. C. Belton. 1956. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. *J Hyg (Lond)* 54 (1):28-36.

Henkel, R. 2008. *Antiviral prophylaxis: implications for determining biosafety containment level and practices*. Bethesda: NIH-RAC.

Repeated Author. 2008. *Research with 1918 influenza virus*. Bethesda: NIH-RAC.

Hensley, L. E., S. M. Jones, H. Feldmann, P. B. Jahrling, and T. W. Geisbert. 2005. Ebola and Marburg viruses: pathogenesis and development of countermeasures. *Curr Mol Med* 5 (8):761-72.

HHS. *HHS to Acquire Anthrax Immune Globulin for Stockpile*, July 28, 2006 2006. Available from http://www.hhs.gov/news/press/2006pres/20060728.html.

Hinnebusch, B. J. 2003. Transmission factors: Yersinia pestis genes required to infect the flea vector of plague. *Adv Exp Med Biol* 529:55-62.

Holty, J. E., D. M. Bravata, H. Liu, R. A. Olshen, K. M. McDonald, and D. K. Owens. 2006. Systematic review: a century of inhalational anthrax cases from 1900 to 2005. *Ann Intern Med* 144 (4):270-80.

Holty, J. E., R. Y. Kim, and D. M. Bravata. 2006. Anthrax: a systematic review of atypical presentations. *Ann Emerg Med* 48 (2):200-11.

Hood, A. M. 2009. The effect of open-air factors on the virulence and viability of airborne Francisella tularensis. *Epidemiol Infect* 137 (6):753-61.

Hoogstraal, H., J. M. Meegan, G. M. Khalil, and F. K. Adham. 1979. The Rift Valley fever epizootic in Egypt 1977-78. 2. Ecological and entomological studies. *Trans R Soc Trop Med Hyg* 73 (6):624-9.

Hooper, J. W., D. M. Custer, J. Smith, and V. Wahl-Jensen. 2006. Hantaan/Andes virus DNA vaccine elicits a broadly cross-reactive neutralizing antibody response in nonhuman primates. *Virology* 347 (1):208-16.

Hooper, J. W., A. M. Ferro, and V. Wahl-Jensen. 2008. Immune serum produced by DNA vaccination protects hamsters against lethal respiratory challenge with Andes virus. *J Virol* 82 (3):1332-8.

Hooper, J. W., T. Larsen, D. M. Custer, and C. S. Schmaljohn. 2001. A lethal disease model for hantavirus pulmonary syndrome. *Virology* 289 (1):6-14.

Hopla, C. E. 1974. The ecology of tularemia. *Adv Vet Sci Comp Med* 18 (0):25-53.

Houhamdi, L., H. Lepidi, M. Drancourt, and D. Raoult. 2006. Experimental model to evaluate the human body louse as a vector of plague. *J Infect Dis* 194 (11):1589-96.

Hsieh, Y. H., C. W. Chen, and S. B. Hsu. 2004. SARS outbreak, Taiwan, 2003. *Emerg Infect Dis* 10 (2):201-6.

Hsu, V. P., M. J. Hossain, U. D. Parashar, M. M. Ali, T. G. Ksiazek, I. Kuzmin, M. Niezgoda, C. Rupprecht, J. Bresee, and R. F. Breiman. 2004. Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* 10 (12):2082-7.

Hufnagel, L., D. Brockmann, and T. Geisel. 2004. Forecast and control of epidemics in a globalized world. *Proc Natl Acad Sci U S A* 101 (42):15124-9.

Huggins, J., Z. X. Zhang, and M. Bray. 1999. Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus in vitro and in a lethal mouse model. *J Infect Dis* 179 Suppl 1:S240-7.

Hugh-Jones, M., and J. Blackburn. 2009. The ecology of Bacillus anthracis. *Mol Aspects Med* 30 (6):356-67.

Hull, H. F., J. M. Montes, and J. M. Mann. 1987. Septicemic plague in New Mexico. *J Infect Dis* 155 (1):113-8.

Human Genome Sciences. *Human Genome Sciences begins delivery of first-in-class anthrax treament to U.S. strategic national stockpile* 2009 [cited January 18, 2010. Available from http://www.hgsi.com/latest/human-genome-sciences-begins-delivery-of-first-in-class-anthrax-treatment-to-u.s.-strategic-national-stoc-4.html.

Hung, I. F., V. C. Cheng, A. K. Wu, B. S. Tang, K. H. Chan, C. M. Chu, M. M. Wong, W. T. Hui, L. L. Poon, D. M. Tse, K. S. Chan, P. C. Woo, S. K. Lau, J. S. Peiris, and K. Y. Yuen. 2004. Viral loads in clinical specimens and SARS manifestations. *Emerg Infect Dis* 10 (9):1550-7.

Hyatt, A. D., P. Daszak, A. A. Cunningham, H. Field, and A. R. Gould. 2004. Henipaviruses: gaps in the knowledge of emergence. *EcoHealth* 1:25-38.

ICTVdB - The Universal Virus Database. 2006. Arenavirus. edited by I. C. o. T. o. Viruses.

Ijaz, M. K., A. H. Brunner, S. A. Sattar, R. C. Nair, and C. M. Johnson-Lussenburg. 1985. Survival characteristics of airborne human coronavirus 229E. *J Gen Virol* 66 (Pt 12):2743-8.

Inegbenebor, U., J. Okosun, and J. Inegbenebor. Prevention of lassa Fever in Nigeria. *Trans R Soc Trop Med Hyg* 104 (1):51-4.

Inglesby, T. V., D. T. Dennis, D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. D. Fine, A. M. Friedlander, J. Hauer, J. F. Koerner, M. Layton, J. McDade, M. T. Osterholm, T. O'Toole, G. Parker, T. M. Perl, P. K. Russell, M. Schoch-Spana, and K. Tonat. 2000. Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 283 (17):2281-90.

Inglesby, T. V., D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. M. Friedlander, J. Hauer, J. McDade, M. T. Osterholm, T. O'Toole, G. Parker, T. M. Perl, P. K. Russell, and K. Tonat. 1999. Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 281 (18):1735-45.

Inglesby, T. V., T. O'Toole, D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. M. Friedlander, J. Gerberding, J. Hauer, J. Hughes, J. McDade, M. T. Osterholm, G. Parker, T. M. Perl, P. K. Russell, and K. Tonat. 2002. Anthrax as a biological weapon, 2002: updated recommendations for management. *JAMA* 287 (17):2236-52.

Isakbaeva, E. T., N. Khetsuriani, R. S. Beard, A. Peck, D. Erdman, S. S. Monroe, S. Tong, T. G. Ksiazek, S. Lowther, I. Pandya-Smith, L. J. Anderson, J. Lingappa, and M. A. Widdowson. 2004. SARS-associated coronavirus transmission, United States. *Emerg Infect Dis* 10 (2):225-31.

Jaax, N., P. Jahrling, T. Geisbert, J. Geisbert, K. Steele, K. McKee, D. Nagley, E. Johnson, G. Jaax, and C. Peters. 1995. Transmission of Ebola virus (Zaire strain) to uninfected control monkeys in a biocontainment laboratory. *Lancet* 346 (8991-8992):1669-71.

Jaax, N. K., K. J. Davis, T. J. Geisbert, P. Vogel, G. P. Jaax, M. Topper, and P. B. Jahrling. 1996. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. *Arch Pathol Lab Med* 120 (2):140-55.

Jackson, D., M. J. Hossain, D. Hickman, D. R. Perez, and R. A. Lamb. 2008. A new influenza virus virulence determinant: the NS1 protein four C-terminal residues modulate pathogenicity. *Proc Natl Acad Sci U S A* 105 (11):4381-6.

Jahrling, P. B., J. B. Geisbert, J. R. Swearengen, T. Larsen, and T. W. Geisbert. 2007. Ebola hemorrhagic fever: evaluation of passive immunotherapy in nonhuman primates. *J Infect Dis* 196 Suppl 2:S400-3.

Jahrling, P. B., J. Geisbert, J. R. Swearengen, G. P. Jaax, T. Lewis, J. W. Huggins, J. J. Schmidt, J. W. LeDuc, and C. J. Peters. 1996. Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. *Arch Virol Suppl* 11:135-40.

Jahrling, P. B., S. Smith, R. A. Hesse, and J. B. Rhoderick. 1982. Pathogenesis of Lassa virus infection in guinea pigs. *Infect Immun* 37 (2):771-8.

Jay, M. T., C. Glaser, and C. F. Fulhorst. 2005. The arenaviruses. *J Am Vet Med Assoc* 227 (6):904-15.

Jeffs, B. 2006. A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa. *Trop Doct* 36 (1):1-4.

Jellison, W. L. 1950. Tularemia; geographical distribution of deerfly fever and the biting fly, Chrysops discalis Williston. *Public Health Rep* 65 (41):1321-9.

Jernigan, J. A., D. S. Stephens, D. A. Ashford, C. Omenaca, M. S. Topiel, M. Galbraith, M. Tapper, T. L. Fisk, S. Zaki, T. Popovic, R. F. Meyer, C. P. Quinn, S. A. Harper, S. K. Fridkin, J. J. Sejvar, C. W. Shepard, M. McConnell, J. Guarner, W. J. Shieh, J. M. Malecki, J. L. Gerberding, J. M. Hughes, and B. A. Perkins. 2001. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg Infect Dis* 7 (6):933-44.

Jia, Q., B. Y. Lee, D. L. Clemens, R. A. Bowen, and M. A. Horwitz. 2009. Recombinant attenuated Listeria monocytogenes vaccine expressing Francisella tularensis IglC induces protection in mice against aerosolized Type A F. tularensis. *Vaccine* 27 (8):1216-29.

Johansson, A., L. Berglund, L. Gothefors, A. Sjostedt, and A. Tarnvik. 2000. Ciprofloxacin for treatment of tularemia in children. *Pediatr Infect Dis J* 19 (5):449-53.

Johnson, E., N. Jaax, J. White, and P. Jahrling. 1995. Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *Int J Exp Pathol* 76 (4):227-36.

Johnson, K.M. 2008. Personal Communication.

Johnson, R. 2006. Epizootiology and ecology of anthrax. edited by A. a. P. H. I. S. United States Department of Agriculture, Veterinary Service.

Repeated Author. 2008. Differentiation of naturally occurring from non-naturally occurring epizootics of anthrax in livestock populations. edited by Department of Agriculture: Animal and Plant Health Inspection Service, Veterinary Service,.

Kaiser, R. 2008. Tick-borne encephalitis. *Infect Dis Clin North Am* 22 (3):561-75, x.

Kammila, S., D. Das, P. K. Bhatnagar, H. H. Sunwoo, G. Zayas-Zamora, M. King, and M. R. Suresh. 2008. A rapid point of care immunoswab assay for SARS-CoV detection. *J Virol Methods* 152 (1-2):77-84.

Kartman, L., S. F. Quan, and H. E. Stark. 1962. Ecological studies of wild rodent plague in the San Francisco Bay Area of California. VII. Effects of plague in nature on Microtus californicus and other wild rodents. *Zoonoses Res* 1:99-119.

Katz, J. . 2008. *Research with 1918 influenza virus*. Bethesda: NIH-RAC.

Kaufmann, A. F., J. M. Boyce, and W. J. Martone. 1980. From the Center for Disease Control. Trends in human plague in the United States. *J Infect Dis* 141 (4):522-4.

Keefer, G. V., G. L. Zebarth, and W. P. Allen. 1972. Susceptibility of dogs and cats to Rift Valley fever by inhalation or ingestion of virus. *J Infect Dis* 125 (3):307-9.

Keim, P., A. Johansson, and D. M. Wagner. 2007. Molecular epidemiology, evolution, and ecology of Francisella. *Ann N Y Acad Sci* 1105:30-66.

Kenyon, R. H., D. E. Green, J. I. Maiztegui, and C. J. Peters. 1988. Viral strain dependent differences in experimental Argentine hemorrhagic fever (Junin virus) infection of guinea pigs. *Intervirology* 29 (3):133-43.

Kenyon, R. H., K. T. McKee, Jr., P. M. Zack, M. K. Rippy, A. P. Vogel, C. York, J. Meegan, C. Crabbs, and C. J. Peters. 1992. Aerosol infection of rhesus macaques with Junin virus. *Intervirology* 33 (1):23-31.

Kenyon, R. H., M. K. Rippy, K. T. McKee, Jr., P. M. Zack, and C. J. Peters. 1992. Infection of Macaca radiata with viruses of the tick-borne encephalitis group. *Microb Pathog* 13 (5):399-409.

Keppie, J., H. Smith, and P. W. Harris-Smith. 1955. The chemical basis of the virulence of Bacillus anthracis. III. The role of the terminal bacteraemia in death of guinea-pigs from anthrax. *Br J Exp Pathol* 36 (3):315-22.

Kerneis, S., L. Koivogui, N. Magassouba, K. Koulemou, R. Lewis, A. Aplogan, R. F. Grais, P. J. Guerin, and E. Fichet-Calvet. 2009. Prevalence and risk factors of Lassa seropositivity in

inhabitants of the forest region of Guinea: a cross-sectional study. *PLoS Negl Trop Dis* 3 (11):e548.

Khan, A.S., Ksiazek, T.G., and C.J. Peters. 1997. Viral hemorrhagic fevers among children. *Seminar of the Pediatric Infectious Disease Journal* 8:64-73.

Khan, S. H., A. Goba, M. Chu, C. Roth, T. Healing, A. Marx, J. Fair, M. C. Guttieri, P. Ferro, T. Imes, C. Monagin, R. F. Garry, and D. G. Bausch. 2008. New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res* 78 (1):103-15.

Kiley, Michael P., Nancy Cox, Luanne H. Elliott, Anthony Sanchez, Ricarda DeFries, Michael J. Buchmeier, Douglas D. Richman, and Joseph B. McCormick. 1988. Physiochemical Properties of Marburg Virus: Evidence for Three Distinct Virus Strains and Their Relationship to Ebola Virus *J. Gen Virol* 69:1957-1967.

King, J.W. 2008. Ebola virus. *eMedicine*, http://emedicine.medscape.com/article/216288-overview

Klein, F., W. Jones Jr, B.G. Mahlandt, and R.E. Lincoln. 1971. Growth of Pathogenic Virus in a Large-Scale Tissue Culture System. *Applied Microbiology* 21 (2):265-271.

Klein, F., B.G. Mahlandt, H.B. Bonner, and R.E. Lincoln. 1971. Ultrafiltration as a Method for Concentrating Rift Valley Fever Virus Grown in Tissue Culture. *Applied Microbiology* 21 (4):758-760.

Klock, L. E., P. F. Olsen, and T. Fukushima. 1973. Tularemia epidemic associated with the deerfly. *JAMA* 226 (2):149-52.

Klontz, K. C., N. A. Hynes, R. A. Gunn, M. H. Wilder, M. W. Harmon, and A. P. Kendal. 1989. An outbreak of influenza A/Taiwan/1/86 (H1N1) infections at a naval base and its association with airplane travel. *Am J Epidemiol* 129 (2):341-8.

Klugman, K. P., C. M. Astley, and M. Lipsitch. 2009. Time from illness onset to death, 1918 influenza and pneumococcal pneumonia. *Emerg Infect Dis* 15 (2):346-7.

Kofler, R. M., J. H. Aberle, S. W. Aberle, S. L. Allison, F. X. Heinz, and C. W. Mandl. 2004. Mimicking live flavivirus immunization with a noninfectious RNA vaccine. *Proc Natl Acad Sci U S A* 101 (7):1951-6.

Kong, W. P., C. Hood, Z. Y. Yang, C. J. Wei, L. Xu, A. Garcia-Sastre, T. M. Tumpey, and G. J. Nabel. 2006. Protective immunity to lethal challenge of the 1918 pandemic influenza virus by vaccination. *Proc Natl Acad Sci U S A* 103 (43):15987-91.

Kool, J. L. 2005. Risk of person-to-person transmission of pneumonic plague. *Clin Infect Dis* 40 (8):1166-72.

Korenberg, E. I., A. A. Pchelkina, and L. N. Spitsina. 1984. Consistent patterns in the contact of domestic animals with tick-borne encephalitis virus in the eastern part of the Russian plain. *J Hyg Epidemiol Microbiol Immunol* 28 (1):73-84.

Ksiazek, T. G., J. G. Olson, G. S. Irving, C. S. Settle, R. White, and R. Petrusso. 1980. An influenza outbreak due to A/USSR/77-like (H1N1) virus aboard a US Navy ship. *Am J Epidemiol* 112 (4):487-94.

Kuiken, T., R. A. Fouchier, M. Schutten, G. F. Rimmelzwaan, G. van Amerongen, D. van Riel, J. D. Laman, T. de Jong, G. van Doornum, W. Lim, A. E. Ling, P. K. Chan, J. S. Tam, M. C. Zambon, R. Gopal, C. Drosten, S. van der Werf, N. Escriou, J. C. Manuguerra, K. Stohr, J. S. Peiris, and A. D. Osterhaus. 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 362 (9380):263-70.

Kumor, L, L. Bates, and S. Stephens. *2005 Anthrax outbreak in Manitoba*. Canadian Food Inspection Agency 2006. Available from http://www.gov.mb.ca/agriculture/livestock/anhealth/pdf/jaa02s00c.pdf.

Kunanusont, C., K. Limpakarnjanarat, and H. M. Foy. 1990. Outbreak of anthrax in Thailand. *Ann Trop Med Parasitol* 84 (5):507-12.

Kunze, U. 2008. Conference report of the 10th meeting of the international scientific working group on tick-borne encephalitis (ISW-TBE): combating tick-borne encephalitis: vaccination rates on the rise. *Vaccine* 26 (52):6738-40.

KuoLee, R., X. Zhao, J. Austin, G. Harris, J. W. Conlan, and W. Chen. 2007. Mouse model of oral infection with virulent type A Francisella tularensis. *Infect Immun* 75 (4):1651-60.

Kuzmin, I. V., M. Niezgoda, R. Franka, B. Agwanda, W. Markotter, R. F. Breiman, W. J. Shieh, S. R. Zaki, and C. E. Rupprecht. Marburg virus in fruit bat, Kenya. *Emerg Infect Dis* 16 (2):352-4.

LaBeaud, A. D., Y. Ochiai, C. J. Peters, E. M. Muchiri, and C. H. King. 2007. Spectrum of Rift Valley fever virus transmission in Kenya: insights from three distinct regions. *Am J Trop Med Hyg* 76 (5):795-800.

Labuda, M., and P. A. Nuttall. 2004. Tick-borne viruses. *Parasitology* 129 Suppl:S221-45.

Lam, W. K., N. S. Zhong, and W. C. Tan. 2003. Overview on SARS in Asia and the world. *Respirology* 8 Suppl:S2-5.

Larrieu, E., G. Cantoni, E. Herrero, A. Perez, G. Talmon, G. Vazquez, O. Arellano, and P. Padula. 2008. Hantavirus antibodies in rodents and human cases with pulmonary syndrome, Rio Negro, Argentina. *Medicina (B Aires)* 68 (5):373-9.

Lau, J. T., M. Lau, J. H. Kim, H. Y. Tsui, T. Tsang, and T. W. Wong. 2004. Probable secondary infections in households of SARS patients in Hong Kong. *Emerg Infect Dis* 10 (2):235-43.

Lázaro, M. E., G. E. Cantoni, L. M. Calanni, A. J. Resa, E. R. Herrero, M. A. Iacono, D. A. Enria, and S. M. Gonzalez Cappa. 2007. Clusters of hantavirus infection, southern Argentina. *Emerg Infect Dis* 13 (1):104-10.

Lázaro, Maria E., Gustavo E. Cantoni, Liliana M. Calanni,‡Amanda J. Resa,§Eduardo R. Herrero, Marisa A. Iacono, Delia A. Enria, and Stella M. González Cappa 2007. Clusters of Hantavirus Infection, Southern Argentina. *Emerging Infectious Diseases* 13 (1):104-110.

Lecompte, E., E. Fichet-Calvet, S. Daffis, K. Koulemou, O. Sylla, F. Kourouma, A. Dore, B. Soropogui, V. Aniskin, B. Allali, S. Kouassi Kan, A. Lalis, L. Koivogui, S. Gunther, C. Denys, and J. ter Meulen. 2006. Mastomys natalensis and Lassa fever, West Africa. *Emerg Infect Dis* 12 (12):1971-4.

LeDuc, J. W. 1989. Epidemiology of hemorrhagic fever viruses. *Rev Infect Dis* 11 Suppl 4:S730-5.

Leendertz, F. H., H. Ellerbrok, C. Boesch, E. Couacy-Hymann, K. Matz-Rensing, R. Hakenbeck, C. Bergmann, P. Abaza, S. Junglen, Y. Moebius, L. Vigilant, P. Formenty, and G. Pauli. 2004. Anthrax kills wild chimpanzees in a tropical rainforest. *Nature* 430 (6998):451-2.

Leendertz, F. H., S. Yumlu, G. Pauli, C. Boesch, E. Couacy-Hymann, L. Vigilant, S. Junglen, S. Schenk, and H. Ellerbrok. 2006. A new Bacillus anthracis found in wild chimpanzees and a gorilla from West and Central Africa. *PLoS Pathog* 2 (1):e8.

Leffel, E. K., and D. S. Reed. 2004. Marburg and Ebola viruses as aerosol threats. *Biosecur Bioterror* 2 (3):186-91.

Leitner, M. *Tick-borne encephalitis; Austria*. International Society for Infectious Disease 2009. Available from

http://www.promedmail.org/pls/apex/f?p=2400:1202:6221312850795987::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,75919.

Lekone, P. E., and B. F. Finkenstadt. 2006. Statistical inference in a stochastic epidemic SEIR model with control intervention: Ebola as a case study. *Biometrics* 62 (4):1170-7.

Lemaitre, M., and F. Carrat. 2010. Comparative age distribution of influenza morbidity and mortality during seasonal influenza epidemics and the 2009 H1N1 pandemic. *BMC Infect Dis* 10:162.

Leong, H. N., K. P. Chan, A. S. Khan, L. Oon, S. Y. Se-Thoe, X. L. Bai, D. Yeo, Y. S. Leo, B. Ang, T. G. Ksiazek, and A. E. Ling. 2004. Virus-specific RNA and antibody from convalescent-phase SARS patients discharged from hospital. *Emerg Infect Dis* 10 (10):1745-50.

Leroy, E. M., B. Kumulungui, X. Pourrut, P. Rouquet, A. Hassanin, P. Yaba, A. Delicat, J. T. Paweska, J. P. Gonzalez, and R. Swanepoel. 2005. Fruit bats as reservoirs of Ebola virus. *Nature* 438 (7068):575-6.

Leroy, E. M., P. Rouquet, P. Formenty, S. Souquiere, A. Kilbourne, J. M. Froment, M. Bermejo, S. Smit, W. Karesh, R. Swanepoel, S. R. Zaki, and P. E. Rollin. 2004. Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 303 (5656):387-90.

Leroy, E. M., P. Telfer, B. Kumulungui, P. Yaba, P. Rouquet, P. Roques, J. P. Gonzalez, T. G. Ksiazek, P. E. Rollin, and E. Nerrienet. 2004. A serological survey of Ebola virus infection in central African nonhuman primates. *J Infect Dis* 190 (11):1895-9.

Leung, G. M., W. W. Lim, L. M. Ho, T. H. Lam, A. C. Ghani, C. A. Donnelly, C. Fraser, S. Riley, N. M. Ferguson, R. M. Anderson, and A. J. Hedley. 2006. Seroprevalence of IgG antibodies to SARS-coronavirus in asymptomatic or subclinical population groups. *Epidemiol Infect* 134 (2):211-21.

Levis, S., Fevillade,M.R., Enria, D.A., Ambrosio,A.M., Briggiler,A.M., McKee,K., and J.I. Maiztegui. 1993. Persistencia de la inmunidad humoral específica en receptors de la vacuna Candid #1 contra la fiebre hemorrhágica argentina (FHA). *Medicina (B Aires)* 53 ((Suppl 2) 271):131-132.

Levis, S., J. E. Rowe, S. Morzunov, D. A. Enria, and S. St Jeor. 1997. New hantaviruses causing hantavirus pulmonary syndrome in central Argentina. *Lancet* 349 (9057):998-9.

Levis, S.C., Briggiler, A.M., Cacass ,M., Peters,C.J., Ksiazek, T.J., Cortes,J., Lázaro, M.E., Resa, A., Rollin, P.E., Pinheiro, F.P., and D. Enriz. 1995. Emergence of hantavirus pulmonary syndrome in Argentina [abstract]. *American Journal of Tropical Medicine and Hygiene* 54 (Suppl:441,).

Liang, W., Z. Zhu, J. Guo, Z. Liu, W. Zhou, D. P. Chin, and A. Schuchat. 2004. Severe acute respiratory syndrome, Beijing, 2003. *Emerg Infect Dis* 10 (1):25-31.

Lillie, R.D., and E. Francis. 1937. The pathology of tularaemia in man (Homo sapiens). Washington, D.C.: US Government Printing Office.

Lim, W., K. C. Ng, and D. N. Tsang. 2006. Laboratory containment of SARS virus. *Ann Acad Med Singapore* 35 (5):354-60.

Limaye, A. P., and C. J. Hooper. 1999. Treatment of tularemia with fluoroquinolones: two cases and review. *Clin Infect Dis* 29 (4):922-4.

Lindquist, L., and O. Vapalahti. 2008. Tick-borne encephalitis. *Lancet* 371 (9627):1861-71.

Linthicum, K. J., A. Anyamba, C. J. Tucker, P. W. Kelley, M. F. Myers, and C. J. Peters. 1999. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science* 285 (5426):397-400.

Lipsitch, M., T. Cohen, B. Cooper, J. M. Robins, S. Ma, L. James, G. Gopalakrishna, S. K. Chew, C. C. Tan, M. H. Samore, D. Fisman, and M. Murray. 2003. Transmission dynamics and control of severe acute respiratory syndrome. *Science* 300 (5627):1966-70.

Lloyd-Smith, J. O., A. P. Galvani, and W. M. Getz. 2003. Curtailing transmission of severe acute respiratory syndrome within a community and its hospital. *Proc Biol Sci* 270 (1528):1979-89.

Lloyd-Smith, J. O., S. J. Schreiber, P. E. Kopp, and W. M. Getz. 2005. Superspreading and the effect of individual variation on disease emergence. *Nature* 438 (7066):355-9.

Lloyd, G., E. T. Bowen, and J. H. Slade. 1982. Physical and chemical methods of inactivating Lassa virus. *Lancet* 1 (8280):1046-8.

Lo, J. Y., T. H. Tsang, Y. H. Leung, E. Y. Yeung, T. Wu, and W. W. Lim. 2005. Respiratory infections during SARS outbreak, Hong Kong, 2003. *Emerg Infect Dis* 11 (11):1738-41.

Lo, M. K., and P. A. Rota. 2008. The emergence of Nipah virus, a highly pathogenic paramyxovirus. *J Clin Virol* 43 (4):396-400.

Longini, I. M., Jr., M. E. Halloran, A. Nizam, and Y. Yang. 2004. Containing pandemic influenza with antiviral agents. *Am J Epidemiol* 159 (7):623-33.

Longini, I. M., Jr., J. S. Koopman, A. S. Monto, and J. P. Fox. 1982. Estimating household and community transmission parameters for influenza. *Am J Epidemiol* 115 (5):736-51.

Loosli, C.G., H.M. Lemon, O.H Roberstson, and E. Appel. 1943. Experimental airborne influenza infection. I. Influence of humidity on survival of virus in air. *Proc. Soc. Exp. Biol.* 53:205-206.

Lopez, N., P. Padula, C. Rossi, M. E. Lazaro, and M. T. Franze-Fernandez. 1996. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. *Virology* 220 (1):223-6.

Lorange, E. A., B. L. Race, F. Sebbane, and B. Joseph Hinnebusch. 2005. Poor vector competence of fleas and the evolution of hypervirulence in Yersinia pestis. *J Infect Dis* 191 (11):1907-12.

Low, J.G.H., and A. Wilder-Smith. 2005. Infectious respiratory illnesses and their impact on health-care workers: a review. *Ann Acad Med Singapore* 34:105-110.

Lowen, A. C., S. Mubareka, J. Steel, and P. Palese. 2007. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* 3 (10):1470-6.

Lowen, A. C., S. Mubareka, T. M. Tumpey, A. Garcia-Sastre, and P. Palese. 2006. The guinea pig as a transmission model for human influenza viruses. *Proc Natl Acad Sci U S A* 103 (26):9988-92.

Lub, MIu, A. N. Sergeev, V. P'Iankov O, G. P'Iankova O, V. A. Petrishchenko, and L. A. Kotliarov. 1995. [Certain pathogenetic characteristics of a disease in monkeys in infected with the Marburg virus by an airborne route]. *Vopr Virusol* 40 (4):158-61.

Luby, S. P., M. J. Hossain, E. S. Gurley, B. N. Ahmed, S. Banu, S. U. Khan, N. Homaira, P. A. Rota, P. E. Rollin, J. A. Comer, E. Kenah, T. G. Ksiazek, and M. Rahman. 2009. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* 15 (8):1229-35.

Luby, S. P., M. Rahman, M. J. Hossain, L. S. Blum, M. M. Husain, E. Gurley, R. Khan, B. N. Ahmed, S. Rahman, N. Nahar, E. Kenah, J. A. Comer, and T. G. Ksiazek. 2006. Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis* 12 (12):1888-94.

Lyons, C.R., and T. H. Wu. 2007. Animal models of Francisella tularensis infection. *Ann N Y Acad Sci* 1105:238-65.

Lytle, C. D., and J. L. Sagripanti. 2005. Predicted inactivation of viruses of relevance to biodefense by solar radiation. *J Virol* 79 (22):14244-52.

Mackenzie, D. 2007. Fruit bats carry deadly Marburg virus. *New Scientist*, 1.

Mahanty, S., and M. Bray. 2004. Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect Dis* 4 (8):487-98.

Mahlandt, B. G., F. Klein, R. E. Lincoln, B. W. Haines, W. I. Jones, Jr., and R. H. Friedman. 1966. Immunologic studies of anthrax. IV. Evaluation of the immunogenicity of three components of anthrax toxin. *J Immunol* 96 (4):727-33.

Maiztegui, J. I., K. T. McKee, Jr., J. G. Barrera Oro, L. H. Harrison, P. H. Gibbs, M. R. Feuillade, D. A. Enria, A. M. Briggiler, S. C. Levis, A. M. Ambrosio, N. A. Halsey, and C. J. Peters. 1998. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis* 177 (2):277-83.

Manchee, R. J., M. G. Broster, A. J. Stagg, and Et al. 1990. Out of Gruinard Island. *Salisbury Medical Bulletin* 68 (special supplement):17-18.

Mann, J. M., L. Shandler, and A. H. Cushing. 1982. Pediatric plague. *Pediatrics* 69 (6):762-7.

Marianneau, P., V. Guillaume, T. Wong, M. Badmanathan, R. Y. Looi, S. Murri, P. Loth, N. Tordo, F. Wild, B. Horvat, and H. Contamin. Experimental infection of squirrel monkeys with nipah virus. *Emerg Infect Dis* 16 (3):507-10.

Markel, H., H. B. Lipman, J. A. Navarro, A. Sloan, J. R. Michalsen, A. M. Stern, and M. S. Cetron. 2007. Nonpharmaceutical interventions implemented by US cities during the 1918-1919 influenza pandemic. *JAMA* 298 (6):644-54.

Markowitz, L.E., Hynes, N.A., de la Cruz, P., et al. 1985. Tick-borne tularemia: an outbreak of lymphadenopthy in children. *JAMA* 254 (20):2922-2925

Martin, J. E., N. J. Sullivan, M. E. Enama, I. J. Gordon, M. Roederer, R. A. Koup, R. T. Bailer, B. K. Chakrabarti, M. A. Bailey, P. L. Gomez, C. A. Andrews, Z. Moodie, L. Gu, J. A. Stein, G. J. Nabel, and B. S. Graham. 2006. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. *Clin Vaccine Immunol* 13 (11):1267-77.

Martina, B. E., B. L. Haagmans, T. Kuiken, R. A. Fouchier, G. F. Rimmelzwaan, G. Van Amerongen, J. S. Peiris, W. Lim, and A. D. Osterhaus. 2003. Virology: SARS virus infection of cats and ferrets. *Nature* 425 (6961):915.

Martinez, V. P., C. Bellomo, J. San Juan, D. Pinna, R. Forlenza, M. Elder, and P. J. Padula. 2005. Person-to-person transmission of Andes virus. *Emerg Infect Dis* 11 (12):1848-53.

Martini, G. A., and H. A. Schmidt. 1968. [Spermatogenic transmission of the "Marburg virus". (Causes of "Marburg simian disease")]. *Klin Wochenschr* 46 (7):398-400.

Martone, W. J., L. W. Marshall, A. F. Kaufmann, J. H. Hobbs, and M. E. Levy. 1979. Tularemia pneumonia in Washington, DC. A report of three cases with possible common-source exposures. *JAMA* 242 (21):2315-7.

Massad, E., M. N. Burattini, F. A. Coutinho, and L. F. Lopez. 2007. The 1918 influenza A epidemic in the city of Sao Paulo, Brazil. *Med Hypotheses* 68 (2):442-5.

Matyas, B. T., H. S. Nieder, and S. R. Telford, 3rd. 2007. Pneumonic tularemia on Martha's Vineyard: clinical, epidemiologic, and ecological characteristics. *Ann N Y Acad Sci* 1105:351-77.

McAuliffe, J., L. Vogel, A. Roberts, G. Fahle, S. Fischer, W. J. Shieh, E. Butler, S. Zaki, M. St Claire, B. Murphy, and K. Subbarao. 2004. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology* 330 (1):8-15.

McBride, B. W., A. Mogg, J. L. Telfer, M. S. Lever, J. Miller, P. C. Turnbull, and L. Baillie. 1998. Protective efficacy of a recombinant protective antigen against Bacillus anthracis challenge and assessment of immunological markers. *Vaccine* 16 (8):810-7.

McCaughey, C., and C. A. Hart. 2000. Hantaviruses. *J Med Microbiol* 49 (7):587-99.

McCormick, J. B. 2004. Ebola Virus Ecology. *The Journal of Infectious Diseases* 190:1893-4.

McCormick, J. B., and S. P. Fisher-Hoch. 2002. Lassa fever. *Curr Top Microbiol Immunol* 262:75-109.

McCormick, J. B., I. J. King, P. A. Webb, K. M. Johnson, R. O'Sullivan, E. S. Smith, S. Trippel, and T. C. Tong. 1987. A case-control study of the clinical diagnosis and course of Lassa fever. *J Infect Dis* 155 (3):445-55.

McCormick, J.B., and S. Fisher-Hoch. 1999. *Level 4: Virus Hunters of the CDC* Updated editon ed: Barnes and Noble Inc.

McCrumb, F. 1961. Aerosol infection of man with *Pasturella tularensis*. *Bacteriological Reviews* 25 (1961):262-267.

McDonald, L.C., A.E. Simor, I-J Su, S. M. Maloney, and et al. 2004. SARS in health-care facilities, Toronto and Taiwan. *Emerg Infect Dis* 10:777-781.

McEachern, J. A., J. Bingham, G. Crameri, D. J. Green, T. J. Hancock, D. Middleton, Y. R. Feng, C. C. Broder, L. F. Wang, and K. N. Bossart. 2008. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* 26 (31):3842-52.

McElroy, A. K., M. Bray, D. S. Reed, and C. S. Schmaljohn. 2002. Andes virus infection of cynomolgus macaques. *J Infect Dis* 186 (12):1706-12.

McGovern, T.W., and A. M. Friedlander. 1997. Plague. In *Textbook of Military Medicine: Medical Aspects of Chemical and Biological Warfare*, edited by R. Zajtchuk and R. F. Bellamy. Washington, D.C.: Office of the Surgeon General, Borden Institute, Walter Reed Army Medical Center.

McIntosh, K., and S. Perlman. 2010. Coroaviruses, including severe acute respiratory syndrom (SARS)-associated coronovirus. In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, edited by G. L. Mandell, J. E. bennett and R. Dolin. Philadelphia: Churchill Livingstone.

McKee, K. T., Jr., J. G. Oro, A. I. Kuehne, J. A. Spisso, and B. G. Mahlandt. 1993. Safety and immunogenicity of a live-attenuated Junin (Argentine hemorrhagic fever) vaccine in rhesus macaques. *Am J Trop Med Hyg* 48 (3):403-11.

McKinney, K. R., Y. Y. Gong, and T. G. Lewis. 2006. Environmental transmission of SARS at Amoy Gardens. *J Environ Health* 68 (9):26-30; quiz 51-2.

Meadors, G. F., 3rd, P. H. Gibbs, and C. J. Peters. 1986. Evaluation of a new Rift Valley fever vaccine: safety and immunogenicity trials. *Vaccine* 4 (3):179-84.

Medina, R. A., F. Torres-Perez, H. Galeno, M. Navarrete, P. A. Vial, R. E. Palma, M. Ferres, J. A. Cook, and B. Hjelle. 2009. Ecology, genetic diversity, and phylogeographic structure of andes virus in humans and rodents in chile. *J Virol* 83 (6):2446-59.

Meegan, J. M. 1979. The Rift Valley fever epizootic in Egypt 1977-78. 1. Description of the epizzotic and virological studies. *Trans R Soc Trop Med Hyg* 73 (6):618-23.

Meegan, J. M., B. Niklasson, and E. Bengtsson. 1979. Spread of Rift Valley fever virus from continental Africa. *Lancet* 2 (8153):1184-5.

Meegan, J.M., and C.L. Bailey. 1989. Rift Valley Fever. In *The Arboviruses:Epidemiology and Ecology*, edited by T. P. Monath. Boca Raton: CRC Press, Inc.

Meltzer, M. I. 2004. Multiple contact dates and SARS incubation periods. *Emerg Infect Dis* 10 (2):207-9.

Meric, M., M. Sayan, A. Willke, and S. Gedikoglu. 2008. [A small water-borne tularemia outbreak]. *Mikrobiyol Bul* 42 (1):49-59.

Merino, C., Arias, A., and C. Castillo. 2002. First case of hantavirus cardiopulmonary syndrome secondary to a rodent bite. *Rev Chil Enf Respir* 18:199-205.

Mertens, P. E., R. Patton, J. J. Baum, and T. P. Monath. 1973. Clinical presentation of Lassa fever cases during the hospital epidemic at Zorzor, Liberia, March-April 1972. *Am J Trop Med Hyg* 22 (6):780-4.

Mertz, G. J., B. Hjelle, M. Crowley, G. Iwamoto, V. Tomicic, and P. A. Vial. 2006. Diagnosis and treatment of new world hantavirus infections. *Curr Opin Infect Dis* 19 (5):437-42.

Meselson, M., J. Guillemin, M. Hugh-Jones, A. Langmuir, I. Popova, A. Shelokov, and O. Yampolskaya. 1994. The Sverdlovsk anthrax outbreak of 1979. *Science* 266 (5188):1202-8.

Meyer, K. F. 1961. Pneumonic plague. *Bacteriol Rev* 25:249-61.

Middleton, D. J., C. J. Morrissy, B. M. van der Heide, G. M. Russell, M. A. Braun, H. A. Westbury, K. Halpin, and P. W. Daniels. 2007. Experimental Nipah virus infection in pteropid bats (Pteropus poliocephalus). *J Comp Pathol* 136 (4):266-72.

Mignani, E., F. Palmieri, M. Fontana, and S. Marigo. 1988. Italian epidemic of waterborne tularaemia. *Lancet* 2 (8625):1423.

Miller, W.S., C.R. Demchak, C.R. Rosenberger, J.W. Dominik, and J.L. Bradshaw. 1963. Stability and infectivity of airborne yellow fever and Rift Valley fever viruses. *American Journal of Hygiene* 77:114-121.

Mills, C. E., J. M. Robins, and M. Lipsitch. 2004. Transmissibility of 1918 pandemic influenza. *Nature* 432 (7019):904-6.

Mills, J. N., J. G. Barrera Oro, D. S. Bressler, J. E. Childs, R. B. Tesh, J. F. Smith, D. A. Enria, T. W. Geisbert, K. T. McKee, Jr., M. D. Bowen, C. J. Peters, and P. B. Jahrling. 1996.

Characterization of Oliveros virus, a new member of the Tacaribe complex (Arenaviridae: Arenavirus). *Am J Trop Med Hyg* 54 (4):399-404.

Mills, J. N., G. E. Calderon, B. A. Ellis, K. T. McKee, T. G. Ksiazek, J. G. Oro, C. J. Peters, J. E. Childs, and J. I. Maiztegui. 1991. [New findings on Junin virus infection in rodents inside and outside the endemic area of hemorrhagic fever in Argentina]. *Medicina (B Aires)* 51 (6):519-23.

Mills, J. N., B. A. Ellis, J. E. Childs, K. T. McKee, Jr., J. I. Maiztegui, C. J. Peters, T. G. Ksiazek, and P. B. Jahrling. 1994. Prevalence of infection with Junin virus in rodent populations in the epidemic area of Argentine hemorrhagic fever. *Am J Trop Med Hyg* 51 (5):554-62.

Mills, J. N., B. A. Ellis, K. T. McKee, Jr., T. G. Ksiazek, J. G. Oro, J. I. Maiztegui, G. E. Calderon, C. J. Peters, and J. E. Childs. 1991. Junin virus activity in rodents from endemic and nonendemic loci in central Argentina. *Am J Trop Med Hyg* 44 (6):589-97.

Mitchell, C.L., and R.L. Penn. 2005. *Francisella tularensis* (tularemia) as an agent of bioterrorism. In *Principles and Practice of Infectious Diseases*, edited by G. L. Mandell, J. E. Bennett and R. Dolin. New York: Elsevier Churchill Livingstone.

Moe, J. B., R. D. Lambert, and H. W. Lupton. 1981. Plaque assay for Ebola virus. *J Clin Microbiol* 13 (4):791-3.

Mollaret, H. H. 1963. [EXPERIMENTAL PRESERVATION OF PLAGUE IN SOIL.]. *Bull Soc Pathol Exot Filiales* 56:1168-82.

Monath, T. P. 1999. Ecology of Marburg and Ebola viruses: speculations and directions for future research. *J Infect Dis* 179 Suppl 1:S127-38.

Montgomery, J. M., T. G. Ksiazek, and A. S. Khan. 2007. Hantavirus pulmonary syndrome: the sound of a mouse roaring. *J Infect Dis* 195 (11):1553-5.

Monto, A. 2008. *Ethical and public health considerations for use of pre-exposure antiviral prophylaxis*: NIH-RAC.

Montville, T. J., R. Dengrove, T. De Siano, M. Bonnet, and D. W. Schaffner. 2005. Thermal resistance of spores from virulent strains of Bacillus anthracis and potential surrogates. *J Food Prot* 68 (11):2362-6.

Morens, D. M., J. K. Taubenberger, and A. S. Fauci. 2008. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 198 (7):962-70.

Morner, T. 1992. The ecology of tularaemia. *Rev Sci Tech* 11 (4):1123-30.

Morrill, J. C., C. A. Mebus, and C. J. Peters. 1997. Safety of a mutagen-attenuated Rift Valley fever virus vaccine in fetal and neonatal bovids. *Am J Vet Res* 58 (10):1110-4.

Morrill, J. C., and C. J. Peters. 2003. Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. *Vaccine* 21 (21-22):2994-3002.

Mubareka, S., A. C. Lowen, J. Steel, A. L. Coates, A. Garcia-Sastre, and P. Palese. 2009. Transmission of influenza virus via aerosols and fomites in the guinea pig model. *J Infect Dis* 199 (6):858-65.

Mungall, B. A., D. Middleton, G. Crameri, K. Halpin, J. Bingham, B. T. Eaton, and C. C. Broder. 2007. Vertical transmission and fetal replication of Nipah virus in an experimentally infected cat. *J Infect Dis* 196 (6):812-6.

Mungall, B. A., N. C. Schopman, L. S. Lambeth, and T. J. Doran. 2008. Inhibition of Henipavirus infection by RNA interference. *Antiviral Res* 80 (3):324-31.

Murphy, B. 2008. *NIH intramural program: proposed biosafety containment and use of prophylaxis for 1918 H1N1*. Bethesda: NIH-RAC.

Repeated Author. 2008. *Pandemic potential of the 1918 H1N1 Virus -Is it the same in 2008?* Bethesda: NIH-RAC.

Murphy, F.A., E.P.J. Gibbs, M.C. Horzinek, and M.J. Studdert. 1994. *Coronaviridae*. In *Veterinary Virology*. New York: Academic Press.

Repeated Author. 1994. *Coronaviridae*. In *Veterinary Virology*. New York: Academic Press.

Repeated Author. 1999. *Rift Valley Fever*. 3rd ed, *Veterinary Virology*. San Diego: Academic Press.

Murúa, R., Gonzalez, L.A., Gonzalez, M., and Y.C. Jofre. 1996. Efectos del florecimiento del arbusto *Chusquea quila* (Poaceae) sobre la demographia de poblaciones de roedores, de los bosques templados frios del sur chileno. *Bol Soc Biol Concepción, Chile* 67:37-42.

Murua, R., M. Navarrete, R. Cadiz, R. Figueroa, P. Padula, L. Zaror, R. Mansilla, L. Gonzalez, and A. Munoz-Pedreros. 2003. [Hantavirus pulmonary syndrome: current situation

among rodent reservoirs and human population in the 10th region, Chile]. *Rev Med Chil* 131 (2):169-76.

Mwengee, W., T. Butler, S. Mgema, G. Mhina, Y. Almasi, C. Bradley, J. B. Formanik, and C. G. Rochester. 2006. Treatment of plague with gentamicin or doxycycline in a randomized clinical trial in Tanzania. *Clin Infect Dis* 42 (5):614-21.

Mwenye, K. S., S. Siziya, and D. Peterson. 1996. Factors associated with human anthrax outbreak in the Chikupo and Ngandu villages of Murewa district in Mashonaland East Province, Zimbabwe. *Cent Afr J Med* 42 (11):312-5.

Ndyabahinduka, D. G., I. H. Chu, A. H. Abdou, and J. K. Gaifuba. 1984. An outbreak of human gastrointestinal anthrax. *Ann Ist Super Sanita* 20 (2-3):205-8.

Nishiura, H. 2007. Time variations in the transmissibility of pandemic influenza in Prussia, Germany, from 1918-19. *Theor Biol Med Model* 4:20.

Normile, D. 2009. Emerging infectious diseases. Scientists puzzle over Ebola-Reston virus in pigs. *Science* 323 (5913):451.

Novel Swine-Origin Influenza, A. Virus Investigation Team, F. S. Dawood, S. Jain, L. Finelli, M. W. Shaw, S. Lindstrom, R. J. Garten, L. V. Gubareva, X. Xu, C. B. Bridges, and T. M. Uyeki. 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 360 (25):2605-15.

Nuzum, E. O., C. A. Rossi, E. H. Stephenson, and J. W. LeDuc. 1988. Aerosol transmission of Hantaan and related viruses to laboratory rats. *Am J Trop Med Hyg* 38 (3):636-40.

Ogbu, O., E. Ajuluchukwu, and C. J. Uneke. 2007. Lassa fever in West African sub-region: an overview. *J Vector Borne Dis* 44 (1):1-11.

Olsuf'ev, N. G., and O. S. Emel'ianova. 1966. [On a study of strains of the tularemia microbe isolated in the south of Europe]. *Zh Mikrobiol Epidemiol Immunobiol* 43 (5):3-4.

Orlando, M.D., R.D. Delauter, and J.M. Riley. 1967. Effect of Virus Input Multiplicity and Tissue Cell Concentration on Growth of Rift Valley Fever Virus. *Applied Microbiology* 15 (3):594-596.

Osorio, M., Y. Wu, S. Singh, T. J. Merkel, S. Bhattacharyya, M. S. Blake, and D. J. Kopecko. 2009. Anthrax protective antigen delivered by Salmonella enterica serovar Typhi Ty21a protects mice from a lethal anthrax spore challenge. *Infect Immun* 77 (4):1475-82.

Osterholm, M. 2008. *Ethical and public health considerations for use of pre-exposure antiviral prophylaxis*. Bethesda: RAC-NIH.

Oswald, W. B., T. W. Geisbert, K. J. Davis, J. B. Geisbert, N. J. Sullivan, P. B. Jahrling, P. W. Parren, and D. R. Burton. 2007. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. *PLoS Pathog* 3 (1):e9.

Overholt, E. L., W. D. Tigertt, P. J. Kadull, M. K. Ward, N. D. Charkes, R. M. Rene, T. E. Salzman, and M. Stephens. 1961. An analysis of forty-two cases of laboratory-acquired tularemia. Treatment with broad spectrum antibiotics. *Am J Med* 30:785-806.

Padula, P., R. Figueroa, M. Navarrete, E. Pizarro, R. Cadiz, C. Bellomo, C. Jofre, L. Zaror, E. Rodriguez, and R. Murua. 2004. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. *J Virol* 78 (21):11972-9.

Padula, P. J., A. Edelstein, S. D. Miguel, N. M. Lopez, C. M. Rossi, and R. D. Rabinovich. 1998. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* 241 (2):323-30.

Paragas, J., and T. W. Geisbert. 2006. Development of treatment strategies to combat Ebola and Marburg viruses. *Expert Rev Anti Infect Ther* 4 (1):67-76.

Park, B. J., A. J. Peck, M. J. Kuehnert, C. Newbern, C. Smelser, J. A. Comer, D. Jernigan, and L. C. McDonald. 2004. Lack of SARS transmission among healthcare workers, United States. *Emerg Infect Dis* 10 (2):244-8.

Parodi, A. S. 2008. Junin virus. *International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates. CDC On-line Edition.*

Parodi, A. S., C. E. Coto, M. Boxaca, S. Lajmanovich, and S. Gonzalez. 1966. Characteristics of Junin virus; etiologic agent of argentine hemorrhagic fever. *Archives of Virology* 19 (4):393-402.

Parodi, A. S., H. R. Rugiero, D. J. Greenway, N. Mettler, and M. Boxaca. 1961. [Isolation of the Junin virus from rodents of non-epidemic areas.]. *Prensa Med Argent* 48:2321-2.

Parodi, A. S., H. R. Rugiero, D. J. Greenway, N. Mettler, A. Martinez, M. Boxaca, and J. M. De La Barrera. 1959. [Isolation of the Junin virus (epidemic hemorrhagic fever) from the mites of the epidemic area (Echinolaelaps echidninus, Barlese).]. *Prensa Med Argent* 46:2242-4.

Parodi, A.S. Greenway, D.J. Rugiero, H.R., Frigerio, M. . 1958. Sobre la etiología del brote epidémico de Junin. . *Dia Médico* 30:2300-2302.

Pasetti, M..F, Cuberos, L., Horn, T.L., et al. 2008. An improved Francisella tularensis live vaccine strain (LVS) is well tolerated and highly immunogenic when administered to rabbits in escalating doses using various immunization routes. *Vaccine* 25 (46 (14)):1773-1785.

Pattyn, SR, ETW Bowen, and PA Webb. *Ebola virus.* (CDC On-line Edition.) 2008. Available from http://www.ncid.cdc.gov/arbocat/catalog-listing.asp?VirusID=137&SI=1

Peck, A. J., E. C. Newbern, D. R. Feikin, E. T. Issakbaeva, B. J. Park, J. Fehr, A. C. LaMonte, T. P. Le, T. L. Burger, L. V. Rhodes, 3rd, A. Weltman, D. Erdman, T. G. Ksiazek, and J. R. Lingappa. 2004. Lack of SARS transmission and U.S. SARS case-patient. *Emerg Infect Dis* 10 (2):217-24.

Pediatrics, American Academy of. 2006. Tularemia. In *2006 Red book: report of the Committee on Infectious Diseases.*, edited by L. K. Pickering, Baker, .CJ., Long, S.S., et al Elk Grove Village: American Academy of Pediatrics.

Peiris, J. S., C. M. Chu, V. C. Cheng, K. S. Chan, I. F. Hung, L. L. Poon, K. I. Law, B. S. Tang, T. Y. Hon, C. S. Chan, K. H. Chan, J. S. Ng, B. J. Zheng, W. L. Ng, R. W. Lai, Y. Guan, and K. Y. Yuen. 2003. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361 (9371):1767-72.

Peiris, J. S., C. M. Chu, V. C. Cheng, K. S. Chan, I. F. Hung, L. L. Poon, K. I. Law, B. S. Tang, T. Y. Hon, C. S. Chan, K. H. Chan, J. S. Ng, B. J. Zheng, W. L. Ng, R. W. Lai, Y. Guan, K. Y. Yuen, and Hku Uch Sars Study Group. 2003. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361 (9371):1767-72.

Penn, R. L. 2010. *Francisella tularensis* (Tularemia). In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseaes*, edited by G. L. Mandell, J. E. Bennett and R. Dolin. Philadelphia: Churchill Livingstone.

Penn, R. L., and G. T. Kinasewitz. 1987. Factors associated with a poor outcome in tularemia. *Arch Intern Med* 147 (2):265-8.

Penn, R.L. 2005. *Franciscella tularensis* (tularemia). In *Principles and Practice of Infectious Diseases*, edited by G. L. Mandell, J. E. Bennett and R. Dolin. New York: Wlsevier Churchill Livingstone.

Perez-Castrillon, J. L., P. Bachiller-Luque, M. Martin-Luquero, F. J. Mena-Martin, and V. Herreros. 2001. Tularemia epidemic in northwestern Spain: clinical description and therapeutic response. *Clin Infect Dis* 33 (4):573-6.

Perlman, S., and A. A. Dandekar. 2005. Immunopathogenesis of coronavirus infections: implications for SARS. *Nat Rev Immunol* 5 (12):917-27.

Perry, R.D., and J.D. Fetherston. 1997. *Yersinia pestis*—Etiologic Agent of Plague. *Clinical Microbiology Review* 10 (1):35-66.

Peters, C. J., and D. M. Hartley. 2002. Anthrax inhalation and lethal human infection. *Lancet* 359 (9307):710-1.

Peters, C. J., P. B. Jahrling, C. T. Liu, R. H. Kenyon, K. T. McKee, Jr., and J. G. Barrera Oro. 1987. Experimental studies of arenaviral hemorrhagic fevers. *Curr Top Microbiol Immunol* 134:5-68.

Peters, C. J., D. Jones, R. Trotter, J. Donaldson, J. White, E. Stephen, and T. W. Slone, Jr. 1988. Experimental Rift Valley fever in rhesus macaques. *Arch Virol* 99 (1-2):31-44.

Peters, C.J. 1991. Arenaviruses. In *Textbook of Human Virology*, edited by R. B. Belshe: Mosby-Year Book.

Petersen, J.M., Stapes, J.E., Kubota, K.A., et al. 2006. Comparative epidemiological and molecular analysis of human tularemia-United States, 1964-2004. *ICEID* Abstract 39.

Peterson, A. T., J. T. Bauer, and J. N. Mills. 2004. Ecologic and geographic distribution of filovirus disease. *Emerg Infect Dis* 10 (1):40-7.

Peterson, A. T., R. R. Lash, D. S. Carroll, and K. M. Johnson. 2006. Geographic potential for outbreaks of Marburg hemorrhagic fever. *Am J Trop Med Hyg* 75 (1):9-15.

Pettersson, L., J. Klingstrom, J. Hardestam, A. Lundkvist, C. Ahlm, and M. Evander. 2008. Hantavirus RNA in saliva from patients with hemorrhagic fever with renal syndrome. *Emerg Infect Dis* 14 (3):406-11.

Pickering, A. K., M. Osorio, G. M. Lee, V. K. Grippe, M. Bray, and T. J. Merkel. 2004. Cytokine response to infection with Bacillus anthracis spores. *Infect Immun* 72 (11):6382-9.

Piercy, T. J., S. J. Smither, J. A. Steward, L. Eastaugh, and M. S. Lever. 2010. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. *J Appl Microbiol* 109 (5):1531-9.

Pike, R. M. 1976. Laboratory-associated infections: summary and analysis of 3921 cases. *Health Lab Sci* 13 (2):105-14.

Repeated Author. 1979. Laboratory-associated infections: incidence, fatalities, causes, and prevention. *Annu Rev Microbiol* 33:41-66.

Pini, N. 2004. Hantavirus pulmonary syndrome in Latin America. *Curr Opin Infect Dis* 17 (5):427-31.

Pini, N., S. Levis, G. Calderon, J. Ramirez, D. Bravo, E. Lozano, C. Ripoll, S. St Jeor, T. G. Ksiazek, R. M. Barquez, and D. Enria. 2003. Hantavirus infection in humans and rodents, northwestern Argentina. *Emerg Infect Dis* 9 (9):1070-6.

Pittman, P. R., C. T. Liu, T. L. Cannon, R. S. Makuch, J. A. Mangiafico, P. H. Gibbs, and C. J. Peters. 1999. Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12-year experience. *Vaccine* 18 (1-2):181-9.

Pitzer, V. E., G. M. Leung, and M. Lipsitch. 2007. Estimating variability in the transmission of severe acute respiratory syndrome to household contacts in Hong Kong, China. *Am J Epidemiol* 166 (3):355-63.

Pokhodiaev, V. A., N. I. Gonchar, and V. A. Pshenichnov. 1991. [An experimental study of the contact transmission of the Marburg virus]. *Vopr Virusol* 36 (6):506-8.

Pons, V. G., J. Canter, and R. Dolin. 1980. Influenza A/USSR/77 (H1N1) on a university campus. *Am J Epidemiol* 111 (1):23-30.

Poponnikova, T. V. 2006. Specific clinical and epidemiological features of tick-borne encephalitis in Western Siberia. *Int J Med Microbiol* 296 Suppl 40:59-62.

Pourrut, X., B. Kumulungui, T. Wittmann, G. Moussavou, A. Delicat, P. Yaba, D. Nkoghe, J. P. Gonzalez, and E. M. Leroy. 2005. The natural history of Ebola virus in Africa. *Microbes Infect* 7 (7-8):1005-14.

Pratt, W. D., D. Wang, D. K. Nichols, M. Luo, J. Woraratanadharm, J. M. Dye, D. H. Holman, and J. Y. Dong. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vaccine Immunol* 17 (4):572-81.

ProMED mail. 2010. Anthrax, human -United Kingdom (11): (Scotland). http://www.promedmail.org/pls/apex/f?p=2400:1202:13131595228855::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,82292.

Pullen, R.L., Stuart, B.M. 1945. Tularemia: analysis of 225 cases. *JAMA* 129 (7):495-500

Qin, C., J. Wang, Q. Wei, M. She, W. A. Marasco, H. Jiang, X. Tu, H. Zhu, L. Ren, H. Gao, L. Guo, L. Huang, R. Yang, Z. Cong, Y. Wang, Y. Liu, Y. Sun, S. Duan, J. Qu, L. Chen, W. Tong, L. Ruan, P. Liu, H. Zhang, J. Zhang, D. Liu, Q. Liu, T. Hong, and W. He. 2005. An animal model of SARS produced by infection of Macaca mulatta with SARS coronavirus. *J Pathol* 206 (3):251-9.

Rabenau, H. F., J. Cinatl, B. Morgenstern, G. Bauer, W. Preiser, and H. W. Doerr. 2005. Stability and inactivation of SARS coronavirus. *Med Microbiol Immunol* 194 (1-2):1-6.

Randolph, S. E. 2008. Tick-borne encephalitis virus, ticks and humans: short-term and long-term dynamics. *Curr Opin Infect Dis* 21 (5):462-7.

Rangel, R. A., and D. A. Gonzalez. 1975. Bacillus anthracic meningitis. *Neurology* 25 (6):525-30.

Rantakokko-Jalava, K., and M. K. Viljanen. 2003. Application of Bacillus anthracis PCR to simulated clinical samples. *Clin Microbiol Infect* 9 (10):1051-6.

Ratsitorahina, M., S. Chanteau, L. Rahalison, L. Ratsifasoamanana, and P. Boisier. 2000. Epidemiological and diagnostic aspects of the outbreak of pneumonic plague in Madagascar. *Lancet* 355 (9198):111-3.

Reed, W. P., D. L. Palmer, R. C. Williams, Jr., and A. L. Kisch. 1970. Bubonic plague in the Southwestern United States. A review of recent experience. *Medicine (Baltimore)* 49 (6):465-86.

Reintjes, R., I. Dedushaj, A. Gjini, T. R. Jorgensen, B. Cotter, A. Lieftucht, F. D'Ancona, D. T. Dennis, M. A. Kosoy, G. Mulliqi-Osmani, R. Grunow, A. Kalaveshi, L. Gashi, and I. Humolli. 2002. Tularemia outbreak investigation in Kosovo: case control and environmental studies. *Emerg Infect Dis* 8 (1):69-73.

Rich, S. M., D. A. Caporale, S. R. Telford, 3rd, T. D. Kocher, D. L. Hartl, and A. Spielman. 1995. Distribution of the Ixodes ricinus-like ticks of eastern North America. *Proc Natl Acad Sci U S A* 92 (14):6284-8.

Richmond, J. K., and D. J. Baglole. 2003. Lassa fever: epidemiology, clinical features, and social consequences. *BMJ* 327 (7426):1271-5.

Riemenschneider, J., A. Garrison, J. Geisbert, P. Jahrling, M. Hevey, D. Negley, A. Schmaljohn, J. Lee, M. K. Hart, L. Vanderzanden, D. Custer, M. Bray, A. Ruff, B. Ivins, A. Bassett, C. Rossi, and C. Schmaljohn. 2003. Comparison of individual and combination DNA vaccines for B. anthracis, Ebola virus, Marburg virus and Venezuelan equine encephalitis virus. *Vaccine* 21 (25-26):4071-80.

Riley, S., C. Fraser, C. A. Donnelly, A. C. Ghani, L. J. Abu-Raddad, A. J. Hedley, G. M. Leung, L. M. Ho, T. H. Lam, T. Q. Thach, P. Chau, K. P. Chan, S. V. Lo, P. Y. Leung, T. Tsang, W. Ho, K. H. Lee, E. M. Lau, N. M. Ferguson, and R. M. Anderson. 2003. Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions. *Science* 300 (5627):1961-6.

Roberts, A., D. Deming, C. D. Paddock, A. Cheng, B. Yount, L. Vogel, B. D. Herman, T. Sheahan, M. Heise, G. L. Genrich, S. R. Zaki, R. Baric, and K. Subbarao. 2007. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathog* 3 (1):e5.

Roberts, A., and C. Kemp. 2002. Lassa fever. *J Am Acad Nurse Pract* 14 (7):289-90.

Rodriguez, L. L., A. De Roo, Y. Guimard, S. G. Trappier, A. Sanchez, D. Bressler, A. J. Williams, A. K. Rowe, J. Bertolli, A. S. Khan, T. G. Ksiazek, C. J. Peters, and S. T. Nichol. 1999. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 179 Suppl 1:S170-6.

Rohrbach, B. W., E. Westerman, and G. R. Istre. 1991. Epidemiology and clinical characteristics of tularemia in Oklahoma, 1979 to 1985. *South Med J* 84 (9):1091-6.

Rose, L. J., R. Donlan, S. N. Banerjee, and M. J. Arduino. 2003. Survival of Yersinia pestis on environmental surfaces. *Appl Environ Microbiol* 69 (4):2166-71.

Rowe, T., G. Gao, R. J. Hogan, R. G. Crystal, T. G. Voss, R. L. Grant, P. Bell, G. P. Kobinger, N. A. Wivel, and J. M. Wilson. 2004. Macaque model for severe acute respiratory syndrome. *J Virol* 78 (20):11401-4.

Russell, P., S. M. Eley, D. L. Bell, R. J. Manchee, and R. W. Titball. 1996. Doxycycline or ciprofloxacin prophylaxis and therapy against experimental Yersinia pestis infection in mice. *J Antimicrob Chemother* 37 (4):769-74.

Ruzek, D., L. Bell-Sakyi, J. Kopecky, and L. Grubhoffer. 2008. Growth of tick-borne encephalitis virus (European subtype) in cell lines from vector and non-vector ticks. *Virus Res* 137 (1):142-6.

Sabbatini, M.S., Barrera Oro,J.G., Maiztegui ,.J.,I Ferradas, B. . 1974. Actividad del virus de la coriomeningitis linfocítica en el area endémica de la fiebre hemorrhágica argentina (FHA). II Aislamiento a partir de un *Mus musculus* campestre capturado en el sudeste de Córdoba. . *Medicina (B Aires)* 34:313-320.

Sabbatini, M.S., Gonzalez de Rios,L.E., Diaz,G., and V.R.Vega. 1977. Infeccion natural y experimental de roedores con virus Junin. *Medicina (B Aires)* 37:149-161.

Safronetz, D., N. R. Hegde, H. Ebihara, M. Denton, G. P. Kobinger, S. St Jeor, H. Feldmann, and D. C. Johnson. 2009. Adenovirus vectors expressing hantavirus proteins protect hamsters against lethal challenge with andes virus. *J Virol* 83 (14):7285-95.

Sagripanti, J. L., and C. D. Lytle. 2007. Inactivation of influenza virus by solar radiation. *Photochem Photobiol* 83 (5):1278-82.

Sahani, M., U. D. Parashar, R. Ali, P. Das, M. S. Lye, M. M. Isa, M. T. Arif, T. G. Ksiazek, and M. Sivamoorthy. 2001. Nipah virus infection among abattoir workers in Malaysia, 1998-1999. *Int J Epidemiol* 30 (5):1017-20.

Said, B., C. Mirrielees, A. Walsh, and D. Morgan. 2007. The survival and transmission potential of CDC category A bioterrorism agents. In *International Meeting on Emerging Diseases and Surveillance 2007*. Vienna, Austria.

Saile, E., and T. M. Koehler. 2006. Bacillus anthracis multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Appl Environ Microbiol* 72 (5):3168-74.

Salkeld, D. J., R. J. Eisen, P. Stapp, A. P. Wilder, J. Lowell, D. W. Tripp, D. Albertson, and M. F. Antolin. 2007. The potential role of swift foxes (Vulpes velox) and their fleas in plague outbreaks in prairie dogs. *J Wildl Dis* 43 (3):425-31.

Saluzzo, J. F., G. W. Anderson, Jr., L. A. Hodgson, J. P. Digoutte, and J. F. Smith. 1989. Antigenic and biological properties of Rift Valley fever virus isolated during the 1987 Mauritanian epidemic. *Res Virol* 140 (2):155-64.

Salvaggio, M. R., and J. W. Baddley. 2004. Other viral bioweapons: Ebola and Marburg hemorrhagic fever. *Dermatol Clin* 22 (3):291-302, vi.

Samoilovich, S. R., M. A. Calello, R. P. Laguens, and M. C. Weissenbacher. 1988. Long-term protection against Argentine hemorrhagic fever in Tacaribe virus infected marmosets: virologic and histopathologic findings. *J Med Virol* 24 (2):229-36.

Samoilovich, S. R., S. N. Rondinone, R. P. Laguens, O. Colillas, M. J. Frigerio, and M. C. Weissenbacher. 1983. [Infection of New World primates with Junin virus. IV. Aotus trivirgatus]. *Rev Argent Microbiol* 15 (4):219-22.

Sanders, C. V., and R. Hahn. 1968. Analysis of 106 cases of tularemia. *J La State Med Soc* 120 (9):391-3.

Saslaw, S., H. T. Eigelsbach, J. A. Prior, H. E. Wilson, and S. Carhart. 1961. Tularemia vaccine study. II. Respiratory challenge. *Arch Intern Med* 107:702-14.

Sawyer, W. D., H. G. Dangerfield, A. L. Hogge, and D. Crozier. 1966. Antibiotic prophylaxis and therapy of airborne tularemia. *Bacteriol Rev* 30 (3):542-50.

Scherer, W.F., Eddy,G.A., Monath,T.P., Walton,T.E., and J.H. Richardson. 1980. Laboratory safety for arboviruses and certain other viruses of vertebrates. *American Journal of Tropical Medicine and Hygiene.* 29:1359-1381.

Schmaljohn, C., D. Custer, L. VanderZanden, K. Spik, C. Rossi, and M. Bray. 1999. Evaluation of tick-borne encephalitis DNA vaccines in monkeys. *Virology* 263 (1):166-74.

Schonrich, G., A. Rang, N. Lutteke, M. J. Raftery, N. Charbonnel, and R. G. Ulrich. 2008. Hantavirus-induced immunity in rodent reservoirs and humans. *Immunol Rev* 225:163-89.

Sebbane, F., N. Lemaitre, D. E. Sturdevant, R. Rebeil, K. Virtaneva, S. F. Porcella, and B. J. Hinnebusch. 2006. Adaptive response of Yersinia pestis to extracellular effectors of innate immunity during bubonic plague. *Proc Natl Acad Sci U S A* 103 (31):11766-71.

Sejvar, J. J., J. Hossain, S. K. Saha, E. S. Gurley, S. Banu, J. D. Hamadani, M. A. Faiz, F. M. Siddiqui, Q. D. Mohammad, A. H. Mollah, R. Uddin, R. Alam, R. Rahman, C. T. Tan, W. Bellini, P. Rota, R. F. Breiman, and S. P. Luby. 2007. Long-term neurological and functional outcome in Nipah virus infection. *Ann Neurol* 62 (3):235-42.

Sejvar, J. J., F. C. Tenover, and D. S. Stephens. 2005. Management of anthrax meningitis. *Lancet Infect Dis* 5 (5):287-95.

Seligman, S.J., and O.V. Morozova. *Tick-borne encephalitis; Austria (02)*. International Society for Infectious Diseases 2009. Available from

http://www.promedmail.org/pls/apex/f?p=2400:1202:3461788566994242::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,75960.

Sertsou, G., N. Wilson, M. Baker, P. Nelson, and M. G. Roberts. 2006. Key transmission parameters of an institutional outbreak during the 1918 influenza pandemic estimated by mathematical modelling. *Theor Biol Med Model* 3:38.

Sewell, D. L. 2003. Laboratory safety practices associated with potential agents of biocrime or bioterrorism. *J Clin Microbiol* 41 (7):2801-9.

Shaman, J., and M. Kohn. 2009. Absolute humidity modulates influenza survival, transmission, and seasonality. *Proc Natl Acad Sci U S A* 106 (9):3243-8.

Shapiro, D. S., and D. R. Schwartz. 2002. Exposure of laboratory workers to Francisella tularensis despite a bioterrorism procedure. *J Clin Microbiol* 40 (6):2278-81.

Shapiro, R. 2008. *Ethical and public health considerations for use of pre-exposure antiviral prophylaxis*. Bethesda: NIH-RAC.

Shen, Z., F. Ning, W. Zhou, X. He, C. Lin, D. P. Chin, Z. Zhu, and A. Schuchat. 2004. Superspreading SARS events, Beijing, 2003. *Emerg Infect Dis* 10 (2):256-60.

Shi, Z., and Z. Hu. 2008. A review of studies on animal reservoirs of the SARS coronavirus. *Virus Res* 133 (1):74-87.

Shimshony, A., and R. Barzilai. 1983. Rift Valley fever. *Adv Vet Sci Comp Med* 27:347-425.

Sidwell, R. W., and D. F. Smee. 2003. Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control. *Antiviral Res* 57 (1-2):101-11.

Siegert, R. , and D.I.H. Simpson. 2008. Marburg virus. *International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates.* (CDC On-line Edition.).

Siegert, R. , and D.I.H. Simpson. *International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates.* 2008. Available from http://www.ncid.cdc.gov/arbocat/catalog-listing.asp?VirusID=286&SI=1.

Sirisanthana, T., and A. E. Brown. 2002. Anthrax of the gastrointestinal tract. *Emerg Infect Dis* 8 (7):649-51.

Sirisanthana, T., N. Navachareon, P. Tharavichitkul, V. Sirisanthana, and A. E. Brown. 1984. Outbreak of oral-oropharyngeal anthrax: an unusual manifestation of human infection with Bacillus anthracis. *Am J Trop Med Hyg* 33 (1):144-50.

Sjostedt, A. 2003. Virulence determinants and protective antigens of Francisella tularensis. *Curr Opin Microbiol* 6 (1):66-71.

Repeated Author. 2007. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann N Y Acad Sci* 1105:1-29.

Slonim, D., and H. Zavadova. 1977. Viremia and serum antibodies in Macacus rhesus monkey after inapparent infection with European tick-borne encephalitis virus. *J Hyg Epidemiol Microbiol Immunol* 21 (4):460-4.

Smyth, H.F. 1941. A 20 year history of anthrax in the U.S.A. Paper read at Symposium on anthrax. , at Harrisburg, Pa.

Spik, K., A. Shurtleff, A. K. McElroy, M. C. Guttieri, J. W. Hooper, and C. SchmalJohn. 2006. Immunogenicity of combination DNA vaccines for Rift Valley fever virus, tick-borne encephalitis virus, Hantaan virus, and Crimean Congo hemorrhagic fever virus. *Vaccine* 24 (21):4657-66.

Stadler, K., A. Roberts, S. Becker, L. Vogel, M. Eickmann, L. Kolesnikova, H. D. Klenk, B. Murphy, R. Rappuoli, S. Abrignani, and K. Subbarao. 2005. SARS vaccine protective in mice. *Emerg Infect Dis* 11 (8):1312-4.

Staples, J. E., K. A. Kubota, L. G. Chalcraft, P. S. Mead, and J. M. Petersen. 2006. Epidemiologic and molecular analysis of human tularemia, United States, 1964-2004. *Emerg Infect Dis* 12 (7):1113-8.

Stenseth, N. C., N. I. Samia, H. Viljugrein, K. L. Kausrud, M. Begon, S. Davis, H. Leirs, V. M. Dubyanskiy, J. Esper, V. S. Ageyev, N. L. Klassovskiy, S. B. Pole, and K. S. Chan. 2006. Plague dynamics are driven by climate variation. *Proc Natl Acad Sci U S A* 103 (35):13110-5.

Stephenson, E. H., E. W. Larson, and J. W. Dominik. 1984. Effect of environmental factors on aerosol-induced Lassa virus infection. *J Med Virol* 14 (4):295-303.

Steward, J., M. S. Lever, P. Russell, R. J. Beedham, A. J. Stagg, R. R. Taylor, and T. J. Brooks. 2004. Efficacy of the latest fluoroquinolones against experimental Yersinia pestis. *Int J Antimicrob Agents* 24 (6):609-12.

Stuart, B.M., and R.L. Pullen. 1945. Tularemic meningitis: review of the literature and report of a case with postmortem observations. . *Arch Intern Med* 76:163-166

Suarez, O. V., G. R. Cueto, R. Cavia, I. E. Gomez Villafane, D. N. Bilenca, A. Edelstein, P. Martinez, S. Miguel, C. Bellomo, K. Hodara, P. J. Padula, and M. Busch. 2003. Prevalence of infection with hantavirus in rodent populations of central Argentina. *Mem Inst Oswaldo Cruz* 98 (6):727-32.

Subbarao, K. 2008. *NIH intramural program: proposed biosafety containment and use of prophylaxis for 1918 H1N1*. Bethesda: NIH-RAC.

Subbarao, K., J. McAuliffe, L. Vogel, G. Fahle, S. Fischer, K. Tatti, M. Packard, W. J. Shieh, S. Zaki, and B. Murphy. 2004. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J Virol* 78 (7):3572-7.

Subbarao, K., and A. Roberts. 2006. Is there an ideal animal model for SARS? *Trends Microbiol* 14 (7):299-303.

Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses. 1980. Laboratory safety for arboviruses and certain other viruses of vertebrates. The Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-Borne Viruses. *Am J Trop Med Hyg* 29 (6):1359-81.

Sullivan, N. J., T. W. Geisbert, J. B. Geisbert, D. J. Shedlock, L. Xu, L. Lamoreaux, J. H. Custers, P. M. Popernack, Z. Y. Yang, M. G. Pau, M. Roederer, R. A. Koup, J. Goudsmit, P. B. Jahrling, and G. J. Nabel. 2006. Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. *PLoS Med* 3 (6):e177.

Suss, J. 2003. Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine* 21 Suppl 1:S19-35.

Süss, J. 2008. Tick-borne encephalitis in Europe and beyond: the epidemiological situation as of 2007. *EUROSURVEILLANCE* 13 (4-6).

Swanepoel, R. 1994. Crimean-Congo Haemorrhagic Fever. In *Infectious Diseases of Livestock, With Special Reference to Southern Africa*, edited by J. A. W. Coetzer, Thomson,G.R., Tustin, R.C. . New York: Oxford University Press.

Swanepoel, R., and J.A.W. Coetzer. 1994. Rift Valley Fever. In *Infectious Diseases of Livestock with Special Reference to Southern Africa*, edited by G. T. JAW Coetzer, & RC Tustin. New York: Oxford University Press.

Swanepoel, R., P. A. Leman, F. J. Burt, N. A. Zachariades, L. E. Braack, T. G. Ksiazek, P. E. Rollin, S. R. Zaki, and C. J. Peters. 1996. Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis* 2 (4):321-5.

Swanepoel, R., S. B. Smit, P. E. Rollin, P. Formenty, P. A. Leman, A. Kemp, F. J. Burt, A. A. Grobbelaar, J. Croft, D. G. Bausch, H. Zeller, H. Leirs, L. E. Braack, M. L. Libande, S. Zaki, S. T. Nichol, T. G. Ksiazek, and J. T. Paweska. 2007. Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis* 13 (12):1847-51.

Swayne, D. E., D. L. Suarez, E. Spackman, T. M. Tumpey, J. R. Beck, D. Erdman, P. E. Rollin, and T. G. Ksiazek. 2004. Domestic poultry and SARS coronavirus, southern China. *Emerg Infect Dis* 10 (5):914-6.

Swenson, D. L., D. Wang, M. Luo, K. L. Warfield, J. Woraratanadharm, D. H. Holman, J. Y. Dong, and W. D. Pratt. 2008. Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections. *Clin Vaccine Immunol* 15 (3):460-7.

Syrjala, H., S. Sutinen, K. Jokinen, P. Nieminen, T. Tuuponen, and A. Salminen. 1986. Bronchial changes in airborne tularemia. *J Laryngol Otol* 100 (10):1169-76.

Tager Frey, M., P. C. Vial, C. H. Castillo, P. M. Godoy, B. Hjelle, and M. G. Ferres. 2003. Hantavirus prevalence in the IX Region of Chile. *Emerg Infect Dis* 9 (7):827-32.

Tai, D.Y.H. . 2006. SARS: How to manage future outbreaks? *Ann Acad Med Singapore* 35:368-373.

Takashima, I., D. Hayasaka, A. Goto, H. Kariwa, and T. Mizutani. 2001. Epidemiology of takc-borne encephalitis (TBE) and phylogenetic analysis of TBE viruses in Japan and Far Eastern Russia. *Jap. J. Infect. Dis.* 54:1-11.

Tan, C. T., and K. B. Chua. 2008. Nipah virus encephalitis. *Curr Infect Dis Rep* 10 (4):315-20.

Tan, C. T., K. J. Goh, K. T. Wong, S. A. Sarji, K. B. Chua, N. K. Chew, P. Murugasu, Y. L. Loh, H. T. Chong, K. S. Tan, T. Thayaparan, S. Kumar, and M. R. Jusoh. 2002. Relapsed and late-onset Nipah encephalitis. *Ann Neurol* 51 (6):703-8.

Tanimura, N., T. Imada, Y. Kashiwazaki, and S. H. Sharifah. 2006. Distribution of viral antigens and development of lesions in chicken embryos inoculated with nipah virus. *J Comp Pathol* 135 (2-3):74-82.

Tarnvik, A., and M. C. Chu. 2007. New approaches to diagnosis and therapy of tularemia. *Ann N Y Acad Sci* 1105:378-404.

Taubenberger, J. 2008. *1918 influenza virus: an overview of the pathogenicity of the N1N1 and virulence factors*. Bethesda MD: NIH-RAC.

Taubenberger, J. K., and D. M. Morens. 2006. 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis* 12 (1):15-22.

ter Meulen, J. 2000. Lassa fever: immuno-epidemiological approach to the study of an endemic viral haemorrhagic fever. *Med Trop (Mars)* 60 (2 Suppl):20-3.

ter Meulen, J., A. B. Bakker, E. N. van den Brink, G. J. Weverling, B. E. Martina, B. L. Haagmans, T. Kuiken, J. de Kruif, W. Preiser, W. Spaan, H. R. Gelderblom, J. Goudsmit, and A. D. Osterhaus. 2004. Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. *Lancet* 363 (9427):2139-41.

Thomas, R. E., R. H. Karstens, and T. G. Schwan. 1990. Experimental infection of Ornithodoros spp. ticks (Acari: Argasidae) with Yersinia pestis. *J Med Entomol* 27 (4):720-3.

Titball, R. W., P. C. Turnbull, and R. A. Hutson. 1991. The monitoring and detection of Bacillus anthracis in the environment. *Soc Appl Bacteriol Symp Ser* 20:9S-18S.

Titball, R. W., and E. D. Williamson. 2001. Vaccination against bubonic and pneumonic plague. *Vaccine* 19 (30):4175-84.

Toro, J., J. D. Vega, A. S. Khan, J. N. Mills, P. Padula, W. Terry, Z. Yadon, R. Valderrama, B. A. Ellis, C. Pavletic, R. Cerda, S. Zaki, W. J. Shieh, R. Meyer, M. Tapia, C. Mansilla, M. Baro, J. A. Vergara, M. Concha, G. Calderon, D. Enria, C. J. Peters, and T. G. Ksiazek. 1998. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. *Emerg Infect Dis* 4 (4):687-94.

Torosian, S. D., P. M. Regan, T. Doran, M. A. Taylor, and A. Margolin. 2009. A refrigeration temperature of 4 degrees C does not prevent static growth of Yersinia pestis in heart infusion broth. *Can J Microbiol* 55 (9):1119-24.

Torres-Perez, F., J. Navarrete-Droguett, R. Aldunate, T. L. Yates, G. J. Mertz, P. A. Vial, M. Ferres, P. A. Marquet, and R. E. Palma. 2004. Peridomestic small mammals associated with confirmed cases of human hantavirus disease in southcentral Chile. *Am J Trop Med Hyg* 70 (3):305-9.

Torres-Perez, F., R. E. Palma, B. Hjelle, M. Ferres, and J. A. Cook. 2009. Andes virus infections in the rodent reservoir and in humans vary across contrasting landscapes in Chile. *Infect Genet Evol*.

Torres-Velez, F. J., W. J. Shieh, P. E. Rollin, T. Morken, C. Brown, T. G. Ksiazek, and S. R. Zaki. 2008. Histopathologic and immunohistochemical characterization of Nipah virus infection in the guinea pig. *Vet Pathol* 45 (4):576-85.

Towner, J. S., B. R. Amman, T. K. Sealy, S. A. Carroll, J. A. Comer, A. Kemp, R. Swanepoel, C. D. Paddock, S. Balinandi, M. L. Khristova, P. B. Formenty, C. G. Albarino, D. M. Miller, Z. D. Reed, J. T. Kayiwa, J. N. Mills, D. L. Cannon, P. W. Greer, E. Byaruhanga, E. C. Farnon, P. Atimnedi, S. Okware, E. Katongole-Mbidde, R. Downing, J. W. Tappero, S. R. Zaki, T. G. Ksiazek, S. T. Nichol, and P. E. Rollin. 2009. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog* 5 (7):e1000536.

Towner, J. S., X. Pourrut, C. G. Albarino, C. N. Nkogue, B. H. Bird, G. Grard, T. G. Ksiazek, J. P. Gonzalez, S. T. Nichol, and E. M. Leroy. 2007. Marburg virus infection detected in a common African bat. *PLoS ONE* 2 (1):e764.

Tumpey, T. 2008. *1918 influenza virus: an overview of the pathogenicity of the H1N1 and virulence factors*. Bethesda: NIH-RAC.

Tumpey, T. M., C. F. Basler, P. V. Aguilar, H. Zeng, A. Solorzano, D. E. Swayne, N. J. Cox, J. M. Katz, J. K. Taubenberger, P. Palese, and A. Garcia-Sastre. 2005. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 310 (5745):77-80.

Tumpey, T. M., A. Garcia-Sastre, A. Mikulasova, J. K. Taubenberger, D. E. Swayne, P. Palese, and C. F. Basler. 2002. Existing antivirals are effective against influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A* 99 (21):13849-54.

Tumpey, T. M., A. Garcia-Sastre, J. K. Taubenberger, P. Palese, D. E. Swayne, and C. F. Basler. 2004. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A* 101 (9):3166-71.

Tumpey, T. M., A. Garcia-Sastre, J. K. Taubenberger, P. Palese, D. E. Swayne, M. J. Pantin-Jackwood, S. Schultz-Cherry, A. Solorzano, N. Van Rooijen, J. M. Katz, and C. F. Basler. 2005. Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice. *J Virol* 79 (23):14933-44.

Turell, M. 2008. Personal communication record regarding TBE-complex viruses and Congo-Crimean viruses. Arlington, October 30, 2008.

Turell, M. J., D. J. Dohm, C. N. Mores, L. Terracina, D. L. Wallette, Jr., L. J. Hribar, J. E. Pecor, and J. A. Blow. 2008. Potential for North American mosquitoes to transmit Rift Valley fever virus. *J Am Mosq Control Assoc* 24 (4):502-7.

Turell, M. J., and G. B. Knudson. 1987. Mechanical transmission of Bacillus anthracis by stable flies (Stomoxys calcitrans) and mosquitoes (Aedes aegypti and Aedes taeniorhynchus). *Infect Immun* 55 (8):1859-61.

Turell, M.J., Bennett,K.E., and W.C. Wilson. 2008. Potential for North American mosquitoes to transmit Rift Valley fever virus. Paper read at American Society of Tropical Medicine and Hygiene annual meeting.

Turnbull, P. C., P. M. Lindeque, J. Le Roux, A. M. Bennett, and S. R. Parks. 1998. Airborne movement of anthrax spores from carcass sites in the Etosha National Park, Namibia. *J Appl Microbiol* 84 (4):667-76.

Turnbull, P.C.B. 1998. Guidelines for the surveillance and control of anthrax in humans and animals. edited by E. a. o. C. D. World Health Organization, Surveillance and Control. Geneva: World Health Organization.

Turnbull, P.C.B., Broster, M.G., Carman, J.A., et al. 1986. Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance to protective immunity. . *Infect Immun* 52:356-363

U.S. Department of Agriculture Forest Service.

Uyeki, T. 2008. *Ethical and public health considerations for use of pre-exposure antiviral prophylaxis*. Bethesda: NIH-RAC.

Vainrub, B., and R. Salas. 1994. Latin American hemorrhagic fever. *Infect Dis Clin North Am* 8 (1):47-59.

Van Hoeven, N., J. A. Belser, K. J. Szretter, H. Zeng, P. Staeheli, D. E. Swayne, J. M. Katz, and T. M. Tumpey. 2009. Pathogenesis of 1918 pandemic and H5N1 influenza virus infections in a guinea pig model: antiviral potential of exogenous alpha interferon to reduce virus shedding. *J Virol* 83 (7):2851-61.

Varia, M., S. Wilson, S. Sarwal, A. McGeer, E. Gournis, E. Galanis, and B. Henry. 2003. Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada. *CMAJ* 169 (4):285-92.

Verreault, D., S. Moineau, and C. Duchaine. 2008. Methods for sampling of airborne viruses. *Microbiol Mol Biol Rev* 72 (3):413-44.

Vial, P. A., F. Valdivieso, G. Mertz, C. Castillo, E. Belmar, I. Delgado, M. Tapia, and M. Ferres. 2006. Incubation period of hantavirus cardiopulmonary syndrome. *Emerg Infect Dis* 12 (8):1271-3.

Viboud, C., T. Tam, D. Fleming, A. Handel, M. A. Miller, and L. Simonsen. 2006. Transmissibility and mortality impact of epidemic and pandemic influenza, with emphasis on the unusually deadly 1951 epidemic. *Vaccine* 24 (44-46):6701-7.

Videla, C., G. Carballal, P. Remorini, and J. La Torre. 1989. Formalin inactivated Junin virus: immunogenicity and protection assays. *J Med Virol* 29 (3):215-20.

von Reyn, C. F., A. M. Barnes, N. S. Weber, T. Quan, and W. J. Dean. 1976. Bubonic plague from direct exposure to a naturally infected wild coyote. *Am J Trop Med Hyg* 25 (4):626-9.

Wacharapluesadee, S., K. Boongird, S. Wanghongsa, N. Ratanasetyuth, P. Supavonwong, D. Saengsen, G. N. Gongal, and T. Hemachudha. 2009. A Longitudinal Study of the Prevalence of Nipah Virus in Pteropus lylei Bats in Thailand: Evidence for Seasonal Preference in Disease Transmission. *Vector Borne Zoonotic Dis*.

Wacharapluesadee, S., B. Lumlertdacha, K. Boongird, S. Wanghongsa, L. Chanhome, P. Rollin, P. Stockton, C. E. Rupprecht, T. G. Ksiazek, and T. Hemachudha. 2005. Bat Nipah virus, Thailand. *Emerg Infect Dis* 11 (12):1949-51.

Wahl-Jensen, V., J. Chapman, L. Asher, R. Fisher, M. Zimmerman, T. Larsen, and J. W. Hooper. 2007. Temporal analysis of Andes virus and Sin Nombre virus infections of Syrian hamsters. *J Virol* 81 (14):7449-62.

Walker, C. M., and G. Ko. 2007. Effect of ultraviolet germicidal irradiation on viral aerosols. *Environ Sci Technol* 41 (15):5460-5.

Wallinga, J., and P. Teunis. 2004. Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures. *Am J Epidemiol* 160 (6):509-16.

Walsh, J. J., N. Pesik, C. P. Quinn, V. Urdaneta, C. A. Dykewicz, A. E. Boyer, J. Guarner, P. Wilkins, K. J. Norville, J. R. Barr, S. R. Zaki, J. B. Patel, S. P. Reagan, J. L. Pirkle, T. A. Treadwell, N. R. Messonnier, L. D. Rotz, R. F. Meyer, and D. S. Stephens. 2007. A case of naturally acquired inhalation anthrax: clinical care and analyses of anti-protective antigen immunoglobulin G and lethal factor. *Clin Infect Dis* 44 (7):968-71.

Wang, J. T., W. H. Sheng, C. T. Fang, Y. C. Chen, J. L. Wang, C. J. Yu, S. C. Chang, and P. C. Yang. 2004. Clinical manifestations, laboratory findings, and treatment outcomes of SARS patients. *Emerg Infect Dis* 10 (5):818-24.

Wang, L. F., and B. T. Eaton. 2007. Bats, civets and the emergence of SARS. *Curr Top Microbiol Immunol* 315:325-44.

Wang, L. F., Z. Shi, S. Zhang, H. Field, P. Daszak, and B. T. Eaton. 2006. Review of bats and SARS. *Emerg Infect Dis* 12 (12):1834-40.

Wang, W., and S. Ruan. 2004. Simulating the SARS outbreak in Beijing with limited data. *J Theor Biol* 227 (3):369-79.

Wang, X. W., J. S. Li, M. Jin, B. Zhen, Q. X. Kong, N. Song, W. J. Xiao, J. Yin, W. Wei, G. J. Wang, B. Y. Si, B. Z. Guo, C. Liu, G. R. Ou, M. N. Wang, T. Y. Fang, F. H. Chao, and J. W. Li. 2005. Study on the resistance of severe acute respiratory syndrome-associated coronavirus. *J Virol Methods* 126 (1-2):171-7.

Warfield, K. L., E. M. Deal, and S. Bavari. 2009. Filovirus infections. *J Am Vet Med Assoc* 234 (9):1130-9.

Warfield, K. L., D. L. Swenson, D. L. Negley, A. L. Schmaljohn, M. J. Aman, and S. Bavari. 2004. Marburg virus-like particles protect guinea pigs from lethal Marburg virus infection. *Vaccine* 22 (25-26):3495-502.

Watanabe, T., S. Watanabe, K. Shinya, J. H. Kim, M. Hatta, and Y. Kawaoka. 2009. Viral RNA polymerase complex promotes optimal growth of 1918 virus in the lower respiratory tract of ferrets. *Proc Natl Acad Sci U S A* 106 (2):588-92.

Wattiau, P., S. R. Klee, D. Fretin, M. Van Hessche, M. Menart, T. Franz, C. Chasseur, P. Butaye, and H. Imberechts. 2008. Occurrence and genetic diversity of Bacillus anthracis strains isolated in an active wool-cleaning factory. *Appl Environ Microbiol* 74 (13):4005-11.

Weber, D. J., and W. A. Rutala. 2001. Risks and prevention of nosocomial transmission of rare zoonotic diseases. *Clin Infect Dis* 32 (3):446-56.

Weber, T. P., and N. I. Stilianakis. 2008. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *J Infect* 57 (5):361-73.

Wein, L. M., D. L. Craft, and Anthrax Modeling Working Group. 2005. Evaluation of public health interventions for Anthrax: a report to the secretary's council on Public Health Preparedness. *Biosecur Bioterror* 3 (4):348-56.

Weinbren, M.P. . 2008. International Catalog of Arboviruses, Including Certain Other Viruses of Vertebrates. In *Rift Valley Fever*, ed CDC. Atlanta: CDC. <http://www2.ncid.cdc.gov/arbocat/catalog-listing.asp?VirusID=397&SI=1>.

Weingartl, H., M. Czub, S. Czub, J. Neufeld, P. Marszal, J. Gren, G. Smith, S. Jones, R. Proulx, Y. Deschambault, E. Grudeski, A. Andonov, R. He, Y. Li, J. Copps, A. Grolla, D. Dick, J. Berry, S. Ganske, L. Manning, and J. Cao. 2004. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. *J Virol* 78 (22):12672-6.

Weingartl, H., S. Czub, J. Copps, Y. Berhane, D. Middleton, P. Marszal, J. Gren, G. Smith, S. Ganske, L. Manning, and M. Czub. 2005. Invasion of the central nervous system in a porcine host by nipah virus. *J Virol* 79 (12):7528-34.

Weingartl, H. M., R. A. Albrecht, K. M. Lager, S. Babiuk, P. Marszal, J. Neufeld, C. Embury-Hyatt, P. Lekcharoensuk, T. M. Tumpey, A. Garcia-Sastre, and J. A. Richt. 2009. Experimental infection of pigs with the human 1918 pandemic influenza virus. *J Virol* 83 (9):4287-96.

Weingartl, H. M., Y. Berhane, and M. Czub. 2009. Animal models of henipavirus infection: a review. *Vet J* 181 (3):211-20.

Weingartl, H. M., J. Copps, M. A. Drebot, P. Marszal, G. Smith, J. Gren, M. Andova, J. Pasick, P. Kitching, and M. Czub. 2004. Susceptibility of pigs and chickens to SARS coronavirus. *Emerg Infect Dis* 10 (2):179-84.

Weis, C. P., A. J. Intrepido, A. K. Miller, P. G. Cowin, M. A. Durno, J. S. Gebhardt, and R. Bull. 2002. Secondary aerosolization of viable Bacillus anthracis spores in a contaminated US Senate Office. *JAMA* 288 (22):2853-8.

Weiss, S. R., and S. Navas-Martin. 2005. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiol Mol Biol Rev* 69 (4):635-64.

Weissenbacher, M. C., M. M. Avila, M. A. Calello, M. S. Merani, J. B. McCormick, and M. Rodriguez. 1986. Effect of ribavirin and immune serum on Junin virus-infected primates. *Med Microbiol Immunol* 175 (2-3):183-6.

Weissenbacher, M. C., M. A. Calello, O. J. Colillas, H. Golfera, S. N. Rondinone, and M. J. Frigerio. 1978. [Infection of New World primates with Junin virus. I. Alouatta caraya]. *Medicina (B Aires)* 38 (5):529-36.

Weissenbacher, M. C., M. A. Calello, O. J. Colillas, S. N. Rondinone, and M. J. Frigerio. 1979. Argentine hemorrhagic fever: a primate model. *Intervirology* 11 (6):363-5.

Weissenbacher, M. C., C. E. Coto, M. A. Calello, S. N. Rondinone, E. B. Damonte, and M. J. Frigerio. 1982. Cross-protection in nonhuman primates against Argentine hemorrhagic fever. *Infect Immun* 35 (2):425-30.

Weissenbacher, M. C., L. B. de Guerrero, and M. C. Boxaca. 1975. Experimental biology and pathogenesis of Junin virus infection in animals and man. *Bull World Health Organ* 52 (4-6):507-15.

Weissenbacher, M. C., E. Edelmuth, M. J. Frigerio, C. E. Coto, and L. B. de Guerrero. 1980. Serological survey to detect subclinical Junin virus infection in laboratory personnel. *J Med Virol* 6 (3):223-6.

Weissenbacher, M. C., M. S. Merani, V. L. Hodara, G. de Villafane, D. C. Gajdusek, Y. K. Chu, and H. W. Lee. 1990. Hantavirus infection in laboratory and wild rodents in Argentina. *Medicina (B Aires)* 50 (1):43-6.

Welch, T. J., W. F. Fricke, P. F. McDermott, D. G. White, M. L. Rosso, D. A. Rasko, M. K. Mammel, M. Eppinger, M. J. Rosovitz, D. Wagner, L. Rahalison, J. E. Leclerc, J. M. Hinshaw, L. E. Lindler, T. A. Cebula, E. Carniel, and J. Ravel. 2007. Multiple antimicrobial resistance in plague: an emerging public health risk. *PLoS ONE* 2 (3):e309.

Welkos, S. L., T. J. Keener, and P. H. Gibbs. 1986. Differences in susceptibility of inbred mice to Bacillus anthracis. *Infect Immun* 51 (3):795-800.

Wells, R. M., S. Sosa Estani, Z. E. Yadon, D. Enria, P. Padula, N. Pini, J. N. Mills, C. J. Peters, and E. L. Segura. 1997. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia. *Emerg Infect Dis* 3 (2):171-4.

Wentworth, D. E., M. W. McGregor, M. D. Macklin, V. Neumann, and V. S. Hinshaw. 1997. Transmission of swine influenza virus to humans after exposure to experimentally infected pigs. *J Infect Dis* 175 (1):7-15.

White, D.O., and F.A. Fenner. 1994. *Coronaviridae*. *Medical Virology*.

White, D.O., and F.J. Fenner. 1994. Rift Valley Fever. *Medical Virology* San Diego, 4th Ed:513-515.

Wild, M. A., T. M. Shenk, and T. R. Spraker. 2006. Plague as a mortality factor in Canada lynx (Lynx canadensis) reintroduced to Colorado. *J Wildl Dis* 42 (3):646-50.

Wild, T. F. 2009. Henipaviruses: a new family of emerging Paramyxoviruses. *Pathol Biol (Paris)* 57 (2):188-96.

Wilkening, D. A. 2006. Sverdlovsk revisited: modeling human inhalation anthrax. *Proc Natl Acad Sci U S A* 103 (20):7589-94.

Willke, A., M. Meric, R. Grunow, M. Sayan, E. J. Finke, W. Splettstosser, E. Seibold, S. Erdogan, O. Ergonul, Z. Yumuk, and S. Gedikoglu. 2009. An outbreak of oropharyngeal tularaemia linked to natural spring water. *J Med Microbiol* 58 (Pt 1):112-6.

Winters, A. M., J. E. Staples, A. Ogen-Odoi, P. S. Mead, K. Griffith, N. Owor, N. Babi, R. E. Enscore, L. Eisen, K. L. Gage, and R. J. Eisen. 2009. Spatial risk models for human plague in the West Nile region of Uganda. *Am J Trop Med Hyg* 80 (6):1014-22.

Won, W. D., and H. Ross. 1966. Effect of diluent and relative humidity on apparent viability of airborne Pasteurella pestis. *Appl Microbiol* 14 (5):742-5.

Wong, J. D., J. R. Barash, R. F. Sandfort, and J. M. Janda. 2000. Susceptibilities of Yersinia pestis strains to 12 antimicrobial agents. *Antimicrob Agents Chemother* 44 (7):1995-6.

Wong, J.D., Shapiro, D.S. 1999. Francisella. In *Manual of clinical microbiology.*, edited by P. R. Murray, Baron, E.J., Pfaller, M.A., et al. Washington, D.C.: American Society for Microbiology Press.

Wong, K. T., I. Grosjean, C. Brisson, B. Blanquier, M. Fevre-Montange, A. Bernard, P. Loth, M. C. Georges-Courbot, M. Chevallier, H. Akaoka, P. Marianneau, S. K. Lam, T. F. Wild, and V. Deubel. 2003. A golden hamster model for human acute Nipah virus infection. *Am J Pathol* 163 (5):2127-37.

Wong, K. T., T. Robertson, B. B. Ong, J. W. Chong, K. C. Yaiw, L. F. Wang, A. J. Ansford, and A. Tannenberg. 2009. Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol Appl Neurobiol* 35 (3):296-305.

Wong, K. T., W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, C. S. Goldsmith, K. B. Chua, S. K. Lam, C. T. Tan, K. J. Goh, H. T. Chong, R. Jusoh, P. E. Rollin, T. G. Ksiazek, and S. R. Zaki. 2002. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol* 161 (6):2153-67.

Wong, K. T., W. J. Shieh, S. R. Zaki, and C. T. Tan. 2002. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer Semin Immunopathol* 24 (2):215-28.

Wong, S.S.Y., and K.Y. Yuen. 2005. The severe acute respiratory syndrome (SARS). *J Neurovirol* 11:455-468.

Woods, C. W., K. Ospanov, A. Myrzabekov, M. Favorov, B. Plikaytis, and D. A. Ashford. 2004. Risk factors for human anthrax among contacts of anthrax-infected livestock in Kazakhstan. *Am J Trop Med Hyg* 71 (1):48-52.

World Health Organization. 2002. Global Soalr UV Index: A Practical Guide.

Repeated Author. 2010. *Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003* 2010 [cited Feb 7 2010 2010]. Available from http://www.who.int/csr/sars/country/table2004_04_21/en/.

Wright, J. G., C. P. Quinn, S. Shadomy, N. Messonnier, Control Centers for Disease, and Prevention. Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 59 (RR-6):1-30.

Yang, C., L. Ye, and R. W. Compans. 2008. Protection against filovirus infection: virus-like particle vaccines. *Expert Rev Vaccines* 7 (3):333-44.

Yang, L., A. Sanchez, J. M. Ward, B. R. Murphy, P. L. Collins, and A. Bukreyev. 2008. A paramyxovirus-vectored intranasal vaccine against Ebola virus is immunogenic in vector-immune animals. *Virology* 377 (2):255-64.

Yang, Z., S. Wang, Q. Li, Y. Li, M. Wei, H. Gao, C. Donovan, and P. P. Wang. 2009. Determining SARS sub-clinical infection: a longitudinal seroepidemiological study in recovered SARS patients and controls after an outbreak in a general hospital. *Scand J Infect Dis* 41 (6-7):507-10.

Yetter, R. A., S. Lehrer, R. Ramphal, and P. A. Small, Jr. 1980. Outcome of influenza infection: effect of site of initial infection and heterotypic immunity. *Infect Immun* 29 (2):654-62.

Yip, C., W. L. Chang, K. H. Yeung, and I. T. Yu. 2007. Possible meteorological influence on the severe acute respiratory syndrome (SARS) community outbreak at Amoy Gardens, Hong Kong. *J Environ Health* 70 (3):39-46.

Yoneda, M., V. Guillaume, F. Ikeda, Y. Sakuma, H. Sato, T. F. Wild, and C. Kai. 2006. Establishment of a Nipah virus rescue system. *Proc Natl Acad Sci U S A* 103 (44):16508-13.

Young, L.S., Bicknell, D.S., Archer, B.G., et al. . 1969. Tularemia epidemic: Vermont 1968. Forty-seven cases linked to contact with muskrats. . *New England Journal of Medicine* 280 (23):1253-1260

Yu, I. T., Y. Li, T. W. Wong, W. Tam, A. T. Chan, J. H. Lee, D. Y. Leung, and T. Ho. 2004. Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N Engl J Med* 350 (17):1731-9.

Zaas, A. K., M. Chen, J. Varkey, T. Veldman, A. O. Hero, 3rd, J. Lucas, Y. Huang, R. Turner, A. Gilbert, R. Lambkin-Williams, N. C. Oien, B. Nicholson, S. Kingsmore, L. Carin, C. W. Woods, and G. S. Ginsburg. 2009. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans. *Cell Host Microbe* 6 (3):207-17.

Zhang, Z. 2007. The outbreak pattern of SARS cases in China as revealed by a mathematical model. *Ecological Modelling* 204:420-426.

Zhong, N. S., and G. W. Wong. 2004. Epidemiology of severe acute respiratory syndrome (SARS): adults and children. *Paediatr Respir Rev* 5 (4):270-4.

Zhou, G., and G. Yan. 2003. Severe acute respiratory syndrome epidemic in Asia. *Emerg Infect Dis* 9 (12):1608-10.

Appendix D.

A Review of Reported Incidents, Exposures and Infections in BSL-3 and BSL-4 Laboratory Facilities

Table of Contents

Note: This appendix is provided as a general indicator of biocontainment safety level (BSL) 3 and 4 laboratory safety experience. As such, it reflects experience with a broad range of microbes in research and clinical laboratories throughout the world. Not all the microbes included herein necessarily will be studied at the NEIDL.

D. A Review of Reported Incidents, Exposure, and Infections in BSL-3 and BSL-4 Laboratory Facilities

D.1 Part I.

D.1.1 Reporting of Laboratory-Associated Infections

Accounts of laboratory-associated infections (LAI) began to appear in the literature in the late 19th century. The earliest incidents involved diphtheria, cholera, typhoid fever, and brucellosis. Infections described in these reports originated by oral, cutaneous, and subcutaneous exposure, and in one instance the route of infection was unknown (Kruse, Puckett, and Richardson 1991). Subsequently, sporadic surveys of LAI in general were published, beginning in 1915 and most recently in 2006, 2008, and 2011 (Harding 2000, 2006; Centers for Disease Control and Prevention 1999; Kimman, Smit, and Klein 2008; Pedrosa and Cardoso 2011). Methods used in those reviews included examining the literature for published reports, submitting questionnaires to thousands of laboratories, and soliciting personal accounts of unpublished incidents. However, there has been no mechanism for centralized and systematic reporting of LAI as a whole. As a result, data that have been collected to date are incomplete.

In the United States, infections in the general population caused by approximately 80 pathogens are voluntarily reported to the Centers for Disease Control and Prevention (CDC) by state health agencies, and these can include infections associated with laboratory activities. In addition, provisions of the Select Agent Program that took effect in 2002 now require laboratories to report infections and other biocontainment releases involving particular pathogens that have been designated as select agents due to their potential impact on human health or agriculture or both (APHIS and CDC 2009). Furthermore, any institution receiving NIH funding is required to report to the NIH any LAI and other biocontainment releases that involve genetically

recombinant pathogens or organisms (National Institutes of Health 2011). Biocontainment releases, including LAI, that involve other pathogens do not have reporting requirements.

It has been suggested that retrospective reviews of reported LAI substantially under-represent the true number of such infections (Harding 2006). There are two reasons for that. First, the findings from the reviews cannot account for subclinical infections that can occur in the laboratory setting. Because such infections are asymptomatic, it is unlikely that they would be detected unless an accompanying breach of biocontainment is suspected or unless periodic serologic surveys of laboratory workers are conducted as part of an ongoing occupational health maintenance program. Second, there can be reluctance by personnel to report laboratory accidents and potential exposures. As noted by Harding and Byers (Harding 2000, 2006), collection of accurate data regarding potential laboratory-related exposures continues to be "…hampered by an indifference to and, frequently, an unwillingness to report these incidents" in part "…due to fear of reprisal and the stigma associated with such events." Recent congressional testimony suggests that a no-fault reporting system for biocontainment failures, similar to that used by the Federal Aviation Administration, could be beneficial to the field of biosafety (Subcommittee on Oversight and Investigations 2007). The potential advantage of such an approach is two-fold. First, incidents could be reported without fear of penalty or stigma for personnel or for the institution. Second, root cause analysis of failures often will demonstrate common factors that, when identified and shared, can be used to reduce the likelihood of similar events in the future.

Comparisons of previous reports with recent literature can be used to consider whether there has been downward trend in the number of reported LAI in general over various periods of time. However, it is important to note that such compilations cover clinical as well as research laboratories, and that BLS-2 laboratory data often are included. At least one comparison based on data reported by Harding and Byers (Harding 2006) led to an inference that LAI reported from these various laboratory settings appeared to have declined for some pathogens (Centers for Disease Control and Prevention 2007). However, a later review by Harding and Byers found such comparisons to be inconclusive because of incomplete data on the total numbers of infections and the numbers of workers at risk (Harding 2006). Other authors have reached the

same conclusion, namely, that information on laboratory incidents is incomplete regarding the total number of infections and the total number of people at risk. Without this information, the actual incidence of LAI cannot be determined (Kimman, Smit, and Klein 2008; Centers for Disease Control and Prevention 2007).

However, information on LAI recently has been compiled from reports on pathogens currently listed as select agents. This 2011 retrospective review showed a marked decrease in reported LAI for these particular pathogens during the years 1930-2009 (Committee on Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents: National Research Council 2011). In their review, the Committee considered three time periods: 1930-1978, 1979-2004, and 2005-2009. Total infections were determined to be 2107, 379, and 8, during the three periods, for a yearly average of 44, 15, and 2 LAI, respectively (Figure D-1a). The Committee attributed the sharp decline in LAI involving select agents to improvements over time in biosafety procedures, primary biocontainment systems, personal protective equipment, and facilities engineering. Examples of these improvements include progress in the design of safety equipment (such as the advent of powered air-purifying respirators for use as personal respiratory protective equipment to be used as part of BSL-3 biocontainment precautions) and laboratory facilities (such as the requirement for HEPA filtration of exhaust air from BSL-3 facilities), the development of safer work practices (such as the elimination or reduction of sharps), improvements in training, and the refinement and implementation of biosafety and biosurety programs (i.e. an emphasis on achieving a biosafety culture).

The Committee also found that, although the number of LAI from select agents has sharply decreased, the number of reports of select agent releases has increased dramatically from 2003 through 2009 (Figure D-1b). The Committee has attributed the increase in release reports "…at least in part, to the broad definition of a release event and to the expansion in Select Agent research since 2001". These two trends reported for select agents by the Committee reflect the widespread expectation among members of the biosafety profession that ongoing progress in the field of biosafety and biocontainment will continue to contribute to the prevention, minimization, and mitigation of laboratory-associated infections.

Figure D-1a. Average annual number of select agent LAI over various time periods.

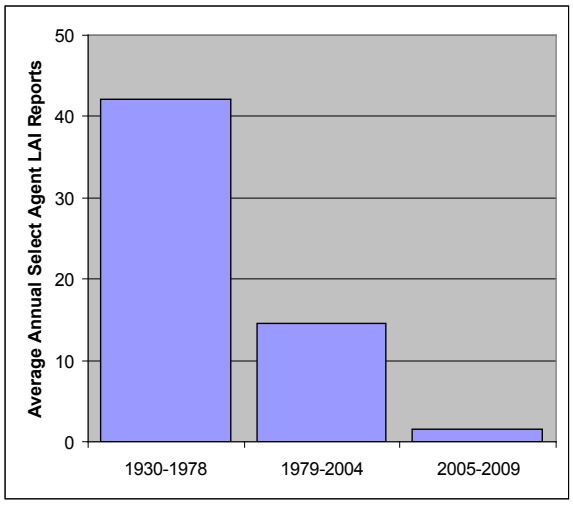
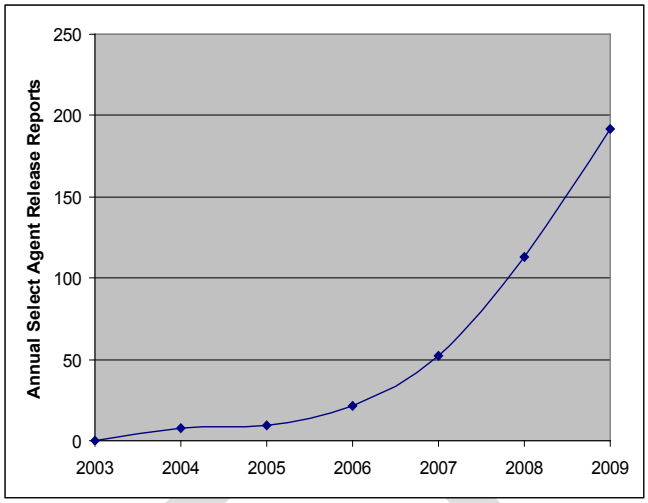


Figure D-1b. Annual number of select agent release reports 2003-2009.



Source: Based on Committee on Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents (Committee on Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents: National Research Council 2011)

It is significant that the operating experience of BSL-4 biocontainment facilities shows there have been no laboratory-associated infections among the public in conjunction with the operation of these facilities (Centers for Disease Control and Prevention 1999) (see Part II. Johnson Reports). As for BSL-3 pathogens, data from the CDC between 1947 and 1973 showed that, for 109 LAI diagnosed among its personnel, no secondary infections occurred among family or community members, and the National Animal Disease Center (Ames, Iowa) similarly found no secondary infections associated with 18 LAI at their institution between 1960 and 1975 (Centers for Disease Control and Prevention 1999). Recently there has been speculation based on strong scientific evidence that the 1977 H1N1 influenza virus outbreak in the Soviet Union and Asia originated from a laboratory source (Zimmer and Burke 2009). These 1977 H1N1 infections could be informative about the potential for a respiratory virus to spread following release from a laboratory setting. However, if speculation about a laboratory origin for this outbreak is true, it must be noted that the extent of any biocontainment precautions that might have been in place are unknown, and it is likely that those would not have consisted of BSL-3 biocontainment precautions applied in a BSL-3 facility. Accordingly, the 1977 H1N1 outbreak is noted here but is not included in the tabulated information on laboratory biosafety incidents that follows.

Operating experience from BSL-3 biocontainment facilities reported since then shows an LAI with SARS-CoV that resulted in seven secondary infections among the public in China (one of which was fatal)(World Health Organization 2004). Reviews conducted after the 2004 SARS incident in China, that would have addressed LAIs linked to BSL-3 or BSL-4 laboratory operations, reported no infections in the community at large (Kimman, Smit, and Klein 2008; Harding 2006; Johnson 2009; (Pedrosa and Cardoso 2011).

D.1.2 BSL-3 And BSL-4 Laboratory Biosafety Experience Summarized Findings From The Johnson Reports 1970-2009

In 2003 Dr. Karl Johnson, the lead scientist who isolated and named Ebola virus in1977, undertook surveys of the biosafety experience for BSL-4 facilities worldwide and for key BSL-3 facilities in the United States. Methodology included on-site review of laboratory and institution records, in-depth interviews with senior scientists, and compilation and assessment of written responses from senior scientists. In 2009 Dr. Johnson updated his report on BSL-4 facilities with data and findings from 2003 through 2009. The findings of those surveys are known as the Johnson Reports. In his review of BSL-4 facilities, Dr. Johnson surveyed the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), the CDC's Special Pathogens Branch, the National Institute for Communicable Diseases in Johannesburg (NICD), the Southwest Foundation for Biomedical Research, and the University of Texas Medical Branch-Galveston. For the five BSL-4 facilities reviewed during 1970–2009, Dr. Johnson determined that no infections occurred during 700,000 worker hours of facility operation. Those finding reflect the efficacy of biocontainment precautions employed at the BSL-4 level. BSL-4 precautions are the most stringent that can be applied to biocontainment of pathogens.

In addition to the surveys of BSL-4 facilities, Dr. Johnson undertook surveys for three BSL-3 biocontainment intramural laboratories of the National Institute of Allergy and Infectious Diseases. Those laboratories composed part of the biological research facilities on the Bethesda and Rockville, Maryland, campuses, and the Rocky Mountain Laboratories in Hamilton, Montana. Those three BSL-3 facilities were reviewed during 1982–2003. In his review, Dr. Johnson found that only one clinical infection and four asymptomatic infections had occurred for 3.2 million worker hours of operation during those years.

The biosafety data compiled in the Johnson Reports for BSL-4 and BSL-3 facilities are compelling evidence of robust and effective engineering controls, training programs, operating procedures, safety awareness, and professional skill in effect at the facilities surveyed. Moreover, the data from those laboratories are particularly informative because they include the number of hours worked for the infections tallied. That allows for facility-specific incidences to be characterized in a form that allows estimation of worker risk for purposes of risk assessment (RA). Because the data include denominators of worker hours, they have been chosen as the best data available for use in estimating frequency of infections in BSL-3 and BSL-4 facilities. Dr. Johnson's findings are detailed in the Supplement to this Appendix, as the Johnson Reports.

D.1.3 Additional Biosafety Experience in Laboratories

Another strength of the data in the Johnson Reports is that the BSL-4 facilities surveyed fully represent the extent of BSL-4 operations in the United States. This is necessarily less true for the BSL-3 facilities, which number in the hundreds in the United States. To address this, other reports from various sources that address BSL-3 and BSL-4 facilities have been compiled and summarized in tabular form (Tables D.1 through D.7). Sources for the data include peer-reviewed scientific literature, reports and presentations from federal governmental agencies, reports from nonprofit entities, and the media in general. The credibility of the accounts and sources has been reviewed carefully, and they serve to expand the data on biosafety in a qualitative manner. However, as discussed previously, reporting of incidents is not mandatory for BSL-3 facilities, and the reporting that does take place is not transparent for the public. As a result, information for additional incidents is incomplete with regard to the total number of infections, the total number of people at risk, and other details, and they do not by themselves represent a sound basis for determining LAI frequency for use in quantitative assessments of risk (Kimman, Smit, and Klein 2008; Centers for Disease Control and Prevention 2007). In that regard, the Johnson Reports offer the best available validated data that can be applied in quantitative RA. The additional materials listed below that concern BSL-4 laboratories are consistent with the findings in the Johnson Reports, and they document three, perhaps four, infections that occurred in Russia and Germany. The additional materials listed below that concern BSL-3 laboratories show several dozen LAI, in contrast to the experience detailed in the

Johnson Reports. As discussed previously, these reports are difficult to interpret on a quantitative basis but can be helpful in understanding the biosafety experience from a qualitative broader viewpoint.

Particular attention in these materials is given to LAI, but biocontainment failures and other protocol lapses that did not result in an LAI are included for certain cases that might have posed potential risk to workers or the community. Also, some incidents reported here under the category of BSL-3 pertain to BSL-2 facilities, either because they involved facilities at Boston University (and therefore are, or could be perceived as, relevant to this RA) or because they are Select Agent pathogens that can be handled using either BSL-2 or BSL-3 biocontainment precautions, depending on circumstances of the work involved. Because U.S. federal requirements for reporting these kinds of incidents exist only for pathogens specified by the Select Agent Program and for incidents that occur in conjunction with research on recombinant DNA, incidents involving other pathogens might be incompletely represented in the tables.

D.1.3.1 BSL-3 Facility Experience at Two NIH Virology Laboratories, 2003-2008 and 1976-2008

Data for the period 2003–2009, collected in the form and manner of those compiled by Dr. Johnson for the three National Institute of Allergy and Infectious Diseases (NIAID) facilities surveyed in 1982–2003, were not available. However, some data concerning two laboratories are available from a recent NIH-sponsored symposium on safe work practices for 1918 H1N1 influenza virus (Subbarao 2008). For one laboratory the reporting period consisted of 111,000 worker hours during the years 2003–2008, and the pathogens involved were highly pathogenic avian influenza virus (HPAIV) and SARS-CoV. No spills or percutaneous exposures were reported. There were five reports of fever from three employees, and laboratory testing for possible infection was indicated in one case. No infections were documented among the workers for a period that equates to 54 person years (Table D-1). For the second laboratory, the reporting period consisted of 203,000 worker hours during the years 1976–2008, and the pathogen involved was HPAIV. Four potential exposures were documented from 2005-2008. Two of the four people received post-exposure Tamiflu and none developed clinical illness. No infections were documented among the workers for a period that equates to 105 person years (Table D-1).

The data from these two laboratories comprise an extremely good biosafety record and are consistent with findings of the Johnson Reports concerning biosafety experience at other NIH BSL-3 laboratories.

Table D-1. Personnel hours worked and accidental exposures to highly pathogenic avian influenza virus (HPAIV) and SARS-CoV for two intramural NIAID laboratories: 1976–2008

	Hours at risk	Accidental exposures
SARS-CoV	41,000	0
HPAIV	273,300	0
Total	**314,300**	**0**

D.1.3.2 BSL-3 Facility Experience at USAMRIID 1989-2004

A recent review of biosafety experience at USAMRIID in 1989 through 2002 was published in 2004. During that time, four (and possibly five) clinical infections from BSL-3 pathogens were detected (Table D-2). There were no secondary infections associated with any of the exposures. These infections, as well as a flooding event concerning *B. anthracis*, are included in Table D-7.

Table D-2. Outcomes of BSL-3 accidental exposures to infectious pathogens: USAMRIID 1989–2002

		Potential exposures	Exposures for which antimicrobial compounds were given	Clinical infections	Infecting agent
Bacterial		150	75	3[a]	*Burkholderia mallei* *Coxiella burnetii* *Yersinia pestis*
Viral		76	2[b]	2	VEE virus[c] Chikungunya virus

Source: (Rusnak, Kortepeter, et al. 2004)

Notes:

a. Infection from Y. pestis was highly likely in one case but confirmatory culture was not performed.

b. Antiviral compounds were not available for most viral pathogens; investigational compounds were administered in two cases.

c. 3VEE = Venezuelan equine encephalitis virus

CDC Select Agent Incident Reports 2003–2009 (NRC (National Research Council) 2011)

Table D-3. Potential release of select agents, 2003–2009, with 7 resulting LAI*

Activity	Number of potential release events
Animal bite or scratch	11
Needlestick or sharps injury	46
Equipment mechanical failure	23
Personal protective equipment failure	12
Loss of containment	196
Procedural issue	30
Spill	77
Total events	395

*As of 9/29/11 details are unavailable, as these data are unpublished, except as Exhibit 13 in Serial No. 110-70 (Subcommittee on Oversight and Investigations 2007). Many of these events and as many as 6 of the LAI are duplicated in the Select Agent data presented before the U.S. House of Representatives on October 4, 2007, which are included in Table D-7 below.

D.1.3.3 BSL-4 Biocontainment Facilities: International

Table D-4. Recent reported incidents involving international BSL-4 biocontainment facilities

Location	Date	Research agent	Description	Results	Action
Vector Laboratory, Novosibirsk, Russia	May 19, 2004	Ebola virus (BSL-4)	A researcher several days earlier had suffered an accidental finger stick with a hypodermic needle that contained the Zaire strain of Ebola virus. She was working with a guinea pig model of the infection at the time (ProMED mail 2004).	The researcher died from Ebola infection on May 19.	No information available.
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (Enserink 2009)	March 12, 2009	Ebola virus	A laboratory researcher wearing protective gloves experienced a needle stick from a syringe suspected to contain Ebola virus (McGroarty 2009).	The researcher was quarantined in Hamburg University Hospital for observation. Treatment included use of the experimental Feldmann vaccine.	The worker did not become ill. No further information concerning potential seroconversion
Vector Laboratory, Novosibirsk, Russia	1990	Marburg virus (BSL-4)	One worker was infected with Marburg virus. Details are not available (ProMED mail 2004)	The infection was not fatal.	No information available.
Vector Laboratory, Novosibirsk, Russia	1988	Marburg virus (BSL-4)	One worker contracted Marburg virus infection. Details are not available (ProMED mail 2004).	The infection was fatal.	No information available.

D.1.3.4 BSL-4 Biocontainment Facilities: United States

Table D-5. Recent reported incidents involving U.S. BSL-4 biocontainment facilities

Location	Date	Research Agent	Description	Results	Action
Centers for Disease Control and Prevention, Atlanta, Georgia Bldg 18	June 8, 2007		A lightning strike knocked out electricity to BSL-3 and unoccupied BSL-4 biocontainment areas. Circuit breakers that should have remained engaged were tripped. (Young 2008; United States Government Accountability Office 2009)	Backup generators failed to start. Negative directional airflow was not maintained. A battery-powered system provided power to lights and doors for 15-20 minutes.	No exposures or infections were reported. Changes to the design of the backup system were discussed and damage to the lightning protection equipment was repaired. CDC has declined to release documents relating to the incident.
University of Wisconsin-Madison, Wisconsin	July 28, 2006	Ebola virus	The Madison IBC sought NIH guidance on whether work they had been conducting during the past year on full length cDNA made from the Ebola virus RNA genome could be continued under BSL-2 rather than BSL-3 biocontainment (Whitney 2006; Associated Press 2006; Hermes 2007).	NIH advised that all work during the 2005-2006 period should have been conducted under BSL-4 biocontainment as specified under Section III-D-2 of NIH guidelines for Research Involving Recombinant DNA Molecules.	The research was halted at Madison and moved to the National Microbiology Laboratory, Winnipeg, Canada, a BSL-4 biocontainment facility.
USAMRIID, Fort Detrick, Frederick, Maryland	February 10, 2004	Ebola virus	A civilian scientist grazed her hand with a hypodermic needle that had been used to inject antibodies into Ebola virus-infected mice (Dishneau 2004; Subcommittee on Oversight and Investigations 2007).	Scientist was sequestered in the biosafety biocontainment care suite until it could be concluded that no infection ensued.	No further information available.
Centers for Disease Control and Prevention, Division of Vector Borne Infectious Diseases, Fort Collins, Colorado	January 2004	Russian spring-summer encephalitis virus	A worker found three broken vials of the virus. Wearing only a laboratory coat and gloves, the worker used tweezers to remove broken glass and then moved the materials to another container (Margasak 2007).	No further information available.	No further information available.

D.1.3.5 BSL-3 Biocontainment Facilities: International

Table D-6. Recent reported incidents involving international BSL-3 biocontainment facilities

Location	Date	Research Agent(s)	Description	Result	Actions
Agence Francaise de Securite Sanitaire des Aliments, Maisons Alfort, France	March 26, 2009	*Bacillus anthracis*	Five technicians potentially were exposed to *B. anthracis*.as a result of a protocol violation. Cultivated *B. anthracis* cells routinely are killed in the BSL-3 area and, after confirmation of death, are moved to the BSL-2 area. In this incident, the cells were moved to the BSL-2 area before confirmatory tests were completed. Test results later showed the cells had not been killed (Nasdala 2009).	Cell suspensions had been handled within a BSC located in the BSL-2 area but, as a precaution, all workers were given prophylactic antibiotic treatment.	No further information available
The National Institute of Virology in Beijing, China	February and April, 2004	Severe acute respiratory syndrome corona virus (SARS-CoV)	Two researchers working with SARS were sickened and diagnosed with the disease 2 weeks apart in April. The identity of these infections was not recognized until the mother of one of the workers sickened as well (World Health Organization 2004).	The mother died and six other persons in contact with the two individuals became infected.	An intense lab investigation revealed that two other workers had experienced SARS-compatible illnesses in Feb. 2004 and were found to have the antibodies of the etiologic SARS-CoV. The investigation also concluded that the infections stemmed from handling of viral material, in a non-BSL-3 laboratory, that had been inadequately inactivated in the facility's BSL-3 laboratory. Efficacy of the inactivation had not been tested prior to leaving the BSL-3 laboratory.
The National Defense University in Taipei, Taiwan	December 10, 2003	SARS-CoV	On Dec. 6, 2003, a senior research scientist working with SARS in a Class III BSC cleaned up waste fluid that leaked from a tightly docked transfer chamber connected to the main cabinet. From the main cabinet he sprayed alcohol into the chamber, waited 10 minutes, opened the chamber to spray more and finally physically cleaned it up. The next day	On Dec. 10, 2003, he noted fever and fatigue, which progressed into a dry cough and severe myalgia. He was hospitalized Dec. 16, and experienced moderately severe clinical illness.	Contacts, especially plane passengers, were monitored or quarantined; no secondary infections occurred. An investigation of the lab revealed that SARS-CoV nucleic acid was on the handle of an alcohol bottle in the transfer chamber and on the light switch in the Class III cabinet

Location	Date	Research Agent(s)	Description	Result	Actions
			he attended a SARS meeting in Singapore (Normile 2004).		
A BSL-3 lab at an Institute, Singapore	August 26, 2003	West Nile virus (WNV) and SARS-CoV	A graduate student working on a virulent recent New York strain of WNV became sick with fever and myalgia after making several passages of the new virus in Vero E6 cells also used to grow SARS-CoV. The student had minimal training and help from an Institute technician (Lim 2004).	On Sept. 3, he was admitted to the hospital with a dry cough and signs of pulmonary inflammation. He was transferred to isolation and developed a moderately severe evolution of the disease. The technician was not infected.	Surveillance and quarantine was maintained on several dozen contacts, but no secondary infections occurred. An investigation of the lab proved that the WNV was contaminated with the SARS-CoV.

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D.1.3.6 BSL-3 Biocontainment Facilities: United States

Table D-7. Recent reported incidents involving U.S. BSL-3 laboratory facilities[a]

Location	Date	Research agent	Description	Results	Action
University of Wisconsin – Madison	October 6, 2010	Influenza A/Brevig Mission/1918H1N1	The intake HEPA filter fell off of the PAPR unit during use and could have been loose for a prolonged period of time. The worker reattached the filter and continued working (NIH 2010).	Respiratory protection was not provided by the PAPR for a short time. There is no mention of whether gloves were changed before handling the filter.	Oseltamivir prophylaxis was initiated. Respiratory specimens collected at 24, 36, and 72 hours were negative (PCR). SOPs were modified to include verification of PAPR HEPA filter before entry into biocontainment areas.
Medical College of Wisconsin, Milwaukee	July 28, 2010	*Mycobacterium tuberculosis* (recombinant strain)	Power failure of a PAPR unit occurred, apparently as a result of its on/off switch unknowingly being pressed against the hard surface of a chair back. Power loss was noticed after a few seconds; power was restored by pressing the on/off switch (NIH 2010).	Respiratory protection was not provided by the PAPR for a short time. Incident details would suggest no exposure occurred.	The worker was evaluated by proper authorities. The manufacture was notified of the deficiency in equipment design. SOP was modified to require placement of PAPR motor unit to the side of the belt when using a chair.
National Institutes of Health	July 18, 2010	Highly pathogenic avian influenza virus	An infected mouse escaped from a BSL-3 facility and later was captured in a BSL-2 facility (National Institutes of Health Office of Biotechnology Activities 2010).	Investigation found inconsistent facility entry logs for the primary animal technician. Review of video surveillance for 3 days prior showed the technician used no personal protective equipment on at least one occasion when entering the BSL-3 facility.	Disinfection of the BSL-2 area was undertaken. The worker who captured the mouse was offered prophylaxis using Tamiflu. Other workers were medically screened. Standard operating procedures were revised and personnel were retrained. The animal care technician was

Location	Date	Research agent	Description	Results	Action
					relieved of duty.
University of Kentucky, Lexington	April 12, 2010	*Yersinia pestis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Colorado State University, Fort Collins	April 2, 2010	Human immunodeficiency virus	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Ch'ldren's Hospital Boston, Massachusetts	May 26, 2010	*Mycobacterium tuberculosis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Virginia, Charlottesville	March 8, 2010	*Francisella tularensis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.

Location	Date	Research agent	Description	Results	Action
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	February 20, 2010	*Bacillus anthracis*	The FBI closed its case files on the 2001 theft and deliberate release of *B. anthracis* spores, concluding that a USAMRIID scientist had acted alone (Brook et al. 2001; Cymet and Kerkvliet 2004; Perez 2010).	At least 22 people were infected and 5 were killed.	No further information is available
The University of North Carolina at Chapel Hill	January 27, 2010	SARS coronavirus	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
East Carolina University, Greenville, North Carolina	January 26, 2010	*Brucella abortus*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Texas at San Antonio	January 14, 2010	*Coccidioides* sp.	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	December 4, 2009	*Francisella tularensis*	A USAMRIID military scientist was reported to have been diagnosed with tularemia, as a result of her work with *F. tularensis* (USAMRIID United States Army Medical Research Institute of Infectious Diseases 2009, 2009; Bhattacharjee 2009).	Oral antibiotics were started on an outpatient basis, followed by inpatient administration of intravenous antibiotics. Recovery was expected	No further information available

Location	Date	Research agent	Description	Results	Action
Indiana University, Bloomington	November 18, 2009	*Yersinia pestis*	Mouse dander was found outside of a mouse cage that was to be completely sealed to contain potentially infectious bioaerosols associated with infected mice (Leonard 2009).	Seven researches were given antibiotic treatment as a precaution.	Work in the BSL-3 lab was halted pending an evaluation. No further information available.
University of Massachusetts Medical School	November 5, 2009	*Mycobacterium tuberculosis*	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Wisconsin at Madison	October 29, 2009	Influenza virus (not further specified)	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Novartis Pharmaceuticals Corporation	October 28, 2009	Venezuelan equine encephalitis virus	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Boston University,Massachusetts	October 25, 2009	*Neisseria meningitidis*	A microbiology researcher at BU sought medical attention for laboratory-acquired bacteremia and meningitis. Molecular typing determined the infecting strain was the same strain he had been working with. Work with *N. meningitis* is conducted at BSL-2 using BSL-3 precautions (respiratory protection provided by Class II	Intravenous antibiotics were administered and the researcher recovered fully.	University experts determined the researcher did not consistently wear appropriate personal protective equipment, and did not consistently follow appropriate safe microbiological practices. It was surmised that the researcher touched his

Location	Date	Research agent	Description	Results	Action
			BSC) (Boston University 2009; Smith 2009, 2009)		gloved hand to his face while working with the bacterium (Ad Hoc Committee 2010). All laboratory members repeated laboratory safety re-training and organism-specific re-training.
The University of Texas Medical Branch at Galveston	September 21, 2009	Rift Valley fever virus	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Wisconsin at Madison	September 11, 2009	*Brucella canis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
University of California, Berkeley	June 26, 2009	*Mycobacterium tuberculosis*	A potential exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
University of Kentucky	June 18, 2009	*Yersinia pestis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.

Location	Date	Research agent	Description	Results	Action
Texas A&M University, College Station	March 5, 2009	*Brucella melitensis*	A breach of containment was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
BD Biosciences, San Jose, California	January 2009	NA	ULPA filters used in the manufacture of aerosol biocontainment enclosures for flow cytometers were found to be faulty. Eight of 10 filters from 3 different lots tested at one site (Yale University, Jan. 22, 2009) failed to meet performance specifications. Seven of 9 units tested at a second site (Duke University, Jan 26, 2009) failed to meet performance criteria. (BD Biosciences 2009; Fontes 2009; Aldermann 2009)	Safety officials of the Universities alerted American Biological Safety Association Listserv subscribers to the issue. There was no mention of possible exposures. Duke U. protocols required use of a walk-in biosafety cabinet as an additional biocontainment feature.	BD Biosciences issued a Field Action Notice asking customers to cease use of the enclosures and await instructions regarding a filter replacement program.
The University of Iowa	November 5, 2008	*Francisella tularensis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Texas at San Antonio	October 17, 2008	*Coccidioides* sp.	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.

Location	Date	Research agent	Description	Results	Action
University of Pittsburgh,Pennsylvania	September 24, 2008	*Mycobacterium tuberculosis*	An animal care technician was bitten by a macaque that was part of a vaccine study of tuberculosis (Templeton 2008).	The technician suffered bone, tendon and nerve damage as well as multiple infections (apparently not related to the research agent).	The University was fined by Occupational Safety and Health Administration (OSHA) for failing to provide training and safety equipment for lab personnel.
The University of Texas at San Antonio	August 21, 2008	*Francisella tularensis*	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Texas Health Sciences Center (not further specified)	July 25, 2008	*Bacillus anthracis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Centers for Disease Control and Prevention, Atlanta, Georgia Bldg 17	July 11, 2008	Program pathogens include influenza, extensively drug-resistant *Mycobacterium tuberculosis*, and rabies	A bird caused a Georgia Power transformer to fail, knocking out electricity to BSL-3 biocontainment areas (Young 2008, 2008, 2008).	Backup generators failed to start, leaving the labs without main electrical power for 75 minutes. CDC personnel did not attempt to override and start the backup generators. Negative directional airflow was not maintained.	No exposures or infections were reported. Backup failure was determined to be due to removal of two generators from service for upgrades. Their absence caused a power fluctuation when main power was lost, resulting in shutdown of the entire backup generator system. The system was tested subsequently on July 21st; CDC officials would not release results of the test. No further information was

Location	Date	Research agent	Description	Results	Action
					available.
The University of North Carolina at Chapel Hill	June 31, 2008	SARS virus	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Wisconsin at Madison	May 22, 2008	*Brucella* sp.	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Texas Health Sciences Center (not further specified)	May 7, 2008	*Bacillus anthracis*	A potential exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of North Carolina at Chapel Hill	April 7, 2008	*Mycobacterium tuberculosis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.

Location	Date	Research agent	Description	Results	Action
St Louis University	April 4, 2008	Yellow fever virus	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Wisconsin at Madison	January 2008	*Brucella* sp.	The University reported conduct of unauthorized experiments with a select agent to the U.S. Dept. of Health and Human Services.	HHS undertook investigation under authority of the Select Agent Program.	In May 2010, the university was fined $40,000 and the Principal Investigator was suspended from related work for 5 years.
Ohio State University	September 27, 2007	*Francisella* sp.	Equipment failure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Texas Health Sciences Center (not further specified)	September 21, 2007	*Bacillus anthracis*	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Lovelace Respiratory Research Institute, Albuquerque, New Mexico	August 28, 2007	*Yersinia pestis*	An employee potentially was exposed by being stuck by a broken scalpel blade (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.

Location	Date	Research agent	Description	Results	Action
University of Mississippi Medical Center, Jackson	August 11, 2007	*Bacillus anthracis*	A graduate student accidentally broke a flask containing *Bacillus anthracis* cells which spilled onto floor (Subcommittee on Oversight and Investigations 2007; Center for Infectious Disease Research & Policy 2007)	The student was exposed to *Bacillus anthracis*.	Procedures for spill biocontainment and decontamination were followed. The student received prophylactic antibacterial therapy.
St. Louis University, Missouri	August 7, 2007	Monkeypox virus	A needle stick with syringe containing the virus was reported (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
University of California-Davis	July 27, 2007	*Brucella abortus*	A needle stick with syringe containing the bacterium was reported (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
Battelle Memorial Institute, Columbus, Ohio	July 23, 2007	Avian influenza virus	A needle stick with syringe containing the virus was reported (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	The matter was referred to USDA APHIS. No further information available.
Bioqual, Inc., Rockville, Maryland	July 23, 2007	Avian influenza virus	An employee was bitten by a ferret inoculated with the virus (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	The matter was referred to USDA APHIS. No further information available.

Location	Date	Research agent	Description	Results	Action
University of New Mexico	July 23, 2007	*Francisella tularensis*	Potential exposure: an employee was bitten by a rat that had been inoculated with the bacterium (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
University of South Alabama	July 19, 2007	*Rickettsia prowazekii*	Potential exposure: an employee dropped a culture vessel of the bacterium, splashing it onto lab coat, pants, and shoes (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
The University of Chicago	July 16, 2007	*Bacillus anthracis*	An employee potentially was exposed via needle stick (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
Lovelace Respiratory Research Institute, Albuquerque, New Mexico	July 10, 2007	*Bacillus anthracis*	A potential loss of containment was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
National Animal Disease Center, Ames, Iowa	July 2, 2007	*Brucella suis*	Potential exposure: a needle stick with syringe containing *B. suis* occurred during necropsy (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.

Location	Date	Research agent	Description	Results	Action
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	June 29, 2007	*Bacillus anthracis*	Potential exposure: environmental surveillance indicated presence of the bacterium on a freezer handle, light switch, and shoes in the hot side of the change room (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
Texas A&M University, College Station	June 26, 2007	*Coxiella burnetii*	Potential exposure to the bacterium was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Centers for Disease Control and Prevention, Atlanta, Georgia Bldg 18	June 8, 2007		A lightning strike knocked out electricity to BSL-3 and unoccupied BSL-4 biocontainment areas. Circuit breakers that should have remained engaged were tripped (Young 2008; Young 2007; Young 2007, 2007, 2007; United States Government Accountability Office 2009).	Backup generators failed to start. Negative directional airflow was not maintained. A battery-powered system provided power to lights and doors for 15-20 minutes. No exposures or infections subsequently were reported.	Changes to the design of the backup system were discussed and damage to the lightning protection equipment was repaired. It was later determined that a ground cable had been cut some time earlier, and prevented the circuit breakers from remaining engaged. CDC has declined to release documents relating to the incident.
National Animal Disease Center, Ames, Iowa	June 4, 2007	*Brucella abortus*	Potential exposure: an employee was scratched by a broken rib during necropsy (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.

Location	Date	Research agent	Description	Results	Action
Mayo Clinic	May 17, 2007	*Coccidioides immitis*	Package shipped via commercial carrier was lost in transit (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
The University of Iowa	May 10, 2007	*Francisella tularensis*	An employee potentially was exposed while working with the bacterium without using BSL-3 biocontainment precautions (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
University of Kentucky, Lexington	May 9, 2007	*Yersinia pestis*	A potential exposure due to a leaking autoclave bag was reported (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
University of North Carolina at Chapel Hill	May 7, 2007	Venezuelan equine encephalitis virus	Potential exposure: a dirty cage from infected mice was dropped inside the BSL-3 suite (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
University of Texas Houston Health Science Center	May 7, 2007	*Bacillus anthracis*	Tube leakage occurred inside a centrifuge used for concentration of *Bacillus anthracis* cells(University of Texas 2007; Subcommittee on Oversight and Investigations 2007) .	Four people potentially were exposed to *Bacillus anthracis*.	Prophylaxis was refused. No infections resulted. Procedures were modified to require that centrifuge buckets be opened only within a BSC, and inspected and decontaminated after each use.

Location	Date	Research agent	Description	Results	Action
The University of North Carolina at Chapel Hill	May 3, 2007	SARS virus	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
Centers for Disease Control and Prevention, Atlanta, Georgia Bldg 18	May 2007	*Coxiella burnetii*	Malfunction of the HVAC system pulled potentially contaminated air out of the BSL-3 biocontainment area and into a clean hallway (Subcommittee on Oversight and Investigations 2007).	Nine workers were tested for possible exposure to the bacterium and no infections were diagnosed.	The HVAC system was brought back into compliance. Duct tape was used to seal the door and remained in place as of June 2008. A self sealing door will be installed by April 2009.
University of Kentucky, Lexington	April 30, 2007	*Yersinia pestis*	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH 2010).	No further information was available.	No further information was available.
The University of Texas at San Antonio	April 20, 2007	*Francisella tularensis*	Personnel entered BSL-3 biocontainment area without wearing required protective equipment (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
Lovelace Respiratory Research Institute, Albuquerque, New Mexico	April 11, 2007	*Yersinia pestis*	An experimentally infected monkey scratched the hand of a laboratory worker (Subcommittee on Oversight and Investigations 2007; The Associated Press 2009).	The skin was broken, potentially infecting the worker.	The worker received medical treatment including antibiotic therapy.

Location	Date	Research agent	Description	Results	Action
University of Virginia, Charlottesville	April 11, 2007	*Francisella tularensis*	Potential exposure from needle stick with syringe that had been used on mice inoculated with the bacterium (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
Boston University, Massachusetts	March 20, 2007	*Francisella tularensis*	Autoclaved biological waste bags could not be removed from the autoclave due to a malfunctioning door. The lack of a service contract caused a 16 day delay in repair. The door was repaired on March 16 but the bags were not removed due to miscommunication (Office of General Services 2007).	The bags combusted in the autoclave sometime during the next 4 days and, upon opening the autoclave door, smoke alarms activated.	Investigation led to numerous recommendations to prevent similar occurrences in the future.
National Animal Disease Center, Ames, Iowa	January 11, 2007	*Brucella suis*	Potential exposure: an employee was bitten by a pig infected with *B. suis* (Subcommittee on Oversight and Investigations 2007).	The worker potentially was exposed to *Brucella* sp. APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
National Animal Disease Center, Ames, Iowa	December 2006	*Brucella suis*	The Center reported leaks of contaminated waste three times in November and December. One worker cut his finger while preparing a pipe for repairs (The Associated Press 2009; Subcommittee on Oversight and Investigations 2007).	The worker potentially was exposed to *Brucella suis*. APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.

Location	Date	Research agent	Description	Results	Action
Texas A & MI University,College Station	December 21, 2006	*Coxiella burnetii*	A mouse infected with *Coxiella burnetii* was found to be unaccounted for, and believed to be missing (Texas A&M University 2006, 2007).	A report to CDC was filed on Dec. 22.	The reason for the discrepancy is unknown.
Armed Forces Institute of Pathology, Washington, D.C.	November 7, 2006	*Bacillus anthracis*	Viable select agent (*B. anthracis*) was taken from BSL-3 biocontainment to BSL-2 biocontainment (Subcommittee on Oversight and Investigations 2007)	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
CDC, Division of Vector Borne Infectious Diseases, Fort Collins, Colorado	November 2006	*Yersinia pestis*	Two culture plates of the bacterium were dropped onto the floor; the lid came off one of the plates (Subcommittee on Oversight and Investigations 2007).	Concluded by CDC to have comprised an unlikely exposure. No further information available.	No further information available.
Lovelace Research Institute, Albuquerque, New Mexico	September 25, 2006	*Yersinia pestis*	An employee was bitten on the hand by an infected monkey. The skin appeared to be broken in two or three places. (Subcommittee on Oversight and Investigations 2007; The Associated Press 2009)	The worker was referred to a physician.	No further information available. Animals infected with *Y pestis* are handled either at ABSL-2 or ABSL-3 depending on the circumstances of the experiment.
University of Virginia, Charlottesville	August 11, 2006	*Francisella tularensis*	Laboratory workers potentially were exposed to the bacterium when a tube of liquid culture in a shaking incubator appeared to be cracked (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.

Location	Date	Research agent	Description	Results	Action
University of Wisconsin – Madison	August 7, 2006	*Brucella melitensis*	Potential exposure to two people when the cap of a liquid culture containing the bacterium came off in a shaking incubator (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
University of Kentucky, Lexington	May 25, 2006	*Yersinia pestis*	A laboratory worker potentially was exposed when opening an autoclave bag, containing the bacterium, that had not been decontaminated (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
University of California-San Diego	May 16, 2006	*Brucella abortus*	Potential exposure: nine individuals worked with the bacterium without using BSL-3 biocontainment precautions (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	Investigation was ongoing. No further information available.
University of Texas at Austin, Texas	April 12, 2006	Recombinant Influenza A H3N2 virus, containing genes from strain H5N1 (bird flu).	A centrifuge secondary container lid broke during centrifugation of virus, causing the rotor to become unbalanced. The researcher noted loss of volume in one viral tube and, suspecting viral leakage, undertook decontamination of centrifuge, centrifuge tube, work area, adjacent equipment and himself (University of Texas at Austin 2006).	A decision was made to treat the researcher empirically using Tamiflu. Secondary decontamination of lab room was undertaken the following day.	Responsible officials maintain that it is unclear whether a leakage of virus occurred. However, their records show that proper decontamination protocol was not followed for the suspected leak.

Location	Date	Research agent	Description	Results	Action
Texas A&M University, College Station	April, 2006	*Coxiella burnetii*	Previously undiagnosed exposures to *C. burnetii* are diagnosed in three laboratory workers by serologic testing (Centers for Disease Control and Prevention 2007, 2007). As many as ten workers might have been infected (further information is unavailable (Subcommittee on Oversight and Investigations 2007).	Responsible officials did not report these infections to federal authorities as required by federal law.	CDC issued a cease and desist order to TAMU on April 20, 2007 that was expanded on June 30 to include work with all Select Agents. Other serious violations were found during a site visit inspection in July 2007.
Texas A&M University, College Station, Texas	February, 2006	*Brucella melitensis*	A researcher contracted undiagnosed brucellosis during improper disinfection of aerosolization chamber. She later required prolonged administration of intravenous and oral antibiotics (Centers for Disease Control and Prevention 2007, 2007; Subcommittee on Oversight and Investigations 2007).	Responsible officials did not report this infection to federal authorities, as required by federal law, until April 11, 2007 in response to an inquiry from the Sunshine Project. (Texas A&M University 2007)	CDC issued a cease and desist order to TAMU on April 20, 2007 that was expanded on June 30 to include work with all Select Agents. Other serious violations were found during a site visit inspection in July 2007.
Walter Reed Army Institute of Research-Naval Medical Research Center, Silver Spring, Maryland	January 2, 2006	*Brucella abortus, B. melitensis, B. suis, Yersinia pestis*	A water supply line burst inside the laboratory causing a flood. Water samples were culture-negative (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
Yeshiva University New York, New York	December 2005	*Mycobacterium tuberculosis*	Two lab workers converted to skin-test positive status, indicating an infection with *M. tuberculosis.* One of these worked in both the BSL-2 and BSL-3 labs, whereas the second worked only in the BSL-2 lab (Yeshiva University 2006).	Infections were sub-clinical. Prophylactic antibiotic treatment was administered.	All other workers were given TB skin tests. Neither of the two positive employees worked with the TB aerosolization animal chamber. No accidents had occurred in the labs.

Location	Date	Research agent	Description	Results	Action
Lawrence Livermore National Laboratory, Livermore, California	September, 2005	*Bacillus anthracis*	1,025 vials were shipped to a Palm Beach, Florida laboratory. Two of the vials were missing caps and a third vial had a loosened cap. A subsequent shipment to a second laboratory contained an incorrect number of vials (Van Derbeken 2007).	Two workers possibly were exposed to *Bacillus anthracis.*	The workers were prophylactically treated with antibiotics. LLNL was fined $450,000 for the violations.
Public Health Research Institute, New Jersey Medical School, Newark	September, 2005	*Yersinia pestis*	Three mice experimentally infected with *Yersinia pestis* went missing from a biocontainment lab (ProMED mail 2005).	No one was infected by the mice. If they had escaped there would have been no public threat according to spokesmen.	An investigation by the CDC and the FBI ruled out theft and concluded that lab error, mouse cannibalism and unauthorized removal all are possibilities.
Medical University of Ohio	September 30, 2005	*Coccidioides immitis*	A student potentially was exposed to infectious aerosols from a broken vial inside a centrifuge (Subcommittee on Oversight and Investigations 2007; Medical University of Ohio 2004 and 2005,).	Medical evaluation was provided.	The BSL-3 facility director, who was in charge of the *C. immitis* research, resigned as Director and was replaced as Animal and Biosafety Protocol Principal Investigator and as advisor to the student (see 2004 *C. immitis* incident elsewhere in table).
University of Chicago Chicago, Illinois	July/August 2005	*Bacillus anthracis* (Subcommittee on Oversight and Investigations 2007)	Percutaneous trauma from an instrument (presumably a hypodermic syringe) that had been used on an infected animal. (University of Chicago IBC 2005)	Possible infection with select agent.	Medical personnel administered antibiotic prophylaxis. Laboratory procedures were reviewed and revised as needed.

Location	Date	Research agent	Description	Results	Action
Walter Reed Army Institute of Research-Naval Medical Research Center, Silver Spring, Maryland	May 2, 2005	*Yesinia pestis*	A laboratory worker sliced through two pair of gloves while handling a rat carcass infected with *Y. pestis* (Subcommittee on Oversight and Investigations 2007).	The worker was sent to a medical emergency room, which released her and asked her to return for a follow-up visit.	No further information available.
Walter Reed Army Institute of Research-Naval Medical Research Center, Silver Spring, Maryland	April 27, 2005	*Yesinia pestis*	A laboratory worker potentially was exposed by a culture plate that was dropped outside of the biological safety cabinet (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available.
The University of North Carolina at Chapel Hill	March 19, 2005	*Mycobacterium tuberculosis* (recombinant)	An exhaust fan servicing two BSC and the general laboratory space failed. Audible alarms on the cabinets and air pressure monitors had been turned off (The University of North Carolina at Chapel Hill Institutional Biosafety Committee 2005).	Loss of primary and secondary biocontainment occurred.	A review of all BSL-3 laboratories was scheduled to identify renovation needs to ensure compliance with current design standards.
University of Iowa, Iowa City	March 11, 2005	*Francisella novicida*	Investigators conducted research on the bacterium without obtaining IBC approval (NIH 2010).	No further information available.	No further information available.
Medical University of Ohio, Ohio	December 8, 2004	*Coccidioides immitis*	An infection, possibly laboratory-acquired, was diagnosed. However, the employee previously resided in an endemic area and previously had worked with *C. immitis* in another laboratory. Therefore, it could not be proved when and how the infection began (Subcommittee	The infected employee was provided with medical treatment and re-assigned to other duties.	Safety policies were revised, video surveillance was installed, serologic monitoring of staff was reviewed (see 2005 *C. immitis* incident elsewhere in this table).

Location	Date	Research agent	Description	Results	Action
			on Oversight and Investigations 2007).		
Cincinnati, Ohio	October, 2004	Non-Contemporary Human Influenza (H2N2)	H2N2 from the 1957-1958 flu pandemic was accidentally distributed to 2,750 labs in the U.S., along with 3,747 labs in 18 different countries, by the College of American Pathologists (CAP). The error was discovered in March by a participating lab in Canada. This virus requires Enhanced BSL-3 biocontainment precautions (ProMED mail 2005; World Health Organization 2005; Center for Infectious Disease Research and Policy 2005).	CAP was requested by the U.S. government to notify all participating labs to destroy the virus, to investigate, and to report to national health authorities any respiratory infection in lab workers.	CAP sent out notifications on April 8 and 12, 2004. No infections were reported as of April 12, 2005.
University of North Carolina at Chapel Hill	September 14, 2004	Venezuelan equine encephalitis virus.	A laboratory worker was found to have a high rise in anti-VEE virus titer. No occupational exposure was confirmed (Subcommittee on Oversight and Investigations 2007).	APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	No further information available
University of Illinois at Chicago	September, 2004	Not specified (redacted by IBC)	Both doors of the double door biocontainment entryway were propped open by laboratory staff while experiments were in progress (University of Illinois at Chicago 2003-2006).	Infectious materials were not being handled at the time.	Principal Investigator was counseled and warned. Staff was re-trained and tested.

Location	Date	Research agent	Description	Results	Action
National Institutes of Health Bethesda, Maryland	July/August, 2004	Various	A waste treatment tank steam valve failed, resulting in severe damage to the maximum biocontainment (BSL-4) laboratory that was being used as a BLS-3 lab at the time (SARS virus research). The NIH Occupational Safety and Health Branch previously had been informed about problems with the valve, but elected to defer repairs (National Institutes of Health 2004).	No exposures resulted.	The building was closed for repairs and renovation.
Oakland, California	June 11, 2004	*Bacillus anthracis*	Children's Hospital and Research Center Southern Research Institute sent live, rather than dead, anthrax samples to researchers in Oakland. The problem was detected after 49 of the 50 research mice quickly died after inoculation with the samples (Centers for Disease Control and Prevention 2005; Miller 2004).	Seven scientists were exposed, but not infected.	No human infections were reported.
Boston University, Massachusetts	May and September, 2004	*Francisella tularensis*	Researchers were working under BSL-2 biocontainment protocol with what was believed to be a non-infectious vaccine strain of the bacterium. Later, it was determined the bacterial culture also contained the infectious wild-type strain that requires BSL-3 biocontainment precautions. Investigation was unable to determine the cause for the mixed culture .(Anonymous 2005; Barry 2005; Lawler 2005; Dalton 2005).	Two researchers became infected with *Francisella tularensis* in May and were not correctly diagnosed until a third scientist became infected with the bacterium in September.	An investigation revealed that researchers had failed to follow proper BSL-2 biocontainment protocol, and that the University failed to identify work-related illness in laboratory staff and failed to immediately report suspicious work-related illness to local and state health departments. Biosafety policies and SOPs were revised accordingly. The

Location	Date	Research agent	Description	Results	Action
					Chief of Infectious Diseases was replaced.
Georgia Public Health Laboratory, Decatur	April 2004	*Brucella* species	A laboratory worker became feverish months after handling a culture of *Brucella* sp (The Associated Press 2009).	Infection was confirmed in July by laboratory testing.	It was determined that employee had handled the culture without using proper biocontainment precautions. The employee eventually returned to work.
Oklahoma State University-Stillwater	After 2003 (not otherwise specified)	Unspecified; possibly *Borrelia burgdorferi*	An experimentally infected mouse died and later could not be accounted for (The Associated Press 2009).	Laboratory personnel suggested the dead mouse was overlooked, was in the cage during cage sterilization, and was discarded with the sterilized bedding.	No further information available.
Bioqual, Inc., Rockville, Maryland	After 2003 (not otherwise specified)	H5N1 influenza A virus	A ferret experimentally infected with H5N1 virus bit a technician on the thumb (The Associated Press 2009; Subcommittee on Oversight and Investigations 2007).	The worker was placed on home quarantine for five days and directed to wear a mask to protect others.	No further information available.
Armed Forces Institute of Pathology, Washington, D.C.	August 15, 2003	*Francisella tularensis* and *Yersinia pestis*	Lost in transit (Subcommittee on Oversight and Investigations 2007).	Investigated by FBI.	Packages discovered and incinerated in Belgium.
Infectious Disease Research, Inc., Seattle, Washington	Late 2003 – March, 2004	*Mycobacterium tuberculosis*	Three researchers became skin-test positive for tuberculosis after using a	Infections were sub-clinical. Prophylactic treatment typically is	Investigation revealed multiple faulty seals in the device, and

Location	Date	Research agent	Description	Results	Action
			newly acquired aerosolization chamber for experimental infection of animals (Washington Department of Labor and Industries 2004).	employed in such cases.	researchers were not fully familiar with proper operation of the device.
Columbus, Ohio	March 1, 2003	West Nile virus (WNV)	An improperly packaged shipment containing dry ice burst. The package was carrying frozen infected bird tissue [b] (New York Times 2003).	Workers at a Federal Express shipping building were potentially exposed to WNV.	Authorities characterized the risk of infection as low.
University of New Mexico, Albuquerque	2003	Redacted by IBC; likely was *Francisella tularensis*	Puncture of thumb with hypodermic needle harboring spores to be used in mouse infections (University of New Mexico IBC 2003) (Subcommittee on Oversight and Investigations 2007).	Worker received prophylactic treatment; no infection resulted.	It was proposed that alternative methods for mouse inoculation be considered.
Texas Tech University , Lubbock, Texas	January 2003	*Yersinia pestis*	30 vials of the bacterium were reported missing by the principal investigator (Leung 2003).	It was not possible to determine what became of the vials.	The investigator was dismissed from the University due to related matters.
United States	October, 2002	West Nile virus (WNV)	A microbiologist working under BSL-3 conditions suffered a finger puncture from a hypodermic needle harboring WNV being harvested from infected mouse brain (Centers for Disease Control and Prevention 2002).	The wound was cleansed and bandaged. Serologic testing showed evidence of acute WNV infection. Mild symptoms developed and resolved.	CDC determined that applicable handling and biocontainment protocols were followed.
United States	August, 2002	West Nile virus (WNV)	A microbiologist, working under BSL-2 conditions using a Class II BSC, lacerated a thumb with a scalpel during necropsy of a bird infected with WNV (Centers for Disease Control and Prevention 2002).	The superficial wound was cleansed and bandaged. Symptoms began 4 days post injury; medical attention was sought 7 days after injury. Infection was self-limiting and was confirmed by serologic	CDC recommends BSL-3 biocontainment measures for WNV. However, CDC does accept BSL-2 biocontainment facilities that incorporate certain elements of BSL-3 biocontainment measures.

Location	Date	Research agent	Description	Results	Action
				testing.	
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	April 20, 2002 April 1, 2002	*Bacillus anthracis*	A researcher tested positive for exposure to anthrax spores, which were also released into a locker room and adjacent hallway. U.S. Army officials reported evidence of a second accidental release of anthrax spores (Perez 2010).	No one was infected in either incident.	The first incident involved a virulent strain. Test samples connected with the second incident tested positive for the attenuated (vaccine) strain.
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	April 8, 2002	*Bacillus anthracis*	A researcher was infected by handling culture flasks that had leaked. Flasks were loosely capped and caps covered by paper, according to protocol. Bacteria had splashed onto the paper, dried, and become airborne upon handling (Rusnak, Boudreau, et al. 2004).	The exposed worker, previously vaccinated, was given a booster injection.	SOP was modified to require use of respiratory protection and filter-lined tightly screwed caps were adopted.
Texas (not otherwise specified)	March 2002	*Bacillus anthracis*	A lab worker used an incorrect disinfectant; failed to wear disposable gloves; and failed to cover a pre-existing skin defect (facial cut from shaving) (Centers for Disease Control and Prevention 2002).	Cutaneous anthrax resulted following skin exposure to a contaminated surface.	Patient was successfully treated using antibiotics. CDC reviewed proper biosafety measures with laboratory personnel.
Rocky Mountain Laboratory, Hamilton, Montana	April 2001	*Yersinia pestis*	A culture container of the bacterium fell off of the shaker during the night. Multiple laboratory workers potentially were exposed to the bacterium when discovered the next morning (Anonymous 2003; Johnson 2009).	The spill was decontaminated. No infections occurred.	No further information is available.

Location	Date	Research agent	Description	Results	Action
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	March 2000	*Burkholderia mallei*	A research microbiologist routinely failed to wear disposable gloves, and became infected. A primary care physician prescribed antibiotics without knowledge of the specific etiology (Srinivasan et al. 2001; Centers for Disease and Prevention 2000).	The patient improved but relapsed to a life-threatening condition. Culture revealed specific etiology and appropriate antibiotics resulted in cure.	A review of laboratory procedures was conducted but no further information is available.
Rocky Mountain Laboratories, Hamilton, Montana	2000	*Mycobacterium tuberculosis*	PPD skin test conversion was noted for a laboratory technician (Johnson 2009).	Source of infection suspected to be from samples sent by outside laboratories. Samples were to have been inactivated prior to receipt, but validation was uncertain.	Policies and SOPs for sampling handling were revised to assume that samples could be infectious. HVAC systems were upgraded. Air flow alarms were added to BSCs. Aerosol-containment centrifuge was added.
Rocky Mountain Laboratories, Hamilton, Montana	1998	*Chlamydia trachomatis*	Researcher was diagnosed with a lung infection soon after working with the pathogen (Johnson 2009).	Policies and SOPs for safe handling of the pathogen were found to be inadequate.	New requirements for PPE (respiratory protection), use of a BSC to open centrifuge rotors/buckets, and correct use of BSC were instituted.
Rocky Mountain Laboratories, Hamilton, Montana	1996	*Mycobacterium tuberculosis*	PPD skin test conversion was noted for a laboratory technician. Source of infection was uncertain (Johnson 2009).	No disease ensued.	Equipment and procedures were revised, including addition of aerosol-containment rotor, upgraded respiratory protective equipment, semiannual chest xrays.
Yale University, New Haven, Connecticut	November 6, 1995	Japanese encephalitis virus	Researchers failed to obtain IBC approval for their work. This was reported to NIH under reporting requirements of the NIH Guidelines for	No further information was available.	No further information was available.

Location	Date	Research agent	Description	Results	Action
			Research Involving Recombinant DNA Molecules (NIH 2010).		
University of Wisconsin – Madison	August 27, 1994	H1N1 influenza A virus (swine)	Two people, working in separate ABSL-3 rooms, each became symptomatic and were diagnosed with influenza 1.5 days after collecting nasal specimens from experimentally infected pigs (Wentworth et al. 1997).	Genetic analyses determined the workers had become infected with the same virus used to infect the pigs.	Investigation determined that an incorrect mask had been supplied to the workers for 1 day, and it is possible this error facilitated infection of personnel.
Yale University, New Haven, Connecticut	August 8, 1994	Sabia virus[c]	A research virologist discovered a leaking vessel upon opening a sealed aerosol biocontainment centrifuge rotor outside of a BSC. Personal respiratory protective equipment consisted of a surgical mask. The incident was not reported (Altman 1994).	Symptoms began 8 days afterward. Two days later the infection was correctly diagnosed.	Antiviral therapy cured the nearly fatal infection. Two external committees strongly criticized the researcher and institution. The university agreed to implement all recommendations. No secondary infections were found among the 142 subsequent human contacts (ref needed)
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland	1989-2002	various	A retrospective review of institute records showed that 67 people were evaluated for likely or highly likely exposure to infectious agents (Rusnak, Kortepeter, et al. 2004)	3 LAI from BSL-3 pathogens were confirmed in 3 cases: (Chikungunya virus, Venezuelan equine encephalitis virus, and *Coxiella burnetii*); LAI was likely in a 4th case (*Yersinia pestis*); post-flooding contamination of a lab with *B. anthracis* was detected.	NA (retrospective review)
Various	1990-1994	*Mycobacterium tuberculosis*	A retrospective survey was sent to 56 state and territorial public health laboratories to	Seven laboratory workers were determined to have	CDC guidelines for preventing LAI tuberculosis, and

Location	Date	Research agent	Description	Results	Action
			determine, by skin tests results, the frequency of probable laboratory-acquired tuberculosis.	laboratory-acquired infections (Kao et al. 1997).	recommendations for regular skin testing of laboratory employees, were re-emphasized.
Royal Oak, Michigan	May-September 1988	*Brucella melitensis*	A laboratory worker thawed a frozen vial of bacterial suspension and inoculated a plate culture on the open bench top instead of within a BSC (Staszkiewicz et al. 1991)	Eight laboratory workers became infected, one being asymptomatic. The outbreak was most consistent with airborne spread.	The 7 symptomatic workers were given antibiotic therapy. One relapsed and required alternative therapy. Enhancements to laboratory SOPs were recommended by the Department of Epidemiology and the Infectious Diseases Division.

Notes:

a Some incidents reported here pertain to BSL-2 facilities, either because they involved facilities at Boston University (and therefore are relevant to this RA) or because they involved Select Agent pathogens that can be handled using either BSL-2 or BSL-3 biocontainment precautions, depending on particular circumstances of the work involved.

b West Nile virus biocontainment recommendations are BSL-3 for dissection of field-collected dead birds. This incident is included here because of public concern surrounding transportation of samples to and from BSL-3 and BSL-4 biocontainment facilities.

c Sabia virus, currently a BSL-4 virus, was not assigned to a biosafety level at the time of the described incident.

D.2 Part II. The Johnson Reports

D.2.1 Biosafety at National Institute of Allergy and Infectious Disease: 1982–2003, Karl M. Johnson, M.D., October 15, 2003

The NIH and CDC first promulgated National Guidelines for safe work with a broad range of infectious organisms in 1980. Four levels of physical containment and work practices were designated for agents with different virulence for humans and relative risk of infection from aerosols induced by laboratory manipulation. BSL-3 is reserved for organisms that cause serious disease and that are known to be infectious via the respiratory route. Examples include *Mycobacterium tuberculosis* and West Nile virus. For such agents, all procedures must be carried out in biosafety cabinets (BSCs) fitted with high-efficiency filters (HEPA). Centrifuges require sealed rotors so that aerosols that ensue if a tube breaks during spinning runs will be contained until the rotor is opened under the BSC. Air in such laboratories is maintained at negative pressure relative to hallways and cannot be blended with air to other laboratories and offices to prevent potential infection to others in the building. More and more, such laboratories also have HEPA filters on laboratory room exhaust.

In addition to agents known to be aerosol transmitted, microbiological science continues to confront newly discovered viruses and bacteria for which aerosol infectiousness is uncertain. The NIAID has adopted a policy for such organisms that stipulates BSL-3 equipment and practices in BSL-2 laboratories with negative pressure. Work with the Human Immunodeficiency Virus (HIV) in the early 1980s led to adoption of that strategy for HIV and its close animal virus relatives, a policy that continues. Similar standards were initiated for work with hepatitis viruses at request of senior investigators, largely because new agents that cause hepatitis continue to emerge and little is known in early years regarding their infectiousness as aerosols.

This review is limited to work done during the past two decades by scientists at intramural laboratories of NIAID on the Bethesda campus, at a neighboring facility in Rockville, Maryland, and at the institute's Rocky Mountain Laboratories in Hamilton, Montana. Senior scientists were interviewed to ascertain agents studied, the variety of research programs that evolved over two decades, animals employed, if any, laboratory space, daily number of

workers in the laboratories, and specific histories of laboratory accidents and consequences. Problems with function of facilities also were solicited and recorded.

Independent records of reported laboratory accidents that might expose workers to infection were reviewed. During the past 21 years, all such accidents were to be reported quickly to the NIH Occupational Medical Service (OMS) for epidemiologic and medical evaluation as well as immediate prophylactic treatment if indicated. Invasive wounds in course of laboratory work and clinical care of persons with chronic HIV infection are of continuing concern. The OMS is now able to provide antiviral therapy within two hours of an accident on a 7-day/24-hour basis when circumstances indicate the need for therapy.

Intake records of all accidents on the NIH campus were initially paper documents. Copies were forwarded to the Occupational Safety and Health Branch (OSHB) in the Director's office for to follow up circumstances of an accident and for remedial action when indicated. In addition to such immediate reaction to accidents and facility emergencies, the OSHB has developed standardized protocols for periodic review of all laboratories for compliance with NIH safety practices. Laboratories at BSL-3 level are reviewed at 6-month intervals; all others are reviewed annually. For the past decade, all records are computerized and electronic copies go from OMS to OSHB instantly. Records for this 21-year interval were cross-checked for details by staff of both offices, together with specific scientist memory, in constructing the biosafety record for NIAID since 1982. Records for the Rocky Mountain Laboratories were reviewed with biosafety and scientific staff at that facility.

The detailed report is organized by Laboratory within the NIAID Division of Intramural Research. Agents, research agendas, containment levels, animal use, location and space for laboratories are presented in tabular form, together with histories of laboratory accidents and of facility problems that have affected work in those laboratories.

By any measure, the safety record at intramural NIAID laboratories, where work is done with the institute's most pathogenic agents, is outstanding. No agent has escaped from any laboratory to cause infection in adjacent civilian communities. Indeed, this record stretches to almost 70

years at RML where several agents now on the national Select List have been studied for decades. If one takes the number of 8-hour person days estimated by senior research staff during direct conversations and translates these into 2,000 person hours per year in exposure to microbial organisms, impressive numbers emerge as shown in the following table.

Personnel hours worked and outcomes of accidental exposures to infectious agents: intramural NIAID 1982–2003

Hours at risk			
	BENCH	Animal	Total
BSL-3	553,000	81,500	634,500
BSL-2/3 P	2,235,500	360,200	2,555,200
Total	2,788,500	441,700	3,189,700

Outcomes of accidental exposures			
	Clinical infections	Silent infections	Other exposures, no infections
BSL-3	1	2	9*
BSL-2/3 P	0	2	15
Total	1	4	24

* One HIV invasive accident treated with anti-retroviral drugs. No infection ensued.

One clinical infection without sequelae and four silent infections in more than 3 million hours of exposure is a remarkable record, especially when continuous exposure of personnel to fluids containing HIV virus over many years is a significant part of that record. Indeed, only a single instance was considered worthy of immediate prophylaxis for that agent and no infection occurred.

Biosafety in NIAID laboratories demands, and receives, constant vigilance. I recommend, however, better documentation of communication between the OSHB and NIH Division of Engineering Services. I was unable to find very many records of specific facility problems and their outcomes. It might be well to have a brief computerized form for registry of each event that requires action, together with follow-up reports that find their way to OSHB.

Another concern is design and function of air handling systems for BSL-3 laboratories. In both Building 10 and the new Building 50, BSC IIB cabinets directly ventilated externally are an essential part of the overall exhaust system that always must be greater than the input air. If room negative pressure diminishes, the BSCs also shut down, a poor condition if aerosols are being generated in course of the work. Much better would be to have IIA BSCs as workstations. These would continue to capture aerosols regardless of overall room negativity. Hoods would not have to run continuously and room failure would not also release aerosols into the laboratory. The Uninterrupted Power Supply installed in Building 50 was a prudent decision. I hope that these questions will be/have been considered in the current renovation of Twinbrook III as BSL-3 laboratory. Finally, it was a pleasure to receive frank, careful responses from all the scientists I approached. They willingly turned from their particular microbial environments to candidly discuss the history of their work from a safety perspective.

D.2.2 Biosafety at BSL-4, More than 20 Years Experience at Three Major Facilities, Karl M. Johnson, M.D., October 15, 2003

What Is BSL-4, And How Did We Get There?

Special containment for work with infectious microbes in the United States originated during World War II in response to intelligence that the German army had a program for development of biological, in additional to chemical weapons that had been used during the first World conflict. Temporary facilities were established in a suburb of Frederick, Maryland, later to become the permanent Fort Detrick. During the 1950s and 1960s several agents, most notably the bacteria that cause plague and anthrax and the rickettsial organism that causes so-called Q fever, were produced in large quantities and in forms with properties that make highly infectious tiny particles in the air. The term used was, and is, *weaponized*.

Infections among those working with these and other microbes were a recurrent problem. Under the inspired leadership of the late Dr. Arnold G. Wedum, recognized today throughout the world as the *Father of Biosafety,* Fort Detrick borrowed technology from the nuclear industry to prevent such infections, especially those induced by small aerosols that arose during the course of routine laboratory manipulations. Stainless steel cabinets (termed Class III) were constructed and assembled in continuous airtight lines. Each had at least one pair of sealed glove ports to

allow manipulation of hazardous materials in a sealed-off environment. Incubators, microscopes, and doors leading directly to autoclaves and to animal cabinets were integral to the cabinet line. The cabinets had a constant supply of filtered air and filtered exhaust fans to remove any particles generated during the work sessions. Air pressure in cabinet lines was negative to the laboratory room and the exhaust was filtered. The room itself also was negative to the rest of the building, and exhaust air was filtered before release to the environment. Thus workers, others in the building, and the outside community, were all protected against aerosol infection from agents otherwise intended for battle.

During these same two decades, new organisms with serious human pathogenicity were discovered in nature on several continents. Most of these, all of which were viruses, caused a syndrome (with variations) known as acute viral hemorrhagic fever (VHF). There was no specific treatment or vaccine available for any of them, except for the classical virus that causes yellow fever. That disease is now recognized as the prototype of VHF. Even more disturbing was the fact that aerosols were infectious for laboratory staff for most of these agents. Virology at Fort Detrick quickly entered the Class III cabinet habitat.

The recognition of Marburg virus in 1967 propelled the CDC into this arena. That agency was asked to help with field studies designed to uncover the African reservoir for the virus, and it was decided that diagnostic reagents were needed. Visions of travelers returning from parts of the globe endemic for HF agents became a chronic concern. A small Class III cabinet laboratory was established in 1970 at the CDC. It had about 70 linear feet of cabinet line and a staff of two persons who tested samples from wild animals for infection and made diagnostic reagents for Marburg and other viruses of concern.

One year previously (1969), President Richard Nixon unilaterally terminated the national program of offensive biowarfare at Fort Detrick. Most of the buildings were given over to the National Cancer Institute. But the Army now expanded its defensive program. A new facility was constructed that became the principal laboratory of the U. S. Army Medical Research Institute for Infectious Diseases (USAMRIID). It opened in early 1971 with a mission to develop technology for detection and identification of potential biowarfare agents, to

understand pathogenesis of the new VHF agents, to search for specific antiviral therapies, and to develop vaccines.

Another VHF agent, Lassa virus, appeared in Nigeria in 1969. When Marburg virus attacked two young Australians traveling in southern Africa in 1975, CDC Director David Sencer decided that it was time to reinforce the nascent Special Pathogens Branch. A surplus large trailer was obtained from NIH and outfitted as a new laboratory for work with VHF agents. It had a Class III cabinet line. Space previously used as offices was redesigned as the first completely suited laboratory and animal room. Workers wore special positive pressured suits that could be hooked up to hoses from the ceiling that provided clean breathing air. Suits came in several sizes and each worker was now able to have gloves that truly fit their hands. All work was to be done in movable Class II laminar flow biosafety cabinets (BSC) that pulled air across the work surface then filtered it, with about half recirculated in the box and the rest released into the laboratory. Similar filtered enclosures were employed to house infected animals. Laboratory exhaust air was twice filtered before release to the environment, all solid wastes were autoclaved in double-door machines installed through a laboratory wall, and all liquid wastes were pressure cooked at high temperature before cool down and released to sanitary sewers. Workers leaving the laboratory stood in a chemical shower to decontaminate the *space* suits before doffing scrub suits and showering before leaving the facility. Various alarms and redundant systems were installed to ensure that power, continuous negative pressure, and breathing air were always available in emergency. Needles and scalpels were used as infrequently as possible and plastic ware replaced glass for almost all procedures.

The new CDC laboratory was opened at the end of 1978. Laboratories using positive pressure suits also were ready at USAMRIID within months. These configurations allowed convenient installation and maintenance of new instruments and other equipment that was being developed for molecular work on viruses. The principles of biocontainment were (1) capture each small particulate aerosol immediately where it is generated, (2) ensure that workers have functional hands, life support, minimum exposure to invasive accidents, and ready access to the tools required for research, and (3) make sure that systems for prevention of escape of aerosolized

viruses to the environment are redundant. The BSC cabinets were the primary containment, the exhaust-filtered laboratories were the secondary, and even these were redundant.

By 1976, some leading molecular microbiologists became worried that new technology could potentially create novel organisms that might conceivably become Andromeda strains. The Director of the NIH ordered new guidelines for standards of microbiological safety for diverse agents with known properties of human pathogenicity and modes of transmission, as well as for newly discovered agents. The first edition of the NIH/CDC guidelines was published in 1980. Most work could be done in ordinary laboratories at BioSafety Level 2 (BSL-2). Others that cause more serious illness in humans, and/or for which no treatment is available, were assigned to BSL-3. All work was to be done in Class II biosafety cabinets. Room air was to be under negative pressure relative to hallways with no recirculation to other space in the building.

BSL-4 was reserved for VHF agents, certain tick-borne encephalitis viruses, and a simian herpesvirus for which human infection is almost universally fatal. At the time, this meant USAMRIID and CDC Special Pathogens, but authorities in South Africa were progressively concerned about VHF on their continent. Ebola virus, an even more virulent relative of Marburg, had been discovered in 1976. Rift Valley fever virus had caused its first-ever epidemic that included hemorrhagic fever. Crimean-Congo virus was a new concern. To meet these challenges, a BSL-4 laboratory, modeled on the Detrick and Atlanta prototypes, was constructed outside Johannesburg and commissioned in 1980. It had both suit and cabinet-line laboratories.

These three laboratories were virtually *the* sites of BSL-4 viral work during the past 22-30 years. With experience over time, most investigators chose to work primarily in the positive-pressure suit environment. Indeed, at the end of the1980s, CDC moved into new large laboratories that were almost devoid of Class III cabinet lines. Moreover, the Johannesburg laboratory, now part of the National Institute for Communicable Diseases (NICD), recently removed its Class III cabinets to expand positive-pressure suit space. Only the British BSL-4 laboratories continue to depend on Class III cabinet line configurations. All recently constructed Level 4 facilities in other countries, as well as those proposed for ours, are positive-pressure suit

labs. Accordingly, this review will not include biosafety at the Porton Down facility. We are concerned principally with the track record of, and a risk analysis for, BSL-4 positive-pressure suit laboratories.

That record is exemplary. Most individuals who begin work in BSL-4 suites are already experienced microbiologists. Specific training for use of the positive-pressure suits and for safe execution of all procedures is standard practice at all of the laboratories. In context of current international concern regarding potential use of some of these viruses as weapons of terror, access to the facilities and to individual laboratories is carefully controlled. At two of the facilities in the United States individual security clearance is required to qualify for work at the BSL-4 level. The viruses under study do not escape, neither by accident nor by covert design. Reviews of individual facilities are summarized below.

D.2.2.1 USAMRIID — 1972-2003

Persons Interviewed:

Drs. Peter Jahrling, Chief Civilian Scientist; Gerald Eddy, retired Chief, Virology Division.

Research Program:

Pathogenesis of viral infections in animal models, including clinical and anatomical pathology. Quantitative susceptibility of animals to aerosol infection by VHF pathogens. Development of diagnostic assays and air sampling detectors. Molecular anatomy and genetics of agents. Drug screening program in search of antiviral compounds. Development of live attenuated, inactivated, and recombinant vaccines.

Agents Studied:

Machupo, Junin, Guanarito, Sabia, and Lassa arenaviruses; Marburg and Ebola; Rift Valley fever and Crimean-Congo hemorrhagic fever viruses; Tick-Borne encephalitis virus. Yersinia pestis and Bacillus anthracis.

Animals Used:

Mice, hamsters, guinea pigs, non-human primates, wild rodents, lambs,

Site:

Two buildings, Fort Detrick, Maryland. Total BSL-4 space: about 6500 sf. One third is animal space and suit/cabinet ratio of lab space is about 2:1.

Time Devoted in BSL-4 Space:

Approximately 343,980 hours. (6.5 persons/8 hour day x 1680 hours/year x 31.5 years).

Laboratory Accidents and Outcomes:

During early years when work was completely in cabinets, invasive accidents resulted in treatment with human plasma containing specific antibodies to virus in question, as well as confinement in an isolation suite in one building that was also set up as an intensive care facility in event that a worker became ill after accidental exposure to an agent. Two invasive accidents were of most concern:

November 1979. Accidental finger puncture with needle on a syringe loaded with Lassa virus. Ribavirin and immune plasma were given. (This was an experimental therapy for monkeys under development at the Institute.) No illness or serological evidence for infection occurred.

December 1982. During autopsy, a bone fragment of a monkey infected with Junin virus punctured a finger. Immune plasma was used and no clinical or subclinical infection ensued.

D.2.2.2 CDC SPECIAL PATHOGENS

Persons Interviewed: Senior Scientists and Author

Research Program:

Development of diagnostic methods and reagents for diagnosis of all BSL-4 agents. Pathogenesis of viral infections in animal models, including natural wild reservoirs. Molecular anatomy and genetics of VHF agents. Limited vaccine development work. Response to VHF epidemics in natural settings. Diagnosis, clinical pathology and virology, discovery of new agents.

Agents Studied:

Five arenaviruses, Marburg, Ebola, Crimean-Congo HF virus, Rift Valley fever virus, Nipah and Hendra viruses, Russian spring summer encephalitis and Tick-Borne encephalitis viruses, Omsk and Kyasanur Forest disease viruses, Hantavirus (animal work only).

Animals Employed:

Mice, hamsters, guinea pigs, non-human primates, rats, five wild rodent species for rodent-borne agents.

Sites:

Building A: 1970-78. About 70 linear feet of Cabinet line.

Building B: 1979-1989. About 900 sf with 30 ft cabinet line, 300 sf positive-pressure suit lab and 200 sf of positive-pressure suit animal space.

Building C: 1990-2003. About 5000 sf of which approximately 30% is animal space. Laboratory is entirely positive-pressure suit operated.

Time Devoted in BSL-4 Space:

120,560 hours.

Laboratory Accidents and Outcomes:

Animal bite; Hantavirus infected rodent, no infection.

Animal bite; animals being inoculated with Hantavirus. Pre-inoculation bite from rat.

Needle stick to worker prior to setting up an inoculum with mouse-adapted Ebola virus. No infection.

Autoclave door interlock failed and a load not autoclaved was opened, but not handled. No infections resulted.

Multiple events over the years of outer gloves or suits developing tears or holes detected during work. Such incidents are evaluated and followed up. No treatments were ever used and no infections resulted.

Facility/System Failures: None of note that caused interruption of work.

D.2.2.3 National Institute for Communicable Diseases, Johannesburg, South Africa, 1980-2003

Person Interviewed:

Dr. Robert Swanepoel, BSL-4 Laboratory Director

Research Program:

Diagnostic reagents and support for all HF outbreaks in Africa and neighboring regions when requested,;pathogenesis of infections in animals, especially candidate wild reservoir species; clinical virology; molecular biology of selected hemorrhagic fever viruses; field investigations of natural history of disease outbreaks; and seroepidemiology of infections in humans and animals.

Agents Studied:

Marburg and Ebola viruses, Rift Valley fever virus, Crimean-Congo HF virus, ten hantaviruses.

Animals Employed:

Mice, guinea pigs, rabbits, bats, tortoises, pigeons, snakes, roaches, spiders, frogs, millipedes, snails, 20 species of wild rodents, hares, hedgehogs, guinea fowl, chickens, etc. Much animal work was devoted to a search for wild reservoirs of Marburg and Ebola viruses.

Site:

Rietfontein, 4500 sf. Space divided into 721 sf positive-pressure suit lab and 222 sf similar animal holding room, plus cabinet lab of 999 sf (now defunct). Remaining 1443 sf devoted to change rooms, showers, and service corridors.

Time Devoted in BSL-4 Space:

Approximately 40,000 hours in nearly 23 years.

Laboratory Accidents and Outcomes:

Bat bite through double gloves. No infection.

Multiple other accidents. Those exposed are monitored closely for 21 days, during which time they are not permitted to leave town—as are all employees after their last day of work inside BSL-4 space. No infections recorded.

Facility/System Failures:

Only one that caused shutdown of operations. About 5 liters of highly concentrated Marburg virus was suddenly aerosolized when worker opened chamber to add a bit more fluid without closing the nitrogen pressure tank and bleeding off pressure. Laboratory was mopped for several hours with glutaraldehyde, and finally decontaminated with formaldehyde gas. No infection occurred in two *exposed* workers. There was no breach in BSL-4 containment, and no infections occurred in neighboring open-air monkey colonies on the campus. This was a maximum challenge to BSL-4 containment, and I am aware of no other event remotely comparable in terms of concentration and volume of a highly lethal virus.

D.2.3 Summary

No clinical infections occurred at three institutions during work with BSL-4 agents, mostly hemorrhagic fever viruses during the past 31 years. Almost half a million hours of laboratory (and field) exposure have been recorded, the majority of which was time spent in positive pressure suits. Nor have there been major defects or incidents in operation of the physical facilities. No escape of any agent with clinical consequences for neighboring communities occurred.

Invasive injuries were infrequent, eloquent testimony to the awareness of the dangers and the daily care observed by workers who volunteer for such duty. One laboratory inadvertently carried out a maximum aerosol challenge to BSL-4 containment with a highly pathogenic hemorrhagic fever virus. Virus did not escape the laboratory, nor was a worker infected.

The zero numerator of infections in these three laboratories and the huge denominator of exposure hours make it impossible to provide a number for 'risk of infection' to either laboratory workers or outside communities. Nevertheless, that number must be small. When the value of diagnosis, treatment, and control of deadly outbreaks of hemorrhagic fever over the past three

decades is added to this equation, risk/benefit clearly comes out in favor of continued operation of BSL-4 laboratories.

Indeed, considering new challenges posed to the world community by these agents, it is fair to conclude that more such facilities are needed. Better therapeutic agents are desperately needed. High priority also must go to the development of vaccines that can protect laboratory and hospital personnel in countries where natural epidemics occur, as well as first responders to intentional aerosol attack on any community.

D.2.4 Biosafety at BSL-4, Experience at Five Facilities, Karl M. Johnson, M.D., 15 May, 2009

This report contains information from an earlier version produced in October 2003 as part of the final EIS for the not yet commissioned NIH Integrated Research Facility at Rocky Mountain Laboratory, Hamilton, Montana. It adds additional experience at two of the laboratoriesreviewed initially, and includes new data from two smaller facilities in the United States. Summarized below are updated reviews of two national facilities since my initial report of July 2003. The third original laboratory (Johannesburg) has been closed for rehabilitation since 2003, so information on that facility is limited to the initial 2003 report. New data are included from laboratories at the University of Texas Medical Branch in Galveston, Texas, and the Southwest Foundation for Biological Research in San Antonio, Texas that began BSL-4 work in the present decade.

D.2.4.1 USAMRIID — 1972-2003 and July 2003-March 2009

Persons Interviewed:

Drs. Peter Jahrling, Chief Civilian Scientist, and Gerald Eddy, retired Chief, Virology Division. Col. Mark Kortepeter, MD, Deputy Commander for the recent interval.

Research Program:

Pathogenesis of viral infections in animal models, including clinical and anatomical pathology. Quantitative susceptibility of animals to aerosol infection by VHF pathogens. Development of diagnostic assays and air sampling detectors. Molecular anatomy and genetics of agents. Drug

screening program in search of anti-viral compounds. Development of live attenuated, inactivated, and recombinant vaccines.

Agents Studied:

Machupo, Junin, Guanarito, Sabia, and Lassa arenaviruses; Marburg and Ebola; Rift Valley fever (now at BSL-3 with worker vaccination) and Crimean-Congo hemorrhagic fever viruses; Tick-Borne encephalitis virus; hantaviruses in animals; SARS coronavirus (animal work).

Animals Employed:

Mice, hamsters, guinea pigs, rabbits, non-human primates, wild rodents, lambs,

Site:

Two buildings, Fort Detrick, Maryland. Only one building since 1999. Total BSL-4 space: about 6500 sf, increasing to ~8,400 sf since 2003. One third is animal space and suit/cabinet ratio of lab space is about 1:1.

Total Hours Devoted 1972-2009: 462,168 in 36.25 yrs.

Approximately 343,980 hours to July, 2003 (31.5 years).

Approximately 118,188 hours from July, 2003, almost 6 yrs.

Laboratory Accidents and Outcomes:

During early years when work was performed completely in cabinets, invasive accidents resulted in treatment with human plasma containing specific antibodies to virus in question, as well as confinement in an isolation suite in one building that was also set up as an intensive care facility in event that a worker became ill after accidental exposure to an agent. Two invasive accidents were of most concern:

November 1979. Accidental finger puncture with needle on a syringe loaded with Lassa virus. Ribavirin and immune plasma were given. (This was an experimental therapy for monkeys under development at the Institute.) No illness or serological evidence for infection occurred.

December 1982. During autopsy, a bone fragment of a monkey infected with Junin virus punctured a finger. Immune plasma was used and no clinical or subclinical infection ensued.

2004. A needle stick occurred inoculation of mice with mouse-adapted Zaire Ebola virus. Exposure was considered probable and risk of infection low to moderate. Worker was isolated at facility for 21 days and did not incur either infection or clinical disease. I consider this event the most potentially serious to have occurred since the original report in 2003.

Six other accidents resulted in daily temperature checks for 21 days, and in three instances, baseline laboratory measurements, but not worker isolation. None led to virus infection or disease:

2005. Ebola and Marburg viruses. Sharp object punctured boot but did not break skin.

2006. Lassa virus. Handled slides not properly irradiated, but acetone fixed.

2007. Marburg virus. Laceration on tip of third finger from contact with animal cage.

2007. Marburg virus. Separation of BSL-4 filter from suit.

2008. Marburg virus. V-shaped rip in suit. Positive airflow inside suit prevents contact with room air.

2008. Lassa virus. Brass connector on suit fell apart.

D.2.4.2 CDC SPECIAL PATHOGENS---1979-2003 and July 2003–March 2009

Persons Interviewed:

Senior Scientists and Author, to 2003.

Dr. Pierre Rollin, Acting Chief Special Pathogens, from 2003.

Research Program:

Development of diagnostic methods and reagents for diagnosis of all BSL-4 agents. Pathogenesis of viral infections in animal models, including natural wild reservoirs. Molecular anatomy and genetics of VHF agents. Limited vaccine development work. Response to VHF epidemics in natural settings. Diagnosis, clinical pathology and virology, discovery of new agents.

Agents Studied:

Six arenaviruses, Marburg, Ebola, Crimean-Congo HF virus, Rift Valley fever virus, Nipah and Hendra viruses, Russian spring summer encephalitis and Tick-Borne encephalitis viruses, Omsk and Kyasanur Forest disease viruses, hantavirus animal work.

Animals Employed:

Mice, hamsters, guinea pigs, non-human primates, rats, five wild rodent species for rodent-borne agents.

Sites:

Building A: 1979-1989. About 900 sf with 30 ft cabinet line, 300 sf positive-pressure suit lab and 200 sf of positive-pressure suit animal space.

Building B: 1990-2009. About 5000 sf of which approximately 30% is animal space. Laboratory is entirely positive-pressure suit operated.

Total Hours Devoted 1979-2009: 153,560 in ~30 years

120,560 hours, to July 2003

33,000 hours since July 2003

Laboratory Accidents and Outcomes:

Animal bite; Hantavirus infected rodent, no infection.

Animal bite; animals being inoculated with Hantavirus. Pre-inoculation bite from rat.

Needle stick to worker prior to setting up an inoculum with mouse-adapted Ebola virus. No infection.

Autoclave door interlock failed and a load not autoclaved was opened, but not handled. No infections resulted.

Multiple events over the years of outer gloves or suits developing tears or holes detected during work. Such incidents are evaluated and followed up. No treatments were ever used and no infections resulted. No events since 2003 except several more glove or suit tears, and disconnects of air supply or filters to suits. No isolation of worker or evidence of infection occurred.

Facility/System Failures: None of note that caused interruption of work.

D.2.4.3 NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES, JOHANNESBURG, SOUTH AFRICA, 1980 -2003

Person Interviewed:

Dr. Robert Swanepoel, BSL-4 Laboratory Director

Research Program:

Diagnostic reagents and support for all HF outbreaks in Africa and neighboring regions when requested. Pathogenesis of infections in animals, especially candidate wild reservoir species, clinical virology. Molecular biology of selected hemorrhagic fever viruses. Field investigations of natural history of disease outbreaks. Seroepidemiology of infections in humans and animals.

Agents Studied:

Marburg and Ebola viruses, Rift Valley fever virus, Crimean-Congo HF virus, ten hantaviruses.

Animals Employed:

Mice, guinea pigs, rabbits, bats, tortoises, pigeons, snakes, roaches, spiders, frogs, millipedes, snails, 20 species of wild rodents, hares, hedgehogs, guinea fowl, chickens, etc. Much animal work was devoted to a search for wild reservoirs of Marburg and Ebola viruses.

Site: Laboratory opened in 1980.

Rietfontein, 4500 sf. Space divided into 721 sf positive-pressure suit lab and 222 sf similar animal holding room, plus cabinet lab of 999 sf (now defunct). Remaining 1443 sf devoted to change rooms, showers, and service corridors.

Total Hours Devoted 1980-2003: 40,000 in almost 23 years

Laboratory has been closed for major refitting since 2003.

Laboratory Accidents and Outcomes:

Bat bite through double gloves. No infection.

Multiple other accidents. Those exposed are monitored closely for 21 days, during which time they are not permitted to leave town—as are all employees after their last day of work inside BSL-4 space. No infections recorded.

Facility/System Failures:

Only one that caused shutdown of operations. About 5 liters of highly concentrated Marburg virus was suddenly aerosolized when worker opened chamber to add a bit more fluid without closing the nitrogen pressure tank and bleeding off pressure. Laboratory was mopped for several hours with glutaraldehyde, and finally decontaminated with formaldehyde gas. No infection occurred in two *exposed* workers. There was no breach in BSL-4 containment, and no infections occurred in neighboring open-air monkey colonies on the campus.

This was the maximum challenge to BSL-4 containment in the past 36+ years, and I am aware of no other event remotely comparable in terms of concentration and volume of a highly lethal virus released as infectious aerosol inside a BSL-4 laboratory. Pressure filtration to concentrate a BSL-4 agent has not been practiced since at any of the facilities covered in this report.

D.2.4.4 SOUTHWEST FOUNDATION FOR BIOMEDICAL RESEARCH, SAN ANTONIO, TEXAS, March 2000 - April 2009

Persons Interviewed:

Dr. Jean Patterson, Chair, Dept. of Virology and Immunology

Dr. Ricardo Carrion Jr. Assistant scientist and BSL-4 lab manager

Research Program:

Pathogenesis of viral hemorrhagic fevers, especially in non-human primates. Development of animal models for studies of infection. Molecular analysis of viral infections and of natural and induced mutant viruses.

Agents Studied:

Marburg and Ebola viruses, Lassa virus and other pathogenic arenaviruses, CCHF virus, SARS virus , herpes B monkey virus.

Animals Employed:

Mice, hamsters, guinea pigs, rabbits, marmosets, cynomolgous monkeys.

Site: Opened in March 2000

Separate building on campus on outskirts of city. Approximately 1200sf suit space, which is one large room for both bench and animal work.

Total Hours Devoted: 11,650 in 9 years.

Laboratory Accidents and Outcomes:

One each glove and suit tear. Workers followed three weeks for fever. No infection.

Facility/System Failures:

Autoclave failure in 2004. Result was overnight flood of entire laboratory to depth of about one foot. Outer door held so no breach in containment occurred. Autoclaves never run after hours now and water supply is turned off before leaving lab each day.

D.2.4.5 UNIVERSITY OF TEXAS MEDICAL BRANCH, July 2004–April 2009

Persons Interviewed:

Ms. Dee Zimmerman, UTMB Biosafety Principal

Dr. Mike Holbrook, University BSL-4 Managing Scientist

Research Program:

Pathogenesis, experimental immunization, diagnostic development. Very active program for training and research among graduate students.

Agents Studied:

Ebola, Marburg, five arenaviruses, Nipah, Hendra, RSSE and Tick-Borne encephalitis, Rift Valley virus, CCHFV, Omsk, Kyanasur Forest Disease virus, Andes hantavirus, H5N1 influenza virus.

Animals Employed:

Mice, hamsters, wild small rodents, guinea pigs, non-human primates.

Site: Opened in July 2004

Small building attached to main Dept. Pathology facility on campus of medical school in residential Galveston, TX. Total 1900 sf, equally divided into bench and animal rooms.

Total Hours devoted: Approximately 25,000 in 5.5 years.

Laboratory Accidents and Outcomes: (All accidents and facility failures reported to CDC/SDAC and Community Liaison Committee).

January 2009. Scientist dropped plate containing Junin virus on floor. Spill was decontaminated and reported after proper procedures were performed. No virus escape, no personnel infection.

Facility/System Failures:

June 2005. Door from fumigation to buffer zone opened spontaneously about 2cm. The lab was shut down for annual maintenance at this time. Equipment and supplies had been surface decontaminated but not yet fumigated. Annual program was continued with full fumigation of all spaces and no untoward event occurred.

February 2008. Door from necropsy room into chemical shower inadvertently opened. Door had to be manually closed through chemical shower, breaking lab containment. Lab subsequently

completely decontaminated and repairs made to malfunctioning door closing device. No human or environmental exposure.

D.2.5 Summary

This report summarizes almost 700,000 hours of worker exposure in BSL-4 laboratories operated for a cumulated 103 facility years at five distinct sites in the United States and South Africa. Not a single clinical infection occurred during this huge work experience over an interval of more than 36 years, nor did any virulent BSL-4 virus escape to cause environmental injury anywhere.

Invasive injuries were infrequent, eloquent testimony to the awareness of the dangers and the daily care observed by workers who volunteer for such duty. Nevertheless, such accidents and potential exposures remain the greatest chronic cause of concern for all those managing such research programs. But it is important to note that infections are almost certain to be recognized even before actual clinical disease occurs and each organization herein cited has written plans and dedicated clinical facilities and staff available for management of patients. This assures that the environment and contiguous human community will not be impacted by any such event.

It also has been gratifying to learn that design, construction, and operation of each of these facilities continue to demonstrate unquestioned competence of thought, and execution of sound principles, use of robust materials, and careful, consistent management over a very long interval. Accidents that release virus aerosols into laboratory space have been infrequent and generally of small volume; but one inadvertent maximum aerosol challenge to BSL-4 containment with a highly pathogenic hemorrhagic fever virus did occur several years ago. Virus did not escape the laboratory, nor was a worker infected, further testimony to the soundness of design and construction of both primary and secondary barriers to aerosol escape.

The zero numerator of infections among laboratorians and the huge denominator of exposure hours make it impossible to provide a number for ‘risk of infection’ to either laboratory workers or outside communities. Nevertheless, that number must be vanishingly small. When the value of diagnosis, treatment, and control of deadly outbreaks of hemorrhagic fever over the past three

decades is added to this equation, risk/benefit clearly comes out in favor of continued operation of BSL-4 laboratories.

Indeed, considering new challenges posed to the world community by these agents, it is no surprise that several more BSL-4 facilities have been designed and/or constructed. Better therapeutic agents are desperately needed. High priority also must go to the development of vaccines that can protect laboratory and hospital personnel in countries where natural epidemics occur, as well as first responders to intentional aerosol attack on any community.

Karl M. Johnson, M.D.
15 May 2009

D.3 REFERNCES

Ad Hoc Committee, Boston University, ,. 2010. Section-2: Detailed Final Incident Report. Boston.

Aldermann, S. 2009. Additional test data: Aerosol Evacuation system; BD flow cytometers. ABSA biosafety forum.

Altman, L.K. 1994. Yale accepts blame for safety lapses linked to lab accident. *The New York Times*, December 13, 1994.

Anonymous. 2003. Hamilton's health no "major" concern. *Missoula Independent*, November 06, 2003.

Repeated Author. 2005. Biosafety Fiasco. *New Scientist* 185 (5):2484.

APHIS and CDC. 2009. *National Select Agent Registry*. APHIS and CDC, 4, Sept. 2009 2009 [cited 11, Oct. 2009 2009]. Available from http://www.selectagents.gov/.

Associated Press. 2006. *Study of Ebola virus in US lab halted*. September 20, 2007. <http://www.msnbc.msn.com/id/20892122/>.

Barry, A.Ml. 2005. Report of pneumonic tularemia in three Boston University researchers. edited by P. H. Commission. Boston.

BD Biosciences. 2009. Aerosol management option (AMO) and ULPA filters from Buffalo Filters used on the BD FACSVantage and BD FACSAria weries flow cytometers. edited by D. Brooks-Smith. San Jose: BD Biosciences.

Bhattacharjee, Y. 2009. Tularemia, laboratory-acquired - USA: (Maryland). *ProMed-Mail* (07-Dec-2009), http://promedmail.oracle.com/pls/otn/f?p=2400:1001:::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000%2C80383.

Boston University. 2009. Boston University School of Medicine reports illness; infection may have been acquired in research laboratory. In *Press release*.

Brook, I., T. B. Elliott, H. I. Pryor, 2nd, T. E. Sautter, B. T. Gnade, J. H. Thakar, and G. B. Knudson. 2001. In vitro resistance of Bacillus anthracis Sterne to doxycycline, macrolides and quinolones. *Int J Antimicrob Agents* 18 (6):559-62.

Center for Infectious Disease Research & Policy. 2007. Student treated after anthrax spill in MIssissippi lab. *CIDRAP News*, http://www.cidrap.umn.edu/cidrap/content/bt/anthrax/news/aug1407anthrax.html.

Center for Infectious Disease Research and Policy. 2005. Pandemic flu virus from 1957 mistakenly sent to labs. *CIDRAP*, http://www.cidrap.umn.edu/cidrap/content/influenza/general/news/april1305h2n2.html.

Centers for Disease Control and Prevention. 2002. Laboratory-acquired West Nile virus infections - United States, 2002. *Morbidity and Mortality Weekly Report* 51 (50):1133-1135.

Repeated Author. 2002. Suspected cutaneous anthrax in a laboratory worker--Texas, 2002. *MMWR Morbidity and Mortality Weekly Report* 51 (13):279-81.

Repeated Author. 2005. Inadvertent laboratory exposure to Bacillus anthracis--California, 2004. *MMWR Morb Mortal Wkly Rep* 54 (12):301-4.

Repeated Author. 2007. Report of Site Visit to Texas A&M University. edited by N. I. o. H. N. Centers for Disease Control and Prevention (CDC), US Department of Health and Human Services.

Repeated Author. 2007. Suspension of Select Agent Work: Texas A&M University. edited by N. I. o. H. N. Centers for Disease Control and Prevention (CDC), US Department of Health and Human Services.

Centers for Disease Control and Prevention, and the National Institutes of Health, ,. 1999. Biosafety in Microbiological and Biomedical Laboratories. edited by U. D. o. H. a. H. S.

Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH).

Repeated Author. 2007. Biosafety in Microbiological and Biomedical Laboratories. edited by H. H. Services.

Centers for Disease, Control, and Prevention. 2000. Laboratory-acquired human glanders--Maryland, May 2000. *MMWR Morb Mortal Wkly Rep* 49 (24):532-5.

Committee on Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents: National Research Council. 2011. Protecting the Frontline in Biodefense Research; The Special Immunizations Program. Washington, D.C.: National Research Council of the National Academies.

Cymet, T. C., and G. J. Kerkvliet. 2004. What is the true number of victims of the postal anthrax attack of 2001? *J Am Osteopath Assoc* 104 (11):452.

Dalton, R. 2005. Infection scare inflames fight against biodefence network. *Nature* 433 (7024):344.

Dishneau, D. 2004. Ebola virus, laboratory accident - USA (Maryland). In *proMED*: ProMED-mail.

Enserink, M. 2009. Ebolavirus, needle stick injury - Germany (02): (Hamburg). *Pro-MED Mail*.

Fontes, B. 2009. High rate of HEPA filter failures in aerosol containment devices built for cell sorter biocontainment. ABSA biosafety forum.

Harding, A.L., Byers, K.B. 2000. *Epidemiology of Laboratory-Associated Infections*. Edited by D. O. Fleming, Hunt, D.L. 3rd ed, *Biological Safety: Principles and Practices*: ASM Press.

Repeated Author. 2006. *Epidemiology of Laboratory-Associated Infections*. Edited by D. O. Fleming, Hunt, D.L. 4th ed, *Biological Safety: Principles and Practices*: ASM Press.

Hermes, J.J. 2007. U. of Wisconsin halted Ebola research after question arose about lab's safety level. *The Chronicle of Higher Education*, September 20, 2007.

Johnson, K.M. 2009. Appendix D - Review of Biocontainment Laboratory Safety Record edited by U. S. D. o. H. a. H. Services. Washington D.C. .

Kao, A. S., D. A. Ashford, M. M. McNeil, N. G. Warren, and R. C. Good. 1997. Descriptive profile of tuberculin skin testing programs and laboratory-acquired tuberculosis infections in public health laboratories. *J Clin Microbiol* 35 (7):1847-51.

Kimman, T. G., E. Smit, and M. R. Klein. 2008. Evidence-based biosafety: a review of the principles and effectiveness of microbiological containment measures. *Clin Microbiol Rev* 21 (3):403-25.

Kruse, R. H., W. H. Puckett, and J. H. Richardson. 1991. Biological safety cabinetry. *Clin Microbiol Rev* 4 (2):207-41.

Lawler, A. 2005. Biodefense labs. Boston University Under Fire for Pathogen Mishap. *Science* 307 (5709):501.

Leonard, M. 2009. Safety breach at IU sends 7 to hospital as a precaution. *Herald-Times*, November 21, 2009.

Leung, R. 2003. The case against Dr. Butler. *60 Minutes*, http://www.cbsnews.com/stories/2003/10/17/60minutes/main578660.shtml.

Lim, P.L., Karup, A., Gopalakrishna, G., et al. 2004. Laboratory acquired sever acute respiratory syndrome. *New England Journal of Medicine* 350:1740-1745.

Margasak, L. 2007. Mishandling of germs on rise at US labs. *Associated Press*, October 2, 2007.

McGroarty, P. 2009. Ebolavirus, needle stick injury Germany (04): Hamburg. *ProMED-mail*.

Medical University of Ohio. 2004 and 2005,. Medical University of Ohio Institutional Biosafety Committee meeting minutes.

Miller, J.D. 2004. US lab is sent live anthrax. *The Scientist* (June 11, 2004).

Nasdala, N. 2009. Anthrax, laboratory exposure - France. *ProMed-Mail*.

National Institutes of Health. 2011. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). edited by Department of Health and Human Services. Washington, D.C.

National Institutes of Health, Institutional Biosafety Committee. 2004. National Institutes of Health Institutional Biosafety Committee meeting minutes.

National Institutes of Health Office of Biotechnology Activities. 2010. Incident report involving recombinant DNA, August 5th, 2010,,. edited by U.S. Department of Health and Human Services. Washington, D.C.

New York Times. 2003. Virus Box Explodes at Ohio FedEx Site. *New York Times*, March 20, 2003.

NIH, National Institutes of Allergy and Infectious Diseases, ,. 2010. Recombinant DNA Incident Report - Medical College of Wisconsin. edited by N. O. o. B. Activities. Washington, D.C.

Repeated Author. 2010. Recombinant DNA Incident Report - University of Wisconsin - Madison. edited by N. O. o. B. Activities. Bethesda.

Repeated Author. 2010. Recombinant DNA Incident Reports 1977-May 2010.

Normile, D. 2004. Second lab accident fuels fears about SARS. *Science* 303 (26).

NRC (National Research Council). 2011. Review of Risk Assessment Work Plan for the Medical Countermeasures Test and Evaluation Facility at For Detrick: a Letter Report. Washington, D.C. : The National Academies Press.

Office of General Services, Boston University Medical Center, ,. 2007. Code Red 700 Albany Street 20 March 2007 After Action Report. Boston: Boston University Medical Center.

Pedrosa, P. B., and T. A. Cardoso. 2011. Viral infections in workers in hospital and research laboratory settings: a comparative review of infection modes and respective biosafety aspects. *Int J Infect Dis* 15 (6):e366-76.

Perez, E. 2010. U.S. closes case in anthrax attacks. *The Wall Street Journal*, February 20, 2010.

ProMED mail. 2004. Ebola, lab accident death - Russia (Siberia).

Repeated Author. 2005. CDC states all H2N2 influenza virus samples destroyed. In *proMED*.

Repeated Author. 2005. Plague, missing laboratory mice - USA (New Jersey). International Society for Infectious Diseases.

Rusnak, J., E. Boudreau, J. Bozue, P. Petitt, M. Ranadive, and M. Kortepeter. 2004. An unusual inhalational exposure to Bacillus anthracis in a research laboratory. *J Occup Environ Med* 46 (4):313-4.

Rusnak, J. M., M. G. Kortepeter, J. Aldis, and E. Boudreau. 2004. Experience in the medical management of potential laboratory exposures to agents of bioterrorism on the basis of risk assessment at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). *J Occup Environ Med* 46 (8):801-11.

Smith, S. 2009. BU grad student develops infection. *The Boston Globe*, October 30, 2009.

Repeated Author. 2009. BU student caught bacterial infection from lab, tests show. *The Boston Globe*, November 10, 2009.

Srinivasan, A., C. N. Kraus, D. DeShazer, P. M. Becker, J. D. Dick, L. Spacek, J. G. Bartlett, W. R. Byrne, and D. L. Thomas. 2001. Glanders in a military research microbiologist. *N Engl J Med* 345 (4):256-8.

Staszkiewicz, J., C. M. Lewis, J. Colville, M. Zervos, and J. Band. 1991. Outbreak of Brucella melitensis among microbiology laboratory workers in a community hospital. *J Clin Microbiol* 29 (2):287-90.

Subbarao, K. 2008. *NIH intramural program: proposed biosafety containment and use of prophylaxis for 1918 H1N1*. Bethesda: NIH-RAC.

Subcommittee on Oversight and Investigations. 2007. Germs, Viruses, and Secrets: The Silent Proliferatioh of Bio-Laboratories in the United States. edited by H. o. R. C. o. E. a. Commerce. Washington, D.C.: U.S. Government Printing Office.

Templeton, D. 2008. Monkey bites Pitt lab technican. *Pittsburg Post-Gazette*, October 3, 2008.

Texas A&M University. 2006. Report of Theft, Loss, or Release of Select Agents or Toxins. In *APHIS/CDC form 3*.

Repeated Author. 2007. Report of theft, loss, or release of select agents and toxins (APHIS/CDC Form 3). edited by U. S. D. o. Agriculture.

Repeated Author. 2007. Report of Theft, Loss, or Release of Select Agents or Toxins.

The Associated Press. 2009. U.S. labs mishandling deadly germs: Number of accidents involving anthrax, other toxins increasing, review finds.

The University of North Carolina at Chapel Hill Institutional Biosafety Committee. 2005. University of North Carolina at Chapel Hill Institutional Biosafety Committee meeting minutes.

United States Government Accountability Office. 2009. High-Containment Laboratories; National Strategy for Oversight is Needed.

University of Texas, Houston, ,. 2007. Environmental Health and Safety Incident/Accident Investigation Report.

University of Chicago IBC. 2005. University of Chicago Institutional Biosafety Committee meeting minutes.

University of Illinois at Chicago. 2003-2006. University of Illinois at Chicago Institutional Biosafety Committee meeting minutes.

University of New Mexico IBC. 2003. University of New Mexico Insitutional Biosafety Committee meeting minutes.

University of Texas at Austin, Institutional Biosafety Committee, ,. 2006. BLS3 Laboratory Incident Report (Draft) regarding H3N2 with H5N1 genes.

USAMRIID United States Army Medical Research Institute of Infectious Diseases. 2009. Press release date December 4, 2009. edited by U. S. A. M. R. I. o. I. Diseases. Frederick MD.

Repeated Author. 2009. Update on USAMRIID scientist, Press release December 9, 2009. edited by U. S. Army. Frederick, MD.

Van Derbeken, J. 2007. Lab fined $450,000 for mishandling anthrax. *San Francisco Chronicle*, October 7, 2007.

Washington Department of Labor and Industries. 2004. Inspection of Infectious Disease Research, Inc. edited by W. D. o. L. a. Industries. Seattle, WA.

Wentworth, D. E., M. W. McGregor, M. D. Macklin, V. Neumann, and V. S. Hinshaw. 1997. Transmission of swine influenza virus to humans after exposure to experimentally infected pigs. *J Infect Dis* 175 (1):7-15.

Whitney, Bruce. 2006. E-mail cummunication to Jan Klein, University of Wisconsin-Madison from Bruce Whitney, NIH.

World Health Organization. 2004. China's recent SARS outbreak: important lessons for global public health. In *proMED; Archive Number 20040704.1792*.

Repeated Author. 2005. International response to the distribution of a H2N2 influenza virus for laboratory testing: Risk considered low for laboratory workers and the public. edited by W. H. Organization.

Yeshiva University, Institutional Biosafety Committee, ,. 2006. Yeshiva University Institutional Biosafety Committee meeting minutes.

Young, A. 2007. CDC lab's backup power fails during storm. *Atlanta Journal-Constitution*, July 7, 2007.

Repeated Author. 2007. CDC lab flaw gets Congress' attention. *Atlanta Journal-Constitution*, August 8, 2007.

Repeated Author. 2007. E-mails outline CDC backup power flaws; One expert: failure to heed warnings a "grave breach of responsibility". *Atlanta Journal-Constitution*, July 24, 2007.

Repeated Author. 2008. CDC safety: Germ lab outages continue. *Atlanta Journal-Constitution*, July 13, 2008.

Repeated Author. 2008. CDC simulates lab blackout. *Atlanta Journal-Constitution*, July 22, 2008.

Repeated Author. 2008. Outage at CDC lab adds to concerns; absence of two generators cited in backup power failure. *Atlanta Jounal-Constitution*, July 19, 2008.

Young, A. . 2007. Outage exposes flaws at CDC lab; World's deadliest germs are kept at DeKalb facility, but security may not be as foolproof as believed, judging from failure of backup generator. *Atlanta Journal-Constitution*, July 20, 2007.

Zimmer, S. M., and D. S. Burke. 2009. Historical perspective--Emergence of influenza A (H1N1) viruses. *N Engl J Med* 361 (3):279-85.

Appendix E.

Identification of Candidate Initiating Events

Contents

E. Identification of Candidate Initiating Events

E.1 Methodology

This risk assessment (RA) selects several of the most common loss of biocontainment initiating events experienced at research laboratories and from those initiating events develops scenarios that account for National Emerging Infectious Disease Laboratory (NEIDL)-specific preventive and mitigative features. This appendix documents the process used to ensure that a broad range of potential initiating events were considered. Multiple sources were used to identify potential initiating events including prior analyses of the NEIDL, analyses of similar laboratories, and reports of incidents at similar facilities.

Prior analyses of the NEIDL reviewed to identify postulated events were the following:

- *Final Environmental Impact Statement, National Emerging Infectious Disease Laboratory, Boston, Massachusetts* (NEIDL FEIS) (NIH and DHHS 2005)
- *Draft Supplementary Risk Assessments and Site Suitability Analyses for the National Emerging Infectious Diseases Laboratory Boston University* (DSRASSA) (NIH 2007)

National Environmental Policy Act (NEPA) documents associated with the analysis of other biosafety laboratories were also reviewed. The review consisted of the following NEPA documents:

- *Final Revised EA for the Proposed Construction and Operation of a Biosafety Level 3 Facility at LLNL* (DOE 2008)
- *Final Environmental Impact Statement For the Galveston National Laboratory for Biodefense and Emerging Infectious Diseases Research Facility in Galveston, Texas* (NIH 2005a)
- *National Bio and Agro-Defense Facility Environmental Impact Statement* (DHS 2008)
- *Final Environmental Impact Statement, Construction and Operation of the New U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Facilities and Decommissioning and Demolition and/or Re-use of Existing UASMRIID Facilities at Fort Detrick, Maryland* (USAMRMC 2006)

- *Evaluation of the Health and Safety Risks of the New USAMRIID High Containment Facilities at Fort Detrick, Maryland* (National Research Council 2010)
- *Final Environmental Impact Statement (FEIS) on the Rocky Mountain Laboratories Integrated Research Facility in Hamilton, Montana* (NIH 2004)

Appendix D (Operating Experience at High Biocontainment Facilities) of this RA provides a listing of some of the relevant incidents that have occurred at other biosafety level (BSL)-3 and BSL-4 laboratories. Numerous other references associated with experiments related to potential laboratory incidents and reports of incidents at other laboratories were reviewed. Many of those incidents in the other sources are also included in Appendix D of this RA. The following documents also provided incidents and postulated accidents that were considered:

- *Subcommittee Hearings on SBIR: Advancing Medical Breakthroughs, Subcommittee On Investigations and Oversight Committee On Small Business United States House of Representatives One Hundred Tenth Congress Second Session* (House Hearing 2008)
- *Epidemiology of Laboratory-Associated Infections*, in *Biological Safety: Principles and Practices* (4th edition) (Harding and Byers 2006)
- *Microbial Aerosol Generation During Laboratory Accidents and Subsequent Risk Assessment* (Bennett and Parks 2006)
- *Risk of Occupationally Acquired Illnesses from Biological Threat Agents in Unvaccinated Laboratory Workers*, Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science, Volume 2, Number 4, 2004 (Rusnak et al. 2004)
- *A Case of Ebola Virus Infection*, British Medical Journal (Emond 1977)
- NIH Office of Biotechnology Activities (OBA) Incident Reporting Template, NIH/NIAID (NIH 2010, NIH 2010a)
- *Fatal Laboratory-Acquired Infection with an Attenuated* Yersinia pestis *Strain - Chicago, Illinois, 2009*, Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report, February 25, 2011 (CDC 2011)
- *NIH Blue Ribbon Panel to Advise on the Risk Assessment for the BU National Emerging Infectious Disease Laboratories* (NIH 2009)
- *Mistakes Happen: Accidents and Security Breaches at Biocontainment Facilities*, The Council for Responsible Genetics. (CRG 2007).

- *Volume V: Anthrax at Sverdlovsk, 1979, U.S. Intelligence On The Deadliest Modern Outbreak* (Wampler and Blanton 2001)

In addition to a review of events identified from other sources, the NEIDL design and operating plans were reviewed to identify events that might be unique to NEIDL or that were not included in other sources.

E.2 Results

A review of the references listed in Section E.1 identified more than 300 incidents and postulated events, but some of them were not reported in sufficient detail to be of value for this analysis and were removed from further consideration. More than 300 incidents and postulated events were found to contain sufficient detail to be of value to this evaluation, and they are listed in Table E-1. Some events are reported in multiple references, but only one reference is listed for each entry. Some details of the scenario descriptions in the table are not fully reflective of NEIDL because of differences in biocontainment features. For example, some incidents occurred before modern biocontainment features and practices were implemented. Also, some incidents involved pathogens or toxins that might not be used in NIEDL. Differences in biocontainment features or pathogens do not mean an initiating event is not relevant for this analysis, only that the than entire event sequences is not relevant. Because this review is focused solely on initiating events, incidents that differ from those possible at NEIDL were retained. Many of the incidents and postulated events have similar initiating events and were grouped accordingly. The last column of the table provides a general candidate grouping, and the grouping was carried forward for use in the initiating event selection process.

1

Table E-1. List of incidents and postulated events

#	Scenario description	Reference	Candidate event group
1	Two steer that had never been inoculated with FMDV were found to be infected. It was determined that FMDV probably came into the room through leaks in the walls, possibly in conjunction with a power failure that could have caused a difference in air pressure between the two rooms. Preventive maintenance of the rooms was conducted to prevent recurrence.	NEIDL RA Appendix D	Breach of containment (wall cracks or open doors)
2	Puncture of thumb through rubber glove while transferring Ebola from liver of infected guinea pig. Immediately removed glove and immersed thumb in hypochlorite solution. Became ill 6 days later.	Emond 1977	Puncture - needlestick
3	Foot and mouth disease virus escaped from the biocontainment facility. The suspected cause was construction work in progress. Cattle outside the laboratory facility were found to be infected with FMDV.	NEIDL RA Appendix D	Contamination outside laboratory
4	Accidental finger puncture with needle on a syringe loaded with Lassa virus. Ribavirin and immune plasma were given. (This was an experimental therapy for monkeys under development at the Institute.) No illness or serological evidence for infection occurred.	NEIDL RA Appendix D	Puncture - needlestick
5	Approximately 9 steer were found to have antibodies to type O FMDV, with no known reason for the unintentional exposure to FMDV. It was surmised that a laboratory worker accidentally could have transmitted the virus to the animals.	NEIDL RA Appendix D	Contamination inside laboratory
6	Four steer vaccinated with FMDV type O were found to be infected with Type A. The cause was not identified, but it was surmised that cross-contamination from another lab area was most likely.	NEIDL RA Appendix D	Contamination inside laboratory
7	During autopsy, a bone fragment of a monkey infected with Junin virus punctured a finger. Immune plasma was used, and no clinical or subclinical infection ensued.	NEIDL RA Appendix D	Puncture - during necropsy
8	Worker punctured finger on broken cover slip in HIV lab. No medical treatment. No infection occurred.	NIH 2004	Puncture - general
9	One heifer without previous inoculation or known exposure to FMDV was found to be infected with type O virus. Type O had been used in nearby rooms, and animals from those rooms had been euthanized and then transported through the corridor.	NEIDL RA Appendix D	Contamination inside laboratory
10	Main electrical power lines to one lab were knocked out by hurricane-force winds. Backup power was unavailable because of deferred repairs on an inoperable emergency transmission line. Disposal systems for virus-laden waste were inoperable for 32 hours, causing a backup of infectious sewage. No infections in animals were reported.	NEIDL RA Appendix D	Loss of power
11	A research virologist discovered a leaking vessel upon opening a sealed aerosol biocontainment centrifuge rotor. Improper PPE[a] was used, and the incident was not reported. Symptoms began 8 days afterward. Two days later, the infection was correctly diagnosed. Antiviral therapy cured the nearly fatal infection. No secondary infections were found.	NEIDL RA Appendix D	Centrifuge release

#	Scenario description	Reference	Candidate event group
12	Two people, working in separate ABSL-3 rooms, each became symptomatic and were diagnosed with influenza 1.5 days after collecting nasal specimens from experimentally infected pigs. Genetic analyses determined that the workers had become infected with the same virus used to infect the pigs. An investigation determined that an incorrect mask had been supplied to the workers for 1 day, and it is possible that error facilitated infection of personnel.	NEIDL RA Appendix D	Inadequate PPE use
13	A research microbiologist routinely failed to wear disposable gloves and became infected. A primary care physician prescribed antibiotics without knowledge of the specific etiology. The patient improved but relapsed to a life-threatening condition. Culture revealed specific etiology and appropriate antibiotics resulted in cure.	NEIDL RA Appendix D	Inadequate PPE use
14	An open container of *Y. pestis* fell off a shaker during the night. Several workers entered the lab next morning and the accident was immediately discovered. Surfaces were decontaminated and lab was closed until a new BSL-3 was available. No infections occurred.	NIH 2004	Spill/splash
15	PPD [purified protein derivative]skin test conversion. Employee treated with isoniazid. No clinical or radiological evidence for disease ensued. Employee worked in the Electronic Microscopy Branch (EMB) in Bldg 5. Work involved prep of samples submitted by outside collaborators in EM. Centrifugation done outside BSC. Although all samples supposedly were inactivated before receipt at RML, suspicion is that residual live bacteria were source of infection. Several modifications to equipment and procedures instituted: (1) A modern sealed centrifuge was installed, (2) bldg 5 air handling was upgraded, and alarms for BSC function were provided, (3) documented inactivation protocols and safety tests must now accompany materials received from outside sources, (4) all samples to be processed as though they still contain viable organisms.	NIH 2004	Pathogen not inactivated
16	Spores were found outside the laboratory.	NRC 2010	Contamination outside laboratory
17	A worker contracted glanders but did not disclose the type of work he was doing when he sought medical attention, which contributed to the delay in his diagnosis. He admitted to not wearing gloves. No other infections occurred.	NRC 2010	Inadequate PPE use
18	A lab worker used an incorrect disinfectant; failed to wear disposable gloves; and failed to cover a preexisting skin defect (facial cut from shaving). Cutaneous anthrax resulted following skin exposure to a contaminated surface. Patient was successfully treated using antibiotics	NEIDL RA Appendix D	Inadequate PPE use
19	A researcher tested positive for exposure to anthrax spores, which were also released into a locker room and adjacent hallway. No one was infected.	NEIDL RA Appendix D	Contamination inside laboratory

#	Scenario description	Reference	Candidate event group
20	A researcher was infected by handling culture flasks that had leaked. Flasks were loosely capped and caps covered by paper, according to protocol. Bacteria had splashed onto the paper, dried, and become airborne upon handling. The exposed worker, previously vaccinated, was given a booster injection. SOP was modified to require use of respiratory protection and filter-lined tightly screwed caps were adopted.	NEIDL RA Appendix D	Container leak/spill/open
21	U.S. Army officials reported evidence of a second accidental release of anthrax spores. No one was infected.	NEIDL RA Appendix D	Contamination inside laboratory
22	Researchers working with an infectious strain of *E. coli* did not use proper protection because they thought they were working with a harmless variety.	CRG 2007	Inadequate PPE use
23	A microbiologist, working under BSL-2 conditions using a Class II BSC, lacerated a thumb with a scalpel during necropsy of a bird infected with WNV. The superficial wound was cleansed and bandaged. Symptoms began 4 days later; medical attention was sought 7 days after injury. Infection was self-limiting and was confirmed by serologic testing.	NEIDL RA Appendix D	Puncture - during necropsy
24	A microbiologist working under BSL-3 conditions suffered a finger puncture from a hypodermic needle harboring WNV being harvested from infected mouse brain. The wound was cleansed and bandaged. Serologic testing showed evidence of acute WNV infection. Mild symptoms developed and resolved.	NEIDL RA Appendix D	Puncture - needlestick
25	Microbiologist contracted WNV after cutting finger with a scalpel used to perform a necropsy on a lab animal.	CRG 2007	Puncture - during necropsy
26	Puncture of thumb with hypodermic needle harboring spores to be used in mouse infections. Worker received prophylactic treatment; no infection resulted. It was proposed that alternative methods for mouse inoculation be considered.	NEIDL RA Appendix D	Puncture - needlestick
27	30 vials of the bacterium were reported missing by the principal investigator. It was not possible to determine the cause.	NEIDL RA Appendix D	Inadequate pathogen accountability
28	An improperly packaged shipment containing dry ice burst. The package was carrying frozen infected bird tissue. Workers at a FedEx shipping building were potentially exposed to WNV. Authorities characterized the risk of infection as low.	NEIDL RA Appendix D	Transportation mishap
29	Inventory discrepancy because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability
30	Inventory discrepancy because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability
31	Academic loss inventory discrepancy because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability
32	Loss in transit while importing select agents. Per FBI, the packages were discovered and incinerated in Belgium.	NEIDL RA Appendix D	Inadequate pathogen accountability
33	A graduate student working on a virulent recent New York strain of WNV became sick with fever and myalgia after making several passages of the new virus in Vero E6 cells also used to grow SARS-CoV. The student had minimal training and help from an institute technician. He was admitted to the hospital with a dry cough and signs of pulmonary inflammation. He was transferred to isolation and developed a moderately severe evolution of the disease. No secondary infections occurred. An investigation of the lab proved that the WNV was contaminated with the SARS-CoV.	NEIDL RA Appendix D	Contamination inside laboratory

#	Scenario description	Reference	Candidate event group
34	An investigation to assess security at Plum Island Animal Disease Center in the aftermath of the 2001 terrorist attacks is concluded. The report enumerated fundamental concerns that pathogens were not adequately secured from unauthorized access.	NEIDL RA Appendix D	Inadequate pathogen accountability
35	A 3-hour power failure undermined containment systems, leading workers to seal windows and doors with duct tape. All three backup generators failed.	CRG 2007	Loss of power
36	A researcher worked with *E. coli* outside a hood and failed to sufficiently sanitize the area afterward, thereby infecting a colleague.	CRG 2007	Contamination inside laboratory
37	A senior research scientist working with SARS in a Class III BSC cleaned up waste fluid that leaked from a tightly docked transfer chamber connected to the main cabinet. From the main cabinet, he sprayed alcohol into the chamber, waited 10 minutes, opened the chamber to spray more and finally physically cleaned it up. The next day he attended a SARS meeting in Singapore. Four days later, he noted fever and fatigue, which progressed into a dry cough and severe myalgia. He was hospitalized Dec. 16, and experienced moderately severe clinical illness. Contacts, especially plane passengers, were monitored or quarantined; no secondary infections occurred. An investigation of the lab revealed that SARS-CoV nucleic acid was on the handle of an alcohol bottle in the transfer chamber and on the light switch in the Class III cabinet.	NEIDL RA Appendix D	Contamination inside laboratory
38	Three researchers were infected with tuberculosis after the seal of a test chamber began leaking. They were not wearing respirators.	NEIDL RA Appendix D	Container leak/spill/open
39	A worker found three broken vials of the virus [Russian spring-summer encephalitis virus]. Wearing only a laboratory coat and gloves, the worker used tweezers to remove broken glass and then moved the materials to another container.	NEIDL RA Appendix D	Puncture - during necropsy
40	FMDV was accidentally transmitted to an animal clean room. Unintentional FMD infection occurred in animals in the nearby clean room as a result.	NEIDL RA Appendix D	Contamination inside laboratory
41	Autoclave failure in 2004. Result was overnight flooding of entire laboratory to depth of about one foot. Outer door held so no breach in containment occurred. Policy and SOP were amended to never allow autoclaves to be in operation after hours, and water supply is turned off before leaving lab each day. The clave door interlock failed, and a load not autoclaved was opened, but not handled. No infections resulted.	NEIDL RA Appendix D	Flooding inside laboratory
42	Unlikely exposure—vials labeled as RSSV, entity could not confirm viability.	House Hearings 2008	Inadequate pathogen accountability
43	A needle stick occurred during inoculation of mice. Exposure was considered probable and risk of infection low to moderate. Worker was isolated at facility for 21 days and did not incur either infection or clinical disease.	NEIDL RA Appendix D	Puncture - needlestick
44	Discrepancy in shipment—2 vials not shipped.	House Hearings 2008	Inadequate pathogen accountability
45	Discrepancy in shipment—shipped empty box.	House Hearings 2008	Inadequate pathogen accountability
46	Inventory discrepancy because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability

#	Scenario description	Reference	Candidate event group
47	A laboratory worker became feverish months after handling a culture of *Brucella* sp. Infection was confirmed in July by laboratory testing. It was determined that the employee had handled the culture without using proper biocontainment precautions. The employee eventually returned to work.	NEIDL RA Appendix D	Inadequate PPE use
48	Shipped inactivated toxin.	House Hearings 2008	Inadequate pathogen accountability
49	Researchers were working under BSL-2 biocontainment protocol with what was believed to be a noninfectious vaccine strain of the bacterium. Later, it was determined that the bacterial culture also contained the infectious wild-type strain that requires BSL-3 biocontainment precautions. The investigation was unable to determine the cause for the mixed culture. Two researchers became infected with *Francisella tularensis* in May and were not correctly diagnosed until a third scientist became infected with the bacterium in September. An investigation revealed that researchers had failed to follow proper BSL-2 biocontainment protocol and that the university failed to identify work-related illness in laboratory staff and failed to immediately report suspicious work-related illness to local and state health departments. Biosafety policies and SOPs were revised accordingly. The chief of Infectious Diseases was replaced.	NEIDL RA Appendix D	Inadequate pathogen accountability
50	Inventory discrepancy—individual failed to note that a vial had been destroyed.	House Hearings 2008	Inadequate pathogen accountability
51	No release occurred because the power outage occurred in the room after the laboratory workers performed necropsy of infected mice and culture tissue inside a BSC that never lost power.	House Hearings 2008	Loss of power
52	A researcher suffered an accidental finger stick with a hypodermic needle that contained the Zaire strain of Ebola virus. She was working with a guinea pig model of the infection. The researcher died several days later.	NEIDL RA Appendix D	Puncture - needlestick
53	Children's Hospital and Research Center Southern Research Institute sent live, rather than dead, anthrax samples to researchers in Oakland. The problem was detected after 49 of the 50 research mice quickly died after inoculation with the samples. Seven scientists were exposed but not infected.	NEIDL RA Appendix D	Inadequate pathogen accountability
54	Two cattle not involved in live virus research were observed with clinical signs of FMD. The FMDV was type O, but not the same type O strain involved in the July 19 incident. No specific explanation for the unintended infections was found. Following that and the July 19 incidents, new animal care protocols were instituted to restrict access to all animal rooms. Restrictions included clothing exchange, mandatory exit showers, and decontamination of all laboratory samples removed from the animal rooms.	NEIDL RA Appendix D	Inadequate pathogen accountability
55	A waste treatment tank steam valve failed, resulting in severe damage to the maximum biocontainment laboratory that was being used as a BSL-3 lab at the time (SARS virus research). The NIH Occupational Safety and Health Branch previously had been informed about problems with the valve but elected to defer repairs. No exposures resulted.	NEIDL RA Appendix D	Flooding inside laboratory
56	A laboratory worker became ill after working with a diagnostic culture that was later identified as a select agent.	House Hearings 2008	Inadequate pathogen accountability

#	Scenario description	Reference	Candidate event group
57	Both doors of the double-door biocontainment entryway were propped open by laboratory staff while experiments were in progress. Infectious materials were not being handled at the time.	NEIDL RA Appendix D	Breach of containment (wall cracks or open doors)
58	Unlikely exposure to biohazardous waste that contained sealed culture plates containing select agent.	House Hearings 2008	Contaminated waste
59	H2N2 from the 1957-1958 flu pandemic was accidentally distributed to 2,750 labs in the United States, along with 3,747 labs in 18 countries. The error was discovered in March by a participating lab in Canada. Sent out notifications on April 8 and 12, 2004. No infections were reported as of April 12, 2005.	NEIDL RA Appendix D	Inadequate pathogen accountability
60	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
61	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
62	Three laboratory workers became ill after working with the wild-type select agent instead of what they believed as the excluded select agent.	NEIDL RA Appendix D	Inadequate pathogen accountability
63	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
64	A sharp object punctured a boot but did not break the skin.	NEIDL RA Appendix D	Puncture - general
65	Potential exposure—individuals were possibly exposed when culture fluid was discovered in the bottom of a centrifuge.	House Hearings 2008	Centrifuge release
66	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
67	A fan servicing two BSCs and the general laboratory space failed. Audible alarms on the cabinets and air pressure monitors had been turned off. Loss of primary and secondary biocontainment occurred.	NEIDL RA Appendix D	HVAC[b] failure
68	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
69	Discrepancy in shipment—3 vials not shipped.	House Hearings 2008	Inadequate pathogen accountability
70	Potential exposure—the BSC fan was turned off while work was being performed in the BSC, and a nasal swab confirmed exposure to an excluded strain.	House Hearings 2008	HVAC failure
71	A laboratory worker potentially was exposed by a culture plate that was dropped outside the BSC.	NEIDL RA Appendix D	Container leak/spill/open
72	A laboratory worker sliced through two pairs of gloves while handling a rat carcass infected with *Y. pestis*. The worker was sent to a medical emergency room, which released her and asked her to return for a follow-up visit.	NEIDL RA Appendix D	Puncture - during necropsy
73	Unlikely exposure—infected lung tissue spattered on disposable lab gown of a laboratory worker vaccinated against the select agent.	House Hearings 2008	Spill/splash
74	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability

#	Scenario description	Reference	Candidate event group
75	The door from a fumigation area to a buffer zone was opened spontaneously approximately 2cm. The lab was shut down for annual maintenance at the time. Equipment and supplies had been surface decontaminated but not yet fumigated. The annual maintenance program was continued with full fumigation of all spaces and no untoward event occurred.	NEIDL RA Appendix D	Breach of containment (wall cracks or open doors)
76	Percutaneous trauma from an instrument (presumably a hypodermic syringe) that had been used on an infected animal. Possible infection with select agent. Medical personnel administered antibiotic prophylaxis.	NEIDL RA Appendix D	Puncture - needlestick
77	Inventory discrepancy—entity could not account for three infected mice. It was determined that the mice were cannibalized by other mice in the cage or buried under the bedding and autoclaved by mistake by the animal care staff.	House Hearings 2008	Inadequate pathogen accountability
78	1,025 vials were shipped to a Palm Beach, Florida, laboratory. Two of the vials were missing caps, and a third vial had a loosened cap. A subsequent shipment to a second laboratory contained an incorrect number of vials. Two workers possibly were exposed to *Bacillus anthracis*. The workers were prophylactically treated with antibiotics	NEIDL RA Appendix D	Container leak/spill/open
79	Three mice experimentally infected with *Yersinia pestis* went missing from a biocontainment lab.	NEIDL RA Appendix D	Inadequate animal control
80	Potential exposure—two laboratory workers opened a package that contained leaking tubes on open bench.	House Hearings 2008	Container leak/spill/open
81	A student potentially was exposed to infectious aerosols from a broken vial inside a centrifuge. Medical evaluation was provided.	NEIDL RA Appendix D	Centrifuge release
82	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
83	Discrepancy in shipment—1 vial not shipped.	House Hearings 2008	Inadequate pathogen accountability
84	Inventory discrepancy—Individual failed to note that a container with a select agent had been destroyed.	House Hearings 2008	Inadequate pathogen accountability
85	Inventory discrepancy—inventory reported as missing because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability
86	Inventory discrepancy—individual failed to note isolates that had been destroyed.	House Hearings 2008	Inadequate pathogen accountability
87	Potential exposure—laboratory workers opened a package that contained an unknown liquid.	House Hearings 2008	Inadequate PPE use
88	Discrepancy in shipment—incorrect number of vials was shipped by sender.	House Hearings 2008	Inadequate pathogen accountability
89	Handled slides not properly irradiated, but acetone fixed.	NEIDL RA Appendix D	Pathogen used with inappropriate biocontainment
90	Unlikely exposure—water supply broke inside lab causing a flood. Water samples were negative.	NEIDL RA Appendix D	Flooding inside laboratory

#	Scenario description	Reference	Candidate event group
91	A researcher contracted brucellosis during improper disinfection of aerosolization chamber. She later required prolonged administration of intravenous and oral antibiotics. Responsible officials did not report the infection to federal authorities, as required by federal law, until April 11, 2007, in response to an inquiry from the Sunshine Project. CDC issued a cease and desist order to TAMU on April 20, 2007, that was expanded on June 30 to include work with all select agents. Other serious violations were found during a site visit inspection in July 2007.	NEIDL RA Appendix D	Pathogen not inactivated
92	Unlikely exposure—entity confirmed no exposure to select agent when a plate containing a select agent dropped to the floor (the plate landed upside down).	House Hearings 2008	Container leak/spill/open
93	Potential exposure to select agent when a laboratory worker failed to turn on an autoclave.	House Hearings 2008	Pathogen not inactivated
94	Inventory discrepancy—inventory reported as missing because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability
95	Potential exposure—laboratory worker working with diagnostic sample on bench top.	House Hearings 2008	Pathogen used with inappropriate biocontainment
96	Potential exposure of five individuals when a broken vial containing a select agent was discovered in a centrifuge.	House Hearings 2008	Centrifuge release
97	Potential exposure—a laboratory worker working with diagnostic sample on bench top.	House Hearings 2008	Pathogen used with inappropriate biocontainment
98	A centrifuge secondary container lid broke during centrifugation of virus, causing the rotor to become unbalanced. The researcher noted loss of volume in one viral tube and, suspecting viral leakage, undertook decontamination of the centrifuge, centrifuge tube, work area, adjacent equipment and himself.	NEIDL RA Appendix D	Centrifuge release
99	Potential exposure—nine individuals worked with the bacterium without using BSL-3 biocontainment precautions. APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	NEIDL RA Appendix D	Inadequate PPE use
100	A laboratory worker potentially was exposed when opening an autoclave bag containing the bacterium. The bag had not been decontaminated.	NEIDL RA Appendix D	Pathogen not inactivated
101	Potential exposure to two people when the cap of a liquid culture containing the bacterium came off in a shaking incubator.	NEIDL RA Appendix D	Container leak/spill/open
102	Laboratory workers potentially were exposed to the bacterium when a tube of liquid culture in a shaking incubator appeared to be cracked.	NEIDL RA Appendix D	Container leak/spill/open
103	Inventory discrepancy—during inventory reconciliation, entity determined that the vial was never filled.	House Hearings 2008	Inadequate pathogen accountability
104	Potential exposure—employee cut arm with box cutter in a room in which select agent work is performed.	House Hearings 2008	Puncture - general
105	Potential exposure—laboratory worker working with diagnostic sample on bench top.	House Hearings 2008	Pathogen used with inappropriate biocontainment
106	Potential exposure—lab worker was bitten by an infected guinea pig.	House Hearings 2008	Animal bite/scratch
107	An employee was bitten on the hand by an infected monkey. The skin appeared to be broken in two or three places.	NEIDL RA Appendix D	Animal bite/scratch

#	Scenario description	Reference	Candidate event group
108	Potential exposure—three laboratory workers exposed while sniffing plates that contained select agents.	House Hearings 2008	Inadequate PPE use
109	Two culture plates of the bacterium were dropped on the floor; the lid came off one of the plates. Concluded by CDC to have been an unlikely exposure.	NEIDL RA Appendix D	Container leak/spill/open
110	Viable select agent (*B. anthracis*) was taken from BSL-3 biocontainment to BSL-2 biocontainment.	NEIDL RA Appendix D	Pathogen used with inappropriate biocontainment
111	Unlikely exposure when a laboratory worker dropped two culture plates containing a select agent (lid intact or plate face down).	House Hearings 2008	Container leak/spill/open
112	Unlikely exposure when notebook was removed from a lab where work was performed on a select agent. The room had been decontaminated with vaporized hydrogen peroxide before removal.	House Hearings 2008	Contamination inside laboratory
113	Potential release due to leakage problem with collection tank that might have contained a select agent.	House Hearings 2008	Liquid waste leak
114	The center reported leaks of contaminated waste three times in November and December. One worker cut his finger while preparing a pipe for repairs. The worker potentially was exposed to *B. suis*. APHIS/CDC form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) was filed.	NEIDL RA Appendix D	Liquid waste leak
115	An inventory discrepancy was noted in the number of mice held for incineration after completing an experiment. Entity determined that the employee left a dead mouse inside a cage and that it was autoclaved and destroyed with the bedding.	House Hearings 2008	Inadequate animal control
116	Inventory discrepancy—an infected mouse was discovered missing. Entity determined that mouse was likely autoclaved with bedding material and disposed of.	House Hearings 2008	Inadequate animal control
117	Laceration on tip of third finger from contact with animal cage.	NEIDL RA Appendix D	Puncture - general
118	Separation of BSL-4 filter from suit.	NEIDL RA Appendix D	PPE failure (excluding PAPR[c])
119	Potential exposure—an employee was bitten by a pig infected with *B. suis*. The worker potentially was exposed to *Brucella* sp.	NEIDL RA Appendix D	Animal bite/scratch
120	An experimentally infected monkey scratched the hand of a laboratory worker. The skin was broken, potentially infecting the worker. The worker received medical treatment including antibiotic therapy.	NEIDL RA Appendix D	Animal bite/scratch
121	Potential exposure from needlestick with a syringe that had been used on mice inoculated with the bacterium.	NEIDL RA Appendix D	Puncture - needlestick
122	Personnel entered BSL-3 biocontainment area without wearing required PPE.	NEIDL RA Appendix D	Inadequate PPE use
123	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
124	Potential exposure—laboratory worker working with a diagnostic sample on bench top.	House Hearings 2008	Pathogen used with inappropriate biocontainment
125	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open

#	Scenario description	Reference	Candidate event group
126	Tube leakage occurred inside a centrifuge used for concentration of *B. anthracis* cells. Four people apparently were exposed to *B. anthracis*. Prophylaxis was refused. No infections resulted. Procedures were modified to require that centrifuge buckets be opened only within a BSC and inspected and decontaminated after each use.	NEIDL RA Appendix D	Centrifuge release
127	Potential exposure—dirty mouse cage dropped inside BSL-3 suite.	NEIDL RA Appendix D	Container leak/spill/open
128	A potential exposure due to a leaking autoclave bag was reported.	NEIDL RA Appendix D	Container leak/spill/open
129	An employee potentially was exposed while working with the bacterium without using BSL-3 biocontainment precautions.	NEIDL RA Appendix D	Inadequate PPE use
130	A package shipped via commercial carrier was lost in transit.	NEIDL RA Appendix D	Transportation mishap
131	Potential exposure when notebooks were removed from a lab where work was performed on a select agent.	House Hearings 2008	Contamination inside laboratory
132	Unlikely exposure—airflow found to be reversed for BSL-3 lab. During the period of reversed airflow, the laboratory was unoccupied.	House Hearings 2008	HVAC failure
133	Potential exposure—an employee was scratched by a broken rib during necropsy.	NEIDL RA Appendix D	Puncture - general
134	A lightning strike knocked out electricity to BSL-3 and unoccupied BSL-4 biocontainment areas. Circuit breakers that should have remained engaged were tripped. Backup generators failed to start. Negative directional airflow was not maintained. A battery-powered system provided power to lights and doors for 15–20 minutes.	NEIDL RA Appendix D	Loss of power
135	Potential exposure when notebooks were removed from a lab where work was performed on a select agent.	House Hearings 2008	Contamination inside laboratory
136	Potential exposure—environmental surveillance indicated presence of the bacterium on a freezer handle, light switch, and shoes in the hot side of the change room.	NEIDL RA Appendix D	Contamination inside laboratory
137	Potential exposure—needlestick with a syringe containing a select agent during necropsy.	NEIDL RA Appendix D	Puncture - needlestick
138	Loss—possible loss of a select agent.	House Hearings 2008	Inadequate pathogen accountability
139	Potential exposure—needlestick with a syringe containing a select agent.	House Hearings 2008	Puncture - needlestick
140	Potential exposure—an employee dropped a culture vessel of the bacterium, splashing it onto a lab coat, pants, and shoes.	NEIDL RA Appendix D	Container leak/spill/open
141	A needle stick with a syringe containing the virus was reported.	NEIDL RA Appendix D	Puncture - needlestick
142	An employee was bitten by a ferret inoculated with a virus.	NEIDL RA Appendix D	Animal bite/scratch
143	Potential exposure—an employee was bitten by a rat that had been inoculated with bacterium.	NEIDL RA Appendix D	Animal bite/scratch
144	Potential exposure—employee stabbed by broken capillary tube that might have been used for PCR testing for *B. anthracis*.	House Hearings 2008	Puncture - general
145	A needle stick with syringe containing the bacterium was reported.	NEIDL RA Appendix D	Puncture - needlestick
146	Three vials discovered missing during inspection of entity.	House Hearings 2008	Inadequate pathogen accountability

#	Scenario description	Reference	Candidate event group
147	An outbreak of foot and mouth disease was confirmed at a farm in Surrey, United Kingdom. It was concluded that the virus likely originated from the nearby Pirbright Research and manufacturing site, because of construction activities surrounding a leaking drainage pipe. A subsequent outbreak on September 12, 2007, was shown by genetic analysis to be unrelated to the Pirbright facilities.	NEIDL RA Appendix D	Contamination outside laboratory
148	A needlestick with syringe containing a virus was reported.	NEIDL RA Appendix D	Puncture - needlestick
149	Potential exposure to a select agent when a laboratory worker failed to turn on an autoclave.	House Hearings 2008	Pathogen not inactivated
150	A graduate student accidentally broke a flask containing *B. anthracis* cells.	NEIDL RA Appendix D	Container leak/spill/open
151	An employee potentially was exposed by being stuck by a broken scalpel blade.	NEIDL RA Appendix D	Puncture - general
152	V-shaped rip in suit. Positive airflow inside the suit prevented contact with room air.	NEIDL RA Appendix D	PPE failure (excluding PAPR)
153	Brass connector on a suit fell apart.	NEIDL RA Appendix D	PPE failure (excluding PAPR)
154	Door from necropsy room into chemical shower inadvertently opened. The door had to be manually closed through the chemical shower, breaking lab containment. The lab was then completely decontaminated and repairs made to the malfunctioning door-closing device. No human or environmental exposure occurred.	NEIDL RA Appendix D	Breach of containment (wall cracks or open doors)
155	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
156	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
157	Inventory discrepancy because of poor record keeping.	House Hearings 2008	Inadequate pathogen accountability
158	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
159	A bird caused a Georgia Power transformer to fail, knocking out electricity to BSL-3 biocontainment areas. Backup generators failed to start, leaving the labs without main electrical power for 75 minutes. CDC personnel did not attempt to override and start the backup generators. Negative directional airflow was not maintained. No exposures or infections were reported. Backup failure was determined to be due to removal of two generators from service for upgrades. Their absence caused a power fluctuation when main power was lost, resulting in shutdown of the entire backup generator system.	NEIDL RA Appendix D	Loss of power
160	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
161	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
162	Inventory discrepancy—entity determined that the vial was left unfilled from an initial spore stock preparation.	House Hearings 2008	Inadequate pathogen accountability

#	Scenario description	Reference	Candidate event group
163	An animal care technician was bitten by a macaque that was part of a vaccine study of tuberculosis.	NEIDL RA Appendix D	Animal bite/scratch
164	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
165	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
166	ULPA [ultra low penetration air] filters used in manufacturing aerosol biocontainment enclosures for flow cytometers were found to be faulty. Eight of 10 filters from 3 different lots tested at one site (Yale University, Jan. 22, 2009) failed to meet performance specifications. Seven of nine units tested at a second site (Duke University, Jan 26, 2009) failed to meet performance criteria.	NEIDL RA Appendix D	HVAC failure
167	Scientist dropped a plate containing the Junin virus on the floor. The spill was decontaminated and reported after proper procedures were performed. No virus escape, no personnel infection.	NEIDL RA Appendix D	Container leak/spill/open
168	An employee found four vials of material that were not in the institution's inventory. A complete inventory was performed resulting in more than 9,000 vials being discovered and reported.	NRC 2010	Inadequate pathogen accountability
169	A mouse escaped during weighing operations and ran up a worker's arm. Located the mouse more than 10 minutes later on left shoulder upon donning PPE. No bites or scratches were noticed. The mouse was determined to not be shedding virus (previously infected with MPXV).	NIH 2011b	Inadequate animal control
170	A laboratory researcher wearing protective gloves experienced a needlestick from a syringe suspected to contain the Ebola virus. The researcher was quarantined in Hamburg University Hospital for observation. Treatment included use of the experimental Feldmann vaccine. The worker did not become ill. No further information concerning potential seroconversion.	NEIDL RA Appendix D	Puncture - needlestick
171	Five technicians potentially were exposed to *B. anthracis* as a result of a protocol violation. Cultivated *B. anthracis* cells routinely are killed in the BSL-3 area and, after confirmation of death, are moved to the BSL-2 area. In this incident, the cells were moved to the BSL-2 area before confirmatory tests were completed. Test results later showed that the cells had not been killed. Cell suspensions had been handled within a BSC in the BSL-2 area, but as a precaution, all workers were given prophylactic antibiotic treatment.	NEIDL RA Appendix D	Pathogen used with inappropriate biocontainment
172	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
173	Scientist working with weakened strain of *Y. Pestis* that was considered harmless to humans died of the plague. Subsequent investigation yielded that the scientist had heochromatosis, which causes an excessive buildup of iron in the body.	CDC 2011	Inadequate PPE use
174	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
175	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open

#	Scenario description	Reference	Candidate event group
176	A microbiology researcher at BU sought medical attention for laboratory-acquired bacteremia and meningitis. Molecular typing determined the infecting strain was the same strain he had been working with. Work with *N. meningitis* is conducted at BSL-2 using BSL-3 precautions (respiratory protection provided by Class II BSC). Intravenous antibiotics were administered, and the researcher recovered fully. University experts determined the researcher did not consistently wear appropriate PPE and did not consistently follow appropriate safe microbiological practices. It was surmised that the researcher touched his gloved hand to his face while working with the bacterium [Ad Hoc Committee, 2010 #16418]. All laboratory members repeated laboratory safety retraining and organism-specific retraining.	NEIDL RA Appendix D	Inadequate PPE use
177	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
178	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
179	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
180	Mouse dander was found outside a mouse cage that was to be completely sealed to contain potentially infectious bioaerosols associated with infected mice. Seven researches were given antibiotic treatment as a precaution. Work in the BSL-3 lab was halted pending an evaluation.	NEIDL RA Appendix D	Contamination inside laboratory
181	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
182	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
183	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
184	The FBI closed its case files on the 2001 theft and deliberate release of *B. anthracis* spores, concluding that a USAMRIID scientist had acted alone. At least 22 people were infected and 5 were killed.	NEIDL RA Appendix D	Malevolent act
185	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
186	A parenteral (injection) exposure was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Puncture - needlestick
187	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open
188	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open

#	Scenario description	Reference	Candidate event group
189	Worker observed an ear-tagged mouse while working in BSL-2 laboratory. The mouse had escaped from BSL-3 biocontainment. Worker caught the mouse and secured it in a flask. The mouse was euthanized inside a BSC. No injuries or illnesses were reported.	NIH 2011a	Inadequate animal control
190	Power failure of a PAPR unit occurred, apparently as a result of its on/off switch unknowingly being pressed against the hard surface of a chair back. Power loss was noticed after a few seconds; power was restored by pressing the on/off switch. Respiratory protection was not provided by the PAPR for a short time. Incident details would suggest no exposure occurred. The worker was evaluated by proper authorities. The manufacture was notified of the deficiency in equipment design. SOP was modified to require placement of PAPR motor unit to the side of the belt when using a chair.	NEIDL RA Appendix D	PAPR failure (addressed separately from other PPE)
191	The intake HEPA filter fell off the PAPR unit during use and could have been loose for a prolonged period. The worker reattached the filter and continued working. Respiratory protection was not provided by the PAPR for a short time. There is no mention of whether gloves were changed before handling the filter. Oseltamivir prophylaxis was initiated. Respiratory specimens collected at 24, 36, and 72 hours were negative (PCR). SOPs were modified to include verification of PAPR HEPA filter before entry into biocontainment areas.	NEIDL RA Appendix D	PAPR failure (addressed separately from other PPE)
192	Exhaust ventilation failed several times in 1980s. Staff was not immunized against *C. burnetti*, so repeated ventilation problems caused sealing of animal room from the laboratory area in 1988. Animal studies were dropped for several years. No infections occurred.	NIH 2004	HVAC failure
193	Two researchers contracted a virus in laboratories became infected after using defective gloves. Exposed through cuts on hands.	CRG 2007	PPE failure (excluding PAPR)
194	Over two dozen agents went missing at USAMRIID. Agents subject to removal without authorization.	CRG 2007	Inadequate pathogen accountability
195	LAIs at USAMRIID. Chikungunya was needlestick; vaccinia was cutaneous.	NRC 2010	Puncture - needlestick
196	Research fellow hospitalized for pneumonia in left lung. *C trachomatis* isolated and specific sero-conversion was documented. Uneventful recovery with antibiotic therapy. Researcher did sonication of cultured organism in Class II BSC 2 days before onset of illness. Three large-scale purifications of organisms done during 3-week period before illness. The specific source of infection remained indeterminate. All procedures were reviewed with staff. Particle masks were adopted for all aerosol-generating procedures and for 30 minutes after completion of those. Centrifuge rotors were to be opened only in BSC, and both the instrument and rotors were to be examined for leaks after each run. Alcohol-soaked sponges were required to surround the sonication tube, and all worker faces were to stay outside the glass front of the BSC. No further infections have occurred since.	NIH 2004	Centrifuge release
197	A spill was reported to NIH under reporting requirements of the NIH Guidelines for Research Involving Recombinant DNA Molecules.	NEIDL RA Appendix D	Container leak/spill/open

#	Scenario description	Reference	Candidate event group
198	An experimentally infected mouse died and later could not be accounted for. Laboratory personnel suggested the dead mouse was overlooked, was in the cage during cage sterilization, and was discarded with the sterilized bedding.	NEIDL RA Appendix D	Inadequate animal control
199	A ferret experimentally infected with H5N1 virus bit a technician on the thumb. The worker was placed on home quarantine for 5 days and directed to wear a mask to protect others.	NEIDL RA Appendix D	Animal bite/scratch
200	Centrifuge was in the room, not in a BSC. A worker was working with one liter of *Coxiella burnetii* slurry. The worker placed 165 mL of slurry into each of six 250-mL polypropylene centrifuge tubes and failed to insert O-rings or tighten the screw-on centrifuge caps.	DOE 2008	Centrifuge release
201	A scientist working with Ebola virus in the maximum containment laboratory setting sustained a percutaneous (through broken skin) exposure to the virus but did not recognize the exposure and thus did not report it.	NIH 2007	Puncture - general
202	During the necropsy of an NHP [non-human primate] infected with the recombinant monkeypox, the virologist sustained an unrecognized percutaneous exposure due to a breach in the integrity of his glove.	NIH 2007	Puncture - general
203	A male virologist-physician removed what he believed to be a BSL-2 arenavirus (Tacaribe) from the laboratory freezer on a Monday morning and expanded the virus in a cell culture system. On Thursday, high-speed centrifugation of cell culture fluids in the laboratory resulted in some spillage through a crack in the seal of a centrifuge bottle. Two other workers were present in the lab but were remote from the centrifuge. The virologist notified his coworkers of the spill and he cleaned it up. Co-worker 1 chose to leave the lab and go on a coffee break, while co-worker 2 remained at the bench remote from the centrifuge during cleanup. The spill was not considered significant by any worker and was not reported.	NIH 2007	Centrifuge release
204	A lab chief who was studying the pathogenesis of the RVFV causing the epidemic in east Africa asked a post-doctoral fellow to package a vial of virus for transportation to a colleague's lab across the country. In doing so, the post-doc placed a small amount of dry ice in the infectious agent shipping container and sealed the container preparing it for courier pickup. The courier placed the package in the back of a step van and proceeded to drive down the street (Albany Street, Tyng Road, or Camp Sargent Road) toward a main road when he hit a pot hole. The jarring of the package caused the over-pressurized shipping container to explode frightening the driver who crashed the van into a utility pole by the road. RVFV was released into the interior of the van. Local emergency responders handle the incident; the driver, while shaken up and experiencing a mild concussion, was not otherwise injured.	NIH 2007	Transportation mishap
205	A 250-mL flask containing 50 mL of the spore suspension was dropped from a height of 0.75 m on the floor.	Bennet and Parks 2006	Container leak/spill/open
206	A universal bottle containing 15 mL of the standard spore suspension was slowly spilled from a height of 0.9 m to simulate the effect of a spill on a bench running on to the laboratory floor.	Bennet and Parks 2006	Container leak/spill/open
207	Dropped three 50-mL bottles, each containing 15 mL of culture. Three bottles in a rack were dropped 1 m to the floor.	Bennet and Parks 2006	Container leak/spill/open

#	Scenario description	Reference	Candidate event group
208	A peristaltic pump was primed from a 50-mL reservoir of the spore suspension. The outlet tubing was then blocked and both the pump and samplers were operated. The increase in back-pressure caused a connector in the outlet tubing to become detached and the suspension was sprayed at the wall of the room.	Bennet and Parks 2006	Spill/splash
209	Four fungal plates with extensive growth of *Penicillium* sp. and heavy surface growth of spores were dropped on the laboratory floor.	Bennet and Parks 2006	Container leak/spill/open
210	An outdated Sorvall GSA rotor had its 'O' ring seal removed and had 10 mL of a 5E9 spore suspension gently pipetted into the rotor chamber. The rotor was accelerated to 4,000 rev/min in a RC5B centrifuge, braked, and the centrifuge lid opened while the air samplers were operated.	Bennet and Parks 2006	Centrifuge release
211	A set of sealed rectangular centrifuge buckets with screw-down lids was tested to find out if they generated microbial aerosols. It was found that the buckets were contained when the bucket seal was in place and applied with silicone grease supplied by the manufacturer. However, aerosols were generated when the seal was not in place. In experiment 9a, the bucket contained two overfilled Falcon centrifuge tubes containing a 9E9 spore/mL suspension and in experiment 9b a 50-mL spill of the same suspension was rolled around inside the bucket so that some of the fluid would rest on the inside walls of the lid before centrifugation.	Bennet and Parks 2006	Centrifuge release
212	Four plates contained 3-day-old colonies of *B. atrophaeus*, which were slightly dry, were dropped on the laboratory floor.	Bennet and Parks 2006	Container leak/spill/open
213	A 1-liter flask containing 200 mL of a 9E8 spore/mL suspension was placed on the rack of a Gallenkamp chest-shaking incubator in a totally unsecured position. The shaker was then operated at 100 rev/min, and the samplers were switched on. The flask smashed almost immediately, and the shaker lid was opened 30 seconds after the broken glass pieces had settled.	Bennet and Parks 2006	Centrifuge release
214	A spill of 18 mL of *B. atrophaeus* spore suspension was created by spilling the contents of a universal container from about 0.8 m. This is a similar scenario to experiment 4, which consisted of a 15-mL spill. The tests were carried out with a 9.1E5 (a), 9.1E6 (b), 9.1E7 (c) and 9.1E8 (d) spore/mL suspension.	Bennet and Parks 2006	Container leak/spill/open
215	A GSA rotor was overfilled with 10 mL of spore suspension as in experiment 8. The centrifuge was accelerated up to 4,700 rev/min within a minute, braked, and the lid opened while the samplers were operated for a 10-minute period. This experiment was carried out with the four different suspensions used in experiment 12.	Bennet and Parks 2006	Centrifuge release
216	Procedure violation creates sharps (scissors, scalpels, sharp lab surfaces, other glass items including reagent bottles, vials, blood tubes, capillary tubes, microscope slides).	Tetra Tech 2009	Puncture - during necropsy
217	Equipment malfunction creates sharps (scissors, scalpels, sharp lab surfaces, other glass items including reagent bottles, vials, blood tubes, capillary tubes, microscope slides).	Tetra Tech 2009	Puncture - during necropsy
218	Procedure violation results in ingestion from inadvertent contact between mucous membranes and contaminated surfaces or hands.	Tetra Tech 2009	Hand to mouth/eyes/nose contamination
219	A laboratory equipment malfunction results in ingestion from inadvertent contact between mucous membranes and contaminated surfaces or hands.	Tetra Tech 2009	Hand to mouth/eyes/nose contamination

#	Scenario description	Reference	Candidate event group
220	Procedure violation results in aerosol production and inhalation (centrifuge, grinding, homogenizing, blending, vigorous shaking or mixing, sonic disruption, cell separator, etc.).	Tetra Tech 2009	Centrifuge release
221	Equipment malfunction results in aerosol production and inhalation (centrifuge, grinding, homogenizing, blending, vigorous shaking or mixing, sonic disruption, cell separator, etc.).	Tetra Tech 2009	Centrifuge release
222	Procedure violation results in aerosol production and inhalation from opening pressurized containers.	Tetra Tech 2009	Centrifuge release
223	Laboratory equipment malfunction results in aerosol production and inhalation from opening pressurized containers.	Tetra Tech 2009	Container leak/spill/open
224	Animal handling procedure violation results in bites, scratches.	Tetra Tech 2009	Animal bite/scratch
225	Animal handling equipment malfunction results in bites, scratches.	Tetra Tech 2009	Animal bite/scratch
226	Animal handling procedure violation results in needlesticks.	Tetra Tech 2009	Puncture - needlestick
227	Animal handling equipment malfunction results in needlesticks.	Tetra Tech 2009	Puncture - during necropsy
228	Animal handling procedure violation results in ingestion from inadvertent contact between mucous membranes and contaminated surfaces or hands.	Tetra Tech 2009	Hand to mouth/eyes/nose contamination
229	Animal handling equipment malfunction results in ingestion from inadvertent contact between mucous membranes and contaminated surfaces or hands.	Tetra Tech 2009	Hand to mouth/eyes/nose contamination
230	Animal handling procedure violation results in aerosol production and inhalation (inoculating animals intranasally, harvesting infected tissue from animals).	Tetra Tech 2009	Animal-related aerosol
231	Animal handling equipment malfunction results in aerosol production and inhalation (inoculating animals intranasal, harvesting infected tissue from animals).	Tetra Tech 2009	Animal-related aerosol
232	Animal handling or insectary procedure violation or equipment malfunction results in escaped animal or arthropod.	Tetra Tech 2009	Animal escape or pathogen release to environment
233	Procedure violation results in incomplete sterilization/ disinfection of solid waste.	Tetra Tech 2009	Contaminated waste
234	Procedure violation results in incomplete sterilization/ disinfection of solid waste.	Tetra Tech 2009	Contaminated waste
235	Procedure violation results in incomplete sterilization/disinfection of liquid waste	Tetra Tech 2009	Contaminated waste
236	Equipment malfunction results in incomplete sterilization/ disinfection of liquid waste; this also includes inadvertent sewage vessel blowdown or overpressure event.	Tetra Tech 2009	Contaminated waste
237	Thermal treatment tanks for accumulated liquid waste fail before treatment in a seismic event; untreated liquid waste enters public system.	Tetra Tech 2009	NPH[d] - earthquake
238	Loss of power and failure of emergency backup causes loss of negative pressure in potentially contaminated areas.	Tetra Tech 2009	Loss of power
239	Severe low-pressure front (hurricane) causes reversal of air flow.	Tetra Tech 2009	NPH - strong wind
240	Incomplete chemical disinfection of HEPA filter plenum during maintenance results in pathogen leak.	Tetra Tech 2009	Pathogen not inactivated

#	Scenario description	Reference	Candidate event group
241	Equipment malfunction during HEPA filter change out during maintenance results in pathogen leak.	Tetra Tech 2009	HVAC failure
242	Seismic event causes loss of power and failure of HEPA filter plenums.	Tetra Tech 2009	NPH - earthquake
243	Loss of power and failure of emergency backup causes loss of negativity in BSL-4 spaces and door seals fail.	Tetra Tech 2009	Loss of power
244	Laboratory supply and exhaust registers both in the ceiling produces incomplete room ventilation and stagnant areas in laboratory; contamination of PPE and LAI results.	Tetra Tech 2009	HVAC failure
245	Re-entrainment of facility exhaust into intake plenum for office spaces and other unfiltered spaces.	Tetra Tech 2009	NPH - strong wind
246	Facility ventilation dP [differential pressure]control based on supply-side parameters fails to react to exhaust damper closing or their failure to open after being closed; this causes facility pressurization and possible loss of containment.	Tetra Tech 2009	HVAC failure
247	Suit breach from crush, pinch, puncture (air-lock doors, quick disconnects, movement of equipment, suit puncture or tear).	Tetra Tech 2009	PPE failure (excluding PAPR)
248	Suit breach from procedural error—inadequate pre-use inspection fails to locate breach in suit.	Tetra Tech 2009	PPE failure (excluding PAPR)
249	Personnel error leads to suit breach from heat/cold, dehydration from physical exertion and dry air supply, hypothermia and hot surfaces from autoclaves and other surfaces, hypoxia (air flow restriction).	Tetra Tech 2009	PPE failure (excluding PAPR)
250	Personnel error leads to suit breach from physical and sensory isolation, claustrophobia.	Tetra Tech 2009	PPE failure (excluding PAPR)
251	Procedure violation during specimen transport inside facility results in spill (slip, trip, fall, drop, jostle, jar, impact).	Tetra Tech 2009	Container leak/spill/open
252	Equipment malfunction during specimen transport/storage inside facility results in spill (slip, trip, fall, drop, jostle, jar, impact) poor or inadequate packaging/transport system, or failure of storage environment.	Tetra Tech 2009	Container leak/spill/open
253	Procedure violation during specimen transport inside facility results in spill (slip, trip, fall, drop, jostle, jar, impact).	Tetra Tech 2009	Container leak/spill/open
254	Equipment malfunction during specimen transport/storage inside facility results in spill (slip, trip, fall, drop, jostle, jar, impact) poor or inadequate packaging/transport system, or failure of storage environment.	Tetra Tech 2009	Container leak/spill/open
255	Procedure violation during specimen transport inside facility results in spill (slip, trip, fall, drop, jostle, jar, impact).	Tetra Tech 2009	Container leak/spill/open
256	Equipment malfunction during specimen transport/storage inside facility results in spill (slip, trip, fall, drop, jostle, jar, impact) poor or inadequate packaging/transport system, or failure of storage environment.	Tetra Tech 2009	Container leak/spill/open
257	Internal flooding from failure of process piping, fire suppression piping, or similar system.	Tetra Tech 2009	Flooding inside laboratory
258	Procedure violation when handling, processing, sterilizing mixed rad/bio-waste/equipment (solid or liquid) resulting in incomplete pathogen destruction.	Tetra Tech 2009	Pathogen not inactivated
259	Equipment malfunction when handling, processing, sterilizing mixed rad/bio waste/equipment (solid or liquid) resulting in incomplete pathogen destruction.	Tetra Tech 2009	Pathogen not inactivated

#	Scenario description	Reference	Candidate event group
260	Procedure violation using radioactive materials creates mechanism for contamination, aerosol generation, ingestion, inoculation.	Tetra Tech 2009	Pathogen not inactivated
261	Procedure violation during necropsy results in LAI because of cut/puncture, ingestion, or inhalation.	Tetra Tech 2009	Puncture - during necropsy
262	Equipment malfunction during necropsy results in LAI because of cut/puncture, ingestion, or inhalation.	Tetra Tech 2009	Puncture - during necropsy
263	Deflagration of natural gas or other flammable process gas leak causing BSC failure, laboratory, or main structure failure fire might result; personnel contamination, room contamination, ventilation system leakage around, through HEPA.	Tetra Tech 2009	Deflagration
264	Overpressure from blockage in steam line leading to autoclave failure or process steam line failure, personnel contamination, room contamination, ventilation system leakage around, through HEPA filters, environmental contamination.	Tetra Tech 2009	Contamination inside laboratory
265	Deflagration of formaldehyde or other flammable agent during laboratory disinfection or sanitization, personnel contamination, room contamination, structural failure, loss of containment, ventilation system leakage around, through HEPA filters.	Tetra Tech 2009	Deflagration
266	Deflagration of unanticipated chemical reaction leading to BSC failure, personnel contamination, room contamination, ventilation system leakage around, through HEPA filters.	Tetra Tech 2009	Deflagration
267	Deflagration/explosion and fire external to the facility involving the supply of diesel, fuel oil, gasoline leading to facility breach, personnel contamination, room contamination, possible environmental contamination.	Tetra Tech 2009	Deflagration
268	Fire from deflagration of natural gas or other flammable process gas leak causing BSC failure, personnel contamination, room contamination, ventilation system leakage around, through HEPA filters.	Tetra Tech 2009	Fire
269	Deflagration of anticipated or unanticipated chemical reaction leading to BSC failure, personnel contamination, room contamination, possible ventilation system leakage around, through HEPA filters.	Tetra Tech 2009	Deflagration
270	Fire from flammable process liquids causing BSC failure, personnel contamination, room contamination, possible ventilation system leakage around, through HEPA filters.	Tetra Tech 2009	Fire
271	Fire from buildup of combustibles (poor combustible control in laboratories) causing BSC failure, personnel contamination, room contamination, possible ventilation system leakage around, through HEPA filters.	Tetra Tech 2009	Fire
272	Fire from fuel accumulation external to the facility; supply of diesel, fuel oil, gasoline burns leading to facility breach, personnel contamination, room contamination, possible environmental contamination.	Tetra Tech 2009	Fire
273	Helicopter or small airplane crash into facility (DOE-STD-3014 scenario) causes structure failure; significant environmental and public contamination.	Tetra Tech 2009	Aircraft crash
274	Wildfire breaches facility boundary and reaches fuel accumulation external to the facility; supply of diesel, fuel oil, gasoline burns leading to facility breach, personnel contamination, room contamination, possible environmental contamination.	Tetra Tech 2009	NPH - other

#	Scenario description	Reference	Candidate event group
275	Liquid waste process piping leak or other source of contamination (equipment malfunction) leads to contamination spread	Tetra Tech 2009	Liquid waste leak
276	Procedure violation during material or waste handling or transfer leads to contamination spread.	Tetra Tech 2009	Contamination inside laboratory
277	Equipment malfunction during material or waste handling or transfer leads to contamination spread.	Tetra Tech 2009	Contamination inside laboratory
278	Procedure violation leads to poor housekeeping and contamination spread.	Tetra Tech 2009	Contamination inside laboratory
279	Procedure violation or equipment failure during shipping-receiving activities (dry ice incident) results in aerosol production and inhalation from opening pressurized containers.	Tetra Tech 2009	Transportation mishap
280	Shipment handling violation results in facility contamination (failure to meet federal biomaterial transportation requirements) results in shipment with broken containers, external contamination, site and personnel contamination.	Tetra Tech 2009	Transportation mishap
281	Misidentification and site contamination (failure to meet federal biomaterial transportation requirements) results in inadequate handling and personnel contamination (high-level pathogen in low-level confinement with inadequate PPE).	Tetra Tech 2009	Pathogen used with inappropriate biocontainment
282	Over-the-road (failure to meet federal biomaterial transportation requirements) results in shipment with broken containers and external contamination not confined to site.	Tetra Tech 2009	Transportation mishap
283	Air cargo contamination (failure to meet federal or international biomaterial transportation requirements) results in shipment with broken containers, external contamination, and greater extent of contamination because of lack of discovery after many airports affected.	Tetra Tech 2009	Transportation mishap
284	Ocean-going contamination (failure to meet federal or international biomaterial transportation requirements) results in shipment with broken containers and external contamination.	Tetra Tech 2009	Transportation mishap
285	Procedure violation and improper intra-site packaging, unpacking, material handling results in broken containers, personnel contamination, and external contamination	Tetra Tech 2009	Container leak/spill/open
286	Seismic event exceeds facility design criteria and structure fails; significant environmental and public contamination.	Tetra Tech 2009	NPH - earthquake
287	Seismic event challenges or exceeds facility design criteria and structure fails, subsequent fire(s) start from ignition sources in laboratories; significant environmental and public contamination.	Tetra Tech 2009	NPH - earthquake
288	High winds (hurricane) challenge or exceed facility design criteria and structure fails; significant environmental and public contamination.	Tetra Tech 2009	NPH - strong wind
289	High winds (hurricane) generate projectiles that challenge or exceed facility design criteria and structure fails; significant environmental and public contamination.	Tetra Tech 2009	NPH - strong wind
290	High water (floods) challenge or exceed facility design criteria and structure damaged; significant environmental and public contamination.	Tetra Tech 2009	NPH - other
291	Snow and ice challenge or exceed facility design criteria and structure fails; significant environmental and public contamination.	Tetra Tech 2009	NPH - other
292	Loss of power from lightning or other source causes loss of negativity; environmental and public contamination.	Tetra Tech 2009	Loss of power

#	Scenario description	Reference	Candidate event group
293	A potential release of a biological agent in the form of a liquid aerosol resulting from a BSL-4 laboratory accident. For the purposes of this MCE analysis, the highest volume used in centrifugation would be six completely filled 250-mL (8.45-fluid-ounce) bottles of cell culture supernatant that contains 108 plaque-forming units (PFUs) per milliliter, for a total of 1.5×10^{11} PFUs. Assuming that all six bottles break, a viral aerosol would be created within the rotor. It is also assumed that the rotor gasket fails to contain any aerosol generated.	USAMRMC 2006	Centrifuge release
294	Escape of an infected animal, terrorist acts, and external acts.	USAMRMC 2006	Inadequate animal control
295	An MCE analysis was developed for potential release of a biological agent in the form of a liquid aerosol resulting from work in BSL-3 facilities. The MCE scenario for a BSL-3 laboratory accident occurs during the processing of a 1-liter (0.26-gallon) slurry. In this scenario, a laboratory worker fails to use rubber O-rings to seal the centrifuge tubes and fails to properly tighten the safety centrifuge caps designed to prevent leakage into the centrifuge compartment that houses the rotor. All six tubes spill slurry into the rotor cups, and some of the slurry leaks into the rotor compartment, which is not sealed against the release of organisms in a small-particle aerosol.	USAMRMC 2006	Centrifuge release
296	A researcher is handling a 15-cc conical tube containing a powder-like preparation of purified *B. anthracis* containing 1×10^{10} spores. The cap fits loosely. The researcher accidentally drops the tube on the bare, stainless steel surface of the properly operating BSC.	NIH 2005	Container leak/spill/open
297	Loss of power.	NIH 2009	Loss of power
298	Malfunction of solid and liquid waste disposal systems.	NIH 2009	Contaminated waste
299	Transportation accident.	NIH 2009	Transportation mishap
300	Site security failure.	NIH 2009	Malevolent act
301	Personnel security failure.	NIH 2009	Malevolent act
302	Fomites bearing transmissible agents.	NIH 2009	Animal escape or pathogen release to environment
303	Vector-borne agent release.	NIH 2009	Animal escape or pathogen release to environment
304	Procedural errors resulting in inadvertent infection (e.g., mislabeled tubes).	NIH 2009	Inadequate pathogen accountability
305	Malevolent actions.	NIH 2009	Malevolent act
306	Suicide bomber/airplane attack/truck with explosives/fire.	NIH 2009	Malevolent act
307	Disgruntled or deranged lab worker spreads agents in community.	NIH 2009	Malevolent act
308	Three researchers became skin-test positive for tuberculosis after using a newly acquired aerosolization chamber for experimental infection of animals.	NEIDL RA Appendix D	Centrifuge release
309	Malfunction of the HVAC system pulled potentially contaminated air out of the BSL-3 biocontainment area and into a clean hallway. Nine workers were tested for possible exposure to the bacterium, and no infections were diagnosed. The HVAC system was brought back into compliance. Duct tape was used to seal the door and remained in place as of June 2008.	NEIDL RA Appendix D	HVAC failure

#	Scenario description	Reference	Candidate event group
310	Researchers were working under BSL-2 biocontainment protocol with what was believed to be a non-infectious vaccine strain of the bacterium. Later, it was determined the bacterial culture also contained the infectious wild-type strain that requires BSL-3 biocontainment precautions. Investigation was unable to determine the cause for the mixed culture. Two researchers became infected with *F. tularensis* in May and were not correctly diagnosed until a third scientist became infected with the bacterium in September. An investigation revealed that researchers had failed to follow proper BSL-2 biocontainment protocol and that the university failed to identify work-related illness in laboratory staff and failed to immediately report suspicious work-related illness to local and state health departments. Biosafety policies and SOPs were revised accordingly.	NEIDL RA Appendix D	Pathogen used with inappropriate biocontainment
311	Bat bite through double gloves. No infection occurred.	NEIDL RA Appendix D	Animal bite/scratch
312	Highly concentrated virus was suddenly aerosolized when worker opened chamber to add a bit more fluid without closing the nitrogen pressure tank and bleeding off pressure. Laboratory was mopped for several hours with glutaraldehyde, and finally decontaminated with formaldehyde gas. No infection occurred in two exposed workers. There was no breach in BSL-4 containment, and no infections occurred in neighboring open-air monkey colonies on the campus.	NEIDL RA Appendix D	Container leak/spill/open
313	One each glove and suit tear. Workers followed for 3 weeks for fever. No infection occurred.	NEIDL RA Appendix D	PPE failure (excluding PAPR)
314	Animal bite; animals being inoculated with Hantavirus. Pre-inoculation bite from rat.	NEIDL RA Appendix D	Animal bite/scratch
315	Needlestick to worker before setting up an inoculum with mouse-adapted Ebola virus. No infection occurred.	NEIDL RA Appendix D	Puncture - needlestick
316	Autoclave door interlock failed, and a load not autoclaved was opened but not handled. No infections resulted.	NEIDL RA Appendix D	Pathogen not inactivated
317	Multiple events over the years of outer gloves or suits developing tears or holes or disconnects of air supply to suits detected during work. Incidents were investigated and followed up. No treatments ever used and no infections resulted.	NEIDL RA Appendix D	PPE failure (excluding PAPR)
318	A laboratory worker thawed a frozen vial of bacterial suspension and inoculated a plate culture on the open bench top instead of within a BSC. Eight laboratory workers became infected, one being asymptomatic. The outbreak was most consistent with airborne spread. The seven symptomatic workers were given antibiotic therapy. One relapsed and required alternative therapy. Enhancements to laboratory SOPs were recommended by the Department of Epidemiology and the Infectious Diseases Division.	NEIDL RA Appendix D	Container leak/spill/open
319	Earthquake up to design basis.	DOE 2008	NPH - earthquake

Notes:
a. PPE = personal protective equipment
b. HVAC = heating, ventilating, and air conditioning system
c. PAPR = powered air-purifying respirator
d. NPH = natural phenomena hazards

As explained previously, the incidents and postulated events identified in Table E-1 were combined into groups of similar initiators, which are identified in the last column of Table E-1. Table E-2 lists the initiating event groups. The candidate initiating events groups were carried forward for further consideration.

Table E-2. Candidate initiating event groups

#	Candidate event group
1	Aircraft crash
2	Animal bite/scratch
3	Animal escape or pathogen release to environment
4	Animal-related infectious aerosol
5	Breach of containment (wall cracks or open doors)
6	Centrifuge release
7	Container leak/spill/open
8	Contaminated waste (e.g., not inactivated)
9	Contamination inside laboratory
10	Contamination outside laboratory
11	Deflagration
12	Fire
13	Flooding inside laboratory
14	Fomite/vector
15	Hand to mouth/eyes/nose contamination
16	HVAC[a] system failure
17	Inadequate animal control
18	Inadequate pathogen accountability
19	Inadequate PPE[b] use
20	Liquid waste leak
21	Loss of power
22	Malevolent act
23	NPH[c]—earthquake
24	NPH—tornado and strong wind
25	NPH—other (flood, snow, etc.)
26	PAPR[d] failure (addressed separately from other PPE)
27	Pathogen not inactivated
28	Pathogen used with inappropriate biocontainment
29	PPE failure (excluding PAPR[c])
30	Puncture—during necropsy
21	Puncture—needlestick
32	Puncture—general
33	Spill/splash
34	Transportation mishap

Notes:
a. HVAC = heating, ventilating, and air conditioning system
b. PPE = personal protective equipment
c. NPH = Natural phenomena hazards
d. PAPR = Powered air-purifying respirator

E.3 References

Bennett, A., and S. Parks 2006. Microbial Aerosol Generation during Laboratory Accidents and Subsequent Risk Assessment. *Journal of Applied Microbiology*, ISSN 1364-5072, accepted October 6, 2005, Volume 100 (2006) pages 658-663. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2005.02798.x/full>. Accessed August 18, 2010.

CDC (Center for Disease Control and Prevention) 2011. *Fatal Laboratory-Acquired Infection with an Attenuated* Yersinia pestis *Strain - Chicago, Illinois, 2009*, Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report, Volume 60, No. 7, February 25, 2011. Available on the internet at: <http://www.cdc.gov/mmwr/pdf/wk/mm6007.pdf>. Accessed February 28, 2011.

CRG 2007. *Mistakes Happen: Accidents and Security Breaches at Biocontainment Facilities*, The Council for Responsible Genetics. (CRG), 2007. http://www.b-safe.ch/downloads/Accidents%20and%20biosecurity%20breaches%20worldwide.pdf

DHS (Department of Homeland Security) 2008. *National Bio and Agro-Defense Facility Environmental Impact Statement,* Science and Technology Directorate, Office of National Laboratories, U.S. Department of Homeland Security, Washington, D.C., Appendix E, December 2008.

DOE (U.S. Department of Energy). 2008, *Final Revised Environmental Assessment for The Proposed Construction and Operation of a Biosafety Level 3 Facility at Lawrence Livermore National Laboratory, Livermore, California*, DOE/EA-1442R, Department of Energy, National Nuclear Security Administration, Livermore Site Office, January 2008. Available on the internet at: < http://cms.doe.gov/sites/prod/files/nepapub/nepa_documents/RedDont/EA-1442-FEA-2008.pdf > Accessed June 22, 2009.

Emond 1977. *A Case of Ebola Virus Infection*, Edmond RTD, Evans B, Bowen ETW, and Lloyd G. British Medical Journal, Volume 2, pages 541-544, August 27, 1077.

Harding, A.L., and K.B. Byers. 2006. Epidemiology of Laboratory-Associated Infections in *Biological Safety: Principles and Practices*, 4th ed., D.O. Fleming and D.L. Hunt, eds. American Society of Microbiology Press, Washington, DC.

House Hearings 2007. Germs, Viruses, and Secrets: The Silent Proliferatioh of Bio-Laboratories in the United States. edited by House of Representatives Committee on. Energy and. Commerce. Washington, D.C.: U.S. Government Printing Office, unpublished Exhibit 13.

National Research Council 2010. *Evaluation of the Health and Safety Risks of the New USAMRIID High Containment Facilities at Fort Detrick, Maryland.* Committee to Review the Health and Safety Risks of High Biocontainment Laboratories at Fort Detrick Board on Life Sciences Division on

Earth and Life Studies, National Research Council of the National Academies, 2010. <http://www.nap.edu/openbook.php?record_id=12871>. Accessed August 26, 2010.

NIH (National Institutes of Health) 2004. *Final Environmental Impact Statement (FEIS) on the Rocky Mountain Laboratories Integrated Research Facility in Hamilton, Montana,* National Institute of Health, National Institute of Allergy and Infectious Diseases (NIAID), Appendix D, 2004.

NIH (National Institutes of Health) 2005. *Final Environmental Impact Statement National Emerging Infectious Diseases Laboratories*. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD. Available on the internet at: <http://www.bu.edu/neidl/files/2010/07/NEIDL-Final-Environmental-Impact-Statement.pdf >. Accessed March 18, 2009.

NIH (National Institutes of Health) 2005a. *Final Environmental Impact Statement for the Galveston National Laboratory for Biodefense and Emerging Infectious Diseases Research Facility in Galveston, Texas*. National Institutes of Health, Division of Occupational Health and Safety, Bethesda, MD, Chapter 3 and Appendices E and F, 2005.

NIH (National Institutes of Health) 2007. *Draft Supplementary Risk Assessments And Site Suitability Analyses for the National Emerging Infectious Diseases Laboratory*. National Institutes of Health, Division of Occupational Health and Safety, Bethesda, MD, 2007. <http://www.nems.nih.gov/aspects/nat_resources/programs/nepa2.cfm>. Accessed July 2, 2011.

NIH (National Institutes of Health) 2009. *NIH Blue Ribbon Panel to Advise on the Risk Assessment for the BU National Emerging Infectious Diseases Laboratories—Teleconference with the National Research Council on Technical Input,* presentation on April 7, 2009, slide 21. <http://nihblueribbonpanel-bumc-neidl.od.nih.gov/docs/2009/April/BRP_NRC_Teleconf_April_7.pdf>. Accessed July 27, 2009.

NIH (National Institutes of Health) 2010. *Template for Reporting Incidents Involving Recombinant DNA to the NIH Office of Biotechnology Activities (OBA)*, National Institutes of Health, Office of Biotechnology Activities, Bethesda, MD, September 25, 2010.

NIH (National Institutes of Health) 2010a. *Template for Reporting Incidents Involving Recombinant DNA to the NIH Office of Biotechnology Activities (OBA)*, National Institutes of Health, Office of Biotechnology Activities, Bethesda, MD, August 5, 2010.

Rusnak 2004. *Risk of Occupationally Acquired Illnesses from Biological Threat Agents in Unvaccinated Laboratory Workers*, Rusnak JM, Kortpeter MG, Hawley RJ, Anderson AO, Boudreau E, and Eitzen E, Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science, Volume 2, Number 4, 2004.

Tetra Tech 2009. *Risk Analyses and Reports on the Operation of the Boston University Medical Center National Emerging Infectious Diseases Laboratories – 25% Draft Risk Assessment*, Tetra Tech Inc., Security and Protective Services Group, April 3, 2009

USAMRIID (U.S. Army Medical Research Institute of Infectious Diseases) 2006, *Final Environmental Impact Statement, Construction and Operation of the New U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Facilities and Decommissioning and Demolition and/or Re-use of Existing UASMRIID Facilities at Fort Detrick, Maryland*, December 2006, Appendix C, Hazard Analyses.

Wampler, R.A., and T.S. Blanton. 2001. *Volume V: Antrax at Sverdlovsk, 1979, U.S. Intelligence on the Deadliest Modern Outbreak*. National Security Archive Electronic Briefing Book No. 61. R.A. Wampler and T.S. Blanton eds. <http://www.gwu.edu/~nsarchiv/NSAEBB/NSAEBB61/index2.html>. Accessed July 20, 2011.

Appendix F. Event Sequence Analysis

Contents

ABREVIATIONS AND ACRONYMS

µm	micrometer
1918 H1N1V	1918 H1N1 influenza virus
AC	aerosol concentration
AIHA	American Industrial Hygiene Association
ANDV	Andes virus
APF	airborne protection factor
B. anthracis	*Bacillus anthracis*
BDB	beyond design basis
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BMC	Boston Medical Center
BR	breathing rate
BRP	Blue Ribbon Panel
BSC	biological safety cabinet
BSL	biosafety level
BU	Boston University
BUMC	Boston University Medical Center
$CCID_{50}$	median cell culture infective doses
CDC	Center for Disease Control and Prevention
CFM	cubic feet per minute
CFU	colony forming units
DOE	U.S. Department of Energy
EBOV	Ebola virus
EPA	Environmental Policy Agency
EX	exposure
F. tularensis	*Francisella tularensis*
FAA	Federal Aviation Administration
FFU	fluorescent focus unit
GAO	General Accounting Office
HVAC	heating, ventilation, and air conditioning
IACS	Innovation Academy Charter School
JUNV	Junín virus
km	kilometer

LAI	laboratory-associated infection
LASV	Lassa virus
LLNL	Lawrence Livermore National Laboratory
LPF	leak path factor
m	meter
MA ESE	Massachusetts Elementary and Secondary Education
MACCS2	MELCOR Accident Consequence Code System, version 2
MARV	Marburg virus
MDOT	Massachusetts Department of Transportation
MEI	maximally exposed individual
mi	mile
$MICLD_{50}$	median mouse intracerebral lethal doses
MID_{50}	median mouse infective dose
mL	milliliter
MRF	maximum reasonably foreseeable
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NEIDL	National Emerging Infectious Diseases Laboratory
NEPA	National Environmental Protection Agency
NHDOT	New Hampshire Department of Transportation
NIH	National Institute of Health
NIPV	Nipah virus
NRC	National Research Council of the National Academies
PAPR	powered air-purifying respirator
PEP	Population Estimate Program
PFU	plaque forming units
PPE	personal protective equipment
RA	*Risk Assessment (RA) of the National Emerging Infectious Diseases Laboratories (NEIDL) at Boston University Medical Center (BUMC)*
RVFV	Rift Valley fever virus
RWDI	Rowan Williams Davies & Irwin Inc
s	second(s)
SARS-CoV	SARS-associated coronavirus
SC	suspension concentration

SECPOP	Sector Population program
SF	spray factor
SOP	standard operating procedure
T	time (duration) of exposure
TBEV-FE	tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus)
$TCID_{50}$	median tissue culture infective dose
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
V	volume
Y. pestis	*Yersinia pestis*
χ/Q	chi over Q—downwind dilution factor from atmospheric dispersion

F. Event Sequence Analysis

F.1 Introduction

F.1.1 Overview

This appendix to the *Risk Assessment (RA) of the National Emerging Infectious Diseases Laboratories (NEIDL) at Boston University Medical Center (BUMC)* provides the analyses of potential loss of biocontainment events selected in Appendix E for analysis. The frequency and exposure categories presented in Appendix E are also used in this appendix. There are several additional analyses that must be performed as inputs to the potential loss of biocontainment analyses. The input analyses provided in this appendix are as follows:

- *Biocontainment features*—The frequency and consequence of an potential loss of biocontainment event is dependent on the biocontainment features. A brief description of the biocontainment features is provided.
- *Inventory*—The pathogen and animal inventories are a required input for all loss of biocontainment analyses.
- *Airborne dispersion analysis*—An analysis of the airborne dispersion is required for all potential aerosol release events (i.e., an earthquake).
- *Population estimates*—Population estimates are required to determine the population consequences for all potential aerosol release events (i.e., an earthquake).

The potential events resulting in a potential loss of biocontainment addressed in this appendix are as follows:

- *Earthquake*—An earthquake was selected as the maximum reasonably foreseeable[1] (MRF) event, defined here as the event that results in the largest release from the facility.
- *Aircraft crash*—The aircraft crash was expected to pose less risk than the earthquake, so this analysis confirms that expectation.
- *Centrifuge release*—The centrifuge release event provides representative results for numerous other events, as explained in Appendix E. Biosafety level (BSL) 3 or BSL-4 laboratory centrifuge release events are analyzed.

[1] The term *reasonably foreseeable* extends to events that may have catastrophic consequences, even if their probability of occurrence is low, provided that the analysis of the impacts is supported by credible scientific evidence, is not based on pure conjecture, and is within the rule of reason (DOE 2002).

- *Needlestick*—The needlestick event provides representative results for numerous other events, as explained in Appendix E. BSL-3 and BSL-4 centrifuge release events are analyzed.
- *Malevolent acts*—Malevolent act scenarios are identified in Chapter 6 are assigned a probability and a determination was made as to whether a release of a pathogen could be reasonably expected to occur if the adversary was successful in accomplishing their mission. The consequences of malevolent acts are also compared to the consequences of accidental loss of biocontainment events, consistent with the recommendations of the DOE NEPA Guidance (DOE 2002).

F.1.2 General Guidance

This RA complies with the National Environmental Policy Act (NEPA) requirements governing disclosure of incomplete and unavailable information (Title 40 of the *Code of Federal Regulations* [CFR] section 1502.22). Consistent with these requirements, this appendix identifies the limitations of the data used, discusses the options and significance of assumptions made, and provides a basis for use of these assumptions and data. The results presented herein reflect the uncertainty in both the data and the models used for this evaluation.

NEPA guidance provided by the U.S. Department of Energy (DOE), *Recommendations for Analyzing Accidents under the National Environmental Policy Act* (DOE 2002, referred to as the DOE NEPA Guidance) was also used as guidance for this analysis. That guidance was selected because it is the most relevant and detailed guidance available for this type of analysis. The National Research Council of the National Academies reviewed the DOE NEPA Guidance and concluded the following (National Research Council 2010, page 15):

> U.S. Department of Energy's (DOE) recommendations for the preparation of EISs contain some of the most detailed explanations and guidelines for discussing human health impacts in an EIS. Although DOE's recommendations for analyzing human health effects are limited to exposure to radiation and chemicals, they also are relevant to pathogen exposures.

A key element of the DOE NEPA Guidance for the accident analyses is the application of a *sliding scale*. This sliding scale allows for adjustment of the level of detail of an accident analysis in accordance with the frequency and consequences of the accident, and the level of information available. While realism is important, the DOE NEPA Guidance also supports use of *bounding* (i.e., analyses that are based on conservative assumptions that envelope potential factors) when its use is consistent with the sliding scale approach. Bounding approaches can have several potential benefits including streamlining the analysis

and potentially being more defensible than more rigorous approaches because they are unlikely to underestimate potential accident consequences (DOE 2002).

F.1.3 Historic Incident Data

Operational incidents have occurred at BSL-3 and BSL-4 facilities (see Appendix D) and the data provide insights into the types of incidents that might occur at NEIDL. Appendix D includes the recent Center for Disease Control and Prevention (CDC) report of 395 "potential release events" and 7 laboratories associated infections (LAIs) from 2003 to 2009 nationwide at laboratories working with select agents (NRC 2011).

The operating experience was used to identify potential initiating events, develop scenarios, and estimate the scenario frequencies. While the operational data were useful, there are a number of limitations of the data that limit its usefulness, especially quantitatively. The incident data cannot be used to estimate the frequency for various events for multiple reasons including the following:

- Biocontainment features and protocols have been improving over time and the historic data do not fully reflect the features in place at the NEIDL.
- Incidents are underreported because of fear of reprisal and the stigma associated with such events (Harding and Byers 2006), with approximately half going unreported (Rosenstock 2000).
- The source of exposure for the majority of laboratory related illness (82 percent) is not known (Harding and Byers 2006). As a result, any derived estimate of the true number of such incidents is uncertain and could be underestimated.
- The number of operational hours associated with the reported incidents is not known.
- The incidents are often not described in sufficient detail to determine the full chain of events. For example, the description "loss of containment" could refer to spills, centrifuge releases, or other types of events.

Therefore, reports of historic incidents were used as an input to identifying candidate events, developing scenarios, and estimating frequency categories in a qualitative manner.

F.1.4 Frequency Categories

The likelihood of events can be described and calculated in several ways. Table F.1-1 compares several equivalent ways of describing (using numbers and measures) the likelihood of events.

Table F.1-1 Measures of likelihood.

Average Return Period[a] (years)	Average Frequency[b] (per year)	Probability / Chance of Occurrence in Facility Lifetime[c] (in 50 years)	
1	1	Virtually 100%	Virtually 100-in-100
10	0.1	99%	99-in-100
100	0.01	39%	1-in-2.5
1,000	0.001	4.9%	1-in-21
10,000	0.0001	0.5%	1-in-200
100,000	0.00001	0.05%	1-in-2000
1,000,000	0.000001	0.005%	1-in-20,000
10,000,000	0.0000001	0.0005%	1-in-200,000

[a] **Average return period in years**: This is the average time, in years, before the event would be expected to occur. If the event was to occur multiple times, this would be the average time between occurrences. This way of describing events is often used in characterizing flood levels; for example, a "1000-year flood" is a water level that is estimated to occur with a 1,000-year average return period or "once per 1,000 years".

[b] **Average frequency per year:** This is the average number of occurrences of the event per year.

[c] **Probability / chanceof occurrence in facility lifetime (50 years)**: This is the chance that the event would occur at least once in a given 50-year period.

The operational data from research laboratories like the NIEDL (see Appendix D) are not adequate for development of quantitative frequency estimates (e.g., mean rate plus uncertainties) for the events analyzed, so alternate approaches were used. A technique commonly used when quantitative estimates of frequency are not possible is the use of categories (i.e., ranges of values), which is used by the U.S. Environmental Protection Agency (EPA), U.S. Department of Energy (DOE), and National Aeronautics and Space Administration (NASA). (EPA 1987, NASA 2005, NASA 2009, DOE 1994) Each event sequence is assigned to a frequency category based the initiating event, and the number and nature of concurrent failures of preventive and mitigative features. The assignment of frequency categories often relies on comparison with events in other industries and use of judgment. For this analysis, when it was not clear which of two frequency categories should be assigned, the higher frequency category was used to avoid underestimating the risk. Table F.1-2 identifies the frequency categories (i.e., A, B, C, and D) used in this RA and provides a verbal description and average return period for each category.

Table F.1-2 Frequency categories

Category	Verbal description	Average return period (1 in "this many" years)
A	An event sequence was assigned to this category if its likelihood is sufficiently high to assume that it will occur during the operational lifetime of the NEIDL (i.e., during 50 years of operation).	1 to 100 years
B	An event sequence was assigned to this category if one or more of the events in this category could occur during the NEIDL's operating life, but any specific event sequence in the group is not expected to occur.	100 to 10,000 years
C	An event sequence was assigned to this category if collectively none of the events in this category is expected to occur during the operating life of the facility, but the events in this category are still reasonably foreseeable.	10,000 to 1 million years
D	An event sequence was assigned to this category if it does not meet the criteria for *reasonably foreseeable*. An event sequence is categorized as category D if it is impossible or highly improbable (i.e., beyond reasonably foreseeable).	>1 million years

F.1.5 Exposure Categories

The exposure categories were defined in terms of the number of people potentially exposed to a pathogen by an event sequence. The number of people potentially exposed can vary depending on the type of event and the operational parameters at the time of the event. For example, the number of people in a room can vary from event to event depending on the activities involved. In addition, some events, such as a needle stick, might affect only one person, while another event such as an aerosolized pathogen release could affect multiple people in the room. Therefore, the exposure categories were generally defined as a range in the number of people potentially exposed. Table F.1-3 defines the exposure categories for each potentially exposed group because the total population and potential for exposure is different for each group.

Table F.1-3. Exposure categories

Exposure category	Laboratory workers	Facility workers	Members of the public (number of people within the radius)
NONE	0	0	≤ 30 m
LOW	A single individual (1)	A few individuals on the same floor (≤10)	> 30 m to ≤ 300 m
MODERATE	Most individuals in the room (1 to 4)	Most individuals on the floor (≤ 87 for BSL-3 and ≤ 30 for BSL-4)	> 300 m to ≤3 km
HIGH	An atypical number of individuals (>4)[a]	Most individuals in the building (≤ 300)	> 3 km

a Although the possibility exists, it is not expected that activities will involve more than four workers in a room (BUMC 2009e).

F.2 BIOCONTAINMENT FEATURES

Biosafety in Microbiological and Biomedical Laboratories (CDC and NIH 2007), referred to as BMBL, defines *containment* (the term biocontainment will be used here) as

> safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

A number of biocontainment features associated with the NEIDL can be grouped as administrative controls (which include laboratory practices and techniques), safety equipment, and the facility design and construction. BMBL (CDC and NIH 2007) and the *BUMC Biosafety Manual* (BUMC 2011) identify and include discussions of the various biocontainment features for each BSL. The biocontainment features that play a key role in these analyses are the following:

Administrative Controls:

1. Procedures
2. Training

Safety Equipment:

3. Container
4. Storage systems
5. Powered air-purifying respirator (PAPR)
6. Positive-pressure suit
7. Centrifuge rotor or buckets/cups
8. Centrifuge chamber
9. Biological safety cabinet (BSC)
10. Personal protective equipment (PPE) (Note: This does not include respiratory protection such as a respirator and a positive-pressure suit, which are addressed separately)

Facility Design and Construction:

11. Heating, ventilation, and air conditioning (HVAC) system
12. Sealed walls, floors, and ceilings
13. Alarms

The following paragraphs describe the NEIDL biocontainment features associated with the analyses in this appendix. Chapter 2 (Facility and Site Description) and Appendix A (Facility Design and Operations) also provide descriptions of these as well as other NEIDL biocontainment features.

1. *Procedures*— – The BUMC Biosafety Manual (BUMC 2011) (Appendix W) provides requirements for development of NEIDL standard operating procedures (SOPs). The BUMC Biosafety Manual and the required NEIDL-specific SOPs minimize the potential for loss of biocontainment and direct the laboratory worker's responses in case of a potential loss of biocontainment. Requirements for safe storage and use of pathogens are a key part of the procedures. Section 2.1.4 of Chapter 2 provides additional details on administrative controls.
2. *Training*—The training requirements for NEIDL laboratory workers are outlined in the BUMC Biosafety Manual Appendix F for BSL-3 and Appendix G for BSL-4. The training program educates employees about the hazards to which they might be exposed and instructs them on the proper protocols to be used for safe operation of the NEIDL. Section 2.1.4 of Chapter 2 provides additional details on administrative controls.
3. *Container*—Pathogens must be stored using double containment. Both the inner and outer containers must be durable and leak-proof (BUMC 2011). Proper storage of pathogens is governed by the Occupational Safety and Health Administration and Commonwealth of Massachusetts regulations (105 CMR 480.000) (BUMC 2011). Containers provide the first (i.e., inner-most) biocontainment barrier.
4. *Storage systems*—Pathogens will be placed in freezers, refrigerators, and other storage systems when not in use, which provides another biocontainment barrier. Frozen samples are far less susceptible to airborne release than unfrozen samples, though the containers may potentially be more susceptible to failure.
5. *PAPR*—Laboratory workers are required to use a hooded, PAPR for all BSL-3 activities. The airborne protection factor (APF) for the PAPR used in NEIDL is 1,000 (BUMC 2010; NIH 2010).
6. *Positive-pressure suit*—In BSL-4, a one-piece totally encapsulating, positive-pressure personnel suit supplied with HEPA-filtered external air is required for all activities, including centrifugation (BUMC 2011).
7. *Centrifuge rotor or buckets/cups*—There are two types of rotors (i.e., fixed angle and swinging bucket) that hold the individual sample containers in the centrifuge. Both fixed-angle rotors and swinging buckets used in the NEIDL BSL-3 and BSL-4 laboratories will have aerosol-tight (i.e., O-ring seals), thereby providing a containment barrier to potential aerosol releases during centrifugation. The rotors or buckets will be prepared for and opened after centrifugation in

BSCs, which includes loading the containers, balancing the rotor or buckets, disinfecting surfaces, and installing the rotor or bucket lids (BUMC 2011, BUMC 2009a).)

8. *Centrifuge chamber*—The centrifuge has an enclosure that surrounds the rotor during centrifugation. The chamber includes a door interlocked with the control system that prevents operation unless the door is closed and prevents opening of the door until the rotor has stopped spinning. The chamber door on all centrifuges can reduce potential aerosol release to some extent (BUMC 2009a).
9. *BSC*—Rotor loading and unloading operations must be performed only inside a BSC (BUMC 2011). The directional air flow of the BSC ensures that any aerosol released when a rotor or bucket is opened is confined by the BSC and filtered by its HEPA filtration. A discussion of BSCs is presented in Appendix A of BMBL and Appendix C of the BUMC Biosafety Manual.
10. *PPE*—The specific PPE used for centrifuge operation is dependent on the pathogen, and concentrations involved, but will always include protective clothing, eye protection (the respirator could serve this function), and double gloves (BUMC 2009a). While respirators are considered to be PPE, they are addressed separately because of their significance for reducing the inhalation hazard and the pathogen-specific requirement to use them.
11. *HVAC*—The HVAC system provides mitigative protection to laboratory workers, facility workers, and the public from potential airborne releases. The HVAC features providing that protection include the following (BUMC 2011, 2009b; CDC and NIH 2007; NIH 2008):
    - Single-pass airflow that does not recycle potentially contaminated air.
    - Directional air flow control for BSL-3 and differential pressure control for BSL-4 to ensure that airborne contamination flows from areas of least biohazard to areas of greatest potential biohazard.
    - A minimum of eight air exchanges per hour for BSL-2, BSL-3, and BSL-4 laboratories, which reduces the concentration of the entrained aerosol over time.
    - HEPA filtration on the inlet for the BSL-4 HVAC system.
    - Exhaust air is filtered before being discharged up the stack. The BSL-3 air is filtered through a single HEPA filter, and the BSL-4 air is filtered through two HEPA filters in series.
    - Backflow prevention on the air supply inlet to prevent release in case of a loss of airflow. BSL-4 dampers are air-tight bubble-dampers and the BSL-3 dampers are low-leakage dampers.
    - Redundant exhaust fans that provide adequate airflow in case of a loss of fan.

- Isolation dampers (airtight dampers for BSL-3 and low-leakage dampers for BSL-3) automatically controlled in case of a loss of directional airflow for BSL-3 or pressure differential for BSL-4.
- Emergency generators and battery-backed power supplies in case of a loss of off-site power.

12. *Sealed walls, floors, and ceilings*—The NEIDL BSL-3 and BSL-4 laboratories are designed and constructed to minimize the potential airborne releases from potentially contaminated areas even in case of a total loss of HVAC airflow. The BSL-3 and BSL-4 areas have sealed floors, ceilings, and walls, including sealed wall penetrations. Access to the laboratories is controlled via interlocked doors that prevent both doors from being opened simultaneously. Airtight doors isolate areas of greatest biohazard in BSL-4 laboratories. Pressure tests in the NEIDL's BSL-4 laboratory show that half of the negative pressure differential will be maintained for 20 minutes in case of a loss of active HVAC. Those features greatly reduce the potential spread of airborne contamination to other areas of the NEIDL or outside the building (BUMC 2011, 2009c; CDC and NIH 2007; NIH 2008).
13. *NEIDL structure*—The NEIDL structure has been designed to withstand severe natural phenomena such as earthquakes. Attachment D of this appendix provides a brief summary of NEIDL structural design features important to this analysis.
14. *Alarm and communications systems*—The NEIDL has alarm and communications systems that alert workers to potentially dangerous systems, such as HVAC upsets.

Biocontainment features can be preventive, mitigative, or a combination of both. Preventive features are structures, systems, or components that prevent the loss of biocontainment, such as an aerosol-tight container. Mitigative features are structures, systems, or components that reduce the consequences given that a loss of biocontainment does occur, such as respiratory protection. The role of each of the individual biocontainment features is specific to each event sequence.

F.3 NEIDL INVENTORY

F.3.1 Introduction

F.3.1.1 Inventory Types

A key factor affecting the consequences associated with potential accidents involving loss of biocontainment at the NEIDL is the pathogen inventory (i.e., quantity, concentration, and form of the pathogen). For example, greater concentrations of pathogens in liquid suspension may result in greater

airborne concentrations for aerosol release events. The inventory is also important because it defines part of the NEIDL operating conditions covered by the RA. Animals (arthropods and mammals) that could be experimentally infected also are important for events that can involve the escape of one or more animals.

NEIDL will contain pathogens in master, seed, and working stock inventories as well as infected animals. In general, a pathogen sample received at NEIDL is used to generate a master stock, which is promptly converted to the seed stock, which in turn is used to generate the working stock as needed. There can be multiple strains of a given pathogen in NEIDL, and each strain would have its own master stock, seed stock, working stock, and infected animal inventories. Those inventory stocks are defined as follows:

***Master stock*—**Upon receipt of a new pathogen sample and after appropriate testing, a master stock is cultured. The master stock has a close and well-documented link to the original sample received at the laboratory and is the source of the seed stock. The identity, purity, and stability of the master stock is verified before being converted to the seed stock. If seed stock inventories become depleted, a new master stock inventory can be cultured from the remaining seed stock, which was potentially derived directly from the original master stock. Generation or regeneration of a seed stock is an infrequent occurrence and is expected to occur about once every one to five years for a pathogen.

***Seed stock*—**The seed stock is created by dividing the master stock into small vials (generally 1 to 2 milliliters [mL]), which are frozen until they used. As the name implies, the seed stock is used to grow the working inventories used in the research activities.

***Working stock*—**When work with a pathogen is to be undertaken, a vial of the seed stock is thawed and used to start a new culture of the pathogen. Working stocks are the cultures used in daily research.

***Infected animal*—**Activities at the NEIDL will include animals (mammals and arthropods) infected with various pathogens as part of the research.

F.3.1.2 Concentration Units of Measure

Various methods are used to quantify the concentration of bacteria and viruses in a liquid suspension. As a result of the differences between those methods, there are different units of measure for the concentration. For bacteria, the most common unit of measure is the colony forming unit (CFU) per milliliter; for viruses, there are multiple different units of measure. The measurement and units of

measure are dependent on the method used to quantitate the virus. Below are the units used to quantify the concentration in this RA.

Colony forming unit (CFU)—A measure of viable cells in which a colony represents growth from either a single cell, or an aggregate of cells, derived from a single progenitor cell. CFU is used to describe the number of viable bacterial cells per unit of measure, e.g., per mL of liquid (Hung et al. 2005).

Fluorescent focus unit (FFU)—The fluorescent-focus assay is a modification of the plaque assay that uses a fluorescent stain to detect virus microscopically and permit quantitation. The FFU assay is useful in detecting viruses that do not destroy host cells (Flint et al. 2009).

Median cell culture infective dose ($CCID_{50}$)—The minimum concentration of virus particles needed to produce, in a given cell line culture under specified conditions, a detectable cytopathic effect in 50 percent of exposed cell cultures (Flint et al. 2009).

Median mouse infective dose (MID_{50})—The minimum concentration of virus required to cause infection in 50 percent of exposed mice (Flint et al. 2009).

Median mouse intracerebral lethal dose ($MICLD_{50}$)—The minimum concentration of virus required to cause death in 50 percent of mice exposed by intracerebral injection (Flint et al. 2009).

Median tissue culture infective dose ($TCID_{50}$)—The terms *cell culture* and *tissue culture* tend to be used synonymously (Spector et al. 2002). See the median cell culture infective dose ($CCID_{50}$).

Plaque forming unit (PFU)—A measure, determined by the technique known as plaque assay, of the quantity of individual and aggregated virus particles that are infectious for a given cell line culture under specified conditions. This measure is expressed as the number of plaques formed per unit volume (liquid) (Flint et al. 2009).

F.3.2 Methodology

The scope of this calculation package is as follows:

- The inventory is provided for the 13 BSL-3 and BSL4 pathogens ultimately evaluated in the RA. The 13 pathogens were selected on the basis of independent review and consideration of

recommendations from the National Institutes of Health (NIH) Blue Ribbon Panel (BRP) and the National Research Council teleconference (NIH 2009). No additional pathogens are considered.

- Estimates are provided for the master, seed, and working stock inventories.
- The experimentally infected animals (mammals and arthropods) likely to be associated with each of the 13 pathogens are identified.
- The inventories presented herein are the culmination of the following activities and sources of information:
  - Discussions and correspondence with representatives of the BUMC provided their expected inventories of the research programs.
  - Literature reviews and surveys of program leaders and senior scientists at other BSL-4 biocontainment facilities were performed.
  - A joint NIH and Tetra Tech review of the collected information was performed at the March 5, 2010, NIH-Tetra Tech Working Group meeting.
  - Subsequent detailed discussions with NEIDL leaders and reviews of available NEIDL operating procedures were used as a final step in resolving specific questions related to operations that affect pathogen inventories.
  - BUMC provided their expected master and working stock volumes (BUMC 2011a).

Those inputs were important factors, but the ultimate basis for the maximum inventory estimates is the NIH and Tetra Tech review and approval process, which is documented by this report.

F.3.3 Results

The seed stock, master stock, working stock, and infected animal inventories are presented below.

F.3.3.1 Master Stock Inventory

Master stock inventories for a given pathogen are present for only a few days while they are being cultured and until they are converted to the seed stock inventory. A new master stock for a given pathogen is likely to be generated no more than once every one to five years. As a result, master stock inventories will be present less than 1 percent of the time throughout the facility operating lifetime because they are used to produce the seed stock. It is possible that research will be conducted on multiple strains of a pathogen, so multiple master stocks could be generated for a pathogen. The maximum NEIDL master stock volumes and concentrations for the facility are presented in Table F.3-1.

Table F.3-1 NEIDL master stock maximum volumes and concentrations

Pathogen[a]	BSL	Maximum volume (mL)	Maximum concentration (/mL)[b]
B. anthracis [c d]	2/3	100[c d]	2.4×10^8 CFU [c d]
F. tularensis [c]	3	100 [c]	2×10^9 CFU [c]
Y. pestis [c]	3	100 [c]	2×10^7 CFU [c]
1918H1N1V	3	500	1×10^8 PFU
SARS-CoV	3	500	1×10^7 PFU
RVFV	3	500	1×10^8 PFU or 1×10^9 $CCID_{50}$ and $MICLD_{50}$ [e]
ANDV	3/4[f]	500	1×10^6 $CCID_{50}$
EBOV	4	500	5×10^7 $CCID_{50}$
MARV	4	500	1×10^7 $CCID_{50}$
LASV	4	500	1×10^7 $TCID_{50}$ and FFU (PFU)
JUNV	4	500	1×10^7 PFU
TBEV-FE	4	500	1×10^8 MID_{50}
NIPV	4	500	2×10^7 PFU and $TCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); Andes virus (ANDV); Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. Suspension concentrations are given in terms of colony forming units (CFU) per milliliter for bacteria and plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), median mouse infective dose (MID_{50}), or median mouse intracerebral lethal dose ($MICLD_{50}$) per milliliter for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. Bacteria will be grown on solid medium plates, which will then be harvested and converted to a liquid suspension for use in either aerosol or subcutaneous animal inoculation. The liquid suspension form is reported here rather than the solid medium form because of the solid medium form has a greatly reduced airborne release potential.

d. In spore form in suspension.

e. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

f. BSL-4 is required when infecting rodent species permissive (susceptible to) for chronic infection.

F.3.3.2 Seed Stock Inventory

There could be multiple 1- to 2-mL vials of each pathogen, and multiple strains of each pathogen, in storage. It is difficult to predict which pathogen species and strains could be present in NEIDL at any time, so generally it should be conservatively assumed that all strains of all pathogen species could be present at all times. The total number of vials of any pathogen is not known but could be 100 or more for each pathogen strain. However, the seed stock is not considered a significant contributor to risk because of the following factors:

- The seed stock is in a frozen form, which is highly resistant to release, especially to aerosol release, and is only thawed before use. Redundancies in the off-site power supply feeds and the

on-site emergency diesel generators minimizing the potential for a loss of power to the freezers (BUMC 2009d, BUMC 2009b). Additionally, the seed stock can remain frozen in the freezers for many hours without power, thereby, allowing time for corrective action.

- Except when being used to produce a working stock, the seed stock is secured in freezers, which protect the individual vials from physical damage and provide an additional barrier to release.
- The volume of each vial is a very small fraction of the total pathogen inventory (e.g., about 1 percent of the working stock volume) and the presence of many vials of a seed stock becoming thawed and breached concurrently is unlikely.

The maximum NEIDL seed stock concentration estimates are presented in Table F.3-2.

Table F.3-2. NEIDL maximum seed stock concentrations

Pathogen[a]	BSL	Maximum concentration (/mL)[b]
B. anthracis	2/3	2.4×10^8 CFU
F. tularensis	3	2×10^9 CFU
Y. pestis	3	2×10^7 CFU
1918H1N1V	3	1×10^8 PFU
SARS-CoV	3	1×10^7 PFU
RVFV	3	1×10^8 PFU or 1×10^9 $CCID_{50}$ and $MICLD_{50}$ [c]
ANDV	3/4[d]	1×10^6 $CCID_{50}$
EBOV	4	5×10^7 $CCID_{50}$
MARV	4	1×10^7 $CCID_{50}$
LASV	4	1×10^7 $TCID_{50}$ and FFU (PFU)
JUNV	4	1×10^7 PFU
TBEV-FE	4	1×10^8 MID_{50}
NIPV	4	2×10^7 PFU and $TCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); Andes virus (ANDV); Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. Suspension concentrations are given in terms of colony forming units (CFU) per milliliter for bacteria and plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), median mouse infective dose (MID_{50}), or median mouse intracerebral lethal dose ($MICLD_{50}$) per milliliter for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

d. BSL-4 is required when infecting rodent species permissive for (susceptible to) chronic infection.

F.3.3.3 Working Stock

The facility working stocks are the inventories used in the daily research experiments. The working stock inventories are a primary focus of the RA because they are in use more frequently than the master stock and are in a liquid suspension, a more releasable form than the frozen seed stock. The working stocks will frequently be in a liquid suspension form; however, virus suspensions may be frozen on occasion and bacteria may be grown on solid media (e.g., agar). For this RA, it is conservatively assumed that the working stocks are always in liquid suspension form because this form has a greater release potential than frozen or solid forms. The working stock concentration used in the RA is the maximum concentration based upon the methodology described in Section F.3.2.

Virus working stock volumes will vary depending on the type of experiments being performed and the quantity of viruses required to carryout those experiments. For bacteria, this RA is based on the assumption that the maximum working stock volumes are present at all times. For viruses, the working stock volumes are considerably greater than the volumes for bacteria. This RA is based on the assumption that for viruses, the typical working stock volume of 150 mL is present at all times. The typical virus working stock volume will be 150 mL; however, some experiments will not require 150 mL and some will require more, with a maximum of 500 mL (BUMC 2011a). The assumption that a 150-mL liquid suspension working stock volume is present for each of the viruses at all times is appropriate and conservative even for the limited instances when the volume may be up to 3-1/3 times larger for the following reasons:

- Active research is likely to be conducted on only a fraction of the viruses at any given time. Therefore, assuming there is a working stock for each virus at all times overstates the overall volume.
- The instances when a working stock exceeds 150 mL are roughly offset by the times when the working stock is less than 150 mL; therefore, the average volume is about 150 mL.
- A liquid suspension working stock for a given actively researched virus will not be present in the laboratory in a vulnerable form (i.e., not frozen and not secured in storage such as a refrigerator) at all times throughout the year. Working stocks will not be present during preparation and clean-up phases of the research. In addition, the working stocks will be frozen or secured in storage such as a refrigerator during large portions of the research period. If a liquid working stock is only out of storage during all normal work hours, it would be at risk for only ¼ of the year.
- The inventory is based on the maximum concentration, which is a large conservative bias, and this conservatism compensates for the occasions when the volume may exceed 150 mL. The

concentration can vary by one or more orders of magnitude while the maximum volume is only a factor of 3-1/3 larger than the typical volume.

The typical working stock volumes and maximum concentrations used for the NEIDL RA for all 13 pathogens being evaluated are presented in Table F.3-3.

Table F.3-3 NEIDL facility working stock typical volumes and concentrations

Pathogen[a]	BSL	Typical volume (mL)	Maximum concentration (/mL)[b]
B. anthracis [c d]	2/3	50[c d]	2.4×10^8 CFU [c d]
F. tularensis [c]	3	1 [c]	2×10^9 CFU [c]
Y. pestis [c]	3	5 [c]	2×10^7 CFU [c]
1918H1N1V	3	150	1×10^8 PFU
SARS-CoV	3	150	1×10^7 PFU
RVFV	3	150	1×10^8 PFU or 1×10^9 $CCID_{50}$ and $MICLD_{50}$ [e]
ANDV	3/4 [f]	150	1×10^6 $CCID_{50}$
EBOV	4	150	5×10^7 $CCID_{50}$
MARV	4	150	1×10^7 $CCID_{50}$
LASV	4	150	1×10^7 $TCID_{50}$ and FFU (PFU)
JUNV	4	150	1×10^7 PFU
TBEV-FE	4	150	1×10^8 MID_{50}
NIPV	4	150	2×10^7 PFU and $TCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); Andes virus (ANDV); Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. Suspension concentrations are given in terms of colony forming units (CFU) per milliliter for bacteria and plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), median mouse infective dose (MID_{50}), or median mouse intracerebral lethal dose ($MICLD_{50}$) per milliliter for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. Bacteria will be grown on solid medium plates, which will then be harvested and converted to a liquid suspension for use in either aerosol or subcutaneous animal inoculation. The liquid suspension form is reported here rather than the solid medium form because of the solid medium form has a greatly reduced airborne release potential.

d. In spore form in suspension.

e. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

f. BSL-4 is required when infecting rodent species permissive (susceptible to) for chronic infection.

It is possible that research will be conducted on multiple strains of a given pathogen at the same time in the same laboratory suite. Based on discussions with BUMC personnel, it is expected that no more than two strains of a given pathogen are likely to be used in a laboratory suite at a given time. However, there are multiple Research Cores housed in BSL-3 or BSL-4 spaces, and concurrent research projects and activities collectively involving multiple pathogens are likely.

F.3.3.4 Infected Animals

The NEIDL will also contain various experimentally infected animals (arthropods and mammals) at any given time. There are multiple ways in which infected animals can expose people including (1) exposure to animals and bedding dust during handling operations, (2) direct exposure of workers and the public by escaped animals, and (3) establishing reservoirs (i.e., domestic and wild animals that are a potential source of infection) after escape. Table F.3-4 identifies the pathogens that could be associated with each type of mammal and arthropod.

Table F.3-4. Animal-pathogen pairing

Pathogen[a]	BSL	Non-human primates	Rodent[b]	Mosquito	Tick
B. anthracis	2/3	✓	✓		
F. tularensis	3	✓	✓		
Y. pestis	3	✓	✓		
1918H1N1V	3	✓	✓		
SARS-CoV	3	✓	✓		
RVFV	3	✓	✓	✓	
ANDV	3/4[c]	✓	✓		
EBOV	4	✓	✓		
MARV	4	✓	✓		
LASV	4	✓	✓		
JUNV	4	✓	✓		
TBEV-FE	4	✓	✓		✓
NIPV	4	✓	✓		

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); Andes virus (ANDV); Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. Includes suckling or weanling mice.

c. BSL-4 is required when infecting rodent species permissive (susceptible to) for chronic infection.

F.3.3.5 Variability and Uncertainty

There are numerous variabilities and uncertainties that apply to the results presented in Sections F.3.1 through F.3.4. Table F.3-5 provides a discussion and an estimate of the potential effect of each of the key variabilities and uncertainties.

1 **Table F.3-5. Summary of key variabilities and uncertainties.**

Variability/ uncertainty	Discussion	Potential effect[a]
Pathogens evaluated	It is possible that not all 13 pathogens evaluated will be used in NEIDL. Consideration of pathogens that might not be used in NEIDL is a conservatism (i.e., overestimate of risk), but the magnitude of the conservatism is unknown. The largest potential conservatism would be if the most affecting pathogens were not ever used in NEIDL.	Conservatism, unknown magnitude
	It is possible that pathogens other the 13 evaluated might be used in NEIDL. Other pathogens could have their own specific characteristics, but the pathogens considered are expected to cover the range of attributes likely to be associated with other pathogens that might be used. To the extent that the 13 pathogens envelope other potential pathogens, research on additional pathogens will not increase risk. If another pathogen is used that exceeds the range/combination of attributes associated with the 13 pathogens, it is possible that this evaluation is non-conservative (i.e., underestimates risk), but the magnitude of the non-conservatism is unknown.	Likely accurate, but potential non-conservatism, unknown magnitude
	At any given moment, research will be conducted on only a fraction of the 13 pathogens. Therefore, some events that are not dependent on laboratory activities (e.g., an earthquake) could overpredict risk.	Conservatism, unknown magnitude
Master stock total volume	The master stock maximum volume estimates might overstate the volumes for actually used for some pathogens. In general, the volumes are expected to be conservative estimates, and actual volumes will be somewhat less.	Likely conservatism, unknown magnitude
Master stock maximum concentration	The master stock concentrations are expected to be maximum values that are not exceeded, but it is possible that those values will be exceeded by a culture inadvertently grown to higher concentrations. If the estimated master stocks concentrations are exceeded, the extent of exposure could increase proportionally to that increase. Because the master stocks are present for only a small fraction of the time, this potential non-conservatism is considered a small risk factor.	Likely conservatism but potential non-conservatism, unknown magnitude
Seed stock total volume	The volume of the seed stock is expected to be 1 to 2 mL per vial and there could be 100 or more vials. A precise estimate of the number of vials is not provided because the frozen seed stock is not considered a large factor in the risk assessment because • The samples are in a frozen form, which is highly resistant to airborne dispersion, • The samples are stored in freezers, which makes them much less vulnerable to airborne release, and • The volume of each vial is a very small fraction of the total pathogen inventory (e.g., often about 1% of the working stock) and a breach of many vials concurrently is unlikely.	Non-conserveratism, but expected to be negligible
Seed stock maximum concentration	The seed stock concentrations are expected to be maximum values, but it is possible that those values will be exceeded or that they overestimate the actual concentrations. However, as explained for the *Seed stock total volume* row above, the risks associated with the seed stock are small so the impact on the overall risk is small.	Possible non-conserveratism, but expected to be negligible
Working stock volume	The maximum working stock volume used for some activities could be larger than the typical volume (up to 500 mL versus 150 mL). As explained in Section F.3.3.3, the typical volumes reflect most activities and the occasions when larger volumes are used are counteracted by the times when smaller volumes are used and the conservatism in the concentration.	Possible non-conservatism, but expected to represent most activities

Variability/ uncertainty	Discussion	Potential effect[a]
Working stock maximum concentration	The working stock concentrations are expected to be maximum values, but it is possible that those values will be exceeded by a culture inadvertently grown to higher concentrations. If the estimated working stocks' concentrations are exceeded, the extent of exposure could increase proportionally to this increase.	Likely conservatism but potential non-conservatism, unknown magnitude
Animal-pathogen pairings: nonhuman primates and rodents	Because all pathogens are selected for each mammal, this is a conservative estimate that cannot be exceeded for the pathogens selected.	Conservatism, unknown magnitude
	It is possible that mammals other than those listed in Table F.3-3 could be used in NEIDL. The risk associated with additional mammals might or might not increase the risk.	Possible non-conservatism, unknown magnitude
Animal-pathogen pairings: mosquito and tick	There is only one pathogen likely to be associated with mosquitoes and one pathogen likely to be associated with ticks. Use of another of the 13 pathogen with either arthropod is possible and this might or might not increase risks.	Possible non-conservatism, unknown magnitude
	It is possible that arthropods (mosquitoes or ticks) other than those listed in Table F.3-3 could be used in NEIDL. Vector-specific SOPs would be required if new vectors are added.	Possible non-conservatism, unknown magnitude

a. This is a qualitative indication of the direction and magnitude of the conservative or non-conservative effect of this factor on risk.

As shown in Table F.3-5, numerous variabilities and uncertainties could affect the results. Some of those potential factors are conservative (i.e., tend to over-estimate frequency or consequence) and some are non-conservative (i.e., tend to under-estimate frequency or consequence). The magnitude of each conservatism and non-conservatism is not known, but the inventories are considered conservative overall because they reflect the maximum expected volumes and concentrations and are appropriate for use in the RA.

F.4 POPULATION ESTIMATES

F.4.1 Introduction

The RA includes consideration of impacts on the general population as a result of postulated airborne pathogen releases. General population estimates for each of the three sites being evaluated (i.e., the urban, suburban, and rural sites) are a necessary input for the population impact assessments. This section describes the methodology and results associated with the development of general population estimates at the three sites. The urban, suburban, and rural sites being evaluated in the RA are described in Appendix B. This analysis addresses only the general population and does not distinguish the medically vulnerable subpopulations or the environmental justice subpopulations, which are addressed in Appendix I and in Appendix M and Chapter 10 of the RA, respectively.

F.4.2 Methodology

This analysis provides estimates of the population surrounding each of the three sites, including both residents and nonresidents. This RA is intended to address known conditions and not to speculate about potential future changes; however, it is important to ensure that foreseeable future population changes do not invalidate the conclusions of this RA. Therefore, following sections address the current resident, current nonresident, and future populations.

F.4.2.1 Current Resident Population

Population estimates for NEPA analyses are typical based on U.S. Census Bureau data, which are residential population values. As explained in Section F.1.2, this analysis follows NEPA guidance. Because of their configuration, Census data are not in a format that is readily usable for population impact analyses, so a computer code was developed by the U.S. Census Bureau to convert the data into an appropriate format. For the calculation, the Census data associated with the three sites were converted to the appropriate format and adjusted to reflect population changes since the last census. This section describes the data set, computer code, and adjustments used to develop the resident population estimates for the three sites.

F.4.2.2 Population Data Set

Census data are collected by the U.S. Census Bureau on a decennial (10-year) basis. Census data are collected and reported on both a county level and a block level. The 2000 Census data set includes almost 8 million census-blocks, one for each block in the continental United States at the time, and more than 3,000 counties (Nuclear Regulatory Commission 2003a). Block-level data are used as the starting point for the analysis because the finer resolution is necessary to obtaining accurate population estimates in close proximity to each site. The 2010 census data have been collected but the block-level data were not available for use at the time of analysis, (i.e., January 2011) so the 2000 census data are used as the starting point for this RA. Use of the 2000 census data as the starting point is adequate because (1) 2010 Massachusetts population is only 3.1 percent larger than the 2000 population, and (2) the 2000 census data are updated to reflect more recent data (i.e., greater of 2009 or 2010 populations).

F.4.2.3 Computer Code

In 1973 the U.S. Census Bureau developed a computer code to calculate population estimates surrounding a site. After release of the 2000 Census data, the U.S. Nuclear Regulatory Commission tasked Sandia National Laboratories with developing an updated version of the U.S. Census Bureau computer code to make it compatible with the new (i.e., 2000) data set and allow it to run on modern personal computer

operating systems. The resulting new software, SECPOP 2000 (Nuclear Regulatory Commission 2003a), was used for this RA.

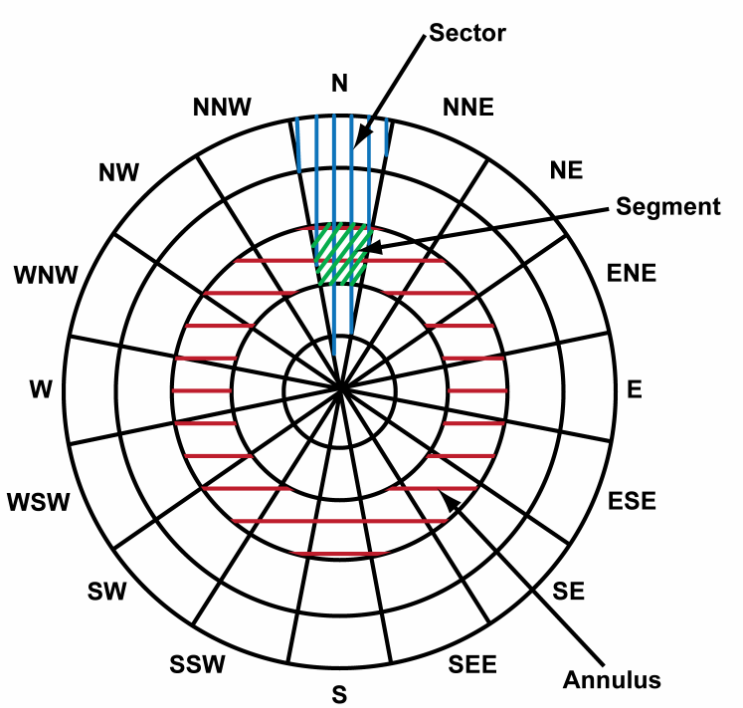


Figure F.4-1. Radial Grid.

The SECPOP 2000 code was used to place the block-level census data into a radial (or polar) grid with sixteen 22.5°-sectors similar to the one shown in Figure F.4-1. The radial grid configuration is used because it is consistent with the configuration used by airborne dispersion computer codes, which are another essential input for the general population impact assessment. SECPOP 2000 provides the population in tabular form by sector for each annular ring. For this analysis, each radial ring is 0.1 km (0.6 mile) wide, and the grid extends to a radius of 1 km. While a radius of 1 km captures the majority of the impacts, the expected number of infections would be somewhat higher if the calculation were performed for a larger radius. There are two competing factors associated with exposures at greater distance, namely, (1) a decrease in the exposure level as the distance increases and (2) a tendency for the number of people per segment to increase at greater radii because the area of each segment increases as the distance from the center increases. The maximum radius of 1 km was selected for several reasons:

- The 1-km radius is consistent with U.S. Nuclear Regulatory Commission recommendations for the NEPA environmental justice evaluation of proposed actions in cities (Nuclear Regulatory Commission 2003).
- The highest density of nonresidents surrounding the urban site is located within 0.5 km of NEIDL and is included in the 1-km radius.
- No high-population resident communities are just beyond the 1-km radius that would significantly affect the results.
- The average exposure levels are extremely low at 1 km and would be even lower for greater distances. The MRF (total collapse) earthquake analysis (see Section F.8.3) estimates the average exposure level to be less than one one-thousandth (1/1,000) of a unit for all but one pathogen and

less than one one-hundredth (1/100) of a unit for all pathogens at 1 km. The calculated exposures at even greater distances would result in even lower exposure levels.

F.4.2.4 Updating of Data

The U.S. Census Bureau Population Estimate Program (PEP) produces estimates of the population for each year after the last published decennial census (i.e., 2000). The PEP estimates are provided at a county level and are based on July 1 of each year. The PEP estimates include the percentage changes from the prior decennial census. The PEP county-level population changes were used to update the 2000 block-level population data to July 2009. Subsequent to the analysis, the 2010 PEP adjustments became available. Table F.4-1 presents the 2009 and 2010 PEP adjustment factors for the counties in which the urban, suburban, and rural sites are located. The analysis was not revised to reflect the 2010 adjustments because the 2009 adjustments are the same or larger for all three sites, which tends to slightly overstate the potential impacts.

Table F.4-1. 2009 and 2010 county population adjustment factors

Site	County	2000 to 2009 adjustment	2000 to 2010 adjustment
Urban	Suffolk, MA	+9.2%	+4.7%
Suburban	Middlesex, MA	+2.6%	+2.6%
Rural	Hillsborough, NH	+6.6%	+5.2%

Source: Census Bureau 2011

F.4.2.5 Averaging

The resident population at the three sites varies from segment to segment within an annular ring. In addition to that direction-dependence of the population, the wind frequency and conditions are direction-dependent. Because both the population and the wind frequency and conditions are direction dependent and are inputs to the population exposure calculations, the approach used to address the direction aspects of the population and wind data is important.

The population exposure calculations can be performed in either a direction-dependent or a direction-independent manner. This analysis was performed using a direction-independent approach (i.e., the population is based on the average population for the 16 segments in each ring) to minimize the effect of the uncertainties associated with both the wind and population estimates. The population estimates are uncertain because of the population movement throughout the day (e.g., residents going to work or stores), which the resident population data set does not take into account. There is also uncertainty

associated with the airborne dispersion calculations because they are based on data from the nearest weather towers, but those towers are as much as 29 km (18 miles) from the corresponding site (see Section F.5). The local weather conditions can be significantly different from the nearest tower conditions, especially with rolling terrain such as the terrain surrounding the rural site. The direction-independent approach is used for this analysis because it minimizes the effects of that uncertainty by averaging the all segment populations in an annular ring. Scoping calculations were performed to consider that effect, and the direction-independent results were found to be about 10 to 20 percent higher than the direction-dependent results for all sites. Therefore, the average population in each annular ring is used for this analysis to reduce the chance that impacts are underestimated.

F.4.2.6 Current Nonresident Population

The Census data include all people residing in the area including student and jail residents; however, the resident population data do not include people frequently near the site but residing elsewhere. Students, staff, patients, and occupants of vehicles are examples of nonresidents near the sites. The following subsections address the methodology for estimating those subpopulations.

F.4.2.7 Students, Staff, and Patients

The student, staff, and patient population estimates were developed for each site by identifying the relevant facilities within 1 km, estimating the number of people at each facility, estimating the fraction of the time they are present, their distance from the site. The average number of people outdoors or inside buildings for each annular ring at each site was estimated using the following methodology:

1. *Number of people*—The total population within 1 km of each was estimated by first identifying the major facilities in the vicinity of each site and then estimating the number of people present at the facilities. The number of people frequenting the facilities was obtained by a combination of reports by the organizations and phone interviews. In general, an influx of employees into an area is largely offset by residents that leave for work in other areas, so this analysis focused on large, nonresident population groups. If estimates could not be obtained for all sectors, estimates for the sectors with the greatest population were used as the basis for other sectors.
2. *Fraction of the time present*—The population estimates are used for analysis of earthquake-caused releases and an earthquake has an equal likelihood of occurring at any time of the year, so it is appropriate to prorate the nonresident population by the fraction of the year each nonresident is present. The fraction of the year that the people are near the sites was estimated on the basis of the nature of the activities near the site. For example, a typical work year was used as a basis for employees, and a typical school year was used for students.

3. *Distance from the site*—The annular ring in which the facilities are located were identified to allocate the population to the appropriate annular ring. When groups were generally beyond 0.5 km, they are assumed to be uniformly distributed throughout the 1-km area. This is a conservative assumption that compensates for potential omissions in the analysis because exposure levels tend to be greater at inner annular rings. When a group is generally closer than 0.5 km but distributed throughout that area, they were assumed to be uniformly distributed throughout the 0.5 km.
4. *Annular ring allocation*—The average population was then allocated to each annular ring surrounding the site. In most cases, the population can be assumed to be uniformly distributed within multiple annular rings, so the population is allocated on the basis of the area of each segment in the rings.

F.4.2.8 Vehicle Occupants

The average number of vehicle occupants traveling within 1 km of each site was also estimated. The average population for each annular ring was based on the number of vehicles in the area, the occupancy rate of the vehicles, and the location of the vehicles relative to the site. The methodology used to estimate this vehicle occupant population was as follows:

- *Vehicle population*—The average vehicle population within 1 km of the site was based on available data. Traffic count data are not available for many, but not all, roadways at each site. The Department of Transportation in Massachusetts and New Hampshire provide traffic count data for many roadways near the sites (MDOT 2011; NHDOT 2011). Those data are provided on 24-hour basis averaged over the year. Vehicle counts are not available for all roadways and two approaches were used in cases where data were not available: (1) estimates of other roadways expected to have similar or greater traffic counts were used as a basis for estimates, and (2) when estimates can be developed for the busiest sectors, this estimate can be used as the basis for the other sectors. The average number of vehicles in a kilometer of roadway can be determined using an assumed traffic speed.
- *Occupancy*—The number of people in a vehicle depends on the vehicle type and the community. The national average occupancy ranges from 1.12 for trucks to 2.35 for vans, with an average of 1.59 for cars (DOE 2010). A value of 2 occupants per vehicle is used for this analysis.
- *Annular ring allocation*—The location of each roadway needs to be considered to allocate the population to each annular ring. A uniform distribution of vehicle occupants throughout the 1-km radius was assumed for this analysis. Use of the uniform distribution assumption is conservative because the vehicle density is low nearest each of the sites, thereby overestimating the vehicle occupants where the exposure levels would be greatest.

F.4.2.9 Future Population Changes

Two potential future changes to the current populations were considered, namely, (1) general growth or decline in the population of the area, and (2) changes in the population that are a direct result of a new laboratory being built.

General population changes—The U.S. Census Bureau has projected that the 2000-to-2030 general population growth will be 10.4 percent for Massachusetts and 33.2 percent for New Hampshire (Census Bureau 2011a), but it does not provide projections at a finer level of resolution (i.e., at a county or block level). Table F.4-2 shows the adjustment that would result if the 2009 populations were increased to the Massachusetts and New Hampshire 2030 projections.

Table F.4-2. Adjustment for 2009 to 2030

Site	2009 to 2030 Adjustment
Urban	1.2%
Suburban	7.8%
Rural	26.6%

Note: The adjustment from 2000 to 2030 is 10.4 percent Massachusetts and 33.2 percent for New Hampshire.

Source: Census Bureau 2011a

The Table F.4-2 adjustments from 2009 to 2030 were not used and would not alter the conclusions of the RA for the following reasons:

- The 2030 projections are statewide averages that do not reflect actual site differences. Therefore, use of these statewide projections would reflect artificial values and would not reflect real differences among the sites. For example, the suburban and rural sites are only about 65 km (40 miles) apart, yet the 2030 projections would result in an 18.8 percent difference in population growth rate.
- The 2030 adjustments are minor. On the basis of the results presented in Section F.4.3.3, the adjustments would increase the average population per sector from 5.8 to 7.3 for the rural site, from 51 to 55 for the suburban site, and from 2,201 to 2,215 for the urban site. Those small changes would not affect the conclusions of this RA.

Effect of a new laboratory—There would be an increase in both the resident and nonresident populations in the areas surrounding the suburban and rural sites if a new laboratory were built there. No discernable changes are expected for the urban site because the facility is already built, and there is a high population

base that is not associated with laboratory. To assess the impact of that population increase on the suburban and rural sites, it is necessary to know both the extent of the increase and the location of this new population.

The population increase at the suburban and rural sites could theoretically be large relative to the current populations, but it is difficult to predict the actual extent of the increase. The population increase would be dependent on such unknowable factors as the number of people who would decide to live near the new laboratory versus those choosing to commute from other towns or cities, the number of other research facilities that might choose to locate near the new laboratory, and the extent of the support infrastructure that would be built around the facility. Projections of those increases are not available and attempts to do so would be speculative; however, the population at the sites would certainly remain only a small fraction of the density near the urban site.

While it is difficult to project the extent of the population increase, it is possible to provide some insight into the location of that growth. The building sites for the suburban and rural sites are owned by Boston University (BU) and are relatively large, so BU can control development near the laboratories. The suburban and rural sites are described as follows:

- The suburban site, formerly the BU Corporate Education Center, is a 210-acre (0.85-km^2) forested site overlooking a private pond (see Chapter 2). The property includes wetlands and a historic site that serve as a buffer zone to limit resident and nonresident populations growth near the new laboratory.
- The rural site, the BU Sargent Center for Outdoor Education, consists of 700 acres (2.8 km^2) of open fields and forested land (see Chapter 2). The buffer zone allows BU to prevent resident and nonresident populations from building close to the new laboratory. The site for the new laboratory is near the center of the site, so if development of the site is not permitted, residents and nonresidents would be 0.5 km to 1 km from the facility.

Airborne pathogen concentrations from a postulated ground-level release decrease dramatically with distance. For example, for ground-level releases, which result in the highest exposure levels, concentrations at 0.5 km are less than 1 percent of the exposure levels at 0.03 km and concentrations at 1.0 km are about a third of those at 0.5 km (see Section F.8). Because those buffer areas restrict nearby population growth and because postulated airborne pathogen concentrations decrease dramatically with distance, population increases beyond 0.5 km are unlikely to result in large impacts.

F.4.3 Results

The resident, nonresident, and combined population estimates are presented in the sections below, consistent with the methodology describe in Section F.4.2. A discussion of the variability and uncertainty associated with key assumptions is also provided.

F.4.3.1 Current Resident Population

Table F.4-3 presents the average current resident population in a segment of each annular ring for the three sites. Those estimates are based on the 2000 U.S. Census Bureau data, as formatted by SECPOP2000, with U.S. Census Bureau PEP adjustments to July 1, 2009. As shown by Table F.4-3, the total urban site population in a segment at a 1-km radius is more than 30 times greater than the suburban site population, and the suburban site population is nearly 20 times the rural site population. That is a broad range in the resident population, which allows the analysis to provide insights into the effects of population density.

Table F.4-3. Segment-averaged resident population as of 2009 by annular rings for the three sites

	Annular ring (km)										
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	Total
Urban	0.0	5.1	26	133	68	155	138	219	275	176	1,194
Suburban	0.0	0.0	3.7	0.0	0.0	11	1.5	10	2.4	8.7	38
Rural	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	2.2

a. The NEIDL facility has an exclusion fence at 0.03 km, and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.2 Nonresident Population

All three sites have significant nonresident populations that need to be considered. A review of facilities near the three sites determined that the largest nonresident population consists of students, employees, patients, and vehicle occupants. It is difficult to comprehensively identify and estimate the entire nonresident population, so conservative estimates of known groups were used to avoid underestimating the total population.

F.4.3.3 Students, Staff, and Patients

The student, staff, and patient population estimates for the three sites are presented below.

F.4.3.3.1 Urban Site

The largest group of nonresidents near the urban site is the student, staff, and patient population associated with BU and the Boston Medical Center (BMC). This nonresident population is highly concentrated and in close proximity to the urban site. The BU and BMC nonresident population is primarily between Harrison Avenue and the Massachusetts Avenue Connector, and between Massachusetts Avenue and East Brookline Street, as shown in Figure F.4-2. The population is most concentrated in 5 sectors of the 16-sector radial grid and within a radius of 0.5 km of urban site; however, it does extend slightly beyond that area. For this analysis, the entire BU/BMC population was conservatively assumed to be in 5 sectors within 0.5 km of the urban site. Because it is difficult to obtain accurate estimates for other sectors and other sectors have lower population densities, the BU/BMC population was used as a conservative basis for estimating the nonresident population in all sectors. The assumption that all 16 sectors have a population density comparable to the BU/BMC nonresident density significantly overstates the actual nonresident population, as can be seen by Figure F.4-2, which shows that the area south and east of the urban site is largely covered by roadways (roadways are addressed in the next section). This conservative basis for estimating the average nonresident population more than compensates for any non-BU/BMC population in the 5 sectors and for any concentrated nonresident populations in the other 11 sectors.

Figure F.4-1. Map of BU and BMC facilities.

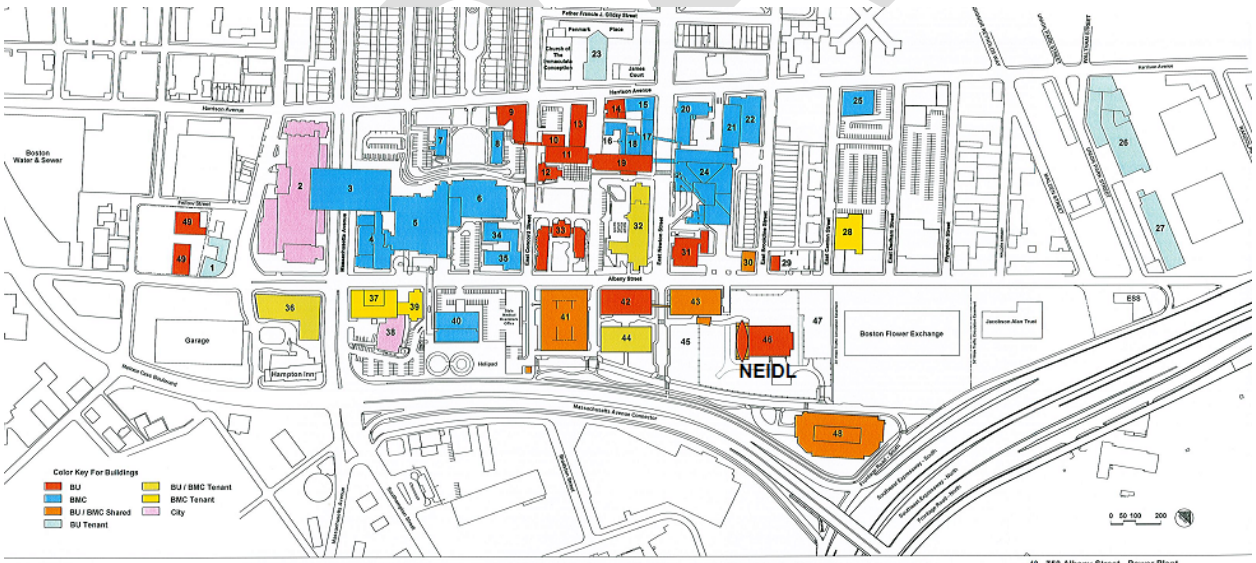


Table F.4-4 itemizes the people associated with BU and BMC that are routinely in the vicinity of the urban site. This nonresident population is only in the area during portions of a year, so population estimates need to account for the time they are present. Table F.4-4 provides an estimate of the fraction of the year each group would be present. Students and employees are assumed to be near urban an average of 25 percent of the year, which is based on 2,000 hours in an 8,760 hour year. This is likely to overestimate the time students and faculty will be present because it does not consider school breaks. The occupancy rate for BMC staffed beds is 76 percent, so it is assumed that a patient occupies each of the 508 staffed beds is occupied 76 percent of the time (BMC 2011). The fraction of the time outpatients are present is based on an average duration of 4 hours per visit in an 8,760-hour year.

Table F.4-4. Student, staff, and patient population for the urban site

Source	Number of people	Fraction of time present	Average population	Comments
Boston University:				
Faculty	1,339	25%	335	Does not account for school breaks.
Students	3,112	25%	778	Some students are also included in the resident population. Does not account for school breaks.
Boston Medical Center:				
Outpatient activity	1,006,356	0.046%	460	Includes clinic visits, ancillary visits, emergency, and ambulatory surgery.
Admitted patients	508	76%	384	The staffed bed occupancy rate is 76% (BMC 2011).
Physicians	1,290	25%	323	
Residents and fellows	791	25%	198	
Nurses	1,506	25%	377	
Employees (full-time equivalent)	4,581	25%	1,145	
Total BU and BMC			3,998	Total in 5 sectors within 0.5 km.
Average per sector			800	Based on a 5-sector area.

Source: BUMC 2008a; BMC 2011

Table F.4-5 shows there is an annual average population of 800 people per sector associated with BU and BMC within a radius of 0.5 km. These people are assumed to be uniformly distributed throughout these sectors up to a radius of 0.5 km and the number of people in each annular ring is ratioed to the area of a segment in each ring. Table F.4-5 provides the segment areas used for allocating the population to each segment. Assuming this population is all within 0.5 km is conservative because the exposure levels from a postulated pathogen release would be lower they were farther from the point of release.

Table F.4-5. Segment-averaged student, staff, and patient population by annular ring—urban site

	Annular ring (km)										
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	Total
Segment area (km^2)	0.0018	0.0059	0.0098	0.0137	0.0177	--	--	--	--	--	0.0489
Population per segment	29	96	161	225	289	--	--	--	--	--	800

a. The NEIDL facility has an exclusion fence at 0.03 km; therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.3.2 Suburban Site

The largest nonresident population within 1 km of the suburban site is associated with the Innovation Academy Charter School (IACS), which now operates what was previously the BU Corporate Education Center. The IACS has 600 middle school and high school students (MA ESE 2011). The IACS high school operates 185 days per year with the school day lasting 7 hours (i.e., from 8:00 a.m. to 3:00 p.m.) (IACS 2010), which means the students are present about 15 percent of the year. To avoid undercounting the nonresident population, it is arbitrarily assumed that there an additional 600 miscellaneous people are present 10 percent of the time within 1 km of the suburban site. That assumed miscellaneous population covers the potential for IACS student extracurricular activities; IACS parents, volunteers, and visitors being at IACS; and other nonresident activities within 1 km of the suburban site. One of the non-IACS-related activities involves the Vesper Country Club, where nine holes, potentially 36 golfers, caddies, grounds-crew, and such, are within the 1-km radius of the rural site. Table F.4-6 provides the basis for the average nonresident population for the suburban site. Using the direction-independent methodology described in Section F.4.2.2.1, the 173 people associated with the IACS are assumed to be uniformly distributed throughout all 16 sectors, which results in a population estimate of 11 people per sector.

Table F.4-6. Student and staff population for the suburban site

Source	Number of people	Fraction of time present	Average population	Comments
Innovation Academy Charter School (IACS):				
Staff	92	25%	23	Does not account for school breaks.
Students	600	15%	90	185 days at 7 hours per day.
Miscellaneous activities	600	10%	60	Extracurricular activities, parents, visitor, volunteers, and non-IACS activities in the area.
Total IACS:			173	Total average population.
Average per sector			11	Total per sector within 1 km.

Source: IACS 2010, 2011

The IACS is more than 0.5 km from the potential laboratory site, but the population is conservatively assumed to uniformly distributed throughout the 1-km area. Therefore, the population per segment is calculated by multiplying the 11 people per sector by the ratio of the segment area in each annular ring to the total sector area. Table F.4-7 provides the segment areas, the total sector area, and the population in each segment.

Table F.4-7. Segment-averaged student and staff population by annular ring—suburban site

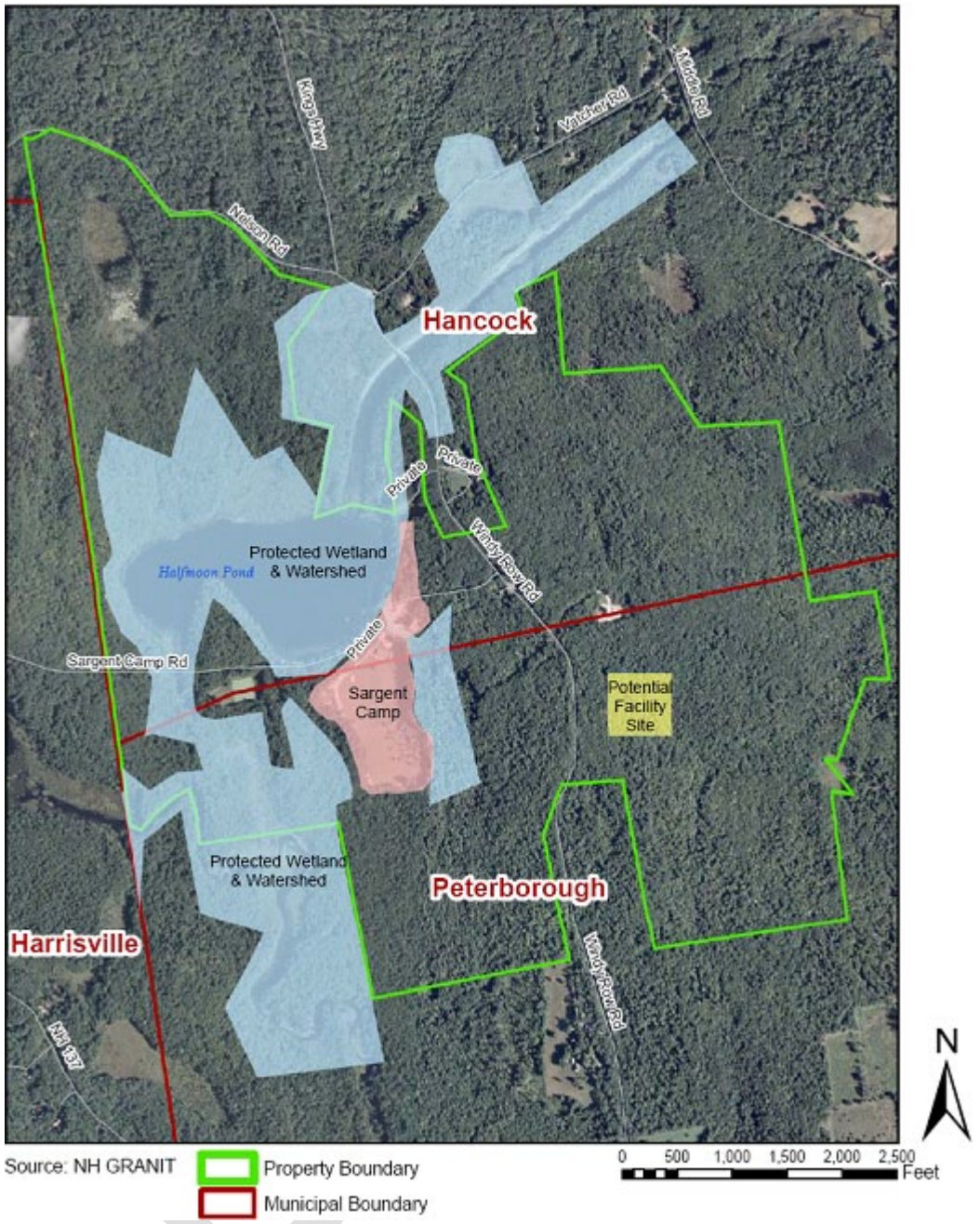
	Annular ring (km)										
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	Total
Segment area (km^2)	0.0018	0.0059	0.0098	0.014	0.018	0.022	0.026	0.029	0.033	0.037	0.20
Population per segment	0.10	0.32	0.54	0.76	0.97	1.2	1.4	1.6	1.8	2.1	10.8

a. The NEIDL facility has an exclusion fence at 0.03 km and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.3.3 Rural Site

As shown in Figure F.4-3, the area surrounding the rural site is largely forested and protected land with a low nonresident population density. The largest nonresident population within 1 km of the rural site is associated with the Nature's Classroom, which now operates what was previously the BU Sargent Center for Outdoor Education (BU 2009). Nature's Classroom offers various 6-day and 13-day summer camp programs from July 3 through August 12 (Nature's Classroom 2011) with a capacity of 200 students (BU 2009). This 40-day period means that students would be at the facility less than 15 percent of the year. To avoid undercounting the nonresident population, it is arbitrarily assumed that there are an additional 200 miscellaneous people present 10 percent of the time within 1 km of the rural site. This assumed miscellaneous population covers the presence of parents and visitors at Nature's Classroom, and other nonresident activities within 1 km of the rural site. Table F.4-8 provides the estimates of the average nonresident population for the rural site.

Figure F.4-3. Rural site location.



Source: NIH 2007

Table F.4-8. Student and staff population for the rural site

Source	Number of people	Fraction of year present	Average population	Comments
Nature's Classroom:				
Staff	15	25%	4	Program only covers 11% of year.
Students	200	15%	30	Program only covers 11% of year.
Miscellaneous activities	200	10%	20	Includes parents and other activities in the area.
Total IACS:			54	Total average population.
Average per sector			3.4	Total per sector within 1 km.

Source: BU 2009; Nature's Classroom 2011

The Nature's Classroom facility is more than 0.5 km from the potential laboratory site, but the students would be hiking throughout the area, so the nonresident population is conservatively assumed to uniformly distributed throughout the 1-km area. Therefore, the population per segment is calculated by multiplying the 3.4 people per sector by the ratio of the segment area in each annular ring to the total sector area. Table F.4-9 provides the segment areas, the total sector area, and the population in each segment

Table F.4-9. Segment-averaged student and staff population by annular ring—rural site

	Annular ring (km)										Total
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	
Segment area (km^2)	0.0018	0.0059	0.0098	0.014	0.018	0.022	0.026	0.029	0.033	0.037	0.20
Population per segment	0.03	0.10	0.17	0.24	0.30	0.37	0.44	0.50	0.57	0.64	3.4

a. The NEIDL facility has an exclusion fence at 0.03 km and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.3.4 Vehicle Occupants

Another nonresident group considered is vehicle occupants traveling in the vicinity of each site. The vehicle occupant is estimated for each of the three sites in the following sections.

F.4.3.3.4.1 Urban Site

The urban site is surrounded by a high traffic area. The Massachusetts Department of Transportation (MDOT) reports vehicle counts for major highways. Unfortunately, MDOT data are not available for all roadways surrounding the site. The highest traffic area within 1 km is the quadrant northeast of the site,

which contains I-93 (Southeast Expressway), the Massachusetts Avenue Connector, and frontage roads. The other three quadrants are busy, but they do not contain as much traffic as the northeastern quadrant. Because sufficient data are available to estimate the traffic in the northeastern quadrant and that is the busiest quadrant, the vehicle occupancy rate for all quadrants is assumed to be the same as the rate for the northeastern quadrant. Table F.4-10 provides the basis and results of the estimate for each roadway and the totals.

Table F.4-10. Average vehicle occupants per sector within 1 km of the urban site

	I-93	Mass. Ave. Connector and Frontage Rd.	Total
24-hr vehicle count	180,700 [a]	180,700 [a]	**361,400**
Average hourly (vehicles)	7,529	7,529	**15,058**
Assumed speed (km/hr)	72	72	**72**
Vehicle per km	104	104	**208**
Roadway distance within 1 km	2	2	**4**
Total vehicles in four sectors within 1 km	208	208	**416**
Occupants per vehicle	2	2	**2**
Vehicle occupants in four sectors	416 [b]	416 [b]	**831**
People per sector			**208**

a. MDOT 2011
b. DOE 2010

The majority of the vehicle traffic is not in close proximity to the site, so assuming that 208 people are distributed uniformly throughout the area is conservative. Table F.4-11 provides the area of a segment in each annular ring, which was used to allocate the 208 people.

Table F.4-11. Segment-averaged nonresident population by annular ring—urban site

	Annular ring (km)										Total
	0.03 [a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	
Segment area (km^2)	0.0018	0.0059	0.0098	0.014	0.018	0.022	0.026	0.029	0.033	0.037	0.20
Vehicle occupants per segment	1.9	6.2	10	15	19	23	27	31	35	40	208

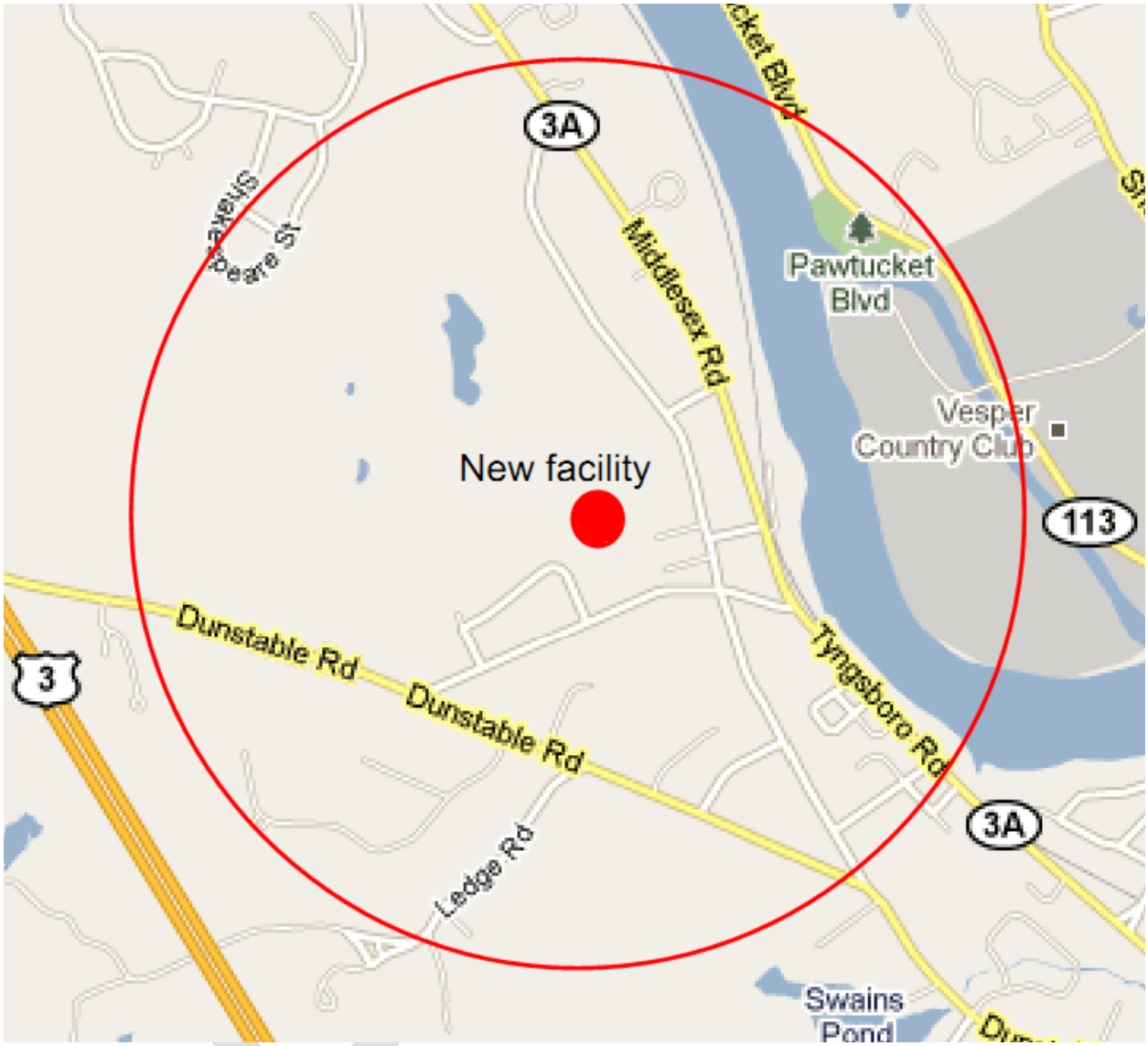
a. The NEIDL facility has an exclusion fence at 0.03 km and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.3.4.2 Suburban Site

The suburban site is in a low vehicle traffic area, especially relative to the urban site. As shown in Figure F.4-4, three roadways are within 1 km of the suburban site; namely, Route 3A (Middlesex Road), Route

113 (Pawtucket Boulevard), and Dunstable Road. The other roadways within 1 km are minor roadways that distribute traffic from those three roadways but do not bring additional traffic into the area.

Figure F.4-4. Roadways within 1 km of the suburban site.



The average number of vehicles associated with each of the three roadways was estimated using the methodology described in Section F.4.2. Table F.4-12 provides the basis and results of the estimate for each roadway and the totals.

Table F.4-12. Average vehicle occupants per sector within 1 km of the suburban site

	Rt. 3A[a]	Rt. 113[b]	Dunstable Road	Total
24-hr count (vehicles)	8,400[c]	5,300[c]	2,900[c]	**16,600**
Average hourly (vehicles)	350	221	121	**692**
Assumed speed km/hour)	64	64	64	**64**
Vehicle density (vehicles per km)	5.4	3.4	1.9	**10.7**
Assumed roadway distance within 1 km (km)	2	1	2	**5**
Total vehicles within 1 km (vehicles)	10.9	3.4	3.8	**18**
Occupants per vehicle (people/vehicle) [d]	2[d]	2[d]	2[d]	**2**[d]
Vehicle occupants in 16 sectors (people)	21.7	6.9	7.5	36
Average over 16 sectors (people)				**2.3**

a. Route 3A, Middlesex Road, south of Westford Street.
b. Pawtucket Boulevard south of Forst Road.
c. MDOT 2011
d. DOE 2010

As shown in Figure F.4-4 both Route 113 and Dunstable Road are beyond 0.5 km of the site, and only a small portion of Route 3A is within 0.5 km of the site. Therefore, the vehicle occupants are almost totally beyond 0.5 km from the site. While the vehicles are mostly beyond 0.5 km from the site, the 2.3 people per sector are assumed to be uniformly distributed throughout the sector. That is a conservative assumption because the level of exposure decreases with distance (see Section F.8), so assuming that occupants are closer than they actually are tends to overstate exposures. Table F.4-13 provides the area of a segment in each annular ring and the number of people per segment based on that allocation by area.

Table F.4-13. Segment-averaged vehicle occupants by annular rings—suburban site

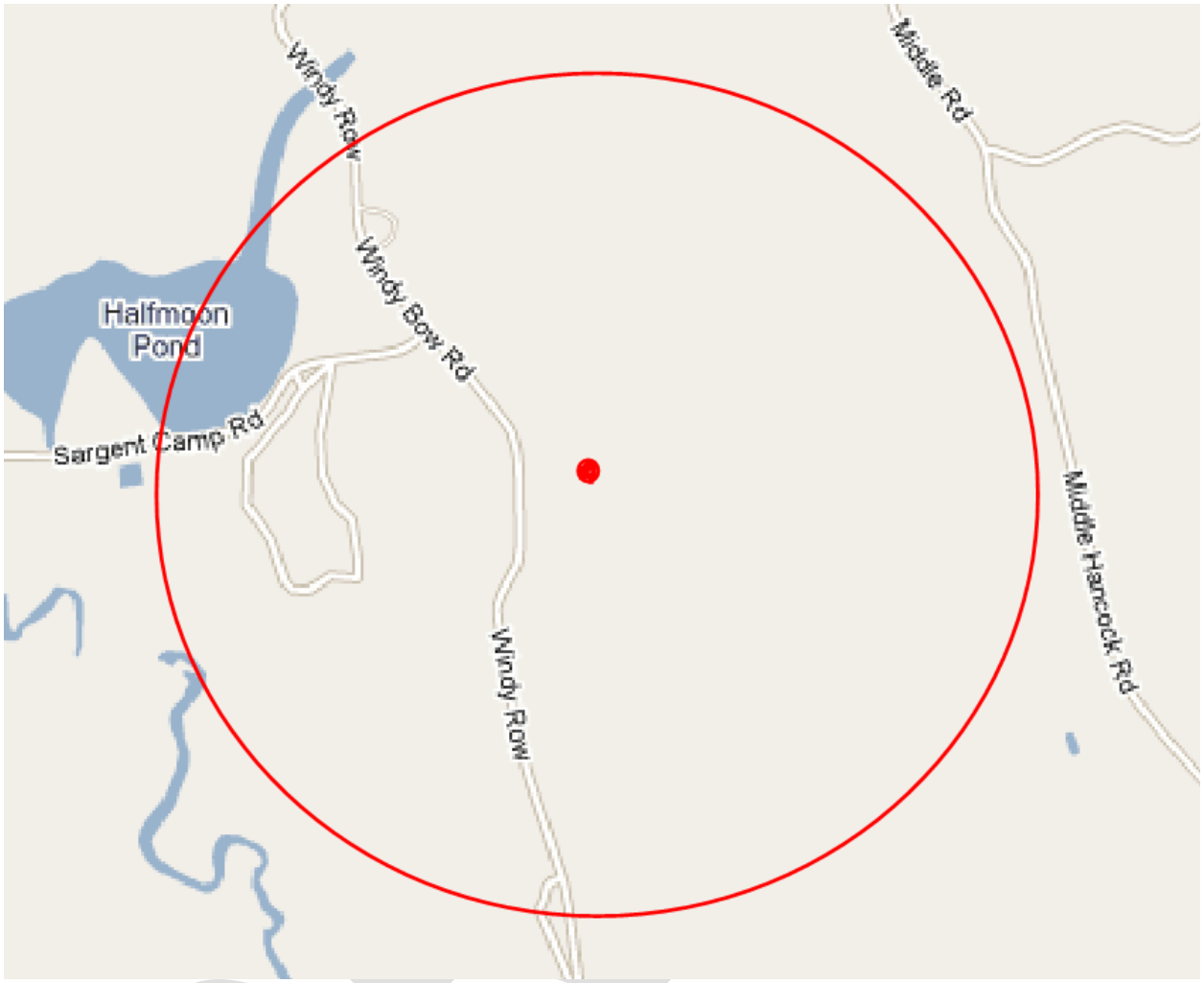
	Annular ring (km)										Total
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	
Segment area (km^2)	0.0018	0.0059	0.0098	0.014	0.018	0.022	0.026	0.029	0.033	0.037	0.20
Vehicle occupants per segment	0.02	0.07	0.11	0.16	0.20	0.25	0.29	0.34	0.38	0.43	2.3

a. The NEIDL facility has an exclusion fence at 0.03 km, and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.3.4.3 Rural Site

The rural site is in a very low vehicle traffic area, especially relative to the urban site. As shown in Figure F.4-5, only two roadways are within 1 km of the rural site; namely, the Windy Row Road and the Sargent Camp Road.

Figure F.4-5. Roadways within 1 km of the rural site.



The average daily vehicle counts for the Windy Row Road and Sargent Camp Road are not available in the New Hampshire Department of Transportation database (NHDOT 2011), so an alternate approach was used to estimate the vehicle counts. Many of the roadways for which traffic counts are available are state or national highways, and they have higher traffic levels than the minor roads. Traffic counts are available for Spring Road, which is south of the site and connects Route 137 to Windy Row Road, as does the Sargent Camp Road. The traffic count for Spring Road in 2008 was 240 vehicles per day (NHDOT 2011). The traffic count for Windy Row Road and Sargent Camp Road are expected to be similar to Spring Road. To ensure that the traffic count is not underestimated, a value of 1,000 vehicles per day is assumed for both Windy Row Road and Sargent Camp Road. Table F.4-14 provides the basis and results of the estimate for each roadway and the totals.

Table F.4-14. Average vehicle occupants per sector within 1 km of the rural site

	Windy Row Road	Sargent Camp Road	Total
Assumed 24-hr count (vehicles)	1,000 [a]	1,000 [a]	
Average hourly (vehicles)	42	42	83
Assumed speed (km/hour)	64	64	64
Vehicle density (vehicles per km)	0.65	0.65	1.3
Assumed roadway distance within 1 km (km)	2	1	3
Total vehicles within 1 km (vehicles)	1.3	0.6	2
Occupants per vehicle (people/vehicle)	2 [a]	2 [a]	2
Vehicle occupants in 16 sectors (people)	2.6	1.3	3.9
Average over 16 sectors (people)			0.24

a Source: NHDOT 2011
b Source: DOE 2010

While most of the rural roadways are more than 0.5 km from the site, the 0.24 person per sector is assumed to be uniformly distributed throughout the sector. Table F.4-15 provides the area of a segment in each annular ring and the number of people per segment based on the allocation by area.

Table F.4-15. Segment-average vehicle occupancy by annular ring for the rural site

	Annular ring (km)										
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	Total
Segment area (km^2)	0.0018	0.0059	0.0098	0.014	0.018	0.022	0.026	0.029	0.033	0.037	0.20
Vehicle occupants per segment	0.00	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.04	0.05	0.24

a. The NEIDL facility has an exclusion fence at 0.03 km, and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.4 Population Summary

Table F.4-16 presents a summary of the resident and nonresident population for all three sites. For every segment and for the total, the urban site population is considerably greater than the population of the suburban site. For the urban site, the resident population is the largest contributor to the total population; however, the BU/BMC population is the dominant contributor within 0.5 km. For most annular rings and for the total, the suburban population is greater than the population of the rural site.

Table F.4-16. Summary of the segment-averaged population by annular ring for the three sites

Population group	Annular ring (km)										Total
	0.03[a] to 0.1	0.1 to 0.2	0.2 to 0.3	0.3 to 0.4	0.4 to 0.5	0.5 to 0.6	0.6 to 0.7	0.7 to 0.8	0.8 to 0.9	0.9 to 1.0	
Urban:											
Residents	0.000	5.1	26	133	68	155	138	219	275	176	1,194
Students, staff, and patients	29	96	161	225	289	n.a.	n.a.	n.a.	n.a.	n.a.	800
Vehicle occupants	1.9	6.2	10	15	19	23	27	31	35	40	208
Total	31	108	196	372	376	178	165	250	310	215	2,201
Suburban:											
Residents	0.0	0.0	3.7	0.0	0.0	11.2	1.5	10.2	2.4	8.7	37.6
Students and staff	0.1	0.3	0.5	0.8	1.0	1.2	1.4	1.6	1.8	2.1	10.8
Vehicle occupants	0.02	0.07	0.11	0.16	0.20	0.25	0.29	0.34	0.38	0.43	2.3
Total	0.1	0.4	4.4	0.9	1.2	12.6	3.2	12.2	4.6	11.1	50.6
Rural:											
Residents	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	2.2
Students and staff	0.03	0.10	0.17	0.24	0.30	0.37	0.44	0.50	0.57	0.64	3.4
Vehicle occupants	0.00	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.04	0.05	0.2
Total	0.03	1.37	0.18	0.25	0.32	0.40	0.47	0.54	1.55	0.69	5.8

a. The NEIDL facility has an exclusion fence at 0.03 km, and it is assumed that a similar exclusion fence would be used at all sites. Therefore, estimates are not provided for distance less than 0.03 km.

F.4.3.5 Variability and Uncertainty

Several assumptions made in this analysis have variabilities and uncertainties associated with them. Table F.4-17 below lists the major assumptions, discusses the variability and uncertainty, and assesses the extent of conservatism or non-conservatism.

Table F.4-17. Summary of key variabilities and uncertainties

Assumption	Discussion	Potential factor
Use of direction-independent model	The population differs by direction, and that difference varies from annular ring to annular ring. Averaging the population over the segments in each annular ring ignores the variability. However, the wind blows more frequently toward sectors with low population density, so use of the direction-independent model is slightly conservative (scoping calculations showed that conservatism to be 10% to 20%).	Slight conservatism overall
Census data were updated to 2009 rather than 2010 for all sites	Retaining the 2009 PEP increases rather than using the 2010 updates results in a slightly higher resident population estimate (4.5% for urban, 0.0% for suburban, and 1.4% for rural sites).	Slight conservatism (< 4.5%)
Future changes in the general population are not included for any site	Population projections for 2030 are available but are not used because the statewide factor does not reflect real differences between the sites and the change would not be large (for additional details, see Section 2.3).	Slight non-conservatism (1.2% to 26.6%, depending on site)
Effect of a new laboratory on suburban and rural sites	As discussed in Section 2.3, this could have a large effect on the more-distant surrounding population, but it would have a minor effect on the population within 0.5 km. Because the exposure level decreases with distance, the effect is muted.	Non-conservatism
Use of the BU and BMC population for all sectors of the urban site	The BU and BMC nonresident estimates are conservative, and those values are used as the basis for all sectors rather than attempting to estimate the nonresident population for all sectors. Using the 5-sector BU and BMC population for all 16 sectors could result in an overestimate of the population by a maximum possible factor of about 3. However, there are nonresidents in the other sectors so the conservatism is less.	Large conservatism for <0.5 km (perhaps factor of 2) at the urban site
Vehicle occupant estimates	The total number of vehicles and occupants per vehicle are overestimated, as discussed in Section F.4-3.3.	Large conservatism for vehicle count, but minor effect on overall population
Vehicle proximity to site	The vehicle occupants are assumed to be uniformly distributed, even though there are few vehicles within 0.5 km of the sites. This results in a much larger exposure level for the average vehicle occupant than would actually result.	Large overestimate in exposure to vehicle occupants

In summary, the overall urban nonresident population estimate is expected to significantly overstate the population within 0.5 km because the high BU and BMC population density was assumed to be the average for all sectors. That intentional overestimate of the population was used to avoid the need to estimate the population in all directions and because the analyses are being performed in a direction-independent manner. The overall suburban and rural nonresident populations might be non-conservative (i.e., underestimate the population). Because the purpose of the RA is at least partially to determine whether the risks would be significantly different if the laboratory were built at a lower population site,

the underestimate bias for the suburban and rural sites will tend to overstate the differences. The suburban and rural site populations might be underestimated, but the extreme difference between the sites and the urban site overwhelms this bias.

F.5 AIRBORNE DISPERSION ANALYSIS

F.5.1 Introduction

This calculation determines the atmospheric dispersion factor (χ/Q or chi over Q) for the NEIDL facility at the Boston site and the two alternate sites. χ/Q, as described later in this report, is the time-integrated air concentration at a given downwind location divided by the source strength of the plume. It typically is expressed in units of seconds per cubic meter (s/m^3). The calculation relies on the requirements and guidance issued by DOE to generate atmospheric dispersion factors suitable to use in a DOE nuclear facility documented safety analysis, and by extension into the risk analysis for the NEIDL. The results of that calculation, together with the source term calculations can provide an estimate of pathogens to a receptor at a given distance from the NEIDL facility, in a postulated accident scenario. Specifically, χ/Q is one term in the equation to estimate downwind dose to a receptor (*Dose = Source Term* × χ/Q × *Breathing Rate*). As stated in *Recommendations for the Preparation of Environmental Assessments and Environmental Impact Statements* (DOE 2004), an accident is, "an unplanned event or sequence of events that results in undesirable consequences."

The MELCOR Accident Consequence Code System, Version 2 (MACCS2) and POSTMAX computer codes were used. MACCS2 is listed in the DOE Central Registry or *toolbox*, and DOE has issued a code guidance document [DOE-EH-4.2.1.4 (DOE 2004a)]. MACCS2 is DOE/Nuclear Regulatory Commission sponsored code that has been used widely in support of probabilistic risk assessments (PRAs) for the nuclear power industry and for consequence analyses for safety documentation throughout the DOE complex. The MACCS2 module used performs all the calculations pertaining to atmospheric transport, dispersion, and deposition, as well as the decay that occurs before release and while the material is in the atmosphere. POSTMAX is a code developed at Los Alamos National Laboratory to facilitate calculation of site-specific consequence metrics from MACCS2 output files. The codes and the parameters used in the analysis are explained later in the calculation.

In addition to calculating 95th percentile χ/Q values, the 50th percentile was also calculated using the guidance in DOE 2002:

> Specifically, avoid compounding conservatisms − evaluating a scenario by using conservative values for multiple parameters will yield unrealistic results.

> For example, in air dispersion modeling, it is nearly always unrealistic to assume only extremely unfavorable meteorological conditions; prevailing (median) meteorological conditions generally should be used. In exceptional cases (e.g., when there is heightened controversy regarding accident risks or to enable a comparison with analysis in another document), it would be appropriate to estimate and present accident consequences for both median conditions and unfavorable conditions. Median conditions are often defined by using 50% meteorology, which represents plume concentrations that are not exceeded 50% of the time for a given direction and distance or receptor location, and are often characterized by stability class D and moderate wind speeds. … Unfavorable conditions are often defined using 95% meteorology, which represents plume concentrations that are not exceeded 95% of the time, and are often characterized by stability class F and low wind speeds.

To be consistent with the above guidance, the median (50^{th} percentile) results were calculated and reported in addition to the 95^{th} percentile results.

When the airborne dispersion analyses were performed, the maximum distance to be included in the analysis was not known, so the calculations were performed to a distance of 20 km. As discussed in Section F.4, the exposure calculations are performed to a distance of only 1 km, so not all the results of this calculation were used in the analyses.

F.5.2 Methodology

The U.S. Environmental Protection Agency (EPA), DOE, and the Nuclear Regulatory Commission have specified in various handbooks, guidance, and standards the use of Gaussian Plume models for the modeling of downwind concentrations of hazardous constituents resulting from an accidental release. In addition, the Defense Threat Reduction Agency also uses a basic Gaussian Plume model to provide estimates of potential down-wind concentrations of biological materials resulting from a release. Because NIH has no standard model or guidance for performing atmospheric dispersion analysis, atmospheric transport modeling using a standard Gaussian Plume approach was used, on the basis of the above government agencies recommendations and standards, to address the potential impacts from the inadvertent release of specified biological agents from the NEIDL. Similar evaluations of the transport of bacterial and viral pathogens have been made using the Gaussian Plume model. For example,

- The 1979 anthrax outbreak in Sverdlovsk, Russia, was modeled using a Gaussian Plume model together with meteorological conditions at the time to conclude that the most likely source of the anthrax release was from a nearby military facility (Meselson et al. 1994).

- Garner 1995 describes the use of a Gaussian Plume model to "the factors affecting the spread and dispersion of virus plumes." The report states that some of the advantages of using this model are
  - It produces results that agree with experimental data;
  - It is relatively straightforward to perform the calculations;
  - It is conceptually appealing;
  - It is consistent with the random nature of turbulence;
  - It is compatible with input weather observations that are readily available; and
  - Results can be obtained quickly to satisfy the demands of emergency decision-making.

The report goes on to state some limitations as

- Distances past 10 km from the source result in less predictable results
- The model must be modified for non-flat terrains (this was done for the MACCS2 code as described below in section 2.1)
- Vertical dispersion might not provide results that are as good as horizontal dispersion (this is not a significant effect for the results provided by MACCS2 for this analysis, which are for ground-level receptors)

Off-site dispersion calculations were performed using a pair of computer codes, the MACCS2 code, and the POSTMAX dispersion analysis postprocessor code. The methodology can be summarized as

1. MACCS2 calculates the ground-level air concentration:
   a. For each hour of the year,
   b. At the specified distances surrounding the release point,
   c. Using the historical weather conditions.
2. For χ/Q calculations, POSTMAX calculates
   a. The ground-level air concentration for the maximally exposed off-site individual for each hour of the year, and
   b. The cumulative distribution function for the ground-level air concentration.
3. From the 95^{th} and 50^{th} percentile ground-level air concentration (and other MACCS2 input values), the 95^{th} and 50^{th} percentile atmospheric dispersion factors (χ/Q) is calculated.

A single year of site-specific meteorological data was obtained for each of the three comparable locations; 5 years of data were obtained for the Boston site for comparison to the single year of data to confirm there were no major discrepancies. The method used to derive the 95^{th} percentile is consistent with the statistical treatment of calculated χ/Q (the normalization of the distribution of spores/particles/and such in

the air, χ, to the spore/particle source strength, Q) values described in Regulatory Position 3 of Nuclear Regulatory Commission Regulatory Guide 1.145 (RG 1.145) for atmospheric dispersion modeling at nuclear power plants. That approach has been adopted for performing the atmospheric dispersion calculations supporting this NEIDL risk assessment.

Gaussian Plume models, such as MACCS2, differ from a single plume model in that the weather meteorological data for an entire year can be used to obtain representative values for the time-integrated dispersion factor, χ/Q, for a given location of a release. That is in contrast to a snapshot in time of χ/Q from just one data set. For example, rain might or might not occur during an actual release; therefore, washout might or might not be a factor in the χ/Q for a plume. The Gaussian model, however, can take into account actual rain duration and rain rate over every hour of a year and integrate the results in a cumulative probability distribution function to determine realistically bounding values of χ/Q (e.g., the 95^{th} or 50^{th} percentile values) for a given distance (x) from the point of release. That is not to say that just because the resulting 95^{th} or 50^{th} percentile χ/Q for one site A is lower than another site B, that if an actual release occurred, the χ/Q at site A will always be lower than site B.

Extreme weather events, such as high winds that disperse pathogens farther (but necessarily more diluted as a result), can always happen. However, using this methodology, results in reasonable and defendable dispersion factors for each site because the calculation methodology uses a year's worth of hourly averaged metrological data for each site, then calculating a χ/Q. The primary output of the MACCS2 model for the purposes of this risk assessment is χ/Q, which is concentration normalized by the source strength. Therefore, for the purposes of this discussion, the absolute value of source strength does not matter.

The methodology is explained further below.

F.5.2.1 MACCS2 Computer Code

The MACCS2 computer code is described and explained in the following documents:

- NUREG/CR-6613 *Code Manual for MACCS2: Volume 1, User's Guide*, Sandia National Laboratory, SAND97-0594, May 1998
- NUREG/CR-4691 *MELCOR Accident Consequence Code System (MACCS), Model Description*, Sandia National Laboratory, SAND86-1562, February 1990
- DOE-EH-4.2.1.4-MACCS-Code Guidance, *MACCS2 Computer Code Application Guidance for Documented Safety Analysis, Final Report*, U.S. Dept. of Energy, June 2004 (DOE 2004a)

The MACCS2 code contains three separate modules to perform transport and dose calculations: ATMOS, EARLY, and CHRONC. For each module used, a different input file is created. Also, depending on the module used, MACCS2 can require other input files such as meteorological data files, a site data file containing the population distribution around the postulated release location, and a nuclide dose-conversion factor file. In this calculation, only the ATMOS module is used and the only additional input files required (for MACCS2) are the meteorological data files. Execution of the EARLY and CHRONC modules is suppressed by setting input variable ENDAT1 (in data group OC) to .TRUE.

The ATMOS module performs all the calculations pertaining to atmospheric transport, dispersion, and deposition, as well as the decay that occurs before release and while the material is in the atmosphere. The results of the calculations are stored for use by the EARLY and CHRONC modules when those modules are included as part of the calculation. The downwind transport of up to four plumes can be modeled. In addition to the air and ground concentrations, ATMOS stores information on wind direction, plume arrival and departure times, and plume dimensions.

The ATMOS module of MACCS2 offers several methodology options. Those options, and their implementation in this calculation, are explained below. The explanations include some discussions of the MACCS2 input parameters.

F.5.2.1.1 Dispersion Parameters

The Gaussian Plume model of atmospheric dispersion uses spatially dependent dispersion parameters, σ_y and σ_z [for a discussion on the terms, see the Code Manual for MACCS2 (NUREG/CR-6613)]. The dispersion parameters can be supplied to MACCS2 in two different ways: as power-law functions or in the form of pre-calculated tables for a lookup-table algorithm.

The power-law functions developed by Tadmor and Gur are used in this calculation for the base case and the ground-level release case. This is the consistent with the guidance in DOE-EH-4.2.1.4 (DOE 2004a) for distances > 100 m from the source. For comparative purposes, the base case was rerun with the dispersion coefficients from Jülich, Germany (hereafter referred to as Jülich). The release height in the Jülich test series (50 m) closely resembles that of the NEIDL stack height (51.5 m); the surface roughness around the NEIDL (urban, which correlates to roughness lengths on the order of 1 to 2 m) is bounded by that of the Jülich test series (0.5 to 3 m); the range of the Jülich test series was 11 km which is greater than the 10 km distance chosen for the last radial zone modeled; and the sampling height from Jülich was between 1–250 m, which is adequate for both ground level and elevated receptors. Per Till and Meyer

(Till and Meyer 1983, p. 2-34), "the diffusion parameters measured in Jülich should be applicable to sites with medium to higher surface roughness, which is due to settlements, vegetation, and other ground obstacles."

F.5.2.1.2 Surface Roughness

The Tadmor and Gur dispersion parameters are based on a surface roughness of 3 cm. For surface roughness lengths (z_0) other than 3 cm, the vertical standard deviation of plume spread is calculated by (DOE-EH-4.2.1.4, p 4-12, p. A-19):

$$\sigma_z (x,z_0) = (z_0/3\text{cm})^{0.2} \times \sigma_z (x,3\text{cm})$$

While MACCS2 does not specifically have an input variable for surface roughness, it does have a scaling factor for σ_z (variable ZSCALE). Therefore, the most appropriate method for modeling the local surface roughness is to set ZSCALE to $(z_0/3\text{cm})^{0.2}$, when using the Tadmor and Gur dispersion parameters. No vertical scaling factor was used for the comparative case with the Jülich dispersion parameters as the roughness length from the test series was on the order of 1–2 m.

F.5.2.1.3 Dry Deposition

MACCS2 has the option of depleting the plume because of dry deposition (i.e., gravitational settling) of particles from the plume. The dry deposition model is used when variable DRYDEP is .TRUE. This calculation recognizes dry deposition as a realistic phenomenon for particulates. Dry deposition is not appropriate for gaseous releases.

F.5.2.1.4 Wet Deposition

MACCS2 has the option of depleting the plume because of wet deposition, that is, as the raindrops, for example, fall through the plume, the raindrops strike and collect particles from the plume. The wet deposition model is used when variable WETDEP is .TRUE. (and not used when WETDEP is .FALSE.). This analysis does not include wet deposition, as recommended in DOE-EH-4.2.1.4 (p. 4-11, 4-41).

F.5.2.1.5 Buoyant Plume Rise

MACCS2 will calculate the buoyant plume rise for heated releases, such as during a fire. For MACCS2 to calculate the buoyant plume rise, the user specifies the plume heat. The cases performed in this analysis conservatively do not consider the plume to be heated.

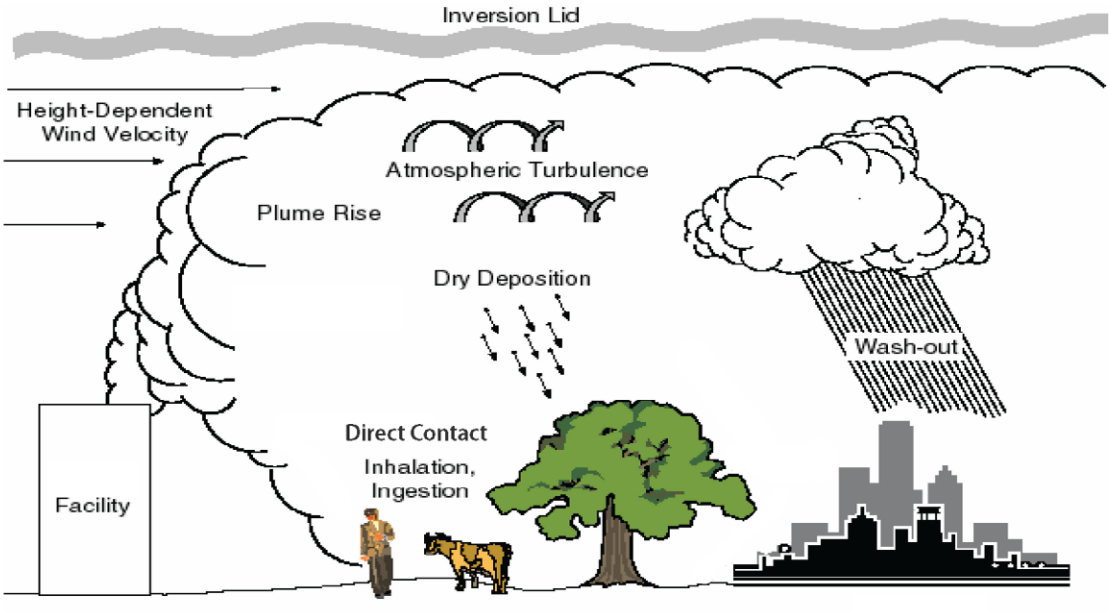
F.5.2.1.6 Plume Rise

A plume rise model is incorporated into MACCS2. There are three basic components of the model: (1) entrainment of buoyant plumes in a building wake, (2) plume rise under unstable and neutral conditions (classes A to D), and (3) plume rise under stable conditions (classes E to F).

In the cases of this analysis, the building wake portion of the plume rise model is suppressed by setting variable SCLCRW to 1.0E6 (the maximum value). MACCS2 allows the user to modify the plume rise model with separate scaling factors for the unstable and neutral conditions (classes A to D), and the stable conditions (classes E to F). This analysis uses the plume rise model as programmed by setting the scaling factors (variables SCLADP and SCLEFP) to 1.0.

Figure F.5-1 below illustrates some of the above phenomenon. The figure is taken from DOE-EH-4.2.1.4 (p. A-9) and modified to make it applicable to any type of facility rather than specifically to nuclear facilities. The figure graphically shows the phenomena of turbulence, plume rise, dry deposition and wet deposition (i.e., washout).

Figure F.5-1. Basic processes occurring during accidental releases and dose pathways.



F.5.2.1.7 Plume Meander

MACCS2 is able to account for the effect of meander during transport of the plume. The plume meander model can be turned off by setting variable TIMBAS to the duration of the plume PLUDUR (NUREG-6613, p. 5-18). This analysis conservatively does not include plume meander.

F.5.2.1.8 Wake Effects

Physically, the initial size of the plume is determined by the width and height of the building wake. The base case in this analysis credits that phenomenon by using the as-built NEIDL dimensions. For the seismic event case, where the building is assumed not to survive, this analysis conservatively assumes a source point release. To model a point source release with MACCS2, the building height (variable BUILDH), the initial σy (variable SIGYINIT), and the initial σz (variable SIGZINIT) are set to their minimum input values (1.0, 0.1, and 0.1 m, respectively).

F.5.2.1.9 Decay

DOE-EH-4.2.1.4 recommends accounting for decay and in-growth if the initial radionuclides involved at the start of the accident condition have half-lives shorter than the travel time to the receptor. In the case of this calculation, decay of biological pathogens is conservatively not considered over the short travel times to the receptors. A long half-life isotope (i.e., C-14) was used to eliminate decay effects for this calculation. C-14 has a half-life of 5700 years; thus, for the relatively short release and transport times, this results in essentially no decay. Any credit for the actual decay of pathogens in the atmosphere will be included in the application of the data in other calculation packages, as appropriate.

F.5.2.1.10 Statistical Sampling

MACCS2 is capable of statistically sampling the input meteorological data file to analyze the plume behavior. However, analyses with POSTMAX do not use statistical sampling. Instead, each hourly observation in the meteorological file is analyzed by MACCS2 to generate the appropriate input for POSTMAX. MACCS2 samples (analyzes) every hour of the year when variable meteorology code (METCOD) is 5 and variable number of samples (NSMPLS) is 24.

F.5.2.2 POSTMAX Computer Code

The POSTMAX computer code was developed at LANL to facilitate calculation of site-specific χ/Q values. Software quality assurance for POSTMAX is documented in LA-UR-09-1601, *POSTMAX V2.0 User's Guide*, Raymond F. Sartor, February 2009 (Sartor 2009).

The desired POSTMAX result is the 95th percentile ground-level concentration for a receptor at the site boundary. Each execution of MACCS2 calculates a ground-level concentration for each hourly weather observation at the spatial grid (distances) specified. The POSTMAX computer code takes the MACCS2 results and (1) statistically accounts for the distance to the site boundary, and (2) builds the cumulative distribution, which identifies the 95th percentile value.

For each MACCS2 input file, POSTMAX generates 8760 ground-level concentration values corresponding to the site boundary (or maximum value beyond the site boundary). Those ground-level concentration values are not for one specific site boundary location, but rather for the worst public location for each hour of the year, i.e., the site boundary location for the wind direction of the hour. The cumulative distribution for the year is developed by sorting the ground-level concentration values in ascending order. POSTMAX can combine the results of up to 6 years. POSTMAX, in effect, combines the results from separate years into one table and sorts the table.

F.5.2.3 Limitations

F.5.2.3.1 Meteorological Data

Hourly averaged values for wind speed, wind direction, atmospheric stability, and precipitation rate were obtained from NOAA's National Climatic Data Center online and then placed into the required year-long weather files format specifically for use in MACCS2 from nearby weather stations with available data for each site. The weather stations chosen were as shown in Table F.5-1, because no meteorological data were found exactly at the three alternate sites that were compatible with the MACCS2 format.

Table F.5-1. Towers used for meteorological data

Site	Meteorological tower used	Approximate distance between site and tower
Urban site (Albany Street, Boston)	Boston Logan Airport	4 miles
Suburban site (Tyng Road, Tyngsboro, MA)	Fitchburg Municipal Airport	18 miles
Rural site (Sargent Camp Road, Hancock, NH)	Jaffrey Municipal Airport	9 miles

The weather conditions at the time of an actual release cannot be predicted. Therefore, the methodology presented below presents both the 50th and 95th percentile χ/Q values to address a broad range of potential conditions.

F.5.2.3.2 Safety Advisory 2009-05

In August 2009, the DOE Office of Health, Safety and Security issued Safety Advisory 2009-05, *Errors in MACCS2 χ/Q Calculations* [DOE 2009]. That advisory identified a problem and corrective action when using a lookup table for sigma-y and sigma-z. Because this calculation uses the power law formula for sigma-y and sigma-z, that issue is not relevant to this calculation. The advisory also recommends turning off the DAY_NIGHT mixing height option when using version 2.4. Because this calculation uses version 1.13.1, that recommendation is not relevant to this calculation.

F.5.2.4 Assumptions

F.5.2.4.1 Release Methodology

Assumption: The material is released from the facility ventilation exhaust stack for operational events and from the ground level as a single point source for the seismic event case.

Justification: This assumption for releases from the exhaust stack is realistic for operational events in which the building integrity and the ventilation system is maintained. For a seismic event, the building is conservatively assumed to fail (e.g., collapse) and the release cannot be assumed to be through the ventilation stack; thus, it is conservative to assume a ground-level point source release because multiple release locations or an area or volume release would disperse and dilute the release.

F.5.2.4.2 Release Duration

Assumption: The release duration is one hour (3,600 seconds).

Justification: The final χ/Q value is calculated from the time-integrated air concentrations given by MACCS2. Because the air concentrations are time-integrated, the release duration does not affect the final χ/Q values. Notice, however, changing the release duration could change the operation of the plume meander model. As stated previously, however, plume meander was turned off, so the value of release duration does not affect the desired results.

F.5.2.4.3 Release Height—Non-buoyant Plumes

Assumption: It is assumed that the plume is buoyantly neutral.

Justification: Buoyancy raises the plume above the ground, which decreases the ground-level concentration and dose consequences. Thus, it is conservative to suppress this option.

F.5.2.4.4 Stability Class

Assumption: Stability class for each hour of the year for each site was determined using the Pasquill stability categories.

Justification: Regulatory Guide 1.23 states that the preferred method for determining Pasquill stability classes is the vertical temperature difference. However, the meteorological data obtained did not have temperature at the recommended heights, so the vertical temperature difference method was unable to be used. Thus, an alternative method to derive the Pasquill stability classes (based on wind speed, insolation and cloud cover) was chosen from available meteorological data is presented in Table F.5-2 (Till and Meyer 1983). The more stable the atmosphere, the less dispersion in the vertical and horizontal directions and, therefore, the higher the concentration of particulates. The atmospheric stability class is a direct input into the MACCS2 model in the meteorological data file. MACCS2 uses stability class in determining plume dispersion using the vertical and horizontal dispersion coefficients σ_y and σ_z.

Table F.5-2. Pasquill stability classes as a function of wind speed, insolation, and cloud cover

Surface wind speed (m/s)	Daytime insolation			Night	
	Strong	Moderate	Slight	Thinly overcast or > 4/8 cloud cover	≤ 3/8 cloud cover
< 2	A	A–B	B	--	--
2-3	A–B	B	C	E	F
3-5	B	B–C	C	D	E
5-6	C	C–D	D	D	D
> 6	C	D	D	D	D

Source: Till and Meyer 1983, Table 2-1

F.5.2.4.5 Building Dimensions

Assumption: If a new laboratory is built at either of the two alternate sites, the building dimensions would be the same as the as-built facility in Boston.

Justification: This assumption is necessary to make meaningful comparisons between the differing topographies and meteorology of the two alternate sites.

F.5.3 Results

F.5.3.1 Description of Cases and Results

The following provides a brief description of the cases run for each of the three cases (i.e., Base Case, Ground-Level Release Case, and Jülich Dispersion Parameters Case). Results are presented for each of

the three sites evaluated (i.e., urban, suburban, and rural sites). The descriptions below correspond to the data presented in the following Tables F.5-3 to F.5-5 and Figures F.5-2 to F.5-5.

Base Case—The first model run (i.e., case) was called the base case; other cases are a variation of this one. As a summary of the above sections on assumptions and inputs, the base case assumed the following: dry deposition velocity of 0.01 m/s; no wet deposition; no buoyant plume (e.g., no fire); an elevated release from the NEIDL exhaust stack; dispersion parameters derived from Tadmor and Gur; and building wake effects turned "on" (e.g., actual NEIDL dimensions used).

As previously described, the base case (and all other cases) was run twice for the Boston site: once using an aggregate of 5 years of meteorological data and once using only one year of meteorological data. For the Boston site, 5 consecutive years of meteorological data were run through MACCS2 and the aggregate 95th percentile χ/Q was determined from POSTMAX. Then the same cases were run with just a single year of meteorological data to compare the results. Results, as shown in tables and graphically in the figures below, indicate that the differences in 95th percentile χ/Q were not significant. Therefore, it was determined that for the two alternate sites (Tyngsboro and Hancock), only single year data was needed because the extra time required for obtaining and formatting meteorological data, running the codes several extra times, and analyzing the extra data did not significantly affect the results.

As can be seen in Table F.5-3 and graphically in Figure F.5-2, the values start on the order of 10 to 5 for receptors close to the release and decrease, as expected, the further one gets from the source. By the time the plume reaches a distance of 20 km, the χ/Q has lowered by two orders of magnitude. Also, the 95th percentile results are approximately a factor of 2 to 4 higher than the 50th percentile results, depending on distance.

Ground-Level Release Case—This case is the same as the Base Case except the source is assumed to be released from the ground instead of from the exhaust stacks. Because this type of release would be realistic under circumstances where the NEIDL facility has failed (e.g., large seismic event), it was also reasonable to assume that building wake effects should be turned off. The 95th percentile χ/Q near the point of release are significantly larger than the Base Case, approximately to two orders of magnitude larger at distances close to the release. This is anticipated as the 95th percentile χ/Q is for a ground-level receptor. At large distances from the release (e.g., 3–4 km) from the release, the 95th percentile χ/Q is lower than the base case; afterwards the two cases approach each other.

Jülich Dispersion Parameters Case—This case is the same as the Base Case except dispersion parameters derived from the Jülich test series were used instead of from Tadmor and Gur and, as a result, no vertical scaling factor was used. The 95th percentile χ/Qs were generally lower than the Base Case at all distances. However, for the two alternate sites the Jülich Case was able to provide a greater fidelity in χ/Q between distances than the base case.

Tables F.5-3 through F.5-5 below summarize the results (50th percentile and 95th percentile) for each of the three sites, respectively (i.e., urban, suburban, and rural). Each table shows the results of each of the three cases (Base Case, Ground-Level, and Jülich case) at each distance modeled. Figures F.5-2 and F.5-3 graphically present the urban site results for all three cases using both 1 year of meteorological data and 5 years of meteorological data. As shown, the results are similar and the 1 year and 5 year data sets. Figures F.5-4 and F.5-5 graphically present the suburban site results for all three cases.

At the extremely close distance of 25 m, the Ground-Level case shows approximately and order of magnitude higher result than even at 100 m. That is expected given the release and the receptor are both assumed to be at ground level. For the base case and the Jülich case, at the extremely close distance of 25 m, the results show approximately the same or sometimes even lower values than the 100-m distance. That is expected because these cases are elevated stack releases, and it takes some plume-travel distance for the plume to spread out enough to reach a ground-level receptor. Because POSTMAX calculates the highest concentration at or beyond the distance requested, often the 100-m distance resulted in higher concentrations because of that phenomenon.

1 **Table F.5-3. 1-Year and 5-year χ/Q (s/m^3) results—urban site**

Distance from release (m)	Base case				Ground-level case		Jülich case	
	50 percentile – 1-Year	50 percentile – 5-Year	95 percentile – 1-Year	95 percentile – 1-year	50 percentile	95 percentile	50 percentile	95 percentile
25	1.96E-05	1.77E-05	4.87E-05	4.01E-05	4.70E-03	4.44E-02	9.23E-6	2.58E-5
100	1.96E-05	1.77E-05	4.87E-05	4.01E-05	4.27E-04	3.39E-03	1.24E-05	3.02E-05
200	1.89E-05	1.77E-05	4.74E-05	3.46E-05	1.29E-04	1.12E-03	1.17E-05	2.72E-05
300	1.67E-05	1.59E-05	4.74E-05	3.40E-05	6.78E-05	5.93E-04	1.05E-05	2.22E-05
400	1.38E-05	1.38E-05	4.55E-05	3.40E-05	4.31E-05	3.77E-04	8.09E-06	1.98E-05
500	1.13E-05	1.22E-05	4.12E-05	3.29E-05	3.03E-05	2.65E-04	6.54E-06	1.60E-05
600	9.95E-06	1.08E-05	3.40E-05	2.90E-05	2.28E-05	1.98E-04	5.38E-06	1.44E-05
700	8.81E-06	9.58E-06	3.36E-05	2.56E-05	1.79E-05	1.55E-04	4.43E-06	1.40E-05
800	7.84E-06	8.52E-06	3.02E-05	2.27E-05	1.45E-05	1.25E-04	3.71E-06	1.28E-05
900	7.61E-06	7.61E-06	2.73E-05	2.02E-05	1.20E-05	1.04E-04	3.34E-06	9.89E-06
1,000	6.30E-06	6.84E-06	2.52E-05	1.81E-05	1.02E-05	8.75E-05	2.71E-06	9.86E-06
2,000	2.79E-06	3.03E-06	1.68E-05	8.00E-06	3.44E-06	2.88E-05	1.05E-06	5.66E-06
3,000	1.60E-06	1.74E-06	1.14E-05	4.71E-06	1.80E-06	1.46E-05	5.50E-07	3.60E-06
4,000	1.06E-06	1.15E-06	8.17E-06	3.16E-06	1.14E-06	4.54E-06	3.55E-07	2.57E-06
5,000	7.61E-07	8.24E-07	6.15E-06	2.29E-06	7.98E-07	3.16E-06	2.51E-07	2.10E-06
10,000	2.67E-07	2.89E-07	1.22E-06	7.17E-07	2.66E-07	9.10E-07	1.12E-07	6.13E-07
15,000	1.43E-07	1.52E-07	5.85E-07	3.79E-07	1.38E-07	4.53E-07	7.65E-08	2.99E-07
20,000	9.00E-08	9.42E-08	3.34E-07	2.41E-07	8.64E-08	2.69E-07	5.93E-08	2.03E-07

Figure F.5-2. Comparison of single year 95 percent cases—urban site.

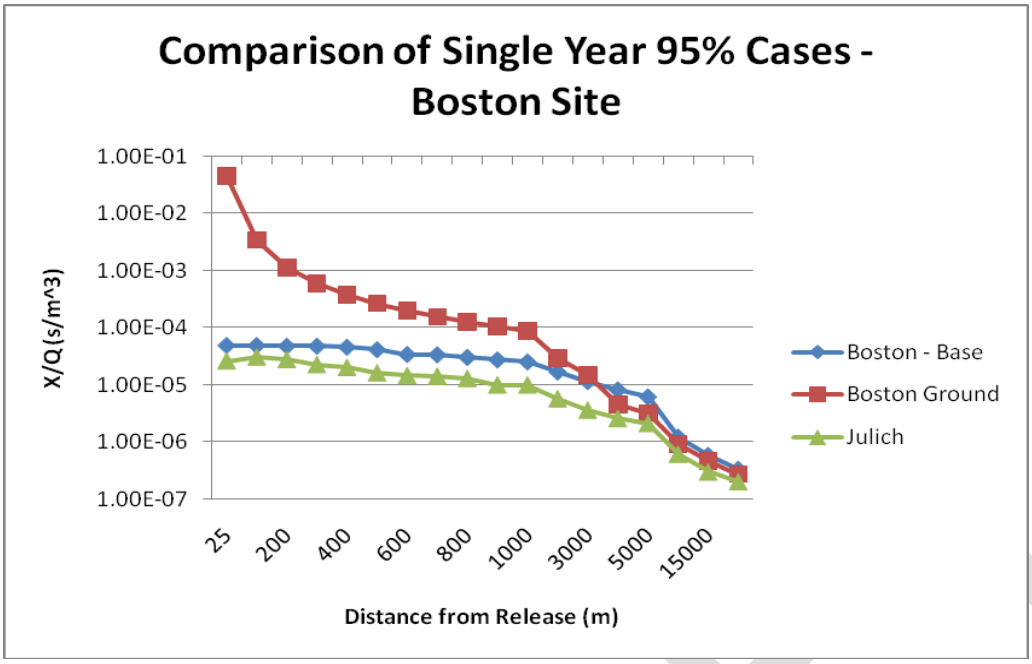


Figure F.5-3. Single-year versus 5-year data comparison—urban site.

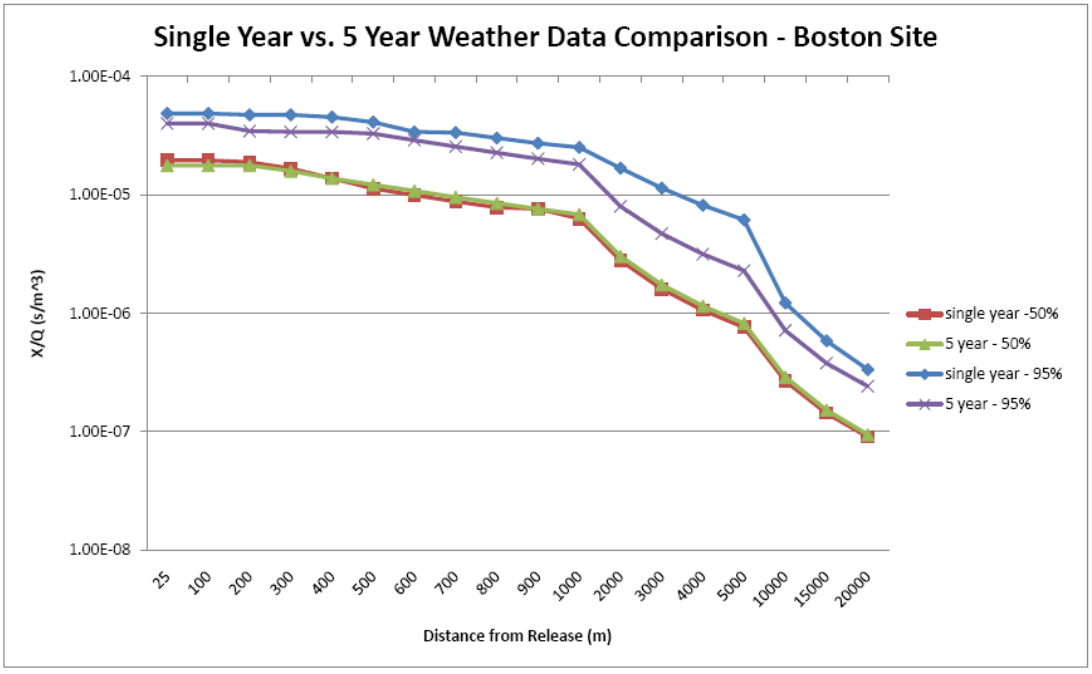


Table F.5-4 –Single-year χ/Q (s/m^3) results—suburban site

Distance from release (m)	Base case		Ground-level case		Jülich case	
	50 percentile	95 percentile	50 percentile	95 percentile	50 percentile	95 percentile
25	2.92E-05	1.97E-04	1.72E-02	2.18E-01	2.04E-05	1.47E-04
100	2.92E-05	1.97E-04	1.22E-03	1.48E-02	2.04E-05	8.64E-05
200	2.67E-05	9.86E-05	3.39E-04	3.92E-03	1.68E-05	3.42E-05
300	2.41E-05	9.86E-05	1.46E-04	1.78E-03	1.30E-05	3.34E-05
400	2.17E-05	9.86E-05	7.06E-05	9.99E-04	1.10E-05	3.31E-05
500	1.88E-05	9.86E-05	4.96E-05	6.32E-04	8.43E-06	3.28E-05
600	1.78E-05	9.86E-05	3.72E-05	4.32E-04	7.69E-06	3.22E-05
700	1.67E-05	9.86E-05	3.20E-05	3.11E-04	6.81E-06	3.16E-05
800	1.56E-05	9.86E-05	2.35E-05	2.33E-04	6.24E-06	3.07E-05
900	1.46E-06	9.86E-05	1.95E-05	1.80E-04	6.11E-06	2.98E-05
1,000	1.28E-05	9.67E-05	1.65E-05	1.50E-04	5.37E-06	2.87E-05
2,000	4.52E-06	6.77E-05	5.52E-06	4.42E-05	1.70E-06	1.85E-05
3,000	2.83E-06	4.40E-05	2.87E-06	2.06E-05	8.94E-07	1.16E-05
4,000	1.72E-06	2.88E-05	1.64E-06	1.19E-05	5.09E-07	7.54E-06
5,000	1.20E-06	1.82E-05	1.14E-06	7.55E-06	3.95E-07	4.78E-06
10,000	4.16E-07	4.34E-06	3.10E-07	2.28E-06	1.56E-07	1.22E-06
15,000	2.02E-07	1.78E-06	1.51E-07	8.90E-07	9.45E-08	5.38E-07
20,000	1.19E-07	8.76E-07	9.47E-08	4.87E-07	6.61E-08	3.07E-07

Figure F.5-4. Comparison of single-year 95 percent cases—suburban site.

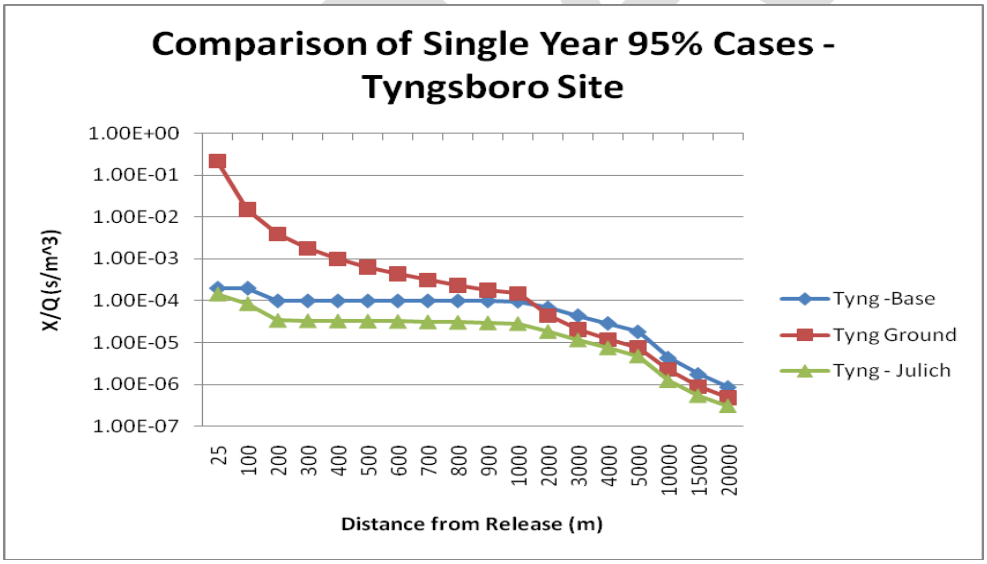


Table F.5.-5. Single-year χ/Q (s/m^3) results—rural site

Distance from release (m)	Base case		Ground-level case		Jülich case	
	50 percentile	95 percentile	50 percentile	95 percentile	50 percentile	95 percentile
25	3.25E-05	1.93E-04	2.21E-02	2.29E-01	2.33E-05	1.47E-04
100	3.25E-05	1.93E-04	1.50E-03	1.52E-02	2.33E-05	1.47E-04
200	3.06E-05	1.59E-04	4.21E-04	3.96E-03	1.86E-05	8.68E-05
300	2.54E-05	1.09E-04	1.87E-04	1.77E-03	1.51E-05	5.25E-05
400	2.33E-05	9.32E-05	1.18E-04	9.88E-04	1.12E-05	3.45E-05
500	2.06E-05	9.32E-05	8.26E-05	6.22E-04	1.06E-05	3.28E-05
600	1.97E-05	9.32E-05	6.16E-05	4.21E-04	8.74E-06	3.22E-05
700	1.89E-05	9.32E-05	4.81E-05	3.01E-04	7.83E-06	3.16E-05
800	1.88E-05	9.32E-05	3.88E-05	2.27E-04	7.70E-06	3.07E-05
900	1.68E-05	9.32E-05	3.21E-05	1.85E-04	7.01E-06	2.98E-05
1,000	1.35E-05	9.17E-05	2.71E-05	1.54E-04	6.34E-06	2.87E-05
2,000	6.79E-06	6.64E-05	7.86E-06	4.48E-05	2.32E-06	1.85E-05
3,000	4.06E-06	4.42E-05	3.27E-06	2.07E-05	1.22E-06	1.16E-05
4,000	2.72E-06	2.94E-05	1.75E-06	1.18E-05	6.49E-07	7.54E-06
5,000	1.72E-06	1.99E-05	1.20E-06	7.63E-06	5.02E-07	5.10E-06
10,000	4.46E-07	4.68E-06	2.49E-07	2.35E-06	1.84E-07	1.28E-06
15,000	2.27E-07	1.88E-06	1.29E-07	8.79E-07	1.09E-07	5.68E-07
20,000	1.29E-07	9.44E-07	8.55E-08	5.00E-07	7.65E-08	3.36E-07

Figure F.5-5. Comparison of single-year 95 percent cases—rural site.

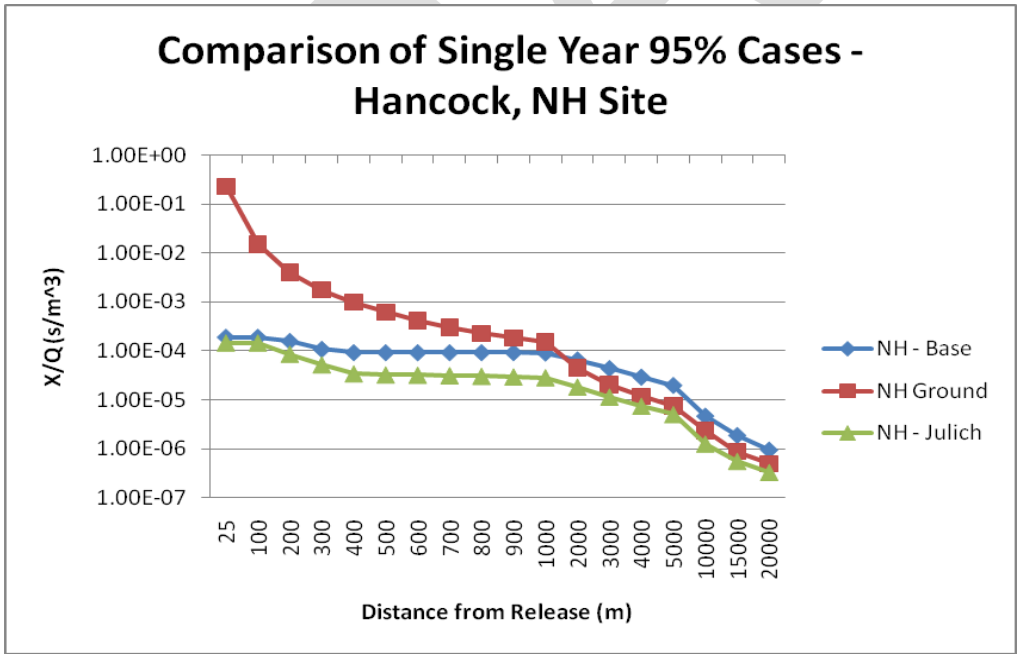


Figure F.5-6 graphically presents the results for all three sites for the Base Case and Figure F.5-7 presents the results for all three sites for the Ground-Level Release Case.

Figure F.5-6. Base case comparison of all three sites.

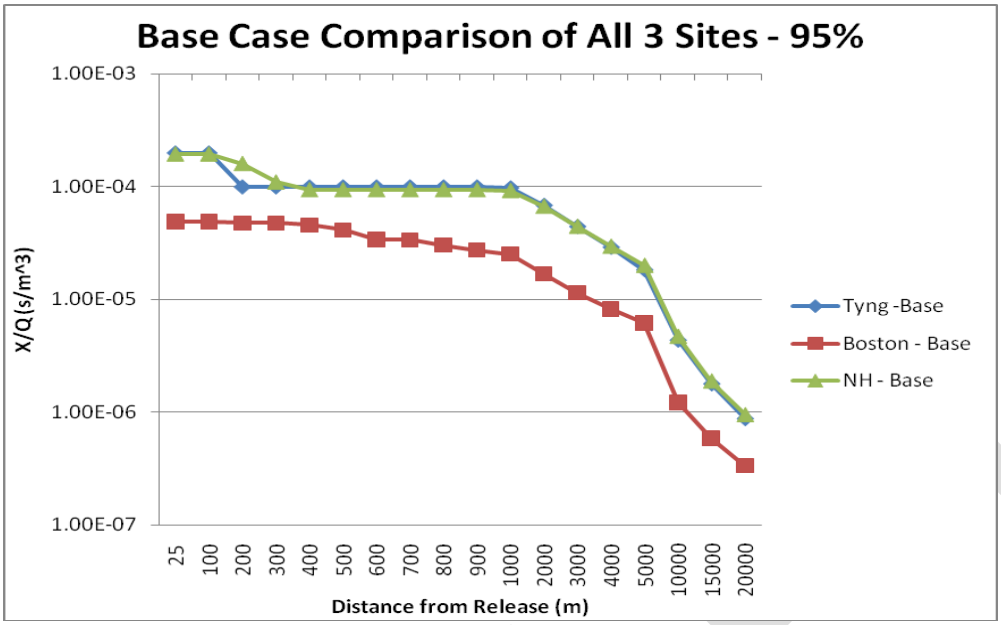
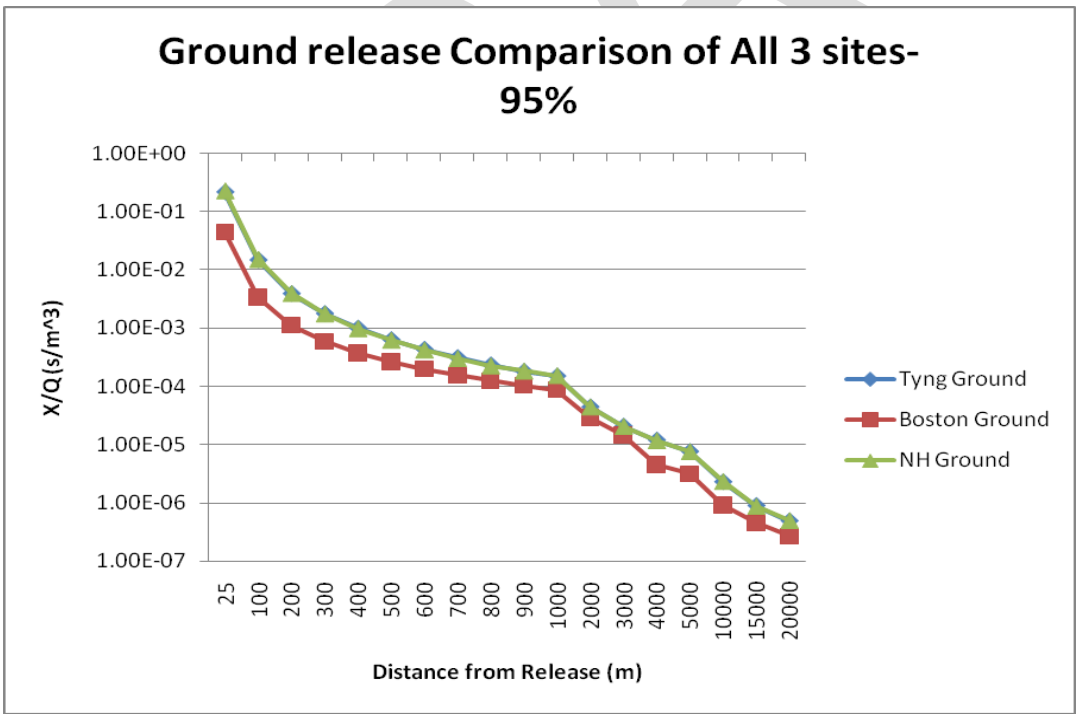


Figure F.5-7. Ground-level release comparison for all three sites (note: Tyng Ground and NH Ground curves are nearly identical).



F.5.3.2 Wind Speed and Direction

In addition to the above dispersion results, the following information is added for illustrative and comparative purposes. One can take the meteorological data for each of the three sites and determine a 50 and 95 percent wind speed. That data can give one an idea of how fast a resulting plume will travel to a receptor after a postulated release. The slower the wind speed the lower the dispersion, thus, the values for 95th percentile are lower than 50th percentile. The results are shown in Table F.5-6 below:

Table F.5.-6. Wind speed for the three sites

Percentile	Boston site	Suburban site	Rural site
50th	5.1 m/s	3.1 m/s	3.1 m/s
95th	1.5 m/s	1.5 m/s	1.5 m/s

Similarly, one can determine the probability of the wind to blow from any wind sector and display it on a wind rose. For example, the urban site wind roses for one year of meteorological data and 5 years of meteorological data are presented Figures F.5-8 and F.5-9, and the data are presented in Table F.5-7 for the sectors and percentage of time that the wind blows in a given direction. As can be shown in Figures F.5-8 and F.5-9, the wind roses of 1 year and 5 years of data are similar.

Figure F.5-8. Wind rose for single-year data—urban site.

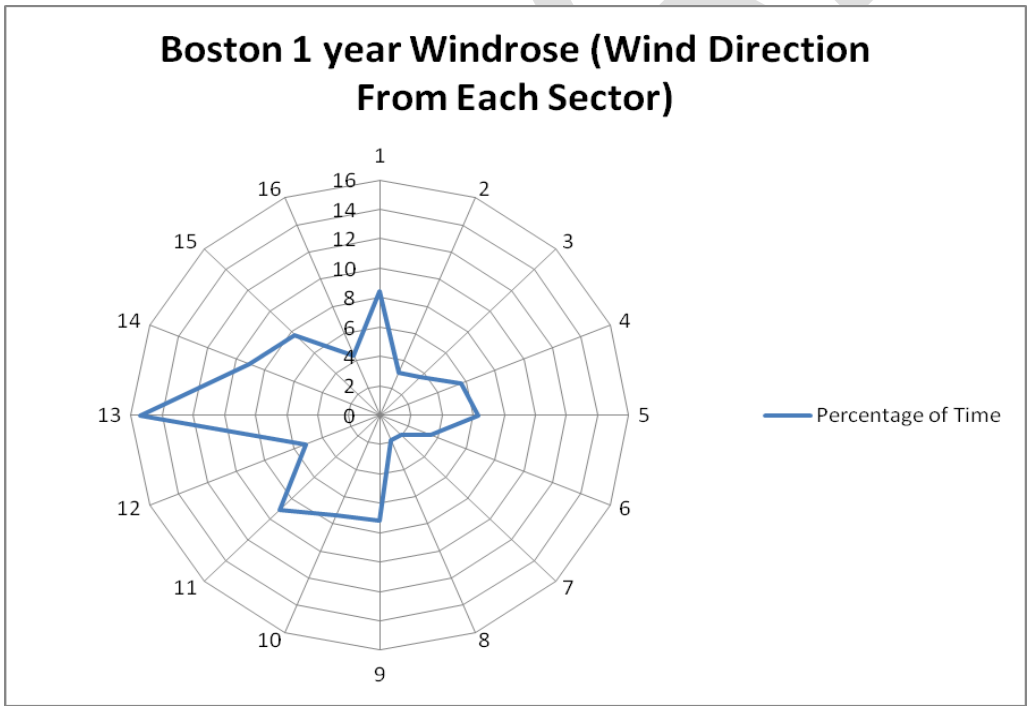


Figure F.5-9. Wind rose for 5-year data—urban site.

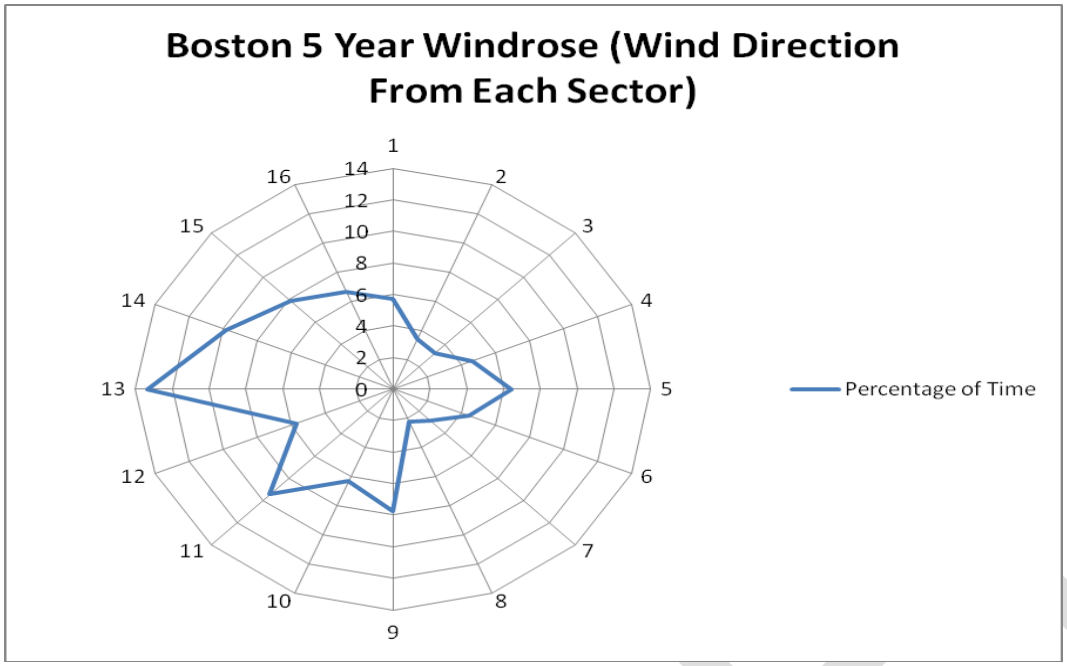


Table F.5-7. Single-year and 5-year wind speed data—urban site

Sector	Percentage of time—1 year of data	Percentage of time—5 years of data
1	8.4%	5.7%
2	3.2%	3.4%
3	3.7%	3.2%
4	5.7%	4.6%
5	6.3%	6.4%
6	3.5%	4.5%
7	1.9%	2.8%
8	1.9%	2.2%
9	7.2%	7.8%
10	7.4%	6.3%
11	9.1%	9.5%
12	5.2%	5.7%
13	15.4%	13.3%
14	9.1%	9.8%
15	7.7%	7.9%
16	4.4%	6.7%

F.5.3.3 Comparison to FEIS Dispersion Calculations

The NEIDL Final Environmental Impact Statement (FEIS) (NIH 2005) included results of a calculation performed for airborne release of 400,000 respirable anthrax spores from the facility exhaust stacks with

varying levels of HEPA filtration (zero HEPAs, 1 stage, and 2 stages). It is noted that the FEIS calculations were performed for highly dispersable anthrax spores, which will not be used at NEIDL, to give upper-bound (*worst-case*) results. The results were presenting using multiple methods, including a wind tunnel and EPA's dispersion code ISC-PRIME. Results were most conservative with the wind tunnel, but for comparative purposes here, the results from the MACCS2 code will be compared with the results from ISC-PRIME. The FEIS does not directly report a χ/Q value, but one can be back calculated with the information given. Namely,

- A release of 400,000 spores
- A breathing rate of 30 liters/minute ($5x10^{-4}$ m^3/sec)
- A calculated maximum number of spores that might be inhaled is 0.1755 spores (zero HEPA filter case)

Thus, the $\chi/Q = \frac{0.1755\ spores}{400000\ spores * 5E-4 \frac{m3}{sec}}$ = 9E-4 sec/m^3

Similarly, the FEIS reports the case with two HEPA filters intact, which is expected for an operational event, will result in a maximum number of spores of 0.036 spores. This corresponds to a χ/Q of 1.8E-4 s/m^3. Those results are consistent with the MACCS2 Boston site Base Case results presented in Table F.5-8. For example the MACCS2 results for a receptor 100 m away is a χ/Q of 4.01E-5 sec/m^3. The results are very close given the difference in the numerical methods (computer codes) chosen and the difference in assumptions. For example, the FEIS assumes an elevated release with an elevated receptor, while the MACCS2 model assumes an elevated release with a ground-level receptor.

Also, for illustrative purposes, if one back-calculates the χ/Q using the FEIS most conservative values percentile χ/Q is 3.39E-3 sec/m^3 and the corresponding 50th percentile χ/Q is 4.27E-4 sec/m^3. Thus, while the results between ISC-PRIME and MACCS2 are not directly comparable, they are very close to one another considering uncertainty in any code and difference in assumptions. The above discussion is summarized in Table F.5-8:

Table F.5-8 Comparison of MACCS2/POSTMAX χ/Q (s/m^3) results to FEIS χ/Q results-urban site

Distance (m)	MACCS2 base case (95th percentile)*	FEIS using ISC-PRIME	MACCS 2 ground-level release (95th percentile)*	MACCS 2 ground-level release (50th percentile)*	FEIS wind tunnel
100	4.01E-05	9E-4**	3.39E-03	4.27E-04	1.5E-3
200	3.46E-05		1.12E-03	1.29E-04	

* Using 5 years of meteorological data

** Distance is not given in the FEIS, although it appears to be less than 100 m. For comparative purposes, both the 100- and 200-m MACCS2 values are used.

In addition, one can compare this to the recommended χ/Q value from DOE-STD-1189 (DOE 2008) (p. A-6); namely, assuming no plume buoyancy, F-stability class, 1.0 m/sec wind speed, a small building size of 10 m × 25 m, and 1 cm/sec deposition velocity, the resulting χ/Q at 100 m is 3.5E-3 s/m^3.

F.6 CENTRIFUGE EVENTS

F.6.1 Introduction

Centrifuges use centrifugal force to separate mixtures of materials having differing densities and will be used in the NEIDL to concentrate infectious pathogen particles in suspensions. NEIDL will use a variety of different types of centrifuges ranging from small, bench-top, low-speed microcentrifuges to large, floor-mounted, ultra-high-speed ultracentrifuges (Rarick 2009). Centrifuges can accept a variety of fixed-angle rotors and swinging buckets/cups, intended for different containers and uses. The specific centrifuge and rotor used for any specific task depends on the purpose of the centrifugation.

A pathogen aerosol release resulting from a centrifuge-related event sequence has been selected as a representative event sequence to be analyzed (see Appendix E) for laboratory-associated infections (LAIs). A centrifuge-related event was selected because it is one of the more frequent sources of aerosol releases in the laboratory setting and aerosol releases pose a significant threat to the laboratory workers.

F.6.2 Methodology

This analysis relies on both generic and NEIDL-specific information. *Biosafety in Microbiological and Biomedical Laboratories* 5th ed. (CDC and NIH 2007), referred to as BMBL, provides generic guidance for the biocontainment of pathogens in laboratories of this type. The *Boston University Medical Center Biosafety Manual* (BUMC 2011), referred to as the BUMC Biosafety Manual, and NEIDL-specific SOPs provide NEIDL-specific procedures.

This analysis focuses on centrifuge operations and potential exposures to a pathogen (or pathogens) that could result. The scope of this centrifuge event sequence analysis is defined as follows:

- Activities directly associated with placing containers into centrifuge rotors or buckets/cups, placing the rotor or buckets/cups into the centrifuge, operating the centrifuge, removing the rotor or buckets/cups from the centrifuge, and removing the containers from the rotor or buckets/cups are included.

  Note: This analysis does not include activities associated with general pathogen sample handling that might be performed for a variety of purposes. For example, general activities associated with

sample preparation, transfer of pathogens from one container to another, and movement of containers within the various laboratory spaces are not considered here. These general activities are addressed in other event sequence evaluations, as appropriate.

- Centrifuge equipment malfunctions, personnel errors, and loss of utilities (e.g., electricity and ventilation) associated with the centrifuge are included.
- The analysis includes progression of the event sequence from the initiating event through to the point of primary exposure.

  Note: This analysis defines the routes of exposure and estimates the number of people exposed, but it does not analyze the health effects from exposures or consider subsequent exposures. The health effects resulting from exposure were evaluated and documented in other calculation packages and reports.
- Aerosol releases from a centrifuge can result in inhalation, ingestion, and direct contact and those three routes of exposure are considered in this RA. Puncture exposures are not relevant for centrifuge releases but are addressed by other analyses.

  Note: For this analysis, it is conservatively assumed that a person potentially inhaling, ingesting, or directly contacting a pathogen is exposed regardless of level of exposure.
- The analysis addresses accidental releases only and does not consider malevolent acts, which are addressed in a separate analysis.

It should also be noted that this analysis does not address event sequences where centrifuge operations are only incidental and are not a key part of the incident. For example, this analysis does not consider centrifuge event sequences caused by an earthquake that coincidentally occurs while a centrifuge is in operation. An earthquake has the potential to cause a release independent of the centrifuge operation. These types of circumstances are addressed as part of other event sequence analyses (e.g., earthquake), as appropriate.

A variety of specific analyses have been performed for the centrifuge release scenarios. Rather than attempt to describe the methodology for each portion of the methodology here, the methodology and results are presented together in the following section.

F.6.3 Results

This section presents the results of the analyses. It begins with the process for selection of event sequences to be analyzed and the presents the analyses of those BSL-3 and BSL-4 event sequences.

F.6.3.1 Selection of Event Sequences

Operations associated with centrifuges have the potential to expose workers as a result of aerosol formation and release. The aerosol has the potential of exposing inadequately protected laboratory workers via inhalation to the lungs, direct contact with mucous membranes and eyes, and ingestion via an open mouth while breathing or speaking (Appendix E).

Factors that were considered in the selection of event sequences for analysis include the following:

- *Detection and reporting*—Event sequences that are more likely to be detected and reported are less likely to result in initial infection and secondary transmission because of the response speed and effectiveness of medical intervention. Therefore, event sequences where early or immediate detection was less likely were selected over similar event sequences that are readily detected.
- *Magnitude of release*—Event sequences that result in greater aerosol releases were selected over event sequences with lesser releases. Other factors being the same, event sequences with greater releases will generally result in the greater consequences and are selected preferentially.

The following paragraphs describe some of the event sequences considered and provide a rationale for their selection or dismissal from further consideration.

- *Dropped container while loading rotor*—A container could be dropped during rotor loading, which could produce an aerosol and potentially result in a release. Rotors are required to be loaded in a BSC (BUMC 2011), which is designed to prevent potential aerosols from being released to the room. The drop height while loading or opening a rotor would likely be no greater than the interior height of the BSC. As a result of the low energy imparted to the container, this event is unlikely to result in large aerosol formation or release. Drop of a container while loading a rotor is similar to other container handling events, which are addressed in other analyses. Because of the low aerosol generation and similarity to general handling activities, such an event sequence was *not selected for analysis*.
- *Dropped rotor during transfer*—A loaded and sealed rotor could be dropped when it is being transferred to the centrifuge, or when it is being removed after centrifugation. This is also a relatively low energy event (the screening evaluation assumed a maximum drop of 4 feet) that was considered unlikely to generate large releases, includes two barriers and is similar to other container handling events addressed in other analyses. Therefore, such an event sequence was *not selected for analysis*.
- *Centrifuge explosion*—There have been several instances of centrifuges *exploding* (e.g., the rotor breaking up because of material fatigue, which results in pieces being ejected at high speeds) (AIHA 2010). While that type of event sequence might have the potential to produce a high

aerosol release fraction, the event sequence would be immediately detected and appropriate mitigative measures (e.g., evacuating the area and notifying the Control Center) would be implemented unless the worker is incapacitated. As a result of the high probability of detection, the risk of subsequent transmission was considered minimal, and such a scenario was *not considered further*.

- *Leak/spill inside rotor*—A leak inside the rotor as a result of human error or container leak is a possible event sequence that might not be detected. The event has the potential to generate significant amounts of aerosol, which could be released if the rotor is not properly sealed or if opened outside a BSC in violation of SOPs. As a result of the potential for aerosol generation without detection, such a scenario is *retained for detailed evaluation*.

The event sequence involving a container leak inside a rotor was selected for analysis for both BSL-3 and BSL-4 laboratories because it has the potential to release aerosols without detection. The consequences of this event sequence are considered to bound the consequences of other event sequences (i.e., its consequences are as severe or more severe). The operating experience includes multiple events that involved leakage from containers during centrifugation (see Attachment A of this appendix) and this experience is used as guidance in development of the event sequence. Respiratory protection (i.e., PAPR for BSL-3 and positive-pressure suits for BSL-4) is required when working with any pathogen (BU 2010) and the event sequences must include this requirement.

The centrifuge event sequences selected for analysis are as follows:

- BSL-3 centrifuge event sequence *with full* respiratory protection—This event was selected to determine the adequacy of the respiratory protection during a release.
- BSL-3 centrifuge event sequence *with partial* respiratory protection—This event was selected to determine the potential consequences if a respirator does not provide full protection.
- BSL-4 centrifuge event sequence *with partial* respiratory protection—This event was selected to determine the potential consequences if a positive-pressure suit does not provide full protection. Analysis of a BSL-4 event with full respiratory protection was not selected because there would be no exposure.

Each of those events was analyzed separately in the following sections.

F.6.3.2 BSL-3 Centrifuge Aerosol Release with Full Respiratory Protection

This section addresses an event sequence involving an aerosol release from a centrifuge in the BSL-3 laboratory with full respiratory protection for the laboratory workers involved. This section describes the event sequence, including the biocontainment features, the frequency category, the consequences, including the exposure category, and the potential extent of exposure.

F.6.3.2.1 Event Sequence Description

A series of experiments designed to mimic the potential event sequences in laboratories was conducted by the Health Protection Agency, an independent organization in the United Kingdom, to obtain data on aerosol releases from those events. The experiments included two centrifuge events, one with a fixed-angle rotor and one with a swinging bucket rotor. The event sequence described here was modeled after the fixed-angle rotor experiment because it resulted in higher aerosol releases and, in fact, the fixed-angle rotor centrifuge experiment resulted in one of the highest aerosol releases of any of the experiments performed. While focusing on the fixed-angle rotor event, this analysis is also applicable to and bounds a swinging bucket event (Bennett and Parks 2006).

The hypothetical scenario evaluated for the NEIDL involves the aerosol release of a pathogen from a bench-top centrifuge and is representative of a variety of centrifuge event sequences that could occur in the BSL-3 laboratory. The following describes the initial conditions and the event sequence.

Initial Conditions—The conditions leading up to this event sequence are as follows.

- A bench-top centrifuge is operated with an aerosol-tight rotor lid or swinging bucket cap is operated outside a BSC. This is a common configuration for centrifugation in newly equipped BSL-3 laboratories.

- The primary containers have caps, but not all the caps identified for centrifugation in NEIDL have aerosol-tight seals.

- The centrifuge contains multiple conical tubes collectively containing the typical working volume for the given pathogen suspension being considered (e.g., multiple tubes with a combined volume of up to 150 mL).

- The pathogen suspension contains the maximum concentration for that pathogen that is expected to be used in NEIDL. Lower concentrations are expected to result in correspondingly lower exposures.

- It is assumed that from one to four laboratory workers in the vicinity of the centrifuge at the time of the event sequence. The number of people in a room during centrifugation is not expected to exceed four (BUMC 2009).

- As required, the laboratory workers in the room are wearing BSL-3 dedicated shoes, shoe covers, scrub suits with elastic cuffs, a back-fastening gown, and double gloves (BUMC 2010). This is the minimum PPE required for all BSL-3 activities.

- It is assumed that the workers are wearing respiratory protection (i.e., a PAPR) as required by the SOPs (BUMC 2010).

A number of biocontainment features prevent or mitigate the consequences of this event sequence. The role of each biocontainment feature for this event sequence is shown in Table F.6-1. Section F.2 provides a description of biocontainment features.

Table F.6-1. Exposed group protected by each biocontainment feature—BSL-3 centrifuge aerosol release with full respiratory protection.

	Admin. controls		Safety equipment							Facility	
Exposed group	Procedures	Training	Container	Rotor/buckets	Centrifuge chamber[a]	BSC	PPE	Respiratory protection	Positive-pressure suit	HVAC system	Sealed walls, ceilings, and floors
Laboratory worker	M[b]/P[c]	M/P	M[e]	M/P	-[d]	-	-	M	-	M	-
Facility worker	P	P	M	M/P	-	-	-	-	-	M	M
Public	P	P	M	M/P	-	-	-	-	-	M	M

a. Centrifuge chambers are not necessarily aerosol-tight and might provide only partial mitigation even when closed. Centrifuge lids provide no mitigation when open, so this is a minor mitigating effect and, therefore, not considered in this evaluation.

b. M signifies a mitigative feature

c. P signifies a preventive feature

d. This biocontainment feature is either not relevant for this event sequence or it does not have a preventive or mitigative role for this exposed group.

e. The cells with cross-hatching indicate a biocontainment feature that is assumed to fail or have partial performance for this event sequence.

Event sequence description—— The event sequence analyzed was modeled in consideration of incidents that have occurred at other facilities (see Attachment A of this appendix) and incident experiments that

have been performed by the Health Protection Agency (Bennett and Parks 2006). The following describe the assumed event sequence and provide the basis for the assumptions:

- A portion of the suspension leaks from the container before or during centrifugation. The leak might be the result of a personnel error or equipment defects, which might include an inadequately sealed container, a defective or missing seal(s) in the lid that is not detected by the worker, use of a leaking container, or a container that cracked during centrifugation. Attachment A of this appendix identifies several incidents involving a container leak associated with centrifugation.
- The suspension leaking into the rotor is assumed to be either the total working volume of the pathogen or 10 mL, whichever is greater. A maximum leakage of 10 mL is used here, consistent with the quantity deemed reasonable by the investigators performing the experiments on which the release calculation is based (Bennett and Parks 2006).
- It is assumed that the centrifuge continues to operate and that the imbalance detection system or operator does not shut off the centrifuge promptly either because (1) the leakage is distributed in a way that does not result in a detectable imbalance, or (2) the imbalance detection system fails.
- The rotor is not aerosol-tight because the seal is either damaged, is installed improperly, or is not installed. This human error is not unexpected at some point in NEIDL operations and is consistent with experiments on which the event sequence is based (Bennett and Parks 2006).
- After centrifugation is completed, the centrifuge chamber door is promptly opened, thereby allowing the aerosol generated to be released from the centrifuge chamber without restriction. This is a conservative assumption (i.e., likely to overstate the result) that is not prevented by procedures. Centrifuge chamber doors are not generally aerosol-tight, but prompt opening of the door increases the release to the room and is consistent with the experiment (Bennett and Parks 2006).
- The laboratory workers remain in the immediate vicinity and are unaware of the aerosol release. No corrective action is assumed to be taken for this event sequence. This is a conservative assumption that is consistent with the failure to detect the leak.
- The HVAC system is operating and has a rate of at least eight air exchanges per hour, which is the minimum design exchange rate (BUMC 2009c). Using the minimum air exchange rate minimizes the aerosol dilution in the room and maximizes worker exposure.

F.6.3.2.2 Frequency Category

The postulated event sequence described above involves an undetected/unreported release because an undetected/unreported event has the greatest potential for secondary transmission to the public. One of the

four centrifuge-related incidents identified in Attachment A was detected but not reported, and the other three were both detected and reported. There was no indication that the workers were wearing or were required to wear respiratory protection during these events. As discussed in Section F.6.2.4.1, the operating incident data is not suitable for use in determining the frequency of this event sequence for several reasons, including the absence of the number of operating hours associated with those incidents (see Section F.1.3).

Modern centrifuges have safety features that minimize the risk of an aerosol release. Those features can include imbalance detection and shutdown circuitry, certified aerosol-tight rotor/bucket seals, incorporation of a fluid containment annulus in the rotor, HEPA-filtered air evacuation systems, and automatic rotor identification systems that prevent operation at speeds beyond the recommendations for the rotor/bucket.

This event sequence requires three distinct events or conditions to occur: (1) a leakage into the rotor, (2) failure of the aerosol-tight rotor seal to contain the aerosol, and (3) a failure to detect or report the incident. If those events or conditions were all independent, the event sequence would be considered to be in frequency category B (1 in 100 to 10,000 years). However, as noted by BMBL (CDC and NIH 2007), "The safety characteristics of modern centrifuges are only effective if the equipment is operated properly." Because the laboratory worker plays a significant role in the prevention and identification of this event sequence, the sequence is conservatively assigned to the frequency category A (1 in 1 to 100 years).

F.6.3.2.3 Exposure Category

The exposure category for this event sequence is addressed below for each potentially exposed group.

Laboratory worker—As discussed in Section 3.2.1, it is expected that there will be from 1 to 4 people in the room at the time of a centrifuge aerosol release event, so the exposure category is MEDIUM. All laboratory workers in the room at the time of the event have the potential of being exposed, depending on their location relative to the room air flow.

***Facility worker*—**The BSL-3 HVAC system is a once-through design, so a release in one room will not be circulated to any other portions of the NEIDL and will not expose facility workers in the rest of the facility. Attachment A identifies six HVAC system faults at BSL-3 laboratories. Only one incident, which is assumed to involve an HVAC system failure though not explicitly stated, resulted in exposure. The other five incidents identified in Attachment A did not result in spread of a pathogen, but could have if an

aerosol release of a pathogen had occurred concurrently. In three of the incidents identified in Attachment A, the HVAC systems failed because of a loss of off-site power and failure of the emergency diesel generators to cycle and accept the load.

For a facility worker to potentially be exposed to the centrifuge aerosol release event described above, the HVAC system would need to both fail to purge air from the room and also fail to isolate the room concurrent with a centrifuge release event. Section A.4 of Attachment A provides a summary of several ventilation system failures at biosafety laboratories. Potential HVAC system failures are addressed below:

1. *Loss of HVAC airflow*——The loss of HVAC flow events identified in Attachment A were the result of a loss of offsite power plus the failure of the emergency diesel generators to provide power. Other mechanism can cause a loss of HVAC airflow such as fires or multiple equipment failures, but this evaluation addresses a loss of power because that is the most frequent cause based on the operational data. For all power to be lost to the HVAC fans, the following must occur:
    a. *Loss of off-site power*—NEIDL has four electrical transformers for offsite power with only one or two required for normal operations, depending on the season and the HVAC requirements. Therefore, there is N + 2 redundancy for offsite power (Tetra Tech 2009a, 2009b).

       For nuclear power plants, the rate for loss of offsite power is 3.59×10^{-2}/yr, which corresponds to 4.1×10^{-6}/hr [$(3.59 \times 10^{-2}$/yr) / (365 days/yr) / (24 hrs/day)]. (NUREG/CR-6928) If one assumes a 1-hour window for loss of HVAC (i.e., loss of power) and concurrent centrifuge release, the unavailability is defined as follows (NUREG/CR-6823):

       $$\begin{aligned} \text{unavailability} &= (\text{outage frequency}) \times (\text{outage duration}) \\ &= (4.1 \times 10^{-6}/\text{hr}) \times (1\ \text{hr}) \\ &= 4.1 \times 10^{-6} \end{aligned}$$

    b. *Failure of emergency diesel generators*—NEIDL has two emergency diesel generators, each of which is capable of providing power to the HVAC blowers (Tetra Tech 2009a, 2009b). For nuclear power plants, the unreliability of emergency diesel generators to supply power is 2.9×10^{-3} per demand (NUREG/CR-6928).
    c. *Loss of both off-site power and emergency diesel generators*—Therefore, on the basis of the nuclear power experience, the conditional probability that off-site power and emergency diesel generators would fail during a 1-hour window of time, for example during an aerosol release from a centrifuge, is 1×10^{-8} [$(4.1 \times 10^{-6}) \times (2.9 \times 10^{-3})$]. On

the basis of that calculation, the loss of all power to the HVAC fans concurrent with a centrifuge aerosol release event is beyond reasonably foreseeable, frequency category D (1 in more than 1 million years).

2. *Loss of HVAC isolation following loss of HVAC airflow*—If the HVAC system is not able to purge air from the laboratories, potentially because of a loss of power as discussed above, the situation would be detected by the NEIDL building automation control systems and appropriate actions would be taken promptly. There are differences between NEIDL BSL-3 and BSL-4 control systems. In the BSL-3 areas, an attempt is made to maintain directional air flow to the extent possible because the isolation dampers are low leakage rather than air-tight dampers. However, if the BSL-3 directional airflow cannot be maintained, the isolation dampers will be closed. In the BSL-4 areas equipped with air-tight dampers, the control system will seal the area on a loss of HVAC. It is estimated failure of the dampers to close on demand is < 0.01 per demand (NUREG/CR-6928) (Tetra Tech 2009, 2009a, 2009b).

A centrifuge-related aerosol release event concurrent with a loss of HVAC flow, loss of HVAC isolation, and flow of contaminated air to uncontaminated areas is beyond reasonably foreseeable, frequency category D (1 in more than 1 million years), as demonstrated by the estimated conditional probability of $< 1 \times 10^{-8}$ (i.e., $1 \times 10^{-8} \times 0.01$) for concurrent loss of off-site power, failure of the emergency diesel generators, and failure to isolate the room.

However, it is also important to consider common-cause failures that would produce both a centrifuge aerosol release and a simultaneous HVAC system fault. Centrifuges are standalone pieces of equipment, and the only common feature they have with the HVAC system is the supply of electrical power, which is unlikely to cause both a centrifuge aerosol release and a failure or reverse-flow of the HVAC system. Because of the independence of the two systems, an infrequent event such as an earthquake is the only common cause that has been identified for this event sequence (the earthquake is analyzed as a separate event).

On the basis of the above evaluation, no reasonable mechanism for exposure of the facility worker was identified, and the facility worker exposure category for a centrifuge aerosol release event is NONE.

Public—Any release from a BSL-3 centrifuge will be drawn into the HVAC system, diluted with air from other portions of the facility (i.e., non-contaminated) and HEPA filtered before discharge. The HVAC system flow rate is 6,900 cubic feet per minute (cfm) for the BSL-3 and the 11,900 cfm for the BSL-4

area (RWDI 2005), thereby reducing the concentration of any entrained pathogen particles by orders of magnitude. The BSL-3 HEPA filter is at least 99.97 percent efficient at removing airborne particles 0.3 micrometer (μm) in diameter (NIH 2008), with higher efficiencies for all other particle sizes, thereby removing nearly all the aerosol particles. The HEPA-filtered air from the HVAC system is ultimately discharged through the stack, where any particles not filtered out will undergo atmospheric dispersion. Atmospheric conditions (e.g., sunlight) will inactivate some infectious particles over time (Bozzette 2011), but that inactivation is conservatively ignored for this analysis. As a result of the dilution, filtration, and dispersion, the public exposure category for a centrifuge aerosol release event is NONE. This category pertains only to direct exposure and the potential for secondary exposures are considered elsewhere.

F.6.3.2.4 Extent of Exposure

Routes of exposure—It is assumed that two to four laboratory workers are in the room at the time of the release and they might all be exposed to the aerosol. The laboratory workers potentially would be exposed via various routes:

1. The laboratory workers could be exposed via inhalation into the lungs. The air would be contaminated with aerosolized particles so breathing could potentially expose the workers via the inhalation route. Inhalation exposure is typically only considered for aerosolized particles with an aerodynamic equivalent diameter no greater than 10 μm (DOE 2000).
2. The laboratory workers could also be exposed via direct contact (i.e., exposure of mucous membranes of the eyes or nasal passages).
3. As a result of breathing or speaking, the worker could also be exposed via the ingestion route.

Laboratory workers are potentially exposed via the inhalation, direct contact, and ingestion routes of exposure. The extent of exposure could be larger for the inhalation route than for the other routes because inhalation involves a continuous exchange of a large volume of air. The extent of exposure is assumed to be the same for each route in this analysis.

Methodology for exposure calculation—The actual exposure for any individual is dependent on many factors including location, response to the incident, and local airflow pattern. The following paragraphs provide a basis for estimating the potential pathogen exposure.

The airborne pathogen concentration in the laboratory is based on experiments that replicated event sequences of this type. The aim of those experiments was to "[t]o quantify microbial aerosols generated

by a series of laboratory accidents and to use these data in risk assessment." (Bennett and Parks 2006) While the experiments were performed with *Bacillus atrophaeus*, the experimental results are assumed to be applicable to the pathogens evaluated for NEIDL because (1) the aim of the experiments and the discussion in the report addresses microorganisms in a general sense rather than *B. atrophaeus* in particular, (2) the report does not specify any limitations on applicability, and (3) the smaller size of viruses provides reason to believe the results are applicable to viruses. The general equation resulting from the experiments is presented below. The spray factor (SF) is the ratio of the pathogen concentration in the room air (i.e., the aerosol concentration, AC) to the pathogen concentration in the liquid suspension (i.e., the suspension concentration, SC). Therefore,

$$AC = SC \times SF \qquad \text{[equation F.6-1]}$$

where

AC The aerosol concentration in a room (per m^3)

SC The suspension concentration (per mL),[2] which are assumed to be the maximum working volumes presented in Section F.3.3.3

For a centrifuge fixed-angle rotor spill, the SF is 4.6×10^{-6} mL/m^3 (Bennett and Parks 2006). A release from a fixed-angle rotor was selected because the SF for a fixed-angle rotor release is more than 20 times greater than the SF from the swinging bucket rotor experiment (4.6×10^{-6} mL/m^3 versus 0.17×10^{-6} mL/m^3). Therefore, the results of this fixed-angle rotor analysis are conservatively applicable to swinging bucket centrifugation operations.

The above equation for calculation of the airborne concentration is a general equation that does not account for variations in the SC. Using the above general equation for a Rift Valley fever virus (RVFV) SC of 1×10^9 $CCID_{50}$/mL and $MICLD_{50}$/mL,[3] the maximum SC in the NEIDL inventory results in the following air concentration:

[2] Suspension concentrations for bacteria are given in terms of colony forming units (CFU). Concentrations for viruses are given in various units, including plaque forming units (PFU), median cell culture infective doses ($CCID_{50}$), and median mouse intracerebral lethal doses ($MICLD_{50}$) per milliliter. Section F.3.1.2 provides some background information on the methods and units associated with the concentration measurements.

[3] The concentrations are reported as both $1 \times x\ 10^9$ $CCID_{50}$/mL and $1 \times x\ 10^9$ $MICLD_{50}$/mL. For the sake of simplicity, the RVFV concentration is reported only in units of $CCID_{50}$ with the understanding that the units of $MICLD_{50}$ also might apply. RVFV concentrations are also given as $1 \times x\ 10^8$ PFU/mL.

$AC = SC \times SF$

$AC = (1 \times 10^{9}\ \text{CCID}_{50}\ /\text{mL}) \times (4.6 \times 10^{-6}\ \text{mL/m}^3)$

$\text{AC} = 4{,}600\ \text{CCID}_{50}/\text{m}^3$

An equation that adjusts the SF for variations in the SC specifically for the fixed-angle rotor release is presented below (Bennett and Parks 2006). Using the same RVFV SC of 1×10^{9} CCID_{50}/mL results in the following result:

$AC = SC^{0.81} / (4.3 \times 10^{3}\ \text{mL/m}^3)$ [equation F.6-2]

$AC = (1 \times 10^{9}\ CCID_{50}/\text{mL})^{0.81} / (4.3 \times 10^{3}\ \text{mL/m}^3)$

$AC = 4{,}500\ CCID_{50}/\text{m}^3$

The above calculations show that the general equation (equation F.6-1) and the equation that adjusts the SF (equation F.6-2) for the solution concentration produce nearly identical results, but the general equation results in slightly higher air concentrations at this maximum solution concentration. The general equation is used for this analysis because of this slight conservative bias at the concentration range of greatest concern.

The potential worker exposure is the airborne pathogen concentration in the air inhaled multiplied by the volume of air inhaled. It is conservatively assumed that all infectious particles inhaled are retained in the body. The pathogen concentration in the inhaled air is the airborne concentration in the room air multiplied by the fraction of aerosol particles that pass through the PAPR filter. The amount of air inhaled is the breathing rate times the effective duration of exposure. Therefore, the average number of infectious particles a worker inhales (N) is equal to the aerosol concentration (AC) times the PAPR leak path factor (LPF) times the breathing rate (BR) multiplied by the time of exposure (T).

$N = AC \times LPF \times BR \times T$ [equation F.6-3]

$N = (SC \times SF) \times LPF \times BR \times T$

where

BR The breathing rate for the worker is assumed to be 3.33E-04 m^3/s or 0.020 m^3/min (DOE 2004a).

LPF The leak path factor is the fraction of airborne particles that would leak past the confinement or filtration system. For this event sequence, the PAPRs will have an APF[4] of 1,000 (29 CFR 1910.134 and NIH 2010), which is the inverse of the LPF of 0.001.

T The time of exposure is the breathing time at the peak concentration. The air exchange rate for NEIDL is at least eight air exchanges per hour (BUMC 2009c), which equates to an air exchange every 7.5 minutes [(1 hr / 8 exchanges) × 60 min/hr = 7.5 min]. The concentration of aerosols in the room will be dependent on the airflow patterns in the room and the nature of the release. The majority of the aerosol would be removed after 1 air exchange (i.e., 7.5 minutes) and 90 percent would be removed after 2.3 air exchanges (i.e., 17.25 minutes = 2.3 × 7.5 minutes) (NIH 2008). A laboratory worker remaining in the area for approximately one hour with the concentration decreasing as the release is purged from the room is approximately the same as a 10-minute exposure at the peak concentration. The exposure is modeled as a 10-minute exposure at peak concentration.

For the previous example involving the centrifuge release of 1×10^9 $CCID_{50}$/mL suspension, the amount of pathogen inhaled is then calculated as follows:

$$N = (SC \times SF) \times LPF \times BR \times T$$

$$N = [(1 \times 10^9\ CCID_{50}/\text{mL}) \times (4.6 \times 10^{-6}\ \text{mL/m}^3)] \times (0.001) \times (0.020\ \text{m}^3/\text{min}) \times (10\ \text{min})$$

$$N = 0.9\ CCID_{50}$$

An average exposure of less than 1 $CCID_{50}$ indicates that although there might be instances where one or more $CCID_{50}$ is inhaled, there could also be instances where zero $CCID_{50}$ are inhaled, for an average inhalation of less than one.

Variability and uncertainty—There are a number of uncertainties associated with the exposure calculation and its applicability to this accident as described below.

- The experiments on which the AC (aerosol concentration) is based were performed in a room without ventilation. The airflow of an operating HVAC system in the NEIDL will continually dilute and purge the aerosol from the room; therefore, AC in the above estimate could be

[4] The APF is the workplace level of respiratory protection that a respirator or class of respirators is expected to provide to employees when the employer implements a continuing, effective respiratory protection program (which includes fit testing and proper training) as specified by this section. (29 CFR 1910.134)

overstated. In addition, concentrations upwind of the release will be lower than concentrations downwind.

- The experiment was performed with a rotor described as *outdated*, which will not be used at NEIDL. It is anticipated that the rotors used in NEIDL will provide greater confinement than the outdated rotors.
- The experiment was performed with a centrifuge operating at 4,700 revolutions per minute. A centrifuge operating at higher speeds could generate more aerosols. Bench-top centrifuges that are planned for use with fixed-angle rotors in NEIDL have speeds of up to 25,000 revolutions per minute (e.g., Beckman Coulter model Avanti J-26 XP).
- If laboratory workers detect the release and evacuate the laboratory promptly, or are upwind from the point of release, the exposure could be zero. Conversely, if a worker does not detect the release and happens to be slightly downwind from the release, the exposure could be greater.
- There is no indication of a particle size cutoff or distribution for the experiments on which this calculation is based, so it is not possible to determine if the SF value only includes aerosol particles with an aerodynamic equivalent diameter no greater than 10 μm. If the calculated AC includes particles greater than 10 μm, the estimated inhalation exposure could be overstated.

Some of the above variables might tend to increase the exposure and some might tend to decrease the exposure relative to the calculated values. To account for those and other variables and uncertainties, the calculated values will be reported as a range. Because the laboratory worker could remain virtually unexposed from a given accident merely by virtue of being upwind of the release, the lower end of the exposure range is assumed to be zero, which means there is no exposure. To account for the variables and uncertainties that might tend to increase the exposure, such as the HVAC system moving the release toward the worker, the upper end of the range is assumed to be 10 times the calculated value. For example, a calculated exposure of 0.9 $CCID_{50}$ would be multiplied by 10 to give 9 $CCID_{50}$. Although that adjustment is not based on a numeric uncertainty analysis because of the lack of data, the addition of this conservatism to account for variability and uncertainty is consistent with the DOE NEPA Guidance.

Exposure range calculation—Table F.6-2 below provides the maximum suspension quantity and concentration for each of the pathogens that might be used in the BSL-3 laboratory and a calculated exposure that results from personnel remaining in the vicinity of a centrifuge aerosol release indefinitely. Pathogens that can be used only in a BSL-4 laboratory are not considered in this analysis. The results presented in Table F.6-2 are applicable for inhalation, direct contact, and ingestion exposure of laboratory

workers. Using the equation presented (equation F.6-3) in the previous subsection and inserting the SC for RVFV (i.e., 1×10^9 $CCID_{50}$/mL from Table F.6-2) yields:

$$N = (SC \times SF) \times LPF \times BR \times T$$

$$N = [(1 \times 10^9\ CCID_{50}/\text{mL}) \times (4.6 \times 10^{-6}\ \text{mL/m}^3)] \times (0.001) \times (0.020\ \text{m}^3/\text{min}) \times (10\ \text{min})$$

$$= 0.9\ CCID_{50}$$

Multiplying that value by 10 (as described previously) results in a value of 9 $CCID_{50}$. The exposure range for RVFV is then reported as 0 to 9 $CCID_{50}$.

Table F.6-2. Laboratory worker exposures—BSL-3 centrifuge aerosol release with full respiratory protection

Pathogen[a]	BSL	Typical volume Volume (mL)[b]	Suspension concentration (/mL)[b c]	Calculated exposure[c]	Exposure range[c]
B. anthracis[d]	3/2	50	2.4×10^8 CFU	0.2 CFU	0–2 CFU
F. tularensis	3	1	2.0×10^9 CFU	0.2 CFU[e]	0–2 CFU
Y. pestis	3	5	2.0×10^7 CFU	0.009 CFU[f]	0–0.09 CFU
1918 H1N1V	3	150	1.0×10^8 PFU	0.09 PFU	0–0.9 PFU
SARS-CoV	3	150	1.0×10^7 PFU	0.009 PFU	0–0.09 PFU
RVFV	3	150	1.0×10^8 PFU or 1.0×10^9 $CCID_{50}$ [g]	0.09 PFU 0.9 $CCID_{50}$ [g]	0–0.9 PFU 0–9 $CCID_{50}$ [g]
ANDV	3/4	150	1.0×10^6 $CCID_{50}$	0.0009 $CCID_{50}$	0–0.009 $CCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b. Based on the maximum working volumes in Section F.3.

c. Concentrations and exposures are given in terms of colony forming units (CFU) for bacteria. For viruses, concentrations and exposures are given in terms of plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$).

d. Spores in a liquid suspension.

e. The calculated exposure includes a factor of 0.1 because the typical volume of 1 mL is 10 percent of the 10-mL leak used in the experiment.

f. The calculated exposure includes a factor of 0.5 because the typical volume of 5 mL is 50 percent of the 10-mL leak used in the experiment.

g. Two values are reported for RVFV with different units. The $CCID_{50}$ value is an order of magnitude greater because this measurement is more sensitive that the PFU measurement. The units of $MICLD_{50}$ also apply to the $CCID_{50}$ value.

The current NEIDL SOPs do not include centrifugation of *F. tularensis* or *Y. pestis* (BUMC 2010b, 2010c) but those pathogens are included in Table F.6-2 because 1) they could be centrifuged at some point in the future, and (2) this analysis is intended to envelope other activities that have the potential to generate aerosols. Therefore, all pathogens being considered in this analysis that might be used in a BSL-3 laboratory are included in Table F.6-2.

The experiment used as the basis for this calculation was performed with a 10-mL suspension leakage in the rotor. The typical volume for *F. tularensis* is only 1 mL and the typical volume for *Y. pestis* is only 5 mL, so it is not possible to have a 10-mL leakage for those pathogens. No experimental data are available for the aerosol generation from smaller leaks, so, so it is assumed that the aerosol concentration is reduced in proportion to the reduced volume for pathogens with a typical volume of less than 10 mL. Therefore, the calculated exposures are multiplied by of 0.1 for *F. tularensis* and 0.5 for *Y. pestis*.

F.6.3.2.5 Summary

Table F.6-3 summarizes the results of the above analysis of the BSL-3 centrifuge aerosol release with full respiratory protection.

Table F.6-3. Summary of results—BSL-3 centrifuge aerosol release with full respiratory protection

Frequency category	Exposed group: category	Route of exposure	Pathogen[a]	Exposure range[b]
A (1 in 1 to 100 years)	Laboratory workers: MODERATE (1-4)	Direct contact Ingestion Inhalation	*B. anthracis*[c]	0–2 CFU
			F. tularensis	0–2 CFU
			Y. pestis	0–0.09 CFU
			1918 H1N1V	0–0.9 PFU
			SARS-CoV	0–0.09 PFU
			RVFV	0–0.9 PFU 0–9 $CCID_{50}$[d]
			ANDV	0–0.009 $CCID_{50}$
	Facility worker: NONE (0)	--	--	--
	Public: NONE (0)	--	--	--

a. *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b. Exposures are given in terms of colony forming units (CFU) for bacteria. For viruses, exposures are given in terms of plaque forming units (PFU), median cell culture infective dose (CCID50), or median mouse intracerebral lethal dose (MICLD50).

c. Spores

d. Two values are reported for RVFV with different units. The CCID50 value is an order of magnitude greater because this measurement is more sensitive that the PFU measurement. The units of MICLD50 also apply to the CCID50 value.

F.6.3.3 BSL-3 Centrifuge Aerosol Release with Partial Respiratory Protection

This section addresses an event sequence involving an aerosol release from a centrifuge in the BSL-3 with only partial respiratory protection (i.e., failure modes as described in Section F.6.3.3.1 below) for one of the laboratory workers. This section describes the event sequence, including the biocontainment features, frequency category, exposure category, and potential extent of exposure. This event sequence is similar to the event sequence analyzed in Section F.6.3.2 with the exception that this event sequence includes the potential for partial loss of respiratory protection. The discussion below does not repeat the common features of the analysis addressed in Section F.6.3.2 but merely addresses the differences.

F.6.3.3.1 Event Sequence Description

This hypothetical scenario involves the aerosol release of a pathogen from a bench-top centrifuge and is representative of a variety of centrifuge event sequences that could occur in the NEIDL BSL-3 laboratories. The following paragraphs describe the initial conditions and the event sequence.

***Initial Conditions*—**The only difference between the initial conditions of this event sequence and the event sequence described in Section F.6.3.2 is that this event sequence includes the potential that one or more laboratory workers in the vicinity of the release have only partial respiratory protection. The PAPR's biocontainment function is to filter potentially infectious particles from the air being inhaled by the worker. Section A.3 of Attachment A provides a summary of PAPR incidents involving reduced respiratory protection. The PAPR failure modes considered here are as follows:

- *Leakage*—Because of the positive pressure within the respirator, any leakage of a PAPR (e.g., leak at fixture or seal) is predominantly outward rather than inward and, thus, is not a threat to the laboratory worker. Therefore, this failure mode is not considered further.
- *Detected reduced airflow*—Some failures, such as loss of power, blower failure, or air hose detachment could result in loss of airflow that is promptly detected. If the airflow is severely reduced, the laboratory worker would be expected to exit the room promptly. A worker could be exposed to the release for a time if the PAPR was removed before exiting the room. A PAPR failing in this mode concurrent with an undetected centrifuge event sequence is beyond reasonably foreseeable, frequency category D (1 in more than 1 million years), and is not considered further.
- *Undetected reduced airflow*—PAPR failures can also result in an undetected reduction of the filtered air supplied to the PAPR. The PAPR failures could result from such problems as loose hose assemblies that leak or crimped hoses that restrict airflow. Such incidents increase the chance for over-breathing and partial reduction in effectiveness, but the PAPR would still provide filtered air and such incidents are not considered further.
- *Reduced filtration efficiency*—There are several conditions that can result in a reduced filtration efficiency for a PAPR. Those failures could result from such conditions as substandard filters, cracked filter assemblies, improperly installed filters, or filter breaches. Substandard filters could have a common cause and all workers could have substandard filters, but the filters are likely to provide nearly full protection and this specific failure is not considered further. A cracked filter housing or breached filter on a single PAPR is the most likely failure of concern and is selected for further analysis.

Therefore, the initial conditions for this event sequence are the same as for the sequence described in Section F.6.3.2 except that one of the laboratory workers has only partial respiratory protection. The likelihood that the reduced respiratory protection will be detected increases as the extent of degradation increases. A small degradation would not be as likely to be detected, but the effect on exposure would be small. Conversely, a large degradation in protection has the potential for a large increase in exposure, but the likelihood of detection is also large. No data were found that would support an estimate in the reduction of the respiratory protection for the described failure modes. While an order of magnitude reduction in effectiveness seems like a reasonable estimate, a two order of magnitude degradation in filter efficiency is conservatively used here to better assess the significance of the respiratory protection. The role of each biocontainment feature for this event sequence, including the PAPRs, is shown in Table F.6-4. The only difference between the biocontainment features in this event sequence and the one addressed in Section F.6.3-2 is the partial performance of the PAPR for one laboratory worker.

Table F.6-4. Exposed group protected by each biocontainment feature—BSL-3 centrifuge aerosol release with partial respiratory protection

	Admin. controls		Safety equipment							Facility	
Exposure group	Procedures	Training	Container	Rotor	Centrifuge chamber[a]	BSC	PPE	PAPR	Positive-pressure suit	HVAC system	Sealed walls, floors, and ceilings
Laboratory worker	M[b]/P[c]	M/P	M[e]	M/P	-[d]	-	-	M[f]	-	M	-
Facility worker	P	P	M	M/P	-	-	-	-	-	M	M
Public	P	P	M	M/P	-	-	-	-	-	M	M

a. Not all centrifuge chambers are aerosol-tight. Centrifuge chambers that are not aerosol-tight provide some mitigation while closed. Centrifuge lids provide no mitigation when open, so this is a minor mitigating effect.

b. M signifies a mitigative feature

c. P signifies a preventive feature

d. This biocontainment feature is not relevant for this event sequence or does not have a preventive or mitigative role for this exposed group.

e. The cells with cross-hatching indicate a biocontainment feature that is assumed to fail or have partial performance for this event sequence.

f. PAPR are assumed to provide only partial respiratory protection for one worker and full protection for any other workers in the room.

Event sequence description—This event sequence proceeds from the initial conditions in the same manner as the event sequence discussed in Section F.6.3.2 with the only exception that the initial

conditions include one laboratory worker with only partial respiratory protection. As a result, the laboratory worker with partial respiratory protection has the potential of a higher exposure to the release.

F.6.3.3.2 Frequency Category

This event sequence involves the combination of a centrifuge aerosol release event sequence concurrent with one laboratory worker with partial respiratory protection. The centrifuge event frequency estimation in Section F.6.3.2.2, frequency category A (1 in 1 to 100 years), is also applicable for the centrifuge aerosol release portion of this event sequence.

This condition requires that the respiratory protection provided by the PAPR be reduced either while in use, such as an impact that damages the housing, or before use. In either case, it is essential that the reduced protection not be detected in check-out, inspection, donning, or use in order for this scenario to occur. NEIDL workers are trained to inspect and test PAPR before entry into the laboratories (BUMC 2010a). The combination of a degradation of this severity in conjunction with a failure to detect makes this conditional improbable. The conditional probability that one of the four or fewer PAPRs in use has an undetected two order of magnitude reduction in filtration is conservatively assumed to be no greater than 1 in 100 (0.01), which equates to a one category reduction in the frequency category. (Note: the results of Appendix K show that this potential reduction in PAPR effectiveness does not significantly affect worker risk.)

Because of the conditional probability of partial respiratory protection for one laboratory worker, this event sequence is considered to be in frequency category B (1 in 100 to 10,000 years).

F.6.3.3.3 Exposure Category

The exposure of laboratory workers, facility workers, and the public for this event sequence is discussed below.

Laboratory worker—As discussed in Section F.6.3.2.3, it is expected that one to four people will be in the room at the time of a centrifuge aerosol release event; however, only one laboratory worker is expected to have a PAPR with reduced respiratory protection. Therefore, the exposure category is LOW (i.e., 1 laboratory worker) for the higher level of exposure and remains at MODERATE for the lower level of exposure.

***Facility worker*—**The potential for facility worker exposure from this event sequence is the same as the potential addressed in Section F.6.3.2.3 because respiratory protection for the worker will have no effect on facility worker exposures. Therefore, the facility worker exposure category for a centrifuge aerosol release event is NONE.

***Public*—**The potential for public exposure from this event sequence is the same as the potential addressed in Section F.6.3.2.3 because respiratory protection for the worker will have no effect on public exposure. As a result of the dilution, filtration, and dispersion, the public exposure category for a centrifuge aerosol release event is NONE.

F.6.3.3.4 Extent of Exposure

***Routes of exposure*—**As discussed in Section F.6.3.2.4, a laboratory worker with partial respiratory protection is also potentially exposed via the inhalation, direct contact, and ingestion routes of exposure. The inhalation route will be used as the basis for estimating the extent of exposure for the other routes.

***Calculated exposure*—**The methodology used to calculate exposure is identical to the calculation performed in Section F.6.3.2.4 with the exception of the difference in the laboratory worker's respiratory protection. For this event sequence, the PAPR LPF for the laboratory worker with reduced respiratory protection is assumed to be 0.1 rather than 0.001. Table F.6-5 below provides the maximum suspension quantity and concentration for each of the pathogens that might be used in the BSL-3 and a calculated exposure that results from a person remaining in the vicinity of a centrifuge aerosol release indefinitely. Pathogens that can be used in a BSL-4 only are not considered in this analysis. The results presented in Table F.6-5 are applicable for inhalation, direct contact, and ingestion exposure of laboratory workers. Using the formula presented in the previous subsection and inserting the SC for RVFV (i.e., 1×10^9/mL from Table F.6-5) yields:

$$N = (1 \times 10^9\ CCID_{50}/\text{mL}) \times (4.6 \times 10^{-6}\ \text{mL/m}^3) \times (0.1) \times (0.020\ \text{m}^3/\text{min}) \times (10\ \text{min})$$
$$= 90\ \text{CCID}_{50}$$

Increasing that value by a factor of 10 to account for uncertainty and variability results in a value of 900 CCID_{50}. The exposure range for RVFV is then reported as 0 to 900 CCID_{50}.

Table F.6-5. Laboratory worker exposures—BSL-3 centrifuge aerosol release with partial respiratory protection

Pathogen[a]	BSL	Suspension concentration (/mL)[b c]	Calculated exposure[c]	Exposure range[c]
B. anthracis[d]	3 / 2	2.4×10^8 CFU	20 CFU	0–200 CFU
F. tularensis	3	2.0×10^9 CFU	20 CFU	0–200 CFU
Y. pestis	3	2.0×10^7 CFU	0.9 CFU	0–9 CFU
1918 H1N1 influenza virus	3	1.0×10^8 PFU	9 PFU	0–90 PFU
SARS-CoV	3	1.0×10^7 PFU	0.9 PFU	0–9 PFU
RVFV	3	1.0×10^8 PFU 1.0×10^9 $CCID_{50}$ [e]	9 PFU 90 $CCID_{50}$ [e]	0–90 PFU 0–900 $CCID_{50}$ [e]
ANDV	3 / 4	1.0×10^6 $CCID_{50}$	0.09 $CCID_{50}$	0–0.9 $CCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b. Taken Section F.4.3.3.

c. Concentrations and exposures are given in terms of colony forming units (CFU) for bacteria. For viruses, concentrations and exposures are given in terms of plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$).

d. Spores in a liquid suspension.

e. Two values are reported for RVFV with different units. The $CCID_{50}$ value is an order of magnitude greater because this measurement is more sensitive that the PFU measurement. The units of $MICLD_{50}$ also apply to the $CCID_{50}$ value.

As discussed in Section F.6.3.2.4, *F. tularensis* and *Y. pestis* might not be centrifuged in NEIDL, so retaining these pathogens in Table F.6-5 is potentially conservative for those pathogens. Also, as explained in Section F.6.3.2.4, the maximum working volume of these two pathogens is less than the 10 mL leakage assumed, so the exposures are scaled accordingly (i.e., a factors of 0.1 for *F. tularensis* and 0.5 for *Y. pestis* were used to adjust for the reduced volumes).

F.6.3.3.5 Summary

Table F.6-6 summarizes the results of the above analysis of the BSL-3 centrifuge aerosol release with partial respiratory protection.

Table F.6-6. Summary of results—BSL-3 centrifuge aerosol release with partial respiratory protection

Frequency category	Exposed group: category	Route of exposure	Pathogen[a]	Exposure range[b]
B (1 in 100 to 10,000 years)	Laboratory workers—full respiratory protection: MODERATE (0-3)[c]	• Direct contact • Ingestion • Inhalation	*B. anthracis*[d]	0–2 CFU[d]
			F. tularensis	0–2 CFU
			Y. pestis	0–0.09 CFU
			1918 H1N1V	0–0.9 PFU
			SARS-CoV	0–0.09 PFU
			RVFV	0–0.9 PFU 0–9 $CCID_{50}$[e]
			ANDV	0–0.009 $CCID_{50}$
	Laboratory workers—partial respiratory protection: LOW (1)	• Direct contact • Ingestion • Inhalation	*B. anthracis*[d]	0–200 CFU[d]
			F. tularensis	0–200 CFU
			Y. pestis	0–9 CFU
			1918 H1N1V	0–90 PFU
			SARS-CoV	0–9 PFU
			RVFV	0–90 PFU 0–900 $CCID_{50}$ [e]
			ANDV	0–0.9 $CCID_{50}$
	Facility worker: NONE (0)	--[a]	--	-
	Public: NONE (0)	--	-	--

a. *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b. Exposures are given in terms of colony forming units (CFU) per milliliter for bacteria. For viruses, exposures are given in terms of plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$).

c. Accounts for the one worker with only partial protection.

d. Spores

e. Two values are reported for RVFV with different units. The $CCID_{50}$ value is an order of magnitude greater because this measurement is more sensitive that the PFU measurement. The units of $MICLD_{50}$ also apply to the $CCID_{50}$ value.

F.6.3.4 BSL-4 Centrifuge Event Sequence with Partial Respiratory Protection

This event sequence is similar to the event sequence analyzed in Section F.6.3.3 with the exception that this event sequence occurs in a BSL-4 laboratory rather than in a BSL-3 laboratory. One piece totally encapsulating positive-pressure suits are required for BSL-4 laboratories, whereas PAPRs are required for BSL-3 laboratories. This event sequence is similar in most respects to the events analyzed for the BSL-3 laboratories, so the discussion below does not repeat the common features of the analysis addressed in Section F.6.3.3; it merely addresses the differences.

F.6.3.4.1 Event Sequence Description

This hypothetical scenario involves the aerosol release of a pathogen from a bench-top centrifuge and is representative of a variety of centrifuge event sequences that could occur in the NEIDL BSL-4 space. This scenario is relevant for any pathogens that might be used in the BSL-4 space, which includes

pathogens requiring BSL-3 biocontainment precautions and pathogens requiring BSL-4 biocontainment precautions because pathogens normally used in BSL-3 space might be used in the BSL-4 area. The following paragraphs describe the initial conditions and the event sequence.

Initial Conditions—The initial conditions of this event sequence include partial respiratory protection for one or more laboratory workers. The respiratory protection for laboratory workers is a positive-pressure suit in BSL-4 space as opposed to the PAPR used in BSL-3 space, which is the difference between this event sequence and the event sequence described in Section F.6.3.3. The biocontainment function of the positive-pressure suit is to supply uncontaminated air from a clean air source to the worker and to provide protection from other contact with the pathogen under study.

Failures of positive-pressure suits do occur, and Section A.2 of Attachment A provides a summary of several positive-pressure suit failures at biosafety laboratories. The potential failure modes considered included the following (Smith and Edwards 2002):

- *Supply of contaminated air*—If an aerosol release occurs, the room air would be drawn into the dedicated BSL-4 HVAC system, which includes double-HEPA filtration. The positive-pressure suits have a dedicated HEPA-filtered air supply that is independent from the BSL-4 HVAC system, so potentially contaminated room air cannot expose the workers. Therefore, a release would need to exit the facility stack and be recycled back into the HVAC inlet to contaminate the workers' breathing air. It is beyond reasonably foreseeable, frequency category D (1 in more than 1 million years), that the worker would be supplied contaminated air, and this possibility is not considered further.
- *Loss of airflow*—Airflow to the positive-pressure suit could be reduced or lost because of such problems as a crimped hose, hose or connector failure, or loss of air supply. The worker can hear the air released from the hood vent during normal airflow, and this sound will be absent if a loss of airflow occurs, thereby alerting the worker to the situation. The suits have a free air volume sufficient for approximately 5 minutes of breathing and are equipped with a life support system (an escape bottle air apparatus). Therefore, the worker has sufficient time to exit the room in case of a total loss of air supply. This failure mode is not considered further because the worker would detect the problem and exit the room without exposure.
- *Small leaks*—The most common mode of air-supplied positive-pressure personnel suit failure is small leaks (Smith and Edwards 2002). Small suit leaks do not pose a risk to laboratory workers because the air flows out of the suit at a tear, thereby preventing potentially contaminated air from entering the suit and exposing the worker. Small leaks are not considered further.

- *Rupture*—The failure mechanism considered here is a severe suit rupture, for example where the umbilical air-supply line connects to the suit. The suit would promptly depressurize, the worker would be aware of the condition and would be expected to promptly exit the room by retreat to the chemical shower airlock. In the process of exiting, the worker could be exposed to some pathogen particles.

The role of each biocontainment feature for this event sequence, including the positive-pressure suits, is shown in Table F.6-7.

Table F.6-7. Exposed group protected by each biocontainment feature—BSL-4 centrifuge event sequence with partial respiratory protection

	Admin. controls		Safety equipment							Facility	
Exposure group	Procedures	Training	Container	Rotor	Centrifuge chamber[a]	BSC	PPE	PAPR	Positive-pressure suit	HVAC system	Sealed walls, floors, and ceilings
Laboratory worker	M[b]/P[c]	M/P	M[e]	M/P	M	-[d]	-	-	M	M	-
Facility worker	P	P	M	M/P	M	-	-	-	-	M	M
Public	P	P	M	M/P	M	-	-	-	-	M	M

a. Not all centrifuge chambers are aerosol-tight. Centrifuge chambers that are not aerosol-tight provide some mitigation while closed. Centrifuge lids provide no mitigation when open, so this is a minor mitigating effect.

b. Mitigative feature

c. Preventive feature

d. This biocontainment feature is not relevant for this event sequence or does not have a preventive or mitigative role for this exposed group.

e. The cells with cross-hatching indicate a biocontainment feature that is assumed to fail or have partial performance for this event sequence.

Event sequence description—This event sequence proceeds from the initial conditions in the same manner as the event sequence discussed in Section F.6.3.2. Unlike the event sequence discussed in Section F.6.3.3, the respiratory protection cannot reasonably be compromised before the release without being detected, so the positive-pressure suit rupture must occur concurrently with the centrifuge aerosol release.

F.6.3.4.2 Frequency

This event sequence involves the combination of a centrifuge aerosol release event sequence concurrent with the rupture of the positive-pressure suit for one laboratory worker. The centrifuge event frequency estimation in Section F.6.3.2.2, frequency category A (1 in 1 to 100 years), is also applicable for the centrifuge aerosol release portion of this event sequence.

A rupture of a positive-pressure suit large enough to cause it to lose pressure and fail to provide clean air to the worker is improbable. Section A.2 of Attachment A lists 3 suit failures that have occurred in biosafety laboratories. However, the three suit rupture incidents did not appear to result in the potential for exposure because the leakage was out of the suit. For the aerosol in the room to enter the positive-pressure suit, the suit would need to be breached and depressurized because, otherwise, the airflow will be out of the suit. A positive-pressure suit breach that depressurizes the suit would clearly be detected immediately by the worker and co-workers.

It is considered beyond reasonably foreseeable, frequency category D (1 in more than 1 million years), that an aerosol release from a centrifuge could occur because a worker exposure requires (1) an undetected aerosol release from a centrifuge, and (2) an undetected positive-pressure suit breach large enough to allow the worker to be exposed. Consistent with guidance (DOE 2002), this event is considered speculative and is not considered further.

F.6.3.4.3 Summary

This analysis did not identify any credible event sequences that result in pathogen exposure to a BSL-4 laboratory worker, facility worker, or the public as a result of an aerosol release from a centrifuge. Because no credible event sequences were identified, there are no exposures, as reported in Table F.6-8.

Table F.6-8. Summary of results—BSL-3 centrifuge aerosol release with partial respiratory protection

Frequency category	Exposed group: category	Route of exposure	Pathogen	Exposure range
D (1 in more than 1 million years))	Laboratory workers: NONE (0)	--[a]	--	-
	Facility worker: NONE (0)	--	--	--
	Public: NONE (0)	--	-	--

a. The dash indicates analysis is not appropriate because exposure, if any, is speculative and would average much less than one particle.

F.6.3.5 Variability and Uncertainty

There are numerous variabilities and uncertainties that apply to the results presented in Sections F.6.3.2 through F.6.3.4. Table F.6-9 identifies the key variabilities and uncertainties, provides a discussion of each, and provides an estimate of the potential effect of each.

Table F.6-9. Summary of key variabilities and uncertainties

Variability/ uncertainty	Discussion	Potential factor[a]
Frequency category	As discussed previously, no data are available suitable for use in estimating the frequency of each event, so there is considerable uncertainty in these estimates. While the estimates are uncertain, they are also considered conservative (i.e., overstate the frequency) because the event sequences analyzed for the release involves multiple failures, and the events are assumed to be undetected/unreported. Such events are expected to be highly improbable given the NEIDL safety program.	Conservatism
Suspension concentration	The exposure calculations were performed using maximum suspension concentrations found in literature reviews and interviews. The applicability of these maximum concentrations to NEIDL operations is uncertain because of potential differences between NEIDL protocols and practices and those on which the literature is based. Because the estimates used for this analysis are expected to be conservative there is no reason to expect that NEIDL protocols will result in higher pathogen concentrations, and, in fact, BUMC personnel expect them to be lower.	Conservatism
Analysis of *Y. pestis* and *F. tularensis*	*Y. pestis* and *F. tularensis* are included in the analysis even though the SOPs do not include centrifugation of those pathogens. This conservatism is appropriate because of the potential that they might be centrifuged in the future and because this analysis is intended to envelope other aerosol-producing events in which those pathogens could be involved.	Conservatism (for frequency)
	The tyical volumes of *F. tularensis* and *Y. pestis* are less than the 10 mL used for the experiments, but the experimental data are not available for smaller leakages. It was assumed that the aerosol concentrations would be scaled down proportionally according to their reduced leakage volumes.	Potential non-conservatism (maximum of 10x for *F. tularensis* and 2x for *Y. pestis)*
10 mL leakage	The analysis is based on a 10-mL leakage of a potentially larger total volume; however, the leakage could be greater or less than 10 mL for those pathogens that have more than a 10-mL volume. However, a larger leak is more likely to result in an imbalanced rotor that would ultimately result in the centrifuge being stopped either automatically by the centrifuge's imbalance control or manually by the laboratory worker, so larger leakages are less likely to occur and more likely to be detected. It is not known whether a larger leakage results in a larger release. Therefore, the frequency could be lower, but the airborne release might or might not be greater.	Unknown (potentially lower frequency and higher release)
SF	The SF is based on experimental data (Bennett and Parks 2006). These experimental results are the best available basis to estimate the exposure, but there is uncertainty associated with this SF. The SF could be different for the NEIDL because of differences in the centrifuge speed and rotor design. The SF used might be conservative or non-conservative.	Unknown

Variability/ uncertainty	Discussion	Potential factor[a]
	It is not known whether the SF is based solely on aerosol particles that are no larger than 10 µm. If the experiments used to determine the SF included particles larger than 10 µm, the SF is conservative and overstates the exposure.	Potential conservatism
LPF	A PAPR APF of 0.001 is used for the full respiratory protection case based on test data. HEPA tests generally reflect the minimum filtration efficiency, but might not reflect HEPA filters in their *as used* condition. Therefore, it is uncertain whether the APF of 0.001 is conservative or non-conservative for the full protection case.	Unknown
	A PAPR APF of 0.1 is used for the partial respiratory protection case. This estimate is not based on data, but is believed to be conservative. The procedures for testing the PAPR before use would detect most severe problems and a two order of magnitude reduction in filtration is very severe for an undetected failure.	Unknown conservatism
BR	The generally accepted and recommended breathing rate is used, but the actual breathing rate varies depending on the level of activity involved. Rather than the 3.33×10^{-4} m^3/s value used, the rate could range from an extreme of 1.25×10^{-4} m^3/s for sleep to 8.33×10^{-4} m^3/s for heavy exercise (DOE 2004a). There is some variability in this value, but it is relatively small compared to the other factors.	Relatively small variation
Number of workers exposed	It is possible for more than four people to be in the room at the time of the release, but that is expected to be an infrequent occurrence (BUMC 2009). If the number of people in the room were to exceed four, the number of people exposed would increase, but the frequency for such an event might decrease. Potential exposure of more than four workers is not expected to be a risk-dominant sequence.	Unknown
Location of workers	The location of the worker(s) relative to the point of release is highly variable and the resulting exposures for any given location are highly uncertain. The lowest end of the exposure range is clearly exposure to 0 organisms. No attempt was made to define locations of individuals or the extent of exposure at any given locations.	Unknown

a. The estimate provided in the analyses (Sections 3.1 and 3.2) would be multiplied by this value to obtain the new value. So a current value of 10 when multiplied by a potential factor of 10 would result in 100 and a current value of 10 when multiplied by a potential factor of 0.1 would result in 1. A factor of 0 means that the result could be 0.

As shown in Table F.6-9, numerous variabilities and uncertainties could affect the results of this analysis, some of which result in a conservative and some in a non-conservative bias. While the net effect of those variabilities and uncertainties is not known, the addition of an order of magnitude increase in the maximum exposure is expected to result in an overall conservative bias. Overall, the results of this analysis are judged to be conservative and an event of this type would likely result in lower exposures.

F.7 NEEDLESTICK EVENTS

F.7.1 Introduction

A needlestick is an inadvertent penetration of the skin by a syringe needle. If the needle contains any pathogenic material, a needlestick in a BSL-3 or BSL-4 laboratory could result in an LAI of a laboratory worker. The BMBL (CDC and NIH 2007) states (p. 12):

> Investigations of LAIs have identified five principal routes of laboratory transmission. These are parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols.

Accidental punctures[5] (note: the term *puncture* is used in this document to generically cover percutaneous[6] routes of exposure) by contaminated sharp objects such as needles or scalpels can expose workers to pathogens through the skin or mucous membranes and result in an infection. Sewell 1995 states, "accidental parenteral inoculation of infectious material is one of the leading causes of laboratory-associated infections. Nearly all microorganisms can produce an infection following penetration of the skin by contaminated needles, scalpels, or broken glass."

Pedrosa and Cardoso 2011 reviewed 141 scientific articles published between 1930[7] and 2008. Results relevant to this analysis include:

- "Of laboratory arboviral infections, 15.7% were acquired percutaneously." Both Rift Valley fever virus (RVFV) and tick-borne encephalitis complex (Russian spring-summer encephalitis virus-Far Eastern type) (TBEV-FE) are arboviruses.
- "Of the 12 infections with viruses with preferential mucocutaneous transmission, seven occurred percutaneously."

While changes in laboratory equipment and procedures have changed during this time period, the fact remains that punctures are a significant cause of LAIs.

Sewell 1995 also states:

> The use of a syringe and needle is the most hazardous laboratory procedure. Infections associated with needles and syringes occur through three routes: (i) inhalation of aerosols, (ii) contamination of fingers and the environment, and (iii) direct inoculation…Direct inoculation of the worker from an accidental needlestick is a leading cause of infections by the blood-borne pathogens.

[5] Puncture: to pierce with or as if with a pointed instrument or object; *puncture* the skin with a needle (www.dictionary.com)

[6] Percutaneous: effected or performed through the skin (www.dictionary.com)

[7] Though some of the articles published before circa 1980 might not be as rigorous or relevant as the more recent articles, the general statements quoted here are judged to still be valid.

Thus, an infection resulting from a puncture by a sharp object, specifically direct inoculation of the worker by a needlestick, has been selected as a representative LAI event to be analyzed for laboratory worker exposure.

This analysis relies on both generic and NEIDL-specific information. BMBL provides generic guidance for the biocontainment of pathogens in laboratories of this type. The BUMC Biosafety Manual, provides NEIDL-specific procedures.

It is reasonable to expect that the majority of the 13 pathogens evaluated could be used to inoculate animals; however, some pathogens are more likely to be used in syringes than others. Wild type SARS-associated coronavirus (SARS-CoV) and 1918 H1N1 influenza virus (1918H1N1V) are not expected to be used as injectable live inocula. However, it is possible that blood samples could be taken from animals infected with those pathogens using needle and syringe, and a needlestick would be possible in that case. If those viruses are used, the animal blood concentration is taken to be the same as the maximum working concentration. There is also a risk of exposure to SARS-CoV and 1918H1N1V via other sharp objects, and a needlestick scenario is used as a surrogate for all sharps exposure.

F.7.2 Methodology

F.7.2.1 Biocontainment Features

There are a number of biocontainment features associated with the NEIDL that can be grouped as administrative controls and safety equipment. The biocontainment features that are considered in this analysis include the following:

Administrative Controls:

- Procedures
- Training

Safety Equipment:

- Containers (including needles/syringes)
- PPE

The following paragraphs describe each of those biocontainment features as they relate to sharp objects in general and to needles in particular as they were used in the analysis of the needlestick event sequence.

Procedures—The BUMC Biosafety Manual (Appendix W) provides requirements for developing NEIDL SOPs, which include safe handling of needles and other sharps. Controls specified by the Biosafety Manual (BUMC 2011) include the following:

- Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.
- Plasticware should be substituted for glassware whenever possible.
- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.
- Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
- Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Syringes that re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.
- Broken glassware must not be handled directly by hand, but must be removed by mechanical means, such as a brush and dustpan, tongs, or forceps.

While the BMBL quote above states, "Needles must not be…recapped," recapping of needles sometimes may be necessary. For example, recapping the needle is appropriate after the syringe and needle are loaded with the inoculums that will later be injected into an animal.

29 CFR 1910.1030, *Bloodborne pathogens*, requires the use of engineering and work practice controls to eliminate or minimize employee exposure to bloodborne pathogens. It also requires employers to keep a sharps injury log for the recording of puncture injuries (i.e., injuries involving broken skin) from contaminated sharps (29 CFR 1910.1030).

***Training*—**The training requirements for NEIDL laboratory workers are outlined in the BUMC Biosafety Manual Appendix F for BSL-3 and Appendix G for BSL-4. The training program educates employees about the hazards to which they might be exposed and instructs them on the proper protocols to be used for safe operation of the NEIDL. An example of training might include not allowing needle guards to be removed or recapped with the worker's teeth.

Containers—Containers provide direct containment of pathogens and consist of tubes, bottles, syringes, flasks, or other types of containers. All containers for liquids used in the BSL-3 and BSL-4 will be resistant to breakage and sealed with either snap or screw caps.

PPE—The specific PPE used is dependent on the pathogen and activity involved but will always include protective clothing, including gloves.

In addition, the needles themselves can provide a mechanism for control against needlesticks. For example, safety syringes that automatically retract or re-shield the needle after use (e.g., no manual recapping is necessary) are available.

F.7.2.2 Extent of Exposure

The consequences of a needlestick involve not only the number of people potentially exposed, which is addressed in the previous section, but also the extent to which those people are exposed. Estimating the specific extent of exposure (e.g., the number of bacteria or virions received) is speculative for needlestick events because it could result in a broad range of pathogen exposures for the laboratory worker. The extent of exposure depends on such factors as the extent to which the needle penetrates the skin, whether pathogen is present in the syringe, the amount and concentration of pathogen present in the syringe, and whether the syringe plunger is depressed. If no pathogens are present, then the extent of exposure is zero. Conversely, if pathogen is present and the syringe plunger is depressed, the exposure could be very large.

To demonstrate that a very small amount of inocula can deliver a very large dose, Attachment B shows the amount of each pathogen that could be injected from a single drop. For example, a single drop of inocula with an SC of 1×10^6 $CCID_{50}$ would deliver an exposure of about 4,500 $CCID_{50}$. That supports the claim from Sewell above that, "Nearly all microorganisms can produce an infection following penetration of the skin by contaminated needled, scalpels, or broken glass."

Instead of speculating on the extent of exposure, it is conservatively assumed that an infectious dose was administered for each needlestick event. This conservatism will result in an overestimate of the number of infections that result from needlestick events. The available data provide limited insight into the likely extent of exposure. Attachment B shows that of the 8 BSL-3 incidents for which detailed data are provided, one incident has been shown to have resulted in an infection, one has been shown to have resulted in no infection, and the balance (6 of 8) of the summaries give no information (e.g., the data only show *potential exposure* and the results indicate *investigation ongoing*).

F.7.3 Results

This section documents the selection and analysis of needlestick event sequences. Separate event sequences are selected and analyzed for the BSL-3 and BSL-4 pathogens.

F.7.3.1 Selection of Event Sequences

Factors that were considered in the selection of event sequences for analysis include the following:

- *Detection and Reporting*—Prompt detection and reporting of a needlestick event can maximize the effectiveness of medical intervention and, thus, potentially lower the likelihood that an initial exposure results in infection (and potentially lower the likelihood of related symptoms) to the worker, and lowers the likelihood of secondary transmissions to other workers or the public. Therefore, event sequences are analyzed both with and without prompt detection and reporting.
- *Type of Pathogen*—As stated previously, BSL-3 and BSL-4 activities will be addressed separately.

Thus, the needlestick event sequences selected for further analysis are as follows:

- BSL-3 needlestick with prompt worker detection and reporting—This event was selected to determine the potential risk at BSL-3 when proper protocols for detection and reporting are followed.
- BSL-3 needlestick without prompt worker detection and/or reporting—This event was selected to determine the potential risk at BSL-3 when proper protocols for detection and reporting are not followed.
- BSL-4 needlestick with prompt worker detection and reporting—This event was selected to determine the potential risk at BSL-4 when proper protocols for detection and reporting are followed.
- BSL-4 needlestick without prompt worker detection or reporting—This event was selected to determine the potential risk at BSL-4 when proper protocols for detection and reporting are not followed.

F.7.3.2 Needlestick in BSL-3 Laboratory with Prompt Detection and Reporting

F.7.3.2.1 Event Sequence Description

***Initial Conditions*—**The conditions leading up to this event sequence are as follows:

- The pathogen suspension contains the maximum concentration for that pathogen that is expected to be used in NEIDL. Lower concentrations are expected to result in correspondingly lower exposures.
- As required, the laboratory workers in the room are wearing BSL-3 dedicated shoes, shoe covers, scrub suits with elastic cuffs, a back-fastening gown and double gloves (BUMC 2011), which are the minimum PPE required for all BSL-3 activities.

Other conditions such as the use of respiratory protection and the number of co-located workers in the laboratory are not important for this scenario as they do not influence the extent of exposure.

***Initiating Events*—**Conditions that could initiate a needlestick event include the following:

- A laboratory worker being startled (e.g., by a co-worker, dropped container, animal)
- Inattention to detail
- Laboratory worker involuntary movement (e.g., sneeze)
- Erratic animal movement

***Contributing Events and Conditions*—**The following events or conditions could influence the likelihood of the event, extent of exposure, and human effects, if any, on the laboratory worker:

- Pathogen present or not—If no pathogen is present, the exposure to the worker is zero. It is assumed that the pathogen is present in this needlestick scenario.
- Skin broken or not—If the needlestick does not penetrate the skin, the chances of an infection decrease considerably (assuming the skin is not already compromised by an existing cut or abrasion). It is assumed that the skin is penetrated for this needlestick scenario.
- Proper use of procedures—Procedures that are followed as written could reduce the frequency of events. It is assumed that procedures are properly implemented for this needlestick scenario.
- Proper availability, selection, and use of PPE—The proper use of PPE can effectively eliminate a variety of potential exposure events; however, the types of PPE available are not always capable of preventing all types of needlesticks given the nature of the work to be performed. For example, the use of metal mesh gloves could prevent punctures from contaminated sharps but might not

prevent a needlestick. Also the use of mesh gloves could make the handling of a syringe/needle unwieldy. It is assumed that PPE does not prevent this needlestick scenario.

- Immediate detection and reporting of potential mishaps and operational upsets—It is assumed that the needlestick is promptly detected and reported for this needlestick scenario.
- Prompt and appropriate medical intervention—It is assumed that appropriate medical intervention is taken for this scenario, which includes such actions as quarantine, as appropriate.

F.7.3.2.2 Frequency Category

The operational incident data (see Attachment C of this appendix and Appendix D) identifies incidents that have occurred at other BSL-3 and BSL-4 facilities and provide insights into the types of incidents that might occur at NEIDL. Section F.1.3 discusses the limitations of the data and explains why it is not suitable for use in developing quantitative estimates of the frequency for needlesticks.

BSL-3 operating experience in Attachment C showing 19 incidents from 2002 to 2010 (about 8 years) at facilities similar to the NEIDL. The number of entities registered with the CDC and USDA Select Agent Program maintaining BSL-3 laboratories increased from 191 in 2004 to 279 in 2008 (GAO 2009). That equates to about 0.01 needlestick incidents per year per facility (19 incidents divided by an average of about 235 facilities divided by 8 years). While this frequency estimate provides insight into the frequency, it may understate or overstate the real value for the following reasons:

- It may understate the historic frequency because incidents have historically been underreported because of fear of reprisal and the source of exposure for over 80 percent of laboratory related illnesses is not known (Harding and Byers 2006). (See Section F.1.3 for details.)
- This historic estimate may overstate the frequency because it may not reflect:
  - The historic decline in LAI rates have declined dramatically over time due to enhancements in biosafety procedures, primary biocontainment systems, personal protective equipment, and facilities engineering, as shown in Section D.1.1 of Appendix D.
  - The sharps safety practices at the NEIDL (see Section F.7.2.1) may significantly reduce the rate of exposures due to sharp objects, including syringe needles, and
  - The enhancements to the NEIDL culture of safety (see Section 2.1 of Chapter 2) may reduce the incident rate.

Therefore, BSL-3 needlesticks events are assigned to frequency category A (1 in 1 to 100 years). Based upon the discussion above, this assignment is considered conservative and an incident rate of greater than once per year is not expected.

F.7.3.2.3 Exposure Category

Because this analysis is limited to the point of primary exposure, exposure categories to facility workers and members of the public are not applicable. For laboratory workers, at most one worker will be exposed from a needlestick event. Therefore, an exposure category of LOW is chosen.

F.7.3.2.4 Extent of Exposure

As explained in Section 7.2.2, all needlesticks are conservatively assumed to result in an infectious exposure. Whether such a postulated event is reported promptly does not affect the laboratory worker's exposure to the likely infectious dose, only the ability for medical intervention to mitigate the possibility of infection to the worker and to prevent secondary exposures.

F.7.3.2.5 Summary

Table F.7-1 summarizes the results of the above analysis for a needlestick in a BSL-3 laboratory with prompt detection and reporting.

Table F.7-1. Summary of results—needlestick in BSL-3 laboratory with prompt detection and reporting

Frequency category	Exposure group category	Pathogen[a]	Exposure
A (1 in 1 to 100 years)	Laboratory workers: LOW (1)	*B. anthracis*	Infection assumed
		F. tularensis	Infection assumed
		Y. pestis	Infection assumed
		1918 H1N1V[b]	Infection assumed
		SARS-CoV[b]	Infection assumed
		RVFV	Infection assumed
		ANDV	Infection assumed
	Facility worker: NONE (0)	--	--
	Public: NONE (0)	--	--

a. *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b. SARS-associated coronavirus, and 1918 H1N1 influenza virus are not expected to be used as wild type inocula but are analyzed here for completeness. Additionally it is assumed that if these viruses are used, then the animal blood concentration is taken to be the same as the maximum working concentration

F.7.3.3 Needlestick in BSL-3 Laboratory without Prompt Detection and Reporting

F.7.3.3.1 Event Sequence Description

The event sequence is the same as described in Section F.7.3.2.1 for a BSL-3 needlestick with prompt reporting except for the following:

- Immediate reporting of potential mishaps and operational upsets is assumed not to occur for this scenario.
- Immediate reporting of symptoms that could be because of an LAI are assumed not to occur for this scenario.

This type of scenario could be representative of a worker who does not feel comfortable reporting the injury for fear of reprisal, believes it was a near-miss event that does not require reporting, or does not notice the needlestick, for example.

F.7.3.3.2 Frequency Category

The available operational experience in Attachment C for BSL-3 facilities indicates that all the events were promptly reported, which implies that the non-reporting rate may be less than about 5% (i.e., all of the 19 incidents were promptly reported). However, as discussed in Section F.3.1.2, there are limitations with the available data; one limitation is that unreported incidents are not included the data and are only likely to be included if an infection resulted later. The likelihood of a needlestick not being reported at the NEIDL is expected to be lower than the historic average for the following reasons:

- The BUMC worker must fail to perform as trained, which includes the requirement to report incidents promptly (BUMC 2011).
- The enhancements to the NEIDL culture of safety (see Section 2.1 of Chapter 2) should encourage reporting and minimize consideration of not reporting.
- The presence of two or more people (i.e., 2-person rule) in the laboratory will discourage any consideration of not reporting incidents.

As described previously in Section F.7.3.2.2, the frequency category for a reported needlestick has been conservatively assigned to frequency category A (1 in 1 to 100 years). The historic data discussed above indicate that the rate of non-reporting considerably lower (e.g., 5%). Therefore, undetected or unreported needlestick incidents are assigned to frequency category B (1 in 100 to 10,000 years) based on the historic data and the enhancements at the NEIDL.

F.7.3.3.3 Exposure Category

Because this analysis is limited to the point of primary exposure, exposure categories to facility workers and members of the public are not applicable. For laboratory workers, at most one worker will be exposed from a needlestick event. Therefore, an exposure category of LOW is chosen.

F.7.3.3.4 Extent of Exposure

As explained in Section F.7.2.2, all needlesticks are conservatively assumed to result in an infectious exposure. Whether such a postulated event is reported promptly does not affect the laboratory worker's exposure to the likely infectious dose, only the ability for medical intervention to mitigate the possibility of infection to the worker and to prevent secondary exposures.

F.7.3.3.5 Summary

Table F.7-2 summarizes the results of the above analysis for a needlestick in a BSL-3 laboratory without prompt detection or reporting.

Table F.7-2. Summary of results—needlestick in BSL-3 laboratory without prompt detection or reporting

Frequency category	Exposure group category	Pathogen[a]	Exposure range
B (1 in 100 to 10,000 years)	Laboratory workers: LOW (1)	*B. anthracis*	Infection assumed
		F. tularensis	Infection assumed
		Y. pestis	Infection assumed
		1918 H1N1V[b]	Infection assumed
		SARS-CoV[b]	Infection assumed
		RVFV	Infection assumed
		ANDV	Infection assumed
	Facility worker: NONE (0)	-	--
	Public: NONE (0)	--	-

a. *Bacillus anthracis* (*B. anthracis*), *Francisella tularensis* (*F. tularensis*), *Yersinia pestis* (*Y. pestis*), 1918 H1N1 influenza virus (1918 H1N1V), SARS-associated coronavirus (SARS-CoV), Rift Valley fever virus (RVFV), and Andes virus (ANDV).

b. SARS coronavirus, and 1918 H1N1 influenza virus are not expected to be used as wild type inocula, but are analyzed here for completeness. Additionally it is assumed that if these viruses are used, then the animal blood concentration is taken to be the same as the maximum working concentration.

F.7.3.4 Needlestick in BSL-4 Laboratory with Prompt Detection and Reporting

F.7.3.4.1 Event Sequence Description

Initial Conditions—The conditions leading up to this event sequence are as follows:

- The pathogen suspension contains the maximum concentration for that pathogen that is expected to be used in NEIDL. As required, the laboratory workers in the room are wearing BSL-4 positive-pressure suits and double gloves (BUMC 2011).

Other conditions such as the use of respiratory protection and the number of co-located workers in the laboratory are not relevant or applicable for this analysis because they do not influence the extent of exposure from this scenario.

***Initiating Events and Contributing Events*—**Conditions that could initiate, prevent, and/or mitigate a needlestick event are similar to those presented in Section F.7.3.2.1 for BSL-3 and are not duplicated here.

F.7.3.4.2 Frequency Category

Background information (unreported incidents) for determining frequency category in both BSL-3 and BSL-4 facilities is presented in Section F.7.3.2.2 and is not duplicated here. Attachment C shows 5 BSL-4 operating experience incidents since 1976 at four different facilities. These five needlestick incidents in over 100 facility-years of operation (see Section D.2.2 of Appendix D) result in a rate of about 0.05 per year. In addition, Appendix D shows for a number of BSL-4 facilities, the following statistics:

- At the U.S. Army Medical Research Institute of Infectious Diseases, between 1972 and 2009, six reported events similar to the needlestick event being evaluated;
- At the CDC Special Pathogens Branch, between 1979 and 2009, one reported needlestick-type like event;
- At the National Institute for Communicable Diseases, between 1980 and 2003, no reported needlestick-type event;
- At the Southwest Foundation for Biomedical Research, between 2000 and 2009, no reported needlestick-type event; and
- At the University of Texas Medical Branch –Galveston, between 2004 and 2009, no reported needlestick-type event.

While this frequency estimate of 0.05 per year provides insight into the frequency, it may understate or overstate the real value for the following reasons:

- It may understate the historic frequency because incidents have historically been underreported because of fear of reprisal and the source of exposure for over 80 percent of laboratory related illnesses is not known (Harding and Byers 2006). (See Section F.1.3 for details.)
- This historic estimate may overstate the frequency because it may not reflect:
  - The historic decline in LAI rates have declined dramatically over time due to enhancements in biosafety procedures, primary biocontainment systems, personal protective equipment, and facilities engineering, as shown in Section D.1.1 of Appendix D.
  - The sharps safety practices at the NEIDL (see Section F.7.2.1) may significantly reduce the rate of exposures due to sharp objects, including syringe needles, and

- The enhancements to the NEIDL culture of safety (see Section 2.1 of Chapter 2) may reduce the incident rate.

Therefore, BSL-4 needlesticks events are assigned to frequency category A (1 in 1 to 100 years). Based upon the discussion above, this assignment is considered conservative and an incident rate of greater than once per year is not expected.

F.7.3.4.3 Exposure Category

Because this analysis is limited to the point of primary exposure, exposure categories to facility workers and members of the public are not applicable. For laboratory workers, at most one worker will be exposed from a needlestick event. Therefore, an exposure category of LOW is chosen.

F.7.3.4.4 Extent of Exposure

As explained in Section 7.2.2, all needlesticks are conservatively assumed to result in an infectious exposure. Whether such a postulated event is reported promptly does not affect the laboratory worker's exposure to the likely infectious dose, only the ability for medical intervention to mitigate the possibility of infection to the worker and to prevent secondary exposures.

F.7.3.4.5 Summary

Table F.7-3 summarizes the results of the above analysis for a needlestick in a BSL-4 laboratory with prompt detection and reporting.

Table F.7-3. Summary of results—needlestick in BSL-4 laboratory with prompt detection and reporting

Frequency category	Exposure group Category	Pathogen[a]	Exposure range
A (1 in 1 to 100 years)	Laboratory workers: LOW (1)	EBOV	Infection assumed
		MARV	Infection assumed
		LASV	Infection assumed
		JUNV	Infection assumed
		TBEV-FE	Infection assumed
		NIPV	Infection assumed
	Facility worker: NONE (0)	--	-
	Public: NONE (0)	--	--

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

F.7.3.5 Needlestick in BSL-4 Laboratory without Prompt Detection and Reporting

F.7.3.5.1 Event Sequence Description

The event sequence is the same as described in section F.7.3.2.1 for a BSL-3 needlestick with prompt reporting except for the following:

- Immediate reporting of potential mishaps and operational upsets (assumed not to occur for this scenario).
- Immediate reporting of symptoms that could be because of an LAI (assumed not to occur for this scenario).

This type of scenario could be representative of a worker who does not feel comfortable reporting the injury for fear of reprisal, believes it was a near-miss event that does not require reporting, or does not notice the needlestick, for example.

F.7.3.5.2 Frequency Category

As discussed in Section 7.3.3.2, the historic data imply that a small fraction (0.05) of the needlestick events go unreported and Attachment C of this appendix gives no indication of unreported needlesticks in BSL-4 facilities. As with BSL-3 (see Section 7.3.3.2) a BSL-4 needlestick event without prompt detection or reporting is assigned to frequency category B (1 in 100 to 10,000 years). This frequency category is considered appropriate or even conservative because: (1) the historic values likely overstate the value for current facilities due to enhanced practices, equipment, and facilities (see Section D.1.1 of Appendix D), (2) and the NEIDL sharps safety program (see Section F.7.2.1) and culture of safety enhancement program (see Section 2.1 of Chapter 2) are expected to reduce rates, and (3) the morbidity and mortality rates of the BSL-4 pathogens provides a greater incentive to report promptly.

F.7.3.5.3 Exposure Category

Because this analysis is limited to the point of primary exposure, exposure categories to facility workers and members of the public are not applicable. For laboratory workers, at most one worker will be exposed from a needlestick event. Therefore, an exposure category of LOW is chosen.

F.7.3.5.4 Extent of Exposure

As explained in Section F.7.2.2, all needlesticks are conservatively assumed to result in an infectious exposure. Whether such a postulated event is reported promptly does not affect the laboratory worker's exposure to the likely infectious dose, only the ability for medical intervention to mitigate the possibility of infection to the worker and to prevent secondary exposures.

F.7.3.5.5 Summary

Table F.7-4 summarizes the results of the above analysis for a needlestick in a BSL-4 laboratory without prompt detection and reporting.

Table F7-4. Summary of results—needlestick in BSL-4 laboratory without prompt detection or reporting

Frequency category	Exposure group category	Pathogen[a]	Exposure range
B (1 in 100 to 10,000 years)	Laboratory workers: LOW (1)	EBOV	Infection assumed
		MARV	Infection assumed
		LASV	Infection assumed
		JUNV	Infection assumed
		TBEV-FE	Infection assumed
		NIPV	Infection assumed
	Facility worker: NONE (0)	--	--
	Public: NONE (0)	--	--

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

F.7.3.6 Variability and Uncertainty

Numerous variabilities and uncertainties apply to the above sections. Table F.7-5 identifies the key variabilities and uncertainties, provides a discussion of each, and provides an estimate of the potential effects of each.

Table F.7-5. Summary of key variabilities and uncertainties

Variability/ uncertainty	Discussion	Potential factor
Frequency Categories	As discussed previously, needlesticks are a relatively common hazard in biocontainment laboratories. However, because of limitations in reporting and uncertainty in the outcomes of events, the frequency estimates are uncertain. That uncertainty is greater for unreported events for obvious reasons. However, on the basis of improving safety (procedures, equipment) and reporting practices, it is anticipated that the frequency categories estimated here will be conservative.	Potential conservatism
Working Concentration	The exposure calculations were performed using the maximum working concentrations as determined in TT-CP-001. Using any lower value than those maximum working concentrations will result in lower exposures to the laboratory worker.	Conservatism
	Because of the uncertain nature of biological activities, it is possible for concentrations to be higher than those assumed here. However, because the upper bound of values in the literature is used herein, it is expected that concentrations would not be much higher.	Potentially non-conservatism (factor undetermined)
SARS-CoV and 1918 H1N1V	SARS-CoV and 1918 H1N1V are not expected to be used as wild-type inocula but were included in this analysis for completeness. It was assumed that the animal blood concentration would equal the maximum working concentration.	Potential conservatism for frequencies
Extent of Exposure	Some needlesticks will involve syringes that are sterile and others will transfer almost no pathogen, therefore assuming an infectious dose is received is conservative. Also, the true infection rate might not be 100% even at relatively high doses.	Known conservatism
Inhalation and ingestion not analyzed	Only direct exposure because of a puncture was analyzed. It is believed that this is appropriate given the nature of needlesticks (i.e., any amount of pathogen available for inhalation or ingestion is negligible).	Potentially non-conservative, but negligible
Needlestick punctures skin	It is assumed that the needlestick punctures the skin and injects pathogen on contact. As stated in the analysis, double gloves are standard operating practice, so a needlestick could penetrate a glove but not the skin.	Conservatism

F.8 EARTHQUAKE EVENTS

F.8.1 Introduction

The NEIDL facility was designed and constructed in compliance with strict seismic criteria. Attachment D of this appendix provides an overview of the relevant design criteria and compliance of the NEIDL design with those criteria. As presented in Attachment D of this appendix, a few features of the NEIDL seismic design are as follows (BUMC 2008; Massachusetts 1997; and NIH 2005):

- The design criteria include compliance with
  - NIH Design Policy and Guidelines, November 2003 Edition (NIH 2003)
  - U.S. Department of Health and Human Services, CDC/NIH, Microbiological and Biomedical Laboratories(BMBL), 5th ed. (CDC and NIH 2007)
  - Massachusetts State Building Code (780 CMR); 6th ed. (Massachusetts 1997)
- The Massachusetts State Building Code establishes Seismic Hazard Exposure Groups and Seismic Performance Categories for buildings depending on the nature of their occupancy. NEIDL is classified as a Seismic Hazard Exposure Group II and is assigned to Seismic Performance Category C.
- As required by the Massachusetts State Building Code, the effective peak velocity-related acceleration and the effective peak acceleration are each 0.12g. For Seismic Performance Category C, the building structure must stay functional after a seismic event (Massachusetts 2009).
- While NEIDL is assigned to Seismic Performance Category C, it complies with Seismic Category D, which includes more stringent criteria. Category D facilities have essential functions that are required for post-earthquake recovery. In addition to the structure remaining intact, Category D facilities must also remain operational following a design basis earthquake.
- The BSL-4 suites are structurally separated from the adjoining floors. Such structural separation allows for movement in an earthquake, while maintaining structural integrity of the BSL-4 suites.
- BSL-4 suites have 12-inch-thick reinforced concrete walls with special epoxy covering that acts as a sealant.
- All fixtures for the BSL-4 suite were designed specifically for the facility, and are Underwriters Laboratories tested, to ensure that the facility retains its air pressurization.

F.8.2 Methodology

The risk associated with an event sequence is defined by the combination of its frequency of occurrence and the consequences of its occurrence (EPA 1987; DOE 2002). This section describes the methodology for determining the frequency and consequence for each event sequence.

F.8.2.1 Radial Grid

The level of exposure is dependent on the amount of pathogen released and how the pathogen is dispersed. Both the airborne dispersion factors and the number of people are site-specific, and they have been developed using the same radial (or polar) grid with sixteen 22.5°-sectors similar to the one shown in Figure F.8-1.

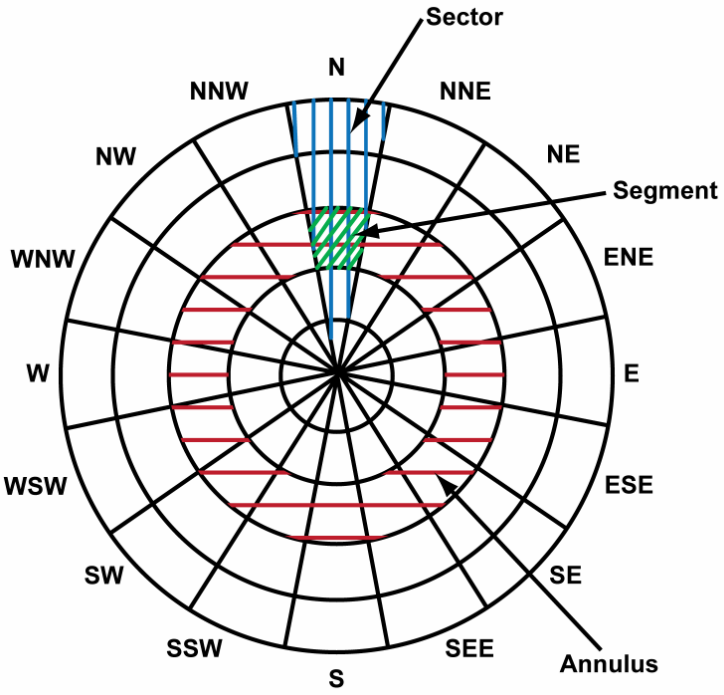


Figure F.8-1. Radial grid.

For this analysis, each annulus is 100-m wide, and the grid extends to a maximum radius of 1 km. While a radius of 1 km captures the majority of the impacts, the expected number of infections would be somewhat higher if the calculation were performed for a larger radius.

There are two competing factors associated with exposures at greater distance, namely, (1) a decrease in the exposure level as the concentration of the pathogen aerosol decreases with distance and (2) a tendency for the number of people per segment to increase because the area of each segment increases as the distance from the center increases. The maximum radius of 1 km (0.6 mile) was selected for several reasons:

- The 1-km radius is consistent with U.S. Nuclear Regulatory Commission recommendations for the NEPA environmental justice evaluation of proposed actions in cities (Nuclear Regulatory Commission 2003).
- The highest density of nonresidents surrounding the urban site is within 0.5 km of NEIDL and is included in the 1-km radius (see Section F.4).

- No high-population communities are just beyond the 1-km radius that would significantly affect the results at any of the sites (see Section F.4).
- The average exposure levels are extremely low at 1 km and would be even lower for greater distances. The MRF (total collapse) earthquake analysis (as shown later) estimates the average exposure level to be less than one one-thousandth (1/1,000) of a unit for all but one pathogen and less than one one-hundredth (1/100) of a unit for all pathogens at 1 km. The calculated exposures at even greater distances would result in even lower exposure levels.

There is limited data on the airborne spread of pathogens, but there is one incident that was considered. The 1979 *B. anthracis* incident in Sverdlovsk, Russia resulted in human infections to a distance of 4 km. However, that incident occurred in a weapons production facility with large quantities of spores released. U.S. biological weapons experts estimate the release to be in the range of one to ten kilograms (Wampler and Blanton 2001). Because the release involved a weaponized pathogen and because the release was many orders of magnitude greater than the entire inventory of NEIDL pathogens, the incident is not relevant to the decision to truncate the exposure calculations at 1 km. Therefore, the 1 km maximum radius is considered appropriate.

F.8.2.2 Atmospheric inactivation

After release from the stack, the pathogen aerosol would be transported and dispersed as a result of winds and meteorological conditions. Atmospheric conditions (e.g., sunlight) would inactivate some infectious particles over time; however, that effect might not be significant for the following reasons:

1. The time required for transport aerosol particles 1 km in the atmosphere is less than 4 minutes at a median wind speed of 5.1 m/second for the urban site (see Section F.5). The median half-life for all pathogens under optimum inactivation conditions is greater than 4 minutes for all pathogens and is 10 to 20 minutes for most pathogens (Bozzette 2011). Therefore, the inactivation is not a major factor for most pathogens under optimum conditions.
2. The earthquake could occur at any time of the day (including nighttime) or of the year (including winter). The half-lives under those unfavorable conditions range from 20 to more than 120 minutes (Bozzette 2011). Therefore, there is virtually no inactivation within 1 km under unfavorable conditions.

Because atmospheric inactivation has a small effect under optimum conditions and virtually no effect under unfavorable conditions (i.e., nighttime and winter), which is a majority of the year, atmospheric inactivation was conservatively not included in this analysis.

F.8.2.3 Airborne dispersion

The airborne dispersion from the point of release is also an essential input to determining the extent of exposure. Section F.5.2 provides the airborne dispersion factors for all three sites. The airborne dispersion factors are calculated for a segment of each annulus of the radial grid to a distance of 1 km. The computer code and parameters used to estimate the airborne dispersion are consistent with guidance (DOE 2002, DOE 2004a) and are addressed in Section F.5.2 of Appendix F. The input parameters for the analyses were the recommended or conservative values (i.e., overestimate aerosol concentrations). For example, wet deposition the dry deposition and building wake effect parameters were the recommended default values. Conservative parameters include the suppression of wet deposition, buoyant plume rise, building wake effect plume rise, and plume meander. The results of these dispersion analyses were compared with the results of other dispersion analyses and wind tunnel tests for NEIDL and were found to be in good agreement. In the case of the MRF (total collapse) earthquake, the assumption of a ground-level release is very conservative and compensates for uncertainties in the analyses. For example at the urban site, concentrations are over 200 times greater at the 30-m exclusion fence for a ground-level release than for an elevated release (see Table F.5.3-1 of Appendix F). See Section F.5 of Appendix F for additional details.

F.8.2.4 Analysis Guidance

DOE NEPA Guidance (DOE 2002) states,

> The key to informative accident analyses is to develop realistic accident scenarios that address a reasonable range of event probabilities and consequences.

In addition to stressing realistic scenarios, DOE NEPA Guidance also notes that *bounding* (i.e., simplified and conservative) approaches may be used for accident analyses, and the bounding approaches might have several distinct advantages (DOE 2002):

> …streamlining an analysis when there are many uncertainties and avoiding the need to prepare more realistic analyses when not warranted. Further, bounding analyses may be more defensible than more realistic approaches because they are unlikely to underestimate potential accident consequences.

Because of the lack of seismic risk information (e.g., seismic hazard curves and NEIDL fragility curves), use of a bounding approach is used for this analysis. However, DOE NEPA Guidance cautions (DOE 2002),

> On the other hand, bounding analyses may mask differences among alternatives and be less informative about the potential need for mitigation. Also, excessive conservatism may result in a misleading presentation of accident risks

Use of a bounding analysis overstates the risks, but it does *not* mask differences between the sites because of the following:

- The same methodology is used for all sites so any conservative bias will be applied equally to all sites.
- The major site differences are the differences in meteorology and population density. Those differences are taken into account with site-specific data, so the site differences are not masked but are accurately reflected in the analysis.

DOE NEPA Guidance (DOE 2002) states, "prevailing (median) meteorological conditions generally should be used." Therefore, median meteorological conditions for each site are used in these analyses.

F.8.3 Results

This section documents the earthquake event selection and the analysis results. The analysis was performed separately for the BSL-3 and BSL-4 areas, although there are many similarities. The variabilities and uncertainties associated with the analyses were also evaluated qualitatively.

F.8.3.1 Scenario Selection

Two earthquakes have been selected for analysis; an earthquake that results in totally facility collapse was selected as the MRF event and a less severe but still beyond design basis (BDB) earthquake that results in minor damage was also analyzed. The following sections discuss those events and the rationale for their selection.

F.8.3.1.1 Maximum Reasonably Foreseeable Earthquake

The MRF event is defined as a reasonably foreseeable accident[8] that results in consequences that are not exceeded by any other reasonably foreseeable event. For this analysis, the MRF event is defined on the basis of direct effects (i.e., pathogen release and direct exposure) on the general public. A severe earthquake has been selected as the MRF event because it:

- Has the potential to affect the entire facility inventory
- Has the potential to compromise all biocontainment features
- Can occur under any meteorological conditions
- Results in higher airborne concentrations than tornadoes and hurricanes, which result in much greater mixing, thereby resulting in much lower concentrations
- Can result in escape of animals (mammals and arthropods)
- Typically *bounds* other natural phenomena events (DOE-HDBK-3010)

F.8.3.1.2 Beyond Design Basis Earthquake

The role of the MRF event is to provide insight into the *worst-case* accident, which was requested by the courts. However, DOE NEPA Guidance (DOE 2002) provides the following caution:

> …in many cases the acceleration forces associated with extremely rare earthquakes (e.g., frequencies of less than 10^{-6} per year) may be so great that destructive impacts unrelated to the proposed action or alternatives would overwhelm impacts associated with the proposed action or alternatives. Such an analysis would not be informative regarding the proposed action or alternatives because a decision maker would be unable to distinguish the consequences resulting from the proposed action or alternatives from the general destructive effects of the earthquake. Analyzing a higher frequency earthquake scenario, however, could be useful in making decisions about the proposed action and alternatives, such as whether a robust earthquake design or alternative location for a proposed facility is warranted.

DOE NEPA Guidance stresses the importance of realism in the analysis and the MRF (total collapse) earthquake is an extremely unlikely event. To put the risks associated with the MRF earthquake into perspective, an earthquake that is less severe and more likely than the MRF earthquake is also analyzed.

[8] "*Reasonably foreseeable*" events include events that mightwhich may have catastrophic consequences, even if their probability of occurrence is low, provided that the analysis of the impacts is supported by credible scientific evidence, is not based on pure conjecture, and is within the rule of reason. ." [Council on Environmental Quality (CEQ) NEPA Regulations, (40 CFR 1502.22)]

Earthquakes that are within the seismic design basis of the facility were not analyzed because the NEIDL structures, systems, and components were designed to perform their functions during and following design basis earthquakes, thereby not resulting in a loss of biological containment. A slightly beyond design basis (BDB) earthquake that is more likely than the MRF (total collapse) earthquake but still beyond the design basis and results in minor damage was selected for analysis. The BDB earthquake is postulated to be slightly beyond the design basis and to result in partially mitigated releases.

F.8.3.2 Beyond Design Basis Earthquake Affecting BSL-3

A BDB (minor damage) earthquake is an earthquake that slightly exceeds the mean seismic ground motion used for the earthquake-resistant design of structures, systems, and components. This section describes the event sequence, frequency category, exposure category, and extent of exposure resulting from a BDB earthquake that results in a partially mitigated the BSL-3 release.

F.8.3.2.1 Event Sequence Description

The initial conditions for the earthquake event sequence are as follows:

- There are no warnings of a potential seismic event and the facility is operating without forewarning of the earthquake.
- Any or all pathogen(s) could be in use in the facility at its typical volumes and maximum concentrations in a liquid suspension. The pathogen could be in one or more containers at the time of the earthquake.
- The facility contains infected animals (mammals and arthropods) at the time.

An earthquake is postulated to occur that involves the following events and conditions:

- The postulated BDB earthquake exceeds the seismic design basis of the NEIDL facility. The NEIDL facility has been demonstrated (Weidlinger 2005) to meet the requirements of the Massachusetts State Building Code (Massachusetts 1997). That code specifies an effective acceleration of 0.12 g (i.e., 0.12 times the acceleration of gravity, or 0.12×32.2 ft/s^2 = 3.86 ft/s^2). As discussed in Attachment E of this appendix, the Richter scale is a measure of the size of the earthquake and does not directly correspond to the effective acceleration at a location.
- While the postulated BDB earthquake exceeds the 0.12 g design basis, accelerations slightly beyond this level are not expected to results in extensive structural damage or collapse. The Massachusetts Building Code (Massachusetts 1997) states the following:

> For ground motions larger than the design levels, the intent of 780 CMR 1612.0 is that there be a low likelihood of building collapse.

Therefore, a total loss of structural integrity would not be expected from the BDB earthquake.

- As a result of the earthquake, one or more containers of pathogens in liquid suspension falls and an airborne release results. Non-breakable containers will be used whenever possible (CDC and NIH 2007), so a broken container is not likely, but a container spill is possible. Containers are allowed to be opened only inside a BSC.
- While loss of structural integrity is not expected, there could be a compromise to other biocontainment features. One of the more affecting potential consequences would be continued operation of the HVAC system with a partial compromise of the HEPA filtration system. That configuration could result in prompt release of the aerosol with a limited attenuation. A loss airflow is also possible, but that would result in a very gradual release and a lower public exposure, so that failure mode is not considered further.
- One or more laboratory workers are in the room at the time of the release and are wearing respiratory protection (i.e., PAPR for BSL-3), as required (BUMC 2010).
- Facility workers would promptly leave the facility and are assumed to remain at the exclusion fenceline for the duration of any release.
- It is assumed that a fire does not result from the earthquake. A fire would inactivate most pathogens and would tend to loft releases over the population, so this assumption results in the highest potential consequences.
- Per guidance, this event occurs during median meteorological conditions (DOE 2002).
- Infected animals (mammals and arthropods) are not expected to escape from a BDB earthquake because there are many barriers to escape, including the cages, interlocking doors to the suits, doors to the floors, and doors to the building.

F.8.3.2.2 Frequency Category

For an exposure to result, a significant vulnerable pathogen inventory (e.g., the working stock) must be present, and it must be release by the BDB (minor damage) earthquake. A rigorous estimate of the frequency is not possible for this analysis because of a lack of information, but the frequency of a BDB earthquake is judged to be in frequency category C (one in 10,000 to 1 million years) for all three candidate sites. This frequency category selection is made because the NEIDL is design to continue to perform its essential functions following a 2-second (effectively the natural frequency of the building) 0.12 g earthquake (BUMC 2005). A 2-second, 0.12 g earthquake has an annual exceedance probability of less than 1×10^{-4} based on the U.S. Geological Survey (USGS) National Seismic Hazard Maps (see Attachment E of this appendix).

F.8.3.2.3 Exposure Category

The exposure category for the laboratory workers is HIGH because all people in all rooms have the potential to be exposed.

As explained in Section F.8.2, the HVAC system protects laboratory workers from potential expose because of the directional airflow; however, workers who evacuate and assemble at the fenceline could be exposed. The exposure category for the facility worker is HIGH.

The estimated exposure category for the public is expected to be LOW. Dispersion calculations indicate that pathogens could be transported beyond the 30-m exclusion zone but are unlikely to spread beyond 300 m. It is possible for pathogen particles to be spread beyond 300 m, but the concentrations would be so low that they would be a minimal risk to health.

F.8.3.2.4 Extent of Exposure

Airborne dispersion of the pathogen organisms can result in inhalation, ingestion, and direct contact routes of exposures. Inhalation exposures are calculated for the laboratory worker and the general public because that is the most likely route of exposure and there is very limited dose-response information for the other routes. The extent of exposure was calculated for the laboratory worker, facility worker, and public. The laboratory worker and facility worker exposures are site-independent because the same facility design and operations are assumed for all three sites. However, the public exposures are site-dependent and are calculated separately for each site.

F.8.3.2.4.1 Laboratory Worker Exposure

A series of experiments designed to mimic the potential event sequences in laboratories was conducted by the Health Protection Agency, an independent organization in the United Kingdom. The purpose of the experiments was "To quantify microbial aerosols generated by a series of laboratory accidents and to use these data in risk assessment." (Bennett and Parks 2006) While the experiments were performed with *B. atrophaeus*, the experimental results are assumed to be applicable to the pathogens evaluated for NEIDL because (1) the aim of the experiments and the discussion in the report addresses microorganisms in a general sense rather than *B. atrophaeus* in particular, (2) the report does not specify any limitations on applicability, and (3) the smaller size of viruses provides reason to believe the results are applicable to viruses. In this analysis, the airborne pathogen concentration in the laboratory room is based on the experiment involving the drop of a 250-mL flask containing 50 mL of pathogen suspension. The experiment is referred to as a *smashed flask*, implying that the flask was glass, while non-breakable

containers will be used whenever possible in NEIDL (CDC and NIH 2007). While the smashed flask likely overestimates the release from a non-breakable container, it is possible that the non-breakable container is not sealed or that it is otherwise breach from a falling object. The general equation resulting from the experiments is presented below.

$$AC = SC \times SF \qquad \text{[equation F.8-1]}$$

where

AC The aerosol concentration in a room (per m^3)

SC The suspension concentration (per mL)[9]

SF The experimentally determined spray factor (mL/m^3)

The above equation is based on the drop of a flask containing 50 mL of suspension, but the maximum working volume for the pathogens being evaluated ranges from 1 mL to 150 mL (see Section F.3). The aerosol concentration in the room is assumed to be proportional to the ratio of the maximum working volume (V_{max}) of the pathogen to the 50 mL used in the experiment. Therefore, AC_{room} is calculated as follows:

$$AC_{room} = SC \times SF \times (V_{max} / 50\ mL) \qquad \text{[equation F.8-2]}$$

Using the same Rift Valley fever virus (RVFV) working stock with an SC of 1×10^9 $CCID_{50}$/mL with a typical volume of 150 mL (see Section F.3) provides in the following example results:

$$AC_{room} = SC \times SF \times (V_{max} / 50\ mL)$$
$$= (1 \times 10^9\ CCID_{50}/mL) \times (5.2 \times 10^{-7}\ mL/m^3) \times (150\ mL / 50\ mL)$$
$$= 1{,}600\ CCID_{50}/m^3$$

Table F.8-1 provides the AC_{room} values for all BSL-3 pathogens.

[9] Suspension concentrations for bacteria are given in terms of colony forming units (CFU). Concentrations for viruses are given in various units, including plaque forming units (PFU), median cell culture infective doses ($CCID_{50}$), and median mouse intracerebral lethal doses ($MICLD_{50}$) per milliliter. Attachment C provides some background information on the methods and units associated with the concentration measurements.

Table F.8-1. BSL-3 room aerosol concentrations from a BDB earthquake

Pathogen[a]	BSL	SC (/mL)[b]		SF (mL/m^3)[c]	V_{max} (mL)	AC_{room} (/m^3)[b]	
B. anthracis[d]	2/3	2.4×10^8	CFU	5.2×10^{-7}	50	120	CFU
F. tularensis	3	2.0×10^9	CFU	5.2×10^{-7}	1	21	CFU
Y. pestis	3	2.0×10^7	CFU	5.2×10^{-7}	5	1.0	CFU
1918H1N1V	3	1.0×10^8	PFU	5.2×10^{-7}	150	160	PFU
SARS-CoV	3	1.0×10^7	PFU	5.2×10^{-7}	150	16	PFU
RVFV[e]	3	1.0×10^8	PFU	5.2×10^{-7}	150	160	PFU
		1.0×10^9	$CCID_{50}$ or $MICLD_{50}$	5.2×10^{-7}	150	1,600	$CCID_{50}$ or $MICLD_{50}$
ANDV	3/4	1.0×10^6	$CCID_{50}$	5.2×10^{-7}	150	1.6	$CCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Suspension concentrations and room aerosol concentrations are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements. Values are taken from the working inventory reported in Section F.3.

c. SF is based on a smashed flask containing 50 mL (Bennett and Parks 2006).

d. In spore form in suspension.

e. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

The worker's potential exposure is the airborne pathogen concentration in the air inhaled multiplied by the volume of air inhaled. It is conservatively assumed that all infectious particles inhaled are retained in the body. The pathogen concentration in the inhaled air is the airborne concentration in the room air multiplied by the fraction of aerosol particles that pass through the PAPR filter. The amount of air inhaled is the breathing rate times the effective duration of exposure. Therefore, the average exposure (i.e., the number of infectious particles a worker inhales) (EX) is equal to the aerosol concentration (AC, which is calculated in equation F.8-2) times the PAPR leak path factor (LPF) times the breathing rate (BR) multiplied by the duration of exposure (T).

$$EX = AC_{room} \times LPF \times BR \times T \qquad \text{[equation F.8-3]}$$

where

LPF The leak path factor is the fraction of airborne particles that would leak past the confinement or filtration system. For this event sequence, the PAPRs will have an APF[10] of 1,000 (29 CFR 1910.134 and NIH 2010), which is the inverse of the LPF of 0.001.

[10] The APF is the workplace level of respiratory protection that a respirator or class of respirators is expected to provide to employees when the employer implements a continuing, effective respiratory protection program (which includes fit testing and proper training) as specified by this section (29 CFR 1910.134).

BR The breathing rate for the worker is assumed to be 3.33E-04 m^3/s or 0.020 m^3/min (DOE 2004a).

T The duration of exposure (T) is the breathing time at the peak concentration. The air exchange rate for NEIDL is at least eight air exchanges per hour (BUMC 2009a), which equates to an air exchange every 7.5 minutes [(1 hr / 8 exchanges) × 60 min/hr = 7.5 min]. The concentration of aerosols in the room will be dependent on the airflow patterns in the room and the nature of the release. The majority of the aerosol would be removed after 1 air exchange (i.e., 7.5 minutes) and 90 percent would be removed after 2.3 air exchanges (i.e., 17.25 minutes = 2.3 × 7.5 minutes) (NIH 2008). A laboratory worker remaining in the area for approximately one hour with the concentration decreasing as the release is purged from the room is approximately the same as a 10–minute exposure at the peak concentration. The exposure is modeled as a 10-minute exposure at peak concentration.

The previous RVFV example that resulted in a AC_{room} value of 1,600 $CCID_{50}/m^3$ is used to as an example of the exposure level calculation:

$$EX = AC_{room} \times LPF \times BR \times T$$
$$= (1{,}600\ CCID_{50}/m^3) \times (0.001) \times (0.020\ m^3/min) \times (10\ min)$$
$$= 0.3\ CCID_{50}$$

An average exposure (*EX*) of less than 1 $CCID_{50}$ indicates that although there might be instances where one or more $CCID_{50}$ is inhaled, there might also be instances where zero $CCID_{50}$ are inhaled, for an average inhalation of less than one. The exposure for all BSL-3 pathogens being evaluated are presented in Table F.8-2.

Table F.8-2. Calculated laboratory worker exposures for BSL-3 pathogens from a BDB earthquake.

Pathogen[a]	AC_{room} ($/m^3$)[b]		LPF	BR (m^3/min)	T (min)	EX[b]	
B. anthracis[c]	2.4×10^8	CFU	0.001	0.020	10	0.02	CFU
F. tularensis	2.0×10^9	CFU	0.001	0.020	10	0.004	CFU
Y. pestis	2.0×10^7	CFU	0.001	0.020	10	0.0002	CFU
1918H1N1V	1.0×10^8	PFU	0.001	0.020	10	0.03	PFU
SARS-CoV	1.0×10^7	PFU	0.001	0.020	10	0.003	PFU
RVFV[d]	1.0×10^8	PFU	0.001	0.020	10	0.03	PFU
	1.0×10^9	$CCID_{50}$ or $MICLD_{50}$	0.001	0.020	10	0.3	$CCID_{50}$ or $MICLD_{50}$
ANDV	1.0×10^6	$CCID_{50}$	0.001	0.020	10	0.0003	$CCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Room aerosol concentrations and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

The laboratory worker exposures resulting from a BDB earthquake are approximately the same as the exposures resulting from the centrifuge release event (see Section F.6). For example, the highest exposure level is for RVFV where the BDB earthquake exposure is 0.3 $CCID_{50}$ and the centrifuge release event exposure is 9 $CCID_{50}$ (see Section F.6), which is a factor of thirty greater than the results above. The centrifuge release event is in frequency category A (1 in 1 to 100 years) and the BDB earthquake is in the frequency category C (1 in 10,000 to 1 million years). The number of infections and secondary transmissions is not calculated for laboratory worker for the following reasons:

- On the basis of the results in RA Chapter 9 for the centrifuge release, the frequency of a laboratory worker infection caused by a BDB earthquake is expected to be either in or on the cusp of the beyond reasonably foreseeable, frequency category D (1 in more than 1 million years). Events that are beyond reasonably foreseeable are dismissed from further consideration.
- The analysis would not provide additional insight into either laboratory worker or public risk because the risk associated with the centrifuge release event is much greater (nominally four orders of magnitude based on the frequency of the release sequence).

F.8.3.2.4.2 Facility Worker

Following an earthquake of this magnitude, it is expected that all facility workers would promptly exit the facility and intuitively move away from the building. However, any facility workers that would remain in the building would not be exposed to the aerosol release because the directional airflow of the HVAC system would keep the aerosol away from facility workers and direct it to the exhaust system.

For this analysis, it is assumed that facility workers exit the building promptly and congregate at the exclusion fenceline (at least 30 m from the NEIDL). A person at the exclusion fence is defined as the maximally exposed individual (MEI), i.e., the hypothetical person receiving the greatest exposure, so the MEI exposure will be used for laboratory workers. The MEI exposure is calculated in the public exposure section below. Approximately 300 workers at NEIDL are present about 25 percent of the year (about 50 weeks at 40 hours per week out of an 8,760 hour year), which results in an average worker population of 75 people. Because of the very low levels of exposure, a facility worker infection is considered to be in frequency category D (1 in more than 1 million years), which is considered beyond reasonably foreseeable and is dismissed from further consideration.

F.8.3.2.4.3 Public Exposure

Following an aerosol release inside a laboratory room, the HVAC system would purge aerosol from the room, filter out aerosol particles, and exhaust the air through the stack. Members of the general public could be exposed to aerosol particles that are not filtered out of the building exhaust. To determine the consequences of that potential exposure, it is necessary to know the number of people exposed and the level of their exposure. The number of people is provided in Section F.4. The level of exposure is dependent on the amount of pathogen released and how the pathogen is dispersed (i.e., the dispersion factor). Both the airborne dispersion factors and the number of people are site-specific and they have been developed using the same radial (or polar) grid with sixteen 22.5°-sectors similar to the one shown in Figure F.8-1.

The stack source term (ST_{stack}), which is the total amount of material released through the stack, is the total amount of aerosol generated times the fraction that is *not* removed by the HEPA filters before discharge through the stack. Assuming a uniform aerosol concentration, the total amount of pathogen released from a dropped flask is the aerosol concentration in the room (AC_{room}) times the volume of the room. The stack source term can be calculated as follows:

$$ST_{stack} = AC_{room} \times V_{room} \times LPF \qquad \text{(equation F.8-4)}$$

where

ST_{stack} The stack source term is the quantity of aerosol released out the stack. The units of the source term are the same as the units used to quantify pathogen concentration, which differs from pathogen to pathogen.

AC_{room} The aerosol concentration in the room is provided in Table F.8-1 above. The units of the source term are the same as the units used to quantify pathogen concentration, which differs from pathogen to pathogen.

V_{room} The volume of the room. Because the aerosol concentration is based on the experiments, this is the volume of the room in which the experiment was performed. The room in which the experiments were performed was 3 m × 3 m × 2 m (Bennett and Parks 2006), which is 18 m^3.

LPF The leak path factor is the fraction of the aerosol that leaks out of the facility (i.e., through the HEPA filtration system).

Under normal conditions, the NEIDL BSL-3 HEPA filters are at least 99.97 percent efficient at removing airborne particles 0.3 μm in diameter with higher efficiencies for all other particle sizes (NIH 2008). Therefore, LPF for HEPA filters is 0.03 percent (i.e., 1–0.9997). However, following a BDB earthquake, it is possible for the HEPA filter efficiency to be reduced because of gasket or media leakage. Several seismic tests of HEPA filters have shown no deterioration in performance; however, actual earthquakes at the Lawrence Livermore National Laboratory (LLNL) indicate a potential increase in filter failures (LLNL 1994). The 1980 earthquake resulted in peak ground accelerations of 0.2 to 0.3 g at LLNL. Tests of 213 HEPA filters over the 12 months following the earthquake showed an increase of about 5 percent in the number of filters experienced leakage beyond the permissible 0.03 percent. Similar tests on 741 HEPA filters were performed following the 1989 earthquake and there was no increase in the number of filters failing the tests. The 1989 earthquake caused peak ground accelerations of 0.1 g at LLNL.

The 1980 earthquake peak ground acceleration of 0.2 to 0.3 g at LLNL was well beyond the NEIDL seismic design basis of 0.12 g, and the 1989 earthquake peak ground acceleration of 0.1 g was slightly less than the NEIDL seismic design basis. If the NEIDL HEPA filters performed as the LLNL filters, one would expect that a BDB earthquake with a peak ground acceleration of slightly greater than 0.12 g would result in between a 0 and 5 percent increase in filter failures. In addition to the number of filter failures, it is also important to consider the severity of the failures. The extent of leakage in the LLNL HEPA filters is not reported, which implies that the severity was not great because that information would have been available and it would be important. However, it was reported that "[n]o contamination was

detected following the earthquakes that would suggest transient releases from the filtration system" (LLNL 1994). On the basis of that experience, it is expected that there would be no or a very slight increase in leakage through the NEIDL HEPA filters as a result of a BDB earthquake. However, to ensure that potential impacts are not underestimated, it was assumed for this analysis that the leakage through the HEPA filters is increased an order of magnitude as a result of the BDB earthquake (i.e., from a leakage rate of 0.03 percent to a leakage rate of 0.3 percent). Therefore, an LPF of 0.3 percent will be used for the fraction of the aerosol in the room that would be released through the stack.

Using equation 3-4 and the RVFV AC_{room} from Table F.8-1 as an example produces the following results:

$$ST_{stack} = AC_{room} \times V_{room} \times LPF$$
$$= (1{,}600\ CCID_{50}/m^3) \times (18\ m^3) \times (0.003)$$
$$= 86\ CCID_{50}$$

Performing the calculation for all BSL-3 pathogens in Table F.8-1 produces the results presented in Table F.8-3. These results are applicable to all three sites because the facility design and operations are assumed to be the same at all sites.

Table F.8-3. Calculated stack source term for BSL-3 pathogens from a BDB earthquake

Pathogen[a]	AC_{room} ($/m^3$)[b]		V_{room} (m^3)	LPF	ST_{stack}[b]	
B. anthracis[c]	120	CFU	18	0.003	7	CFU
F. tularensis	21	CFU	18	0.003	1	CFU
Y. pestis	1.0	CFU	18	0.003	0.06	CFU
1918H1N1V	160	PFU	18	0.003	8	PFU
SARS-CoV	16	PFU	18	0.003	0.8	PFU
RVFV[d]	160	PFU	18	0.003	8	PFU
	1,600	$CCID_{50}$ or $MICLD_{50}$	18	0.003	80	$CCID_{50}$ or $MICLD_{50}$
ANDV	1.6	$CCID_{50}$	18	0.003	0.08	$CCID_{50}$

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Room aerosol concentrations and stack source term are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

The inhalation exposure of a member of the public downwind from the release is calculated by the following formula, which is adapted from the airborne dispersion code guide by deleting the radiological factors that are not relevant here (DOE 2004a):

$$EX = (ST_{stack}) \times (\chi/Q) \times (BR) \qquad \text{(equation F.8-5)}$$

where

EX The inhalation exposure (*EX*) in to a person at that location. The units of the exposure are the same as the units used to quantify pathogen concentration, which differs from pathogen to pathogen.

ST_{stack} The stack source term is the quantity of aerosol released out the stack. The units of the source term are the same as the units used to quantify pathogen concentration, which differs from pathogen to pathogen.

χ/Q The downwind dilution factor (chi over Q) from atmospheric dispersion, which represents the time-integrated concentration at a specific downwind location that is normalized by the quantity released to the atmosphere, with typical units of s/m^3. χ/Q is site-specific and changes with the distance from the point of release. Therefore, site and distance specific χ/Q values were developed in Section F.5. As discussed in Section F.8.2.7, the DOE guidance recommends, "…prevailing (median) meteorological conditions generally should be used." Therefore, median values are used in this analysis. The NEIDL stack is assumed to remain intact because structural failures are not expected until well beyond the design basis (see Section F.8.3.2.1).

BR The breathing rate (BR) of the individual exposed to the plume of released radiological material, with typical units of m^3/s. The recommended value for BR is 3.33×10^{-4} m^3/s (DOE 2004a), a value which applies for all three sites being evaluated.

Using the RVFV at 0.3 m for the MEI as an example results in the following:

$$EX = (ST_{stack}) \times (\chi/Q) \times (BR)$$
$$= (1{,}600\ CCID_{50}) \times (1.96 \times 10^{-5}\ s/m^3) \times (3.33 \times 10^{-4}\ m^3/s)$$
$$= 5.5 \times 10^{-7}\ CCID_{50}$$

The average exposures were calculated for each annulus in the same manner shown above for an individual located at the midpoint of each segment. The χ/Q value for each annulus was based on the midpoint χ/Q, which was assumed to be the average of the values for the inner and outer radii. Using that average value for the midpoint χ/Q slightly underestimates the dispersion (i.e., overstates the exposure)

because a χ/Q versus distance plot is a concave curve. The midpoint χ/Q values are assumed to apply to the entire segment even though the concentrations on either side of the plume centerline will be lower. Use of the plume centerline values for the entire segment is another conservatism of this calculation.

Tables F.8-4a through F.8-4c are a composite of inputs and results for the urban, suburban, and rural sites. The first four rows of each table present input values used for the calculation, including the BR and the inner, outer, and midpoint χ/Q values for each annulus. The table also presents the exposures for each pathogen for each annulus. The units for the exposure values are the same as the units for the ST_{stack} values. The tables also present the average segment population for each annulus as calculated in Section F.5. The exposure values are calculated for the plume centerline, which is the highest concentration in the sector, so use of these exposure values for all people in the segment slightly overstates the risk.

Table F.8-4a. Average public exposures to BSL-3 pathogens resulting from a BDB earthquake for the urban site

			Annulus (km)										
			0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[e] and BR inputs:													
BR (m^3/s)			3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner			1.96E-05	1.96E-05	1.96E-05	1.89E-05	1.67E-05	1.38E-05	1.13E-05	9.95E-06	8.81E-06	7.84E-06	7.61E-06
χ/Q (s/m^3)—Outer			1.96E-05	1.96E-05	1.89E-05	1.67E-05	1.38E-05	1.13E-05	9.95E-06	8.81E-06	7.84E-06	7.61E-06	6.30E-06
χ/Q (s/m^3)—Midpoint			1.96E-05	1.96E-05	1.93E-05	1.78E-05	1.53E-05	1.26E-05	1.06E-05	9.38E-06	8.33E-06	7.73E-06	6.96E-06
Pathogen[a]:	**ST_{stack}[b]**		**Plume Centerline Exposure (same units as ST_{stack})[b]**										
B. anthracis[c]	7	CFU	4.4E-08	4.4E-08	4.3E-08	4.0E-08	3.4E-08	2.8E-08	2.4E-08	2.1E-08	1.9E-08	1.7E-08	1.6E-08
F. tularensis	1	CFU	7.3E-09	7.3E-09	7.2E-09	6.7E-09	5.7E-09	4.7E-09	4.0E-09	3.5E-09	3.1E-09	2.9E-09	2.6E-09
Y. pestis	0.06	CFU	3.7E-10	3.7E-10	3.6E-10	3.3E-10	2.9E-10	2.3E-10	2.0E-10	1.8E-10	1.6E-10	1.4E-10	1.3E-10
1918H1N1V	8	PFU	5.5E-08	5.5E-08	5.4E-08	5.0E-08	4.3E-08	3.5E-08	3.0E-08	2.6E-08	2.3E-08	2.2E-08	2.0E-08
SARS-CoV	0.8	PFU	5.5E-09	5.5E-09	5.4E-09	5.0E-09	4.3E-09	3.5E-09	3.0E-09	2.6E-09	2.3E-09	2.2E-09	2.0E-09
RVFV[d]	8	PFU	5.5E-08	5.5E-08	5.4E-08	5.0E-08	4.3E-08	3.5E-08	3.0E-08	2.6E-08	2.3E-08	2.2E-08	2.0E-08
	80	$CCID_{50}$ or $MICLD_{50}$	5.5E-07	5.5E-07	5.4E-07	5.0E-07	4.3E-07	3.5E-07	3.0E-07	2.6E-07	2.3E-07	2.2E-07	2.0E-07
ANDV	0.08	$CCID_{50}$	5.5E-10	5.5E-10	5.4E-10	5.0E-10	4.3E-10	3.5E-10	3.0E-10	2.6E-10	2.3E-10	2.2E-10	2.0E-10
Segment population:				31	108	196	372	376	178	165	250	310	215

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Stack source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

e. χ/Q values are the Base Case values presented in Section F.5.

Table F.8-4b. Average public exposures to BSL-3 pathogens resulting from a BDB earthquake for the suburban site

Pathogen	ST_{stack}	Annulus (km)										
		0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[e] and BR inputs:												
BR (m^3/s)		3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner		2.92E-05	2.92E-05	2.92E-05	2.67E-05	2.41E-05	2.17E-05	1.88E-05	1.78E-05	1.67E-05	1.56E-05	1.46E-06
χ/Q (s/m^3)—Outer		2.92E-05	2.92E-05	2.67E-05	2.41E-05	2.17E-05	1.88E-05	1.78E-05	1.67E-05	1.56E-05	1.46E-06	1.28E-05
χ/Q (s/m^3)—Midpoint		2.92E-05	2.92E-05	2.80E-05	2.54E-05	2.29E-05	2.03E-05	1.83E-05	1.73E-05	1.62E-05	8.53E-06	7.13E-06
Pathogen[a]:	**ST_{stack}[b]**	**Plume Centerline Exposure (same units as ST_{stack})[b]**										
B. anthracis[c]	7 CFU	6.6E-08	6.6E-08	6.3E-08	5.7E-08	5.1E-08	4.5E-08	4.1E-08	3.9E-08	3.6E-08	1.9E-08	1.6E-08
F. tularensis	1 CFU	1.1E-08	1.1E-08	1.0E-08	9.5E-09	8.6E-09	7.6E-09	6.8E-09	6.5E-09	6.0E-09	3.2E-09	2.7E-09
Y. pestis	0.06 CFU	5.5E-10	5.5E-10	5.2E-10	4.8E-10	4.3E-10	3.8E-10	3.4E-10	3.2E-10	3.0E-10	1.6E-10	1.3E-10
1918H1N1V	8 PFU	8.2E-08	8.2E-08	7.8E-08	7.1E-08	6.4E-08	5.7E-08	5.1E-08	4.8E-08	4.5E-08	2.4E-08	2.0E-08
SARS-CoV	0.8 PFU	8.2E-09	8.2E-09	7.8E-09	7.1E-09	6.4E-09	5.7E-09	5.1E-09	4.8E-09	4.5E-09	2.4E-09	2.0E-09
RVFV[d]	8 PFU	8.2E-08	8.2E-08	7.8E-08	7.1E-08	6.4E-08	5.7E-08	5.1E-08	4.8E-08	4.5E-08	2.4E-08	2.0E-08
	80 $CCID_{50}$ or $MICLD_{50}$	8.2E-07	8.2E-07	7.8E-07	7.1E-07	6.4E-07	5.7E-07	5.1E-07	4.8E-07	4.5E-07	2.4E-07	2.0E-07
ANDV	0.08 $CCID_{50}$	8.2E-10	8.2E-10	7.8E-10	7.1E-10	6.4E-10	5.7E-10	5.1E-10	4.8E-10	4.5E-10	2.4E-10	2.0E-10
Segment population:			0.1	0.4	4.4	0.9	1.2	12.6	3.2	12.2	4.6	11.1

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Stack source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

e. χ/Q values are the Base Case values presented in Section F.5.

Table F.8-4c. Average public exposures to BSL-3 pathogens resulting from a BDB earthquake for the rural site

Pathogen	ST_{stack}	Annulus (km)										
		0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[e] and BR inputs:												
BR (m^3/s)		3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner		3.25E-05	3.25E-05	3.25E-05	3.06E-05	2.54E-05	2.33E-05	2.06E-05	1.97E-05	1.89E-05	1.88E-05	1.68E-05
χ/Q (s/m^3)—Outer		3.25E-05	3.25E-05	3.06E-05	2.54E-05	2.33E-05	2.06E-05	1.97E-05	1.89E-05	1.88E-05	1.68E-05	1.35E-05
χ/Q (s/m^3)—Midpoint		3.25E-05	3.25E-05	3.16E-05	2.80E-05	2.44E-05	2.20E-05	2.02E-05	1.93E-05	1.89E-05	1.78E-05	1.52E-05
Pathogen[a]:	**ST_{stack}[b]**	**Plume Centerline Exposure (same units as ST_{stack})[b]**										
B. anthracis[c]	7 CFU	7.3E-08	7.3E-08	7.1E-08	6.3E-08	5.5E-08	4.9E-08	4.5E-08	4.3E-08	4.2E-08	4.0E-08	3.4E-08
F. tularensis	1 CFU	1.2E-08	1.2E-08	1.2E-08	1.0E-08	9.1E-09	8.2E-09	7.5E-09	7.2E-09	7.1E-09	6.7E-09	5.7E-09
Y. pestis	0.06 CFU	6.1E-10	6.1E-10	5.9E-10	5.2E-10	4.6E-10	4.1E-10	3.8E-10	3.6E-10	3.5E-10	3.3E-10	2.8E-10
1918H1N1V	8 PFU	9.1E-08	9.1E-08	8.9E-08	7.9E-08	6.8E-08	6.2E-08	5.7E-08	5.4E-08	5.3E-08	5.0E-08	4.2E-08
SARS-CoV	0.8 PFU	9.1E-09	9.1E-09	8.9E-09	7.9E-09	6.8E-09	6.2E-09	5.7E-09	5.4E-09	5.3E-09	5.0E-09	4.2E-09
RVFV[d]	8 PFU	9.1E-08	9.1E-08	8.9E-08	7.9E-08	6.8E-08	6.2E-08	5.7E-08	5.4E-08	5.3E-08	5.0E-08	4.2E-08
	80 $CCID_{50}$ or $MICLD_{50}$	9.1E-07	9.1E-07	8.9E-07	7.9E-07	6.8E-07	6.2E-07	5.7E-07	5.4E-07	5.3E-07	5.0E-07	4.2E-07
ANDV	0.08 $CCID_{50}$	9.1E-10	9.1E-10	8.9E-10	7.9E-10	6.8E-10	6.2E-10	5.7E-10	5.4E-10	5.3E-10	5.0E-10	4.2E-10
Segment population:			0.03	1.37	0.18	0.25	0.32	0.4	0.47	0.54	1.55	0.69

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Stack source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3.1.2 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

e. χ/Q values are the Base Case values presented in Section F.5.

F.8.3.2.5 Escaped Animals

The NEIDL could contain a number of infected animals (mammals and arthropods) at any time [see Section F.3.3.4). For an animal to escape the facility, it would need to escape from its enclosure, escape through interlocking laboratory doors, make its way to the ground floor through additional doors, and escape the building. That is highly unlikely for an earthquake slightly beyond the design basis. The potential impact of the infected escaped animals is analyzed in Chapter 7.

F.8.3.3 Beyond Design Basis Earthquake Affecting BSL-4

This section describes the event sequence, frequency category, exposure category, and extent of exposure resulting from a BDB (minor damage) earthquake that damages the BSL-4 areas of the facility. As explained in Section F.8.1.2, the BSL-4 area is isolated from the BSL-3 areas so an earthquake that fails the BSL-3 area might not fail the BSL-4 area. There are many similarities between the BSL-3 and the BSL-4 event sequences, but because of this BSL-4 isolation and because the pathogens that might be used are different, the BSL-3 and BSL-4 areas are analyzed separately.

F.8.3.3.1 Event Sequence Description

While the BSL-4 laboratories are isolated from the rest of the building, the seismic design information available (see Section F.8.1.2 and Attachment D) does not distinguish between the BSL-3 and BSL-4 areas when addressing the design criteria or seismic capacity. Therefore, there is no basis for distinguishing between the effects of a BDB earthquake on BSL-3 and BSL-4 areas, even though it is expected that the BSL-4 has a higher seismic capacity. Because there is no basis for distinguishing between the two areas, this event sequence is also assumed to fail all biocontainment features with the exception that BSL-4 requires positive-pressure suits for laboratory workers rather than PAPR. As a result, the BSL-3 discussion of the event sequence description (Section F.8.3.2.1) applies for the BSL-4 in its entirety and is not repeated here.

F.8.3.3.2 Frequency Category

The seismic capacity of the BSL-4 laboratories is expected to be greater than the seismic capacity of the BSL-3 laboratories; thus, the frequency should be lower than the frequency for the BSL-3 BDB earthquake. However, the BSL-4 has not been demonstrated to have a great capacity than the BSL-3 nor is the extent of this increased capacity known. Therefore, it is conservatively assumed that the seismic capacity of the BSL-4 laboratories is the same as the capacity of the BSL-3 laboratories and the discussion in Section F.8.3.2.2 and Attachment E are applied directly for the BSL-4 and are not repeated

here. As a result, the BSL-4 earthquake event sequence is placed in frequency category C (1 in 10,000 to 1 million years).

F.8.3.3.3 Exposure Category

The exposure category for the BSL-4 is comparable to the exposure category for the BSL-3, which is presented in Section F.8.3.2.3 and is not repeated here. The estimated exposure category for the laboratory worker is HIGH (more than 4), facility worker is HIGH, and public is LOW (30 to 300 m).

F.8.3.3.4 Extent of Exposure

F.8.3.3.4.1 Laboratory Worker

As discussed in the Section F.6.3.4, the positive-pressure suits provide clean external air to the BSL-4 laboratory workers so the laboratory workers in the room will not be exposed the aerosol released into the room. For an exposure to result, the following must all occur concurrently:

1. A BDB earthquake, which is assigned to the frequency category C (1 in 10,000 to 1 million years)
2. A large aerosol release in a room
3. A large breach in a positive-pressure suit in the specific room with the aerosol release
4. The laboratory worker with the breached suit remains in the room long enough to receive a significant exposure

The combination of all those events occurring is considered beyond reasonably foreseeable, frequency category D (1 in more than 1 million years), and the exposure for the BSL-4 laboratory worker is dismissed from further consideration.

F.8.3.3.4.2 Facility Worker

The BDB earthquake discussion for the BSL-3 also applies to the BSL-4 and is not repeated here. Therefore, a facility worker infection is considered beyond reasonably foreseeable, frequency category D (1 in more than 1 million years), and is dismissed from further consideration.

F.8.3.3.4.3 Public

The same methodology used in Section F.8.3.2.4 is applied to the BSL-4 analysis as well because it is assumed that the release phenomena are the same. This methodology begins by estimating the aerosol concentration in the room, which uses equation (F.8-2) from Section F.8.3.2.4. Table F.8-5 provides the

details of the source term calculation for the BSL-4 laboratory. Table F.8-5 is applicable for all three sites because the facility and its operations are assumed to be the same for all 3 sites.

Table F.8-5. BSL-4 room aerosol concentrations from a BDB earthquake

Pathogen[a]	BSL	SC (/mL)[b]		SF (mL/m^3)[c]	V_{max} (mL)	AC_{room} ($/m^3$)[b]	
EBOV	4	5.0E+07	$CCID_{50}$	5.2×10^{-7}	150	78	$CCID_{50}$
MARV	4	1.0E+07	$CCID_{50}$	5.2×10^{-7}	150	16	$CCID_{50}$
LASV	4	1.0E+07	$TCID_{50}$ or FFU (PFU)	5.2×10^{-7}	150	16	$TCID_{50}$ or FFU (PFU)
JUNV	4	1.0E+07	PFU	5.2×10^{-7}	150	16	PFU
TBEV-FE	4	1.0E+08	MID50	5.2×10^{-7}	150	160	MID50
NIPV	4	2.0E+07	$TCID_{50}$ or PFU	5.2×10^{-7}	150	31	$TCID_{50}$ or PFU

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. Room aerosol concentrations and stack source term are given in terms of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).

c. SF is based on a *smashed flask* containing 50 mL (Bennett and Parks 2006).

Then, using equation F.8-4 from Section F.8.3.2.4.3 to calculate the stack source term (ST_{stack}) produces the results presented in Table F.8-6. The BSL-4 HVAC system has two HEPA filters in series rather than the single HEPA filter in the BSL-3 HVAC. As discussed in Section F.8.3.2.4.3, the filtration efficiency of each HEPA filter is assumed to be reduced by an order of magnitude (i.e., from a leakage rate of 0.0003 to a leakage rate of 0.003). Therefore, an LPF of 9×10^{-6} (i.e., 0.003×0.003) will be used for the fraction of the aerosol in the room that would be released through the stack.

Table F.8-6. Calculated stack source term for BSL-4 pathogens from a BDB earthquake

Pathogen[a]	AC_{room} ($/m^3$)[b]		V_{room} (m^3)	LPF	ST_{stack}[b]	
EBOV	78	$CCID_{50}$	18	9×10^{-6}	0.01	$CCID_{50}$
MARV	16	$CCID_{50}$	18	9×10^{-6}	0.003	$CCID_{50}$
LASV	16	$TCID_{50}$ or FFU (PFU)	18	9×10^{-6}	0.003	$TCID_{50}$ or FFU (PFU)
JUNV	16	PFU	18	9×10^{-6}	0.003	PFU
TBEV-FE	160	MID50	18	9×10^{-6}	0.03	MID50
NIPV	31	$TCID_{50}$ or PFU	18	9×10^{-6}	0.005	$TCID_{50}$ or PFU

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. Room aerosol concentrations and stack source term are given in terms of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).

As shown in Table F.8-6, all stack source term values are much less than 1 unit. That means that less than one unit of pathogen would be expected to be released from the MRF earthquake. As shown in Table F.8-4 for the BSL-3 impacts, the airborne dispersion will reduce exposures by at least three orders of magnitude. As a result, the average public exposures would be extremely small, and a significant exposure is considered beyond reasonably foreseeable, frequency category D (1 in more than 1 million years). Therefore, public exposures from a BDB earthquake are dismissed from further consideration.

F.8.3.3.5 Escaped Animals

The NEIDL might contain a number of infected animals (mammals and arthropods) at any time [see the NEIDL Inventory report (Tetra Tech 2011a)]. For an animal to escape the facility, it would need to escape from its enclosure, escape through interlocking laboratory doors, make its way to the ground floor through additional doors, and escape the building. That is highly unlikely for an earthquake slightly beyond the design basis. The potential impact of those infected escaped animals is analyzed in RA Chapter 7.

F.8.3.4 Maximum Reasonably Foreseeable Earthquake Affecting BSL-3

Consistent with Section F.8.2.7, a bounding analysis was performed for the earthquake event sequence. *American National Standard Categorization of Nuclear Facility Structures, Systems, and Components for Seismic Design,* (ANSI 2004) provides guidance specifically for analysis of earthquake events. That document is based on the methodology developed by DOE and is intended for facility design purposes; however, that methodology is frequently used in safety analyses and for environmental impact statements (EISs). The relevant guidance provided for the impact analysis includes the following recommendations:

1. "The unmitigated consequence analysis shall be performed considering only the inherent physical or chemical characteristics of the hazardous material and the energy sources for dispersing the material."
2. The "…engineered mitigating features shall be assumed not to function unless the robustness of each mitigating feature can be demonstrated to survive the postulated event."
3. ANSI/ANS-5.10-1998, *Airborne Release Fractions at Non-Reactor Nuclear Facilities* provides guidance concerning mechanisms for release of the hazardous material into the air or water and shall be used to support similar calculations required by this standard." Because the document referred to is based on DOE-HDBK-3010 (DOE 2000) and because DOE-HDBK-3010 is more comprehensive, this analysis uses DOE-HDBK-3010 as its basis.
4. The "…consequence analysis shall strive to use mean values for the parameters related to material release, dispersal in the environment, and health consequences."

In summary, this guidance results in development of bounding scenarios with the use of median factors when analyzing these bounding scenarios. This is an extremely severe scenario that is extremely unlikely to occur and whose consequences are highly unlikely to be exceeded.

The sections below describe the event sequence, frequency category, exposure category, and extent of exposure resulting from a MRF (total collapse) earthquake that severely damages the BSL-3.

F.8.3.4.1 Event Sequence Description

The initial conditions for the earthquake event sequence are as follows:

- There are no warnings of potential seismicity, and the facility is operating without forewarning of the earthquake.
- Any or all pathogen(s) could be in use in the facility at its typical volumes and maximum concentrations in a liquid suspension. The pathogen could be in one or more containers at the time of the earthquake.
- The facility contains infected animals (mammals and arthropods) at the time.

A severe earthquake is postulated to occur that involves the following events and conditions:

- The postulated severe earthquake exceeds the seismic capacity of the NEIDL facility. The NEIDL facility has been demonstrated (Weidlinger 2005) to meet the requirements of the Massachusetts State Building Code (Massachusetts 1997). That code requires design to an effective acceleration of 0.12 g (i.e., 0.12 times the acceleration of gravity, or 0.12×32.2 ft/s^2 = 3.86 ft/s^2). As discussed in Attachment E, the Richter scale is a measure of the size of the earthquake and does not directly correspond to the effective acceleration at a location.
- The postulated earthquake exceeds 0.12 g; however, the failure point for the NEIDL facility has not been determined. Therefore, the integrity of biocontainment features has not been demonstrated for the postulated earthquake, and the structure is assumed to fail. Consistent with the guidance provided in Section F.8.3.4 above, it is assumed that the biocontainment features do not provide biocontainment. Therefore, it is assumed that as a result of the severe earthquake, the NEIDL structure suffers a catastrophic failure, and all biocontainment is lost. Such a loss of biocontainment includes failure of the HVAC and its HEPA filtration and biocontainment provided by the facility walls. A total collapse of the facility would be the extreme case of this structural failure and a total loss of biocontainment.

- It is assumed that a fire does not result from the earthquake. A fire would inactivate most pathogens and would tend to loft releases over the population, so this assumption results in the highest potential consequences.
- As a result of falling debris from the earthquake, the container(s) of pathogens in liquid suspension are breached and released to the environment. Containers of frozen pathogen suspensions could be breached, but their release is minimal because they are initially frozen, and a large-scale, prompt release is unlikely; therefore, they are dismissed from further consideration for this analysis.
- Per guidance, this event occurs during median meteorological conditions.
- Infected animals (mammals and arthropods) could escape from the facility.

F.8.3.4.2 Frequency Category

A rigorous estimate of the frequency is not possible for this analysis because of a lack of information, but the frequency of an earthquake that causes failure of the NEIDL structure is judged to be in the frequency category C (1 in 10,000 to 1,000,000 years) for all three candidate sites. This selection is made because the NEIDL structure will continue to perform its functions following a 2-second shaking period, 0.12 g earthquake (BUMC 2005). A 2-second, 0.12 g earthquake has a return period in excess of 10,000 years or a frequency of less than 1×10^{-4} based on the USGS National Seismic Hazard Maps (see Attachment E).

F.8.3.4.3 Exposure Category

The exposure category for the laboratory workers is HIGH because all people in all rooms have the potential to be exposed.

The exposure category for the facility workers is HIGH because all people in the facility have the potential to be exposed.

The estimated exposure category for the public is expected to be MODERATE. Aerosol particles could be dispersed beyond 300 m, but concentrations would be extremely low beyond 3 km.

F.8.3.4.4 Extent of Exposure

The DOE NEPA Guidance (DOE 2002) provides the following guidance:

> …in many cases the acceleration forces associated with extremely rare earthquakes (e.g., frequencies of less than 10^{-6} per year) may be so great that destructive impacts unrelated to the proposed action or alternatives would overwhelm impacts associated with the

> proposed action or alternatives. Such an analysis would not be informative regarding the proposed action or alternatives because a decision maker would be unable to distinguish the consequences resulting from the proposed action or alternatives from the general destructive effects of the earthquake.

This caution is certainly applicable for the MRF earthquake because of the low frequency of the event and the extremely conservative assumptions used for the analyses. However, the exposures are estimated for this event because the scenario provides insight into the maximum consequences that could reasonably be expected from operation of the facility. Analysis of this extreme event also provides insight into potential site differences.

F.8.3.4.4.1 Laboratory Worker

If a severe earthquake were to result in total structural failure of the NEIDL, it is unlikely that laboratory workers would survive if they were in the BSL-3 laboratories. Estimation of exposures to workers in a collapsed building provides no insight into worker risk from this event. Therefore, the laboratory workers are assumed to have escaped the building and congregate at the NEIDL exclusion fence for the duration of the release. In such a case, the laboratory workers are no different than any other facility worker and are included in the following exposure estimate.

F.8.3.4.4.2 Facility Worker

If a severe earthquake were to result in total structural failure of the NEIDL, it is unlikely that facility workers inside the facility would survive. Estimation of exposures to workers in a collapsed building provides no insight into worker risk from this event. Therefore, the laboratory workers are assumed to be at the NEIDL exclusion fence for the duration of the release, as described in Section F.8.3.2.4.2.

For this analysis, it is assumed that facility workers exit the building promptly and congregate at the exclusion fenceline (at least 30 m from the NEIDL). A person at the exclusion fence is defined as the MEI, so the MEI exposure will be used for laboratory workers. The MEI exposure is calculated in the public exposure section below. Approximately 300 workers at NEIDL are present about 25 percent of the year (about 50 weeks at 40 hours per week out of an 8,760 hour year), which results in an average worker population of 75 people.

F.8.3.4.4.3 Public

Airborne dispersion of the pathogen organisms can result in inhalation, ingestion, and direct contact routes of exposures. Exposure due to puncture by contaminated sharp objects is also possible, especially for workers that might survive the event. The sections below provide the exposure estimates for the public, laboratory worker, and members of the public.

The source term (ST) is the amount of material released into the air to which a person can be exposed. *Airborne Release Fractions/Rates and Respirable Fractions for Nonreactor Nuclear Facilities*, DOE-HDBK-3010 (DOE 2000) equation (1-1) provides the typical formula for estimating the ST:

$$ST = (MAR) \times (DR) \times (ARF) \times (RF) \times (LPF) \qquad \text{(equation F.8.3-6)}$$

where

ST The source term is the amount of respirable material, in organisms, released to the air.

MAR The material at risk amount of pathogenic materials (e.F., the number of organisms) available to be acted on by a given physical stress. For this analysis, the MAR is the product of the suspension volume (*V*) times the SC. The maximum inventory is provided in the *Pathogen Inventory* (Tetra Tech 2011. The seed stock is not included in the MAR because it is generally a small fraction of the working stock (e.g., 2 mL versus 150 mL) and the frozen form has release fractions that are expected to be orders of magnitude lower. Excluding the frozen seed stock does not significantly affect the results.

DR The damage ratio is the fraction of MAR affected by the conditions under evaluation. A DR of 1, the maximum value, means that the entire inventory is at risk.

ARF The airborne release fraction is the coefficient used to estimate the amount of a material that is suspended in air and made available for airborne transport under a specific set of induced physical stresses. Attachment F provides the basis for selection of the ARF value.

RF The respirable fraction is the fraction of airborne particles that can be transported through air and inhaled into the human respiratory system and is commonly assumed to include particles 10-µm Aerodynamic Equivalent Diameter (AED) and less. Attachment F provides the basis for selection of the RF value.

LPF The Section F.8.2.7 methodology used here does not include mitigation by any biocontainment features that cannot be demonstrated to survive the event. Therefore, the analysis does not include consideration of the HVAC HEPA filtration system,

sealed walls, or an elevated release. This analysis is based on a ground-level release. Assumption of a ground-level release is conservative (i.e., overestimates the exposure) because it results in less vertical mixing. While the LPF does not include any biocontainment features, even a totally collapsed building will provide some confinement. Structural debris is estimated to reduce the rate of release by a factor of 10 (DOE 2000), so the LPF is 0.1.

Substituting $V \times SC$ for MAR, the source term equation becomes

$$ST = [(V) \times (SC)] \times (DR) \times (ARF) \times (RF) \times (LPF) \quad \text{(equation 3-7)}$$

Table F.8-7 provides the details of the source term calculation for all BSL-3 pathogens. Table F.8-7 is applicable for all three sites because the facility and its operations are assumed to be the same for all three sites. Using equation F.8-7 and the RVFV as an example, the calculation is as follows:

$$ST = (150\ \text{mL}) \times (1\text{E+}09\ \text{CCID}_{50}/\text{mL}) \times (1) \times (4\text{E-}05) \times (0.7) \times (1)$$

$$ST = 4.2\text{E+}06\ \text{CCID}_{50}$$

Table F.8-7. Calculated source term for BSL-3 pathogens from an MRF earthquake

Pathogen[a]	BSL[b]	V (mL)[b]	SC (/mL)[b]		DR[b]	ARF[c]	RF[c]	LPF[d]	ST[e]	
B. anthracis[e]	2 / 3	50	$2.4\text{x}10^{8}$	CFU[e]	1	$4\text{x}10^{-5}$	0.7	0.1	$3.4\text{x}10^{4}$	CFU[e]
F. tularensis	3	1	$2.0\text{X}10^{9}$	CFU	1	$4\text{x}10^{-5}$	0.7	0.1	$5.6\ \text{x}10^{3}$	CFU
Y. pestis	3	5	$2.0\text{X}10^{7}$	CFU	1	$4\text{x}10^{-5}$	0.7	0.1	$2.8\ \text{x}10^{2}$	CFU
1918H1N1V	3	150	$1.0\text{X}10^{8}$	PFU	1	$4\text{x}10^{-5}$	0.7	0.1	$4.2\ \text{x}10^{4}$	PFU
SARS-CoV	3	150	$1.0\text{X}10^{7}$	PFU	1	$4\text{x}10^{-5}$	0.7	0.1	$4.2\ \text{x}10^{3}$	PFU
RVFV	3	150	$1.0\text{X}10^{8}$	PFU	1	$4\text{x}10^{-5}$	0.7	0.1	$4.2\ \text{x}10^{4}$	PFU
			$1.0\text{X}10^{9}$	CCID_{50} or MICLD_{50}	1	$4\text{x}10^{-5}$	0.7	0.1	$4.2\ \text{x}10^{5}$	CCID_{50} or MICLD_{50}
ANDV	3 / 4	150	$1.0\text{X}10^{6}$	CCID_{50}	1	$4\text{x}10^{-5}$	0.7	0.1	$4.2\ \text{x}10^{2}$	CCID_{50}

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. This value is taken from the working stock inventory in Section F.3.

c. The maximum value of 1 is used, which puts the entire inventory of that working stock at risk.

d. This is the mean ARF for a 3-m spill, as selected in Attachment F.

e. A value of 0.1 is used to account for the release reduction due to structural debris (DOE 2000).

f. In spore form in suspension.

After release to the air, the source term can be transported and dispersed as a result of winds and meteorological conditions. The inhalation exposure of a member of the public downwind from the release is calculated by equation F.8.3-5 (see Section F.8.3.2.4.3) (DOE 2004a):

$$EX = (ST) \times (\chi/Q) \times (BR)$$

As discussed in Section F.8.2.7, the DOE guidance recommends that, "prevailing (median) meteorological conditions generally should be used." Therefore, median ground-level meteorological conditions are used in these analyses. The ground-level χ/Q values result in pathogen concentrations that are significantly greater (as much as 200 times greater at the nearest annulus) that the corresponding concentrations from the Base Case (i.e., an elevated release). The median meteorological conditions can be different at the various sites, so site-specific meteorological data are used to calculate exposures.

The DOE guidance for use of the MACCS2 code (DOE 2004a) notes that, "like all Gaussian models, MACCS2 is not well suited for modeling dispersion close to the source (less than 100 meters from the source)." While that limitation is noted, the downwind dilution factor is calculated for distances less than 100 m to account for the potentially higher concentrations. Section F.5 provides a comparison of the MACCS2 results with the wind tunnel tests and independent dispersion calculation results performed for NEIDL. That comparison showed that the MACCS2 results are very close to the other results and supports the reasonableness of its use in this analysis.

Tables F.8-8a through F.8-8c are a composite of inputs and results for the urban, suburban, and rural sites. The first four rows of each table present input values used for the calculation, including the BR and the inner, outer, and midpoint χ/Q values for each annulus. The tables also present the exposures for each pathogen for each annulus. The units for the exposure values are the same as the units for the ST values. The tables also present the average segment population for each annulus as calculated in Section F.4. The exposure values are calculated for the plume centerline, which is the highest concentration in the sector, so use of those exposure values for all people in the segment overstate the risk.

Table F.8-8a. Average public exposures to BSL-3 pathogens resulting from an MRF earthquake for the urban site

			Annulus (km)										
			0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[e] and BR inputs:													
BR (m^3/s)			3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner			4.70E-03	4.70E-03	4.27E-04	1.29E-04	6.78E-05	4.31E-05	3.03E-05	2.28E-05	1.79E-05	1.45E-05	1.20E-05
χ/Q (s/m^3)—Outer			4.70E-03	4.27E-04	1.29E-04	6.78E-05	4.31E-05	3.03E-05	2.28E-05	1.79E-05	1.45E-05	1.20E-05	1.02E-05
χ/Q (s/m^3)—Midpoint			4.70E-03	2.56E-03	2.78E-04	9.84E-05	5.55E-05	3.67E-05	2.66E-05	2.04E-05	1.62E-05	1.33E-05	1.11E-05
Pathogen[a]:	**ST[b]**		**Plume Centerline Exposure (same units as ST_{stack})[b]**										
B. anthracis[c]	3.4E+04	CFU[e]	5.3E-02	2.9E-02	3.1E-03	1.1E-03	6.2E-04	4.1E-04	3.0E-04	2.3E-04	1.8E-04	1.5E-04	1.2E-04
F. tularensis	5.6E+03	CFU	8.8E-03	4.8E-03	5.2E-04	1.8E-04	1.0E-04	6.8E-05	5.0E-05	3.8E-05	3.0E-05	2.5E-05	2.1E-05
Y. pestis	2.8E+02	CFU	4.4E-04	2.4E-04	2.6E-05	9.2E-06	5.2E-06	3.4E-06	2.5E-06	1.9E-06	1.5E-06	1.2E-06	1.0E-06
1918H1N1V	4.2E+04	PFU	6.6E-02	3.6E-02	3.9E-03	1.4E-03	7.8E-04	5.1E-04	3.7E-04	2.8E-04	2.3E-04	1.9E-04	1.6E-04
SARS-CoV	4.2E+03	PFU	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
RVFV[d]	4.2E+04	PFU	6.6E-02	3.6E-02	3.9E-03	1.4E-03	7.8E-04	5.1E-04	3.7E-04	2.8E-04	2.3E-04	1.9E-04	1.6E-04
	4.2E+05	$CCID_{50}$ or $MICLD_{50}$	6.6E-01	3.6E-01	3.9E-02	1.4E-02	7.8E-03	5.1E-03	3.7E-03	2.8E-03	2.3E-03	1.9E-03	1.6E-03
ANDV	4.2E+02	$CCID_{50}$	6.6E-04	3.6E-04	3.9E-05	1.4E-05	7.8E-06	5.1E-06	3.7E-06	2.8E-06	2.3E-06	1.9E-06	1.6E-06
Segment population:				31	108	196	372	376	178	165	250	310	215

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The $CCID_{50}$ and $MICLD_{50}$ units are an order of magnitude greater because this measurement is more sensitive.

e. χ/Q values are the Ground Level values presented in Section F.5.

Table F.8-8b. Average public exposures to BSL-3 pathogens resulting from an MRF earthquake for the suburban site

Pathogen	ST_{stack}	Annulus (km)										
		0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[e] and BR inputs:												
	BR (m^3/s)	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
	χ/Q (s/m^3)—Inner	1.72E-02	1.72E-02	1.22E-03	3.39E-04	1.46E-04	7.06E-05	4.96E-05	3.72E-05	3.20E-05	2.35E-05	1.95E-05
	χ/Q (s/m^3)—Outer	1.72E-02	1.22E-03	3.39E-04	1.46E-04	7.06E-05	4.96E-05	3.72E-05	3.20E-05	2.35E-05	1.95E-05	1.65E-05
	χ/Q (s/m^3)—Midpoint	1.72E-02	9.21E-03	7.80E-04	2.43E-04	1.08E-04	6.01E-05	4.34E-05	3.46E-05	2.78E-05	2.15E-05	1.80E-05
Pathogen[a]:	**ST_{stack}[b]**	**Plume Centerline Exposure (same units as ST_{stack})[b]**										
B. anthracis[c]	3.4E+04 CFU	1.9E-01	1.0E-01	8.7E-03	2.7E-03	1.2E-03	6.7E-04	4.9E-04	3.9E-04	3.1E-04	2.4E-04	2.0E-04
F. tularensis	5.6E+03 CFU	3.2E-02	1.7E-02	1.5E-03	4.5E-04	2.0E-04	1.1E-04	8.1E-05	6.5E-05	5.2E-05	4.0E-05	3.4E-05
Y. pestis	2.8E+02 CFU	1.6E-03	8.6E-04	7.3E-05	2.3E-05	1.0E-05	5.6E-06	4.0E-06	3.2E-06	2.6E-06	2.0E-06	1.7E-06
1918H1N1V	4.2E+04 PFU	2.4E-01	1.3E-01	1.1E-02	3.4E-03	1.5E-03	8.4E-04	6.1E-04	4.8E-04	3.9E-04	3.0E-04	2.5E-04
SARS-CoV	4.2E+03 PFU	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
RVFV[d]	4.2E+04 PFU	2.4E-01	1.3E-01	1.1E-02	3.4E-03	1.5E-03	8.4E-04	6.1E-04	4.8E-04	3.9E-04	3.0E-04	2.5E-04
	4.2E+05 $CCID_{50}$ or $MICLD_{50}$	2.4E+00	1.3E+00	1.1E-01	3.4E-02	1.5E-02	8.4E-03	6.1E-03	4.8E-03	3.9E-03	3.0E-03	2.5E-03
ANDV	4.2E+02 $CCID_{50}$	2.4E-03	1.3E-03	1.1E-04	3.4E-05	1.5E-05	8.4E-06	6.1E-06	4.8E-06	3.9E-06	3.0E-06	2.5E-06
Segment population:			0.1	0.4	4.4	0.9	1.2	12.6	3.2	12.2	4.6	11.1

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

e. χ/Q values are the Ground Level values presented in Section F.5.

Table F.8-8c. Average public exposures to BSL-3 pathogens resulting from an MRF earthquake for the rural site

Pathogen	ST_{stack}	Annulus (km)										
		0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[e] and BR inputs:												
BR (m^3/s)		3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner		2.21E-02	2.21E-02	1.50E-03	4.21E-04	1.87E-04	1.18E-04	8.26E-05	6.16E-05	4.81E-05	3.88E-05	3.21E-05
χ/Q (s/m^3)—Outer		2.21E-02	1.50E-03	4.21E-04	1.87E-04	1.18E-04	8.26E-05	6.16E-05	4.81E-05	3.88E-05	3.21E-05	2.71E-05
χ/Q (s/m^3)—Midpoint		2.21E-02	1.18E-02	9.61E-04	3.04E-04	1.53E-04	1.00E-04	7.21E-05	5.49E-05	4.35E-05	3.55E-05	2.96E-05
Pathogen[a]:	**ST_{stack}[b]**	**Plume Centerline Exposure (same units as ST_{stack})[b]**										
B. anthracis[c]	3.4E+04 CFU	2.5E-01	1.3E-01	1.1E-02	3.4E-03	1.7E-03	1.1E-03	8.1E-04	6.1E-04	4.9E-04	4.0E-04	3.3E-04
F. tularensis	5.6E+03 CFU	4.1E-02	2.2E-02	1.8E-03	5.7E-04	2.8E-04	1.9E-04	1.3E-04	1.0E-04	8.1E-05	6.6E-05	5.5E-05
Y. pestis	2.8E+02 CFU	2.1E-03	1.1E-03	9.0E-05	2.8E-05	1.4E-05	9.4E-06	6.7E-06	5.1E-06	4.1E-06	3.3E-06	2.8E-06
1918H1N1V	4.2E+04 PFU	3.1E-01	1.7E-01	1.3E-02	4.3E-03	2.1E-03	1.4E-03	1.0E-03	7.7E-04	6.1E-04	5.0E-04	4.1E-04
SARS-CoV	4.2E+03 PFU	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
RVFV[d]	4.2E+04 PFU	3.1E-01	1.7E-01	1.3E-02	4.3E-03	2.1E-03	1.4E-03	1.0E-03	7.7E-04	6.1E-04	5.0E-04	4.1E-04
	4.2E+05 $CCID_{50}$ or $MICLD_{50}$	3.1E+00	1.7E+00	1.3E-01	4.3E-02	2.1E-02	1.4E-02	1.0E-02	7.7E-03	6.1E-03	5.0E-03	4.1E-03
ANDV	4.2E+02 $CCID_{50}$	3.1E-03	1.7E-03	1.3E-04	4.3E-05	2.1E-05	1.4E-05	1.0E-05	7.7E-06	6.1E-06	5.0E-06	4.1E-06
Segment population:			0.03	1.37	0.18	0.25	0.32	0.4	0.47	0.54	1.55	0.69

a. *Bacillus anthracis* (*B. anthracis*); *Francisella tularensis* (*F. tularensis*); *Yersinia pestis* (*Y. pestis*); 1918 H1N1 influenza virus (1918H1N1V); SARS-associated coronavirus (SARS-CoV); Rift Valley fever virus (RVFV); and Andes virus (ANDV).

b. Stack source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), median cell culture infective dose ($CCID_{50}$), or median mouse intracerebral lethal dose ($MICLD_{50}$) for viruses. Section F.3 provides background information on the methods and units associated with the concentration measurements.

c. In spore form in suspension.

d. Two values are reported for RVFV with different units. The CCID50 and MICLD50 units are an order of magnitude greater because this measurement is more sensitive.

e. χ/Q values are the Ground Level values presented in Section F.5.

F.8.3.4.5 Escaped Animals

The NEIDL could contain a number of infected animals (mammals and arthropods) at any time. The earthquake could damage the enclosures, resulting in the potential escape of infected animals. The animals and the pathogen with which they might be infected are identified in Section F.3. The potential impact of those infected escaped animals is analyzed in RA Chapter 7.

F.8.3.5 Maximum Reasonably Foreseeable Earthquake Affecting BSL-4

The general approach describe in Section F.8.3.4 for the BSL-3 laboratories is also used for the BSL-4 laboratories and is not repeated here. This section describes the event sequence, frequency category, exposure category, and extent of exposure resulting from an earthquake that damages the BSL-4 areas of the facility.

F.8.3.5.1 Event Sequence Description

The seismic design information available (see Section F.8.1.2 and Attachment D of this appendix) does not distinguish between the BSL-3 and BSL-4 areas when addressing the design criteria or seismic capacity, so there is no basis for distinction of the two events, even though it is expected that the BSL-4 has a higher seismic capacity. Because there is no basis for distinguishing between the two areas, this event sequence is also assumed to fail all biocontainment features. As a result, the BSL-3 discussion of the event sequence description (Section F.8.3.4.1) applies for the BSL-4 in its entirety and is not repeated here.

F.8.3.5.2 Frequency Category

The seismic capacity of the BSL-4 laboratories is expected to be greater than the seismic capacity of the BSL-3 laboratories; thus, the frequency should be lower than the frequency for the BSL-3 MRF earthquake. However, the BSL-4 has not been demonstrated to have a great capacity than the BSL-3 nor is the extent of this increased capacity known. Therefore, it is conservatively assumed that the seismic capacity of the BSL-4 laboratories is the same as the capacity of the BSL-3 laboratories and the discussion in Section F.8.3.2.2 and Attachment E are applied directly for the BSL-4 and are not repeated here. As a result, the BSL-4 earthquake event sequence is placed in frequency category C (1 in 10,000 to 1 million years).

F.8.3.5.3 Exposure Category

The exposure category for the laboratory workers is HIGH because all people in all rooms have the potential to be exposed.

The exposure category for the facility workers is HIGH because all people in the facility have the potential to be exposed.

The estimated exposure category for the public is expected to be MODERATE. Aerosol particles could be dispersed beyond 300 m, but concentrations would be extremely low beyond 3 km.

F.8.3.5.4 Extent of Exposure

As discussed in Section F.8.3.4.4, DOE NEPA Guidance (DOE 2002) recommends caution when evaluating events like the MRF earthquake where the impacts with and without the proposed action are indistinguishable because of the magnitude of the unrelated impacts. However, the exposures are estimated for this event because this scenario provides insight into the maximum consequences that could reasonably be expected from operation of the facility. Analysis of this extreme event also provides insight into potential site differences.

F.8.3.5.4.1 Laboratory Worker

If a severe earthquake were to result in total structural failure of the NEIDL, it is unlikely that laboratory workers would survive if they were in the BSL-3 laboratories. Estimation of exposures to workers in a collapsed building provides no insight into worker risk from this event. Therefore, the laboratory workers are assumed to have escaped the building and congregate at the NEIDL exclusion fence for the duration of the release. In such a case, the laboratory workers are no different than any other facility worker and are included in the following exposure estimate.

F.8.3.5.4.2 Facility Worker

If a severe earthquake were to result in total structural failure of the NEIDL, it is unlikely that facility workers inside the facility would survive. Estimation of exposures to workers in a collapsed building provides no insight into worker risk from this event. Therefore, the laboratory workers are assumed to be located at the NEIDL exclusion fence for the duration of the release, as described in Section F.8.3.2.4.2.

For this analysis, it is assumed that facility workers exit the building promptly and congregate at the exclusion fenceline (at least 30 m from the NEIDL). A person located at the exclusion fence is defined as the MEI, so the MEI exposure will be used for laboratory workers. The MEI exposure is calculated in the public exposure section below. There are approximately 300 workers at NEIDL that are present about 25

percent of the year (about 50 weeks at 40 hours per week out of an 8,760 hour year), which results in an average worker population of 75 people.

F.8.3.5.4.3 Public

The calculation of public exposures for the BSL-4 affected release are performed using the same methodology described in Section F.8.3.4.4.3 (i.e., the equation F.8.3-7) with the only difference being the pathogens involved and their inventories. Table F.8-9 provides the source term for the BSL-4 event.

Table F.8-9. Calculated source term for BSL-4 pathogens from an MRF earthquake

Pathogen[a]	BSL[b]	V (mL)[b]	SC (/mL)[bc]		DR[d]	ARF[e]	RF[e]	LPF[f]	ST[c]	
EBOV	4	150	5.0E+07	$CCID_{50}$	1	4E-05	0.7	0.1	2.1E+04	$CCID_{50}$
MARV	4	150	1.0E+07	$CCID_{50}$	1	4E-05	0.7	0.1	4.2E+03	$CCID_{50}$
LASV	4	150	1.0E+07	$TCID_{50}$ or FFU (PFU)	1	4E-05	0.7	0.1	4.2E+03	$TCID_{50}$ or FFU (PFU)
JUNV	4	150	1.0E+07	PFU	1	4E-05	0.7	0.1	4.2E+03	PFU
TBEV-FE	4	150	1.0E+08	MID50	1	4E-05	0.7	0.1	4.2E+04	MID50
NIPV	4	150	2.0E+07	$TCID_{50}$ or PFU	1	4E-05	0.7	0.1	8.4E+03	$TCID_{50}$ or PFU

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. This value is taken from the working stock values of Section F.3.

c. Suspension concentrations and source term values are given in terms of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).

d. The maximum value of 1 is used, which puts the entire inventory of that working stock at risk.

e. This is the mean ARF and RF for a 3-m spill, as selected in Attachment F.

f. A value of 0.1 is used to account for the release reduction because of structural debris (DOE 2000).

Tables F.8-10a through F.8-10c are a composite of inputs and results for the urban, suburban, and rural sites. The first four rows of each table present input values used for the calculation, including the BR and the inner, outer, and midpoint χ/Q values for each annulus. The table also presents the exposures for each pathogen for each annulus. The units for the exposure values are the same as the units for the ST values. The tables also present the average segment population for each annulus as calculated in Section F.4. The exposure values are calculated for the plume centerline, which is the highest concentration in the sector, so use of those exposure values for all people in the segment overstate the risk.

Table F.8-10a. Average public exposures to BSL-4 pathogens resulting from an MRF earthquake for the urban site

			Annulus (km)										
			0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[c] and BR inputs:													
BR (m^3/s)			3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner			4.70E-03	4.70E-03	4.27E-04	1.29E-04	6.78E-05	4.31E-05	3.03E-05	2.28E-05	1.79E-05	1.45E-05	1.20E-05
χ/Q (s/m^3)—Outer			4.70E-03	4.27E-04	1.29E-04	6.78E-05	4.31E-05	3.03E-05	2.28E-05	1.79E-05	1.45E-05	1.20E-05	1.02E-05
χ/Q (s/m^3)—Midpoint			4.70E-03	2.56E-03	2.78E-04	9.84E-05	5.55E-05	3.67E-05	2.66E-05	2.04E-05	1.62E-05	1.33E-05	1.11E-05
Pathogen[a]:	**ST[b]**		**Plume Centerline Exposure (same units as ST_{stack})[b]**										
EBOV	2.1E+04	$CCID_{50}$	3.3E-02	1.8E-02	1.9E-03	6.9E-04	3.9E-04	2.6E-04	1.9E-04	1.4E-04	1.1E-04	9.3E-05	7.8E-05
MARV	4.2E+03	$CCID_{50}$	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
LASV	4.2E+03	$TCID_{50}$ or FFU (PFU)	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
JUNV	4.2E+03	PFU	6.6E-03	3.6E-03	3.9E-04	1.4E-04	7.8E-05	5.1E-05	3.7E-05	2.8E-05	2.3E-05	1.9E-05	1.6E-05
TBEV-FE	4.2E+04	MID_{50}	6.6E-02	3.6E-02	3.9E-03	1.4E-03	7.8E-04	5.1E-04	3.7E-04	2.8E-04	2.3E-04	1.9E-04	1.6E-04
NIPV	8.4E+03	$TCID_{50}$ or PFU	1.3E-02	7.2E-03	7.8E-04	2.8E-04	1.6E-04	1.0E-04	7.4E-05	5.7E-05	4.5E-05	3.7E-05	3.1E-05
Segment population:				31	108	196	372	376	178	165	250	310	215

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. The source term and exposure values are given in terms of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).b Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), or median cell culture infective dose ($CCID_{50}$). Section F.3 provides background information on the methods and units associated with the concentration measurements.

c. χ/Q values are the Ground Level values presented in Section F.5.

Table F.8-10b. Average public exposures to BSL-4 pathogens resulting from an MRF earthquake for the suburban site

Pathogen	ST_{stack}	Annulus (km) 0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[c] and BR inputs:												
	BR (m^3/s)	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
	χ/Q (s/m^3)—Inner	1.72E-02	1.72E-02	1.22E-03	3.39E-04	1.46E-04	7.06E-05	4.96E-05	3.72E-05	3.20E-05	2.35E-05	1.95E-05
	χ/Q (s/m^3)—Outer	1.72E-02	1.22E-03	3.39E-04	1.46E-04	7.06E-05	4.96E-05	3.72E-05	3.20E-05	2.35E-05	1.95E-05	1.65E-05
	χ/Q (s/m^3)—Midpoint	1.72E-02	9.21E-03	7.80E-04	2.43E-04	1.08E-04	6.01E-05	4.34E-05	3.46E-05	2.78E-05	2.15E-05	1.80E-05
Pathogen[a]:	**ST_{stack}[b]**	**Plume Centerline Exposure (same units as ST_{stack})[b]**										
EBOV	2.1E+04 $CCID_{50}$	1.2E-01	6.4E-02	5.5E-03	1.7E-03	7.6E-04	4.2E-04	3.0E-04	2.4E-04	1.9E-04	1.5E-04	1.3E-04
MARV	4.2E+03 $CCID_{50}$	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
LASV	4.2E+03 $TCID_{50}$ or FFU (PFU)	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
JUNV	4.2E+03 PFU	2.4E-02	1.3E-02	1.1E-03	3.4E-04	1.5E-04	8.4E-05	6.1E-05	4.8E-05	3.9E-05	3.0E-05	2.5E-05
TBEV-FE	4.2E+04 MID_{50}	2.4E-01	1.3E-01	1.1E-02	3.4E-03	1.5E-03	8.4E-04	6.1E-04	4.8E-04	3.9E-04	3.0E-04	2.5E-04
NIPV	8.4E+03 $TCID_{50}$ or PFU	4.8E-02	2.6E-02	2.2E-03	6.8E-04	3.0E-04	1.7E-04	1.2E-04	9.7E-05	7.8E-05	6.0E-05	5.0E-05
Segment population:			0.1	0.4	4.4	0.9	1.2	12.6	3.2	12.2	4.6	11.1

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. The source term and exposure values are given in terms of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).b Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), or median cell culture infective dose ($CCID_{50}$). Section F.3 provides background information on the methods and units associated with the concentration measurements.

c. χ/Q values are the Ground Level values presented in Section F.5.

Table F.8-10c. Average public exposures to BSL-4 pathogens resulting from an MRF earthquake for the rural site

Pathogen	ST_{stack}	Annulus (km)										
		0.03 (MEI)	0.03–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
χ/Q[c] and BR inputs:												
BR (m^3/s)		3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04	3.33E-04
χ/Q (s/m^3)—Inner		2.21E-02	2.21E-02	1.50E-03	4.21E-04	1.87E-04	1.18E-04	8.26E-05	6.16E-05	4.81E-05	3.88E-05	3.21E-05
χ/Q (s/m^3)—Outer		2.21E-02	1.50E-03	4.21E-04	1.87E-04	1.18E-04	8.26E-05	6.16E-05	4.81E-05	3.88E-05	3.21E-05	2.71E-05
χ/Q (s/m^3)—Midpoint		2.21E-02	1.18E-02	9.61E-04	3.04E-04	1.53E-04	1.00E-04	7.21E-05	5.49E-05	4.35E-05	3.55E-05	2.96E-05
Pathogen[a]:	**ST_{stack}[b]**	**Plume Centerline Exposure (same units as ST_{stack})[b]**										
EBOV	2.1E+04 $CCID_{50}$	1.5E-01	8.3E-02	6.7E-03	2.1E-03	1.1E-03	7.0E-04	5.0E-04	3.8E-04	3.0E-04	2.5E-04	2.1E-04
MARV	4.2E+03 $CCID_{50}$	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
LASV	4.2E+03 $TCID_{50}$ or FFU (PFU)	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
JUNV	4.2E+03 PFU	3.1E-02	1.7E-02	1.3E-03	4.3E-04	2.1E-04	1.4E-04	1.0E-04	7.7E-05	6.1E-05	5.0E-05	4.1E-05
TBEV-FE	4.2E+04 MID_{50}	3.1E-01	1.7E-01	1.3E-02	4.3E-03	2.1E-03	1.4E-03	1.0E-03	7.7E-04	6.1E-04	5.0E-04	4.1E-04
NIPV	8.4E+03 $TCID_{50}$ or PFU	6.2E-02	3.3E-02	2.7E-03	8.5E-04	4.3E-04	2.8E-04	2.0E-04	1.5E-04	1.2E-04	9.9E-05	8.3E-05
Segment population:			0.03	1.37	0.18	0.25	0.32	0.4	0.47	0.54	1.55	0.69

a. Ebola virus (EBOV); Marburg virus (MARV); Lassa virus (LASV); Junín virus (JUNV); tick-borne encephalitis virus, Far Eastern sub-type, formerly known as tick-borne encephalitis complex (Russian spring-summer encephalitis virus) (TBEV-FE); and Nipah virus (NIPV).

b. The source term and exposure values are given in terms of plaque forming units (PFU), median tissue culture infective dose ($TCID_{50}$), fluorescent focus units (FFU), median cell culture infective dose ($CCID_{50}$), or median mouse infective dose (MID_{50}).b Source term and exposures are given in terms of colony forming units (CFU) for bacteria and plaque forming units (PFU), or median cell culture infective dose ($CCID_{50}$). Section F.3 provides background information on the methods and units associated with the concentration measurements.

c. χ/Q values are the Ground Level values presented in Section F.5.

F.8.3.5.5 Escaped Animals

The NEIDL might contain a number of infected animals (mammals and arthropods) at any time. The earthquake could damage the enclosures, resulting in the potential escape of infected animals. The animals and the pathogen with which they might be infected are identified in Section F.3. The potential impact of those infected escaped animals is analyzed in RA Chapter 7.

F.8.3.6 Variability and Uncertainty

Numerous variabilities and uncertainties apply to the results presented in Sections F.8.3.1 through F.8.3.5. Table F.8-11 identifies the key variabilities and uncertainties, provides a discussion of each, and provides an estimate of the potential effect of each.

Table F.8-11. Summary of key variabilities and uncertainties

Variability/ uncertainty	Discussion	Potential factor[a]
Frequency category	*BDB earthquake*—The design has been demonstrated by analysis to survive the 0.12 g event, but the maximum acceleration it can withstand or the extent of damage at any point beyond is uncertain. The BDB earthquake would have a frequency slightly below the frequency for the 0.12 g event.	Unknown
	MRF earthquake—A complete structural failure is highly unlike at accelerations just beyond the design point, but the point at which complete structural failure occurs is not known, but failure is expected to be beyond 0.12 g.	Conservatism (potentially large)
MAR	The MAR was developed on the basis of typical volume and concentration expected according to literature reviews. At any time, not all pathogens will be used in active research. For those used in active research, the volumes will vary from volumes less than to perhaps 3-1/3 larger than the typical volume	Potential conservatism or non-conservatism (0x to 3-1/3x)
	The frozen seed stock was excluded from consideration because (1) it is almost always secured in freezers, which would impede release; and (2) the release fraction for frozen liquids is not provided or discussed in DOE-STD-3010 (DOE 2000), but it is expected to be orders of magnitude lower than the release fractions for liquids.	Slight non-conservatism
DR	The entire MAR is not likely to be involved in an earthquake release, but the exact DR is uncertain. Even in a catastrophic building failure, individual containers in a room might not be damaged or compromised and, if compromised, not all the material they contain will necessarily be released. DR actually ranges from 0 to 1.	Conservatism (0x to 1x)
Release phenomenon	*BDB earthquake*—The release is modeled as the release from a *smashed flask*. Because NEIDL requires use of non-breaking containers, this release mechanism is conservative.	Conservatism (potentially large)
	MRF earthquake—The release is modeled as a 3-m, free-fall spill. There are multiple different types of phenomena that could result, but this was selected because it results in a larger release than other expected phenomena (see Attachment F of this appenidx). The actual phenomena that would occur are uncertain but are unlikely to result in a higher release.	Reasonable estimate

Variability/ uncertainty	Discussion	Potential factor[a]
ARF	*BDB*—The release factors are good estimates for the phenomena selected, so the conservatism is in the phenomena selection.	Reasonable estimate
	MRF earthquake—The release phenomenon selected is addressed above, and it largely determines the ARF. However, both bounding and mean values are available for the spill phenomena. The mean ARF was selected using the guidance for such analyses (ANSI 2004). Use of the bounding value would increase the release by a factor of 5 (4.00E-05 mean versus 2.00E-04 bounding per Attachment F of this appendix) if the bounding release value were used. The ARF is based on a 3-m, free-fall spill. A spill would likely be from a lower height, perhaps 1 m, which would reduce the potential energy to 1/3 and would reduce the ARF by an unknown amount.	Unknown
RF	*BDB earthquake*—The ARF and RF values are assumed to be integrated into one value in the Bennett and Parks (2006) data. The aerosol particle size is not reported.	Unknown
	MRF earthquake—The RF value was selected on the same basis used for the ARF. There is uncertainty in the actual RF, and it could even be pathogen dependent (e.g., the tendency of organisms to agglomerate can differ). The RF value paired with the bounding ARF is actually less than the value paired with the mean value (i.e., 0.7 versus 0.5 per Attachment F of this appendix). The RF is based on a 3-m, free-fall spill. A spill would likely be from a lower height, perhaps 1 m, which would reduce the potential energy to 1/3 and would reduce the RF by an unknown amount.	Unknown (potentially 0.7x)
LPF	*BDB earthquake*—The LLNL data (LLNL 1994), there was no observed release following the event. Therefore, use of the factor of 10 increase in the release for each HEPA filter, the estimates could be an order of magnitude high for each HEPA filter. Because the BSL-4 has two HEPA filters in series, it could be a factor of 100 high.	Conservatism (potentially 10x for BSL-3 and 100x for BSL-4)
	MRF earthquake—The LPF of 0.1 is based on structural debris. A large pile of debris, which would result from the catastrophic failure of a building the size of NEIDL with multiple internal walls, could result in a lower LPF.	Conservatism (potentially orders of magnitude)
χ/Q	*Decay*—Pathogens exposed to the outdoor environment would be expected to undergo some biological decay (i.e., inactivation). The extent of this inactivation depends on the pathogen and the environmental conditions. Inactivation was not included in this analysis.	Conservatism (generally minor magnitude)
	Washout and plume meander—The airborne dispersion calculations did not include washout due to precipitation or plume meander, which would reduce exposures. These are both conservatisms, the magnitude of which is dependent on the conditions at the time.	Conservatism (unkown magnitude)
	BDB earthquake—The elevated release with median dispersion provides the expected results. Actual conditions could result in greater dispersion (lower concentrations) or less dispersion (greater concentrations).	Potentially conservative or non-conservative
	MRF earthquake—A ground-level release is assumed for this analysis consistent with the DOE guidance. An elevated release would result in a decrease in the concentration, especially at the very closest distances. If the building remains standing, which would be the case for all but the most severe failure case, this is a large conservatism. Concentrations resulting from an elevated release are distance dependent but can be 1 to 2 orders of magnitude lower for locations very close to the point of release (Tetra Tech 2011b).	Conservatism (0.01x to 1x)
	MRF earthquake—Median meteorology values were used, per DOE recommendation (DOE 2002). The concentrations would be about 9 times higher if the 95% conditions were used instead of the 50%. Use of the ground-level release values has a greater effect than use of 95% meteorology for locations near the point of release (Tetra Tech 2011b).	Potentially conservative or non-conservative

Variability/ uncertainty	Discussion	Potential factor[a]
	MRF earthquake-χ/Q was calculated via the MACCS2 computer code, which is a DOE-approved code for this type of analysis. Alternate codes would have provided different values. A direct comparison of the MACCS2 calculations with another code is not available, but a general comparison was performed and it is concluded that the results are very close (Tetra Tech 2011b).	unknown
BR	The generally accepted and recommended BR is used, but the BR varies depending on the level of activity involved. Rather than the 3.33×10^{-4} m3/s value used, the rate could range from 1.25×10^{-4} m3/s for sleep to 8.33×10^{-4} m3/s for heavy exercise (DOE 2004a). It could vary depending on the size, age, etc., of the person potentially exposed.	0.38x to 2.5x

a. The estimate provided in the analyses (Sections F.8.3.2 through F.8.3.5) would be multiplied by this value to obtain the new value. So a current value of 10 when multiplied by a potential factor of 10x would result in 100, and a current value of 10 when multiplied by a potential factor of 0.1x would result in 1. A 0x factor means that the result could be 0.

As shown in Table F.8-11, numerous variabilities and uncertainties could affect the results. Overall, the results of this analysis are judged to be conservative for the BDB (minor damage) earthquake and very conservative for the MRF (total collapse) earthquake (i.e., an event of that type would likely result in considerably lower exposures).

While the variabilities and uncertainties are potentially quite large, especially for the MRF earthquake, they do not invalidate the conclusions regarding site differences because the analyses are performed were the same for all three sites. Therefore, the variabilities and uncertainties would merely tend to shift the results for all three sites similarly.

F.9 AIRCRAFT CRASH

F.9.1 Introduction

An aircraft crash into the NEIDL is a postulated externally initiated accident scenario for the potential release of pathogens from the NEIDL facility to the public. This analysis demonstrates that the risk of an aircraft crash is bounded by the risk of other analyzed accident scenarios, namely the MRF (total collapse) earthquake event, and therefore does not necessitate a detailed analysis. Risk is a function of (1) the frequency of an adverse event and (2) the consequences of the adverse event. Thus, the frequency and consequence of a postulated aircraft crash scenario will be compared to that of the MRF earthquake.

This scenario involves an accidental aircraft crashing into the NEIDL facility resulting in an aerosol release of a pathogen and potentially exposing the public. Only the Boston site is analyzed because Boston Logan International Airport has a far more flights than the municipal airports nearby the two comparable sites and, therefore, a higher anticipated frequency for such an event. An aircraft crash

scenario initiated by a malevolent act is not specifically addressed by this section; however, the consequences of an aircraft crash initiated by a malevolent act would be comparable to the consequence addressed below.

F.9.2 Methodology

The DOE has detailed guidance for estimating the frequency of an aircraft crash probability at a given location, as opposed to crash frequencies on a per flight basis. The DOE guidance, *Accident Analysis for Aircraft Crash into Hazardous Facilities* (DOE-STD-3014-2006, DOE 2006), was used in this evaluation.

DOE 2006 uses a four-factor formula to estimate the annual aircraft crash frequency at a location. DOE 2006 cautions,

> It should be noted that there is uncertainty associated with the frequency estimates produced using the four-factor formula, caused by the need to model complex physical processes using parameters that are based on limited historical data. Experience-based judgments have been made as needed to supplement historical data, introducing additional uncertainties. This standard does not provide a quantitative estimate of the uncertainties involved; rather, the mathematical formulations and supporting parameter estimates have been made so as to provide a reasonable point estimate of the frequency of aircraft crash impacts into specified facilities.

The estimates provided by the DOE guidance are considered adequate for this screening assessment. The four factors are (1) the number of aircraft operations; (2) the probability that an aircraft will crash; (3) given a crash, the probability that the aircraft will crash into a one square mile (mi^2) area where a facility is located; and (4) the size of the facility. This is expressed mathematically as follows:

$$F = \sum_{i,j,k} N_{ijk} \times P_{ijk} \times f_{ijk}(x,y) \times A_{ij} \qquad \text{[equation F.9-1]}$$

where

- F Estimated annual aircraft crash impact frequency for the facility of interest (number/year)
- N_{ijk} Annual number of site-specific airport operations (i.e., takeoffs, landings, in-flights) for each applicable summation parameter (number/year)
- P_{ijk} Aircraft crash rate per takeoff or landing for near-airport phases and per flight for the in-flight (non-airport) phase of operation for each applicable summation parameter
- f_{ijk} (x,y) Aircraft crash location conditional probability (per square mile) given a crash evaluated at the facility location for each applicable summation parameter

	A_{ij}	Site-specific effective area for the facility of interest that includes skid and fly-in effective areas (square miles) for each applicable summation parameter, aircraft category or subcategory, and flight phase for military aviation
	i	(index for flight phases): i = 1, 2, and 3 (takeoff, in-flight, and landing)
	j	(index for aircraft category or subcategory): j = 1, 2,…, 11
	k	(index for flight source): k = 1, 2,…, k (there could be multiple runways, and non-airport operations)

F.9.3 Results

F.9.3.1 Frequency

An airport *operation* can include more than takeoffs and landings; for example, it can include such activities as an aircraft contacting the tower for a change of vector. For the purposes of this analysis, airport operations will be conservatively assumed to only mean takeoffs and landings (i.e, parameter i = 1 and 3 in the equation above). The definition of airport operations from DOE 2006 is, "As defined by the Federal Aviation Administration (FAA), the number of arrivals and departures from the airport at which the airport traffic control tower is located." Therefore, in-flight (non-airport operations, parameter i = 2) are discussed later because they do not meet that definition.

F.9.3.1.1 Airport Operation Frequency Estimation

Each of the four factors in equation F.9-1 is discussed below for airport operations.

N_{ijk} (Number of airport operations)

Data for number of airport operations at the Boston Logan International Airport were obtained from the US. Department of Transportation Federal Aviation Administration's Airport Master Record (FAA 2010). Data from that website states that a total of 208,494 operations involving air carriers, 113,175 operations involving air taxis, and 15,560 general aircraft operations occurred during the most recently reported 12 months (FAA 2010). The data do not discriminate between takeoffs and landings, so the conservative assumption that 50 percent of all operations are takeoffs and 50 percent are landings is used (DOE-STD-3014-2006, p. 40). That assumption results in "very conservative numbers because total operations include activities other than takeoff and landing, such as an aircraft contacting the tower for a change of vector" (DOE 2006). As stated above non-airport operations are not included here because they do not meet the definition of *airport operation*; the annual crash frequency of non-airport operations is discussed later in this document. The data included in the calculation for airport operations are in Table F.9-1.

Table F.9-1. Number of airport operations and crash rates

Aircraft operation	$\sum N_{ijk}$ (Number of operations per year)	P (Crash rate[a])
Commercial aviation -air carrier (takeoff)	104,247	1.90×10^{-7}
Commercial aviation—air carrier landing)	104,247	2.80×10^{-7}
Commercial aviation—air taxi (takeoff)	56,587.5	1.00×10^{-6}
Commercial aviation—air taxi (landing)	56,587.5	2.30×10^{-6}
General aviation (takeoff)	7,780	1.10×10^{-5}
General Aviation (landing)	7,780	2.00×10^{-5}

a. Table B-1 of DOE STD 3014-2006 (p. B-3)

P_{ijk} (Aircraft Crash Rate per Takeoff, Landing, and In-flight)

For airport operations (takeoff and landing), the value of P, Crash rate, is taken from Table B-1 of DOE-STD-3014-2006 (p. B-2). Those values are shown in Table F.9-1.

A – Effective Area

The effective area is calculated using the methodology in Section B.4 of DOE-STD-3014-2006. Specifically, three equations are used:

A_{eff} = Effective Fly-in Area plus Effective Skid Area = $A_f + A_s$

$A_f = (WS + R) \times H \cot\varphi + 2 \times (L \times W \times WS) / R + L \times W$

$A_s = (WS + R) \times S$

where

R	$(L^2+W^2)^{0.5}$ = diagonal length of facility
L	Length of facility = 120 ft (Payne 2009)
W	Width of facility = 226 ft (Payne 2009)
cot φ	Mean of the cotangent of the aircraft impact angle (Table B-17, of DOE-STD-3014-2006, p. B-29)
S	Mean aircraft skid distance (Table B-18, of DOE-STD-3014-2006, p. B-29)
WS	Aircraft wingspan (provided in Table B-16, of DOE-STD-3014-2006, p. B-28)
H	Facility height = 139 ft (Payne 2009)

The calculation of effective area for each aircraft type is given in Table F.9-2.

Table F.9-2. Effective area calculation

Variable	Commercial air carrier	Commercial air taxi	General aviation
WS (ft)[a]	98	59	50
L (ft)	120	120	120
W (ft)	226	226	226
H (ft)	139	139	139
R (ft)	255.88	255.88	255.88
cot (Φ)*	10.2	10.2	8.2
S (ft)*	1,440	1,440	60
A_f (mi^2)	1.97×10^{-2}	1.74×10^{-2}	1.39×10^{-2}
A_s (mi^2)	1.83×10^{-2}	1.63×10^{-2}	6.58×10^{-4}
Aeff (mi^2)	3.80×10^{-2}	3.37×10^{-2}	1.45×10^{-2}

a. From Tables B-16, B-17, and B-18, respectively, of DOE STD 3014-2006

$F_{ijk}(x,y)$ (Conditional Probability per Square Mile)

For airport operations, the conditional probabilities were taken from Tables B-2 through B-5 of DOE-STD-3014-2006 (p. B-12 to B-15). To use these tables, the standard specifies that the orthnormal distances (Cartesian distance in both the x and y directions) must be determined from the facility's closest point to the center of each runway. As described in the standard (p. B-4), the "x axis coincides with the extended runway centerline; the positive direction is the direction of flight. The y axis is perpendicular to the x axis with the positive direction created by a 90-degree counterclockwise rotation of the positive x axis." There are multiple runways at Boston Logan International Airport, but only four (4) different directions in which the various runways are oriented: 150°, 220°, 270°, and 320°. The runway number is "approximately one-tenth of the angle that the extended runway direction makes with magnetic north" (DOE 2006). Thus runways labeled 22 equates to 220°. Also note that runways can use multiple numbers: 4/22, equating to 40° and 220°; those are identical because they are 180° out of phase. The distance from the NEIDL facility to intersection of two runways was determined to be approximately as 3.75 miles at an angle of 59°.

DOE 2006 gives two relationships to determine x and y:

$$x = -R \cos(\Theta - \Phi)$$

and

$$y = R \sin(\Theta - \Phi)$$

where

R = distance from the facility (miles) = 3.75

Θ = bearing from the facility to the airport = 59°

Φ = runway bearing as an angle with respect to magnetic north (runway number times ten) = 150°, 220°, and 270°

Thus x and y are calculated for each runway, and the results are shown in Table F.9-3:

Table F.9-3. Distances from NEIDL to each Boston Logan runway

Φ (runway bearing, degrees)	x (miles)	y (miles)
150°	0.07	-3.75
220°	3.55	-1.22
270°	3.21	1.93
320°	0.59	3.70

Once the x and y direction distances were determined, Tables B-2 and B-5 of DOE-STD-3014-2006 were used to look up values of F(x,y); results are shown in the tables below for each runway. By inspection runway 27 (270°) should yield the highest crash probabilities because it is the closest direction to the line from NEIDL to/from the airport. The results of Table F.9-1 are input into Tables F.9-4 through F.9-7, which provide confirm that runway yields the highest crash probabilities.

Table F.9-4. Aircraft crash frequency calculations for airport operations—Runway 15 (150°).

Aircraft operation	$\sum N_{ijk}$ (Number of operations per year)	P (Crash rate[a])	X (Distance) (mi)	Y (Distance) (mi)	f(x,y) (Aircraft crash location probability[b])	A_{eff} (Effective area [mi^2])	F (Impact frequency per year)
Commercial aviation—air carrier (takeoff)	104,247	1.90×10^{-7}	0.07	-3.75	4.50×10^{-5}	3.80×10^{-2}	3.39×10^{-8}
Commercial aviation—air carrier (landing)	104,247	2.80×10^{-7}	-0.07	3.75	$0.00 \times 10^{+0}$	3.80×10^{-2}	$0.00 \times 10^{+0}$
Commercial aviation—air taxi (takeoff)	56,587.5	1.00×10^{-6}	0.07	-3.75	4.50×10^{-5}	3.37×10^{-2}	8.58×10^{-8}
Commercial aviation—air taxi (landing)	56,587.5	2.30×10^{-6}	-0.07	3.75	$0.00 \times 10^{+0}$	3.37×10^{-2}	$0.00 \times 10^{+0}$
General aviation (takeoff)	7,780	1.10×10^{-5}	0.07	-3.75	1.80×10^{-4}	1.45×10^{-2}	2.23×10^{-7}
General aviation (landing)	7,780	2.00×10^{-5}	-0.07	3.75	7.60×10^{-4}	1.45×10^{-2}	1.71×10^{-6}
Total crash frequency (per year)							**2.06×10^{-6}**

a. Table B-1 of DOE STD 3014-2006 (p. B-3)
b. Tables B-2 through B-5 of DOE STD 3014-2006(p. B-12 to B-15)

Table F.9-5. Aircraft crash frequency calculations for airport operations—Runway 22 (220°).

Aircraft Operation	$\sum N_{ijk}$ (Number of operations per year)	P (Crash rate[a])	X (Distance) (mi)	Y (Distance) (mi)	f(x,y) (Aircraft crash location probability[b])	A_{eff} (Effective area [mi^2])	F (Impact frequency per year)
Commercial aviation—air carrier (takeoff)	104,247	1.9×10^{-7}	3.55	-1.22	2.6×10^{-3}	3.80×10^{-2}	1.96×10^{-6}
Commercial aviation—air carrier (landing)	104,247	2.8×10^{-7}	-3.55	1.22	4.3×10^{-4}	3.80×10^{-2}	4.77×10^{-7}
Commercial aviation—air taxi (takeoff)	56,587.5	1.0×10^{-6}	3.55	-1.22	2.6×10^{-3}	3.37×10^{-2}	4.96×10^{-6}
Commercial aviation—air taxi (landing)	56,587.5	2.3×10^{-6}	-3.55	1.22	4.3×10^{-4}	3.37×10^{-2}	1.89×10^{-6}
General aviation (takeoff)	7,780	1.1×10^{-5}	3.55	-1.22	5.2×10^{-4}	1.45×10^{-2}	6.45×10^{-7}
General aviation (landing)	7,780	2.0×10^{-5}	-3.55	1.22	1.1×10^{-3}	1.45×10^{-2}	2.48×10^{-6}
Total crash frequency (per year)							**1.24×10^{-5}**

a. Table B-1 of DOE STD 3014-2006 (p. B-3)
b. Tables B-2 through B-5 of DOE STD 3014-2006(p. B-12 to B-15)

Table F.9-6. Aircraft crash frequency calculations for airport operations—Runway 27 (270°)

Aircraft operation	$\sum N_{ijk}$ (Number of operations per year)	P (Crash rate[a])	X (Distance) (mi)	Y (Distance) (mi)	f(x,y) (Aircraft crash location probability[b])	A_{eff} (Effective area [mi^2])	F (Impact frequency per year)
Commercial aviation—air carrier (takeoff)	104,247	$1.9x10^{-7}$	0.07	1.93	$2.6x10^{-3}$	$3.80x10^{-2}$	$3.39x10^{-8}$
Commercial aviation—air carrier (landing)	104,247	$2.8x10^{-7}$	-0.07	3.75	$4.3x10^{-4}$	$3.80x10^{-2}$	$0.00x10^{+0}$
Commercial aviation—air taxi (takeoff)	56,587.5	$1.0x10^{-6}$	0.07	1.93	$2.6x10^{-3}$	$3.37x10^{-2}$	$8.58x10^{-8}$
Commercial aviation—air taxi (landing)	56,587.5	$2.3x10^{-6}$	-0.07	3.75	$4.3x10^{-4}$	$3.37x10^{-2}$	$0.00x10^{+0}$
General aviation (takeoff)	7,780	$1.1x10^{-5}$	3.21	1.93	$2.0x10^{-3}$	$1.45x10^{-2}$	$2.48x10^{-6}$
General aviation (landing)	7,780	$2.0x10^{-5}$	-3.21	-1.93	$1.0x10^{-3}$	$1.45x10^{-2}$	$2.26x10^{-6}$
Total crash frequency (per year)							**$1.40X10^{-5}$**

a. Table B-1 of DOE STD 3014-2006 (p. B-3)

b. Tables B-2 through B-5 of DOE STD 3014-2006(p. B-12 to B-15)

Table F.9-7. Aircraft crash frequency calculations for airport operations—Runway 32 (320°)

Aircraft operation	$\sum N_{ijk}$ (Number of operations per year)	P (Crash rate[a])	X (Distance) (mi)	Y (Distance) (mi)	f(x,y) (Aircraft crash location probability[b])	A_{eff} (effective area [mi^2])	F (Impact frequency per year)
Commercial aviation—air carrier (takeoff)	104,247	$1.9x10^{-7}$	0.59	3.70	$4.5x10^{-5}$	$3.80x10^{-2}$	$3.39x10^{-8}$
Commercial aviation—air carrier (landing)	104,247	$2.8x10^{-7}$	-0.59	-3.70	$0.0x10^{+0}$	$3.80x10^{-2}$	$0.00x10^{+0}$
Commercial aviation—air taxi (takeoff)	56,587.5	$1.0x10^{-6}$	0.59	3.70	$4.5x10^{-5}$	$3.37x10^{-2}$	$8.58x10^{-8}$
Commercial aviation—air taxi (landing)	56,587.5	$2.3x10^{-6}$	-0.59	-3.70	$0.0x10^{+0}$	$3.37x10^{-2}$	$0.00x^{+0}$
General aviation (takeoff)	7,780	$1.1x10^{-5}$	0.59	3.70	$1.8x10^{-4}$	$1.45x10^{-2}$	$2.23x10^{-7}$
General aviation (landing)	7,780	$2.0x10^{-5}$	-0.59	-3.70	$3.9x10^{-4}$	$1.45x10^{-2}$	$8.80x10^{-7}$
Total crash frequency (per year)							**$1.22x10^{-6}$**

a. Table B-1 of DOE STD 3014-2006 (p. B-3)

b. Tables B-2 through B-5 of DOE STD 3014-2006(p. B-12 to B-15)

F.9.3.1.2 Non-Airport Operations Crash Frequency Estimation

Non-airport operations (e.g., in-flight, parameter i, from four factor formula = 2) crash frequency is also estimated using the four-factor formula, however the first three terms are combined into one term, $N_{Pf}(x,y)$, for the estimated number of crashes per square mile per year (mi^2/yr). This value is taken from Tables B-14 and B-15 of DOE-STD-3009-2006, depending on the type of aircraft operations, conservatively using the maximum values listed. To determine the impact frequency into the facility, one simply multiplies these values by the effective area discussed above. The calculation is shown in Table F.9-8 with the resulting total of 4.38×10^{-5} per year. It should be noted that general aviation flights by far dominate this non-airport risk. General aviation planes include small planes that are unlikely to penetrate the NEIDL walls.

Table F.9-8. Non-airport operations crash frequency calculations

Type of aircraft	NPf(x,y) number of crashes per sq mile per year[a]	A_{eff} effective area (mi^2)	Non-airport crash frequency per year [NPf(x,y) × A_{eff}]
Commercial aviation—air carrier	2.00×10^{-6}	3.80×10^{-2}	7.60×10^{-8}
Commercial aviation—air taxi	8.00×10^{-6}	3.37×10^{-2}	2.70×10^{-7}
General aviation	3.00×10^{-3}	1.45×10^{-2}	4.35×10^{-5}
	Total crash frequency (per year)		4.38×10^{-5}

a. Maximum values from Tables B-14 and B-15 of DOE STD-3014-2006

F.9.3.1.3 Total Aircraft Crash Frequency

The resulting total estimated aircraft crash frequency is just the summation of the calculated frequencies for airport operations assuming all flights are associated with that runway plus non-airport operations. This is summarized for each of the runways in Table F.9-9 below:

Table F.9-9. Total annual crash frequency for each Boston Logan runway

Runway direction (degrees)	Total crash frequency for airport operations (/year)	Total crash frequency for non-airport operations (/year)	Total crash frequency (/year)
150°	2.06×10^{-6}	4.38×10^{-5}	4.6×10^{-5}
220°	1.24×10^{-5}	4.38×10^{-5}	5.6×10^{-5}
270°	1.40×10^{-5}	4.38×10^{-5}	5.8×10^{-5}
320°	1.22×10^{-6}	4.38×10^{-5}	4.5×10^{-5}

Thus, as expected, the largest contributor is runway 270 with a total crash frequency of approximately 6×10^{-5}/yr. The frequency of 6×10^{-5}/yr (return period of ~16,700 years) places the aircraft crash into frequency category C (1 in 10,000 to 1 million years). However, that is the frequency of an aircraft crashing into NEIDL and it does not take into account the conditional probability of conditions that must

exist for a pathogen release to occur. For an aircraft crash to result in a pathogen release, the following conditions are necessary:

1. The aircraft crashing must have sufficient energy (speed and mass of a projectile) to penetrate the building. It is not known which aircraft would be capable of penetrating the walls of the building exterior and the laboratory walls (BSL-3 or BSL-4), but not all are likely to be capable of penetrating both walls. That is especially true of the general aviation flights, which dominate the frequency for non-airport operations, because they include small planes.
2. The angle of impact must be sufficiently perpendicular for penetration to result. Impacts at lower angles can result in the aircraft ricocheting off the building, or hitting with a grazing blow, without penetrating the interior laboratory spaces.
3. The impact must be at a location that results in a pathogen release. An aircraft impact into administrative areas is not likely to result in a pathogen release. The BSL-3 and BSL-4 areas compose 29 percent of the facility floor space (13 percent BSL-3 and 16 percent BSL-4) (BUMC 2008a). The laboratory rooms will contain pathogens in a releasable form (i.e., a liquid suspension) for only a fraction of the time. Rooms where pathogens are stored are not in an easily releasable form (i.e., frozen).

On the basis of the conditional probabilities, the frequency of an aircraft crash that results in a pathogen release is judged to be in frequency category C (1 in 10,000 to 1 million years), but it is in the low-frequency (high return period) end of that category. The MRF earthquake scenario is also in frequency category C (1 in 10,000 to 1 million years). However, given the multiple extreme conservative assumptions for the calculation of the aircraft crash frequency (e.g., all planes take off and land in the direction to/from NEIDL) and the conditional probabilities stated above, it is judged that that aircraft crash frequency is considered comparable to or lower than the MRF earthquake frequency.

F.9.3.2 Consequences

While a structural evaluation of aircraft crash per the guidance of DOE-STD-3014-2006 has not been performed, the consequences (i.e., the number and extent of potential exposures) to the public are qualitatively judged to be less than that of an MRF earthquake event for the following reasons:

1. The MRF earthquake event assumes total collapse of the NEIDL building [i.e., all available material at risk (MAR) has the potential to be release and appropriate release factors applied to the entire inventory]. An aircraft crashing into the building will likely only impact the immediate portion of the building that is involved at point of impact, and to a lesser degree, the surrounding areas. That means that only a portion of the available MAR has the potential to be released in a

crash. In the unlikely event of a total facility collapse after an aircraft collision (similar to that of the September 11, 2001, terrorist strikes on the World Trade Center and the Pentagon) could impact all the available MAR. In such a case, the amount of MAR impacted would be equal to, but not exceed, that assumed in the MRF earthquake event.

2. An aircraft provides a considerable fuel source and an ignition potential when crashed into a building, thus the potential of a fire exists. A fire would raise the local and surrounding temperatures of the crash site (i.e., areas where MAR is impacted), thus inactivating the pathogens before or during their release. An inactive pathogen presents no hazard to a public receptor.
3. NEIDL rooms containing pathogens are in the interior of the facility and there is at least one exterior wall plus one primary containment wall protecting the pathogens. Therefore, an aircraft projectile would have to penetrate two walls to impact the pathogens and result in a potential airborne release. In addition, the BSL-4 area is constructed as a box-within-a-box with interior walls that are more robust than (and seismically independent from) the rest of the building structure.
4. The BSL-3 and BSL-4 laboratories are several stories above ground level, so any release would be an elevated release that would allow dilution as the plume is dispersed, while the MRF earthquake scenario was analyzed on the basis of a ground-level release. Because a ground level release will tend to result in higher concentrations in the respirable zones/altitudes than an elevated release, the MRF earthquake scenario will tend to bound the aircraft crash scenario.

Therefore, the consequences of an aircraft crash are expected to be less than the consequence estimates for the MRF earthquake.

F.9.3.3 Summary

Both the frequency and consequences of an aircraft crashing are shown to be less than or no greater than the frequency and consequences of the MRF earthquake as analyzed. Therefore, the MRF earthquake analysis is considered bounding for the aircraft crash and further detailed aircraft crash analysis is not deemed to be necessary.

The frequency of an aircraft carrying live pathogens and resulting in a leak to the environment was demonstrated to be less than the threshold of frequency category D (1 in more than 1 million years) beyond reasonably foreseeable, and is dismissed from further evaluation.

F.10 REFERENCES

AIHA (American Industrial Hygiene Association). 2010. *Centrifuge Explosion*. <http://www.aiha.org/insideaiha/volunteergroups/labHandScommittee/Pages/CentrifugeExplosion.aspx>. Accessed June 4, 2010.

ANSI (American National Standards Institute). 2004. *American National Standard Categorization of Nuclear Facility Structures, Systems, and Components for Seismic Design,* prepared by the American Nuclear Society Standards Committee Working Group, ANS-2.26-2004, approved December 2, 2004.

Bennett, A., and S. Parks 2006. Microbial Aerosol Generation during Laboratory Accidents and Subsequent Risk Assessment. *Journal of Applied Microbiology*, ISSN 1364-5072, accepted October 6, 2005, Volume 100 (2006) pages 658-663. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2005.02798.x/full>. Accessed August 18, 2010.

BMC (Boston Medical Center). 2011. Boston Medical Center Facts. <http://bmc.org/Documents/BMC-Facts-2010.pdf>. Accessed June 22, 2011.

Bozzette, S. 2011. *Final Project Report: Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment*, February 28, 2011. Interdisciplinary Health Sciences Advisors, Inc., San Diego, CA.

BU (Boston University). 2009. BU Today, *Sargent Camp Revives,* July 1, 2009. <http://www.bu.edu/today/node/9134>. Accessed November 29, 2011.

BUMC (Boston University Medical Center). 2005. NEIDL Seismic Design, file BU_NEIDL01R5 Design Notes 01.doc.

BUMC (Boston University Medical Center). 2008. *Boston Public Health Commission Biological Laboratory Safety Permit Application: Boston University Medical Center National Emerging Infectious Diseases Laboratories,* September 3, 2008 Draft.

BUMC (Boston University Medical Center). 2008a. Boston University Medical Campus Fact Sheet. <http://www.bumc.bu.edu/FactSheet.html>. Assessed June 7, 2011.

BUMC (Boston University Medical Center). 2009. *Estimated Total Workforce of the NEIDL,* Item #16 of BUMC Files Received on July 13, 2009.

BUMC (Boston University Medical Center). 2009a. Telephone Meeting Notes, Centrifuges, Conversation with Tetra Tech, Inc. July 07, 2009.

BUMC (Boston University Medical Center). 2009b. Telephone Meeting Notes, Off-site Power, Fire Alarm, Detection, and Suppression System, and Air Systems, Conversation with Tetra Tech, Inc. June 26, 2009.

BUMC (Boston University Medical Center). 2009c. Telephone Meeting Notes, HVAC/Ventilation System Overview, Conversation with Tetra Tech, Inc. June 16, 2009.

BUMC (Boston University Medical Center). 2009d. Telephone Meeting Notes, UPS EDG and Spill Response, Conversation with Tetra Tech, Inc. June 19, 2009.

BUMC (Boston University Medical Center). 2010. Personal Protective Equipment Matrix for BSL-3, provided by BUMC about 1/6/2010.

BUMC (Boston University Medical Center). 2010a. R. L. Morales, BUMC, e-mail to J. P. Khoshbin, NIH, *FW: PAPR Testing,* 5/24/2010.

BUMC (Boston University Medical Center). 2010b. M. S. Klempner, BUMC, e-mail to W. A. Schell, Tetra Tech, *Re: SOPs,* August 30, 2010, including attachments and previous exchanges.

BUMC (Boston University Medical Center). 2010c. M. S. Klempner, BUMC, e-mail to R. Hirschberg, NIH, Y pestis *cultures,* March 03, 2010, including attachments and previous exchanges.

BUMC (Boston University Medical Center). 2011. *Boston University Medical Center Biosafety Manual*, September 2011. < http://www.bu.edu/orccommittees/files/2010/07/Boston-University-Biosafety-Manual-Revised-September-2011.pdf>. Accessed November 19, 2011.

BUMC (Boston University Medical Center). 2011a. T.J. Moore, BUMC, e-mail to K. Fennington, NIH, "master and working stocks, August 22, 2011.

CDC (Centers for Disease Control and Prevention) and NIH (National Institutes of Health). 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. U.S. Government Printing Office, Printing Office, Washington, DC.

Census Bureau. 2011. State and County Quick Facts. <http://quickfacts.census.gov/qfd/index.html>. Accessed March 29, 2011 and June 14, 2011.

Census Bureau. 2011a. *State Interim Population Projections by Age and Sex: 2004–2030, Table 1,* <http://www.census.gov/population/www/projections/projectionsagesex.html>. Accessed June 15, 2011.

DOA (Department of the Army). 1969. *Technical Study 67, Containers for Chemical/Biological Agents Drop-Tested from Aircraft*, M. Barbeito and A. Wedum, March 1969. Department of the Army, Fort Detrick, MD.

DOE (U.S. Department of Energy). 2000. *DOE Handbook –Airborne Release Fractions/Rates and Respirable Fractions for Nonreactor Nuclear Facilities,* DOE-HDBK-3010-94, U.S. Department of Energy. <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/hdbk3010/h3010v1.pdf>. Accessed 09/11/2010. Also, Change Notice 01 dated March 2000 <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/hdbk3010/hdbk301094_cn.pdf> was reviewed but it does not affect the portions used in this analysis.

DOE (U.S. Department of Energy). 2002. *Recommendations For Analyzing Accidents Under The National Environmental Policy Act,* U.S. Department of Energy, Office of Environment, Safety and

Health, Environment, Safety and Health, Office of NEPA Policy and Compliance July 2002. <http://nepa.energy.gov/nepa_documents/TOOLS/GUIDANCE/Volume2/2-10-greenbook-recommendations.pdf>. Accessed 8/16/2010.

DOE (U.S. Department of Energy). 2004 . *Recommendations for the Preparation of Environmental Assessments and Environmental Impact Statements,* Second Edition, U.S. Department of Energy, Office of NEPA Policy Compliance, December 2004. <http://nepa.energy.gov/documents/green_book2004_12_30_final.pdf>. Accessed 11/10/2009.

DOE (U.S. Department of Energy). 2004a. MACCS2 Computer Code Application Guidance for Documented Safety Analysis, Final Report, DOE-EH-4.2.1.4-MACCS2-Code Guidance, U.S. Department of Energy, Office of Environment, Safety and Health, June 2004. <http://www.hss.doe.gov/nuclearsafety/qa/sqa/central_registry/MACCS2/Final_MACCS2_Guidance_Report_June_1_2004.pdf>. Accessed November 10, 2009.

DOE (U.S. Department of Energy). 2005. *Respiratory Protection Incidents,* DOE/EH-0697, U.S. Department of Energy, Office of Environment, Safety and Health, September 2005. <http://www.hss.energy.gov/CSA/csp/safety_bulletins/2005-14.pdf >. Accessed November 25, 2009.

DOE (U.S. Department of Energy). 2006. DOE STD 3014-2006, Accident Analysis for Aircraft Crash into Hazardous Facilities, U.S. Department of Energy. <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/std3014/std3014.pdf>. Accessed September 28, 2010.

DOE (U.S. Department of Energy). 2008. *Integration of Safety into the Design Process*, DOE-STD-1189-2008, March 2008. <http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/std1189/DOE-STD-1189-2008.pdf>. Accessed September 28, 2010.

DOE (U.S. Department of Energy). 2009.. Safety Advisory 2009-05, *Errors in MACCS2 χ/Q Calculations*, SA_2009-05, U.S. Department of Energy, DOE Office of Health, Safety and Security, August 2009. Available on the internet at: <http://www.hss.doe.gov/csa/csp/advisory/SA_2009-05.pdf>. Accessed December 06, 2011.

DOE (U.S. Department of Energy). 2010. *March 8, 2010 Vehicle Occupancy Rates,* U.S. Department of Energy, Vehicle Technologies Program. <http://www1.eere.energy.gov/vehiclesandfuels/facts/2010_fotw613.html>. Accessed June 16, 2011.

EPA (U.S. Environmental Protection Agency). 1987. *Technical Guidance for Hazards Analysis—Emergency Planning for Extremely Hazardous Substances,* U.S. Environmental Protection Agency, Federal Emergency Management Agency, and U.S. Department of Energy. <http://www.epa.gov/oem/docs/chem/tech.pdf>. Accessed June 25, 2010.

EPA (U.S. Environmental Protection Agency). 1995. *Guidance for Risk Characterization,* Science Policy Council, U.S. Environmental Protection Agency, February 1995. <http://www.epa.gov/spc/pdfs/rcguide.pdf >. Accessed June 26, 2010.

FAA (Federal Aviation Administration). 2010. *Airport Master Record*. U.S. Department of Transportation, Federal Aviation Administration. <http://gcr1.com/5010web>. Accessed September 23, 2010.

Flint, S.J., L.W. Enquist,V.R. Racaniello, and A.M. Skalka. 2009. *Principles of Virology*. 3rd ed., Vol. I: Molecular Biology. ASM Press, Washington, DC.

GAO U.S. Government Accountability Office). 2009. *High-Containment Biosafety Laboratories, National Strategy for Oversight Is Needed*. GAO-09-574. <http://www.gao.gov/new.items/d09574.pdf>. Accessed June 9, 2011.

Garner, M.G., and R.M. Cannon. 1995. *Potential for Wind-Borne Spread of Foot and Mouth Disease Virus in Australia*. Commonwealth of Australia, Bureau of Resource Sciences. <http://www.daff.gov.au/__data/assets/pdf_file/0007/159541/fmdwind.pdf>. Accessed June 13, 2011.

Harding, A.L., and K.B. Byers. 2006. Epidemiology of Laboratory-Associated Infections in *Biological Safety: Principles and Practices*, 4th ed., D.O. Fleming and D.L. Hunt, eds. American Society of Microbiology Press, Washington, DC.

Hung, L., J.D. Miller, and K.H. Dillon. 2005. *Field Guide for the Determination of Biological Contaminants in Environmental Samples*, 2nd ed. American Industrial Hygiene Association, Fairfax, VA.

IACS (Innovation Academy Charter School). 2010. *Innovation Academy Charter School—High School Student and Family Handbook, 2010-2011*. <https://603671056090493229 4-a-innovationcharter-org-s-sites.googlegroups.com/a/innovationcharter.org/iacssite/current-students-and-families/high-school/hs_handbook.pdf?attachauth=ANoY7cpWPJPvfJKUV74sfb6PaFbejHl7ATYriSUAkuf5iQFiOB9yU4na1Yu7FrrUXPjt5uocci_7sHnlJlsf_C5AJHl-Vc1JTC2tj0jAGpVjoEagH_DhaDHEioaBagwTstCvxX9XKCBLHX5h23DElB0RtKVCaL40oiQjva8nJhcNMExaAOc68TBEtYqdWHNU6LZxwS0DYdZmzPTHjI6JZg6ycPU4W-1N7_gV5fwR5nclP4X5aj1vACGmKNFznQHyXTouLxrWmZgkX_hqTCFpblTOtxgu8UCrxA%3D%3D&attredirects=0>. Accessed July 7, 2011.

IACS (Innovation Academy Charter School). 2011. Staff Directory. <http://www.innovationcharter.org/current-students-and-families/staff-directory>. Accessed June 30, 2011.

Jungwirth and Tobias. Specific Ion Effects at the Air/Water Interface, P. Jungwirth and D. Tobias. <http://marge.uochb.cas.cz/~jungwirt/paper104.pdf>. Accessed October 20, 2010.

LLNL (Lawrence Livermore National Laboratory). 1994. W. Bergman, J. Elliott, and K. Wilson, *Performance of HEPA Filters at LLNL Following the 1980 and 1989 Earthquakes*. Lawrence Livermore National Laboratory, UCRL-JC-115890. <http://www.osti.gov/bridge/purl.cover.jsp?purl=/10196612-g5DqNQ/webviewable/>. Accessed 06/06/2011.

MA ESE (Massachusetts Department of Elementary and Secondary Education). 2011. *Charter School Pre-Enrollment for the 2011-2012 School Year, by Charter School.* Massachusetts Department of Elementary and Secondary Education. <http://www.doe.mass.edu/charter/enrollment/FY12.html>. Accessed July 7, 2011.

Massachusetts 1997. Massachusetts State Building Code, 6th ed. 780 CMR 1612.0 Earthquake Loads. <http://www.mass.gov/Eeops/docs/dps/BuildingCode/780016PT4.pdf>. Accessed 10/31/2010.

MDOT (Massachusetts Department of Transportation, Highway Division). 2011. *Traffic Volume Counts*. Excel spreadsheet titled BOOK2009a.XLS <http://www.mhd.state.ma.us/default.asp?pgid=content/traffic01&sid=about>. Under *Available Reports*, City/Town Traffic Volume Count Listing. Accessed June 16, 2011.

Meselson, M., et al. 1994. The Sverdlovsk Anthrax Outbreak of 1979. *Science* Volume 266, pages 1202–1208. Available on the internet at: < http://www.vaccines.mil/documents/library/Sverdlovsk.pdf >. Accessed February 10, 2011.

NASA (National Aeronautics and Space Administration). 2005. *Development of Risk Assessment Matrix for NASA Engineering and Safety Center, Kelly D. Moses*. Futron Corporation, and Roy W. Malone, Jr. NASA Marshall Space Flight Center, 2005. <http://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20050123548_2005093494.pdf>. Accessed June 4, 2010.

NASA (National Aeronautics and Space Administration). 2009. *Guidelines for Risk Management*. S3001, Revision B, NASA Independent Verification & Verification Program, Effective Date March 25, 2009. <http://www.nasa.gov/centers/ivv/pdf/209213main_S3001.pdf>. Accessed June 15, 2010.

National Research Council. 2010. *Evaluation of the Health and Safety Risks of the New USAMRIID High Containment Facilities at Fort Detrick, Maryland.* Committee to Review the Health and Safety Risks of High Biocontainment Laboratories at Fort Detrick Board on Life Sciences Division on Earth and Life Studies, National Research Council of the National Academies, 2010. <http://www.nap.edu/openbook.php?record_id=12871>. Accessed August 26, 2010.

Nature's Classroom 2011. *Adventure Camp 2011* brochure by Nature's Classroom. <http://www.naturesclassroom.org/sargent/Adventure%20Camp/AC2011-0.html>. Accessed July 3, 2011.

NHDOT (New Hampshire Department of Transportation). 2011. *Average Annual Daily Traffic for Selected Station.* New Hampshire Department of Transportation, Bureau of Traffic. <http://www.nh.gov/dot/org/operations/traffic/tvr/locations/documents/peterborough.pdf>. Accessed July 4, 2011.

NIH (National Institutes of Health). 2002. *Compliance with the NIH Guidelines For Research Involving Recombinant DNA Molecules*. Notice: NOT-OD-02-052, Office of Biotechnology Activities, National Institutes of Health, Release Date: May 28, 2002. <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-052.html>. Accessed 6/26/2010.

NIH (National Institutes of Health). 2003. *NIH Design Policy and Guidelines*. National Institute of Health, Office of Research Facilities, Bethesda, MD. Available on the internet at: <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/policy-index.htm >. Accessed 12/04/2011.

NIH (National Institutes of Health). 2005. *Final Environmental Impact Statement National Emerging Infectious Diseases Laboratories*. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD. Available on the internet at: <http://www.bu.edu/neidl/files/2010/07/NEIDL-Final-Environmental-Impact-Statement.pdf >. Acessed March 18, 2009.

NIH (National Institutes of Health). 2007. *Draft Supplementary Risk Assessments And Site Suitability Analyses for the National Emerging Infectious Diseases Laboratory*. National Institutes of Health, Division of Occupational Health and Safety, Bethesda, MD, 2007. <http://www.nems.nih.gov/aspects/nat_resources/programs/nepa2.cfm>. Accessed July 2, 2011.

NIH (National Institutes of Health). 2008. *National Institutes of Health (NIH) Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities (DRM)*, National Institutes of Health, Division of Technical Resources, Bethesda, MD August 27, 2008. <http://orf.od.nih.gov/PoliciesAndGuidelines/BiomedicalandAnimalResearchFacilitiesDesignPoliciesandGuidelines/DesignRequirementsManualPDF.htm>. Accessed November 2, 2009.

NIH (National Institutes of Health). 2009. *NIH Blue Ribbon Panel to Advise on the Risk Assessment for the BU National Emerging Infectious Diseases Laboratories—Teleconference with the National Research Council on Technical Input,* presentation on April 7, 2009, slide 21. <http://nihblueribbonpanel-bumc-neidl.od.nih.gov/docs/2009/April/BRP_NRC_Teleconf_April_7.pdf>. Accessed July 27, 2009.

NIH (National Institutes of Health). 2010. E-mail from J. P. Khoshbin, NIH to K. D. Bulmahn, Tetra Tech, *PAPR APF,* dated May 18, 2010.

Nuclear Regulatory Commission 1983. *Atmospheric Dispersion Models for Potential Accident Consequence Assessments at Nuclear Power Plants*, Regulatory Guide 1.145, Rev. 1, U.S. Nuclear Regulatory Commission, Office of Regulatory Research, Washington, D.C., November 1982, revised February 1983.

Nuclear Regulatory Commission 1983a. Till, J.E., and H.Robert Meyer. *Radiological Assessment—A Textbook on Environmental Dose Analysis*. NUREG/CR-3332, U.S. Nuclear Regulatory Commission, Office of Nuclear Reactor Regulation, Washington, D.C., September 1983. Available on the internet at: <http://www.gl.iit.edu/govdocs/resources/NUREGCR3332.pdf>. Accessed May 18, 2011.

Nuclear Regulatory Commission 1990.. H-N. Jow, et al., *MELCOR Accident Consequence Code System (MACCS). Model Description*, NUREG/CR-4691, SAND86-1562. Sandia National Laboratory, Albuquerque, NM. Available on the internet at: <http://pbadupws.nrc.gov/docs/ML0635/ML063560409.pdf>. Accessed December 05, 2011.

Nuclear Regulatory Commission. 1998. D. Chanin, C*ode Manual for MACCS2: Volume 1, User's Guide*. NUREG/CR-6613, SAND97-0594. Sandia National Laboratory, Albuquerque, NM. Available on the internet at: <http://www.doeal.gov/SWEIS/OtherDocuments/481%20MACCS2%20Vol%201.pdf>. Accessed December 05, 2011.

Nuclear Regulatory Commission.. 2002. C. L. Atwood, et al., *Handbook of Parameter Estimation for Probabilistic Risk Assessment*. NUREG/CR-6823. U.S. Nuclear Regulatory Commission, Office of Regulatory Research Washington, D.C.,. Available on the internet at: <http://www.nrc.gov/reading-rm/doc-collections/nuregs/contract/cr6823/cr6823.pdf>. Accessed June 11, 2010.

Nuclear Regulatory Commission. 2003. *Environmental Review Guidance for Licensing Actions Associated with NMSS Programs*, NUREG-1748. U.S. Nuclear Regulatory Commission, Office of Nuclear Material Safety and Safeguards, Washington, D.C. Available on the internet at: <http://www.nrc.gov/reading-rm/doc-collections/nuregs/staff/sr1748/>. Accessed June 14, 2011.

Nuclear Regulatory Commission. 2003a. N.E. Bixler, et al., *SECPOP 2000: Sector Population, Land Fraction, and Economic Estimation Program*, NUREGE/CR-6525, Revision 1, U.S. Nuclear Regulatory Commission, Office of Nuclear Regulatory Research, Washington, D.C., August 2003. <http://www.nrc.gov/reading-rm/doc-collections/nuregs/contract/cr6525/>. Accessed March 1, 2011.

Nuclear Regulatory Commission. 2007. S.A. Eide, et al., *Industry-Average Performance for Components and Initiating Events at U.S. Commercial Nuclear Power Plants*., NUREG/CR-6928. U.S. Nuclear Regulatory Commission, Office of Regulatory Research, Washington, D.C., published February 2007. Available on the internet at: <http://www.nrc.gov/reading-rm/doc-collections/nuregs/contract/cr6928/cr6928.pdf>. Accessed June 11, 2010.

Payne, M. 2009. Personal communication (e-mail) between M. Payne, Boston University, and Heath McLaughlin, January 20, 2009.

Pedrosa, P., and T. Cardoso. 2011. *Viral infections in workers in hospitals and research laboratory settings: a comparative review of infection modes and respective biosafety aspects,* International Journal of Infectious Diseases, accepted 21 March 2011. Available on the internet at: < http://www.sciencedirect.com/science/article/pii/S1201971211000555>.

Pelican. 2011. Pelican 0340 Protector 18" Cube Case, Description and Specifications, <http://pelican.com/cases_detail.php?Case=0370>. Accessed May 14, 2011.

Rarick, M.D. 2009. Spreadsheet, Tetra-Tech-Centrifuge-Info.xls transmitted by e-mail on June 25, 2009, from Boston University to Craig Klein, Tetra Tech, Inc.

Rosenstock 2000. Rosenstock, L., *Statement for the Record on Needlestick Injuries,* Before the House Subcommittee on Workforce Protections Committee on Education and the Workforce, 22 June 2000. <http://www.hhs.gov/asl/testify/t000622a.html

RWDI (Rowan Williams Davies & Irwin). 2005. *Exhaust Re-entrainment Study, NIH National Emerging Infectious Diseases Laboratory, Boston, Massachusetts,* RDDI Reference Number #04-1288A, Rowan Williams Davies & Irwin Inc., Guelph, Ontario, Canada, April 08, 2005.

SARSTEDT. 2011. *SARSTEDT Screw Cap Micro Tube Description*. Available on the internet at: <http://www.sarstedt.com/php/tube.php?artnr=72.694.006&lnr=>. Accessed May 10, 2011.

Sartor 2009. *POSTMAX V2.0 User's Guide*, LA-UR-09-1601, Raymond F. Sartor, Los Alamos National Laboratory, February 2009. Available on the internet at: < https://sbts.lanl.gov/POSTMAX_Install.shtml > Accessed December 04, 2011.

Sewell, D.L. 1995. *Laboratory-Associated Infections and Biosafety.* Clinical Microbiology Reviews 8(3): 389–405. Available on the internet at: <http://cmr.asm.org/cgi/reprint/8/3/389.pdf>. Accessed December 23, 2009.

Sigma-Aldrich, Syringe Needle Gauge Chart, Sigma-Aldrich. <http://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/needle-gauge-chart.html>. Accessed October 30, 2010.

Smith, P.L., and S. Edwards. 2002. Working at Biosafety Level 4—Contain the Operator or Contain the Bug? In *Anthology of Biosafety V. BSL-4 Laboratories*. American Biological Safety Association, Mundelein, IL.

Spector, S., R.L. Hodinka, D.L. Wiedbrauk, and S.A. Young. 2002. *Diagnosis of Viral Infections*, Clinical Virology, 2nd ed. Richman D.D., Whitely R.J., and Hayden F.G. eds. ASM Press, Washington, DC.

Tetra Tech 2009. *HVAC/ Ventilation system overview,* Telephone Meeting Notes for call on June 16, 2009.

Tetra Tech 2009a. *Review of previous Ventilation System call, UPS, Emergency on-site Power, and Spill Response,* Telephone Meeting Notes for call on June 19, 2009.

Tetra Tech 2009b. *Off-Site Power, Fire Alarm, Detection, Suppression, Air Systems (Breathing, Instrument, Gases)*. Telephone Meeting Notes for call on June 26, 2009.

The Engineering Toolbox, Surface Tension of Water in Contact with Air, The Engineering Toolbox, <http://www.engineeringtoolbox.com/water-surface-tension-d_597.html>. Accessed October 30, 2010.

USGS (U.S. Geological Survey). 2008. 2008 U.S. Geological Survey National Seismic Hazard Maps, <http://earthquake.usgs.gov/hazards/products/conterminous/2008/maps/>. Accessed 10/31/2010.

USGS (U.S. Geological Survey). 2010. Earthquake Hazards Program, Massachusetts Earthquake History, <http://earthquake.usgs.gov/hazards/products/conterminous/2008/maps/>. Accessed 10/31/2010.

USGS (U.S. Geological Survey). 2010a. Earthquake Hazards Program, Magnitude/Intensity Comparison, <http://earthquake.usgs.gov/learn/topics/mag_vs_int.php>. Accessed 10/31/2010.

Wampler, R.A., and T.S. Blanton. 2001. *Volume V: Antrax at Sverdlovsk, 1979, U.S. Intelligence on the Deadliest Modern Outbreak*. National Security Archive Electronic Briefing Book No. 61. R.A. Wampler and T.S. Blanton eds. <http://www.gwu.edu/~nsarchiv/NSAEBB/NSAEBB61/index2.html>. Accessed July 20, 2011.

Weidlinger. 2005. *BU_NEIDL, Structural Seismic, Structural Design of Containment,* Mohammad Haidar, Weidlinger Associates, Inc., letter to Kevin Jelinek, CUH2A, October 6, 2005.

Woodward,R. P., Surface Tension Measurements Using the Drop Shape Method, Available online at: http://www.firsttenangstroms.com/pdfdocs/STPaper.pdf

ATTACHMENT A. SUMMARY OF INCIDENTS RELATED TO A CENTRIFUGE RELEASE

This attachment focuses on the historic incident data provided in Appendix D of the RA and provides a summary of the incidents relevant to this analysis. Incidents considered here include those associated with centrifuge aerosol releases, reduced PAPR performance, loss of positive-pressure suit confinement, and failure of ventilation systems.

A.1 Centrifuge Incidents

The RA (Appendix D) identifies four centrifuge-related incidents at BSL-3 facilities, which are summarized below. No centrifuge-related incidents are identified in the RA Appendix D for BSL-4 facilities.

1. Location: University of Texas Houston Health Science Center, Houston, Texas
   Date: May 7, 2007
   Research Agent: *Bacillus anthracis* (anthrax)
   Description: Tube leakage occurred inside a centrifuge used for concentration of *B. anthracis* cells.
   Results: Four people apparently were exposed to *B. anthracis*.
   Action: Prophylaxis was refused. No infections resulted. Procedures were modified to require that centrifuge buckets be opened only within a BSC, and inspected and decontaminated after each use.

2. Location: University of Texas at Austin, Texas
   Date: April 12, 2006
   Research Agent: Recombinant Influenza A H3N2 virus, containing genes from strain H5N1 (avian influenza virus).
   Description: A centrifuge secondary container lid broke during centrifugation of virus, causing the rotor to become unbalanced. The researcher noted loss of volume in one viral tube and, suspecting viral leakage, undertook decontamination of centrifuge, centrifuge tube, work area, adjacent equipment, and himself.
   Results: A decision was made to treat the researcher empirically using Tamiflu©® [Oseltamivir]. Secondary decontamination of laboratory room was undertaken the following day.

Action: Responsible officials maintain that it is unclear whether a leakage of virus occurred. However, their records show that proper decontamination protocol was not followed for the suspected leak.

3. Location: Medical University of Ohio, Toledo, Ohio
   Date: September 30, 2005
   Research Agent: *Coccidioides immitis*
   Description: A student potentially was exposed to infectious aerosols from a broken vial inside a centrifuge.
   Results: Medical evaluation was provided.
   Action: The BSL-3 facility director, who was in charge of the *C. immitis* research, resigned as director and was replaced as animal and biosafety protocol principal investigator and as advisor to the student.

4. Location: New Haven, Connecticut
   Date: August 8, 1994
   Research Agent: Sabia virus[11]
   Description: A research virologist discovered a leaking vessel on opening a sealed aerosol biocontainment centrifuge rotor. Improper PPE was used, and the incident was not reported.
   Results: Symptoms began 8 days afterward. Two days later, the infection was properly diagnosed.
   Action: Antiviral therapy cured the nearly fatal infection. Two external committees strongly criticized the researcher and institution. The university agreed to implement all recommendations.

The above incident data can be summarized as follows:

- There have been four potential centrifuge-related release incidents in BSL-3 facilities and none in BSL-4 facilities.
- Three incidents resulted in potential exposure of one individual each, and one incident involved the exposure of four people.
- Only one of the incidents resulted in infection and only one individual was infected.

[11] Sabia virus, a BSL-4 virus, was not classified by BMBL 3rd edition regarding biocontainment precautions at the time of this incident

- Only one incident was undetected or unreported. This incident involved a failure to report the incident until after the researcher became ill.

A.2 Positive-Pressure Suit Incidents

Appendix D of the RA identifies three positive-pressure suit failure incidents in BSL-4 facilities. These incidents are identified below, along with the pathogens involved.

- Marburg virus—Separation of BSL-4 filter from suit.
- Marburg virus—V-shaped rip in a suit. Positive airflow inside the suit prevented contact with room air.
- Lassa virus—Brass connector on suit fell apart.

There is no indication that any of those incidents prevented the suits from providing the necessary protection, in fact one of the descriptions states that continued protection was provided.

A.3 PAPR Incidents

Two incidents related to PAPR are summarized below.

1. Location: Medical College of Wisconsin
   Date: July 28, 2010
   Research Agent: *Mycobacterium tuberculosis*
   Description: During activities, the worker backed into a doorway and bumped the power pack on the PAPR unit. The PAPR power was inadvertently turned off as a result.
   Results: The worker noticed the air was not flowing into the hood and immediately turned the unit back on. It was estimated that the PAPR was not operating for a few seconds.
   Action: No other actions were taken.

2. Location: Medical College of Wisconsin
   Date: October 6, 2010
   Research Agent: Influenza A/Brevig Mission/1918
   Description: The individual was centrifuging washed cells (i.e., cells contained in sealed cups inside the centrifuge) when the HEPA filter fell off the PAPR belt. It was

unknown whether the filter was loose for a prolonged period or was knocked off at that moment.

Results: The individual picked up the filter, reconnected it to the PAPR, and finished the work.

Action: The individual was given Tamiflu and quarantined but showed no symptoms.

A.4 Ventilation System Incidents

Six ventilation incidents at BSL-3 facilities were identified in Appendix D of the RA and are summarized below.

1. Location: Centers for Disease Control and Prevention, Atlanta, Georgia, Bldg 17
   Date: July 11, 2008
   Research Agent: Program pathogens included influenza virus, extensively drug-resistant *Mycobacterium tuberculosis*, and rabies virus.
   Description: A bird caused a Georgia Power transformer to fail, knocking out electricity to BSL-3 biocontainment areas.
   Results: Backup generators failed to start, leaving the laboratories without main electrical power for 75 minutes. CDC personnel did not attempt to override and start the backup generators. Negative directional airflow was not maintained.
   Action: No exposures or infections were reported. Backup failure was determined to be due to removal of two generators from service for upgrades. Their absence caused a power fluctuation when main power was lost, resulting in shutdown of the entire backup generator system. The system was tested subsequently on July 21; CDC officials would not release results of the test. No further information was available.

2. Location: Centers for Disease Control and Prevention, Atlanta, Georgia, Building 18
   Date: June 8, 2007
   Research Agent: Not applicable
   Description: A lightning strike knocked out electricity to BSL-3 and unoccupied BSL-4 biocontainment areas. Circuit breakers that should have remained engaged were tripped.

Results: Backup generators failed to start. Negative directional airflow was not maintained. A battery-powered system provided power to lights and doors for 15–20 minutes. No exposures or infections subsequently were reported.

Action: Changes to the design of the backup system were discussed and damage to the lightning protection equipment was repaired. It was later determined that a ground cable had been cut some time earlier, and prevented the circuit breakers from remaining engaged. CDC has declined to release documents relating to the incident.

3. Location: Centers for Disease Control and Prevention, Atlanta, Georgia, Building 18
   Date: May 2007
   Research Agent: Not applicable
   Description: Malfunction of the HVAC system pulled potentially contaminated air out of the BSL-3 biocontainment area and into a clean hallway.
   Results: Nine workers were tested for possible exposure to the bacterium and no infections were diagnosed.
   Action: The HVAC system was brought back into compliance. Duct tape was used to seal the door and remained in place as of June 2008. A self sealing door will be installed by April 2009.

4. Location: The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina
   Date: March 19, 2005
   Research Agent: *Mycobacterium tuberculosis* (recombinant)
   Description: An exhaust fan servicing two BSCs and the general laboratory space failed. Audible alarms on the cabinets and air pressure monitors had been turned off.
   Results: Loss of primary and secondary biocontainment occurred.
   Action: A review of all BSL-3 laboratories was scheduled to identify renovation needs to ensure compliance with current design standards.

5. Location: University of Illinois at Chicago, Chicago, Illinois
   Date: September, 2004
   Research Agent: Not specified (redacted by IBC)
   Description: Both doors of the double door biocontainment entryway were propped open by laboratory staff while experiments were in progress.

Results:	Infectious materials were not being handled at the time.
Action:	Principal investigator was counseled and warned. Staff was retrained and tested.

6. Location: Fort Detrick, Maryland
   Date: April 1, 2002
   April 20, 2002
   Research Agent: *Bacillus anthracis* (anthrax)
   Description: A researcher tested positive for exposure to anthrax spores, which were also released into a locker room and adjacent hallway.
   U.S. Army officials reported evidence of a second accidental release of anthrax spores.
   Results: No one was infected in either incident.
   Action: The first incident involved a virulent strain. Test samples connected with the second incident tested positive for the attenuated (vaccine) strain.

ATTACHMENT B. ESTIMATE OF THE AMOUNT OF PATHOGEN IN A DROP OF SUSPENSION

The purpose of this attachment is to get first order estimate as to the amount of pathogen toward which a worker could be exposed. It is first assumed that 1 molar saline-water is the solution used inside of a syringe. For purposes of this example analysis, a single drop is the basic unit used to determine how much pathogen could be injected into the worker. The laboratory worker could be exposed to multiple drops or a fraction of a drop, but a single drop is used as a foundation for this analysis. The effect of multiple drops or fractions of a drop can be calculated by extension, if desired.

A relationship for the mass of a drop of water hanging from the end of a tube is (Woodward)

$$W = 2 \times \pi \times r \times \gamma \qquad \text{[equation B-1]}$$

where

W weight of the drop (N) = m × g

m mass of water drop (kg)

g acceleration due to gravity (9.8 m/s^2)

r tube radius (m) = 0.5 × tube diameter

γ surface tension (N/m)

Assuming room temperature is approximately 20 °C (68 °F), the surface tension of water is 7.28×10^{-2} N/m (The Engineering Toolbox). Jungwirth and Tobias (Figure 13c) show relationships of saline solutions relative to pure water for several salts. The general trend is a higher surface tension with increasing molarity, but the results are minor. For example, for one molar sodium chloride (NaCl), the surface tension increases approximately 1.5×10^{-3} N/m. Thus, the total surface tension is 7.43×10^{-2} N/M. Assuming a hypodermic needle that is 27 gauge (typical gauge for injecting mice), a nominal inner diameter of 0.19 mm (1.9×10^{-4} m) is used (WWW 3).

Solving equation B-1 for the mass of water in the drop gives the following:

$$m = 4.5 \times 10^{-6} \text{ kg} = 4.5 \times 10^{-3} \text{ grams}$$

Assuming a nominal density of water was assumed at 1 g/cc, this is equivalent to 4.5×10^{-3} cc or 4.5×10^{-3} mL (4.5 micro-liters). An exposure to 4.5×10^{-3} mL at a concentration of 1×10^{6} $CCID_{50}$ results in an exposure to 4,500 $CCID_{50}$. Other pathogens have higher SCs so the exposure levels would be correspondingly higher.

ATTACHMENT C. SUMMARY OF INCIDENTS RELATED TO NEEDLESTICK

This attachment provides an overview of historic needlestick incidents at BSL-3 and BSL-4 facilities

BSL-3: Literature searches identified eight needlestick-related incidents at BSL-3 facilities, which are summarized below. In addition, NIH has supplied 11 other BSL-3 events from RDNA incidents (NIH RDNA 2010), which are summarized below in lesser detail.

1. Location: United States of America
   Date: October 2002
   Research Agent: West Nile virus, genus *Flavivirus* (WNV)
   Description: A microbiologist working under BSL-3 conditions suffered a finger puncture from a hypodermic needle harboring WNV being harvested from infected mouse brain (Centers for Disease Control and Prevention, 2002 #16273).
   Results: The wound was cleansed and bandaged. Serologic testing showed evidence of acute WNV infection. Mild symptoms developed and resolved.
   Action: CDC determined that applicable handling and biocontainment protocols were followed.

2. Location: University of New Mexico, Albuquerque, New Mexico
   Date: 2003
   Research Agent: Redacted by IBC; likely was *Francisella tularensis*
   Description: Puncture of thumb with hypodermic needle harboring spores to be used in mouse infections (University of New Mexico IBC, 2003 #4024) (Subcommittee on Oversight and Investigations, 2007 #16247).
   Results: Worker received prophylactic treatment; no infection resulted.
   Action: It was proposed that alternative methods for mouse inoculation be considered.

3. Location: University of Virginia
   Date: April 11, 2007
   Research Agent: *Francisella tularensis*
   Description: Potential exposure—Needlestick with syringe that had been in contact with mice. Inoculated with select agent.
   Results: Unknown
   Action: No further information available.

4. Location: National Animal Disease Center
   Date: July 2, 2007
   Research Agent: *Brucella suis*
   Description: Potential exposure—needlestick with syringe containing select agent during necropsy
   Results: Unknown
   Action: No further information available.

5. Location: The University of Chicago
   Date: July 18, 2007
   Research Agent: *Bacillus anthracis*
   Description: Potential exposure—needlestick with syringe containing select agent during necropsy
   Results: Unknown
   Action: No further information available.

6. Location: Battelle Memorial Institute
   Date: July 23, 2007
   Research Agent: Avian influenza virus
   Description: Potential exposure—needlestick with syringe containing select agent.
   Results: USDA select agent, referred to APHIS
   Action: No further information available.

7. Location: University of California, Davis
   Date: July 27, 2007
   Research Agent: *Brucella abortus*
   Description: Potential exposure—needlestick with syringe containing select agent.
   Results: Unknown
   Action: No further information available.

8. Location: St. Louis University
   Date: August 7, 2007
   Research Agent: Monkeypox virus
   Description: Potential exposure—needlestick with syringe containing select agent.

Results: Unknown

Action: No further information available.

Table C-1. External parenteral exposures in BSL-3

Institution	Date	Agent
Colorado State University	4/2/10	HIV
University of Rochester	1/20/10	Lentivirus
University of Texas—San Antonio	1/14/10	*Coccidioides* sp.
University of Massachusetts Medical School	11/5/09	Mycobacterium tuberculosis
Novartis	10/28/09	VEE and Sindbis
University of North Carolina Chapel Hill	11/13/08	Ross River virus
University of Texas—San Antonio	8/21/08	*Francisella tularensis*
University of Wisconsin—Madison	5/22/08	*Brucella* sp.
University of Colorado—Denver	4/16/08	*Mycobacterium tuberculosis*
St Louis University	4/4/08	Yellow Fever virus
University of Texas Health Science Center	9/21/07	*Bacillus anthracis*

Source: NIH RDNA 2010

BSL-4: There have been five needlestick related incidents in BSL-4 facilities, which are summarized below.

1. Location: U.S. Army Medical Research Institute For Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland
   Date: November, 1979
   Research Agent: Lassa virus
   Description: Accidental finger puncture with needle on a syringe loaded with Lassa virus.
   Results: No illness or serological evidence for infection occurred
   Action: Ribavirin and immune plasma were given. (This was an experimental therapy for monkeys under development at the Institute.)

2. Location: U.S. Army Medical Research Institute For Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland
   Date: February 18, 2004
   Research Agent: Ebola virus
   Description: A civilian scientist grazed her hand with a hypodermic needle that had been used to inject antibodies into Ebola virus-infected mice (Dishneau, 2004 #4037)(Subcommittee on Oversight and Investigations, 2007 #16247)

Results: Scientist was sequestered in the biosafety biocontainment care suite until it could be concluded that no infection ensued.

Action: No further information available.

3. Location: Vector Laboratory, Novosibirsk, Russia

Date: May 19, 2004

Research Agent: Ebola virus

Description: A researcher several days earlier had suffered an accidental finger stick with a hypodermic needle that contained the Zaire strain of Ebola virus. She was working with a guinea pig model of the infection at the time.

Results: The researcher died from Ebola infection on May 19.

Action: No further information available.

4. Location: Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (Enserink, 2009 #16252)

Date: March 12, 2009

Research Agent: Ebola virus

Description: A laboratory researcher wearing protective gloves experienced a needlestick from a syringe suspected to contain Ebola virus

Results: The researcher was quarantined in Hamburg University Hospital for observation. Treatment included use of the experimental Feldmann vaccine.

Action: The worker did not become ill. No further information concerning potential seroconversion. (McGroarty, 2009 #16253)

5. Location: CDC Special Pathogens Branch

Date: 2003 or earlier

Research Agent: Ebola virus, Zaire strain, mouse-adapted

Description: Needlestick to worker before setting up an inoculum with mouse-adapted Ebola virus

Results: No infection resulted

Action: No further information available

ATTACHMENT D. SUMMARY OF NEIDL DESIGN

This attachment presents excerpts from various design documents and summarizes the NEIDL design with respect to seismic, wind, blast effects, and security. The wind, blast, and security design information is potentially, but not necessarily, relevant to the earthquake analysis, but they are presented here to provide all potentially relevant design information for the earthquake event sequence analysis in one location.

D.1 General Design

The following excerpts apply to the general design of NEIDL:

1. "The NEIDL structure consists of a seven-story structural steel composite frame. The BSL-4 laboratory facility is contained in a concrete structure (referred to as the Containment Box) and is supported on level 2. The floors above the containment box will be used for various mechanical, BSL-2 and BSL-3 laboratories and office spaces.

   In accordance with the basis of design report (BOD) for this project, the structural design is in compliance with the 6th edition of 780 CMR." (Weidlinger 2005)

2. "The overall facility design and construction is in conformance with the applicable codes, including:

   Code Compliance

   - The NIH Design Policy and Guidelines; November 2003 Edition
   - The U.S. Department of Health and Human Services, CDC/NIH, Biosafety In Microbiological and Biomedical Laboratories
   - The Massachusetts State Building Code (780 CMR); 6th Edition (BMBL)" (BUMC 2008)

D.2 Seismic Design

The following excerpts apply specifically to the seismic design of NEIDL (Weidlinger 2005).

1. According to the Rolf Jensen & Associates, Inc., August 19, 2005, Code Compliance Report, the project was designed in accordance with the requirements of Use Group Classification B (Business) occupancy for laboratory and office areas. Per the report, this understanding is based on the use and storage of hazardous materials below allowable exempt quantities per control area. In addition, "materials in storage and in use in the BSL-4 areas are not classified as highly toxic,

as defined by MSBC 307.2, because they are biological in nature." This use group classification translates to a Seismic user Group II (which corresponds to Seismic performance category C). A more stringent seismic performance category (Seismic Performance Category D) is also specified in 780 CMR which corresponds to User Group III. At the request of the project team, Weidlinger Associates, Inc. (WAI) performed an evaluation of the current containment box structural design when subjected to the seismic loads and deformation requirements of 780 CMR for seismic category D. In accordance with 780 CMR, WAI has performed a dynamic modal analysis and generated a new lateral load distribution for each level of the building. The modal analysis was based on the base shear value obtained from the equivalent lateral load procedure. The modal analysis is an alternate method to generate lateral loads that is known to yield more accurate results than the more conservative equivalent lateral load procedure (used in the current design for seismic category C). The structural design of the containment was then subjected to the new generated lateral loads and tested in accordance with the deformation requirements of Category D.

Based on the recently performed modal analysis, it is WAI's conclusion that the design of the containment structure will not change if a Category D design were to be specified and that the current design meets the load and deflection criteria for this requirement.

2. The design and construction includes the following (BUMC 2008):
    - The NEIDL is designed according to Massachusetts Building Codes for Seismic Category D.
    - The BSL-4 suites are structurally separated from the adjoining floors. This structural separation allows for movement in the event of an earthquake, while maintaining structural integrity of the BSL-4 suites.
    - All fixtures for the BSL-4 suite designed specifically for the facility, and are Underwriters Laboratories (UL) tested, to ensure the facility retains its air pressurization.

ATTACHMENT E. ESTIMATE OF FREQUENCY

This attachment presents the basis for the frequency category assignment for an earthquake that would cause failure of the NEIDL structure. This attachment provides background information, summarizes the NEIDL design criteria, presents U.S. Geologic Survey (USGS) seismic hazard data, and estimates the frequency of an earthquake that could fail the NEIDL structure.

E.1 Introduction

Several different scales are used to measure characterize earthquakes. The Richter scale is a well-known measure of an earthquake's magnitude. The Richter scale is a logarithmic earthquake magnitude scale for measuring the size of the earthquake, it is not used to express damage or effect. Another measure of an earthquake is the Modified Mercalli (MM) intensity, which is a measure of the effect observed from the earthquake. Table E-1 below provides a comparison of the Richter and MM scales.

Table E-1. Richter magnitude and MM intensity earthquake measures

Richter magnitude	Typical maximum MM intensity
1.0–3.0 (Micro)	I
3.0–3.9 (Minor)	II–III
4.0–4.9 (Light)	IV–V
5.0–5.9 (Moderate)	VI–VII
6.0–6.9 (Strong)	VII–IX
7.0 and higher (Major or Great)	VIII or higher

Source: USGS 2010a

Table E-2 provides a description of each MM intensity level to provide some understanding of the effects.

Table E-2. Description of MM intensities

Level	Description
I	Not felt except by a very few under especially favorable conditions.
II	Felt only by a few persons at rest, especially on upper floors of buildings.
III	Felt quite noticeably by persons indoors, especially on upper floors of buildings. Many people do not recognize it as an earthquake. Standing motor cars might rock slightly. Vibrations similar to the passing of a truck. Duration estimated.
IV	Felt indoors by many, outdoors by few during the day. At night, some awakened. Dishes, windows, doors disturbed; walls make cracking sound. Sensation like heavy truck striking building. Standing motor cars rocked noticeably.
V	Felt by nearly everyone; many awakened. Some dishes, windows broken. Unstable objects overturned. Pendulum clocks might stop.
VI	Felt by all, many frightened. Some heavy furniture moved; a few instances of fallen plaster. Damage slight.
VII	Damage negligible in buildings of good design and construction; slight to moderate in well-built ordinary structures; considerable damage in poorly built or badly designed structures; some chimneys broken.

Level	Description
VIII	Damage slight in specially designed structures; considerable damage in ordinary substantial buildings with partial collapse. Damage great in poorly built structures. Fall of chimneys, factory stacks, columns, monuments, walls. Heavy furniture overturned.
IX	Damage considerable in specially designed structures; well-designed frame structures thrown out of plumb. Damage great in substantial buildings, with partial collapse. Buildings shifted off foundations.
X	Some well-built wooden structures destroyed; most masonry and frame structures destroyed with foundations. Rails bent.
XI	Few, if any (masonry) structures remain standing. Bridges destroyed. Rails bent greatly.
XII	Damage total. Lines of sight and level are distorted. Objects thrown into the air.

Source: USGS 2010a

The following is a USGS summary of earthquakes in Massachusetts (USGS 2010).

> Nineteen earthquakes, intensity V or greater, have centered in Massachusetts. A number of other earthquakes were centered off the coast of Massachusetts and affected the eastern portion of the State. A shock in 1755 reached intensity VIII at Boston and was felt across the State. In addition, Massachusetts was affected by some of the more severe Canadian shocks plus the earthquake of 1929 that centered on Grand Banks of Newfoundland.

Table E-3 provides a list of the more significant earthquakes in Massachusetts.

Table E-3. Earthquakes in Massachusetts

Location	Date	MM intensity or Richter magnitude
Northern Cape Ann region, east of Newbury, Massachusetts	November 10, 1727	MM Intensity VII
Southern Cape Ann, Massachusetts region	June 14, 1744	MM Intensity VI
Cape Ann, Massachusetts	November 18, 1755	MM Intensity VIII (the largest earthquake recorded in Massachusetts)
Near the coast of Massachusetts	July 22, 2003	Richter Magnitude 3.6

The Richter magnitude and the MM intensity are not used when assessing a building's seismic capacity; instead, the spectral acceleration is used. The spectral acceleration is approximately the acceleration experienced by the building and accounts for the frequency of the shaking. The Richter magnitude and the MM intensity cannot be related to the spectral acceleration. So it is not possible to identify the largest historic spectral acceleration for Massachusetts.

E.2 NEIDL Design

The NEIDL was designed in accordance with the Massachusetts Building Code (Massachusetts 1997) which requires the following:

> The effective peak velocity-related acceleration (Av) and the effective peak acceleration (Aa) shall each be taken as 0.12g throughout Massachusetts for the purposes of seismic design in accordance with 780 CMR.

NEIDL was designed to withstand the 0.12 g acceleration (i.e., 0.12×32.2 ft/s^2 = 3.86 ft/s^2) (BUMC 2005). The fundamental period (essentially the inverse of the natural frequency) of the NEIDL structure is 1.866 seconds in the X direction and 1.964 seconds in the Y direction (BUMC 2005). An earthquake with a shaking period approximately equal to the fundamental period of the NEIDL structure, approximately 2 seconds, has the greatest potential to cause damage to the facility.

The Massachusetts Building Code (Massachusetts 1997) states the following:

> For ground motions larger than the design levels, the intent of 780 CMR 1612.0 is that there be a low likelihood of building collapse.

In summary, the NEIDL structure is expected to withstand an earthquake with an acceleration of 0.12 g with a shaking period of approximately 2 seconds (approximately the natural frequency of the NEIDL structure) and for ground motions larger than the design levels, there is a low likelihood of a building collapse.

E.3 USGS Seismic Hazard Data

The USGS provides seismic hazard maps (USGS 2008), which report the spectral acceleration at various periods for portions of the country. Table E-4 below provides the spectral acceleration the corresponding likelihoods for 1 and 2 second shaking periods (i.e., the time between peak acceleration cycles). The data are applicable for northeastern Massachusetts, which includes the area surrounding the three candidate sites. The 2-second shaking period is quite close to the NEIDL fundamental periods of 1.866 and 1.964 seconds and is the period of greatest concern for NEIDL. Though the 1-second data are not important for this analysis because they are not close to the 2-second fundamental period of the NEIDL structure, they do provide insight into the shape of the curve, while the two data points from USGS maps for a 2-second period do not allow the shape of the curve to be determined. The footnotes below Table E-4 provide the identifiers listed on the USGS seismic hazard website (USGS 2008) for the specific maps from which the data were taken.

Table E-4. USGS Seismic Hazard Data

Likelihood	Spectral acceleration	
	1-second period	2-second period
10% in 50 years	0.02 to 0.03 g[a]	0.01 to 0.02 g[b]
5% in 50 years	0.04 to 0.06 g[c]	not available
2% in 50 years	0.06 to 0.08 [d]	0.04 to 0.05 [e]

a. Conterminous U.S., 10% in 50 Years, 1.0 Second
b. Conterminous U.S., 10% in 50 Years, 2.0 Seconds
c. Conterminous U.S., 5% in 50 Years, 1.0 Second
d. Conterminous U.S., 2% in 50 Years,1.0 Second)
e. Conterminous U.S., 2% in 50 Years, 2.0 Seconds

Figure E-1 below provides a graph of the data in Table E-4 using the maximum spectral acceleration of each range (e.g., 0.03 g is used for the 0.02 to 0.03 g range). The likelihood values in Table E-4 (i.e., a percentage in 50 years) are presented as annual probability of exceedance values; so, the 10 percent in 50 years value is converted to an annual exceedance probability of 2×10^{-3} (i.e., 0.10 / 50 years) and the 2 percent in 50 years value is converted to 4×10^{-4} (i.e., 0.02 / 50 years). Dashed lines connect the points for each data set and are extrapolated beyond the end points in Figure E-1 to help visualize the potential extension. The three points for the 1-second shaking period data show that the relationship is not linear and that the curves bends downward, as shown by the thee point for the 1-second shaking period curve. Figure E-1 also identifies the design basis for the NEIDL facility (i.e., 0.12 g spectral acceleration), which is the acceleration that NEIDL was designed to withstand without loss of function. Using a linear extrapolation, which is conservative, Figure E-1 shows that a 2-second 0.12 g spectral acceleration earthquake is expected to have an annual exceedance probability of about 1×10^{-5}.

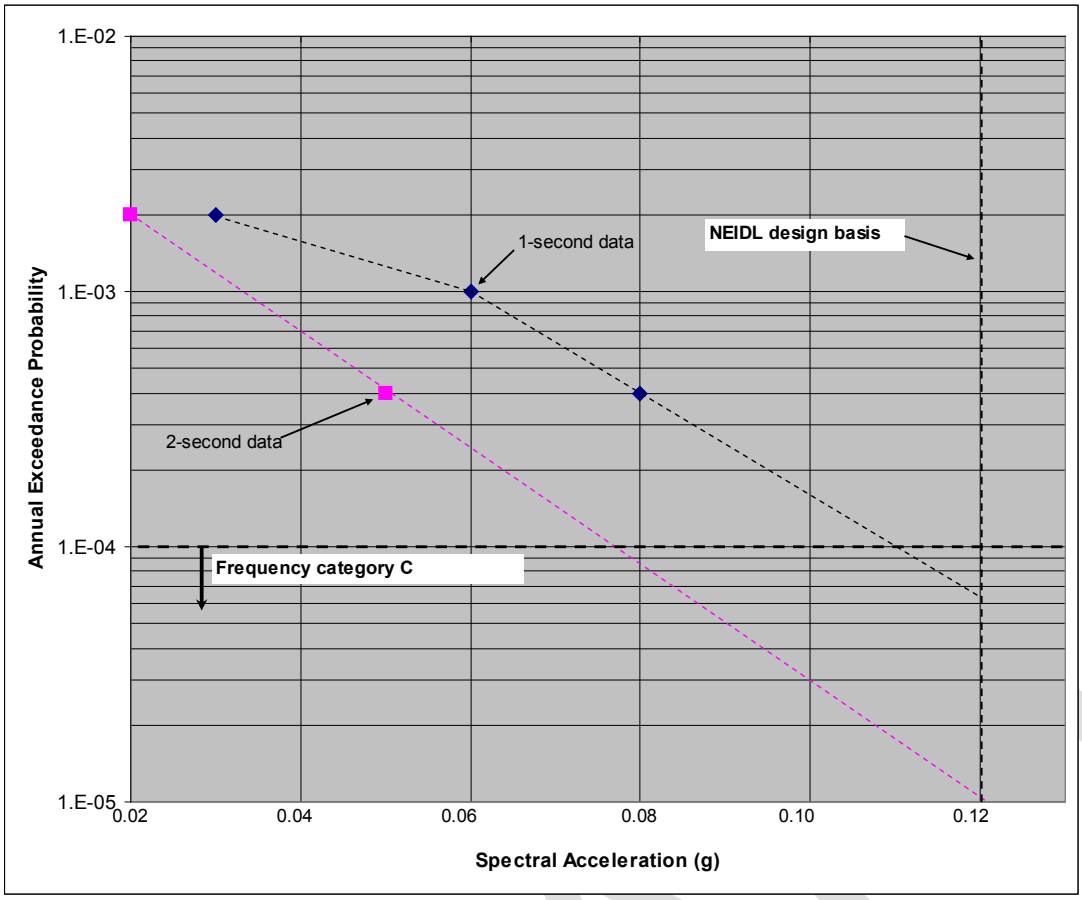


Figure E-1. Chart of frequency and acceleration for 1- and 2-second shaking periods.

E.4 Frequency Category

On the basis of a visual examination of Figure E-1, a 2-second shaking period 0.12 g spectral acceleration earthquake is expected to have an annual exceedance probability of about 1 x 10^{-5} for NEIDL. Because NEIDL has been determined to remain functional after a 2-second 0.12 g earthquake, the frequency category for an earthquake that fails the NEIDL structure, either total or partial failure, is expected to have an annual exceedance probability of less than 1 x 10^{-5}. In the case of the MRF (total collapse) earthquake, Section E.2, the Massachusetts State Building Code (780 CMR) states that for ground motions larger than the design levels, there is a low likelihood of a building collapse. Therefore, an earthquake beyond the NEIDL design basis, either the BDB (minor damage) or the MRF (total collapse) earthquake, is assigned to frequency category C (1 in 10,000 to 1 million years).

E.5 Variability and Uncertainty

Numerous variabilities and uncertainties affect the results in Section E.3. Table E-5 identifies the key variabilities and uncertainties, provides a discussion of each, and provides an estimate of the potential effect of each.

Table E-5. Summary of key variabilities and uncertainties.

Variability/ uncertainty	Discussion	Potential effect
Seismic capacity of the NEIDL structure	BSL-3 structure-The structure has been analyzed and demonstrated to comply with the 0.12 g seismic requirement. It was also determined that the fundamental period is slightly under 2 seconds. (BUMC 2005) Therefore, the seismic capacity is expected to be somewhat beyond this level; however, there is always a some chance of failure at lower levels.	Expected conservatism
	BSL-4 structure-The BSL-4 structure is isolated from the BSL-3 structure and expected to have a greater seismic capacity than the BSL-3 areas, but this has not been demonstrate and the extent to which the BSL-4 capacity is greater is unknown.	Potentially large conservatism
Annual exceedance probability of a 2-second 0.12 g earthquake	Interpretation of the USGS seismic hazard maps-The maps show ranges of spectral accelerations and the highest acceleration of each range was used; therefore, this value may be conservative.	Potential conservatism
	Linear extension of the curve generated from the USGS seismic hazard maps-A linear extrapolation of the 1-second curve was used even though the 2-second curve demonstrates that the curve bends downward. Therefore, this is a conservative approach, but the magnitude of the conservatism cannot be determined.	Potential conservatism
Frequency category for the BDB earthquake	BDB earthquake-The frequency category of C was assigned based on the projected annual exceedance probability of 1×10^{-5}, which fall in the middle of frequency category C. However, it is possible that the frequency of an earthquake beyond the design basis or a conditional probability of structural failure at a lower acceleration is greater or lower than expected.	Potentially conservative or non-conservative
	MRF earthquake- The frequency category of C was assigned based on the projected annual exceedance probability of 1×10^{-5}, which fall in the middle of frequency category C. This is considered conservative because for ground motions larger than the design levels, there is a low likelihood of a building collapse (780 CMR).	Conservatism

As shown in Table E-5, there are a number of uncertainties and variabilities associated with the frequency category of the earthquake; however, the assignment of frequency category C (1 in 10,000 to 1 million years) is considered conservative and appropriate. A frequency category for the MRF earthquake is considered especially conservative because total collapse of the NEIDL structure is expected to require a much more severe earthquake, which would have a lower frequency.

ATTACHMENT F. SELECTION OF AIRBORNE RELEASE AND RESPIRABLE FRACTIONS FOR THE MAXIMUM REASONABLY FORESEEABLE EARTHQUAKE

Per the guidance provided in Section F.8.2, this analysis did not include confinement by any biocontainment features anticipated to be present at the NEIDL. The only limitation on the respirable airborne release is the initial form, quantity, and concentration of the pathogens and the selection of airborne release fractions and respirable fractions. To ensure the proper selection of airborne release and respirable fractions, a comprehensive review of potential values was performed. All airborne release and respirable fractions associated with liquids in Section 3.1 of *DOE Handbook –Airborne Release Fractions/Rates and Respirable Fractions for Nonreactor Nuclear Facilities* (DOE 2000) were reviewed and evaluated. Table F-1 lists all release phenomena associated with liquids, lists the airborne release fraction and respirable fraction, and provides a basis for selecting or not selecting the phenomena.

The phenomenon selected for analysis is a spill. This phenomenon was selected because

- A spill is likely to result from an earthquake, while the occurrences of other phenomena (e.g., explosive stress) are more speculative.
- The respirable airborne release fraction for a spill is comparable to or greater than the fraction for most other phenomenon.
- A spill does not involve high temperatures, which could inactivate most pathogens. The few phenomena that have a higher respirable airborne release involve high temperatures (e.g., boiling phenomenon).

Therefore, this analysis is performed on the basis of spill phenomena. Per the guidance discussed in Section 2, the median release fraction and respirable fraction is used. Therefore, the airborne release fraction used in this analysis is 4×10^{-5} and the respirable fraction is 0.7.

Table F-1. Basis for selection of release fractions

Release phenomena	Release/respirable fraction	Basis for selection or non-selection
Thermal stress		
Non-boiling	Mean = ARF[a] 6E-7; RF[b] 1.0 Bounding = ARF 3E-5 / RF 1.0	This ARF × RF is less than the value for free-fall spill. In addition, given sufficient time the temperatures associated with near-boiling phenomena would inactivate most pathogens. Therefore, this phenomenon is dismissed from further consideration.
Boiling	Mean = ARF 1E-3; RF 1.0 Bounding = ARF 2E-3; RF 1.0	The ARF × RF is greater than the value for free-fall spill, but the temperatures that result in boiling will also inactivate most pathogens. There are no gas lines in the BSL-3 or BSL-4 rooms, so a severe fire is not expected. Therefore, this phenomenon is dismissed from further consideration.
Explosive stress		
Shock effect	Case-specific	Detonation phenomena require very specific conditions and are highly unlikely to occur. There are no gas lines in the BSL-3 or BSL-4 rooms, so there are no large fuel sources within the biocontainment spaces. Therefore, this phenomenon is dismissed from further consideration.
Blast effect	ARF 4E-3/hour (for duration of pulse)	Any blast effect would be a very short-duration spike (fractions of a second); therefore, this phenomenon is dismissed from further consideration.
High-pressure (200 psi) venting below the liquid surface level	Bounding = ARF 1E-3; RF 1.0	The pathogens are not stored in a pressurized configuration; therefore, this phenomenon is dismissed from further consideration.
Venting of low pressure liquids (< 0.35 MPa or 50 psi)	Bounding = ARF 5E-5; RF 0.8	This ARF × RF is less than the free-fall spill release factors (comparing bounding to bounding); therefore, this phenomenon is dismissed from further consideration.
Depressurization of liquid above boiling point	Bounding = ARF ≤ 1E-2; RF ≥ 0.6	Most pathogens would be inactivated at these superheated temperatures; therefore, this phenomenon is dismissed from further consideration.
Free-fall spill		
Aqueous solution [c]	**Mean = ARF 4E-5; RF 0.7** **Bounding = ARF 2E-4; RF 0.5**	**This is for a spill from a height of 3 m, which is greater than the room height.** ***This is the phenomena selected for use in the analyses.***
Slurries	Mean = ARF 2E-5; RF 0.7 Bounding = ARF 5E-5; RF 0.8	This ARF × RF is less than the value for aqueous solution spills and the suspensions do not have 40% solid content; therefore, this phenomenon is dismissed from further consideration.
Viscous solutions	Mean = ARF 4E-5; RF 0.7 Bounding = ARF 2E-4; RF 0.5	This ARF × RF is less than the value for aqueous solution spills and the suspensions anticipated at the NEIDL are unlikely to be this viscous; therefore, so this phenomenon is dismissed from further consideration.
Aerodynamic entrainment and re-suspension		
Indoors, heterogeneous surface, low airspeeds	ARR 4E-7; hr; RF 1.0	This value is much lower than the aqueous, free-fall spill for any reasonably time period, so it is dismissed from further consideration.
Indoors, heterogeneous surface, covered with debris, static conditions	ARR 4E-8/hr; RF 1.0	This value is much lower than the aqueous free-fall spill for any reasonably time period, so it is dismissed from further consideration.

Release phenomena	Release/respirable fraction	Basis for selection or non-selection
Outdoors, higher wind-speeds (30 mph)	ARR 4E-6/hr; RF 1.0	This value for about 8 hours duration is equivalent to the aqueous free-fall spill. Wind speeds of this magnitude occur less than 1% of the time at the urban site and would result in much lower concentrations than could result from an earthquake. Therefore, this phenomenon is dismissed from further consideration.
Outdoors, higher wind-speeds (50 mph)	ARR 9E-5/hr; RF 1.0	This ARF × RF is somewhat greater than the value for aqueous free-fall spill, but this is far from typical meteorological conditions. DOE NEPA Guidance recommends use of typical meteorology and these conditions are extreme, which makes the event beyond reasonably foreseeable. This phenomenon is dismissed from further consideration.

a. Airborne release fraction (ARF)

b. Respirable fraction (RF)

c. Release fractions for an aqueous free-fall spill were selected for the analysis.

1

Contents

G. TRANSPORTATION ANALYSIS

G.1 INTRODUCTION

NEIDL operations will include incoming and outgoing shipments of infectious pathogen samples during the life of the facility. Those infectious pathogen shipments could involve both truck and air modes of transportation. This analysis addresses the potential risks (i.e., frequency and consequences) to members of the public because of a loss of an infectious pathogen release resulting from a transportation accident. The risks associated with both truck and air shipment of infectious pathogens are estimated for members of the public in the vicinity of the three sites being evaluated (i.e., the urban, suburban, and rural sites).

G.1.1 Transportation Analysis Guidance

There is no directly applicable guidance pertaining to the analysis of accidents associated with transportation of infectious pathogens to the NEIDL. The NRC has observed the lack of guidance for facilities similar to NEIDL and concluded the following (NRC 2010):

> U.S. Department of Energy's (DOE) recommendations for the preparation of EISs contain some of the most detailed explanations and guidelines for discussing human health impacts in an EIS. Although DOE's recommendations for analyzing human health effects are limited to exposure to radiation and chemicals, they also are relevant to pathogen exposures.

DOE provides detailed guidance for transportation analyses in *A Resource Handbook on DOE Transportation Risk Assessment* (DOE 2002). The DOE transportation analysis guidance was written specifically for transportation of radioactive material, but the general methodology and some of the data are applicable for any transportation analysis and were used to guide this analysis.

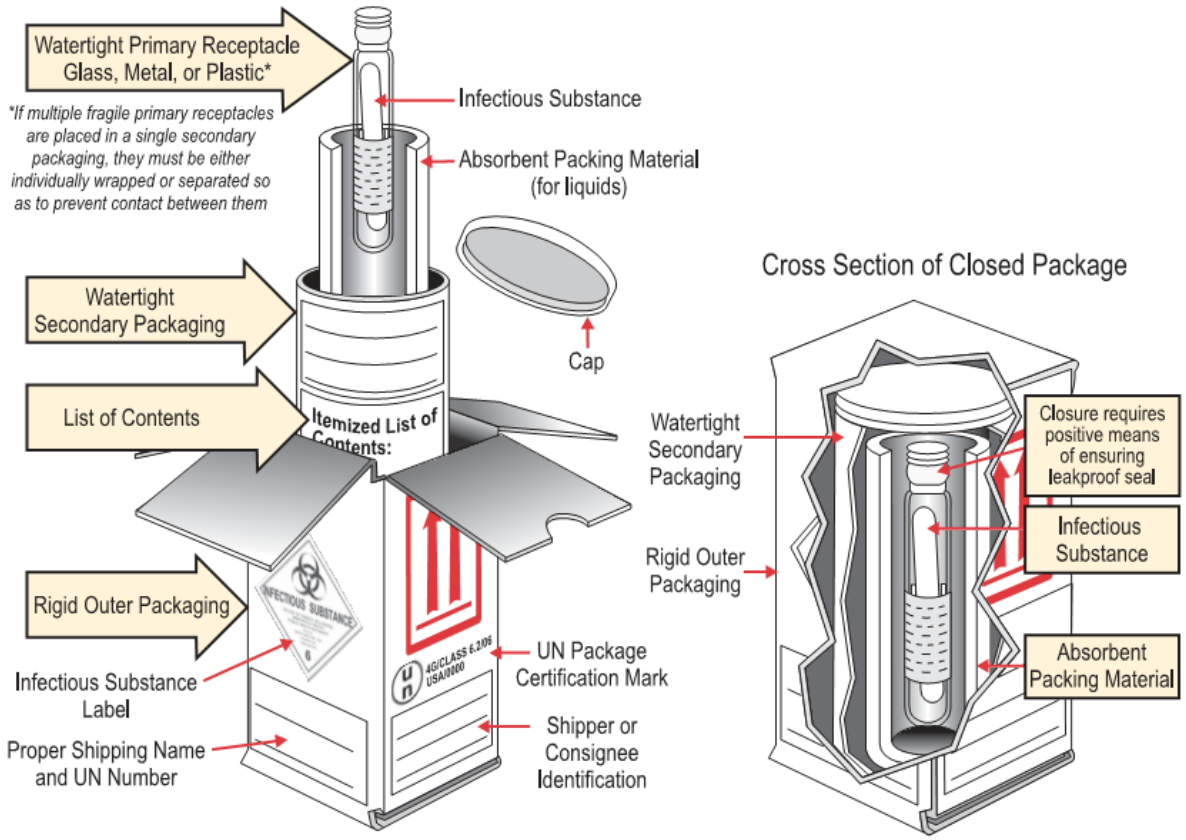
G.1.2 Packaging Requirements

The DOT Hazardous Material Regulations (49 CFR Parts 171-180) govern the transportation of infectious substances. The BSL-3 and BSL-4 pathogens being evaluated for NEIDL are classified as Category A Infectious Substances. The packaging requirements for those pathogens are provided in 10 CFR 173.196, *Category A infectious substances*. Category A infectious substances must be triple-packed, which includes the following [49 CFR 171.196(a)]:

- A leak-proof primary receptacle.
- A leak-proof secondary packaging. If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either wrapped individually or separated to prevent contact between them.

- A rigid outer packaging of adequate strength for its capacity, mass and intended use. The outer packaging must measure not less than 100 mm (3.9 inches) at its smallest overall external dimension.

An example of the triple-packaging required by 49 CFR 171.196 for infectious substance shipments is shown in Figure G-1.



Note 1: *The smallest external dimension of the outer packaging must not be less than 100 mm (3.9 inches)*
Note 2: *The primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa*
Note 3: *Follow package manufacturer's closure instructions*

Source: DOT 2006

Figure G-1. Example packaging and markings for category A pathogens.

DOT testing requirement for infectious substance packaging are provided in 49 CFR 178.609. Some of the requirements are dependent on the materials of construction for the packaging. Assuming that the primary and secondary containers are constructed of plastic, which is expected to be the case for NEIDL, the requirements are as follows (summarized from 40 CFR 178.609):

- *Impact test*—Samples must be subjected to free-fall drops onto a rigid, non-resilient, flat, horizontal surface from a height of 9 m (30 feet) in various orientations with no leakage from the primary container. This drop test must be performed promptly after conditioning the container at –18 °C (0 °F).
- *Puncture test*—Samples must be subjected to steel rod puncture test with a 38 mm (1.5 inches) diameter cylindrical steel rod. The mass for this test must be 7 kg (15 pounds) or the mass of the sample, whichever is greater. The test must be conducted with a vertical free fall from a height of 1 m (3 feet). One sample must be placed on its base and a second sample must be placed in an orientation perpendicular to that used for the first. In each instance, the steel rod must be aimed to impact the primary receptacle(s). For a successful test, there must be no leakage from the primary receptacle(s) following each impact.

G.1.3 Description of NEIDL Shipments

The likelihood and consequences of a transportation mishap are dependent on details of the shipment. All NIEDL shipments must comply with the DOT regulations addressed in Section G.1.2; however, additional shipment details are important to this analysis. The following paragraphs describe those details, some of which apply to all shipments and some of which apply to only truck or air shipments. The descriptions apply to all BSL-3 and BSL-4 shipments to or from NEIDL.

G.1.3.1 All Shipments

Pathogen configuration—Bacteria could be shipped in a liquid, frozen, or solid form (e.g., agar in a tube). For this analysis, all bacteria are assumed to be shipped in a liquid suspension, such as a 10–15 percent glycerol (aqueous) at ambient temperature. The liquid form is assumed here for bacteria because that form is more vulnerable to release, which is a conservative assumption (i.e., tends to overestimate the risk). If bacteria are shipped in solid form, the consequences of any packaging breach would be lower because a solid form (e.g., agar in a tube or frozen liquid) would have lower release fractions.

Viruses will be sent shipped in a tissue culture broth after harvesting, and it is likely that they will be shipped in a frozen form. A frozen sample would have a lower release fraction than a liquid, so releases could be higher if viruses are shipped in liquid form. This analysis considers both the liquid and the frozen forms.

Pathogens per shipment—Only one infectious pathogen sample is assumed to be included in each shipment, which is expected to be the case. The analysis is not particularly sensitive to that assumption

because synergistic effects from concurrent exposure to multiple infectious pathogens are considered minimal.

Primary container—The DOT-compliant primary (innermost) containers for shipments to and from NEIDL are assumed to be sealed, shatter-resistant, 2-mL containers similar to the one shown in Figure G-2. Experience at similar facilities indicates that 2-mL tubes are typically used for shipments. The example container is made of polypropylene with an approximate length of 50 mm and a diameter of about 11 mm. The container has a screw cap, which will be reinforced with adhesive tape. Containers of this type are extremely robust and can withstand forces associated with accelerations of 20,000 g (Sarstedt 2011).

Source: Sarstedt 2011

Figure G-2. Example primary container.

Secondary container—The DOT-compliant secondary container serves multiple functions: it contains absorbent material that will absorb liquids potentially leaking from the primary container, provides a leak-proof secondary container, shields the primary container from external forces, and, when multiple primary containers are shipped in the same secondary container, includes a means of separating primary containers from each other. Either the primary or the secondary container must be capable of withstanding an internal pressure of 95 kPa (approximately 14 psi) without leaking (10 CFR 173.196).

Tertiary container—The DOT-compliant rigid, tertiary packaging must be at least 100 mm (4 in) in its smallest external dimension. Substances shipped frozen must carry the refrigerant outside the secondary container. If dry ice is used to keep the infectious pathogen frozen, the packaging must permit the release of carbon dioxide gas (10 CFR 173.196).

Over-packing—The DOT-compliant triple packaging will be placed in a non-crushable, liquid-tight, solid container for an added layer of safety (Robbins 2011). A Pelican™ 0370, 24-inch Cube Case meets that requirement and is shown in Figure G-3. The Pelican case provides an additional measure of safety beyond those provided by the packaging required by the DOT regulations. The Pelican case has been independently tested for vibration, low temperature, dry heat, impact, dust, and water immersion and found to meet various international specifications (Pelican 2011); however, the capacity of the case to withstand crushing loads is not known.

Figure G-3. Pelican case

G.1.3.2 Truck Shipments

Carrier—Shipments will be made via carriers specializing in expedited, increased security shipping services accustomed to handling Category A hazardous materials. Those carriers will provide exclusive-use vehicles (i.e., vehicles that do not contain cargo from any other shipper) (Robbins 2011). The shipping agent (i.e., the shipper) would fully comply with all the provisions of the federal and international regulations and requirements for transportation.

Vehicle—It is assumed that shipments will be made via a large truck (e.g., 10,000 lbs. or larger) with an enclosed bed (Robins 2011).

Loading—The over-pack is then secured to the middle of the truck bed away from the walls (Robins 2011), thereby, protecting it from impacts with the walls or from other vehicles that could impact the walls in a collision.

Global positioning system (GPS) tracking—Shippers must have the ability to provide GPS tracking of packages or vehicles as determined appropriate and approved by Boston University Mecical Center (BUMC) (Robins 2011). GPS tracking provides for prompt detection of potential mishaps, thereby ensuring for prompt emergency response and plus the opportunity to provide emergency responders with important guidance.

Number of shipments—Per BUMC policy, all shipments to or from locations in the United States will be made via truck whenever feasible. For this analysis, it is assumed an average of one truck shipment per year to the NEIDL would occur for each of the 13 pathogens, for a total of 13 truck shipments (Robbins 2011).

In addition to solely truck shipments, there could also be air shipments of infectious pathogens to or from NEIDL. Those air shipments would include a truck-based component that delivers the package from (or to) Boston Logan International Airport to the laboratory. For this analysis it is assumed that there would be one incoming and one outgoing air shipment each year, so there would be two truck shipments between Boston Logan International Airport and each of the three sites (Robbins 2011).

Origin and destination of shipments—Most infectious pathogen shipments are expected to originate at Bethesda, Maryland; Frederick, Maryland, Hamilton, Montana, and Atlanta, GAGeorgia. The number of shipments from each location is not known and is not an important factor for this analysis.

Driver—Trained professional drivers dedicated to the shipment will operate the cargo vehicles.

G.1.3.3 Air Shipments

Number of shipments—Per BUMC policy, all shipments to or from locations in the United States will be made via truck where feasible; however, there is a potential for air-based shipments to or from the NEIDL. For this analysis, it is assumed that an average of one ground shipment per year to and one from the NEIDL will occur (Robins 2011). For this analysis, it is assumed the shipment could involve any of the 13 pathogens.

Origin and destination of shipments—The air-based shipments were assumed have Boston Logan International Airport their origin or destination when evaluating each of the three sites.

G.1.4 Department of the Army Packaging Testing

Triple-packaged systems similar to required for all Category A pathogen shipments were tested by the Department of the Army (DOA) in the 1960s (DOA 1969). The tests consisted of a series of drop-tests from heights of 500 and 1,000 ft with varying packaging configurations including glass and plastic bottles. The drop heights (i.e., 500 and 1,000 ft) are well beyond the 30-ft (9-m) drop test requirements (49 CFR 178.609). The bottles were over-packed in two outer containers (metal and fiberboard) with either cotton or vermiculite as packaging between the containers. The packages were dropped onto soil, concrete, and macadam surfaces. The purpose of the tests was to determine which packaging configurations could survive such drops. Despite the extreme test conditions, several of the packaging configurations resulting in one or more of the three nested containers remaining leak-tight. In addition, the DOA report states that "containers similar to those tested in May 1961 later withstood the crash of a C-119 aircraft at 138 miles per hour" into a concrete wall (DOA 1969).

Two of the packaging systems tested had plastic primary containers with metal secondary containers, different packing materials (cotton and vermiculite), and different outer containers (metal and fiberboard). Both of those systems passed the 500-ft drop onto soil and one passed the 1,000-ft test onto soil. The packages tested by DOA differ in several important respects from the packages used for shipping pathogens to NEIDL but the test can be used to gain insight into the survivability of NEIDL packages:

- The primary container volumes in the DOA tests ranged from 450 to 1,300 mL, while the NEIDL primary containers are expected to be 2-mL tubes. The 2-mL tubes have much less mass, so the impact loads would be much smaller than those used in the DOA tests. In addition to the lower impact loads, the 2-mL containers will tend to have greater strength-to-weight ratio.
- The plastic primary containers in the DOA tests were made of polyethylene, while the containers expected to be used for NEIDL are made of polypropylene. Polyethylene is softer and not as

strong as polypropylene, so the NEIDL containers are less likely to eject caps or breach.

- The Pelican case provides a large margin of safety for the packages. The shell and foam liner of the case would absorb and distribute much of the impact loads.

On the basis of the partial survival of the DOA packages, it is expected that the NEIDL packaging (triple packaging plus non-crushable liquid tight solid container) would survive both the 500-ft and 1,000-ft DOA drop tests, as well as the aircraft crash test without leakage from the primary container.

G.2 METHODOLOGY

The infectious pathogen release frequency is dependent on the truck or aircraft crash frequency and the conditional probability that a package will breach in a crash. The conditional probability of a breach in a crash is dependent on the ability of the package to withstand the conditions that could be associated with a severe crash. A qualitative assessment of the ability to withstand a severe crash is presented below. In addition, the methodology used to estimate the frequency of a release is presented below.

G.2.1 Testing Requirements for Severe Hypothetical Accidents

The triple-packaging must pass the requirement of 49 CFR 173.196, as discussed in Section G.1.2, however, that does not ensure that the packaging will survive all potential accident conditions. The requirements for testing of Type B radioactive material shipping containers are provided in 10 CFR 71.73 are intended to simulate the conditions of severe hypothetical conditions and those test go beyond the requirements of 49 CFR 173.196. While the NEIDL packaging is not required to comply with them, the requirements of 10 CFR 71.73 do provide insight into the type of severe hypothetical conditions that could occur. Those tests include consideration of fire, impact loads, puncture, and crushing loads on the package. The following paragraphs identify the 10 CFR 71.73 testing requirements and discuss expected performance of NEIDL packages for each test to provide some insights into the survivability of the NEIDL packaging.

Free drop—This test consists of a free drop of the package from a height of 9 m (30 ft) onto a flat, essentially unyielding, horizontal surface striking in an orientation that is expected to produce the maximum damage. This 10 CFR 71.71 requirement is equivalent to the requirement for packaging of infectious substances (i.e., 49 CFR 173.196), as discussed in Section 1.2. The Pelican case might or might not be damaged in the test, but the case will distribute and absorb much of the impact load. The NEIDL packaging is expected to pass this test because the triple-packaging must pass the equivalent 49 CFR 173.196 test and the Pelican case will likely enhance its ability to withstand the free drop.

Crush—This test consists of dropping a 500-kg (1,100-lb) plate with a cross-section of 1 m (40 in) by 1 m (40 in) from a height of 9 m (30) onto the package. This test is not required for all radioactive material packages but would be required for containers that are the size of the NEIDL package. The Pelican case is unlikely to survive the test without extensive damage. The survivability of the primary container depends on the amount of dynamic energy absorbed by the Pelican case and the extent to which the case distributes the static load. While the survival of the primary container is unknown, it is unlikely that any aerosol or liquid would be released from the test because the Pelican case foam liner would compress around the inner packaging and prevent any leakage. Therefore, the NEIDL packaging could be breached but there might not be any release.

Puncture—This test consists of free dropping a package 1 m (40 in) in the position of maximum expected damage onto the upper end of a 15-cm (6-in) diameter bar. The size of the bar is smaller in the 49 CFR 173.198 test, so the triple packaging is expected to survive this test. The Pelican case might or might not survive this test.

Thermal—This test consists of a fully engulfing fire with a flame temperature of at least 800 °C (1,475 °F) for 30 minutes. The NEIDL packaging would not withstand the test; however, the test is not relevant for infectious pathogen shipments because the pathogens would be inactivated at temperatures much lower than those. The NEIDL packaging would fail the test, but there would be no risk to the public or the environment because the infectious pathogens would be inactivated.

Immersion—The package must be subjected to water pressures of 150 kPa (21.7 lbf/in2) gauge, which is equivalent to immersion in 15 m (50 ft) of water. 40 CFR 173.196 does not require a similar test for packaging of infectious substances. The primary or secondary container is required to withstand an internal pressure of 95 kPa (approximately 14 psi), which is about 60 percent of this criterion. The 2-mL primary containers are expected to survive the test according to their ability to withstand 20,000 g of acceleration (see Section G.1.3.1).

On the basis of the 10 CFR 71.73 criteria for severe hypothetical accident conditions, free drop, puncture, immersion, and thermal conditions are of minimal survival concern for NEIDL packages; the greatest concern is a crushing load. Therefore, this analysis will focus primarily on crushing loads for severe accident conditions.

G.2.2 Truck Shipments

Consistent with DOE NEPA guidance and the DOE transportation analysis guidance, this analysis used a sliding scale that "analyzes issues and impacts with the amount of detail that is commensurate with their

importance" (DOE 2004, 2002). Because the risks are relatively low and to maximize transparency, this analysis did not use the DOE computer codes (e.g., routing or risk analysis codes), but instead used the general methodology and input parameters provided by DOE for transportation analyses (DOE 2002) to perform simplified and more transparent calculations.

The frequency for ground accidents of a specific type is calculated by the following equation:

$$F = D \times R \times P \qquad \text{Equation G-1}$$

where

- F is the frequency of the event (/yr)
- D is the travel distance (km/yr)
- R is the accident, injury, or fatality rate (/km)
- P is the conditional probability, or series of conditional probabilities, that lead to the outcome being considered

Transportation-related injury, fatality, and infectious pathogen release frequencies were calculated using Equation G-1. Accident, injury, and fatality rates associated with heavy trucks (i.e., greater than 10,000 lbs) are provided in the DOE transportation analysis guidance (DOE 2002) and are presented in Table G-1. Those transportation accident rates include accidents that do not necessarily result in injuries or fatalities, but might result in vehicle damage only.

Table G.2-1. National average accident rates

Road type	Accident rate (accidents/km)	Injury rate (injuries/km)	Fatality rate (fatalities/km)
Interstate highways	3.00×10^{-7}	2.25×10^{-7}	9.60×10^{-9}
Primary highways	2.78×10^{-7}	2.17×10^{-7}	1.78×10^{-8}
Other highways	4.56×10^{-7}	3.33×10^{-7}	1.71×10^{-8}
Combined total	3.21×10^{-7}	2.39×10^{-7}	1.42×10^{-8}

Source: DOE 2002, Tables 6-20, 6.38, and 6.39

The DOE transportation analysis guidance notes that those rates are based on shipment of all types of goods, but the rates for shipment of radioactive material, and presumably other hazardous materials, have lower accident rates. The pathogens addressed in this analysis are categorized as Class 6, Division 6.2 materials (i.e., biohazards) hazardous materials by DOT, Federal Motor Carrier Safety Administration (49 CFR 173.176). The accident rate for Class 6 materials is 2.29576×10^{-7} accidents per mile (Battelle 2001) or 1.43×10^{-7} accidents per km. The accident rate of 1.43×10^{-7} accidents per km was used for this analysis because it is the most relevant basis, but the injury and fatality rates of Table G.2-1 were used.

The conditional probabilities are for release are scenario dependent and are presented with the results.

G.2.3 Air Shipments

The guidance provided in *Accident Analysis for Aircraft Crash into Hazardous Facilities*, DOE STD 3014-2006 (DOE 2006) is used as the basis for this analysis of aircraft crash. The aircraft is assumed to be carrying an infectious pathogen to or from the Boston Logan International Airport, which is the airport expected to be used for all three sites being evaluated. The infectious pathogen is assumed to be transported via a certified commercial air carrier (i.e., a plane with 30 seats or more or a maximum payload of 3,401 kg [7,500 lbs] or more) in accordance with 14 CFR 121. Table G.2-2 provides the crash rate for commercial air carriers.

Table G.2-2. Aircraft crash rates

Aircraft operation	Crash rate
Commercial aviation—air carrier (takeoff)	1.9×10^{-7}
Commercial aviation—air carrier (landing)	2.8×10^{-7}

Source: Table B-1, page B-3, of DOE STD 3014-2006 (DOE 2006)

G.3 RESULTS

This section documents the frequency and consequence analyses for both truck and air shipments events that have the potential to result in an infectious pathogen release.

G.3.1 Truck Shipments

G.3.1.1 Frequency

As discussed in Section G.1.3, an average of 15 infectious pathogen shipments per year are assumed, 13 that are solely truck shipments and 2 that involve a combination of truck and air. To allow direct comparison of the sites, it is assumed that those same 15 shipments per year are associated with each of the three sites.

The frequency of a release in the vicinity of each site is calculated on the basis of a travel distance of 10 km (6 miles). The 10-km distance is greater than the distance recommended by the U.S. Nuclear Regulatory Commission to define the vicinity of a site for environmental justice purposes (Nuclear Regulatory Commission 2003). Therefore, the total distance for truck shipments is 150 km.

The injury and fatality rates for *other highways* was used for this analysis for all three sites because a portion of the travel at all three sites would be on other highways (i.e., non-interstate and non-primary highways), and it is the highest rate provided in Table G.2-1.

On the basis of the evaluation of the likely survivability of the NEIDL packaging (see Section G.2.1) the only type of truck accidents that have the potential to breach all containers and result in an infectious pathogen release are those that result in severe crushing forces. A review of the National Transportation Safety Board characteristics of truck accidents (NTSB 2002, Table B-7) identified only two categories of accidents that are judged to have the potential to result in severe crushing forces, namely, overturns (rollover) and collisions involving trains. The data for intrastate travel were selected because they include a greater fraction of travel on roadways other than interstate highways. The fraction of accidents that involved an overturn (rollover) was 2.9 percent and those involving collision with a train was 0.2 percent, for a combined total of 3.1 percent of all accidents.

The frequencies of accidents, injuries, and fatalities were calculated using equation G-1 and the values identified above. Those frequencies are provided in Table G.2-3.

Table G.2-3. Accident, injury, and fatality frequencies for truck shipments

Road type	Accidents	Injuries	Fatalities
Other highway rate (/km)	4.56×10^{-7}	3.33×10^{-7}	1.71×10^{-8}
Distance traveled (km/yr)	150	150	150
Conditional probability of overturn or train collision	0.031	0.031	0.031
Frequency (/yr)	2.1×10^{-6}	1.5×10^{-6}	8.0×10^{-8}

Only a small fraction of those overturns would result in an infectious pathogen release. There are no data to allow estimation of the conditional probability of a release, but the potential was qualitatively evaluated. In most cases, the truck is expected to merely roll onto its side, which does not challenge the pathogen packaging. In complete or multiple rollover events, the bed enclosure and the truck cab would both continue to protect the NEIDL package from crushing forces, even if they were severely damaged. Similarly, many collisions involving a train would not compromise the package because the truck would be slid along the tracks without any crushing forces being applied to the package. The fraction of the overturns and train collisions that result in severe crushing forces that might breach the NEIDL package is not known but is expected to be quite small. The humans involved in those events are far more vulnerable to impact loads than the NEIDL package, so the frequency of fatalities from overturns or train collisions is used as upper bound on the frequency of infectious pathogen releases. Therefore, the frequency of a

truck accident resulting in an infectious pathogen release is estimated to be less than 8×10^{-8}/yr, which is in the BEYOND REASONABLY FORESEEABLE frequency category ($< 10^{-6}$/yr).

The accident, injury, and fatality frequencies for the entire distance (e.g., from CDC in Atlanta to NEIDL) of all truck shipments would have higher frequencies. Those frequency calculations were not performed for the following reasons:

- The majority of the routes would be identical for all sites and, therefore, such calculations would not provide insights into the risks to the population in the vicinity of the laboratory or the differences between sites.
- No specific scenarios were identified that could credibly lead to an aerosol release because of the multilayered packaging system. The analysis above uses the frequency of a fatality as the upper bound for the frequency of an aerosol release, but only a small fraction of the fatal crashes have the potential to result in a release. It would be speculative to estimate the actual conditional probability of an aerosol release because there are no tests or empirical data to support such an analysis.

G.3.1.2 Consequences

Because the frequency is in the BEYOND REASONABLY FORESEEABLE frequency category, detailed consequence calculations were not performed for truck shipment accidents. However, the consequences of a truck shipment accident are qualitatively considered to ensure that the consequences are not grossly larger than more frequent events.

Crushing forces capable of breaching the inner container will not result in public exposures because the foam interior of the Pelican case will confine any liquid or aerosol release. If the inner container is breached after being ejected from the Pelican case, a highly improbable event, then the potential exists for an aerosol release. The virus samples are expected to be frozen, which not result in a significant aerosol release. The consequence calculations for the maximum reasonably foreseeable (MRF) release earthquake (see Section F.8.3.4 of Appendix F) estimated that on average, a person 30 m from a ground-level release of the aerosol resulting from a 150-mL liquid suspension spill at any of the three sites would receive three units (the units were defined on a pathogen-specific bases in Section F.3 of Appendix F) for the pathogen resulting in the highest exposure. The exposure level for all other pathogens would be one to three orders of magnitude less. The transportation packages contain 2-mL samples and the exposure levels would scale approximately proportionally to the volume, so the 2-mL spill would result in exposures that are about 1.3 percent of the value for the MRF earthquake. Therefore, the average exposure 30 m from a

hypothetical aerosol release from a 2-mL spill is less than 0.4 (i.e., 3 × 0.013) unit for all pathogens. The average exposure levels would likely be greater at closer distances.

G.3.2 Air Shipments

G.3.2.1 Frequency

One inbound and one outbound pathogen air shipments are estimated per year (see Section G.1.3). The crash rate is 1.9×10^{-7} for takeoffs and 2.8×10^{-7} for landings, as shown in Table G.2-2. Therefore, the crash rate for inbound flights carrying a pathogen is 1.9×10^{-7}/yr and for outbound flights carrying a pathogen is 2.8×10^{-7}/yr, for a total of 3.7×10^{-7}/yr for all air shipment of a pathogen. Therefore, a pathogen-carrying aircraft crash is in the BEYOND REASONABLY FORESEEABLE frequency category ($< 10^{-6}$/yr).

An aircraft crash does not necessarily result in an infectious pathogen release. On the basis of the Army tests results discussed in Section G.1.4, the NEIDL packaging is expected to survive drops from both 500 ft and 1,000 ft, as well as the aircraft crash test without leakage from the primary container (see Section G.1.4). Therefore, a pathogen-carrying aircraft crash that results in an infectious pathogen release is certainly in the BEYOND REASONABLY FORESEEABLE frequency category ($< 10^{-6}$/yr).

G.3.2.2 Consequences

Because the frequency is in the BEYOND REASONABLY FORESEEABLE frequency category, consequence calculations were not performed for an air shipment accident; however, the consequences of an air shipment accident are qualitatively considered. The aerosol release and exposure from an aircraft crash are expected to be similar to those of a truck crash, so the discussion in Section G.3.1.2 is applicable, and exposure levels would average a fraction of a unit for all pathogens.

G.3.3 Summary

This analysis demonstrates that truck and air shipment accidents near each of the three sites do not pose a significant risk to the public within 10 km of any of the three sites for the following reasons:

- The accident and injury frequency for a truck crash within 10 km of any of the three sites is in the LOW frequency category ($< 10^{-4}$ to e 10^{-6}/yr). That would be an unperceivable increase in the number of crashes and injuries.
- Conservative calculations show that the frequency of an air or truck shipment crash that results in a loss of biocontainment within 10 km of any of the three sites evaluated is BEYOND REASONABLY FORESEEABLE frequency category ($< 10^{-6}$/yr).

- If a crash occurs that breaches the triple packaging and the Pelican case, the multiple layers of packaging are expected to confine all or nearly all the release.
- If the multiple layers of packaging do not confine the release, the exposure from a transportation accident is about 1.3 percent of the exposure calculated for the MRF release earthquake. Therefore, the MRF release earthquake frequency and consequence are bounded by those of a transportation accident.

G.4 REFERENCES

10 CFR 71.73 — *Packaging and Transportation or Radioactive Materials*, 10 CFR Part 71, Subpart F, §71.73, Hypothetical Accident Conditions. Available on the web at: http://www.nrc.gov/reading-rm/doc-collections/cfr/part071/part071-0073.html Accessed 05/20/2011.

49 CFR 173.196 — *Category A infectious substances,* 49 CFR 173.196. Available on the web at: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=853b7a2b2c39c2d408629eaa641281f9&rgn=div8&view=text&node=49:2.1.1.3.9.5.25.31&idno=49. Accessed May 24, 2011.

49 CFR 178.609 — *Test requirements for packagings for infectious substances.,* 49 CFR 178.609. Available on the web at: http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=19e6ff1e9aaa247970b66046d3520827&rgn=div8&view=text&node=49:2.1.1.3.14.10.39.10&idno=49. Accessed May 24, 2011.

Battelle 2001 — *Comparative Risks of Hazardous Materials and Non-Hazardous Materials Truck Shipment Accidents/Incidents,* Battelle Memorial Institute, prepared for the Federal Motor Carrier Safety Administration, March 2001. Available on the internet at: http://www.fmcsa.dot.gov/documents/hazmatriskfinalreport.pdf Accessed 6/26/2011.

DOA 1969 — Department of the Army, Fort Detrick, Technical Study 67, Containers for Chemical/Biological Agents Drop-Tested from Aircraft, M. Barbeito and A. Wedum, March 1969. Available on the web at http://www.dtic.mil/cgi-bin/GetTRDoc?Location=U2&doc=GetTRDoc.pdf&AD=AD0686313 . Accessed 05/20/2011.

DOE 2002 — *A Resource Handbook on DOE Transportation Risk Assessment,* DOE/EM/NTP/HB-01, July 2002. Available on the web at: http://nepa.energy.gov/documents/DOETransportationRiskAssessment.pdf Accessed 05/20/2011.

DOE 2004 — *Recommendations for the Preparation of Environmental Assessments and Environmental Impact Statements,* Second Edition, December 2004. Available on the web at:

http://nepa.energy.gov/nepa_documents/TOOLS/GUIDANCE/Volume2/2-10-greenbook-recommendations.pdf. Accessed 05/20/2011.

DOE 2006 — DOE STD 3014-2006, Accident Analysis for Aircraft Crash into Hazardous Facilities, U.S. Department of Energy, available online at http://www.hss.doe.gov/nuclearsafety/ns/techstds/standard/std3014/std3014.pdf . Accessed 03/26/2011.

DOT 2006 — Transporting Infectious Substances Safely, Guide to Changes, Effective October 1, 2006. Available on the web at: http://www.aps.anl.gov/Safety_and_Training/Hazardous_Materials/Transporting%20Infectious%20Substances%20Safely.pdf.pdf . Accessed May 24, 2011.

NRC 2010 — *Evaluation of the Health and Safety Risks of the New USAMRIID High Containment Facilities at Fort Detrick, Maryland*. Committee to Review the Health and Safety Risks of High Biocontainment Laboratories at Fort Detrick Board on Life Sciences Division on Earth and Life Studies, National Research Council of the National Academies, 2010. Available on the web at http://www.nap.edu/openbook.php?record_id=12871 . Accessed August 26, 2010.

NTSB 2002 — *Analysis of Intrastate Trucking Operations,* Safety Report NTSB/SR-02/01, National Transportation Safety Board, Washington, DC, Adopted March 28, 2002. Available on the internet at: http://www.ntsb.gov/doclib/safetystudies/SR0201.pdf Accessed 05/23/2011.

Nuclear Regulatory Commission 2003 — *Environmental Review Guidance for Licensing Actions Associated with NMSS Programs,* NUREG-1748. Available on the internet at: <http://www.nrc.gov/reading-rm/doc-collections/nuregs/staff/sr1748/>. Accessed April 2011.

Pelican 2011 — Pelican 0340 Protector 18-inch Cube Case, Description and Specifications, available on-line at http://www.pelican-case.com/0340.html . Accessed May 14, 2011.

Robbins 2011 — T. G. Robbins, Executive Director of Public Safety, Boston University letter to F. Gallegos, Tetra Tech, dated July 18, 2011.

Sarstedt 2011 Sarstedt Screw Cap Micro Tube Description, available online at http://www.sarstedt.com/php/tube.php?artnr=72.694.006&lnr=

Revised 20 November 2011

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Appendix H

Final Project Report: Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment

Summary

The NIH is sponsoring a risk assessment of the National Emerging Infectious Diseases Laboratories (NEIDL) at Boston University Medical Center. The risk assessment will include a comprehensive qualitative analysis as well as a quantitative component with a focus on 13 pathogens selected by a Blue Ribbon Panel. Because information in the literature on four key parameters is not fully adequate for direct use by the quantitative component of the assessment, the NIH convened a distinguished expert panel and conducted an expert elicitation. The elicitation was conducted using a standard methodology, the modified Delphi, in a manner consistent with accepted practice. Panelists were asked to estimate the infectivity of aerosols for the 13 pathogens; estimate the half-life of the 13 pathogens in an aerosol on a cool humid night and a dry sunny day; estimate the percentage increase in vulnerability to disease and death among five population subgroups; and corroborate the TetraTech team's choices of the basic reproduction number (R_0) for five of the 13 pathogens. Through careful application of a widely-used and accepted technique for expert elicitation in biomedicine, the exercise provided defensible supplementary inputs to quantitative component of the comprehensive NEIDL risk assessment.

Introduction

The NIH, with the scientific and technical advice of a Blue Ribbon Panel, is sponsoring a risk assessment of the National Emerging Infectious Diseases Laboratories (NEIDL) at Boston University Medical Center.* The risk assessment is being conducted by TetraTech Inc. Part of the assessment will include analyses of an array of pathogens and the events leading to exposure of individuals to these pathogens, including an assessment of potential differences at three different locations. The following 13 pathogens were selected for analysis by the Blue Ribbon Panel to cover a range of relevant characteristics:

* The full name of the Blue Ribbon Panel is the NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratories at Boston University Medical Center. See http://nihblueribbonpanel-bumc-neidl.od.nih.gov/default.asp

- SARS-associated coronavirus,
- 1918 pandemic influenza virus,
- Andes hantavirus,
- Junin virus,
- Lassa fever virus,
- Marburg virus,
- Ebola virus,
- Nipah virus,
- Rift Valley
- Fever virus,
- Tick-borne encephalitis complex virus,
- *Yersinia pestis,*
- *Francisella tularensis,* and
- *Bacillus anthracis*.

In addition to a comprehensive qualitative analysis, the NIH believes that selected quantitative analyses are important to a thorough assessment and for fulfilling commitments made by the NIH and the Blue Ribbon Panel to the Boston community and other stakeholders. For this reason, the NIH has elected to include high quality quantitative modeling in the risk assessment. Mathematical models will estimate the risk of initial infection after release for all 13 pathogens. In addition, mathematical models will estimate the potential secondary spread of five representative pathogens for which human transmission data are sufficient to support modeling. These are:

- SARS-associated coronavirus,
- 1918 pandemic influenza virus,
- Rift valley fever virus,
- Ebola virus, and
- *Yersinia pestis*.

The quantitative component of this risk assessment, like all infectious disease modeling, necessarily entails making a number of assumptions. The evidence for these assumptions varies, but it is rare to have adequate empirical data that bear directly on the case to be modeled. In some cases, data are lacking. For example, there are no adequate data on dose-response for infection by the pathogens under study over the full range of likely exposure. In other cases, several sources of data - none of which may strictly apply to the population being modeled - may need to be reconciled. For example, several estimates are often available for the basic reproduction number (R_0), a key parameter for mathematical modeling of secondary transmission.[†] Therefore, expert judgment is necessary to establish reasonable initial values for these and other essential parameters needed for modeling of initial infection and secondary transmission.

The need for expert judgment is not confined to parameter estimates for the quantitative element; it is an integral part of this and every risk assessment. The required judgment is often applied quite

[†] The basic reproduction number is defined as “the mean number of secondary cases generated by a single infective case in a totally susceptible population" (Gail)

informally by an individual or small group of professionals - for example, in choosing model structure and certain parameters. Informal approaches are also the most common way of estimating baseline parameters in infectious disease modeling. Many studies have based parameter estimates on the investigators' existing knowledge supplemented by literature searches and/or consensus reached in meetings, by telephone, or by other means (Halloran and personal communication, Steven Eubank). Because of the importance of the NEIDL risk assessment, the NIH elected to go beyond informal expert judgment and conduct a formal elicitation of outside expert opinion to estimate parameter values for which adequate empirical data is lacking.

The formal elicitation of expert opinion is widely endorsed as a means of filling knowledge gaps, and is routinely used in a number of fields. The Nuclear Regulatory Commission has recommended that "expert elicitation should be considered" when "Empirical data are not reasonably obtainable, or the analyses are not practical to perform"(Kotra). The Environmental Protection Agency has stated, "In general, experts can be used to quantify the probability distributions of key parameters and relationships"(Expert Elicitation Task Force). In one report, the National Research Council endorsed "models based not only on available data but also on expert judgment" and in another it stated, "the rigorous use of expert elicitation for the analyses of risks is considered to be quality science" (Committee on Estimating the Health-Risk-Reduction Benefits, Committee on Risk Assessment of Hazardous Air Pollutants).

The NIH convened a distinguished expert panel and conducted an expert elicitation using the modified Delphi technique, a group process that is standard in biomedicine and many other settings (Kotra, Office of Management and Budget). This expert elicitation focused on four groups of needed parameters:

- infectivity,
- atmospheric stability,
- population vulnerability, and
- rate of spread.

Infectivity: The first area requiring expert elicitation relates to the likelihood of initial infection. Determining the likelihood of infection after exposure to a specified dose of a pathogen is an essential element in establishing the risk of that exposure and, therefore, in modeling the consequences of an event-pathogen pair on the public. This property, which can be termed infectivity, depends on many factors including the properties of the organism, the route of exposure, and the characteristics of the host. The traditional measure of infectivity is the Infectious Dose 50 (ID_{50}), which is the dose necessary to infect 50% of subjects (ID_{50}).

Atmospheric stability: The second area requiring expert elicitation is the stability of organisms in the atmosphere. Estimating the stability of a pathogen in the air is an essential element in determining the dose of that pathogen received after an aerosol exposure. However, the literature provides little information regarding the question of atmospheric stability for the pathogens under study in the NEIDL risk assessment (Appendix 1).

Population vulnerability: The third area requiring expert elicitation relates to the possibility that a release of a pathogens may affect medically vulnerable subpopulations (e.g., the elderly) than the general population. These subpopulations are thought to be more likely to contract infections and have severe or fatal outcomes from infectious diseases. This is a concern for the NEIDL risk assessment because persons in medically vulnerable subpopulations are overrepresented in rural areas and in environmental justice communities such as South Boston. However, how much more vulnerable these populations are to various diseases is not well known. This is true even for HIV/AIDS, where estimates are highly dependent on the usually unknown distribution of CD4 cells in a population (Kaplan). Moreover, it is especially true for the pathogens studied in the NEIDL risk assessment because very little is known about differential vulnerability to exotic and aggressive diseases.

Rate of spread: The fourth area requiring expert opinion is the rate of spread as described by the basic reproduction number or R_0. Several estimates are available in the literature for the five pathogens that will undergo full mathematical modeling of secondary transmission (Appendix 1). The risk assessment team used this literature in selecting values for use in modeling, but external corroboration of their choices is desirable.

Approach

While expert opinion can be obtained in many ways, formal elicitation processes are preferred in order to maximize validity, reliability, and consistency. This project sought to take advantage of an expert panel's group wisdom with minimal interpretation by moderators or other staff. Therefore, the NIH preferred a modified Delphi exercise to individual interviews, a simple consensus meeting or call, or other approaches.

The Delphi method is the most widely accepted group process used for expert elicitation (Brook, Dalkey 1962, Dalkey 1963, Fink, Jones, Linstone, Powell). In 1974, it was estimated that approximately 10,000 studies on technological forecasting and policy analysis employed this approach (Linstone). The U.S. Office of Management and Budget accepts that "expert judgment as revealed, for example, through Delphi methods" is an acceptable approach to probability and parameter estimation (Office of Management and Budget).

The use of the Delphi technique is extremely common in biomedicine. "Delphi Technique" is a unique National Library of Medicine Medical Subject Heading (MeSH) term, and a PubMed keyword search on "Delphi" yields over 3,000 entries. Common uses relate to health services, nursing, and education (Anandarajah, Brook, Hewlett, Mangione-Smith, Powell, Richards, Shekelle, Smith). Clinical and biological issues are also common subjects, with topics including diagnosis, treatment, and outcomes for diverse diseases such as trauma, glaucoma, malaria, tuberculosis, and others, and, in at least one case, biological aspects of risk assessment (Adkinal, Appropriateness Study Group, Koplan, Milholland, Sudre).

The Delphi technique is an iterative process of soliciting anonymous input in repeated rounds with feedback of the group's aggregate responses and each expert's own responses between rounds. As outlined by Helmer, the classical steps in conducting a Delphi exercise are:

- selecting issues and knowledgeable experts,
- familiarizing experts with the problems and questions,
- elicitation of experts,
- aggregation and presentation of estimates as medians and ranges,
- review of results and initial answers by the experts,
- repeating elicitations for several cycles until convergence occurs, and
- summarizing results.

There are no consensus guidelines for the use of the Delphi technique in biomedicine and implementation varies substantially, but the focus is almost exclusively on estimation (Thomson). In the classical technique, there is no contact between panelists, but it is now common to convene panels for in-person or electronic meetings as part of the process (Brook, Shekelle). The number of rounds of estimation varies. Some studies iterate until formal criteria for convergence are met, but it is most common to conduct 2 to 4 rounds (Linstone). The amount of information distributed before and during the process also varies, with some studies distributing none and other providing exhaustive literature searches and/or training (Brook, Sudre). Finally, the complexity of the queries varies, with some studies asking for simple numerical estimates or agreement with a few statements, while others include evaluations of 1000 or more complex scenarios (Appropriateness Study Group, Sudre).

Implementation

Many groups have provided guidance in the conduct of expert elicitation in the context of risk assessment. While much of the guidance is aimed primarily at individual rather than group approaches and at complex rather than straightforward questions, the steps recommended for the process are useful to consider. For example, the Nuclear Regulatory Commission has recommended the following steps (Kotra):

- definition of objectives,
- selection of experts,
- refinement of issues,
- assembly and dissemination of basic information,
- pre-elicitation training,
- elicitation of judgments,
- post-elicitation feedback,
- aggregation of judgments, and
- documentation.

This elicitation used a process that generally followed the steps above and falls well within the standards used in biomedical research. The process, organized according to Nuclear Regulatory Commission's recommended steps, is presented below.

Definition of objectives.

This modified Delphi exercise sought to elicit expert opinion on poorly known parameters deemed essential to the modeling element of the biomedical component for the comprehensive NEIDL risk assessment. Objectives included obtaining straightforward numerical estimates of two parameters for the pathogens under study: infectivity and atmospheric stability. The process also sought estimates of increased vulnerability to infection and death for certain population subgroups and endorsement of the risk assessment team's estimates of the rates of spread.

Selection of experts.

As has been recommended, a formal process was used to select the experts in order to have "more relevant expertise, more explicitness, greater potential for reproducibility, more ease of execution, less sponsor control, and more balance than would result from a purely informal approach." (Hawkins). However, Hawkins and others also indicate that sponsor involvement in selection should be considered on a case-by-case basis (Hawkins, Expert Elicitation Task Force). In this case, the sponsors and their advisors were well positioned to identify individuals who were "at the forefront of knowledge in their field, and ... recognized as leaders by their peers" (Keeney).

The criteria used for selection of subject-matter experts were aligned with Nuclear Regulatory Commission recommendations that "The subject-matter experts selected for elicitation should be individuals who: (a) possess the necessary knowledge and expertise; (b) have demonstrated their ability to apply their knowledge and expertise..." (Kotra). With respect to conflict of interest, persons with direct ties to Boston University or the NEIDL were excluded. However, "if experts were always excluded from participation due to potential conflicts of interest, for some disciplines there might be few or no experts available to participate" (Expert Elicitation Task Force). In this case, it was inevitable that true experts would have ties to NIH and, in some sense, would be competitors with Boston University investigators.

The NIH developed a preliminary list of seven areas of expertise to be included in the panel and later removed three that were less relevant to the project's developing objectives. The remaining four areas were:

- Bacteriology,
- Biosafety,
- High Biocontainment Experience, and
- Virology.

At a face-to-face meeting on 19 March 2010, a group from the NIH and TetraTech developed an initial list of nominees. This list was supplemented by discussion with the NIH Blue Ribbon Panel and additional knowledgeable individuals then cut to 14 candidates based on "best-fit" with the desired areas of expertise. Invitations were issued in rank order within the four areas of expertise to fill a panel of 8 persons. The members are listed in the box below; biographic sketches are provided in Appendix 2.

Arturo Casadevall, M.D., Ph.D. Professor of Microbiology and Immunology and	**Charles N. Haas, Ph.D.** L.D. Betz Chair Professor of Environmental

Medicine Chair, Department of Microbiology and Immunology Albert Einstein College of Medicine	Engineering Head -- Department of Civil, Architectural & Environmental Engineering Drexel University
Joseph Kanabrocki, Ph.D., C.B.S.P. Assistant Dean for Biosafety Associate Professor of Microbiology Biological Sciences Division University of Chicago	**James W. LeDuc, Ph.D.** Professor, Microbiology and Immunology Robert E. Shope M.D. and John S. Dunn Distinguished Chair in Global Health Director, Galveston National Laboratory University of Texas Medical Branch
Alison D. O'Brien, Ph.D. Professor and Chair Department of Microbiology and Immunology Uniformed Services University of the Health Sciences	**Jean Patterson, Ph.D.** Scientist and Chair Department of Virology and Immunology Southwest Foundation for Biomedical Research
C.J. Peters, M.D. John Sealy Distinguished University Chair in Tropical and Emerging Virology Professor, Departments of Microbiology and Immunology & Pathology Director for Biodefense, Center for Biodefense and Emerging Infectious Diseases University of Texas Medical Branch	**Wayne Thomann, Ph.D**. Director, Occupational and Environmental Safety Assistant Research Professor Duke University Medical Center

Refinement of issues.

Infectivity: The goal was not to generate dose-response curves *per se*, but to generate inputs to models that would estimate dose-response curves. The TetraTech team planned to fit three theory-based models including a one parameter exponential model and two parameter log-probit and beta Poisson models. In order to avoid placing undue burden on the panelists, queries were limited to the values necessary to fit the models; specifically estimates of the ID_{10}, ID_{50}, and ID_{90}. The unit used for the estimation was number of organisms. This straightforward approach has some limitations, but exploits the principle that one infectious particle is, put simply, one infectious particle.

Background information for this parameter was substantial, but most studies were of little or no relevance to human infectious doses in the required ranges (Appendix 1). This value would ideally be calculated from experimental data from unprotected humans but, with the exception of *F. Tularensis* - for which older aerosol challenge data are available - such data are lacking for the agents studied in the NEIDL risk assessment and cannot be obtained for ethical reasons (Appendix 1). Extrapolation from animal experiments is risky because of interspecies differences and because of the specialized inbred nature of many laboratory animals. Few studies are dose-ranging as investigators are most often using

doses expected to infect all unprotected animals. Finally, many available studies involve attenuated strains of the pathogens or vaccines (Appendix 1).

Atmospheric Stability: Estimates are needed to assess the potential that atmospheric conditions might inactivate some portion of airborne pathogens. Differences in pathogens warranted separate estimates for each. The task was simplified by asking for estimates that encompass the range of inactivation rates: a cold humid night and a hot dry sunny day.[‡] In order to have a common and commonly understood metric, the panelists were asked to express their estimates as half-lives in minutes.

Background information for this parameter was scanty, and often difficult to interpret. Much of the data were on organisms that were not in an aerosolized state and very few of the test conditions reflected normal atmospheric conditions. Several were expressed as D37, or the fluence of ultraviolet light that reduces the abundance to 37% of its original level. The latter were converted to half-lives prior to inclusion in the evidence tables (Appendix 1).

Population Vulnerability: The goal was to obtain coarse grain estimates of the degree of increased vulnerability to infection and to severe disease that could be used as a starting point for analyses of differential health effects. A distinction was made between medical vulnerability and environmental justice, with the former being the topic of this estimation. The literature on medical vulnerability to infection is fragmented, and extrapolation from less to more aggressive pathogens or from (often inbred) laboratory animal to humans is difficult (Appendix 1). However, drawing on analogies from the medical literature and the experience of Infectious Diseases physicians at the NIAID, in the risk assessment team, and expert consultants, five groups were selected for consideration.

The five groups included those 5 years of age or younger, those 65 years of age or older, and pregnant women because all three groups have increased vulnerability to influenza and certain bacteria; diabetics because of increased vulnerability to pneumonia and other infections; and those with HIV/AIDS because of increased vulnerability to a number of opportunistic infections (Brabin, High, Kaplan, Monto, Muller, Rothberg, Siston, Teo). Inclusion of transplantation recipients was considered, but as less than 0.01% of the population receives a transplant each year, there cannot be a meaningful differential impact on health outcomes (National Center for Health Statistics). Inclusion of persons with asthma was also considered, but evidence of an association between asthma and increased vulnerability to infection is lacking (Busse).[§] Specific data on the prevalence of these conditions are available for use by the risk assessment from sources such as the Current Population Survey and the Health of Boston 2010 report (Boston Public Health Commission, US Census Bureau).

Querying about differential vulnerability to each of the 13 specific pathogens was deemed impractical and, given the similarity of defenses for similar organisms and the extreme aggressiveness of most of the pathogens studied, unnecessary. Nonetheless, some distinctions are informative. In general, defenses against bacterial and viral pathogens are quite distinct (Kohlmeier, Munford). For these

[‡] Increasing temperature and UV exposure generally decrease the half-life of viable organisms.

[§] The relationship between asthma and respiratory infections is complex. Respiratory infections contribute to the development of asthma and trigger asthmatic episodes, and certain viral infections may be more likely to progress to pneumonia. However, asthmatics do not appear to experience more respiratory infections (Busse).

reasons, experts were not asked about each bacterium or virus, but separately about bacteria and viruses in general. In addition, it is desirable to capture information on potential increased severity of disease. Severity is a complex issue; markers of disease severity and staging systems vary across diseases and might include specific physiologic (e.g., hypoxia, hypotension) and/or more general measures (e.g., hospitalization, intensive care unit admission). Moreover, information on sub-lethal severity is generally sparse. For these reasons, and because the most interest for these serious diseases lies in the most serious outcomes, mortality rate was used as the measure of severity.

Rate of Spread: The reproduction rate or R_o is estimated using epidemiologic data from real outbreaks (see above). TetraTech researchers found multiple estimates of R_o in the literature for the five organisms that will undergo modeling of secondary transmission (Appendix 1). From these, they chose existing consensus estimates when available and developed a synthesis of the reported values when not. These were presented to the panel for affirmation or rejection.

Assembly and dissemination of basic information.

The TetraTech team prepared extensive background materials based on TetraTech's electronic search of scientific literature as supplemented by selected hand searches and additional literature suggested by the NIH and panelists. The literature provided to panelists from this review was not intended to be truly comprehensive but rather to be a large representative sample of the most informative articles.[**] The included scientific literature was presented in evidence tables and reference lists with abstracts (Appendix 1). In addition, the full text of all referenced papers was made available electronically to panelists.

For infectivity and population vulnerability, information was generally provided without further processing. For atmospheric stability, information in the literature was supplemented by calculations intended to convert that information into a common scale. For rate of spread, information from the literature was synthesized and candidate values were presented for the panel to affirm or disagree with.

Pre-elicitation training.

Two orientation calls were held. As part of the preparation for the calls, panelists were provided with an agenda, roster, the draft elicitation questionnaire, and an introductory slide deck (Appendix 2, Appendix 3). During the calls:

- the context of the development of the NEIDL risk assessment was reviewed,
- the goals of the exercise were presented,
- the Delphi process and panelists' role in it were explained,
- the questionnaire was shown, and the steps for completing it demonstrated, and
- questions were taken and discussions held.

[**] The National Research Council committee reviewing the NEIDL risk assessment elected to review the preliminary Delphi report. The committee suggested a re-examination of the material on *F. tularensis* and *B. anthracis*, and provided additional articles. After independent evaluation by TetraTech, the NIAID, and the NIH consultant, it was concluded that one of these articles was relevant but consistent with material already provided while the others were either not relevant or already included. One additional article was found upon re-review, but this too was judged to be consistent with material already provided.

Elicitation of opinion.

The group elicitation was conducted using the Delphi technique of informed iterative confidential estimation with feedback, with typical modifications of allowing interaction among panelists and not requiring convergence of estimates. Three rounds of data collection were conducted, which is consistent with common practice (Linstone). The steps in the elicitation process were as follows:

- orientation calls (discussed above),
- distribution of background materials (discussed above),
- completion of a standardized electronic questionnaire for Round 1,
- face-to-face meeting consisting of :
    - feedback of round one results including the distribution of the group estimates and, for panelists who completed round one, that panelist's own responses,
    - general discussion,
    - detailed review and discussion of the background evidence table for each organism, and
    - completion of a paper questionnaire for round 2,
- round 2 feedback of group responses and each panelist's own responses,
- completion of the standardized electronic questionnaire for Round 3, and
- aggregation and analysis.

Post-elicitation feedback.

At the end of the exercise, brief individual semi-structured qualitative interviews with the panelists were conducted by telephone.

Aggregation of judgments.

Votes to agree or disagree with the TetraTech estimates for R_o were tabulated. Numeric estimates were combined mathematically using equal weights in calculating and reporting the geometric mean, median, and range of the estimates. The use of varying weights was rejected because of the difficulty of assigning "quality" or "knowledgeability" weights on an organism-by-organism basis and because many authors advise equal weights. As is the case in many areas of social science, these authors have found that "simple combination procedures, such as an equally weighted average, produce combined probabilities [values] that perform as well as those from more complex aggregation models" and that "the benefits of imposing the equal weights constraint often exceed the costs" (Clement, Clement and Winkler) .

Documentation.

This report will be made widely available by the NIH.

Process

Round 1.

The first or preliminary round was conducted electronically. Panel members were sent instructions, background materials, and an electronic questionnaire by email and asked to record their preliminary responses. After attending an orientation call, five of eight returned acceptably completed forms. These were processed and a feedback document prepared that summarized the aggregated results as

histograms and tabulations for categorical data, and the low, high, median, and geometric mean for numeric data. These are provided in Appendix 4. In addition, panelists' own responses were reformatted for confidential feedback to that panelist.

Round 2.

The second round was conducted at a meeting in Bethesda MD on 18 May 2010. The agenda and materials are provided in Appendix 5. Six of eight panel members attended in person and one participated by telephone. Each participant was given an additional copy of the background materials, the summary of the first round responses, and, for those who returned forms in the round one, their own initial responses.

The meeting started with an overview of the Delphi process and of the ways in which the responses would be used, with associated discussions. The rest of the meeting was devoted to sections on infectivity, rate of spread, population vulnerability, and atmospheric stability. The format for each section was:

- brief introductory presentation and general discussion,
- detailed review and discussion of the summary background tables,
- review of round one responses, and
- round two estimation/voting.

Discussions of infectivity included comments on the listed data (e.g., how to interpret the susceptibility of lab animals), unpublished data and anecdotes (especially related to laboratory experience), and related data (especially regarding outbreaks, epidemics, and epizootics). There was also discussion of the ID_{10} and ID_{90} estimates with some thought that subpopulations with extreme vulnerability or resistance coupled with a poor evidentiary base made these estimates particularly challenging. Others thought that these issues could be incorporated by adjusting one's estimates.

The panelists had few comments on rate of spread and population vulnerability. Discussions of atmospheric stability centered on unpublished data, anecdotal experiences in the laboratory, and questioning of the usefulness of available data (especially those studies on organisms in liquid media but also all data not gathered under atmospheric conditions). There was also discussion of day-to-day variations in conditions and the likely mechanisms of release from a laboratory, but the panelists concluded that the Delphi questions would capture the extremes for the organisms being assessed.

Following the meeting, the materials and a transcript of the meeting were transmitted to the one panel member who attended by telephone and to the one member who was not present, and their responses taken. The results of round two contained a small number of inconsistencies in the estimates for infectivity that were resolved by simple queries.[††] After these were resolved, the results of the round two responses from eight of eight panelists were processed as before. Results are presented in Appendix 5.

[††] These were simple number errors or transpositions (e.g., a power of 10 error resulted in an ID_{10} that was higher than the ID_{50} for the same organism).

Round 3 (final round).

Panelists were sent round 2 aggregate results and their own responses, and a third round of electronic responses requested. The results of round three also contained a small number of inconsistencies similar to those found in the second round. After these were resolved, results were processed as before. The raw and tabulated data for eight of eight panel members are presented in Appendix 6, and summarized below under Results.

Post-elicitation interviews.

Consistent with best practices, brief semi-structured follow-up interviews with panelists were conducted during January 2011. Seven of eight panelists participated. The following topics were included.

Panel membership: Four of the seven panelists agreed that the panel membership was a strength. Comments from these panelists included that the membership was "outstanding," that the panelist's "expertise was a big plus," that the membership was "as appropriate as possible," that those who worked with the pathogens were "highly respected," and that “generalists would be useless.” One panelist thought that the specific fund of relevant knowledge was "not great" for some panelists while another thought that the expertise represented was not broad enough. The latter also thought that there were "too many MD's."

Literature Review: None of the panelists found the literature review deficient, although five expressed dissatisfaction with the state of knowledge. One panelist said, "(I) wish that it would have been possible to provide additional relevant literature" but the inadequate literature "is what it is." Two panelists noted that they had contributed papers to the literature review. One indicated that all of the key papers in their field were included. Another indicated that some potentially useful older papers were missing, but that their inclusion would not have changed outcomes. Several indicated that they learned from the material provided.

Role of face-to-face discussions: Five of the seven panelists found the discussions useful. Two of these indicated that they were highly influenced by the discussions. One indicated that the discussions provided background but did not affect responses and one wished that there was more discussion. The panelist who joined by telephone did not find it useful nor did another who indicated, "best practices would have included more interchange of ideas."

Estimation: All seven panelists indicated that they used their own prior knowledge as a primary source for at least some of the opinions rendered, including one panelist who estimated the ID_{10} and ID_{90} values by mathematical means. Four panelists indicated that the face-to-face meeting was at least somewhat influential, especially the discussions of unpublished and anecdotal information. Three indicated that the background material was also highly influential and one indicated that he queried his most knowledgeable colleague about each organism prior to responding.

Overall impression: Five of seven panelists had an overall positive impression. Several of the panelists were uncomfortable with aspects of the process but felt that it was "the best that could be done given the state of the literature." One panelist characterized it as "scientific." Another thought it "interesting, useful, even valuable." A third was initially skeptical but "became enamored" with the effort. Two

panelists had unfavorable impressions. One was uncomfortable with using opinion generally and the iterative process specifically because of a concern that the latter could lead to false precision. The other thought it "an interesting process but not well executed."

Results of the Delphi Exercise

The summary results for aerosol infectivity, expressed as number of airborne organisms, were as follows for the ID_{50}:

ID_{50}	Median	Low	High	Geometric Mean
B. anthracis	10,000	5,000	30,000	9,949
F. tularensis	40	10	500	42
Y. pestis	4,000	500	100,000	4,349
Andes hantavirus	400	10	1,000	308
Ebola virus	250	120	1,000	331
Marburg virus	175	100	1,000	255
Junin virus	50	15	1,000	84
Lassa fever virus	65	18	30,000	134
Nipah virus	1,000	500	30,000	1,715
Rift Valley fever virus	1,000	40	5,000	678
Russian spring-summer encephalitis virus	200	71	10,000	441
SARS virus	3,500	283	8,000	2,197
1918 influenza virus	850	100	250,000	1,497

ID_{10} and ID_{90} estimates are intended to help determine the shape of the dose-response curve, and to allow fitting of multi-parameter models. The results for the $ID_{10,}$ expressed as number of airborne organisms, were:

ID_{10}	Median	Low	High	Geometric Mean
B. anthracis	750	200	4,000	709
F. tularensis	8	2	100	9
Y. pestis	200	100	700	202
Andes hantavirus	55	1	160	42
Ebola virus	45	19	100	49
Marburg virus	20	10	100	25
Junin virus	20	2	100	17
Lassa fever virus	15	3	300	17
Nipah virus	100	75	1,000	180
Rift Valley fever virus	100	10	1,000	79
Russian spring-summer encephalitis virus	25	11	1,000	48
SARS virus	100	43	1,000	156
1918 influenza virus	55	10	50,000	93

The results for the ID_{90}, expressed as number of airborne organisms, were:

ID_{90}	Median	Low	High	Geometric Mean
B. anthracis	100,000	10,000	300,000	86,028
F. tularensis	200	41	1,000	245
Y. pestis	35,000	1,500	1,000,000	32,993
Andes hantavirus	4,500	100	30,000	3,980
Ebola virus	3,000	400	10,000	2,847
Marburg virus	2,000	400	10,000	2,707
Junin virus	1,000	49	10,000	1,026
Lassa fever virus	1,000	59	3,000,000	1,792
Nipah virus	10,000	1,650	500,000	15,919
Rift Valley fever virus	10,000	300	50,000	5,148
Russian spring-summer encephalitis virus	2,500	230	1,000,000	6,384
SARS virus	40,000	950	300,000	24,861
1918 influenza virus	15,000	3,000	1,000,000	30,941

The results for atmospheric stability, expressed as a half-life in minutes, were estimated for a humid, cool night and a warm, dry, sunny day. For night, the estimates of the half-life in minutes were:

Night	Median	Low	High	Geometric Mean
B. anthracis	**Indefinite**			
F. tularensis	**48**	**20**	**60**	**41**
Y. pestis	**25**	**8**	**60**	**21**
Andes hantavirus	**48**	**30**	**60**	**44**
Ebola virus	**20**	**2**	**60**	**15**
Marburg virus	**18**	**10**	**60**	**20**
Junin virus	**20**	**10**	**30**	**19**
Lassa fever virus	**22**	**15**	**60**	**24**
Nipah virus	**18**	**5**	**20**	**14**
Rift Valley fever virus	**23**	**20**	**60**	**27**
Russian spring-summer encephalitis virus	**28**	**20**	**120**	**36**
SARS virus	**125**	**60**	**200**	**117**
1918 influenza virus	**55**	**30**	**300**	**68**

For day, the estimates of the half-life in minutes were:

Day	Median	Low	High	Geometric Mean
B. anthracis	**Indefinite**			
F. tularensis	**20**	**3**	**25**	**15**
Y. pestis	**10**	**2**	**15**	**8**
Andes hantavirus	**10**	**10**	**30**	**13**
Ebola virus	**10**	**1**	**30**	**7**
Marburg virus	**10**	**2**	**30**	**9**

Junin virus	**10**	**5**	**10**	**9**
Lassa fever virus	**10**	**5**	**30**	**11**
Nipah virus	**5**	**2**	**10**	**6**
Rift Valley fever virus	**10**	**10**	**20**	**13**
Russian spring-summer encephalitis virus	**11**	**10**	**60**	**15**
SARS virus	**25**	**10**	**60**	**25**
1918 influenza virus	**20**	**5**	**30**	**20**

Increased vulnerability was divided into increased vulnerability to disease and to death from disease, each expressed as the percentage of increase in vulnerability. For increased vulnerability to disease, the results were:

Disease (% increase in vulnerability)		**Median**	**Low**	**High**	**Geometric Mean**
Young	**Bacteria**	**18%**	**5%**	**33%**	**15%**
	Viruses	**20%**	**0%**	**33%**	**9%**
Older	**Bacteria**	**28%**	**5%**	**50%**	**23%**
	Viruses	**23%**	**5%**	**50%**	**21%**
Diabetes	**Bacteria**	**20%**	**5%**	**30%**	**16%**
	Viruses	**15%**	**5%**	**25%**	**13%**
HIV	**Bacteria**	**30%**	**10%**	**40%**	**26%**
	Viruses	**30%**	**10%**	**40%**	**28%**
Pregnancy	**Bacteria**	**5%**	**0%**	**30%**	**3%**
	Viruses	**5%**	**2%**	**50%**	**8%**

For increased vulnerability to death from disease, the results were:

Death (% increase in vulnerability)		**Median**	**Low**	**High**	**Geometric Mean**
Young	**Bacteria**	**10%**	**5%**	**20%**	**11%**
	Viruses	**10%**	**5%**	**20%**	**12%**
Older	**Bacteria**	**10%**	**5%**	**50%**	**14%**
	Viruses	**15%**	**5%**	**50%**	**16%**
Diabetes	**Bacteria**	**10%**	**5%**	**25%**	**10%**
	Viruses	**10%**	**5%**	**25%**	**10%**
HIV	**Bacteria**	**25%**	**10%**	**30%**	**22%**
	Viruses	**25%**	**10%**	**30%**	**22%**
Pregnancy	**Bacteria**	**5%**	**1%**	**20%**	**5%**
	Viruses	**4%**	**1%**	**20%**	**5%**

A total of 40 'votes' were cast regarding the TetraTech team's estimates for R_o. Of these, 36 were to affirm those estimates. This included eight of eight for SARS virus and seven of eight for Y pestis, Ebola virus, Rift Valley Fever virus, and 1918 Influenza. The four disagree votes came from two panelists. One panelist disagreed with estimates for *Y. pestis* (1.3), Ebola virus (1.5), and Rift Valley Fever virus (1.2),

thinking them too low. A different panelist disagreed with the estimate for 1918 influenza (3.0 with a range of 1.5-4), thinking it too high.

Number agreeing/total panelists	
Y. pestis	**7/8**
Ebola virus	**7/8**
Rift Valley fever virus	**7/8**
SARS virus	**8/8**
1918 influenza virus	**7/8**

Conclusions:

The NIH believes that adding a quantitative component to the qualitative components of the NEIDL risk assessment is important to a comprehensiveness that will satisfy stakeholder expectations. The NIH, its consultants, TetraTech, and the Blue Ribbon Panel all agreed that the expert opinion was needed to complete the modeling. The NIH preferred formal to informal approaches for obtaining expert opinion. It convened a distinguished expert panel and conducted a formal expert elicitation. The elicitation used the widely accepted Delphi technique and a careful standard process. Panelists were asked to:

- estimate the infectivity of aerosols for the 13 pathogens studied in the NEIDL risk assessment in terms of number of organisms for the ID_{10}, ID_{50}, and ID_{90},
- estimate the half-life in minutes of the 13 pathogens in an aerosol on a cool humid night and a dry sunny day,
- estimate the percentage increase in vulnerability to disease and death among five groups: those 5 years of age or younger, those 65 years of age or older, those with diabetes mellitus, those with HIV/AIDS, and those who are pregnant, and
- corroborate the TetraTech team's choices for R_0 in the base case for the five pathogens undergoing modeling of secondary spread.

The distribution of responses was reasonably balanced with geometric means approximating the medians for a large majority of the estimates. Results seem consistent with the fragmentary literature, biomedical experience, and known differences between experimental and actual circumstances.

The Delphi technique is the most widely used approach to expert elicitation in biomedicine, and the majority of the panelists viewed the exercise favorably. The NIH, its consultants, TetraTech, and the Blue Ribbon Panel found the process and the estimates highly credible. The results of the elicitation provide defensible supplemental inputs to the NEIDL risk assessment.

List of Appendices

Citations

Adkin A, Matthews D, Hope J, Maddison BC, Somerville RA, Pedersen J. Risk of escape of prions in gaseous emissions from on-farm digestion vessels. Vet Rec. 2010 Jul 3;167(1):28-9.

Anandarajah G, Craigie F Jr, Hatch R, Kliewer S, Marchand L, King D, Hobbs R 3rd, Daaleman TP Toward competency-based curricula in patient-centered spiritual care: recommended competencies for family medicine resident education. Acad Med. 2010 Dec;85(12):1897-904.

Appropriateness of Treating Glaucoma Suspects RAND Study Group. For which glaucoma suspects is it appropriate to initiate treatment? Ophthalmology. 2009 Apr;116(4):710-6, 716.e1-82

Boston Public Health Commission, Research and Evaluation. Health of Boston 2010. Web. Accessed 16 Jan 2011. http://www.bphc.org/about/research/hob2010/Pages/Home.aspx

Brabin BJ. (1985). Epidemiology of infection in pregnancy. Rev Infect Dis. 7(5):579-603.

Brook RH, Chassin MR, Fink A, Solomon DH, Kosecoff J, Park RE. A method for the detailed assessment of the appropriateness of medical technologies. Int J Technol Assess Health Care 1986;2:53-63

Busse WW, Lemanske RF, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet 2010; 376: 826–34

Clemen, RT. Combining Forecasts: A Review and Annotated Bibliography. International Journal of Forecasting. 1989;5:559-583.

Clemen RT, Winkler RL Combining Probability Distributions From Experts in Risk Analysis. Risk Analysis. 1999;2:187-203.

Committee on Estimating the Health-Risk-Reduction Benefits of Proposed Air Pollution Regulations. Estimating the Public Health Benefits of Proposed Air Pollution Regulations. Washington, DC: National Academy Press, 2002.

Committee on Risk Assessment of Hazardous Air Pollutants. Science and Judgment in Risk Assessment. Washington, DC: National Academy Press, 1994.

Dalkey N, Crolee N, Helmer-Hirschberg O. An Experimental Application of the Delphi Method to the Use of Experts (RM-727/1-ABR). Santa Monica: The RAND Corporation, 1962

Dalkey N, Helmer O. An Experimental Application of the Delphi Method to the Use of Experts. Management Science. 1963;9(3):458-67.

Expert Elicitation Task Force. Expert Elicitation Task Force White Paper. Science Policy Council. U.S. Environmental Protection Agency, 2009

Fink A, Kosecoff J, Chassin M, Brook RH. Consensus methods: characteristics and guidelines for use. Am J Public Health. 1984;74(9):979-83.

Gail MH, Benichou J, eds. Encyclopedia of Epidemiologic Methods. Chichester, England: John Wiley and Sons Ltd, 2000.

Halloran, ME, Ferguson NM, Eubank S, Longini IM, Cummings DA, Lewis B, Xu S, Fraser C, Vullikanti A, Germann TC, Wagener D, Beckman R, Kadau K, Barrett C, Macken CA, Burke DS, Cooley P. Modeling targeted layered containment of an influenza pandemic in the United States. PNAS USA 2008;105:4639-44.

High KP, Bradley S, Loeb M, Palmer R, Quagliarello V, Yoshikawa T (2005). A new paradigm for clinical investigation of infectious syndromes in older adults: assessment of functional status as a risk factor and outcome measure. Clinical Infectious Diseases 40:114–22

Hawkins NC, Graham JD. Expert Scientific Judgment And Cancer Risk Assessment: A Pilot Study Of Pharmacokinetic Data. Risk Analysis 1988;8:615-25

Helmer O. Analysis of the Future: The Delphi Method, and The Delphi Method An Illustration in Bright J (ed.), Technological Forecasting for Industry and Government. Englewood Cliffs, NJ: Prentice Hall, 1968

Jones J, Hunter D. Qualitative Research: Consensus methods for medical and health services research. BMJ 1995;311:376-380

Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H; Centers for Disease Control and Prevention (CDC); National Institutes of Health; HIV Medicine Association of the Infectious Diseases Society of America. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep. 2009;58(RR-4):1-207

Keeney RL, von Winterfeldt D. Eliciting Probabilities from Experts in Complex Technical Problems. IEEE Transactions on Engineering Management. 1991;38:191-201

Kok J, Tudo K, Blyth CC, Foo H, Hueston L, Dwyer DE. Pandemic (H1N1) 2009 Influenza Virus Seroconversion Rates in HIV-infected Individuals. J Acquir Immune Defic Syndr 2011;56:91–94 (Epub 13 Nov 2010)

Kohlmeier JE, Woodland DL. Immunity to Respiratory Viruses. Annu. Rev. Immunol. 2009;27:61–82

Koplan JP, Farer LS. Choice of preventive treatment for isoniazid-resistant tuberculous infection. Use of decision analysis and the Delphi technique. JAMA. 1980;244(24):2736-40.

Kotra JP, Lee MP, Eisenberg NA, DeWispelare AR. Branch Technical Position on the Use of Expert Elicitation in the High-Level Radioactive Waste Program (NUREG-1563). United States Nuclear Regulatory Commission, 1996.

Linstone H. A., and Turoff, M., 1975. The Delphi Method, Techniques and Applications. Reading MA: Addison Wesley, 1975. (Available at http://is.njit.edu/pubs/delphibook/)

Mangione-Smith R, DeCristofaro AH, Setodji CM, Keesey J, Klein DJ, Adams JL, Schuster MA, McGlynn EA. The quality of ambulatory care delivered to children in the United States. N Engl J Med. 2007;357(15):1515-23

ID50. Merriam-Webster's Medical Dictionary. Merriam-Webster, Inc. Web. Accessed: January 18, 2011. http://www2.merriam-webster.com/cgi-bin/mwmedsamp

Munford RS, Suffredini AF. Normal Host Responses to Infection. In Mandel GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases-7th ed. Philadelphia PA, Churchill Livingstone (Elsevier), 2010.

Monto AS. Epidemiology of viral respiratory infections. Am J Med. 2002;112 Suppl 6A:4S-12S.

Milholland AV, Wheeler SG, Heieck JJ. Medical assessment by a Delphi group opinion technique. N Engl J Med. 1973;288(24):1272-5.

Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, Rutten GE. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis. 2005;41(3):281-8.

National Center for Health Statistics. Health United States, 2009. Web. Accessed 14 Jan 2011 <http://www.cdc.gov/nchs/index.htm>

Office of Management and Budget. Circular A-4. USOMB, 2003

Powell C. The Delphi technique: myths and realities. J Adv Nurs. 2003;41(4):376-82.

Shekelle PG, Kahan JP, Bernstein SJ, Leap LL, Kamberg CJ, Park RE. The Reproducibility of a Method to Identify the Overuse and Underuse of Medical Procedures. N Engl J Med 1998;338:1888-1895

Richards GA, Sprung CL; European Society of Intensive Care Medicine's Task Force for intensive care unit triage during an influenza epidemic or mass disaster. Recommendations and standard operating procedures for intensive care unit and hospital preparations for an influenza epidemic or mass disaster. Intensive Care Med. 2010;36 Suppl 1:S70-9.

Rothberg MB, Haessler SD, Brown RB. Complications of viral influenza. Am J Med. 2008;121(4):258-64.

Siston AM, Rasmussen SA, Honein MA, Fry AM, Seib K, et al. Pandemic H1N1 Influenza in Pregnancy Working Group. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA. 2010;303(15):1517-25.

Smith DR. Establishing national priorities for Australian occupational health and safety research. J Occup Health. 2010;52(4):241-8. Epub 2010 May 6

Sudre P, Breman JG, Koplan JP. Delphi survey of malaria mortality and drug resistance in Africa. Lancet. 1990;335(8691):722

Teo SS, Nguyen-Van-Tam JS, Booy R. Influenza burden of illness, diagnosis, treatment, and prevention: what is the evidence in children and where are the gaps? Arch Dis Child. 2005;90(5):532-6.

Thompson M. Considering the implication of variations within Delphi research. Family Practice 2009;26: 420–424.

US Census Bureau and the Bureau of Labor Statistics. Current Population Survey (CPS). Web. Accessed 16 Jan 2011. http://www.census.gov/cps/

Attachment H-1: Background Materials Prepared by Tetra Tech Inc.

(Completed 26 April 2010)

A: Tables

Section 1: Infectiousness

Part A. Dose-Response

Estimating the proportion of persons exposed to an organism who will become infected requires an estimate of the dose-response curve for infection. We will use traditional measures of infectivity to elucidate points on these curves:

- the Infectious Dose 50 (ID50), or the minimum dose required to infect 50% of a susceptible population;
- the Infectious Dose 10 (ID10), or the minimum dose required to infect 10% of a susceptible population;
- the Infectious Dose 90 (ID90), or the minimum dose required to infect 90% of a susceptible population.

Once the expert panel has reached consensus on these 3 values for each organism, we will mathematically fit curves to the points, thereby interpolating values for intermediate doses.

Table 1.A below contains information on infectious doses, human infectious doses (HID), and lethal doses (LD) for the 13 organisms being studied in the NEIDL Risk Assessment.

Table 1.A. Prior information: infectious doses[1]

	Human	Non-human primate	Other animal	Values used in prior models
Bacillus anthracis	8-50 x 10^3 spores estimated HID_{50} (Franz, Jahrling et al. 1997) 8,600 spores estimated HID_{50} (Wilkening 2006)	As few as 100 spores (Brachman, Kaufman et al. 1966) LD_{50} of 4,130 (95% C.I. 1,980-8,630) spores (Glassman 1966); rough estimates derived from incomplete data description by author: LD_{10} of 50 (95% C.I. 14-112),		"Model A": ID_{10} of 110 spores; ID_{50} of 8,600 spores, ID_{90} of 700,000 spores; "Model D": ID_{10} of 1,300 spores; ID_{50} of 8,600 spores, ID_{90} of 29,000 spores

	100 spores estimated LD_{10} and 2.5-55 x 10^3 spores estimated LD_{50} (Peters and Hartley 2002)	LD_{90} of 340,000 (95% C.I. 153,000-1,200,000) ID_{50} 61,800 (34,000-110,000) CFU, aerosol, in macaques (Vasconcelos, Barnewall et al. 2003) LD_{50} of 96,800 spores (95% C.I. 70,7000-136,000), LD_{10} of 14,700 spores (95% C.I. 10,800-20,700), LD_{90} of 322,000 spore (95% C.I. 235,000-451,000) calculated by Haas (Haas 2002) using data from rhesus monkeys obtained by Druett et al (Druett, Henderson et al. 1953). LD_{50} of 45,000 spores, aerosol, in rhesus monkeys (Druett, Henderson et al. 1953)		(Wilkening 2006) ID_{10} of 150 spores; ID_{50} of 10,000 spores, ID_{90} of 680,000 spores (Baccam and Boechler 2007)
Francisella tularensis	HID_{78} (14/18) for aerosolized challenge doses of 10-52 CFU (Saslaw, Eigelsbach et al. 1961) 200 aerosolized cells infected 2/2 volunteers (McCrumb 1961)	LD_{50} as little as 14 cells in primates (Lyons and Wu 2007) ID_{100} from 10 cells aerosol, type B strain (Lyons and Wu 2007)	Mouse, rat, guinea pig, rabbit data available	
Yersinia pestis (pneumonic)	100-500 cells in humans as "infective dose" (% not specified) (Franz, Jahrling et al. 1997)	LD_{50} of 2.3 x 10^4 (range 1-3.3 x 10^4) CFU via aerosol in rhesus monkeys (Speck and Wolochow 1957) LD_{100} of 1.4 x 10^2 (lower doses not tested) via aerosol in African green monkeys (Davis, Fritz et al. 1996) 120-270 cells, intratracheal dose, cells caused lethal infection in 8 of 12 rhesus monkeys (Ehrenkranz and Meyer 1955)	Rats, LD_{50} of 1.6 x 10^3 (Agar, Sha et al. 2009) Mice, Swiss-Webster & BALB/c, intranasal LD50 of 340 and 100 cells, respectively (Sha, Agar et al. 2008)	
Andes hantavirus				

Ebola virus		2 of 2 rhesus monkeys rapidly killed by 400 pfu (Johnson, Jaax et al. 1995) 7 of 7 *Macaca fascicularis* infected by 1,000 PFU intramuscular (Geisbert, Geisbert et al. 2009) 4 of 4 *Macaca fascicularis,* and 3 of 3 rhesus monkeys, infected by 1,000 PFU aerosolized or intramuscular (Pratt, Wang et al.)		
Marburg virus		0.1 to 0.003 guinea pig LD50 killed 6 of 10 African green monkeys (Leffel and Reed 2004) 1of 1 *Macaca fascicularis* infected by 1,000 PFU intramuscular (Geisbert, Geisbert et al. 2009)	1,000 PFU, intraperitoneal, uniformly fatal (10/10 BABL/c mice); intranasal, subcutaneous, or intramuscular injection of the same dose was not fatal (Warfield, Bradfute et al. 2009)	
Junin virus (Argentine hemorrhagic fever virus)			ID_{50} (considered synonymous with LD_{50}) of 15 PFU in outbred guinea pigs (Peters, Jahrling et al. 1987) ID_{66} (66% infection, no mortality) for an intranasal dose of $10^{3.5}$ $TCID_{50}$ in guinea pigs (Peters, Jahrling et al. 1987) In guinea pig, some Junin virus strains required < 1 PFU to produce an LD_{50} while other strains killed 20% regardless of dose (Peters, Jahrling et al. 1987)	
Lassa fever virus		Macaques, aerosol doses 2.7 – 4.5 log_{10} PFU - all died (Peters, Jahrling et al. 1987)	Median infectious dose (ID_{50}) for guinea pigs was 15 PFU	Model values (best fit) derived from previously

			(Stephenson, Larson et al. 1984) LD_{50} in guinea pigs as low as 0.3 PFU depending on virus strain and host strain (Peters, Jahrling et al. 1987)	published guinea pig data: ID_{10}, ID_{50}, and ID_{90} = 2.7, 18, and 59 PFU, respectively (Tamrakar and Haas 2008)
Nipah virus		Squirrel monkeys (*Saimiri sciureus*), IV or intranasal inoc of 10^3 or 10^7 PFU (3 animals per group); 10^3 PFU intranasal failed to infect (Marianneau, Guillaume et al.),	MID_{50} (minimum ID_{50}) in ferrets 500 $TCID_{50}$ by oro-nasal route (Bossart, Zhu et al. 2009). LD_{50} in golden hamster model of 270 & 47,000 PFU for intraperitoneal and intranasal routes, respectively (Wong, Grosjean et al. 2003) LD_{100} of 10^4 $TCID_{50}$ in golden hamster, intraperitoneal (Freiberg, Worthy et al.)	
Rift Valley fever virus		4 rhesus monkeys inoc with $10^{5.3}$ PFU: viremia in 3 of 4 (Peters, Jones et al. 1988)	Susceptible rat strains develop fulminate disease when inoculated with less that 5 PFU (ZH-501 viral strain), while resistant strains become infected asymptomatically following inoculation with as much as 500,000 PFU virus dose (Shimshony and Barzilai 1983) Other animal studies published; results are highly strain-host variable.	
Russian spring-summer encephalitis virus (TBE complex virus)				
SARS-associated coronavirus		ID_{63} (5/8 rhesus macaques)	10^3- 10^6 intranasal, mice (Cheng, Lau et al. 2007)	

		Used 10^3, 10^5,10^7 $TCID_{50}$ in rhesus macaques; found 10^5 to be "optimum". All 8 animals had brief fever but neutalizing antibodies were found only in 5. (Qin, Wang et al. 2005) 10^3- 10^7 intranasal(Cheng, Lau et al. 2007)	10^3 intranasal, golden Syrian hamster (Cheng, Lau et al. 2007) 10^6 intratracheal, marmosets (Cheng, Lau et al. 2007) 10^6 intratracheal, cats (Cheng, Lau et al. 2007) 10^6 intranasal or intratracheal, ferrets (Cheng, Lau et al. 2007) 10^6 intranasal or intratracheal, civets (Cheng, Lau et al. 2007)	
1918 Influenza virus	Estimated "infectious dose" 1-10 virions (Subbarao) 9/17 (53%) infected via intranasal instillation; inocula: 10^2 , 10^3 , 10^4, or 10^5 TCID50 H3N2 (Zaas, Chen et al. 2009) A2 virus, aerosol challenge of 5-126 TCID50; 7/23 volunteers infected = HID30 (Alford, Kasel et al. 1966) 362/532 (68%) infected via intranasal instillation; inocula		1918 H1N1 LD50 in ferrets is 10^6 PFU. The same dose in susceptible mouse strain is 100% lethal (Tumpey 2008) 1918 H1N1 LD50 in susceptible mouse strain is 3.5 log PFU50 or EID50 (Tumpey 2008) H3N2 ID_{50} in guinea pig is 5 pfu (Lowen, Mubareka et al. 2006) H0N1 ID_{50} in mice is 0.079 - 5 EID_{50} (Yetter, Lehrer et al. 1980)	Not 1918 virus: 0.671 $TCID_{50}$ and 500 $TCID_{50}$ for respiratory and nasal epithelium, respectively (Atkinson and Wein 2008)

	range: 10^3-10^7.2 TCID50 of H1N1 (not 1918) (Carrat, Vergu et al. 2008) 11/15 (73%) infected with 10^4 TCID50 of H1N1 (not 1918); H3N2 testing infected 93%; all via intranasal instillation (Clements, Betts et al. 1986)			

[1] Data are representative of the open literature as of January 2009, but are not necessarily exhaustive. A cursory update from the open literature was performed in April, 2010. Additional relevant data may exist under classified status. Experimental data are presented when possible. Data presented for *F. tularensis,Y. pestis,* and Lassa virus are consistent with data derived by modeling (Sinclair, Boone et al. 2008).

Part B: R_0

Modeling of secondary spread requires an estimate of an infection's propensity to transmit in a population. The Basic Reproduction Number (R_0) is often used as a measure of this. It is the expected number of secondary infections caused by a typical primary case in a fully susceptible population, in the absence of interventions to control the infection and its transmission. While some researchers have attempted to quantify R_0 directly from contact tracing data from the early stages of real outbreaks, more often researchers estimate R_0 indirectly by using more easily observed data from outbreaks, such as case incidence rates, incubation period, and generation time. Usually, this information is then translated into R_0 mathematically.

Table 1B outlines estimates of R_0 from the literature for each of the 5 pathogens that will undergo transmission modeling, and the modeling team's proposal for the nominal value to be used in modeling. Note that in all cases, the values only apply before control measures are put in place. Also note that we will be analyzing the sensitivity of model outputs to the value of R_0 by assessing a range of values around the proposed nominal value.

Table 1.B. Prior information: R_0[1]

	Epidemiologically-derived values/ Values used in prior models	Initial estimates (without intervention)
		Ro
Yersinia pestis (pneumonic)	1.3 (model-derived estimate) (Gani and Leach 2004) 1.32 (90% confidence interval: (1.01-1.61)) estimated from six historical outbreaks (Lloyd-Smith, Schreiber et al. 2005)	1.3
Ebola virus	Estimated to be 1.83 (SD 0.06) and 1.34 (SD 0.03) for the 1995 Congo and 2000 Uganda outbreaks, respectively. Alternate analysis gives median 1.89 (Interquartile range 1.66-2.28) based on empirical data from Zaire 1976 outbreak (Chowell, Hengartner et al. 2004) 1.36 (SD 0.13) estimated from 1995 Congo outbreak (Lekone and Finkenstadt 2006) 1.50 (90% confidence interval: (0.85-2.08)) estimated from 2000 Uganda outbreak (Lloyd-Smith, Schreiber et al. 2005)	1.5
Rift Valley fever virus	Predicted by one modeling effort to have a mean of 1.193 (95% CI 1.177-1.209), (median 1.113, max. 3.743, min. 0.037) (Gaff, Hartley et al. 2007). Note this is an estimate for R_0 for spread through a mosquito population via mosquito-mosquito and mosquito-livestock-mosquito transmission. It is not a prediction for R_0 for human transmission.	1.2 (for mosquitoes)
SARS-associated coronavirus	1.63 (90% confidence interval: 0.54-2.65) for first three generations of transmission in Singapore 2003; 2.55 (90% confidence interval: 0.50-4.50) for first two generations of transmission in Singapore 2003. Third generation occurred before centralized control measures were in place, but after WHO's global alert on SARS, so drop in R0 estimate could be explained by informal behavior changes or informal increased isolation of patients. These are direct estimates of R in Singapore from contact tracing data (Lloyd-Smith, Schreiber et al. 2005) 1.88 (90% confidence interval 0.41-3.32) for first two generations of transmission in Beijing 2003; 0.94 (90% confidence interval 0.27-1.51) for only second generation of transmission in Beijing 2003. The first generation consisted of a single infected individual who directly transmitted to 33 others. These are direct estimates of R in Beijing from contact tracing data (Lloyd-Smith, Schreiber et al. 2005). 3.6 (95% confidence interval 3.1-4.2) for Hong Kong. 2.4 (95% confidence interval 1.8-3.1) for Vietnam.	3.0 - consensus estimate determined by review paper (Bauch, Lloyd-Smith et al. 2005). Simulation of control measures will decrease R value as outbreak progresses. Individual variation will be incorporated as in Lloyd-Smith et

	3.1 (95% confidence interval 2.3-4.0) for Singapore. 2.7 (95% confidence interval 1.8-3.6) for Canada. These are estimates inferred from epidemic curves and generation interval data from each city, using a likelihood-based estimation procedure (Wallinga and Teunis 2004) Estimated 0.86 (0.24-1.18) for Toronto, 1.70 (0.44-2.29) for Hong Kong, 1.83 (0.47-2.47) for Singapore (Chowell, Castillo-Chavez et al. 2004). Ranges are the inter-quartile range. These are model-based estimates assuming a portion of population is less susceptible, which explains why these are lower than other estimates. For example, with uniform susceptibility, estimate for Hong Kong increases to 2.6 (Bauch, Lloyd-Smith et al. 2005) 2.7 (95% confidence interval 2.2-3.7) for Hong Kong (Riley, Fraser et al. 2003). This excluded super-spreading events. Including them may increase the estimate to about 3.2 (Bauch, Lloyd-Smith et al. 2005). Estimate based on fitting stochastic spatial simulation to case-incidence data. 3.5 (90% confidence interval 1.5-7.7) for Singapore using Bayesian procedure. Range of 2.2-3.6 using deterministic model estimate (Lipsitch, Cohen et al. 2003) 4.80 for Toronto; 3.60 for Hong Kong; 5.04 for Singapore; 4.91 for Beijing (Gumel, Ruan et al. 2004). These are deterministic dynamic model-based estimates. The reason they are higher than other estimates may be because of unrealistic assumptions about the efficacy of control measures (Bauch, Lloyd-Smith et al. 2005) 4.2 for Taiwan (Hsieh, Chen et al. 2004). May be higher than other estimates because of uncertainties in model infectiousness parameters and/or unusually high rate of hospital transmission in Taiwan. 2.1 for Hong Kong (Zhou and Yan 2003). Lower because estimate includes data from after implementation of control measures (Bauch, Lloyd-Smith et al. 2005) 1.5 for Toronto, 2.0 for Hong Kong (Choi 2003). Estimates are too low because of authors' interpretation of generation time parameter. After adjusting for this, results are 2.5 for Toronto and 3.4 for Hong Kong (Bauch, Lloyd-Smith et al. 2005) 1.1-3.3 for Beijing (Wang and Ruan 2004). Lower end of range assumes no transmission after entering hospital, highly unrealistic (Bauch, Lloyd-Smith et al. 2005).	al (Lloyd-Smith, Schreiber et al. 2005) and James et al. (James, Shindo et al. 2006).

	2.23 for Taiwan (Bombardt 2006) using approach similar to Wallinga and Teunis (Wallinga and Teunis 2004) 2.87 for mainland China (Zhang 2007) 3 (range 1.5-5)) (Lloyd-Smith, Galvani et al. 2003) 2-3 (Anderson, Fraser et al. 2004) 2.5-2.9 Hong Kong (Fang, Chen et al. 2005)	
1918 Influenza virus	2.2 (95% C.I. 1.7-2.7) extreme at 3.5, Iceland (Gottfredsson, Halldorsson et al. 2008) 1.33-1.86, England and Wales (Chowell, Bettencourt et al. 2008) 2.0-5.4 (summer), 1.2-1.6 (fall), Scandinavia: (Andreasen, Viboud et al. 2008) "Initial R" = 2.0 (Interquartile range 1.7-2.3); "Extreme R" = 2.7 (Interquartile range 2.3-3.4); Maximum R = 6.5 (upper bound); estimate over 45 U.S. cities (Mills, Robins et al. 2004) 2.1, England and Wales (Viboud, Tam et al. 2006) 1.49 (95% CI 1.45-1.53) spring wave; 3.75 (95% CI 3.57-3.93) fall wave, Geneva (Chowell, Ammon et al. 2006) 1.79 – 2.1, estimates from eight model variants over 16 U.S. cities; largest confidence interval 1.3-3.2 (Bootsma and Ferguson 2007) 1.58 – 3.41 for Prussia, Germany (Nishiura 2007) 1.3 – 3.1, New Zealand(Sertsou, Wilson et al. 2006) 2.68, Sao Paulo, Brazil (Massad, Burattini et al. 2007) 1.7-2.0, 83 cities in U.K. (Ferguson, Cummings et al. 2006) 1.70, UK and Wales (Gani, Hughes et al. 2005)	3.0 as nominal value. Will investigate range 1.5-4.0. Will incorporate control measures and individual variation as appropriate.

	2.4, 3.5, San Francisco (Chowell, Nishiura et al. 2007)	

[1] Data are representative of the open literature as of January 2009, but are not necessarily exhaustive. A cursory update from the open literature was performed in April, 2010. Additional relevant data may exist under classified status. Experimental data are presented when possible. Data presented for *F. tularensis,Y. pestis,* and Lassa virus are consistent with data derived by modeling (Sinclair, Boone et al. 2008).

Part C: Infection in the vulnerable.

The Risk Assessment will take into account the differential vulnerability of certain groups of persons to disease and death. The groups have been dictated in part by the level of data available for the sites being evaluated. The groups are persons are children younger than 5, adults older than 65, persons with diabetes, persons with HIV infection, and pregnant women.

There are published papers on increased risk of specific infections such as influenza in the young and elderly, pandemic influenza in pregnancy, tuberculosis in HIV/AIDS patients and pneumonia in diabetics. From these it is inferred that there is an increased risk of infection in these vulnerable groups. However, there are few published reports on the increased risk to infections in general in these groups. References are provided are for increased morbidity and mortality in the elderly (High 2004), diabetics (Muller 1985) and pregnant women (Brabin 1985; Jamieson 2006).

Section 2: Atmospheric decay

Determining the probabilities of infection in certain circumstances requires estimates of the rate at which pathogens would be inactivated by atmospheric factors such as sunlight, humidity, and temperature during airborne transport of aerosols. More specifically, this is needed to estimate the dose of organisms received by persons situated at various distances from an external release.

The various references found during a literature search on the topic use multiple different units to describe the inactivation of pathogens in airborne transport. These various units of measure include: time for a 3-log decay, time for a 1-log decrease, %/min reduction, and D_{37}(the amount of ultraviolet light needed to reduce the number of organisms to 37% of the original value). In order to compare these values, we present both the raw data and the same information using a common unit of measure. The half-life (HL), or period of time it takes for a substance undergoing decay to decrease by half, was selected as the common unit of measure.

Table 2 contains information regarding viability of organisms under various conditions. In the table, times are converted to half-lives (HL), which are noted in [square brackets]. D_{37} values were also converted to a HL using the estimated midday effective solar flux for Boston.

Table 2. Prior information: Stability in ambient environmental conditions[1,2]

	Experimental data/Estimations
Bacillus anthracis[3]	Spores seeded into a plant/soil model had survival ratio at day 2 and day 4 of approx. 60% and 53%, respectively, and the percentage of cells that were vegetative was 47% and 43% , respectively (Saile and Koe**HL**er 2006). Spores resist drying, heat, UV light, and some disinfectants (Turnbull 1998). Anthrax spores can survive for months or even decades depending on pH, temperature, and nutrients in the soil (Lewerin, Elvander et al.; Manchee, Broster et al. 1990; Montville, Dengrove et al. 2005).
Francisella tularensis	Viability of *F. tularensis* aerosols in a chamber ventilated with outdoor air ranged from 7% (45 minutes at 79-82% RH) [**HL**=11.7 min] to 25% (30 minutes at 73% relative humidity) [**HL**=15min] (Hood 2009). Oxygen toxicity is minimal for *F. tularensis* (Cox 1995)
Yersinia pestis	About 3-log decay over 90 minutes [**HL**[4]=9 min] using an avirulent strain in aerosolized heart infusion broth at conditions of 26C and RH varying from 20-50% (Won and Ross 1966). WHO estimated that aerosolized *Y. pestis* would remain viable for 1 hour (Borio and Hynes 2010). Laboratory surface stability varies: steel (6h), glass (7h), polyethylene (24h), paper (5d) (Rose, Donlan et al. 2003). Can survive days to weeks in flea feces, tissues of dead animals, and up to 40 weeks in some soils (Drancourt, Houhamdi et al. 2006; Ayyadurai, Houhamdi et al. 2008; Eisen, Petersen et al. 2008)
Andes hantavirus[5]	The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for hanta virus was calculated to be 12 [**HL**=28 min at maximum solar radiation] (Lytle and Sagripanti 2005).
Ebola virus[5]	Predicted inactivation of a filovirus was calculated for six geographic points for as many as five times of year. The time for a 1 log decrease varied from 20 to 100 minutes[**HL** range of 6 to 30 min] (Lytle and Sagripanti 2005). The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for filovirus was calculated to be 7.3 [**HL**=17 min at maximum solar radiation] (Lytle and Sagripanti 2005). Filoviruses are relatively stable in aerosols, retain virulence after lyophilization, and can persist for long periods on contaminated surfaces (Leffel and Reed 2004) Filoviruses retain infectivity @ room temperature on environmental surfaces; thus, fomites may be sources of transmission,

	e.g., blankets and sleeping mats identified (Salvaggio and Baddley 2004)
Marburg virus[5]	Predicted inactivation of a filovirus was calculated for six geographic points for as many as five times of year. The time for a 1 log decrease varied from 20 to 100 minutes [**HL** range of 6 to 30 min] (Lytle and Sagripanti 2005). The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for filovirus was calculated to be 7.3 [**HL**=17 min at maximum solar radiation] (Lytle and Sagripanti 2005). Survives up to 5 days on contaminated surfaces; unstable in aerosols with specific rate of inactivation= 0.05/min (Belanov, Muntianov et al. 1996) [assumed for purposes herein to be 5%/min, resulting in **HL**=13.5 min] Filoviruses are relatively stable in aerosols, retain virulence after lyophilization, and can persist for long periods on contaminated surfaces (Leffel and Reed 2004) Filoviruses retain infectivity @ room temperature on environmental surfaces; thus, fomites may be sources of transmission, e.g., blankets and sleeping mats identified (Salvaggio and Baddley 2004)
Junin virus (Argentine hemorrhagic fever virus)[5]	The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for Junin was calculated to be 13 [**HL** = 30 min at maximum solar radiation] (Lytle and Sagripanti 2005),. Inactivation at 37^0C is 47%, 59%, and 99.9% at timepoints of 1, 6, and 26 hours [**HL** of 65,280, and 156 min], respectively. At 25^0C, inactivation was 75% and 99% at 26 and 72 hours [**HL** of 780 and 650 min], respectively (Parodi, Coto et al. 1966)
Lassa fever virus[5]	Biological half-lives of the virus in aerosols at both 24 and 32°C ranged from 10.1 to 54.6 min, and were sufficient for aerosol dispersion of virus to considerable distances in natural situations (Stephenson, Larson et al. 1984) The D_{37} value (the dose of UV_{254} in J/m^2 that reduces the surviving virus to 37% of its original) for Lassa was calculated to be 13 [**HL** = 30 min at maximum solar radiation] (Lytle and Sagripanti 2005).
Nipah virus	"Survival of henipaviruses in the environment is hig**HL**y sensitive to temperature and desiccation. Under most conditions survival time was brief, with half-lives limited to a few hours, indicating that transmission to a new host requires close contact with an infected animal or exposure to contaminated material shortly after excretion. However, under optimal conditions henipaviruses can persist for a number of days and under these circumstances, vehicle-borne transmission may be possible." (Fogarty, Halpin et al. 2008) Henipavirus viable >4 days in bat urine and viable 1-4 days in various fruit juices@22°C; tolerates a wide range of pH. It is inactivated in 1 day @37°C and is hig**HL**y susceptible to dessication (Fogarty, Halpin et al. 2008)
Rift Valley fever virus[5]	Geometric mean biological decay rate 2.3% per minute over range of 50 and 80% relative humidity; aerosol stability not influenced by relative humidity [**HL**=30 min] (Miller, Demchak et al. 1963) Viral half life of 77, 15.8, and 6.9 min for 30%, 55% and 80% relative humidity, respectively (Brown, Dominik et al. 1982)

	The D_{37} value (the dose of UV_{254} in J/m^2) that reduces the surviving virus to 37% of its original) for Rift Valley fever virus was calculated to be 12 [**HL**=28 min at maximum solar radiation] (Lytle and Sagripanti 2005). Virus very stable in aerosols @23°C and 50-85% relative humidity (Shimshony and Barzilai 1983; Swanepoel and Coetzer 1994)
Russian spring-summer encephalitis virus (TBE complex virus)[5]	The D_{37} value for West Nile virus was calculated to be 24 [**HL**=56 min at maximum solar UV conditions](Lytle and Sagripanti 2005). RSSE virus and West Nile virus are classified in the genus *Flavivirus*. Stable for at least 6 h in liquid aerosol suspension @ room temperature and 23–80% humidity (Gritsun, Lashkevich et al. 2003)
SARS-associated coronavirus[5]	The D_{37} value for BEV (Berne) was calculated to be 3.1 [**HL**=7 min at maximum solar radiation] (Lytle and Sagripanti 2005). BEV and SARS-CoV are classified in the family *Coronaviridaae.* Virus was inactivated by ultraviolet light (UV) at 254 nm (UVC wavelength). UVA had no significant effect. Details as follows: virus suspension 1 cm deep in 24-well plates, UV source at height of 3 cm from the bottom of the wells. UVC (254 nm) at 4016 $\mu W/cm^2$ (where $\mu W = 10^{-6}$ J/s) and UVA (365 nm) at 2133 $\mu W/cm^2$. UVA had no significant effect. UVC resulted in a 1 log reduction at 1 minute, a 2 log reduction at 2 minutes, a 3 log reduction at 3 minutes, a 3.5 log reduction at 4 minutes, a 4 log reduction at 6 minutes, complete inactivation at 15 minutes (Darnell, Subbarao et al. 2004). However, note that UVC radiation consists of wavelengths of 100-280 nm and is absorbed by the ozone layer; a negligible amount [of UV 254 nm] reaches earth's surface (World Health Organization 2002). Titer decreased from $10^{7.5}$ $CCID_{50}$ to $10^{3.2}$ $CCID_{50}$ within 5 days @ room temperature (details not available) (from Bao, et al, 2003 [Chinese]) (Wang, Li et al. 2005) In vitro, virus persists for 2 days in hospital wastewater, domestic sewage and dec**HL**orinated tap water; 3 days in feces, 14 days in PBS, 17 days in urine at 20 ◦C; @ 4◦C, virus persists for 14 days in wastewater and >17 days in feces or urine: free c**HL**orine inactivates virus better than c**HL**orine dioxide; free residue c**HL**orine over 0.5 mg/L for c**HL**orine or 2.19 mg/L for c**HL**orine dioxide in wastewater ensures complete inactivation (Wang, Li et al. 2005) Virus survived at RT for 9 days in cell culture suspension and 6 days in dried state on plastics (Rabenau, Cinatl et al. 2005) Virus persists in the environment-- 1+ day on surfaces and 4 days in feces (McKinney, Gong et al. 2006) On dry surfaces@ room temperature, virus survives for 2-3 days; in fecal samples, survival for 2-4 days (Wong and Yuen 2005)

	At Amoy Gardens, optimum environmental temperature range associated with SARS cases was 16-28°C [~61-82F] (Yip, Chang et al. 2007) At Amoy Gardens, virus is believed to have spread between apartment block buildings via aerosols from sewage system carried by ambient wind (Yip, Chang et al. 2007) Virus can survive in respiratory samples for 5 days @ room temperature (Lim, Ng et al. 2006) In diarrheal feces, can survive for a few days @ room temperature; fecal droplets with a high titer of virus, 10^6 $CCID_{50}$/mL, can remain infectious for 4-5 days (Lim, Ng et al. 2006)
1918 Influenza virus[5]	The D_{37} value for influenza A virus was calculated to be 7.5 [**HL**=18 min at maximum solar conditions] (Lytle and Sagripanti 2005). Solar radiation-induced infectivity reduction for influenza A virus has been reported to range from 0.1 to 7.5 $\log_{10}$ /day in U.S. locations depending on latitude and season (Sagripanti and Lytle 2007) "The study of the inactivation of influenza virus as a function of relative humidity and temperature has produced contradictory results." "Maximum survival times vary between 1 h (80% RH) and 24 h (20% RH)." (Weber and Stilianakis 2008) Effects of humidity on the ability of influenza viruses to infect mice in a nonventilated room with constantly agitated air have been studied (Loosli, Lemon et al. 1943). At a relative humidity of 17%–24%, animals became infected with influenza as late as 24 h after the virus was first aerosolized into the room, although the proportion of animals infected decreased over time. Infectivity was enhanced at 22h after influenza virus was introduced, when the floor was vigorously swept, suggesting that desiccation of the virus does not eliminate infectivity. Whether sufficient numbers of virus laden particles can remain viable to infect humans in a similar setting is unknown (Bridges, Kuehnert et al. 2003). Human influenza viruses can survive on a variety of surfaces at 35%–49% humidity and a temperature of 28 degrees C. Both influenza A and B viruses were cultured from experimentally contaminated, nonporous surfaces, such as steel and plastic, up to 24–48 h after inoculation, and from cloth, paper, and tissues up to 8–12 h after inoculation (Bean, Moore et al. 1982). "Avian influenza viruses can be isolated from natural, open fresh water." (Weber and Stilianakis 2008)

[1] Data are representative of the open literature as of January 2009, but are not necessarily exhaustive. A cursory update from the open literature was performed in April, 2010. Additional relevant data may exist under classified status. Experimental data are presented when possible. Data presented for *F. tularensis,Y. pestis,* and Lassa virus are consistent with data derived by modeling (Sinclair, Boone et al. 2008).

[2] "Viral agents are hardier and reach further into the environment than previously expected. For example, an information leak in 2002 from the former Soviet Union reported an accidental infection in 1971 of naval personnel 11 miles offshore from a smallpox testing site in the Soviet city of Aralsk (Tucker and Zilinskas 2003)" (Lytle and Sagripanti 2005).

[3] Few data are published for survival of vegetative cells in the environment (Saile and Koehler 2006). At 26 ^{0}C in the laboratory, sporulation begins no earlier that 24, 28, 40,and 60 hours, under relative humidities of 90-100%,, 80%, 50-70%, and 20-40%, respectively (Davis 1960).

[4] Half-life values were calculated and inserted.

[5] Virtually all data on UV inactivation of various viruses have been generated using UV_{254} radiation from an artificial source (mercury lamp). However, this wavelength does not reach the surface of the earth (World Health Organization 2002; Lytle and Sagripanti 2005). In the absence of experimental data on aerosolized pathogens exposed under actual solar conditions, estimation of virus inactivation by other wavelengths of solar exposure via extrapolation has been attempted for viruses relevant to biodefense (Lytle and Sagripanti 2005). It is important to note these values were determined based on the minimum solar zenith angle, that is, the time of day when UV radiation is most intense. Validation of the extrapolation method was attempted using data from two experiments using TMV virus and bacteriophages T*1* and T*7*. Predicted inactivation of a filovirus, as an example, was calculated for six geographic points for as many as five times of year. The time for a 1 log decrease in filovirus viability varied from 20 to 100 minutes.

Appendix 2: References and Abstracts (Prepared by Tetra Tech)

B: Abstracts

Reprints are available on the Tetra Tech Inc. SharePoint site; information on accessing this resource sent separately.

Agar, S. L., J. Sha, et al. (2009). "Characterization of the rat pneumonic plague model: infection kinetics following aerosolization of Yersinia pestis CO92." Microbes Infect **11**(2): 205-14.

Yersinia pestis, the causative agent of human bubonic and pneumonic plague, is spread during natural infection by the fleas of rodents. Historically associated with infected rat fleas, studies on the kinetics of infection in rats are surprisingly few, and these reports have focused mainly on bubonic plague. Although the natural route of primary infection results in bubonic plague in humans, it is commonly thought that aerosolized Y. pestis will be utilized during a biowarfare attack. Accordingly, based on our previous characterization of the mouse model of pneumonic plague, we sought to examine the progression of infection in rats exposed in a whole-body Madison chamber to aerosolized Y. pestis CO92. Following an 8.6 LD(50) dose of Y. pestis, injury was apparent in the rat tissues based on histopathology, and chemokines and cytokines rose above control levels (1h post infection [p.i.]) in the sera and organ homogenates over a 72-h infection period. Bacteria disseminated from the lungs to peripheral organs, with the largest increases in the spleen, followed by the liver and blood at 72h p.i. compared to the 1h controls. Importantly, rats were as sensitive to pneumonic plague as mice, having a similar LD(50) dose by the intranasal and aerosolized routes. Further, we showed direct transmission of plague bacteria from infected to uninfected rats. Taken together, the data allowed us to characterize for the first time a rat pneumonic plague model following aerosolization of Y. pestis.

Alford, R. H., J. A. Kasel, et al. (1966). "Human influenza resulting from aerosol inhalation." Proc Soc Exp Biol Med **122**(3): 800-4.

Anderson, R. M., C. Fraser, et al. (2004). "Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic." Philos Trans R Soc Lond B Biol Sci **359**(1447): 1091-105.

This paper reviews current understanding of the epidemiology, transmission dynamics and control of the aetiological agent of severe acute respiratory syndrome (SARS). We present analyses of data on key parameters and distributions and discuss the processes of data capture, analysis and public health policy formulation during the SARS epidemic are discussed. The low transmissibility of the virus, combined with the onset of peak infectiousness following the onset of clinical symptoms of disease, transpired to make simple public health measures, such as isolating patients and quarantining their contacts, very effective in the control of the SARS epidemic. We conclude that we were lucky this time round, but may not be so with the next epidemic outbreak of a novel aetiological agent. We present analyses that help to further understanding of what intervention measures are likely to work best with infectious agents of defined biological and epidemiological properties. These lessons learnt from the SARS experience are presented in an epidemiological and public health context.

Andreasen, V., C. Viboud, et al. (2008). "Epidemiologic characterization of the 1918 influenza pandemic summer wave in Copenhagen: implications for pandemic control strategies." J Infect Dis **197**(2): 270-8.

BACKGROUND: The 1918-1919 A/H1N1 influenza pandemic killed approximately 50 million people worldwide. Historical records suggest that an early pandemic wave struck Europe during the summer of 1918. METHODS: We obtained surveillance data that were compiled weekly, during 1910-1919, in Copenhagen, Denmark; the records included medically treated influenza-like illnesses (ILIs), hospitalizations, and deaths by age. We used a Serfling seasonal regression model to quantify excess morbidity and mortality, and we estimated the reproductive number (R) for the summer, fall, and winter pandemic waves. RESULTS: A large epidemic occurred in Copenhagen during the summer of 1918; the age distribution of deaths was characteristic of the 1918-1919 A/H1N1 pandemic overall. That summer wave accounted for 29%-34% of all excess ILIs and hospitalizations during 1918, whereas the case-fatality rate (0.3%) was many-fold lower than that of the fall wave (2.3%). Similar patterns were observed in 3 other Scandinavian cities. R was substantially higher in summer (2.0-5.4) than in fall (1.2-1.6) in all cities. CONCLUSIONS: The Copenhagen summer wave may have been caused by a precursor A/H1N1 pandemic virus that transmitted efficiently but lacked extreme virulence. The R measured in the summer wave is likely a better approximation of transmissibility in a fully susceptible population and is substantially higher than that found in previous US studies. The summer wave may have provided partial protection against the lethal fall wave.

Atkinson, M. P. and L. M. Wein (2008). "Quantifying the routes of transmission for pandemic influenza." Bull Math Biol **70**(3): 820-67.

Motivated by the desire to assess nonpharmaceutical interventions for pandemic influenza, we seek in this study to quantify the routes of transmission for this disease. We construct a mathematical model of aerosol (i.e., droplet-nuclei) and contact transmission of influenza within a household containing one infected. An analysis of this model in conjunction with influenza and rhinovirus data suggests that aerosol transmission is far more dominant than contact transmission for influenza. We also consider a separate model of a close expiratory event, and find that a close cough is unlikely (approximately 1% probability) to generate traditional droplet transmission (i.e., direct deposition on the mucous membranes), although a close, unprotected and horizontally-directed sneeze is potent enough to cause droplet transmission. There are insufficient data on the frequency of close expiratory events to assess the relative importance of aerosol transmission and droplet transmission, and it is prudent to leave open the possibility that droplet transmission is important until proven otherwise. However, the rarity of close, unprotected and horizontally-directed sneezes-coupled with the evidence of significant aerosol and contact transmission for rhinovirus and our comparison of hazard rates for rhinovirus and influenza-leads us to suspect that aerosol transmission is the dominant mode of transmission for influenza.

Ayyadurai, S., L. Houhamdi, et al. (2008). "Long-term persistence of virulent *Yersinia pestis* in soil." Microbiology **154**(Pt 9): 2865-71.

Plague is characterized by geographical foci from which it re-emerges after decades of silence, a fact currently explained by enzootic and epizootic cycles between plague-susceptible and plague-resistant rodents. To assess the potential role of soil in plague epidemiology, we experimentally investigated whether Yersinia pestis could persist alive and virulent in soil. Sterilized soil inoculated with virulent Y. pestis biotype Orientalis was regularly sampled for 40 weeks in duplicate. Each sample was observed by acridine orange staining and immunofluorescence using an anti-Y. pestis polyclonal antibody, and DNA was extracted for PCR

amplification and sequencing of the Y. pestis ureD, caf1 and pla genes. All samples were inoculated onto selective agar, and samples from soil that had been incubated for 10, 60, 165, 210 and 280 days were also inoculated into each of two BALB/c female mice. The mouse experiment was performed in triplicate. Non-inoculated, sterilized soil samples were used as negative controls. Micro-organisms fluorescing orange and detected by immunofluorescence were identified as Y. pestis in all samples. They were recovered in pure agar cultures for up to 30 weeks but thereafter were contaminated with Pseudomonas spp. Soil that had been inoculated with Y. pestis proved to be fully virulent in mice, which died with Y. pestis septicaemia and multiple organ involvement. Negative control mice showed no signs of disease. These data indicate that Y. pestis biotype Orientalis can remain viable and fully virulent after 40 weeks in soil. This study is a first step on which to base further investigations of a potential telluric reservoir for Y. pestis, which could represent an alternative mechanism for the maintenance of plague foci.

Baccam, P. and M. Boechler (2007). "Public health response to an anthrax attack: an evaluation of vaccination policy options." Biosecur Bioterror **5**(1): 26-34.

A discrete-time, deterministic, compartmental model was developed and analyzed to provide insight into how the use of anthrax vaccine before or after a large-scale attack can reduce casualties. The model accounts for important response and protection factors such as antibiotic and vaccine efficacy, the protective effects of buildings, the timing of emergency response, and antibiotic adherence and vaccine coverage in the population prior to the attack. The relative benefit of pre- versus post-exposure vaccination is influenced by the timing of the post-exposure antibiotic distribution campaign as well as assumptions of antibiotic adherence. The results indicate that, regardless of which vaccination policy is adopted, a rapid and effective post-attack medical response has a large impact on the number of lives that can be saved by a post-exposure prophylaxis (PEP) campaign. A sensitivity analysis of the model indicates that uncertainty in medical efficacy and the time to initiate a PEP campaign are the model parameters that have the greatest impact on the number of predicted deaths. It is shown that for each day that a mass prophylaxis campaign is delayed, more casualties and deaths result than for each day that the completion of the campaign is delayed.

Bauch, C. T., J. O. Lloyd-Smith, et al. (2005). "Dynamically modeling SARS and other newly emerging respiratory illnesses: past, present, and future." Epidemiology **16**(6): 791-801.

The emergence and rapid global spread of the severe acute respiratory syndrome (SARS) coronavirus in 2002-2003 prompted efforts by modelers to characterize SARS epidemiology and inform control policies. We overview and discuss models for emerging infectious diseases (EIDs), provide a critical survey of SARS modeling literature, and discuss promising future directions for research. We reconcile discrepancies between published estimates of the basic reproductive number R0 for SARS (a crucial epidemiologic parameter), discuss insights regarding SARS control measures that have emerged uniquely from a modeling approach, and argue that high priorities for future modeling of SARS and similar respiratory EIDs should include informing quarantine policy and better understanding the impact of population heterogeneity on transmission patterns.

Bean, B., B. M. Moore, et al. (1982). "Survival of influenza viruses on environmental surfaces." J Infect Dis **146**(1): 47-51.

To investigate the transmission of influenza viruses via hands and environmental surfaces, the survival of laboratory-grown influenza A and influenza B viruses on various surfaces was studied.

Both influenza A and B viruses survived for 24-48 hr on hard, nonporous surfaces such as stainless steel and plastic but survived for less than 8-12 hr on cloth, paper, and tissues. Measurable quantities of influenza A virus were transferred from stainless steel surfaces to hands for 24 hr and from tissues to hands for up to 15 min. Virus survived on hands for up to 5 min after transfer from the environmental surfaces. These observations suggest that the transmission of virus from donors who are shedding large amounts could occur for 2-8 hr via stainless steel surfaces and for a few minutes via paper tissues. Thus, under conditions of heavy environmental contamination, the transmission of influenza virus via fomites may be possible.

Belanov, E. F., V. P. Muntianov, et al. (1996). "[Survival of Marburg virus infectivity on contaminated surfaces and in aerosols]." Vopr Virusol **41**(1): 32-4.

Marburg virus was shown to survive for up to 4-5 days on contaminated surfaces. In aerosol it was not stable, the specific rate of its inactivation being 0.05 min-1. This brought the authors to a conclusion that a relatively close contact is needed for virus transmission from man to man, although the possibility of aerosol transmission of the infection may be appreciably increased in case of the hemorrhagic syndrome with a high level of viremia.

Bombardt, J. N. (2006). "Congruent epidemic models for unstructured and structured populations: analytical reconstruction of a 2003 SARS outbreak." Math Biosci **203**(2): 171-203.

Both the threat of bioterrorism and the natural emergence of contagious diseases underscore the importance of quantitatively understanding disease transmission in structured human populations. Over the last few years, researchers have advanced the mathematical theory of scale-free networks and used such theoretical advancements in pilot epidemic models. Scale-free contact networks are particularly interesting in the realm of mathematical epidemiology, primarily because these networks may allow meaningfully structured populations to be incorporated in epidemic models at moderate or intermediate levels of complexity. Moreover, a scale-free contact network with node degree correlation is in accord with the well-known preferred mixing concept. The present author describes a semi-empirical and deterministic epidemic modeling approach that (a) focuses on time-varying rates of disease transmission in both unstructured and structured populations and (b) employs probability density functions to characterize disease progression and outbreak controls. Given an epidemic curve for a historical outbreak, this modeling approach calls for Monte Carlo calculations (that define the average new infection rate) and solutions to integro-differential equations (that describe outbreak dynamics in an aggregate population or across all network connectivity classes). Numerical results are obtained for the 2003 SARS outbreak in Taiwan and the dynamical implications of time-varying transmission rates and scale-free contact networks are discussed in some detail.

Bootsma, M. C. and N. M. Ferguson (2007). "The effect of public health measures on the 1918 influenza pandemic in U.S. cities." Proc Natl Acad Sci U S A **104**(18): 7588-93.

During the 1918 influenza pandemic, the U.S., unlike Europe, put considerable effort into public health interventions. There was also more geographic variation in the autumn wave of the pandemic in the U.S. compared with Europe, with some cities seeing only a single large peak in mortality and others seeing double-peaked epidemics. Here we examine whether differences in the public health measures adopted by different cities can explain the variation in epidemic patterns and overall mortality observed. We show that city-specific per-capita excess mortality in 1918 was significantly correlated with 1917 per-capita mortality, indicating some intrinsic variation in overall mortality, perhaps related to sociodemographic factors. In the subset of 23 cities for which we had partial data on the timing of interventions, an even stronger correlation

was found between excess mortality and how early in the epidemic interventions were introduced. We then fitted an epidemic model to weekly mortality in 16 cities with nearly complete intervention-timing data and estimated the impact of interventions. The model reproduced the observed epidemic patterns well. In line with theoretical arguments, we found the time-limited interventions used reduced total mortality only moderately (perhaps 10-30%), and that the impact was often very limited because of interventions being introduced too late and lifted too early. San Francisco, St. Louis, Milwaukee, and Kansas City had the most effective interventions, reducing transmission rates by up to 30-50%. Our analysis also suggests that individuals reactively reduced their contact rates in response to high levels of mortality during the pandemic.

Borio, L. and N. A. Hynes (2010). Plague as a Bioterrorism Weapon. Principles and Practice of Infectious Diseases. G. L. Mandell, J. E. Bennett and R. Dolan. Philadelphia, Churchill Livingston Elsevier**:** 3965-3970.

Bossart, K. N., Z. Zhu, et al. (2009). "A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection." PLoS Pathog **5**(10): e1000642.

Nipah virus is a broadly tropic and highly pathogenic zoonotic paramyxovirus in the genus Henipavirus whose natural reservoirs are several species of Pteropus fruit bats. Nipah virus has repeatedly caused outbreaks over the past decade associated with a severe and often fatal disease in humans and animals. Here, a new ferret model of Nipah virus pathogenesis is described where both respiratory and neurological disease are present in infected animals. Severe disease occurs with viral doses as low as 500 TCID(50) within 6 to 10 days following infection. The underlying pathology seen in the ferret closely resembles that seen in Nipah virus infected humans, characterized as a widespread multisystemic vasculitis, with virus replicating in highly vascular tissues including lung, spleen and brain, with recoverable virus from a variety of tissues. Using this ferret model a cross-reactive neutralizing human monoclonal antibody, m102.4, targeting the henipavirus G glycoprotein was evaluated in vivo as a potential therapeutic agent. All ferrets that received m102.4 ten hours following a high dose oral-nasal Nipah virus challenge were protected from disease while all controls died. This study is the first successful post-exposure passive antibody therapy for Nipah virus using a human monoclonal antibody.

Brabin BJ. (1985). "Epidemiology of infection in pregnancy. Rev Infect Dis. 7(5):579-603.

In this article the immunologic, clinical, and epidemiologic evidence for altered host susceptibility to infection during pregnancy is reviewed in an attempt to determine general principles that can be applied to interpret the wide range of information available and that can be utilized for epidemiologic analysis and study design. Gestational changes in immunity are related to the maternal history of infection during pregnancy. Primary infections are distinguished from recurrent infections, and the different patterns of recurrent infection in pregnancy are defined. This classification system is then used to interpret a wide range of data. The impact of infection in pregnancy on the offspring is discussed in relation to vertical transmission and pregnancy immune status: in pregnant women the clearance, if not the incidence, of infection is similar to that in nonpregnant women; maternal susceptibility to infection alters early in gestation (at less than 12 weeks); the degree of maternal recovery from early gestational infection affects vertical transmission rates; there are few data on how patterns of infection with the major tropical parasites during pregnancy relate to vertical transmission.

Brachman, P. S., A. F. Kaufman, et al. (1966). "Industrial inhalation Anthrax." Bacteriol Rev **30**(3): 646-59.

Bridges, C. B., M. J. Kuehnert, et al. (2003). "Transmission of influenza: implications for control in health care settings." Clin Infect Dis **37**(8): 1094-101.

Annual influenza epidemics in the United States result in an average of >36,000 deaths and 114,000 hospitalizations. Influenza can spread rapidly to patients and health care personnel in health care settings after influenza is introduced by visitors, staff, or patients. Influenza outbreaks in health care facilities can have potentially devastating consequences, particularly for immunocompromised persons. Although vaccination of health care personnel and patients is the primary means to prevent and control outbreaks of influenza in health care settings, antiviral influenza medications and isolation precautions are important adjuncts. Although droplet transmission is thought to be the primary mode of influenza transmission, limited evidence is available to support the relative clinical importance of contact, droplet, and droplet nuclei (airborne) transmission of influenza. In this article, the results of studies on the modes of influenza transmission and their relevant isolation precautions are reviewed.

Brown, J. L., J. W. Dominik, et al. (1982). Airborne survival of Rift Valley fever virus. U.S. Army Medical Research Institute of Infectious Diseases Aerobiology Division. Frederick, U.S. Department of Defense**:** 1-11.

The aerosol stability characteristics of an Egyptian isolate of Rift Valley fever virus (ZH-501 strain) were determined in a static aerosol chamber. Aerosolized particles had a mass median diameter of 4.0 pm. At 30, 55, 80% relative humidity (RH) the biological decay rate was 0.9, 4.1,and 10.1% per min, respectively. The decay rate data tested significantly different ($P < 0.001$) at each RH. The biological half-life values were 6.9 min at 80% RH, 15.8 min at 55% RH, and 77.0 min at 30% RK. Comparable decay rates were obtained with a strain (SA-51) isolated in South Africa in 1951.

Carrat, F., E. Vergu, et al. (2008). "Time lines of infection and disease in human influenza: a review of volunteer challenge studies." Am J Epidemiol **167**(7): 775-85.

The dynamics of viral shedding and symptoms following influenza virus infection are key factors when considering epidemic control measures. The authors reviewed published studies describing the course of influenza virus infection in placebo-treated and untreated volunteers challenged with wild-type influenza virus. A total of 56 different studies with 1,280 healthy participants were considered. Viral shedding increased sharply between 0.5 and 1 day after challenge and consistently peaked on day 2. The duration of viral shedding averaged over 375 participants was 4.80 days (95% confidence interval: 4.31, 5.29). The frequency of symptomatic infection was 66.9% (95% confidence interval: 58.3, 74.5). Fever was observed in 37.0% of A/H1N1, 40.6% of A/H3N2 ($p = 0.86$), and 7.5% of B infections ($p = 0.001$). The total symptoms scores increased on day 1 and peaked on day 3. Systemic symptoms peaked on day 2. No such data exist for children or elderly subjects, but epidemiologic studies suggest that the natural history might differ. The present analysis confirms prior expert opinion on the duration of viral shedding or the frequency of asymptomatic influenza infection, extends prior knowledge on the dynamics of viral shedding and symptoms, and provides original results on the frequency of respiratory symptoms or fever.

Cheng, V. C., S. K. Lau, et al. (2007). "Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection." Clin Microbiol Rev **20**(4): 660-94.

Before the emergence of severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) in 2003, only 12 other animal or human coronaviruses were known. The discovery of this virus was

soon followed by the discovery of the civet and bat SARS-CoV and the human coronaviruses NL63 and HKU1. Surveillance of coronaviruses in many animal species has increased the number on the list of coronaviruses to at least 36. The explosive nature of the first SARS epidemic, the high mortality, its transient reemergence a year later, and economic disruptions led to a rush on research of the epidemiological, clinical, pathological, immunological, virological, and other basic scientific aspects of the virus and the disease. This research resulted in over 4,000 publications, only some of the most representative works of which could be reviewed in this article. The marked increase in the understanding of the virus and the disease within such a short time has allowed the development of diagnostic tests, animal models, antivirals, vaccines, and epidemiological and infection control measures, which could prove to be useful in randomized control trials if SARS should return. The findings that horseshoe bats are the natural reservoir for SARS-CoV-like virus and that civets are the amplification host highlight the importance of wildlife and biosecurity in farms and wet markets, which can serve as the source and amplification centers for emerging infections.

Choi, B. C. K., Pak, A. W. P. (2003). "A simple approximate mathematical model to predict the number of severe acute respiratory syndrome cases and deaths
" J Epidemiol Community Health **57**: 831-835.

Background: Severe acute respiratory syndrome (SARS) is currently spreading in many countries. This paper proposes a simple approximate mathematical model for public health practitioners to predict the number of SARS cases and deaths. Methods: The model is based on four parameters: Ro (basic reproductive number), F (case-fatality rate), i(incubation period), and d (duration of disease). The calculations can be done by hand or by using a computer spreadsheet. Results: The best parameters to fit Canadian data as of 6 April 2003 (before infection controls took effect) are Ro = 1.5, F = 30%, i = 5 days, d = 14 days. On 6 April (day 40) there were 74 cases and 7 deaths. If this trend continues, SARS numbers in Canada are predicted to be as follows: 387 cases and 34 deaths by 26 April (day 60), 4432 cases and 394 deaths by 26 May (day 90), and 50 500 cases and 4489 deaths by 25 June (day 120). By comparison, the best parameters to fit Hong Kong data as of 10 April 2003 are Ro = 2.0, F = 20%, i = 5 days, d = 14 days. Conclusions: Using the proposed mathematical model, it was estimated that about 1.5 to 2 new infectious cases were produced per infectious case every five days. Also, about 20% to 30% of the cases die within 14 days. The case-fatality may therefore be considerably higher than initially thought. The model indicates that SARS can spread very fast when there are no interventions.

Chowell, G., C. E. Ammon, et al. (2006). "Transmission dynamics of the great influenza pandemic of 1918 in Geneva, Switzerland: Assessing the effects of hypothetical interventions." J Theor Biol **241**(2): 193-204.

Recurrent outbreaks of the avian H5N1 influenza virus in Asia represent a constant global pandemic threat. We characterize and evaluate hypothetical public health measures during the 1918 influenza pandemic in the Canton of Geneva, Switzerland. The transmission rate, the recovery rate, the diagnostic rate, the relative infectiousness of asymptomatic cases, and the proportion of clinical cases are estimated through least-squares fitting of the model to epidemic curve data of the cumulative number of hospital notifications. The latent period and the case fatality proportion are taken from published literature. We determine the variance and identifiability of model parameters via a simulation study. Our epidemic model agrees well with the observed epidemic data. We estimate the basic reproductive number for the spring wave R1;=1.49 (95% CI: 1.45-1.53) and the reproductive number for the fall wave R2;=3.75 (95% CI: 3.57-3.93). In addition, we estimate the clinical reporting for these two waves to be 59.7% (95%

CI: 55.7-63.7) and 83% (95% CI: 79-87). We surmise that the lower reporting in the first wave can be explained by a lack of initial awareness of the epidemic and the relative higher severity of the symptoms experienced during the fall wave. We found that effective isolation measures in hospital clinics at best would only ensure control with probability 0.87 while reducing the transmission rate by >76.5% guarantees stopping an epidemic.

Chowell, G., L. M. Bettencourt, et al. (2008). "The 1918-1919 influenza pandemic in England and Wales: spatial patterns in transmissibility and mortality impact." Proc Biol Sci **275**(1634): 501-9.

Spatial variations in disease patterns of the 1918-1919 influenza pandemic remain poorly studied. We explored the association between influenza death rates, transmissibility and several geographical and demographic indicators for the autumn and winter waves of the 1918-1919 pandemic in cities, towns and rural areas of England and Wales. Average measures of transmissibility, estimated by the reproduction number, ranged between 1.3 and 1.9, depending on model assumptions and pandemic wave and showed little spatial variation. Death rates varied markedly with urbanization, with 30-40% higher rates in cities and towns compared with rural areas. In addition, death rates varied with population size across rural settings, where low population areas fared worse. By contrast, we found no association between transmissibility, death rates and indicators of population density and residential crowding. Further studies of the geographical mortality patterns associated with the 1918-1919 influenza pandemic may be useful for pandemic planning.

Chowell, G., C. Castillo-Chavez, et al. (2004). "Model parameters and outbreak control for SARS." Emerg Infect Dis **10**(7): 1258-63.

Control of the 2002-2003 severe acute respiratory syndrome (SARS) outbreak was based on rapid diagnosis coupled with effective patient isolation. We used uncertainty and sensitivity analysis of the basic reproductive number R0 to assess the role that model parameters play in outbreak control. The transmission rate and isolation effectiveness have the largest fractional effect on R0. We estimated the distribution of the reproductive number R0 under perfect isolation conditions. The distribution lies in the interquartile range 0.19-1.08, with a median of 0.49. Even though the median of R0 is <1, we found that 25% of our R0 distribution lies at R0 > 1, even with perfect isolation. This implies the need to simultaneously apply more than one method of control.

Chowell, G., N. W. Hengartner, et al. (2004). "The basic reproductive number of Ebola and the effects of public health measures: the cases of Congo and Uganda." J Theor Biol **229**(1): 119-26.

Despite improved control measures, Ebola remains a serious public health risk in African regions where recurrent outbreaks have been observed since the initial epidemic in 1976. Using epidemic modeling and data from two well-documented Ebola outbreaks (Congo 1995 and Uganda 2000), we estimate the number of secondary cases generated by an index case in the absence of control interventions R0. Our estimate of R0 is 1.83 (SD 0.06) for Congo (1995) and 1.34 (SD 0.03) for Uganda (2000). We model the course of the outbreaks via an SEIR (susceptible-exposed-infectious-removed) epidemic model that includes a smooth transition in the transmission rate after control interventions are put in place. We perform an uncertainty analysis of the basic reproductive number R0 to quantify its sensitivity to other disease-related parameters. We also analyse the sensitivity of the final epidemic size to the time interventions begin and provide a distribution for the final epidemic size. The control measures implemented during these two outbreaks (including education and contact tracing followed by quarantine)

reduce the final epidemic size by a factor of 2 relative the final size with a 2-week delay in their implementation.

Chowell, G., H. Nishiura, et al. (2007). "Comparative estimation of the reproduction number for pandemic influenza from daily case notification data." J R Soc Interface **4**(12): 155-66.

The reproduction number, R, defined as the average number of secondary cases generated by a primary case, is a crucial quantity for identifying the intensity of interventions required to control an epidemic. Current estimates of the reproduction number for seasonal influenza show wide variation and, in particular, uncertainty bounds for R for the pandemic strain from 1918 to 1919 have been obtained only in a few recent studies and are yet to be fully clarified. Here, we estimate R using daily case notifications during the autumn wave of the influenza pandemic (Spanish flu) in the city of San Francisco, California, from 1918 to 1919. In order to elucidate the effects from adopting different estimation approaches, four different methods are used: estimation of R using the early exponential-growth rate (Method 1), a simple susceptible-exposed-infectious-recovered (SEIR) model (Method 2), a more complex SEIR-type model that accounts for asymptomatic and hospitalized cases (Method 3), and a stochastic susceptible-infectious-removed (SIR) with Bayesian estimation (Method 4) that determines the effective reproduction number Rt at a given time t. The first three methods fit the initial exponential-growth phase of the epidemic, which was explicitly determined by the goodness-of-fit test. Moreover, Method 3 was also fitted to the whole epidemic curve. Whereas the values of R obtained using the first three methods based on the initial growth phase were estimated to be 2.98 (95% confidence interval (CI): 2.73, 3.25), 2.38 (2.16, 2.60) and 2.20 (1.55, 2.84), the third method with the entire epidemic curve yielded a value of 3.53 (3.45, 3.62). This larger value could be an overestimate since the goodness-of-fit to the initial exponential phase worsened when we fitted the model to the entire epidemic curve, and because the model is established as an autonomous system without time-varying assumptions. These estimates were shown to be robust to parameter uncertainties, but the theoretical exponential-growth approximation (Method 1) shows wide uncertainty. Method 4 provided a maximum-likelihood effective reproduction number 2.10 (1.21, 2.95) using the first 17 epidemic days, which is consistent with estimates obtained from the other methods and an estimate of 2.36 (2.07, 2.65) for the entire autumn wave. We conclude that the reproduction number for pandemic influenza (Spanish flu) at the city level can be robustly assessed to lie in the range of 2.0-3.0, in broad agreement with previous estimates using distinct data.

Clements, M. L., R. F. Betts, et al. (1986). "Resistance of adults to challenge with influenza A wild-type virus after receiving live or inactivated virus vaccine." J Clin Microbiol **23**(1): 73-6.

The efficacy of live attenuated cold-adapted (ca) reassortant influenza A H3N2 and H1N1 virus vaccines against experimental challenge with homologous wild-type virus 7 months after vaccination was compared with that of licensed inactivated virus vaccine in 106 seronegative (hemagglutination-inhibiting antibody titer less than or equal to 1:8) college students. The live attenuated virus vaccines induced as much resistance against illness as did the inactivated vaccine. Vaccine efficacy, measured by reduction in febrile or systemic illness in vaccines, compared with that in controls was 100% for ca H3N2 vaccine, 84% for inactivated H3N2 vaccine, 79% for ca H1N1 vaccine, and 67% for inactivated H1N1 vaccine. Less protection was conferred against upper respiratory tract illness; there was 50 and 77% protection in ca and inactivated H3N2 vaccines, respectively, but there was no protection in ca or inactivated H1N1 vaccinees. The duration, but not the magnitude, of H1N1 wild-type virus shedding in both ca and inactivated vaccinees was significantly reduced compared with controls. In contrast, a significant

reduction in the duration and magnitude of H3N2 virus shedding was observed in ca vaccinees but not in inactivated vaccines. After wild-type virus challenge, live ca virus vaccinees demonstrated resistance at least as great 7 months postvaccination as did inactivated virus vaccinees. These observations indicate that live virus vaccines may be a satisfactory alternative to inactivated vaccines for healthy persons.

Cori, A., P. Y. Boelle, et al. (2009). "Temporal variability and social heterogeneity in disease transmission: the case of SARS in Hong Kong." PLoS Comput Biol **5**(8): e1000471.

The extent to which self-adopted or intervention-related changes in behaviors affect the course of epidemics remains a key issue for outbreak control. This study attempted to quantify the effect of such changes on the risk of infection in different settings, i.e., the community and hospitals. The 2002-2003 severe acute respiratory syndrome (SARS) outbreak in Hong Kong, where 27% of cases were healthcare workers, was used as an example. A stochastic compartmental SEIR (susceptible-exposed-infectious-removed) model was used: the population was split into healthcare workers, hospitalized people and general population. Super spreading events (SSEs) were taken into account in the model. The temporal evolutions of the daily effective contact rates in the community and hospitals were modeled with smooth functions. Data augmentation techniques and Markov chain Monte Carlo (MCMC) methods were applied to estimate SARS epidemiological parameters. In particular, estimates of daily reproduction numbers were provided for each subpopulation. The average duration of the SARS infectious period was estimated to be 9.3 days (+/-0.3 days). The model was able to disentangle the impact of the two SSEs from background transmission rates. The effective contact rates, which were estimated on a daily basis, decreased with time, reaching zero inside hospitals. This observation suggests that public health measures and possible changes in individual behaviors effectively reduced transmission, especially in hospitals. The temporal patterns of reproduction numbers were similar for healthcare workers and the general population, indicating that on average, an infectious healthcare worker did not infect more people than any other infectious person. We provide a general method to estimate time dependence of parameters in structured epidemic models, which enables investigation of the impact of control measures and behavioral changes in different settings.

Cox, C. S. (1995). Stability of airborne microbes and allergens. Bioaerosols Handbook. C. S. Cox and C. M. Wathes. Boca Raton, Lewis PUblishers**:** 77-99.

Darnell, M. E., K. Subbarao, et al. (2004). "Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV." J Virol Methods **121**(1): 85-91.

Severe acute respiratory syndrome (SARS) is a life-threatening disease caused by a novel coronavirus termed SARS-CoV. Due to the severity of this disease, the World Health Organization (WHO) recommends that manipulation of active viral cultures of SARS-CoV be performed in containment laboratories at biosafety level 3 (BSL3). The virus was inactivated by ultraviolet light (UV) at 254 nm, heat treatment of 65 degrees C or greater, alkaline (pH > 12) or acidic (pH < 3) conditions, formalin and glutaraldehyde treatments. We describe the kinetics of these efficient viral inactivation methods, which will allow research with SARS-CoV containing materials, that are rendered non-infectious, to be conducted at reduced safety levels.

Davis, D. G. (1960). "The influence of temperature and humidity on spore formation and germination in *Bacillus anthracis.*" The Journal of Hygiene **58**(2): 177-186.

Davis, K. J., D. L. Fritz, et al. (1996). "Pathology of experimental pneumonic plague produced by fraction 1-positive and fraction 1-negative Yersinia pestis in African green monkeys (Cercopithecus aethiops)." Arch Pathol Lab Med **120**(2): 156-63.

OBJECTIVE: The protein capsule of Yersinia pestis, known as Fraction 1 or F1, is a protective immunogen and is an assumed, but not proven, virulence factor. Our objectives were to determine if inhaled F1-negative and/or F1-positive strains of Y pestis were virulent in the African green monkey and, if so, to differentiate F1-negative from F1-positive monkeys. Because F1-negative strains have been isolated from natural sources and have caused experimental fatal disease, we felt that this information was crucial to the development of future vaccines and diagnostic tests. MATERIALS AND METHODS: Adult African green monkeys were exposed by aerosol to F1-positive (CO92, n=15) or F1-negative (CO92-C12, n=6; Java-9, n=2) Y pestis strains. RESULTS: All monkeys died 4 to 10 days postexposure and had lesions consistent with primary pneumonic plague. Antibodies to F1 antigen and other Y pestis antigens allowed us to differentiate F1-positive from F1-negative Y pestis strains in fixed tissues. CONCLUSIONS: In this study, F1 antigen was not a required virulence factor. Therefore, there may be a need for vaccines and diagnostic assays that are not solely based on the F1 antigen.

Drancourt, M., L. Houhamdi, et al. (2006). "*Yersinia pestis* as a telluric, human ectoparasite-borne organism." Lancet Infect Dis **6**(4): 234-41.

The classic epidemiological model of plague is an infection of rodents that is transmitted to human beings by rodent ectoparasites. This model fits with observations of sporadic and limited outbreaks, but hardly explains the persistence of plague foci for millennia or the epidemiological features drawn from the descriptions of historical pandemics. A comprehensive review of the published data, including scientific papers published in France between 1920 and 1940, allows the completion of the epidemiological chain by introducing soil as a reservoir, burrowing rodents as a first link, and human ectoparasites as the main driving force for pandemics. Modern studies are needed to confirm the validity of this controversial model and to assess the relative contribution of each link in the various epidemiological presentations of plague. If confirmed, these data should be taken into account to update public-health policies and bioterrorism risk management, particularly among ectoparasite-infested people.

Druett, H. A., D. W. Henderson, et al. (1953). "Studies on respiratory infection. I. The influence of particle size on respiratory infection with anthrax spores." J Hyg (Lond) **51**(3): 359-71.

Ehrenkranz, N. J. and K. F. Meyer (1955). "Studies on immunization against plague. VIII. Study of three immunizing preparations in protecting primates against pneumonic plague." J Infect Dis **96**(2): 138-44.

Eisen, R. J., J. M. Petersen, et al. (2008). "Persistence of Yersinia pestis in soil under natural conditions." Emerg Infect Dis **14**(6): 941-3.

As part of a fatal human plague case investigation, we showed that the plague bacterium, Yersinia pestis, can survive for at least 24 days in contaminated soil under natural conditions. These results have implications for defining plague foci, persistence, transmission, and bioremediation after a natural or intentional exposure to Y. pestis.

Enria, D. A. and J. G. Barrera Oro (2002). "Junin virus vaccines." Curr Top Microbiol Immunol **263**: 239-61.

Fang, H., J. Chen, et al. (2005). "Modelling the SARS epidemic by a lattice-based Monte-Carlo simulation." Conf Proc IEEE Eng Med Biol Soc **7**: 7470-3.

We have analyzed the SARS data and the effect of the control measure in HongKong, based on a spatial Monte-Carlo model (SEIR) with susceptibles, exposed(latent), infective, and recovered. The SARS data can be well fit by numerical simulations. The control measure is effective to decreasing the transmission by reducing the contact rate. The average value of the reproductive number is consistent with many of the previous models.

Ferguson, N. M., D. A. Cummings, et al. (2006). "Strategies for mitigating an influenza pandemic." Nature **442**(7101): 448-52.

Development of strategies for mitigating the severity of a new influenza pandemic is now a top global public health priority. Influenza prevention and containment strategies can be considered under the broad categories of antiviral, vaccine and non-pharmaceutical (case isolation, household quarantine, school or workplace closure, restrictions on travel) measures. Mathematical models are powerful tools for exploring this complex landscape of intervention strategies and quantifying the potential costs and benefits of different options. Here we use a large-scale epidemic simulation to examine intervention options should initial containment of a novel influenza outbreak fail, using Great Britain and the United States as examples. We find that border restrictions and/or internal travel restrictions are unlikely to delay spread by more than 2-3 weeks unless more than 99% effective. School closure during the peak of a pandemic can reduce peak attack rates by up to 40%, but has little impact on overall attack rates, whereas case isolation or household quarantine could have a significant impact, if feasible. Treatment of clinical cases can reduce transmission, but only if antivirals are given within a day of symptoms starting. Given enough drugs for 50% of the population, household-based prophylaxis coupled with reactive school closure could reduce clinical attack rates by 40-50%. More widespread prophylaxis would be even more logistically challenging but might reduce attack rates by over 75%. Vaccine stockpiled in advance of a pandemic could significantly reduce attack rates even if of low efficacy. Estimates of policy effectiveness will change if the characteristics of a future pandemic strain differ substantially from those seen in past pandemics.

Fogarty, R., K. Halpin, et al. (2008). "Henipavirus susceptibility to environmental variables." Virus Res **132**(1-2): 140-4.

The routes of henipavirus transmission between hosts are poorly understood. The purpose of this study was to measure the persistence of henipaviruses under various environmental conditions and thereby gain an insight into likely mechanisms of transmission. Henipaviruses survived for more than 4 days at 22 degrees C in pH-neutral fruit bat urine but were sensitive to higher temperatures and pH changes. On mango flesh, survival time varied depending on temperature and fruit pH, ranging from 2h to more than 2 days. Desiccation of viruses substantially reduced survival time to less than 2h. The sensitivity of henipaviruses to pH, temperature and desiccation indicates a need for close contact between hosts for transmission to occur, although under ideal conditions henipaviruses can persist for extended periods facilitating vehicle-borne transmission.

Franz, D. R., P. B. Jahrling, et al. (1997). "Clinical recognition and management of patients exposed to biological warfare agents." JAMA **278**(5): 399-411.

Concern regarding the use of biological agents--bacteria, viruses, or toxins--as tools of warfare or terrorism has led to measures to deter their use or, failing that, to deal with the consequences. Unlike chemical agents, which typically lead to violent disease syndromes within

minutes at the site of exposure, diseases resulting from biological agents have incubation periods of days. Therefore, rather than a paramedic, it will likely be a physician who is first faced with evidence of the results of a biological attack. We provide here a primer on 10 classic biological warfare agents to increase the likelihood of their being considered in a differential diagnosis. Although the resultant diseases are rarely seen in many countries today, accepted diagnostic and epidemiologic principles apply; if the cause is identified quickly, appropriate therapy can be initiated and the impact of a terrorist attack greatly reduced.

Freiberg, A. N., M. N. Worthy, et al. "Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection." J Gen Virol **91**(Pt 3): 765-72.

Hendra virus (HeV) and Nipah virus (NiV) are recently emerged, closely related and highly pathogenic paramyxoviruses that cause severe disease such as encephalitis in animals and humans with fatality rates of up to 75 %. Due to their high case fatality rate following human infection and because of the lack of effective vaccines or therapy, they are classified as Biosafety Level 4 pathogens. A recent study reported that chloroquine, an anti-malarial drug, was effective in preventing NiV and HeV infection in cell culture experiments. In the present study, the antiviral efficacy of chloroquine was analysed, individually and in combination with ribavirin, in the treatment of NiV and HeV infection in in vivo experiments, using a golden hamster model. Although the results confirmed the strong antiviral activity of both drugs in inhibiting viral spread in vitro, they did not prove to be protective in the in vivo model. Ribavirin delayed death from viral disease in NiV-infected hamsters by approximately 5 days, but no significant effect in HeV-infected hamsters was observed. Chloroquine did not protect hamsters when administered either individually or in combination with ribavirin, the latter indicating the lack of a favourable drug-drug interaction.

Gaff, H. D., D. M. Hartley, et al. (2007). "An epidemiological model of Rfit Valley fever." Electronic Journal of Differential Equations **115**: 1-12.

Gani, R., H. Hughes, et al. (2005). "Potential Impact of Antiviral Drug Use during Influenza Pandemic." Emerging Infectious Diseases **11**(9): 1355-1362.

The recent spread of highly pathogenic strains of avian influenza has highlighted the threat posed by pandemic influenza. In the early phases of a pandemic, the only treatment available would be neuraminidase inhibitors, which many countries are considering stockpiling for pandemic use. We estimate the effect on hospitalization rates of using different antiviral stockpile sizes to treat infection. We estimate that stockpiles that cover 20%–25% of the population would be sufficient to treat most of the clinical cases and could lead to 50% to 77% reductions in hospitalizations. Substantial reductions in hospitalization could be achieved with smaller antiviral stockpiles if drugs are reserved for persons at high risk.

Gani, R. and S. Leach (2004). "Epidemiologic determinants for modeling pneumonic plague outbreaks." Emerg Infect Dis **10**(4): 608-14.

Pneumonic plague poses a potentially increasing risk to humans in plague nonendemic regions either as a consequence of an aerosolized release or through importation of the disease. Pneumonic plague is person-to-person transmissible. We provide a quantitative assessment of transmissibility based on past outbreaks that shows that the average number of secondary cases per primary case (R0) was 1.3 (variance = 3.1), assuming a geometric probability distribution, prior to outbreak control measures. We also show that the latent and infectious periods can be approximated by using lognormal distributions with means (SD) of 4.3 (1.8) and 2.5 (1.2) days.

Based on this parameter estimation, we construct a Markov-chain epidemic model to demonstrate the potential impact of delays in implementing outbreak control measures and increasing numbers of index cases on the incidence of cases in simulated outbreaks.

Geisbert, T. W., J. B. Geisbert, et al. (2009). "Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of ebola virus." J Virol **83**(14): 7296-304.
The filoviruses Marburg virus and Ebola virus cause severe hemorrhagic fever with high mortality in humans and nonhuman primates. Among the most promising filovirus vaccines under development is a system based on recombinant vesicular stomatitis virus (VSV) that expresses a single filovirus glycoprotein (GP) in place of the VSV glycoprotein (G). Here, we performed a proof-of-concept study in order to determine the potential of having one single-injection vaccine capable of protecting nonhuman primates against Sudan ebolavirus (SEBOV), Zaire ebolavirus (ZEBOV), Cote d'Ivoire ebolavirus (CIEBOV), and Marburgvirus (MARV). In this study, 11 cynomolgus monkeys were vaccinated with a blended vaccine consisting of equal parts of the vaccine vectors VSVDeltaG/SEBOVGP, VSVDeltaG/ZEBOVGP, and VSVDeltaG/MARVGP. Four weeks later, three of these animals were challenged with MARV, three with CIEBOV, three with ZEBOV, and two with SEBOV. Three control animals were vaccinated with VSV vectors encoding a nonfilovirus GP and challenged with SEBOV, ZEBOV, and MARV, respectively, and five unvaccinated control animals were challenged with CIEBOV. Importantly, none of the macaques vaccinated with the blended vaccine succumbed to a filovirus challenge. As expected, an experimental control animal vaccinated with VSVDeltaG/ZEBOVGP and challenged with SEBOV succumbed, as did the positive controls challenged with SEBOV, ZEBOV, and MARV, respectively. All five control animals challenged with CIEBOV became severely ill, and three of the animals succumbed on days 12, 12, and 14, respectively. The two animals that survived CIEBOV infection were protected from subsequent challenge with either SEBOV or ZEBOV, suggesting that immunity to CIEBOV may be protective against other species of Ebola virus. In conclusion, we developed an immunization scheme based on a single-injection vaccine that protects nonhuman primates against lethal challenge with representative strains of all human pathogenic filovirus species.

Glassman, H. N. (1966). "Discussion: Industrial inhalation anthrax." Bacteriological Reviews **30**(3): 657-659.

Gottfredsson, M., B. V. Halldorsson, et al. (2008). "Lessons from the past: familial aggregation analysis of fatal pandemic influenza (Spanish flu) in Iceland in 1918." Proc Natl Acad Sci U S A **105**(4): 1303-8.
The pandemic influenza of 1918 (Spanish flu) killed 21-50 million people globally, including in Iceland, where the characteristics and spread of the epidemic were well documented. It has been postulated that genetic host factors may have contributed to this high mortality. We identified 455 individuals who died of the Spanish flu in Iceland during a 6-week period during the winter of 1918, representing >92% of all fatal domestic cases mentioned by historical accounts. The highest case fatality proportion was 2.8%, and peak excess mortality was 162/100,000/week. Fatality proportions were highest among infants, young adults, and the elderly. A genealogical database was used to study relatedness and relative risk (RR) of the fatal influenza victims and relatives of their unaffected mates. The significance of these RR computations was assessed by drawing samples randomly from the genealogical database matched for age, sex, and geographical distribution. Familial aggregation of fatalities was seen, with RRs for death ranging from 3.75 for first-degree relatives ($P < 0.0001$) to 1.82 ($P = 0.005$), 1.12 ($P = 0.252$), and 1.47 ($P = 0.0001$) for second- to fourth-degree relatives of fatal influenza

victims, respectively. The RRs within the families of unaffected mates of fatal influenza victims were 2.95 (P < 0.0001), 1.27 (P = 0.267), 1.35 (P = 0.04), and 1.42 (P = 0.001), for first- to fourth-degree relatives, respectively. In conclusion, the risk of death from the Spanish flu was similar within families of patients who succumbed to the illness and within families of their mates who survived. Our data do not provide conclusive evidence for the role of genetic factors in susceptibility to the Spanish flu.

Gritsun, T. S., V. A. Lashkevich, et al. (2003). "Tick-borne encephalitis." Antiviral Res **57**(1-2): 129-46.
Tick-borne encephalitis (TBE) is one of the most dangerous human infections occurring in Europe and many parts of Asia. The etiological agent Tick-borne encephalitis virus (TBEV), is a member of the virus genus Flavivirus, of the family Flaviviridae. TBEV is believed to cause at least 11,000 human cases of encephalitis in Russia and about 3000 cases in the rest of Europe annually. Related viruses within the same group, Louping ill virus (LIV), Langat virus (LGTV) and Powassan virus (POWV), also cause human encephalitis but rarely on an epidemic scale. Three other viruses within the same group, Omsk hemorrhagic fever virus (OHFV), Kyasanur Forest disease virus (KFDV) and Alkhurma virus (ALKV), are closely related to the TBEV complex viruses and tend to cause fatal hemorrhagic fevers rather than encephalitis. This review describes the clinical manifestations associated with TBEV infections, the main molecular-biological properties of these viruses, and the different factors that define the incidence and severity of disease. The role of ticks and their local hosts in the emergence of new virus variants with different pathogenic characteristics is also discussed. This review also contains a brief history of vaccination against TBE including trials with live attenuated vaccine and modern tendencies in developing of vaccine virus strains.

Gumel, A. B., S. Ruan, et al. (2004). "Modelling strategies for controlling SARS outbreaks." Proc Biol Sci **271**(1554): 2223-32.
Severe acute respiratory syndrome (SARS), a new, highly contagious, viral disease, emerged in China late in 2002 and quickly spread to 32 countries and regions causing in excess of 774 deaths and 8098 infections worldwide. In the absence of a rapid diagnostic test, therapy or vaccine, isolation of individuals diagnosed with SARS and quarantine of individuals feared exposed to SARS virus were used to control the spread of infection. We examine mathematically the impact of isolation and quarantine on the control of SARS during the outbreaks in Toronto, Hong Kong, Singapore and Beijing using a deterministic model that closely mimics the data for cumulative infected cases and SARS-related deaths in the first three regions but not in Beijing until mid-April, when China started to report data more accurately. The results reveal that achieving a reduction in the contact rate between susceptible and diseased individuals by isolating the latter is a critically important strategy that can control SARS outbreaks with or without quarantine. An optimal isolation programme entails timely implementation under stringent hygienic precautions defined by a critical threshold value. Values below this threshold lead to control, but those above are associated with the incidence of new community outbreaks or nosocomial infections, a known cause for the spread of SARS in each region. Allocation of resources to implement optimal isolation is more effective than to implement sub-optimal isolation and quarantine together. A community-wide eradication of SARS is feasible if optimal isolation is combined with a highly effective screening programme at the points of entry.

Haas, C. N. (2002). "On the risk of mortality to primates exposed to anthrax spores." Risk Anal **22**(2): 189-93.

Current events have heightened the importance of understanding the risks from inhalation exposure to small numbers of spores of Bacillus anthracis. Previously reported data sets have not been fully assessed using current understanding of microbial dose response. This article presents an assessment of the reported primate dose-response data. At low doses, the risk to large populations of low doses of inhaled spores (e.g., < 100) is not insignificant.

High KP, Bradley S, Loeb M, Palmer R, Quagliarello V, Yoshikawa T (2005). "A new paradigm for clinical investigation of infectious syndromes in older adults: assessment of functional status as a risk factor and outcome measure." Clinical Infectious Diseases 40:114–22

Adults aged >or=65 years comprise the fastest-growing segment of the United States population, and older adults experience greater morbidity and mortality due to infection than do young adults. Although age is well established as a risk factor for infection, most clinical investigations of infectious diseases in older adults focus on microbiology and on crude end points of clinical success, such as cure rates or death; however, they often fail to assess functional status, which is a critical variable in geriatric care. Functional status can be evaluated either as a risk factor for infectious disease or as an outcome of interest after specific interventions using well-validated instruments. This article outlines the currently available data that suggest an association between infection, immunity, and impaired functional status in elderly individuals, summarizes the instruments commonly used to determine specific aspects of functional status, and provides recommendations for a new paradigm in which clinical trials that involve older adults include assessment of functional status.

Hood, A. M. (2009). "The effect of open-air factors on the virulence and viability of airborne Francisella tularensis." Epidemiol Infect **137**(6): 753-61.

Unidentified open-air factors (OAFs) found to be adverse to the survival of microorganisms suspended on microthreads were investigated for their effect on realistic aerosols of Francisella tularensis in an open-air environment. This organism was chosen because it is probably the most infectious organism known to be capable of infecting both animals and man via the respiratory route, hence its potential use as a bioterrorist agent. A direct correlation was found between an open-air adverse effect on viability and virulence of airborne particles of <3 microm via the respiratory route in guinea pigs. One viable organism was sufficient to initiate an infection that resulted in a fatal tularaemia infection. The lethal effect of OAFs on F. tularensis was found to vary from day to day and was related to the source of the air in the UK. The adverse effect on viability was associated with an inverse effect according to the size of the airborne particle.

Hsieh, Y. H., C. W. Chen, et al. (2004). "SARS outbreak, Taiwan, 2003." Emerg Infect Dis **10**(2): 201-6.

We studied the severe acute respiratory syndrome (SARS) outbreak in Taiwan, using the daily case-reporting data from May 5 to June 4 to learn how it had spread so rapidly. Our results indicate that most SARS-infected persons had symptoms and were admitted before their infections were reclassified as probable cases. This finding could indicate efficient admission, slow reclassification process, or both. The high percentage of nosocomial infections in Taiwan suggests that infection from hospitalized patients with suspected, but not yet classified, cases is a major factor in the spread of disease. Delays in reclassification also contributed to the problem. Because accurate diagnostic testing for SARS is currently lacking, intervention measures aimed at more efficient diagnosis, isolation of suspected SARS patients, and reclassification procedures could greatly reduce the number of infections in future outbreaks.

Inglesby, T. V., T. O'Toole, et al. (2002). "Anthrax as a biological weapon, 2002: updated recommendations for management." JAMA **287**(17): 2236-52.

OBJECTIVE: To review and update consensus-based recommendations for medical and public health professionals following a Bacillus anthracis attack against a civilian population. PARTICIPANTS: The working group included 23 experts from academic medical centers, research organizations, and governmental, military, public health, and emergency management institutions and agencies. EVIDENCE: MEDLINE databases were searched from January 1966 to January 2002, using the Medical Subject Headings anthrax, Bacillus anthracis, biological weapon, biological terrorism, biological warfare, and biowarfare. Reference review identified work published before 1966. Participants identified unpublished sources. CONSENSUS PROCESS: The first draft synthesized the gathered information. Written comments were incorporated into subsequent drafts. The final statement incorporated all relevant evidence from the search along with consensus recommendations. CONCLUSIONS: Specific recommendations include diagnosis of anthrax infection, indications for vaccination, therapy, postexposure prophylaxis, decontamination of the environment, and suggested research. This revised consensus statement presents new information based on the analysis of the anthrax attacks of 2001, including developments in the investigation of the anthrax attacks of 2001; important symptoms, signs, and laboratory studies; new diagnostic clues that may help future recognition of this disease; current anthrax vaccine information; updated antibiotic therapeutic considerations; and judgments about environmental surveillance and decontamination.

James, L., N. Shindo, et al. (2006). "Public health measures implemented during the SARS outbreak in Singapore, 2003." Public Health **120**(1): 20-6.

The SARS outbreak hit Singapore between March and May 2003. Public health control measures were applied along three fronts; prevention and control within healthcare settings, community and at the borders. Nosocomial spread composed majority of SARS cases in Singapore. To prevent infection within healthcare facilities, cases were centralized in a SARS-designated hospital, a no-visitors rule was applied and movement of patients and healthcare staff were restricted. For triaging purposes, fever clinics were established. A dedicated ambulance service was used to transport possible cases to the SARS-designated hospital. Hospitals were surveyed for fever clusters. The challenge was to identify cases with atypical presentation. Effective and safe discharge criteria were established from the lessons learnt. To prevent community spread, contacts of cases were stringently traced, quarantined in their homes and monitored daily. For prompt identification of a case and to reduce the time between onset of symptoms and isolation, the Infectious Diseases Act was amended. A large wholesale market closure resulted in massive quarantine thereby limiting the spread of infection. A mass education campaign was implemented in order to educate and raise awareness of the public. At all air, sea and land points-of-entry, exit and entry screening took place that resulted in zero importation and exportation of SARS cases after implementation of screening. Coordinated effort of the cross sectional inter-ministerial collaboration and strong coordination by the Task Force and commitment from different professionals made it possible to conquer the disease.

Jamieson DJ, Theiler RN, Rasmussen SA (2006). Emerging infections and pregnancy. Emerg Infect Dis. 12(11):1638-43

A key component of the response to emerging infections is consideration of special populations, including pregnant women. Successful pregnancy depends on adaptation of the woman's immune system to tolerate a genetically foreign fetus. Although the immune system changes are not well understood, a shift from cell-mediated immunity toward humoral immunity is

believed to occur. These immunologic changes may alter susceptibility to and severity of infectious diseases in pregnant women. For example, pregnancy may increase susceptibility to toxoplasmosis and listeriosis and may increase severity of illness and increase mortality rates from influenza and varicella. Compared with information about more conventional disease threats, information about emerging infectious diseases is quite limited. Pregnant women's altered response to infectious diseases should be considered when planning a response to emerging infectious disease threats.

Johnson, E., N. Jaax, et al. (1995). "Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus." Int J Exp Pathol **76**(4): 227-36.

The potential of aerogenic infection by Ebola virus was established by using a head-only exposure aerosol system. Virus-containing droplets of 0.8-1.2 microns were generated and administered into the respiratory tract of rhesus monkeys via inhalation. Inhalation of viral doses as low as 400 plaque-forming units of virus caused a rapidly fatal disease in 4-5 days. The illness was clinically identical to that reported for parenteral virus inoculation, except for the occurrence of subcutaneous and venipuncture site bleeding and serosanguineous nasal discharge. Immunocytochemistry revealed cell-associated Ebola virus antigens present in airway epithelium, alveolar pneumocytes, and macrophages in the lung and pulmonary lymph nodes; extracellular antigen was present on mucosal surfaces of the nose, oropharynx and airways. Aggregates of characteristic filamentous virus were present within type I pneumocytes, macrophages, and air spaces of the lung by electron microscopy. Demonstration of fatal aerosol transmission of this virus in monkeys reinforces the importance of taking appropriate precautions to prevent its potential aerosol transmission to humans.

Leffel, E. K. and D. S. Reed (2004). "Marburg and Ebola viruses as aerosol threats." Biosecur Bioterror **2**(3): 186-91.

Ebola and Marburg viruses are the sole members of the genus Filovirus in the family Filoviridae. There has been considerable media attention and fear generated by outbreaks of filoviruses because they can cause a severe viral hemorrhagic fever (VHF) syndrome that has a rapid onset and high mortality. Although they are not naturally transmitted by aerosol, they are highly infectious as respirable particles under laboratory conditions. For these and other reasons, filoviruses are classified as category A biological weapons. However, there is very little data from animal studies with aerosolized filoviruses. Animal models of filovirus exposure are not well characterized, and there are discrepancies between these models and what has been observed in human outbreaks. Building on published results from aerosol studies, as well as a review of the history, epidemiology, and disease course of naturally occurring outbreaks, we offer an aerobiologist's perspective on the threat posed by aerosolized filoviruses.

Lekone, P. E. and B. F. Finkenstadt (2006). "Statistical inference in a stochastic epidemic SEIR model with control intervention: Ebola as a case study." Biometrics **62**(4): 1170-7.

A stochastic discrete-time susceptible-exposed-infectious-recovered (SEIR) model for infectious diseases is developed with the aim of estimating parameters from daily incidence and mortality time series for an outbreak of Ebola in the Democratic Republic of Congo in 1995. The incidence time series exhibit many low integers as well as zero counts requiring an intrinsically stochastic modeling approach. In order to capture the stochastic nature of the transitions between the compartmental populations in such a model we specify appropriate conditional binomial distributions. In addition, a relatively simple temporally varying transmission rate function is

introduced that allows for the effect of control interventions. We develop Markov chain Monte Carlo methods for inference that are used to explore the posterior distribution of the parameters. The algorithm is further extended to integrate numerically over state variables of the model, which are unobserved. This provides a realistic stochastic model that can be used by epidemiologists to study the dynamics of the disease and the effect of control interventions.

Lewerin, S. S., M. Elvander, et al. "Anthrax outbreak in a Swedish beef cattle herd--1st case in 27 years: Case report." Acta Vet Scand **52**: 7.

After 27 years with no detected cases, an outbreak of anthrax occurred in a beef cattle herd in the south of Sweden. The outbreak was unusual as it occurred in winter, in animals not exposed to meat-and-bone meal, in a non-endemic country. The affected herd consisted of 90 animals, including calves and young stock. The animals were kept in a barn on deep straw bedding and fed only roughage. Seven animals died during 10 days, with no typical previous clinical signs except fever. The carcasses were reportedly normal in appearance, particularly as regards rigor mortis, bleeding and coagulation of the blood. Subsequently, three more animals died and anthrax was suspected at necropsy and confirmed by culture and PCR on blood samples. The isolated strain was susceptible to tetracycline, ciprofloxacin and ampicillin. Subtyping by MLVA showed the strain to cluster with isolates in the A lineage of Bacillus anthracis. Environmental samples from the holding were all negative except for two soil samples taken from a spot where infected carcasses had been kept until they were picked up for transport. The most likely source of the infection was concluded to be contaminated roughage, although this could not be substantiated by laboratory analysis. The suspected feed was mixed with soil and dust and originated from fields where flooding occurred the previous year, followed by a dry summer with a very low water level in the river allowing for the harvesting on soil usually not exposed. In the early 1900s, animal carcasses are said to have been dumped in this river during anthrax outbreaks and it is most likely that some anthrax spores could remain in the area. The case indicates that untypical cases in non-endemic areas may be missed to a larger extent than previously thought. Field tests allowing a preliminary risk assessment of animal carcasses would be helpful for increased sensitivity of detection and prevention of further exposure to the causative agent.

Lim, W., K. C. Ng, et al. (2006). "Laboratory containment of SARS virus." Ann Acad Med Singapore **35**(5): 354-60.

Following the severe acute respiratory syndrome (SARS) outbreak in 2003, a large number of clinical and environmental samples containing/potentially containing SARS coronavirus (SARSCoV) as well as SARS-CoV stocks were retained in clinical and research laboratories. The importance of laboratory biosafety was demonstrated by the occurrence of laboratory incidents in Singapore, Taiwan and Beijing. It is imperative that safe practice and techniques, safety equipment and appropriate facility design should be in place to reduce or eliminate exposure of laboratory workers, other persons and the outside environment to SARS-CoV containing materials. Discussion on laboratory containment of SARS-CoV was initiated in Hong Kong in August 2003. It was agreed that an inventory of all specimens with the potential presence of SARS-CoV collected for any diagnostic or research purposes from November 2002 to July 2003 should be established in each laboratory. They should be stored in a secure place at the appropriate biosafety level with access control. Un-needed samples collected during the period should be destroyed. These laboratories should be audited to ensure inventories are updated. The audit should include safety and security measures to detect irregularities. Any laboratory accidents involving materials suspected of containing SARS-CoV should be reported to the

authorities and all personnel exposed closely followed medically. A contingency plan should be in place in the laboratory and a drill conducted regularly to test its efficacy. By January 2004, all clinical laboratories performing SARS-CoV testing in Hong Kong set up inventories to document location and types of SARS-CoV containing materials retained in their laboratory. Audits of these laboratories in 2004 showed that laboratory safety and containment requirements as recommended were generally met.

Lipsitch, M., T. Cohen, et al. (2003). "Transmission dynamics and control of severe acute respiratory syndrome." Science **300**(5627): 1966-70.

Severe acute respiratory syndrome (SARS) is a recently described illness of humans that has spread widely over the past 6 months. With the use of detailed epidemiologic data from Singapore and epidemic curves from other settings, we estimated the reproductive number for SARS in the absence of interventions and in the presence of control efforts. We estimate that a single infectious case of SARS will infect about three secondary cases in a population that has not yet instituted control measures. Public-health efforts to reduce transmission are expected to have a substantial impact on reducing the size of the epidemic.

Lloyd-Smith, J. O., A. P. Galvani, et al. (2003). "Curtailing transmission of severe acute respiratory syndrome within a community and its hospital." Proc Biol Sci **270**(1528): 1979-89.

Severe acute respiratory syndrome (SARS) has been transmitted extensively within hospitals, and healthcare workers (HCWs) have comprised a large proportion of SARS cases worldwide. We present a stochastic model of a SARS outbreak in a community and its hospital. For a range of basic reproductive numbers (R(0)) corresponding to conditions in different cities (but with emphasis on R(0) approximately 3 as reported for Hong Kong and Singapore), we evaluate contact precautions and case management (quarantine and isolation) as containment measures. Hospital-based contact precautions emerge as the most potent measures, with hospital-wide measures being particularly important if screening of HCWs is inadequate. For R(0) = 3, case isolation alone can control a SARS outbreak only if isolation reduces transmission by at least a factor of four and the mean symptom-onset-to-isolation time is less than 3 days. Delays of a few days in contact tracing and case identification severely degrade the utility of quarantine and isolation, particularly in high-transmission settings. Still more detrimental are delays between the onset of an outbreak and the implementation of control measures; for given control scenarios, our model identifies windows of opportunity beyond which the efficacy of containment efforts is reduced greatly. By considering pathways of transmission in our system, we show that if hospital-based transmission is not halted, measures that reduce community-HCW contact are vital to preventing a widespread epidemic. The implications of our results for future emerging pathogens are discussed.

Lloyd-Smith, J. O., S. J. Schreiber, et al. (2005). "Superspreading and the effect of individual variation on disease emergence." Nature **438**(7066): 355-9.

Population-level analyses often use average quantities to describe heterogeneous systems, particularly when variation does not arise from identifiable groups. A prominent example, central to our current understanding of epidemic spread, is the basic reproductive number, R(0), which is defined as the mean number of infections caused by an infected individual in a susceptible population. Population estimates of R(0) can obscure considerable individual variation in infectiousness, as highlighted during the global emergence of severe acute respiratory syndrome (SARS) by numerous 'superspreading events' in which certain individuals infected unusually large numbers of secondary cases. For diseases transmitted by non-sexual

direct contacts, such as SARS or smallpox, individual variation is difficult to measure empirically, and thus its importance for outbreak dynamics has been unclear. Here we present an integrated theoretical and statistical analysis of the influence of individual variation in infectiousness on disease emergence. Using contact tracing data from eight directly transmitted diseases, we show that the distribution of individual infectiousness around R(0) is often highly skewed. Model predictions accounting for this variation differ sharply from average-based approaches, with disease extinction more likely and outbreaks rarer but more explosive. Using these models, we explore implications for outbreak control, showing that individual-specific control measures outperform population-wide measures. Moreover, the dramatic improvements achieved through targeted control policies emphasize the need to identify predictive correlates of higher infectiousness. Our findings indicate that superspreading is a normal feature of disease spread, and to frame ongoing discussion we propose a rigorous definition for superspreading events and a method to predict their frequency.

Loosli, C. G., H. M. Lemon, et al. (1943). "Experimental airborne influenza infection. I. Influence of humidity on survival of virus in air." Proc. Soc. Exp. Biol. **53**: 205-206.

Lowen, A. C., S. Mubareka, et al. (2006). "The guinea pig as a transmission model for human influenza viruses." Proc Natl Acad Sci U S A **103**(26): 9988-92.

The severity of epidemic and pandemic influenza outbreaks is dictated in part by the efficiency with which the causative strain transmits between human hosts. The mechanisms underlying influenza virus spread are poorly understood, in part because of the lack of a convenient animal model to study this phenomenon. Indeed, despite extremely efficient transmission among humans and virulence in the mouse model, we have shown that even the 1918 pandemic influenza virus does not transmit between mice. We therefore evaluated the guinea pig as a model mammalian host for influenza virus. Using the recent human isolate A/Panama/2007/99 (Pan/99) (H3N2) virus, we found that guinea pigs were highly susceptible to infection with the unadapted virus (ID(50) = 5 plaque-forming units). Pan/99 virus grew to high titers in the upper respiratory tract and was shed in nasal washings of infected animals. Moreover, influenza virus was transmitted from infected guinea pigs to noninfected guinea pigs housed in the same cage, an adjacent cage, and a cage placed 91 cm away. Our results demonstrate that influenza virus can pass between guinea pigs by means of droplet spread and thereby establish the suitability of the guinea pig as a model host for influenza virus transmission studies.

Luby, S. P., M. J. Hossain, et al. (2009). "Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007." Emerg Infect Dis **15**(8): 1229-35.

Human Nipah outbreaks recur in a specific region and time of year in Bangladesh. Fruit bats are the reservoir host for Nipah virus. We identified 23 introductions of Nipah virus into human populations in central and northwestern Bangladesh from 2001 through 2007. Ten introductions affected multiple persons (median 10). Illness onset occurred from December through May but not every year. We identified 122 cases of human Nipah infection. The mean age of case-patients was 27 years; 87 (71%) died. In 62 (51%) Nipah virus-infected patients, illness developed 5-15 days after close contact with another Nipah case-patient. Nine (7%) Nipah case-patients transmitted virus to others. Nipah case-patients who had difficulty breathing were more likely than those without respiratory difficulty to transmit Nipah (12% vs. 0%, p = 0.03). Although a small minority of infected patients transmit Nipah virus, more than half of identified cases result from person-to-person transmission. Interventions to prevent virus transmission from bats to humans and from person to person are needed.

Lyons, C. R. and T. H. Wu (2007). "Animal models of Francisella tularensis infection." Ann N Y Acad Sci **1105**: 238-65.

The increased incidence of emerging infections has caused a resurgence in the development of animal models in order to study their pathophysiology and develop therapeutics against them. Optimizing these models and improving our ability to extrapolate information from animals to humans is critical because in many cases the animal model will represent the only modality for efficacy testing. Francisella tularensis (F. tularensis) is an emerging pathogen that fits this category. While there is a significant body of literature that has examined infections with F. tularensis in a variety of species, the optimal small animal model has yet to be defined. A vast majority of studies have used two strains of F. tularensis, the more virulent type A strain commonly found in North America and the less virulent type B strain common to Europe. None of the small animal models described in the literature thus far behave in a fashion identical to humans with respect to their sensitivity to SCHU S4 (type A) or live vaccine strains (LVS) (attenuated type B) and an ability of LVS vaccination to consistently protect against a SCHU S4 aerosol challenge, suggesting that significant work on animal model development still remains. This report briefly describes the parameters important for animal model development and reviews the literature related to animal models of F. tularensis, including the human model, and the characterization performed for those models.

Lytle, C. D. and J. L. Sagripanti (2005). "Predicted inactivation of viruses of relevance to biodefense by solar radiation." J Virol **79**(22): 14244-52.

UV radiation from the sun is the primary germicide in the environment. The goal of this study was to estimate inactivation of viruses by solar exposure. We reviewed published reports on 254-nm UV inactivation and tabulated the sensitivities of a wide variety of viruses, including those with double-stranded DNA, single-stranded DNA, double-stranded RNA, or single-stranded RNA genomes. We calculated D(37) values (fluence producing on average one lethal hit per virion and reducing viable virus to 37%) from all available data. We defined "size-normalized sensitivity" (SnS) by multiplying UV(254) sensitivities (D(37) values) by the genome size, and SnS values were relatively constant for viruses with similar genetic composition. In addition, SnS values were similar for complete virions and their defective particles, even when the corresponding D(37) values were significantly different. We used SnS to estimate the UV(254) sensitivities of viruses for which the genome composition and size were known but no UV inactivation data were available, including smallpox virus, Ebola, Marburg, Crimean-Congo, Junin, and other hemorrhagic viruses, and Venezuelan equine encephalitis and other encephalitis viruses. We compiled available data on virus inactivation as a function of wavelength and calculated a composite action spectrum that allowed extrapolation from the 254-nm data to solar UV. We combined our estimates of virus sensitivity with solar measurements at different geographical locations to predict virus inactivation. Our predictions agreed with the available experimental data. This work should be a useful step to understanding and eventually predicting the survival of viruses after their release in the environment.

Manchee, R. J., M. G. Broster, et al. (1990). "Out of Gruinard Island." Salisbury Medical Bulletin **68**(special supplement): 17-18.

Marianneau, P., V. Guillaume, et al. "Experimental infection of squirrel monkeys with nipah virus." Emerg Infect Dis **16**(3): 507-10.

We infected squirrel monkeys (Saimiri sciureus) with Nipah virus to determine the monkeys' suitability for use as primate models in preclinical testing of preventive and therapeutic treatments. Infection of squirrel monkeys through intravenous injection was followed by high death rates associated with acute neurologic and respiratory illness and viral RNA and antigen production.

Massad, E., M. N. Burattini, et al. (2007). "The 1918 influenza A epidemic in the city of Sao Paulo, Brazil." Med Hypotheses **68**(2): 442-5.

The 1918 pandemic H1N1 outbreak in the city of Sao Paulo is revisited. The outbreak lasted for 10 weeks and reached 116,771 officially recorded cases amongst 523,194 inhabitants. The total number of deaths summed up to 5331, with a lethality rate of 4.5% and an overall mortality rate of around 1%. We propose a mathematical model that tallies available data with good accuracy and allows the estimation of the basic reproductive number, R(0). The model showed a remarkably good accuracy in retrieving the real data from Sao Paulo city outbreak considering the total number of recorded cases and deaths and the timing of the outbreak. The basic reproduction number calculated of 2.68 can be compared to estimates carried out for other flu strains, like the estimates for H3N2, whose values ranged from 1.5 to 2.5. We hypothesize that the Southern parts of the world in which there was relatively little impact of the Great War, like South America, suffered a much lower H1N1 influenza mortality as compared with that reported for the Northern hemisphere heavily affected by the I World War.

McCrumb, F. (1961). "Aerosol infection of man with *Pasturella tularensis*." Bacteriological Reviews **25**(1961): 262-267.

McKinney, K. R., Y. Y. Gong, et al. (2006). "Environmental transmission of SARS at Amoy Gardens." J Environ Health **68**(9): 26-30; quiz 51-2.

Recent investigations into the March 2003 outbreak of SARS in Hong Kong have concluded that environmental factors played an important role in the transmission of the disease. These studies have focused on a particular outbreak event, the rapid spread of SARS throughout Amoy Gardens, a large, private apartment complex. They have demonstrated that, unlike a typical viral outbreak that is spread through person-to-person contact, the SARS virus in this case was spread primarily through the air. High concentrations of viral aerosols in building plumbing were drawn into apartment bathrooms through floor drains. The initial exposures occurred in these bathrooms. The virus-laden air was then transported by prevailing winds to adjacent buildings at Amoy Gardens, where additional exposures occurred. This article reviews the results of the investigations and provides recommendations for maintenance and other measures that building owners can take to help prevent environmental transmission of SARS and other flulike viruses in their buildings.

Meselson, M., J. Guillemin, et al. (1994). "The Sverdlovsk anthrax outbreak of 1979." Science **266**(5188): 1202-8.

In April and May 1979, an unusual anthrax epidemic occurred in Sverdlovsk, Union of Soviet Socialist Republics. Soviet officials attributed it to consumption of contaminated meat. U.S. agencies attributed it to inhalation of spores accidentally released at a military microbiology facility in the city. Epidemiological data show that most victims worked or lived in a narrow zone extending from the military facility to the southern city limit. Farther south, livestock died of anthrax along the zone's extended axis. The zone paralleled the northerly wind that prevailed

shortly before the outbreak. It is concluded that the escape of an aerosol of anthrax pathogen at the military facility caused the outbreak.

Miller, W. S., C. R. Demchak, et al. (1963). "Stability and infectivity of airborne yellow fever and Rift Valley fever viruses." American Journal of Hygiene **77**: 114-121.

Mills, C. E., J. M. Robins, et al. (2004). "Transmissibility of 1918 pandemic influenza." Nature **432**(7019): 904-6.

The 1918 influenza pandemic killed 20-40 million people worldwide, and is seen as a worst-case scenario for pandemic planning. Like other pandemic influenza strains, the 1918 A/H1N1 strain spread extremely rapidly. A measure of transmissibility and of the stringency of control measures required to stop an epidemic is the reproductive number, which is the number of secondary cases produced by each primary case. Here we obtained an estimate of the reproductive number for 1918 influenza by fitting a deterministic SEIR (susceptible-exposed-infectious-recovered) model to pneumonia and influenza death epidemic curves from 45 US cities: the median value is less than three. The estimated proportion of the population with A/H1N1 immunity before September 1918 implies a median basic reproductive number of less than four. These results strongly suggest that the reproductive number for 1918 pandemic influenza is not large relative to many other infectious diseases. In theory, a similar novel influenza subtype could be controlled. But because influenza is frequently transmitted before a specific diagnosis is possible and there is a dearth of global antiviral and vaccine stores, aggressive transmission reducing measures will probably be required.

Montville, T. J., R. Dengrove, et al. (2005). "Thermal resistance of spores from virulent strains of Bacillus anthracis and potential surrogates." J Food Prot **68**(11): 2362-6.

The objective of this study was to determine the thermal resistance of spores of Bacillus anthracis and potential surrogates. The heat resistance of spores suspended in buffer (pH 7.0 or 4.5), milk, or orange juice was determined at 70, 80, and 90 degrees C. D-values for B. anthracis strains Sterne, Vollum, and Pasteur ranged from < 1 min at 90 degrees C to approximately 200 min at 70 degrees C and were lower under acidic than under neutral conditions. The D-values for B. anthracis spores fell within the range obtained for spores from eight strains of Bacillus cereus, Bacillus thuringiensis, Bacillus mycoides, and Bacillus subtilis. However, there were significant differences ($P < 0.001$) among the D-values of the strains. The z-values in pH 7.0 buffer and milk averaged approximately 10.5 degrees C and were not significantly different among strains ($P < 0.05$). The z-values in pH 4.5 buffer and orange juice averaged 12.9 and 13.9 degrees C, respectively, significantly ($P < 0.05$) higher than those obtained in milk or in pH 7.0 buffer. The significance of this difference was driven by large differences among a few strains. The z-values for B. anthracis strain Pasteur were twice as high in the acid media than in the neutral media. This study confirms that B. anthracis spores are not unusually heat resistant and that spores from validated Bacillus species are appropriate surrogates for thermal resistance studies.

Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, Rutten GE (2005). Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis. 1;41(3):281-8.

BACKGROUND: Clinical data on the association of diabetes mellitus with common infections are virtually lacking, not conclusive, and often biased. We intended to determine the relative risks of common infections in patients with type 1 and type 2 diabetes mellitus (DM1 and DM2,

respectively). METHODS: In a 12-month prospective cohort study conducted as part of the Second Dutch National Survey of General Practice, we compared 705 adult patients who had DM1 and 6712 adult patients who had DM2 with 18,911 control patients who had hypertension without diabetes. Outcome measures were medically attended episodes of infection of the respiratory tract, urinary tract, and skin and mucous membranes. We applied multivariable and polytomous logistic regression analysis to determine independent risks of infections and their recurrences in patients with diabetes, compared with control patients. RESULTS: Upper respiratory infections were equally common among patients with diabetes and control patients. Patients with diabetes had a greater risk of lower respiratory tract infection (for patients with DM1: adjusted odds ratio [AOR], 1.42 [95% confidence interval {CI}, 0.96-2.08]; for patients with DM2: AOR, 1.32 [95% CI, 1.13-1.53]), urinary tract infection (for patients with DM1: AOR, 1.96 [95% CI, 1.49-2.58]; for patients with DM2: AOR, 1.24 [95% CI, 1.10-1.39]), bacterial skin and mucous membrane infection (for patients with DM1: AOR, 1.59 [95% CI, 1.12-2.24]; for patients with DM2: AOR, 1.33 [95% CI, 1.15-1.54]), and mycotic skin and mucous membrane infection (for patients with DM1: AOR, 1.34 [95% CI, 0.97-1.84]; for patients with DM2: AOR, 1.44 [95% CI, 1.27-1.63]). Risks increased with recurrences of common infections. CONCLUSIONS: Patients with DM1 and DM2 are at increased risk for lower respiratory tract infection, urinary tract infection, and skin and mucous membrane infection. Studies are warranted into management of such infections in patients with diabetes.

Nishiura, H. (2007). "Time variations in the transmissibility of pandemic influenza in Prussia, Germany, from 1918-19." Theor Biol Med Model **4**: 20.

BACKGROUND: Time variations in transmission potential have rarely been examined with regard to pandemic influenza. This paper reanalyzes the temporal distribution of pandemic influenza in Prussia, Germany, from 1918-19 using the daily numbers of deaths, which totaled 8911 from 29 September 1918 to 1 February 1919, and the distribution of the time delay from onset to death in order to estimate the effective reproduction number, Rt, defined as the actual average number of secondary cases per primary case at a given time. RESULTS: A discrete-time branching process was applied to back-calculated incidence data, assuming three different serial intervals (i.e. 1, 3 and 5 days). The estimated reproduction numbers exhibited a clear association between the estimates and choice of serial interval; i.e. the longer the assumed serial interval, the higher the reproduction number. Moreover, the estimated reproduction numbers did not decline monotonically with time, indicating that the patterns of secondary transmission varied with time. These tendencies are consistent with the differences in estimates of the reproduction number of pandemic influenza in recent studies; high estimates probably originate from a long serial interval and a model assumption about transmission rate that takes no account of time variation and is applied to the entire epidemic curve. CONCLUSION: The present findings suggest that in order to offer robust assessments it is critically important to clarify in detail the natural history of a disease (e.g. including the serial interval) as well as heterogeneous patterns of transmission. In addition, given that human contact behavior probably influences transmissibility, individual countermeasures (e.g. household quarantine and mask-wearing) need to be explored to construct effective non-pharmaceutical interventions.

Parodi, A. S., C. E. Coto, et al. (1966). "Characteristics of Junin virus; etiologic agent of argentine hemorrhagic fever." Archives of Virology **19**(4): 393-402.

Peters, C. J. and D. M. Hartley (2002). "Anthrax inhalation and lethal human infection." Lancet **359**(9307): 710-1.

Peters, C. J., P. B. Jahrling, et al. (1987). "Experimental studies of arenaviral hemorrhagic fevers." Curr Top Microbiol Immunol **134**: 5-68.

Peters, C. J., D. Jones, et al. (1988). "Experimental Rift Valley fever in rhesus macaques." Arch Virol **99**(1-2): 31-44.

Rift Valley fever (RVF) is a major cause of human morbidity and mortality in endemic areas of sub-Saharan Africa and has the potential to cause epidemic disease in receptive areas world-wide. In this study, a RVF viral isolate from the 1977 Egyptian epidemic (ZH-501) inoculated intravenously into rhesus macaques caused a benign viremic infection in most, but resulted in the hemorrhagic fever syndrome in 20 per cent (3 of 15). Serious disease of this type has not previously been observed in nonhuman primates inoculated with RVF virus and may be a consequence of the viral strain used or the route of inoculation. Severe disease was accompanied by extensive liver necrosis, disseminated intravascular coagulation, and microangiopathic hemolytic anemia. We also attempted to prevent RVF by passive transfer of serum from vaccinated rhesus monkeys (plaque-reduction neutralization test titer 1:2,560). As little as 0.025 ml/kg prevented the development of viremia in naive rhesus monkeys after subcutaneous inoculation of virus. The monkey model should be helpful in understanding the pathogenesis and prevention of human RVF.

Pratt, W. D., D. Wang, et al. "Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector." Clin Vaccine Immunol **17**(4): 572-81.

Ebola viruses are highly pathogenic viruses that cause outbreaks of hemorrhagic fever in humans and other primates. To meet the need for a vaccine against the several types of Ebola viruses that cause human diseases, we developed a multivalent vaccine candidate (EBO7) that expresses the glycoproteins of Zaire ebolavirus (ZEBOV) and Sudan ebolavirus (SEBOV) in a single complex adenovirus-based vector (CAdVax). We evaluated our vaccine in nonhuman primates against the parenteral and aerosol routes of lethal challenge. EBO7 vaccine provided protection against both Ebola viruses by either route of infection. Significantly, protection against SEBOV given as an aerosol challenge, which has not previously been shown, could be achieved with a boosting vaccination. These results demonstrate the feasibility of creating a robust, multivalent Ebola virus vaccine that would be effective in the event of a natural virus outbreak or biological threat.

Qin, C., J. Wang, et al. (2005). "An animal model of SARS produced by infection of Macaca mulatta with SARS coronavirus." J Pathol **206**(3): 251-9.

A new SARS animal model was established by inoculating SARS coronavirus (SARS-CoV) into rhesus macaques (Macaca mulatta) through the nasal cavity. Pathological pulmonary changes were successively detected on days 5-60 after virus inoculation. All eight animals showed a transient fever 2-3 days after inoculation. Immunological, molecular biological, and pathological studies support the establishment of this SARS animal model. Firstly, SARS-CoV-specific IgGs were detected in the sera of macaques from 11 to 60 days after inoculation. Secondly, SARS-CoV RNA could be detected in pharyngeal swab samples using nested RT-PCR in all infected animals from 5 days after virus inoculation. Finally, histopathological changes of interstitial pneumonia were found in the lungs during the 60 days after viral inoculation: these changes were less marked at later time points, indicating that an active healing process together with resolution of an acute inflammatory response was taking place in these animals. This animal model should

provide insight into the mechanisms of SARS-CoV-related pulmonary disease and greatly facilitate the development of vaccines and therapeutics against SARS.

Rabenau, H. F., J. Cinatl, et al. (2005). "Stability and inactivation of SARS coronavirus." Med Microbiol Immunol **194**(1-2): 1-6.

The SARS-coronavirus (SARS-CoV) is a newly emerged, highly pathogenic agent that caused over 8,000 human infections with nearly 800 deaths between November 2002 and September 2003. While direct person-to-person transmission via respiratory droplets accounted for most cases, other modes have not been ruled out. Faecal shedding is common and prolonged and has caused an outbreak in Hong Kong. We studied the stability of SARS-CoV under different conditions, both in suspension and dried on surfaces, in comparison with other human-pathogenic viruses, including human coronavirus HCoV-229E. In suspension, HCoV-229E gradually lost its infectivity completely while SARS-CoV retained its infectivity for up to 9 days; in the dried state, survival times were 24 h versus 6 days. Thermal inactivation at 56 degrees C was highly effective in the absence of protein, reducing the virus titre to below detectability; however, the addition of 20% protein exerted a protective effect resulting in residual infectivity. If protein-containing solutions are to be inactivated, heat treatment at 60 degrees C for at least 30 min must be used. Different fixation procedures, e.g. for the preparation of immunofluorescence slides, as well as chemical means of virus inactivation commonly used in hospital and laboratory settings were generally found to be effective. Our investigations confirm that it is possible to care for SARS patients and to conduct laboratory scientific studies on SARS-CoV safely. Nevertheless, the agents tenacity is considerably higher than that of HCoV-229E, and should SARS re-emerge, increased efforts need to be devoted to questions of environmental hygiene.

Riley, S., C. Fraser, et al. (2003). "Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions." Science **300**(5627): 1961-6.

We present an analysis of the first 10 weeks of the severe acute respiratory syndrome (SARS) epidemic in Hong Kong. The epidemic to date has been characterized by two large clusters-initiated by two separate "super-spread" events (SSEs)-and by ongoing community transmission. By fitting a stochastic model to data on 1512 cases, including these clusters, we show that the etiological agent of SARS is moderately transmissible. Excluding SSEs, we estimate that 2.7 secondary infections were generated per case on average at the start of the epidemic, with a substantial contribution from hospital transmission. Transmission rates fell during the epidemic, primarily as a result of reductions in population contact rates and improved hospital infection control, but also because of more rapid hospital attendance by symptomatic individuals. As a result, the epidemic is now in decline, although continued vigilance is necessary for this to be maintained. Restrictions on longer range population movement are shown to be a potentially useful additional control measure in some contexts. We estimate that most currently infected persons are now hospitalized, which highlights the importance of control of nosocomial transmission.

Rose, L. J., R. Donlan, et al. (2003). "Survival of Yersinia pestis on environmental surfaces." Appl Environ Microbiol **69**(4): 2166-71.

The survival of two strains of Yersinia pestis (avirulent A1122 and virulent Harbin) on the surfaces of four materials was investigated. Viability was evaluated with epifluorescence microscopy by using the metabolic stain cyanoditolyl tetrazolium chloride and plate counts. Small numbers of cells suspended in phosphate buffer survived 2 to 4 h after visible drying on

stainless steel, polyethylene, or glass and beyond 48 h on paper. Cells suspended in brain heart infusion broth (BHI) persisted more than 72 h on stainless steel, polyethylene, and glass. Small numbers of cells suspended in BHI were still viable at 120 h on paper. These data suggest that Y. pestis maintains viability for extended periods (last measured at 5 days) under controlled conditions.

Sagripanti, J. L. and C. D. Lytle (2007). "Inactivation of influenza virus by solar radiation." Photochem Photobiol **83**(5): 1278-82.

Influenza virus is readily transmitted by aerosols and its inactivation in the environment could play a role in limiting the spread of influenza epidemics. Ultraviolet radiation in sunlight is the primary virucidal agent in the environment but the time that influenza virus remains infectious outside its infected host remains to be established. In this study, we calculated the expected inactivation of influenza A virus by solar ultraviolet radiation in several cities of the world during different times of the year. The inactivation rates reported here indicate that influenza A virions should remain infectious after release from the host for several days during the winter "flu season" in many temperate-zone cities, with continued risk for reaerosolization and human infection. The correlation between low and high solar virucidal radiation and high and low disease prevalence, respectively, suggest that inactivation of viruses in the environment by solar UV radiation plays a role in the seasonal occurrence of influenza pandemics.

Saile, E. and T. M. Koehler (2006). "Bacillus anthracis multiplication, persistence, and genetic exchange in the rhizosphere of grass plants." Appl Environ Microbiol **72**(5): 3168-74.

Bacillus anthracis, the causative agent of anthrax, is known for its rapid proliferation and dissemination in mammalian hosts. In contrast, little information exists regarding the lifestyle of this important pathogen outside of the host. Considering that Bacillus species, including close relatives of B. anthracis, are saprophytic soil organisms, we investigated the capacity of B. anthracis spores to germinate in the rhizosphere and to establish populations of vegetative cells that could support horizontal gene transfer in the soil. Using a simple grass plant-soil model system, we show that B. anthracis strains germinate on and around roots, growing in characteristic long filaments. From 2 to 4 days postinoculation, approximately one-half of the B. anthracis CFU recovered from soil containing grass seedlings arose from heat-sensitive organisms, while B. anthracis CFU retrieved from soil without plants consisted of primarily heat-resistant spores. Co-inoculation of the plant-soil system with spores of a fertile B. anthracis strain carrying the tetracycline resistance plasmid pBC16 and a selectable B. anthracis recipient strain resulted in transfer of pBC16 from the donor to the recipient as early as 3 days postinoculation. Our findings demonstrate that B. anthracis can survive as a saprophyte outside of the host. The data suggest that horizontal gene transfer in the rhizosphere of grass plants may play a role in the evolution of the Bacillus cereus group species.

Salvaggio, M. R. and J. W. Baddley (2004). "Other viral bioweapons: Ebola and Marburg hemorrhagic fever." Dermatol Clin **22**(3): 291-302, vi.

The term viral hemorrhagic fever refers to a clinical syndrome characterized by acute onset of fever accompanied by nonspecific findings of malaise, prostration, diarrhea,and headache. Patients frequently show signs of increased vascular permeability, and many develop bleeding diatheses. The hemorrhagic fever viruses represent potential agents for biologic warfare because of capability of aerosol transmission, high morbidity,and mortality associated with infection, and ability to replicate in cell culture in high concentrations. Herein we discuss the Filoviridae, the agents of Ebola and Marburg hemorrhagic fevers.

Saslaw, S., H. T. Eigelsbach, et al. (1961). "Tularemia vaccine study. II. Respiratory challenge." Arch Intern Med **107**: 702-14.

Sertsou, G., N. Wilson, et al. (2006). "Key transmission parameters of an institutional outbreak during the 1918 influenza pandemic estimated by mathematical modelling." Theor Biol Med Model **3**: 38.

AIM: To estimate the key transmission parameters associated with an outbreak of pandemic influenza in an institutional setting (New Zealand 1918). METHODS: Historical morbidity and mortality data were obtained from the report of the medical officer for a large military camp. A susceptible-exposed-infectious-recovered epidemiological model was solved numerically to find a range of best-fit estimates for key epidemic parameters and an incidence curve. Mortality data were subsequently modelled by performing a convolution of incidence distribution with a best-fit incidence-mortality lag distribution. RESULTS: Basic reproduction number (R0) values for three possible scenarios ranged between 1.3, and 3.1, and corresponding average latent period and infectious period estimates ranged between 0.7 and 1.3 days, and 0.2 and 0.3 days respectively. The mean and median best-estimate incidence-mortality lag periods were 6.9 and 6.6 days respectively. This delay is consistent with secondary bacterial pneumonia being a relatively important cause of death in this predominantly young male population. CONCLUSION: These R0 estimates are broadly consistent with others made for the 1918 influenza pandemic and are not particularly large relative to some other infectious diseases. This finding suggests that if a novel influenza strain of similar virulence emerged then it could potentially be controlled through the prompt use of major public health measures.

Sha, J., S. L. Agar, et al. (2008). "Braun lipoprotein (Lpp) contributes to virulence of yersiniae: potential role of Lpp in inducing bubonic and pneumonic plague." Infect Immun **76**(4): 1390-409.

Yersinia pestis evolved from Y. pseudotuberculosis to become the causative agent of bubonic and pneumonic plague. We identified a homolog of the Salmonella enterica serovar Typhimurium lipoprotein (lpp) gene in Yersinia species and prepared lpp gene deletion mutants of Y. pseudotuberculosis YPIII, Y. pestis KIM/D27 (pigmentation locus minus), and Y. pestis CO92 with reduced virulence. Mice injected via the intraperitoneal route with 5 x 10(7) CFU of the Deltalpp KIM/D27 mutant survived a month, even though this would have constituted a lethal dose for the parental KIM/D27 strain. Subsequently, these Deltalpp KIM/D27-injected mice were solidly protected against an intranasally administered, highly virulent Y. pestis CO92 strain when it was given as five 50% lethal doses (LD(50)). In a parallel study with the pneumonic plague mouse model, after 72 h postinfection, the lungs of animals infected with wild-type (WT) Y. pestis CO92 and given a subinhibitory dose of levofloxacin had acute inflammation, edema, and masses of bacteria, while the lung tissue appeared essentially normal in mice inoculated with the Deltalpp mutant of CO92 and given the same dose of levofloxacin. Importantly, while WT Y. pestis CO92 could be detected in the bloodstreams and spleens of infected mice at 72 h postinfection, the Deltalpp mutant of CO92 could not be detected in those organs. Furthermore, the levels of cytokines/chemokines detected in the sera were significantly lower in animals infected with the Deltalpp mutant than in those infected with WT CO92. Additionally, the Deltalpp mutant was more rapidly killed by macrophages than was the WT CO92 strain. These data provided evidence that the Deltalpp mutants of yersiniae were significantly attenuated and could be useful tools in the development of new vaccines.

Shimshony, A. and R. Barzilai (1983). "Rift Valley fever." Adv Vet Sci Comp Med **27**: 347-425.

Sinclair, R., S. A. Boone, et al. (2008). "Persistence of category A select agents in the environment." Appl Environ Microbiol **74**(3): 555-63.

Speck, R. S. and H. Wolochow (1957). "Studies on the experimental epidemiology of respiratory infections. VIII. Experimental pneumonic plague in Macacus rhesus." J Infect Dis **100**(1): 58-69.

Stephenson, E. H., E. W. Larson, et al. (1984). "Effect of environmental factors on aerosol-induced Lassa virus infection." J Med Virol **14**(4): 295-303.

Previous studies suggested that the most frequent means of transmission of Lassa virus was by either direct or indirect contact with infectious material. Aerosol stability and respiratory infectivity of the Josiah strain of Lassa virus were assessed to determine the effect of environmental factors on aerosol-induced infection. The stability of the virus in aerosol, particularly at low relative humidity (30% RH), plus the ability of the virus to infect guinea pigs and monkeys via the respiratory route emphasize the potential for aerosol transmission of Lassa virus. Biological half-lives at both 24 and 32 degrees C ranged from 10.1 to 54.6 min, and were sufficient for aerosol dispersion of virus to considerable distances in natural situations. Infectivity of Lassa virus in small particle aerosol was demonstrated in outbred guinea pigs and cynomolgus monkeys using dynamic aerosol equipment. Monkeys exposed to inhaled doses to 465 PFU were infected and died. The median infectious dose (ID50) for guinea pigs was 15 PFU, yet a definitive median lethal aerosol dose (LD50) could not be established. Organ tropism of aerosol-induced Lassa virus infections in outbred guinea pigs was similar to that previously reported for inbred guinea pigs infected by subcutaneous inoculation.

Subbarao, K. (2008). NIH intramural program: proposed biosafety containment and use of prophylaxis for 1918 H1N1. Safety symposium on public health and biosafety practices for research with 1918 H1N1 influenza virus. Bethesda, NIH-RAC.

Swanepoel, R. and J. A. W. Coetzer (1994). Rift Valley Fever. Infectious Diseases of Livestock with Special Reference to Southern Africa. G. T. JAW Coetzer, & RC Tustin. New York, Oxford University Press. **1:** 688-717.

Tamrakar, S. B. and C. N. Haas (2008). "Dose-response model for Lassa virus." Human and Ecological Risk Assessment **14**: 742-752.

This article develops dose-response models for Lassa fever virus using data sets found in the open literature. Dose-response data were drawn from two studies in which guinea pigs were given subcutaneous and aerosol exposure to Lassa virus. In one study, six groups of inbred guinea pigs were inoculated subcutaneously with doses of Lassa virus and five groups of out-bred guinea pigs were similarly treated. We found that the out-bred subcutaneously exposed guinea pig did not exhibit a dose-dependent trend in response. The inbred guinea pigs data were best fit by an exponential dose- response model. In a second study, four groups of out-bred guinea pigs were exposed to doses of Lassa virus via the aerosol route. In that study, aerosol diameter was less than 4.5 µm and both mortality and morbidity were used as endpoints. The log-probit dose-response model provided a somewhat better fit than the Beta-Poisson model for data with mortality as the endpoint, but the Beta-Poisson is considered the best fit model because it can be derived using biological considerations. Morbidity data were best fit with an exponential dose-response model.

Tucker, J. B. and R. A. Zilinskas (2003). "The 1971 smallpox outbreak in the Soviet city of Aralsk: implications for Variola virus as a bioterrorist threat. Introduction." Crit Rev Microbiol **29**(2): 81-95.

Tumpey, T. (2008). 1918 influenza virus: an overview of the pathogenicity of the H1N1 and virulence factors. Safety symposium on public health and biosafety practices for research with 1918 H1N1 influenza virus. Bethesda, NIH-RAC.

Turnbull, P. C. B. (1998). Guidelines for the surveillance and control of anthrax in humans and animals. E. a. o. C. D. World Health Organization, Surveillance and Control. Geneva, World Health Organization**:** 1-106.

Vasconcelos, D., R. Barnewall, et al. (2003). "Pathology of inhalation anthrax in cynomolgus monkeys (Macaca fascicularis)." Lab Invest **83**(8): 1201-9.

Anthrax is considered a serious biowarfare and bioterrorism threat because of its high lethality, especially by the inhalation route. Rhesus macaques (Macaca mulatta) are the most commonly used nonhuman primate model of human inhalation anthrax exposure. The nonavailability of rhesus macaques necessitated development of an alternate model for vaccine testing and immunologic studies. This report describes the median lethal dose (LD(50)) and pathology of inhalation anthrax in cynomolgus macaques (Macaca fascicularis). Gross and microscopic tissue changes were reviewed in 14 cynomolgus monkeys that died or were killed after aerosol exposure of spores of Bacillus anthracis (Ames strain). The LD(50) and 95% confidence intervals were 61800 (34000 to 110000) colony-forming units. The most common gross lesions were mild splenomegaly, lymph node enlargement, and hemorrhages in various organs, particularly involving the meninges and the lungs. Mediastinitis, manifested as hemorrhage or edema, affected 29% of the monkeys. Microscopically, lymphocytolysis occurred in the intrathoracic lymph nodes and spleens of all animals, and was particularly severe in the spleen and in germinal centers of lymph nodes. Hemorrhages were common in lungs, bronchial lymph nodes, meninges, gastrointestinal tract, and mediastinum. These results demonstrate that the Ames strain of B. anthracis is lethal by the inhalation route in the cynomolgus macaque. The LD(50) of the Ames strain of B. anthracis was within the expected experimental range of previously reported values in the rhesus monkey in an aerosol challenge. The gross and microscopic pathology of inhalation anthrax in the cynomolgus monkey is remarkably similar to that reported in rhesus monkeys and humans. The results of this study are important for the establishment of an alternative nonhuman primate model for evaluation of medical countermeasures against inhalational anthrax.

Viboud, C., T. Tam, et al. (2006). "Transmissibility and mortality impact of epidemic and pandemic influenza, with emphasis on the unusually deadly 1951 epidemic." Vaccine **24**(44-46): 6701-7.

There are important gaps in our current understanding of the influenza virus behavior. In particular, it remains unclear why some inter-pandemic seasons are associated with unusually high mortality impact, sometimes comparable to that of pandemics. Here we compare the epidemiological patterns of the unusually deadly 1951 influenza epidemic (A/H1N1) in England and Wales and Canada with those of surrounding epidemic and pandemic seasons, in terms of overall mortality impact and transmissibility. Based on the statistical and mathematical analysis of vital statistics and morbidity epidemic curves in these two countries, we show that the 1951 epidemic was associated with both higher mortality impact and higher transmissibility than the 1957 and 1968 pandemics. Surprisingly in Liverpool, considered the 'epicenter' of the severe 1951 epidemic, the mortality impact and transmissibility even surpassed the 1918 pandemic.

Wallinga, J. and P. Teunis (2004). "Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures." Am J Epidemiol **160**(6): 509-16.

Severe acute respiratory syndrome (SARS) has been the first severe contagious disease to emerge in the 21st century. The available epidemic curves for SARS show marked differences between the affected regions with respect to the total number of cases and epidemic duration, even for those regions in which outbreaks started almost simultaneously and similar control measures were implemented at the same time. The authors developed a likelihood-based estimation procedure that infers the temporal pattern of effective reproduction numbers from an observed epidemic curve. Precise estimates for the effective reproduction numbers were obtained by applying this estimation procedure to available data for SARS outbreaks that occurred in Hong Kong, Vietnam, Singapore, and Canada in 2003. The effective reproduction numbers revealed that epidemics in the various affected regions were characterized by markedly similar disease transmission potentials and similar levels of effectiveness of control measures. In controlling SARS outbreaks, timely alerts have been essential: Delaying the institution of control measures by 1 week would have nearly tripled the epidemic size and would have increased the expected epidemic duration by 4 weeks.

Wang, W. and S. Ruan (2004). "Simulating the SARS outbreak in Beijing with limited data." J Theor Biol **227**(3): 369-79.

We propose a mathematical model to simulate the SARS outbreak in Beijing. The model consists of six subpopulations, namely susceptible, exposed, quarantined, suspect, probable and removed, as China started to report SARS cases as suspect and probable separately from April 27 and cases transferred from suspect class to probable class from May 2. By simplifying the model to a two-compartment suspect-probable model and a single-compartment probable model and using limited data, we are able to simulate the SARS outbreak in Beijing. We estimate that the reproduction number varies from 1.0698 to 3.2524 and obtain certain important epidemiological parameters.

Wang, X. W., J. S. Li, et al. (2005). "Study on the resistance of severe acute respiratory syndrome-associated coronavirus." J Virol Methods **126**(1-2): 171-7.

In this study, the persistence of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) was observed in feces, urine and water. In addition, the inactivation of SARS-CoV in wastewater with sodium hypochlorite and chlorine dioxide was also studied. In vitro experiments demonstrated that the virus could only persist for 2 days in hospital wastewater, domestic sewage and dechlorinated tap water, while 3 days in feces, 14 days in PBS and 17 days in urine at 20 degrees C. However, at 4 degrees C, the SARS-CoV could persist for 14 days in wastewater and at least 17 days in feces or urine. SARS-CoV is more susceptible to disinfectants than Escherichia coli and f2 phage. Free chlorine was found to inactivate SARS-CoV better than chlorine dioxide. Free residue chlorine over 0.5 mg/L for chlorine or 2.19 mg/L for chlorine dioxide in wastewater ensures complete inactivation of SARS-CoV while it does not inactivate completely E. coli and f2 phage.

Warfield, K. L., S. B. Bradfute, et al. (2009). "Development and characterization of a mouse model for Marburg hemorrhagic fever." J Virol **83**(13): 6404-15.

The lack of a mouse model has hampered an understanding of the pathogenesis and immunity of Marburg hemorrhagic fever (MHF), the disease caused by marburgvirus (MARV), and has created a bottleneck in the development of antiviral therapeutics. Primary isolates of the

filoviruses, i.e., ebolavirus (EBOV) and MARV, are not lethal to immunocompetent adult mice. Previously, pathological, virologic, and immunologic evaluation of a mouse-adapted EBOV, developed by sequential passages in suckling mice, identified many similarities between this model and EBOV infections in nonhuman primates. We recently demonstrated that serially passaging virus recovered from the liver homogenates of MARV-infected immunodeficient (SCID) mice was highly successful in reducing the time to death in these mice from 50 to 70 days to 7 to 10 days after challenge with the isolate MARV-Ci67, -Musoke, or -Ravn. In this study, we extended our findings to show that further sequential passages of MARV-Ravn in immunocompetent mice caused the MARV to kill BALB/c mice. Serial sampling studies to characterize the pathology of mouse-adapted MARV-Ravn revealed that this model is similar to the guinea pig and nonhuman primate MHF models. Infection of BALB/c mice with mouse-adapted MARV-Ravn caused uncontrolled viremia and high viral titers in the liver, spleen, lymph node, and other organs; profound lymphopenia; destruction of lymphocytes within the spleen and lymph nodes; and marked liver damage and thrombocytopenia. Sequencing the mouse-adapted MARV-Ravn strain revealed differences in 16 predicted amino acids from the progenitor virus, although the exact changes required for adaptation are unclear at this time. This mouse-adapted MARV strain can now be used to develop and evaluate novel vaccines and therapeutics and may also help to provide a better understanding of the virulence factors associated with MARV.

Weber, D. J. and W. A. Rutala (2001). "Risks and prevention of nosocomial transmission of rare zoonotic diseases." Clin Infect Dis **32**(3): 446-56.

Americans are increasingly exposed to exotic zoonotic diseases through travel, contact with exotic pets, occupational exposure, and leisure pursuits. Appropriate isolation precautions are required to prevent nosocomial transmission of rare zoonotic diseases for which person-to-person transmission has been documented. This minireview provides guidelines for the isolation of patients and management of staff exposed to the following infectious diseases with documented person-to-person transmission: Andes hantavirus disease, anthrax, B virus infection, hemorrhagic fevers (due to Ebola, Marburg, Lassa, Crimean-Congo hemorrhagic fever, Argentine hemorrhagic fever, and Bolivian hemorrhagic fever viruses), monkeypox, plague, Q fever, and rabies. Several of these infections may also be encountered as bioterrorism hazards (i.e., anthrax, hemorrhagic fever viruses, plague, and Q fever). Adherence to recommended isolation precautions will allow for proper patient care while protecting the health care workers who provide care to patients with known or suspected zoonotic infections capable of nosocomial transmission.

Weber, T. P. and N. I. Stilianakis (2008). "Inactivation of influenza A viruses in the environment and modes of transmission: a critical review." J Infect **57**(5): 361-73.

OBJECTIVES: The relative importance of airborne, droplet and contact transmission of influenza A virus and the efficiency of control measures depends among other factors on the inactivation of viruses in different environmental media. METHODS: We systematically review available information on the environmental inactivation of influenza A viruses and employ information on infectious dose and results from mathematical models to assess transmission modes. RESULTS: Daily inactivation rate constants differ by several orders of magnitude: on inanimate surfaces and in aerosols daily inactivation rates are in the order of 1-10(2), on hands in the order of 10(3). Influenza virus can survive in aerosols for several hours, on hands for a few minutes. Nasal infectious dose of influenza A is several orders of magnitude larger than airborne infectious dose. CONCLUSIONS: The airborne route is a potentially important transmission pathway for

influenza in indoor environments. The importance of droplet transmission has to be reassessed. Contact transmission can be limited by fast inactivation of influenza virus on hands and is more so than airborne transmission dependent on behavioral parameters. However, the potentially large inocula deposited in the environment through sneezing and the protective effect of nasal mucus on virus survival could make contact transmission a key transmission mode.

Wilkening, D. A. (2006). "Sverdlovsk revisited: modeling human inhalation anthrax." Proc Natl Acad Sci U S A **103**(20): 7589-94.

Several models have been proposed for the dose-response function and the incubation period distribution for human inhalation anthrax. These models give very different predictions for the severity of a hypothetical bioterror attack, when an attack might be detected from clinical cases, the efficacy of medical intervention and the requirements for decontamination. Using data from the 1979 accidental atmospheric release of anthrax in Sverdlovsk, Russia, and limited nonhuman primate data, this paper eliminates two of the contending models and derives parameters for the other two, thereby narrowing the range of models that accurately predict the effects of human inhalation anthrax. Dose-response functions that exhibit a threshold for infectivity are contraindicated by the Sverdlovsk data. Dose-dependent incubation period distributions explain the 10-day median incubation period observed at Sverdlovsk and the 1- to 5-day incubation period observed in nonhuman primate experiments.

Won, W. D. and H. Ross (1966). "Effect of diluent and relative humidity on apparent viability of airborne Pasteurella pestis." Appl Microbiol **14**(5): 742-5.

Airborne Pasteurella pestis (A-1122) at low humidities [20 to 50% relative humidity (RH)] exhibited exponential decay when either 1% peptone or Heart Infusion Broth (HIB) was used as the diluent in the viable assay system. At higher RH values (65 and 87%), however, the 1% peptone diluent adversely affected the viability assay. In contrast, HIB as diluent was remarkably effective in demonstrating a higher number of viable cells in aerosols held at high RH values. Similarly, with HIB as diluent, aerosols were shown to contain viable cells during 90 min of observation; with 1% peptone, viability was not detectable after 20 min in the airborne state.

Wong, K. T., I. Grosjean, et al. (2003). "A golden hamster model for human acute Nipah virus infection." Am J Pathol **163**(5): 2127-37.

A predominantly pig-to-human zoonotic infection caused by the novel Nipah virus emerged recently to cause severe morbidity and mortality in both animals and man. Human autopsy studies showed the pathogenesis to be related to systemic vasculitis that led to widespread thrombotic occlusion and microinfarction in most major organs especially in the central nervous system. There was also evidence of extravascular parenchymal infection, particularly near damaged vessels (Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR, Nipah Virus Pathology Working Group: Nipah virus infection: Pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 2002, 161:2153-2167). We describe here a golden hamster (Mesocricetus auratus) model that appears to reproduce the pathology and pathogenesis of acute human Nipah infection. Hamsters infected by intranasal or intraperitoneal routes died within 9 to 29 days or 5 to 9 days, respectively. Pathological lesions were most severe and extensive in the hamster brain. Vasculitis, thrombosis, and more rarely, multinucleated endothelial syncytia, were found in blood vessels of multiple organs. Viral antigen and RNA were localized in both vascular and extravascular tissues including neurons, lung, kidney, and spleen, as demonstrated by immunohistochemistry and in situ hybridization, respectively.

Paramyxoviral-type nucleocapsids were identified in neurons and in vessel walls. At the terminal stage of infection, virus and/or viral RNA could be recovered from most solid organs and urine, but not from serum. The golden hamster is proposed as a suitable model for further studies including pathogenesis studies, anti-viral drug testing, and vaccine development against acute Nipah infection.

Wong, S. S. Y. and K. Y. Yuen (2005). "The severe acute respiratory syndrome (SARS)." J Neurovirol **11**: 455-468.

World Health Organization (2002). Global Soalr UV Index: A Practical Guide.

Yetter, R. A., S. Lehrer, et al. (1980). "Outcome of influenza infection: effect of site of initial infection and heterotypic immunity." Infect Immun **29**(2): 654-62.

An infection established throughout the total respiratory tract of mice with a highly lung adapted influenza virus (H0N1) led to death from viral pneumonia. The 50% lethal dose (LD(50)) was approximately the same as the 50% infectious dose (ID(50)). An infection with the same virus initiated in the nasal mucosa spread to the trachea and lungs over a 3- to 5-day period but was not lethal except at very high infecting doses. The LD(50) was 30,000 times the ID(50). Mice that had recovered from a prior infection with A/PC/73(H3N2) demonstrated enhanced recovery (heterotypic immunity) when challenged with A/PR/8/34(H0N1). Heterotypically immune mice infected while anesthetized with this potentially lethal virus stopped shedding virus from the nose, trachea, and lungs by day 7 and recovered. Heterotypically immune mice, infected awake, stopped shedding virus from the nose by day 5, and, in fact, the virus did not spread to the trachea or lungs. Thus, some of the variation in the severity of influenza infections may be explained by two factors: the site of initial infection and previous infection with heterotypic influenza virus.

Yip, C., W. L. Chang, et al. (2007). "Possible meteorological influence on the severe acute respiratory syndrome (SARS) community outbreak at Amoy Gardens, Hong Kong." J Environ Health **70**(3): 39-46.

The largest community outbreak of Severe Acute Respiratory Syndrome (SARS) occurred in the Amoy Gardens residential estate in Hong Kong, in March and April of 2003. It affected more than 300 residents, or 1.7 percent of the total Amoy Gardens population. An airborne pathway has been hypothesized as a possible mode for the spread of the disease. If that hypothesis is correct, meteorological factors may have played a contributory role; the virus-laden aerosols may have been transported between apartment blocks by the ambient wind, low mixing heights may have prevented the efficient dispersion of the aerosols, and a fall in temperature may have fostered the survival of the virus or increased the susceptibility of the exposed population. This information, used in combination with weather forecasts available several days ahead from meteorological services, should be useful for mitigation considerations in the unlikely event of a similar occurrence.

Zaas, A. K., M. Chen, et al. (2009). "Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans." Cell Host Microbe **6**(3): 207-17.

Acute respiratory infections (ARIs) are a common reason for seeking medical attention, and the threat of pandemic influenza will likely add to these numbers. Using human viral challenge studies with live rhinovirus, respiratory syncytial virus, and influenza A, we developed peripheral blood gene expression signatures that distinguish individuals with symptomatic ARIs from uninfected individuals with >95% accuracy. We validated this "acute respiratory viral" signature-

encompassing genes with a known role in host defense against viral infections-across each viral challenge. We also validated the signature in an independently acquired data set for influenza A and classified infected individuals from healthy controls with 100% accuracy. In the same data set, we could also distinguish viral from bacterial ARIs (93% accuracy). These results demonstrate that ARIs induce changes in human peripheral blood gene expression that can be used to diagnose a viral etiology of respiratory infection and triage symptomatic individuals.

Zhang, Z. (2007). "The outbreak pattern of SARS cases in China as revealed by a mathematical model." Ecological Modelling **201**: 420-426.

Since it first appeared in China's Guangdong Province, Severe Acute Respiratory Syndrome (SARS) has caused serious damages to many parts of the world, especially Asia. Little is known about its epidemiology. We developed a modified discrete SIR model including susceptible individuals, non-hospitalized SARS persons; hospitalized patients, cured hospital patients, and those who have died due to SARS infection. Here,we demonstrate the effective reproduction number is determined by infection rates and infectious period of hospitalized and non-hospitalized SARS patients. Both infection rate and the effective reproductive number of the SARS virus are significantly negatively correlated with the total number of cumulative cases, indicating that the control measures implemented in China are effective, and the outbreak pattern of accumulative SARS cases in China is a logistic growth curve. We estimate the basic reproduction number R0 of SARS virus is 2.87 in mainland of China, very close to the estimations in Singapore and Hong Kong.

Zhou, G. and G. Yan (2003). "Severe acute respiratory syndrome epidemic in Asia." Emerg Infect Dis **9**(12): 1608-10.

We analyzed the dynamics of cumulative severe acute respiratory syndrome (SARS) cases in Singapore, Hong Kong, and Beijing using the Richards model. The predicted total SARS incidence was close to the actual number of cases; the predicted cessation date was close to the lower limit of the 95% confidence interval.

Attachment H-2: Expert Panel; Orientation call materials

Arturo Casadevall, MD, PhD is the Leo and Julia Forchheimer Professor of Microbiology & Immunology at the Albert Einstein College of Medicine of Yeshiva University in the Bronx, New York. He is Chairman of the Department of Microbiology and Immunology and served as Director of the Division of Infectious Diseases at the Montefiore Medical Center at the Albert Einstein College of Medicine from 2000-2006. Dr Casadevall received both his MD and PhD (biochemistry) degrees from New York University in New York, New York. Subsequently, he completed internship and residency in internal medicine at Bellevue Hospital in New York, New York. Later he completed subspecialty training in Infectious Diseases at the Montefiore Medical Center and Albert Einstein College of Medicine. Dr. Casadevall major research interests are in fungal pathogenesis and the mechanism of antibody action. In the area of Biodefense Dr. Casadevall has an active research program to understand the mechanisms of antibody-mediated neutralization of Bacillus anthracis toxins. He has authored over 410 scientific papers. Dr. Casadevall was elected to membership in the American Society for Clinical Investigation, the American Academy of Physicians, and the American Academy of Microbiology. He was elected a fellow of the American Academy for the Advancement of Science and has received numerous honors including the Solomon A Berson Medical Alumni Achievement Award in Basic Science from the NYU School of Medicine, the Maxwell L. Littman Award (mycology award), the Rhoda Benham Award from Medical Mycology Society of America, and the Kass Lecture of the Infectious Disease Society of America. Dr. Casadevall is an editor of Infection and Immunity and serves in the editorial board of the Journal of Clinical Investigation and the Journal of Experimental Medicine. He is Editor-in-Chief of mBio, the first NIH open access general journal. He has served in numerous NIH committees including those that drafted the NIAID Strategic Plan and the Blue Ribbon Panel on Biodefense Research. He was a member of the National Academy Committee that reviewed the FBI Amerithrax Investigation. He is currently a member of the National Science Advisory Board for Biosecurity and co-chairs the NIAID Board of Scientific counselors.

Since he joined the AECOM faculty in 1992 Dr. Casadevall has mentored dozens of graduate students, postdoctoral fellows, and junior faculty. Many of his trainees have gone on to have highly successful careers in science and several have currently AECOM faculty. From 2000-2006 Dr. Casadevall was director of the Division of Infectious Diseases at AECOM-Montefiore and oversaw the expansion of its research program. He is highly regarded as a teacher and was elected to the Davidoff Society. In 2001 Dr. Casadevall received the Samuel M. Rosen outstanding teacher award and in 2008 he was recognized the American Society of Microbiology with the William Hinton Award for mentoring scientists from underrepresented groups. Dr. Casadevall has organized numerous symposia and conference and was the Chair of the Program committee of the Infectious Disease Society of America in 2006. Dr. Casadevall has taken a national leadership role in postgraduate education and chairs the Career Development committee of the American Society of Microbiology.

Charles N. Haas, PhD is the L.D. Betz Professor of Environmental Engineering and head of the Department of Civil, Architectural and Environmental Engineering, at Drexel University, where he has been since 1991. He directs the Drexel Engineering Cities Initiative. He also has an adjunct appointment in the Department of Emergency Medicine of the Drexel University College of Medicine. He received his BS (Biology) and MS (Environmental Engineering) from the Illinois Institute of Technology and his PhD in Environmental Engineering from the University of Illinois at Urbana-Champaign. He has served on the faculties of Rensselaer Polytechnic Institute and the Illinois Institute of Technology prior to joining Drexel. He co-directs the USEPA/DHS University Cooperative Center of Excellence – Center for Advancing Microbial Risk Assessment (CAMRA). He is a fellow of the American Academy for the Advancement of Science, the Society for Risk Analysis, and the American Academy of Microbiology. He is a Board Certified Environmental Engineering Member by eminence of the American Academy of

Environmental Engineers. For over 30 years, Professor Haas has specialized in the assessment of risk from and control of human exposure to pathogenic microorganisms, and in particular the treatment of water and wastewater to minimize microbial risk to human health. Professor Haas has served on numerous panels of the National Research Council.. He is a past member of the Water Science and Technology Board of the National Academies, and is on the US EPA Board of Scientific Counselors.

Jim LeDuc, PhD is the director of the Galveston National Laboratory (GNL) located on the campus of the University of Texas Medical Branch in Galveston, Texas. He formerly served as the GNL's Deputy Director (2008-2010) and Associate Director for Program Development (2006-2008). He also currently serves as the Director for Global Health in the Institute for Human Infections and Immunity and holds the inaugural Robert E. Shope, MD and John S. Dunn Distinguished Chair in Global Health.

Dr. LeDuc relocated to UTMB in late 2006 from the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, where he was the Influenza Coordinator. He also served as Director, Division of Viral and Rickettsial Diseases (2000-2005), coordinating research activities, prevention initiatives and outbreak investigations for viral and rickettsial pathogens of global importance, including viral hemorrhagic fevers, influenza and other respiratory infections, childhood viral diseases, and newly emerging diseases such as SARS. He served as the Associate Director for Global Health (1996-2000) in the Office of the Director, National Center for Infectious Diseases at CDC, and was a Medical Officer in charge of arboviruses and viral hemorrhagic fevers at the World Health Organization in Geneva, Switzerland (1992-1996). He also held leadership positions during a 23-year career as a U.S. Army officer in the medical research and development command, with assignments in Brazil, Panama and at various locations in the United States, including the Walter Reed Army Institute of Research and the U.S. Army Medical Research Institute of Infectious Diseases. His professional career began as a field biologist working for the Smithsonian Institution in West Africa.

He is a member of various professional organizations, has published over 200 scientific articles and book chapters, and is well recognized as an expert in virus diseases, biodefense and global health. Dr. LeDuc is a native of southern California and earned his masters and doctoral degrees from the University of California at Los Angeles. He and his wife Maryellen reside in Galveston and have three grown children and five grandchildren.

Alison O'Brien, PhD received her B.A. in Biology and Bacteriology from The University of California at Davis in 1969. She graduated Phi Beta Kappa. She then trained as a medical technologist for one year and worked for two years in bacteriology, chemistry, and hematology sections of diagnostic laboratories. She received her Ph.D. in Pathogenic Bacteriology from the Department of Medical Microbiology at The Ohio State University in 1976. She was awarded graduate student research awards from the College of Letters and Science in 1975 and 1976. Her graduate research project focused on the role of the Staphylococcus aureus delta toxin in the pathogenesis of staphylococcal enterocolitis. In 1976, Dr. O'Brien was awarded a National Research Council postdoctoral fellowship to study a newly described toxin of Shigella. Her project was conducted under the sponsorship of Dr. Samuel Formal at the Walter Reed Army Institute, Washington, D.C. In 1978, Dr. O'Brien became an Assistant Professor of Microbiology at Uniformed Services University of the Health Sciences (USUHS). She was promoted to an Associate Professor in 1981, a Professor in 1985, and Chair of Microbiology and Immunology in 1996. Dr. O'Brien's research areas include: the pathogenic mechanisms of Shiga toxin-producing E. coli, the contributions of Cytotoxic Necrotizing Factor and Hemolysins to infections caused by Uropathogenic E. coli, and development of therapeutics against Bacillus anthracis and B. cereus. Her research has been

funded by the National Institutes of Health (NIH), the United States Department of Agriculture, and the Agency for International Development. She also serves as the Editor in Chief of one of the major journals in pathogenesis, Infection and Immunity, and as a member of the U.S. Department of Agriculture's National Advisory Committee on Microbiological Criteria for Foods. She was a member of the NIH Bacteriology and Mycology Study Section 1 and the Chair of the Food and Drug Administration (FDA) Advisory Panel on Vaccines and Related Projects. She previously served as President of the American Society for Microbiology.

Jean Patterson, PhD is currently Chairman of the Department of Virology and Immunology at the Southwest Foundation for Biomedical Research (SFBR). Her major research interests are the molecular biology of viruses infecting protozoan parasites, transcription of bunyaviruses, mechanisms of action of antiviral agents, and therapeutics and vaccine development for biodefense agents. She is the author or coauthor of more than 75 peer-reviewed publications. Dr. Patterson holds the E.M. Stevens Chair for Biomedical Research and is an Adjunct Professor in the Department of Microbiology at the University of Texas Health Science Center at San Antonio (UTHSCSA). Before joining the Foundation, she was an Associate Professor in the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts. Other prior positions include Scientific Associate, Department of Medicine (Infectious Diseases), Children's Hospital, Boston, Massachusetts; Assistant Professor of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; and Instructor, Department of Microbiology and Molecular Genetics, Harvard Medical School. Dr. Patterson is a reviewer for the *Journal of Virology; Virology; Journal of General Virology; Molecular and Biochemical Parasitology; Experimental Parasitology; Antiviral Research;* and the American Institute for Biological Sciences. In addition, she is a member of the Study Group on Protozoal Viruses of the International Committee on Taxonomy of Viruses and numerous National Biodefense Analysis and Countermeasures Center panels. Dr. Patterson has more than 20 years of teaching experience, primarily at the University of Notre Dame and Harvard Medical School, and most recently at the UTHSCSA. She is a member of the design team that completed the construction of a 36,000 square-foot laboratory building that holds 12 Biosafety Level 2, three Biosafety Level 3, and one Biosafety Level 4 laboratories at SFBR. She also serves as a consultant for the development of the Montana BSL4 laboratory facility.

C. J. Peters, MD received his B.A. degree (summa cum laude) in chemistry at Rice University, Houston, TX in 1962 and his M.D. degree from Johns Hopkins, Baltimore, M.D. (alpha omega alpha) in 1966. After being on the Parkland Hospital, Dallas, TX internal medicine house staff for two years he became a NIAID Research Associate at the Middle America Research Unit in the Canal Zone. He extended past his two year obligation for a total of 5 years and acquired an interest in tropical diseases, chronic virus infections, arenaviruses, and ecological determinants of disease transmission. He then went to the University of California in San Diego to finish his internal medicine training and become board certified. Subsequently he spent 3 years as an immunology fellow at the Scripps Clinic and Research Foundation.

From 1977-1991 Dr. Peters was at the U.S.Army Medical Research Institute for Infectious Diseases where he began working as a research scientist and later became Division Director and Deputy Commander. While there he worked with biothreats, biodefense, and emerging diseases, particularly hemorrhagic fevers. In 1992 he moved to the Centers for Disease Control and Prevention where he was head of Special Pathogens Branch in the National Center of Infectious Diseases and was involved in epidemiological investigations of hemorrhagic fevers and other emerging infections. He moved to the University of Texas Medical Branch in 2001, where he is professor of Pathology and of Microbiology and

Immunology as well as being Director for Biodefense of the Center for Biodefense and Emerging Infectious Diseases.

Some of the emerging infectious diseases he has been closely involved with are Rift Valley fever virus, arenavirus hemorrhagic fevers, Ebola virus, Nipah virus, and hantavirus pulmonary syndrome. Because of the aerosol infectiousness of many of these agents they are commonly implicated as causing laboratory infections and suspected as potential agents of bioterrorism or large scale biological warfare. He has published more than 300 scientific articles, reviews, and chapters. He has served on numerous committees, including NAS committees on emerging microbial threats and misuse of biotechnology.

His current research interests are Rift Valley fever biology and vaccines, Phlebovirus pathogenesis, SARS coronavirus, and monoclonal antibodies for therapy. Peters is deeply involved in qualifying a live-attenuated vaccine for human licensure and a related vaccine for domestic animal use.

Wayne Thomann, PhD is Director of Occupational and Environmental Safety at Duke University Medical Center. He is also Assistant Clinical Professor in the Division of Occupational and Environmental Medicine, Department of Community and Family Medicine, and Assistant Professor in the Nicholas School of the Environment and Earth Sciences. Dr. Thomann received B.S. and M.S. degrees in microbiology from Florida Atlantic University in Boca Raton, Florida. He received his doctor of public health degree from the School of Public Health at the University of North Carolina at Chapel Hill. He has been researching the identification and control of bioaerosols for more than 20 years and is a member of ASHRAE Standard 62.1 Committee on Ventilation for Indoor Air Quality. He teaches graduate courses in occupational health and safety at the Duke University Nicholas School of the Environment and Earth Sciences and lectures extensively about indoor air quality.

Expert Panel on HID50

Teleconference
April 8, 2010
10:00 – 10:30 AM

Phone Number: 1-888-552-2815 Passcode: 149213

AGENDA

10:00 AM Welcome and Roundtable Introductions
Sam Bozzette, M.D., Ph.D., Consultant to NIH, Professor of Medicine, University of California at San Diego, Senior Natural Scientist, The RAND Corporation
Adi Gundlapalli, M.D., Ph.D., M.S., Assistant Professor, Departments of Internal Medicine, Pathology and Biomedical Informatics, University of Utah School of Medicine

10:10 AM Development of the Supplementary Risk Assessment of the National Emerging Infectious Diseases Laboratories at Boston University Medical Center

10:15 AM Goals for Expert Consultation

10:20 AM Review of the Delphi Process

10:25 AM Questions and Discussion

10:30 AM Adjourn

Expert Consultation for the Risk Assessment of the National Emerging Infectious Diseases Laboratory at Boston University

Orientation Call

Background: Lab Capacity

- 2002: BSL-4/3 laboratory space deemed insufficient for biodefense
- 2003: Funding awarded for 2 national labs
  - Galveston National Laboratory
    - BSL-3 operational now
    - BSL-4 operational soon
  - National Emerging Infectious Diseases Laboratories at Boston University (NEIDL)
    - construction started in 2006
    - 98+% complete but not operational

Background: NEIDL

- Difficulties with Environmental Impact Statements
  - Found inadequate in state and federal courts
- Supplemental Risk Assessment performed
  - Unfavorably reviewed by a National Research Council committee
- New risk assessment being performed
  - Contractor is TetraTech
  - Guidance from a NIH Blue Ribbon Panel
- RA divided into 3 elements
  - Threat assessment
  - Risk and amount of exposure
  - Initial and secondary infections

Goals for Consultation

- Modeling exposure requires estimates of atmospheric decay
  - Half-life under specified conditions
- Estimating infections requires estimates of dose-response and transmissibility
  - Dose-response: amount needed to infect specific proportions of specific populations (ID10, ID50, ID90)
  - Transmissibility: average number of infections transmitted by an infected person in a susceptible population (R_o)

Modified Delphi Method

- Efficiently gains consensus
- Gives all a voice
- Our implementation:
  - Review of provided information
  - Voting
  - Feedback:
    - aggregated group results
    - your own individual responses
  - Discussion at single meeting
    - voting
  - Feedback and re-voting

Example of Questionnaire: Dose-Response for Infection

		Number	x	Power (of 10)
1. B. anthracis	ID50	5,000	x	
	ID10	1	x	2
	ID90	5	X	4

		Number	x	Power (of 10)
2. F. tularensis	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
3. Y. pestis	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
4. SARS virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
5. 1918 Influenza virus	ID50		x	
	ID10		x	
	ID90		x	

Example of Questionnaire: Vulnerability

1. How much more vulnerable to infection do you judge a child younger than 5 to be?
Please enter a percentage | 10 | Percent more vulnerable

2. How much more vulnerable to infection do you judge a person older than 65 to be?
Please enter a percentage | | Percent more vulnerable

3. How much more vulnerable to infection do you judge a person with diabetes to be?
Please enter a percentage | | Percent more vulnerable

4. How much more vulnerable to infection do you judge a person with HIV/AIDS to be?
Please enter a percentage | | Percent more vulnerable

Example of Questionnaire: Transmissibility

	Estimated Range for Ro	Do you concur with this estimate? (enter Y or N)
1. Y. pestis	1 – 2.5	Y
2. SARS virus		
3. 1918 Influenza virus		
4. Rift Valley Fever virus		
5. Ebola virus		

Example of Questionnaire: Atmospheric Decay

	Cold humid night (minutes)	Hot dry sunny day (minutes)
6. Lassa Fever virus	720	10
7. Rift Valley Fever virus		
8. Andes hantavirus		
9. Junin HF virus		
10. Russian Spring-Summer Encephalitis virus		

Attachment H-3: Paper Version of Questionnaire

Infectivity and Decay Expert Consultation **Round 1 Voting**

Please enter last name:

Thank you for agreeing to participate in this exercise. In formulating your answers to the questions below, you should draw on the material provided, other relevant information known to you, discussions with other panel members and colleagues, and (especially) your own judgment. Note that there are 2 sections and 5 pages to this questionnaire.

Section 1: Infectivity

The purpose of this section is to estimate the dose-response curve for infection with agents being evaluated as part of the NEIDL Risk Assessment. It is necessary for you to fill all of the fields in this section, even if you are extremely uncertain about a particular response.

Part A: For each organism, we are asking you to make three dose estimates: the minimum number of organisms necessary to infect 50% of susceptible individuals, or the ID50; the minimum number of organisms necessary to infect 10% of susceptible individuals, or the ID10; and the minimum number or organisms necessary to infect 90% of susceptible individuals, or the ID90.

For this set of questions, you may enter a single digit in the "Number" field and a power of 10 in the "Power" field. For example, 10,000 would be entered as '1' in the Number field and '4' in the Power field, 500,000 would be '5' in the Number field and '5' in the Power field, and 20 would be '2' in the Number field and '1' in the Power field. Alternatively, you may enter your entire estimate in only the "Number" field, leaving the "Power" field blank.

		Number	x	Power (of 10)
1. B. anthracis	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
2. F. tularensis	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
3. Y. pestis	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
4. Andes hantavirus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
5. Ebola virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
6. Marburg virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
7. Junin virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
8. Lassa fever virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
9. Nipah virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
10. Rift Valley fever virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
11. Russian spring-summer encephalitis virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)

12. SARS virus	ID50		x	
	ID10		x	
	ID90		x	

		Number	x	Power (of 10)
13. 1918 influenza virus	ID50		x	
	ID10		x	
	ID90		x	

Part B. In this part, we are asking for your concurrence with estimates of infectivity that we have derived from the literature. The measure of infectivity that we are using is Ro, or the average number of infections that would arise from each infected person in a susceptible population.

	Estimated Base Value for Ro		Do you concur with this estimate? (enter Y or N)
1. Y. pestis	1.3		
2. Ebola virus	1.5		
3. Rift Valley fever virus	1.2		
4. SARS virus	3.0		
5. 1918 influenza virus	3.0 (1.5-4)		

Part C. In this part, we are asking you to estimate how much *more* vulnerable to disease and death that certain compromised subgroups are likely to be. The groups are the very young, the very old, those with diabetes, those with HIV infection, and women who are pregnant.

We will not be asking for separate values for each organism, but for a factor to apply to all. Specifically, we are asking you to make 4 judgments for each group: vulnerability to bacterial disease, vulnerability to viral disease, mortality from bacterial disease, and mortality from viral disease.

For these question, please enter a number corresponding to the percentage increase in vulnerability. For example, if your judgment is 10% more vulnerable, enter "10."

How much more vulnerable do you judge the following to be?

			To disease	To mortality
1. A child younger than 5?	(Please enter a percentage)	Bacteria		
		Viruses		

			To disease	To mortality
2. An adult older than 65?	(Please enter a percentage)	Bacteria		
		Viruses		

			To disease	To mortality
3. A person with diabetes?	(Please enter a percentage)	Bacteria		
		Viruses		

			To disease	To mortality
4. A person with HIV infection?	(Please enter a percentage)	Bacteria		
		Viruses		

			To disease	To mortality
5. A pregnant woman?	(Please enter a percentage)	Bacteria		
		Viruses		

Section 2: Atmospheric Decay

The purpose of this section is to develop estimates of the stability in the atmosphere of the 13 agents being evaluated as part of the as part of the NEIDL Risk Assessment. As above, it is necessary for you to fill all of the fields in this section, even if you are extremely uncertain about a particular response.

For each organism, we are asking you to make two estimates: one for the half-life in a cold humid night and another for a hot sunny dry day.

For this set of questions, please enter your estimate, in minutes, of the nighttime and daytime half-life of viable organisms in the fields below. For example, if you estimate that the number of virulent organisms would be halved every quarter hour, you would enter "15" minutes. Note that we are not asking you to estimate the viability of *B. anthracis* spores.

	Cold humid night	Hot dry sunny day
1. B. anthracis	Indefinite	Indefinite

	Cold humid night (minutes)	Hot dry sunny day (minutes)
2. *F. tularensis*		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
3. Y. pestis		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
4. Andes hantavirus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
5. Ebola virus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
6. Marburg virus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
7. Junin virus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
8. Lassa fever virus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
9. Nipah virus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
10. Rift Valley fever virus		

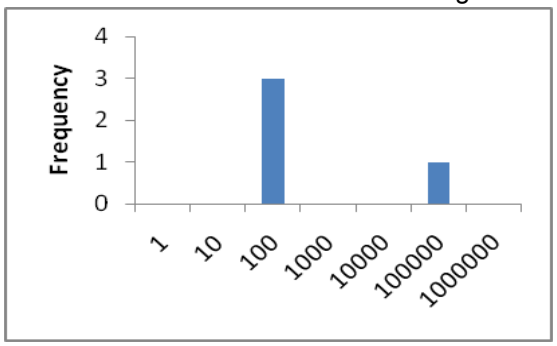
	Cold humid night (minutes)	Hot dry sunny day (minutes)
11. Russian spring-summer encephalitis virus		

	Cold humid night (minutes)	Hot dry sunny day (minutes)
12. SARS virus		

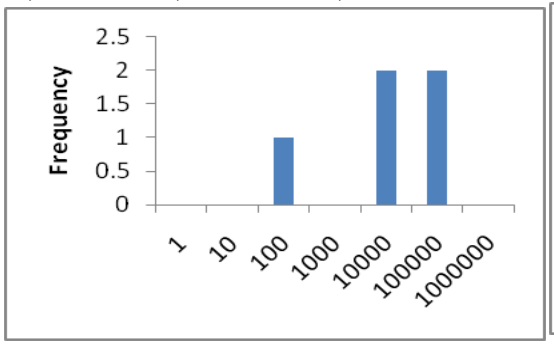
	Cold humid night (minutes)	Hot dry sunny day (minutes)
13. 1918 influenza virus		

Attachment H-4: Results of Round 1

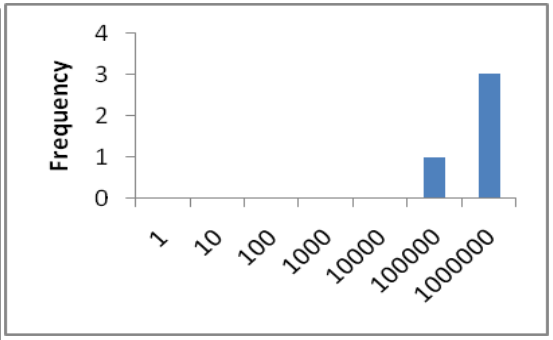
Organism		ID10	ID50	ID90
1. B. anthracis	Geo Mean	349	8,425	236,828
	Median	100	10,000	510,500
	Low	100	100	20,000
	High	14,800	94,300	700,000



ID10

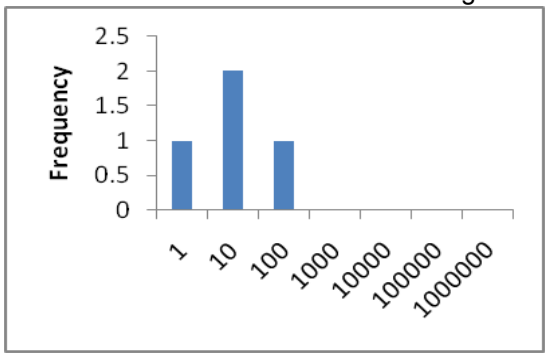


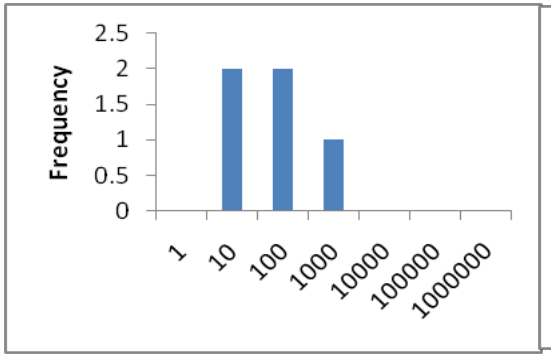
ID50

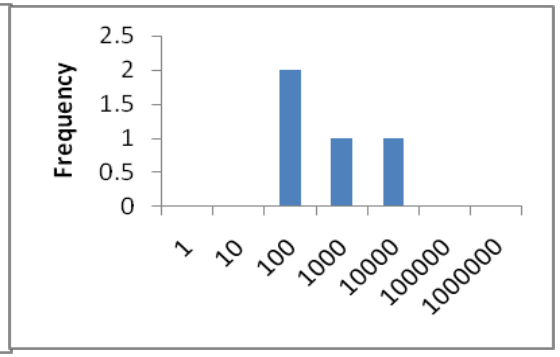


ID90

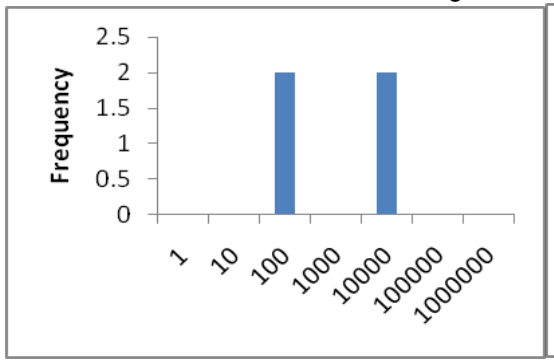
Organism		ID10	ID50	ID90
2. F. tularensis	Geo Mean	4	36	253
	Median	2	12	150
	Low	1	10	41
	High	100	1,000	5,000

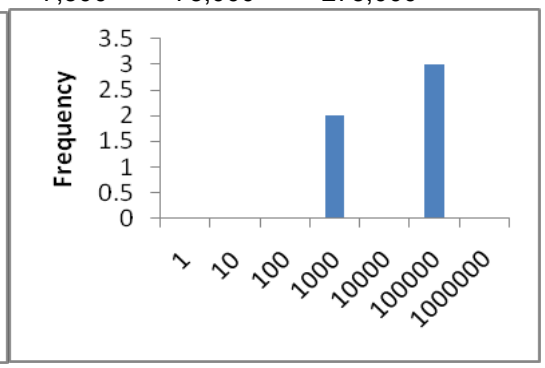


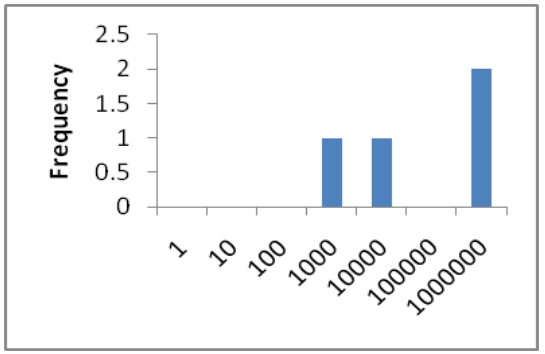




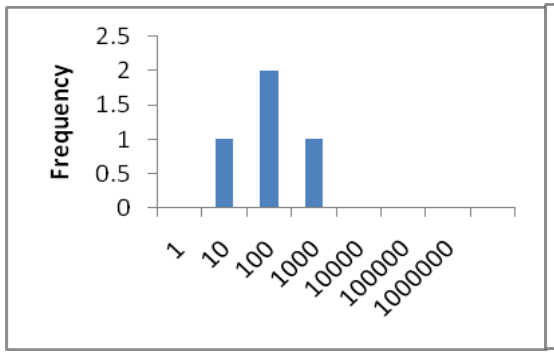
Organism		ID10	ID50	ID90
	Geo			
3. Y. pestis	Mean	479	5,543	28,795
	Median	750	15,166	130,000
	Low	50	200	1,000
	High	7,500	75,000	275,000

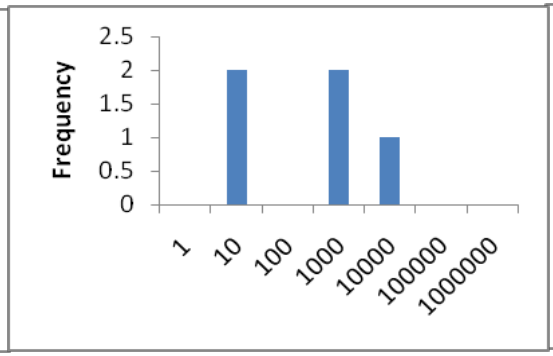


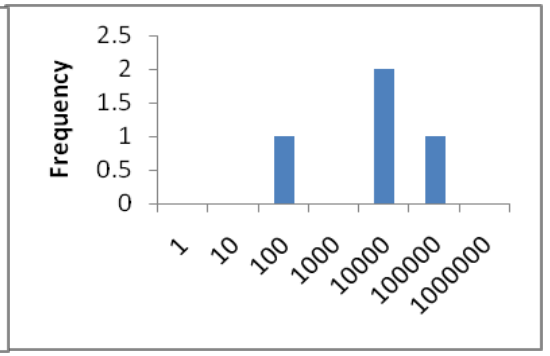




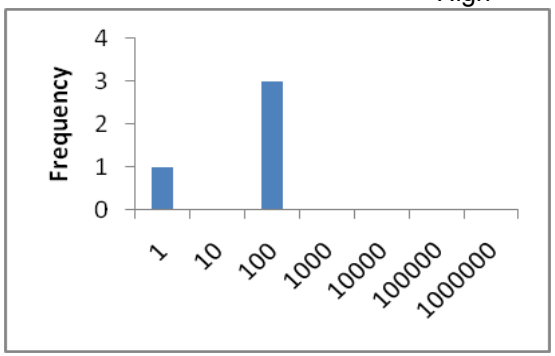
Organism		ID10	ID50	ID90
	Geo			
4. Andes hantavirus	Mean	64	213	3,091
	Median	100	1,000	10,000
	Low	2	8	50
	High	814	5,500	18,250

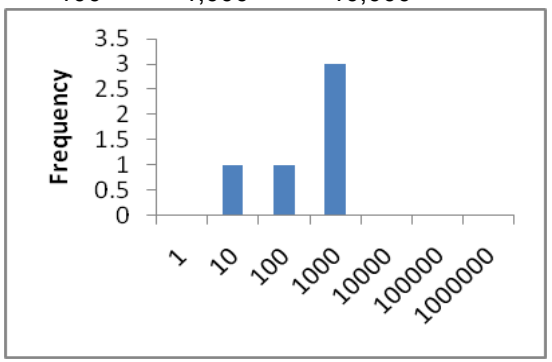


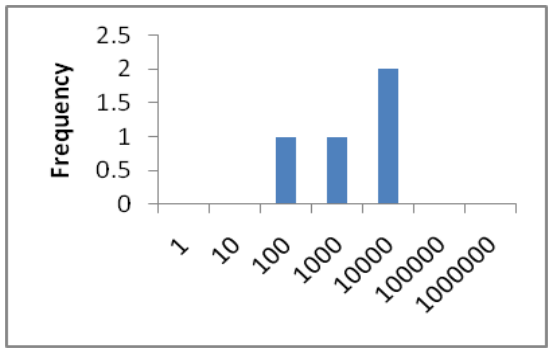




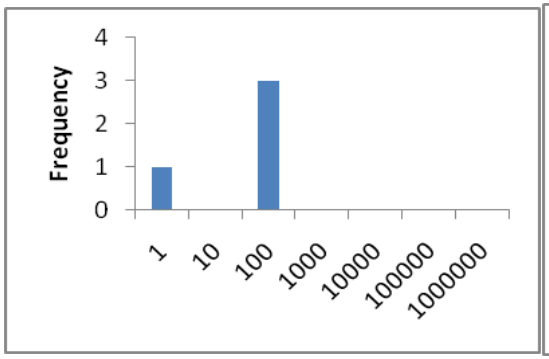
Organism		ID10	ID50	ID90
5. Ebola virus	Geo Mean	18	137	1,000
	Median	35	120	2,700
	Low	1	10	50
	High	100	1,000	10,000

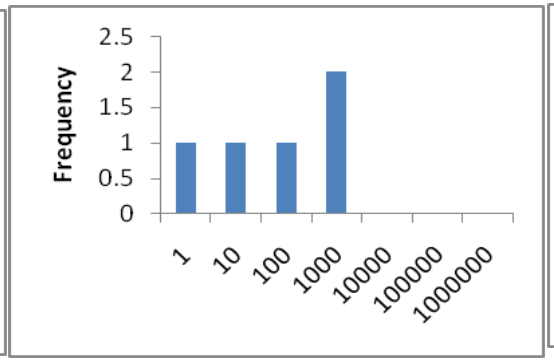


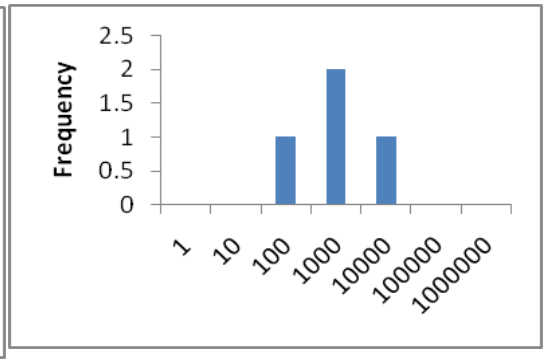




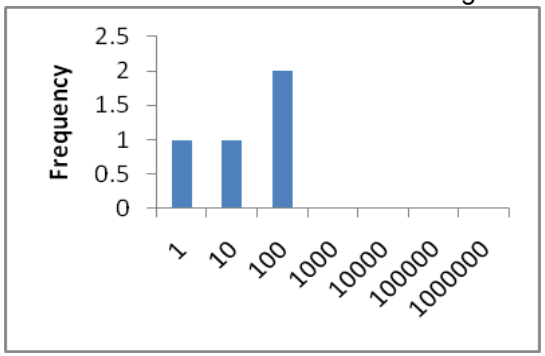
Organism		ID10	ID50	ID90
6. Marburg virus	Geo Mean	18	41	669
	Median	35	100	700
	Low	1	1	50
	High	100	1,000	10,000

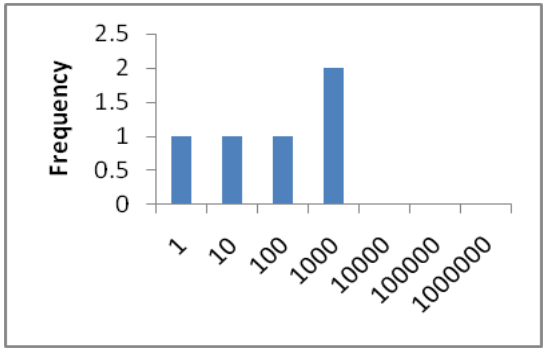


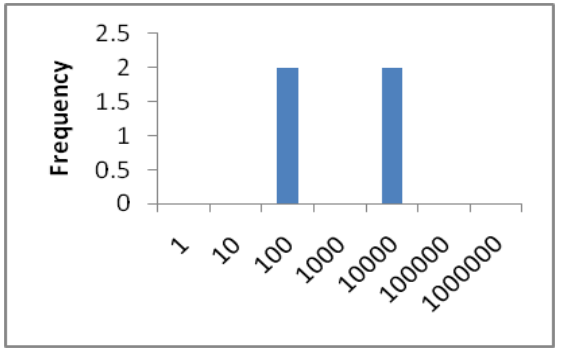




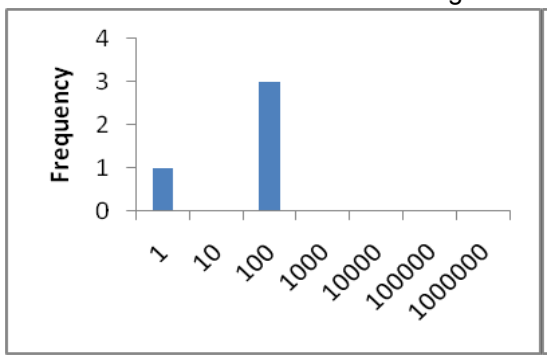
Organism		ID10	ID50	ID90
7. Junin virus	Geo Mean	12	43	592
	Median	51	15	2,525
	Low	1	1	49
	High	100	1,000	10,000

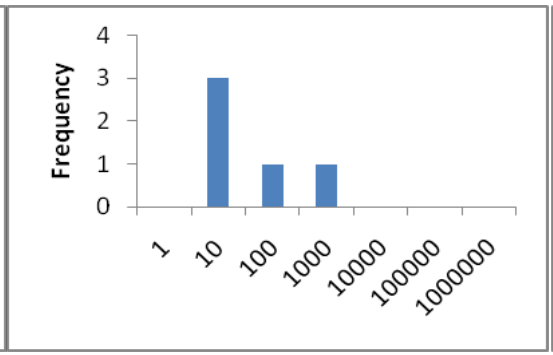


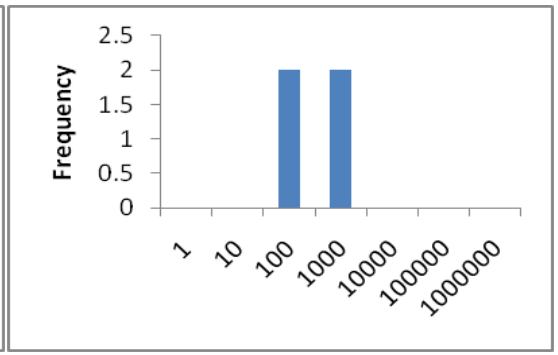




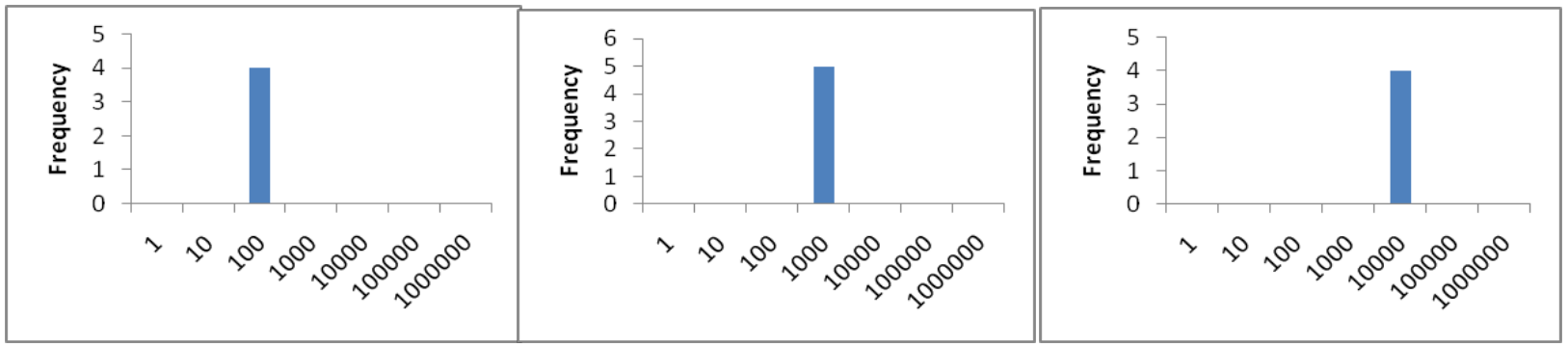
Organism		ID10	ID50	ID90
8. Lassa fever virus	Geo Mean	15	19	233
	Median	34	10	530
	Low	1	3	50
	High	50	300	1,000



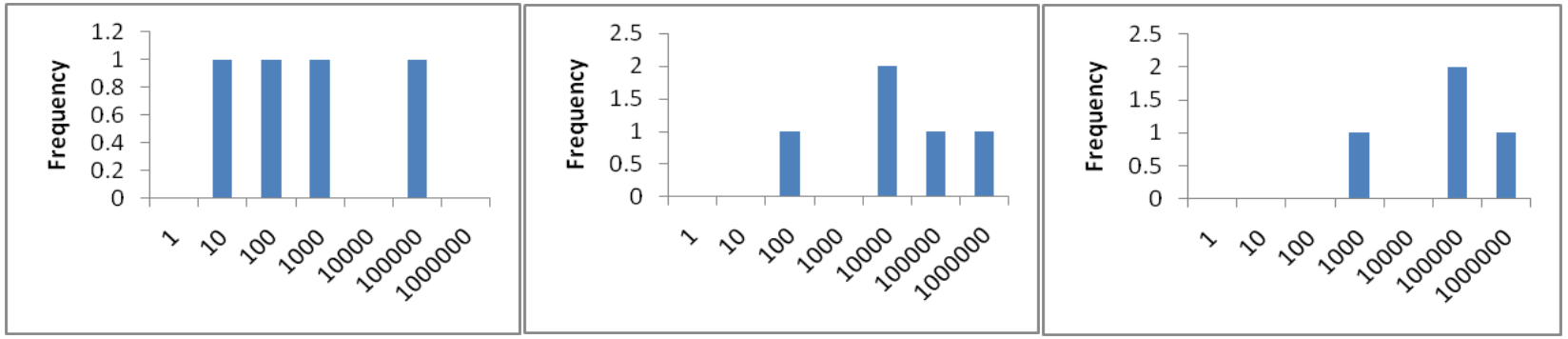




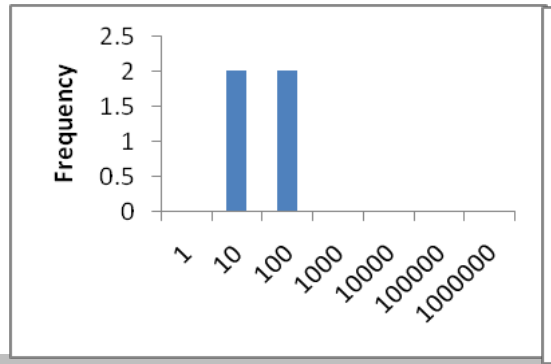
Organism		ID10	ID50	ID90
9. Nipah virus	Geo Mean	78	758	6,028
	Median	88	1,000	9,000
	Low	50	500	1,650
	High	100	1,000	10,000

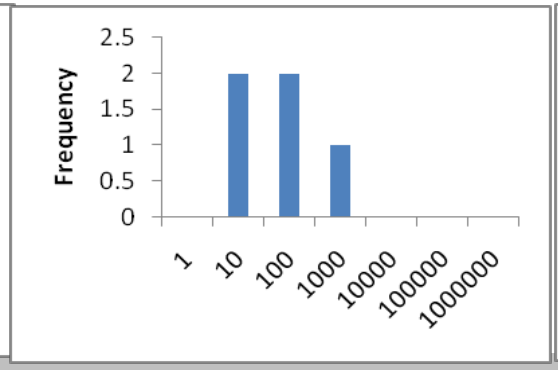


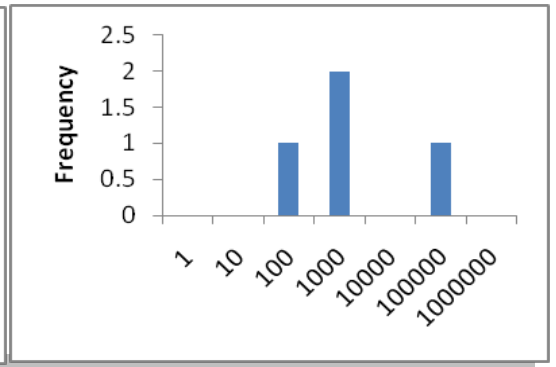
Organism		ID10	ID50	ID90
10. Rift Valley fever virus	Geo Mean	351	15,817	35,799
	Median	550	10,000	100,000
	Low	10	100	500
	High	15,200	1,000,000	328,500



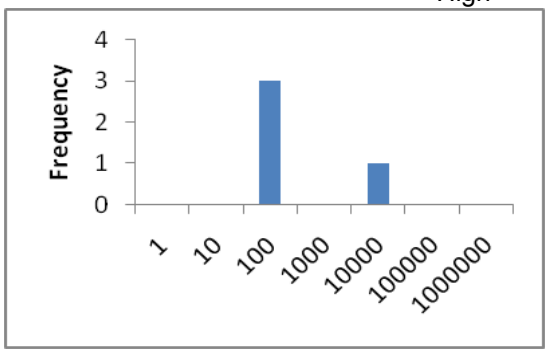
Organism		ID10	ID50	ID90
11. Russian spring-summer encephalitis virus	Geo Mean	12	56	1,036
	Median	11	71	615
	Low	2	8	50
	High	100	1,000	100,000

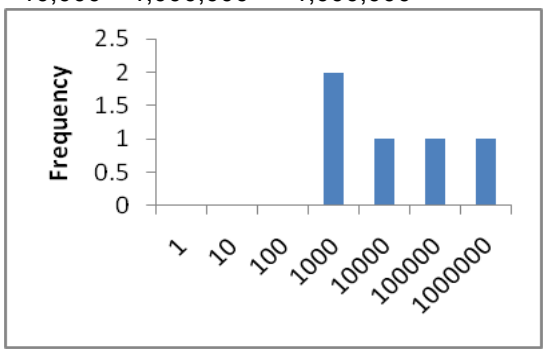


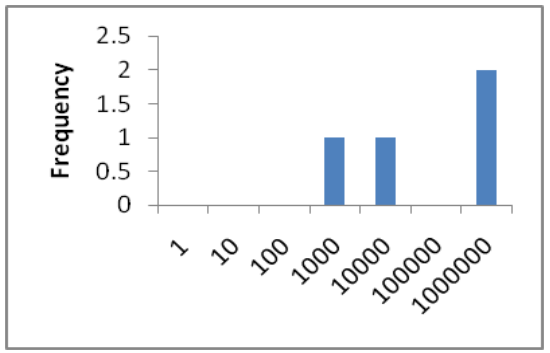




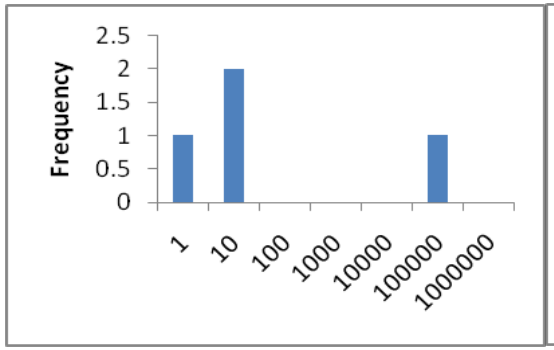
Organism		ID10	ID50	ID90
12. SARS virus	Geo Mean	256	12,313	46,685
	Median	100	10,000	255,000
	Low	43	283	950
	High	10,000	1,000,000	1,000,000

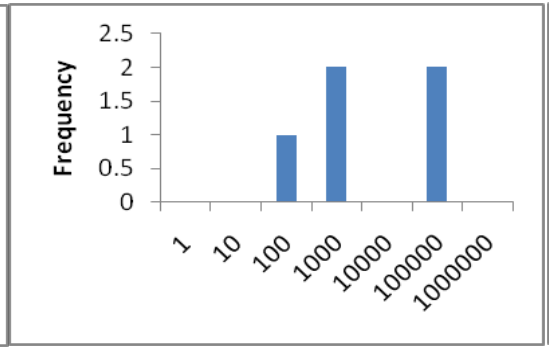


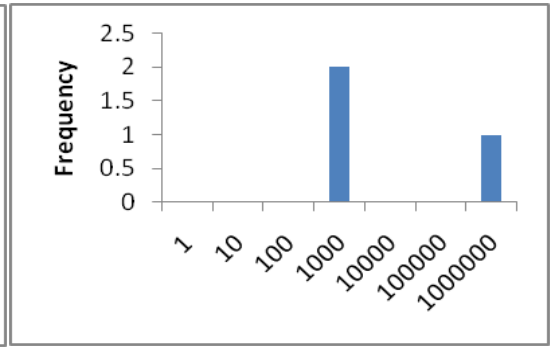




Organism		ID10	ID50	ID90
13. 1918 influenza virus	Geo Mean	35	2,724	56,234
	Median	7	500	500,500
	Low	1	100	1,000
	High	50,000	100,000	10,000,000

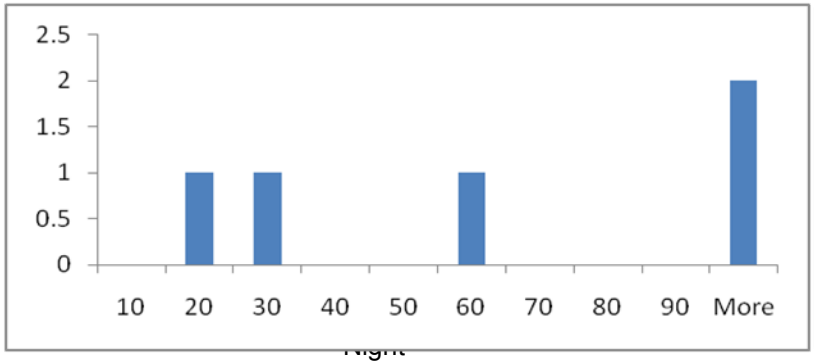


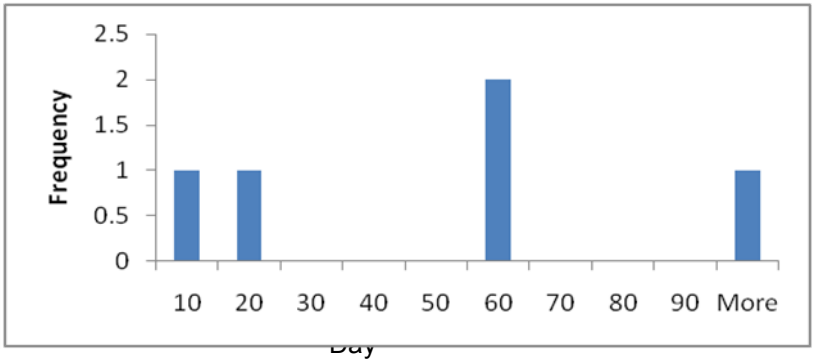




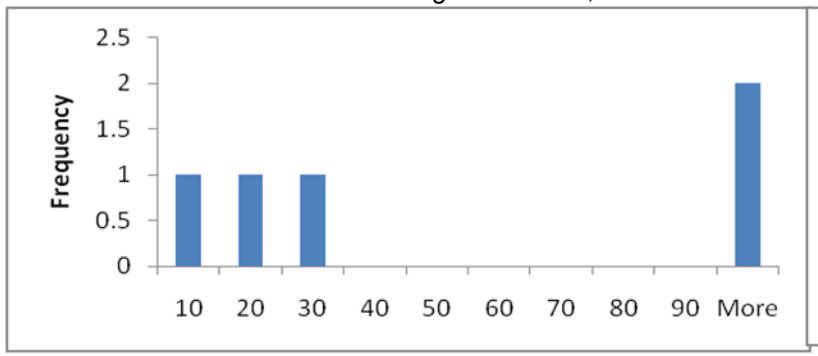
		Stability	
		Night	Day
1. B. anthracis	Geo Mean	NA	NA
	Median	NA	NA
	Low		
	High		

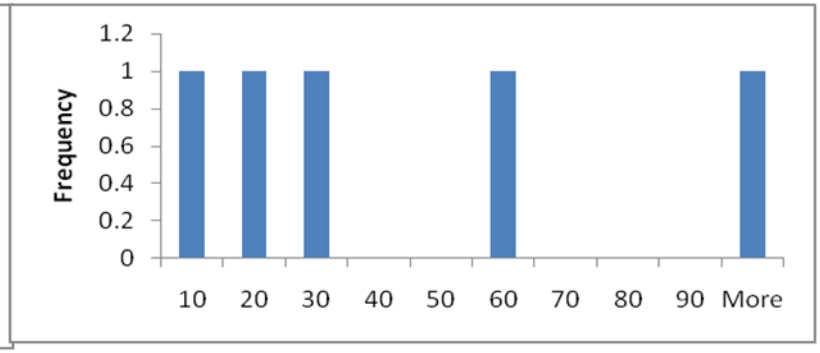
		Night	Day
2. F. tularensis	Geo Mean	167	30
	Median	60	60
	Low	15	3
	High	40,000	120



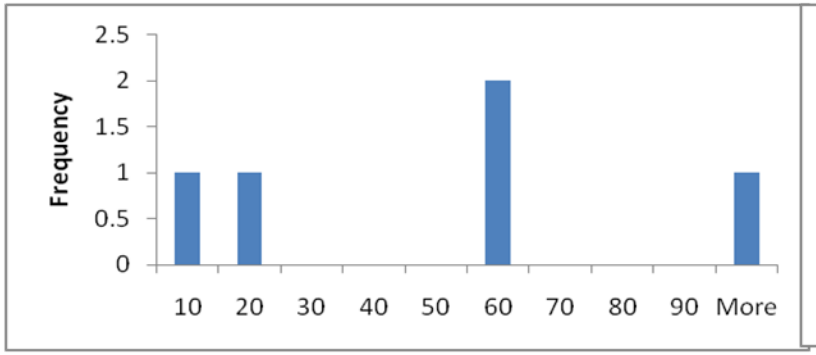


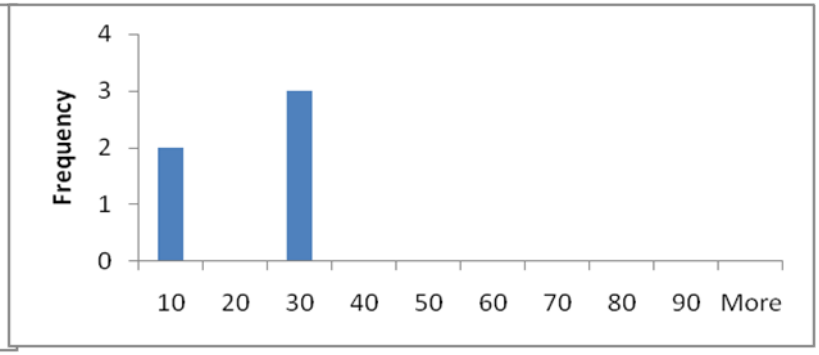
		Night	Day
3. Y. pestis	Geo Mean	105	21
	Median	30	30
	Low	6	1
	High	40,000	120



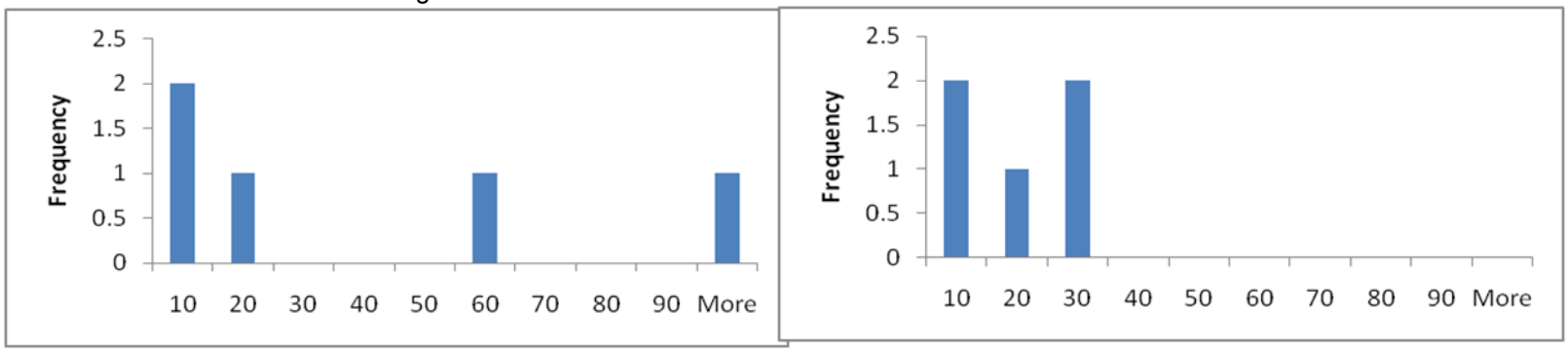


		Night	Day
4. Andes hantavirus	Geo Mean	45	19
	Median	60	28
	Low	10	10
	High	250	30

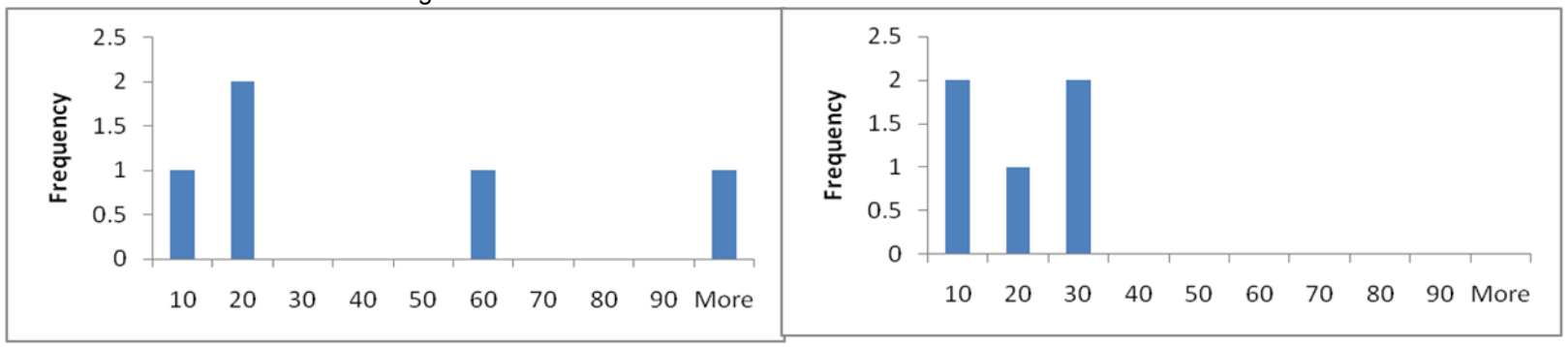




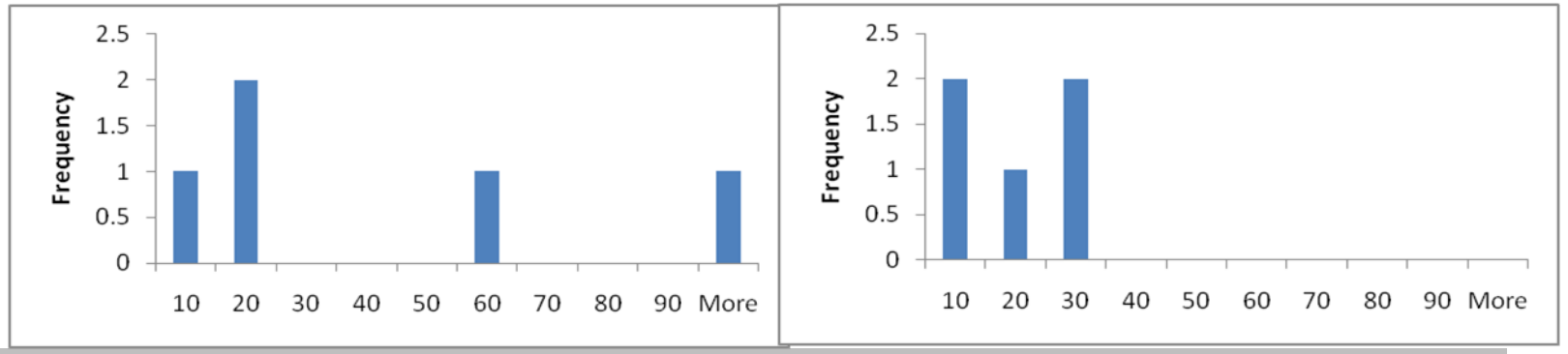
		Night	Day
	Geo		
5. Ebola virus	Mean	21	9
	Median	15	15
	Low	2	1
	High	240	30



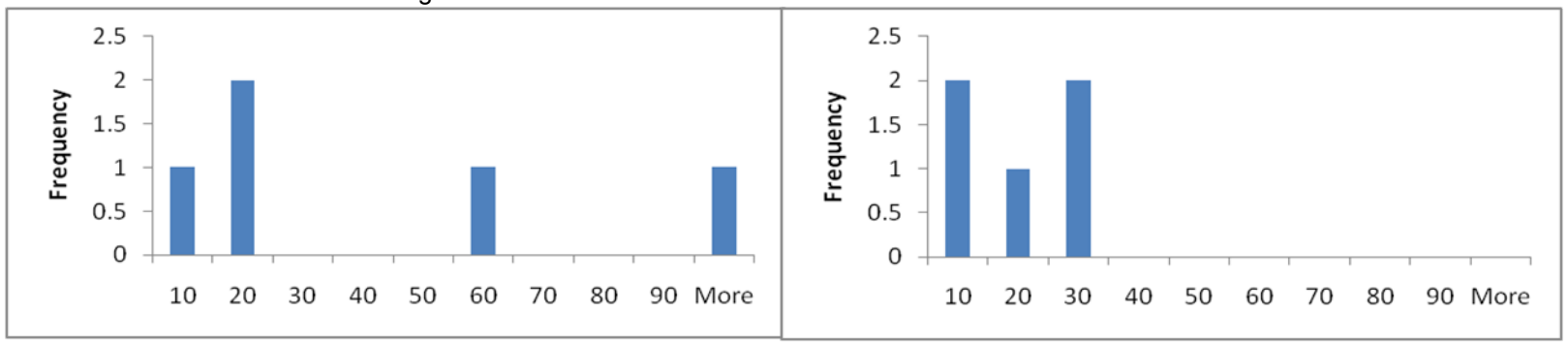
		Night	Day
	Geo		
6. Marburg virus	Mean	32	12
	Median	15	15
	Low	10	2
	High	240	30



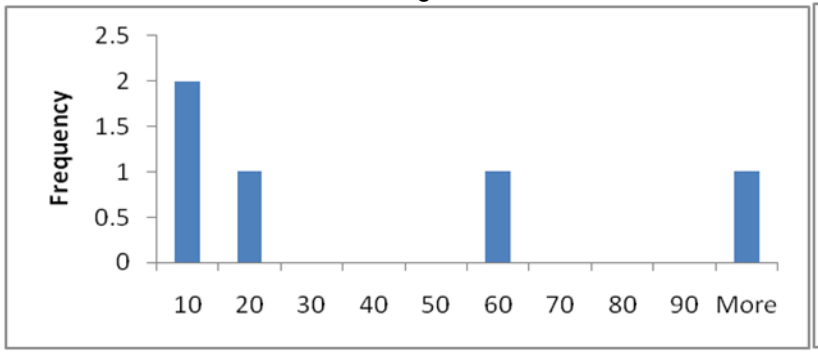
		Night	Day
7. Junin virus	Geo		
	Mean	35	15
	Median	20	15
	Low	10	5
	High	300	30

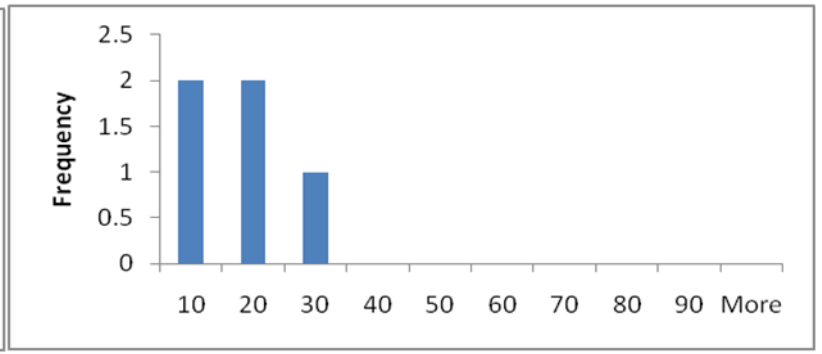


		Night	Day
8. Lassa fever virus	Geo		
	Mean	35	15
	Median	20	15
	Low	10	5
	High	300	30

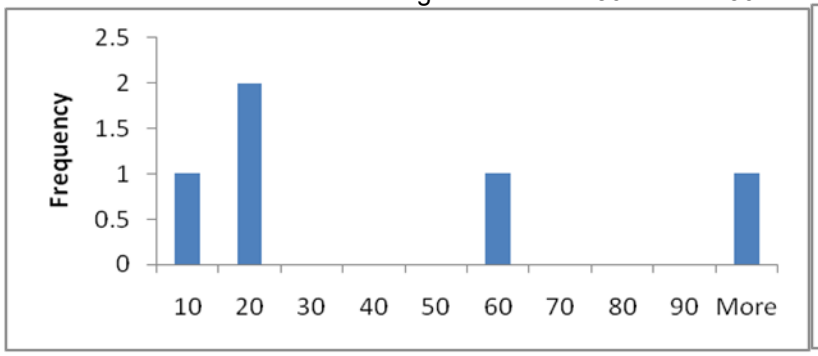


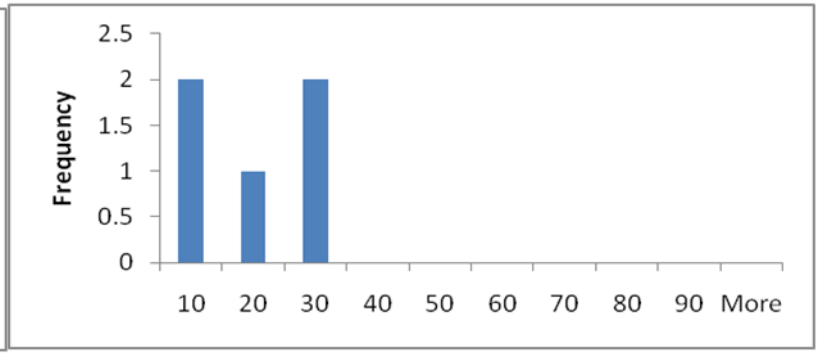
		Night	Day
9. Nipah virus	Geo Mean	42	11
	Median	15	15
	Low	5	2
	High	2,880	30



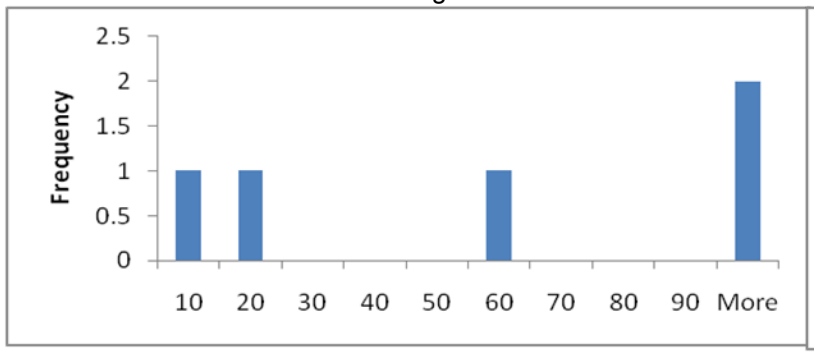


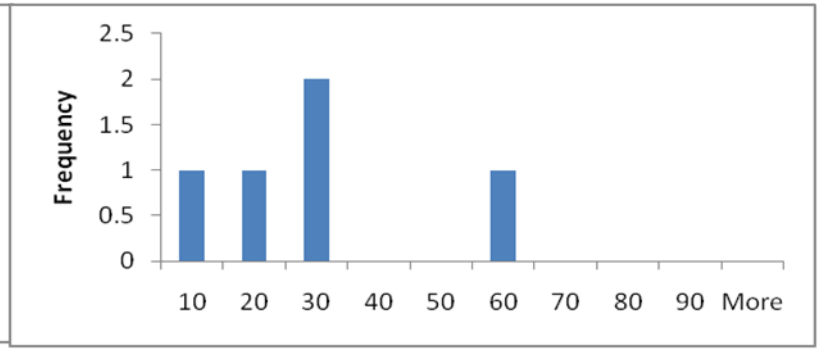
		Night	Day
10. Rift Valley fever virus	Geo Mean	35	17
	Median	20	15
	Low	10	10
	High	280	30



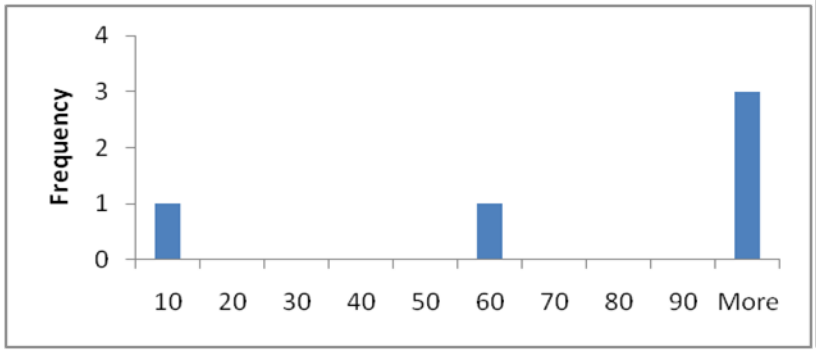


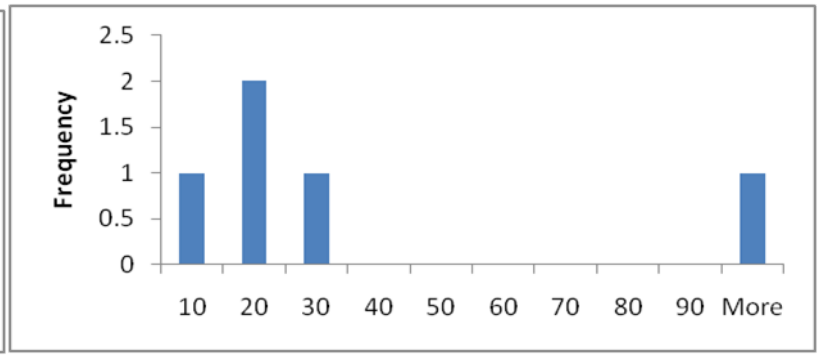
		Night	Day
11. Russian spring-summer encephalitis virus	Geo Mean	44	24
	Median	60	30
	Low	5	10
	High	360	56



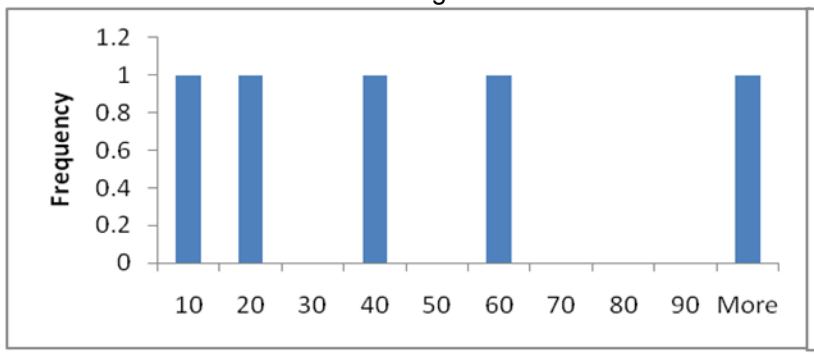


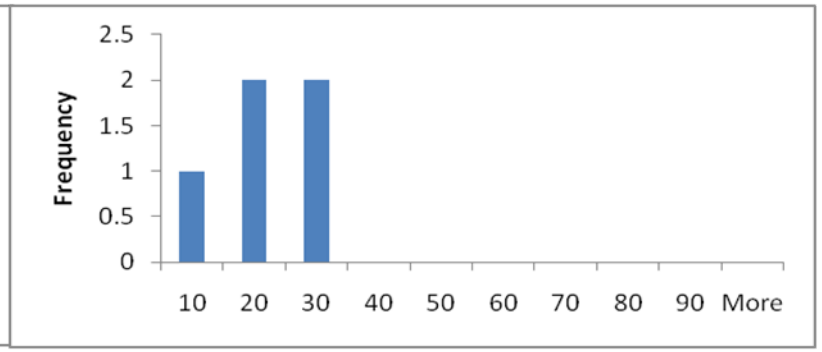
		Night	Day
12. SARS virus	Geo Mean	166	31
	Median	120	20
	Low	10	7
	High	3,600	480



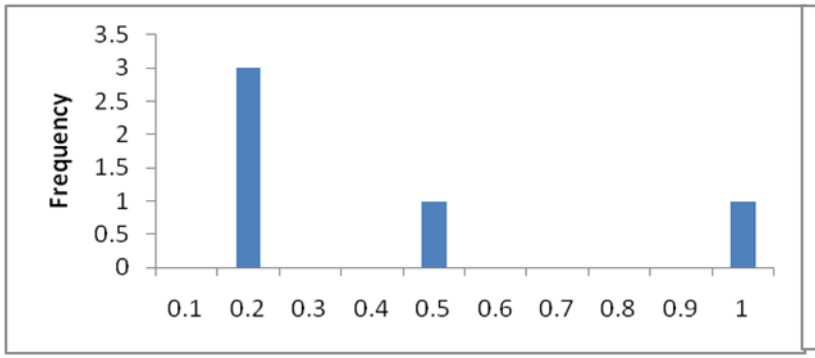


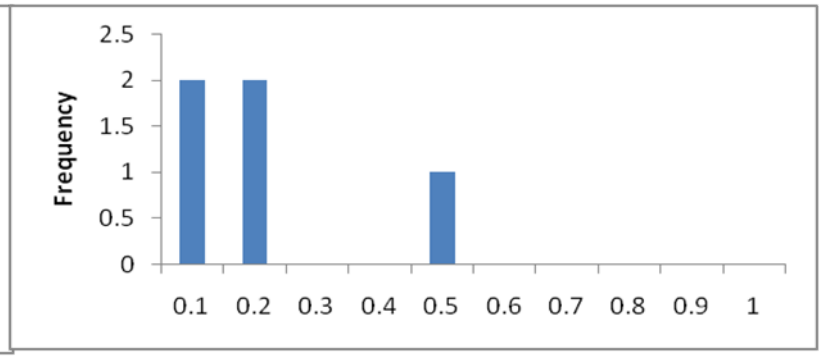
		Night	Day
13. 1918 influenza virus	Geo Mean	40	17
	Median	36	20
	Low	10	5
	High	300	30





Vulnerability Young	Bacterial Disease	Mortality
Geo Mean	33%	11%
Median	20%	20%
Low	20%	1%
High	100%	50%

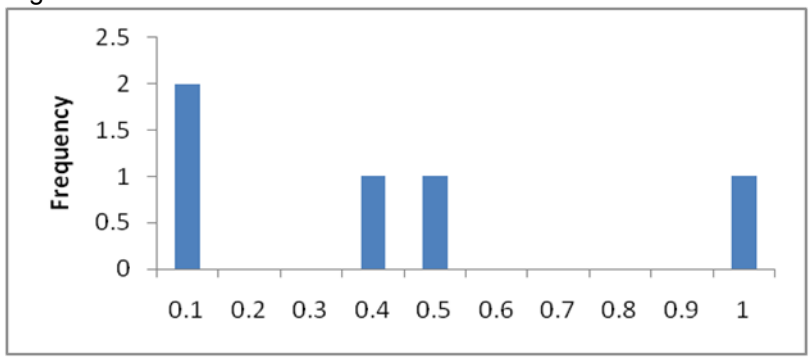


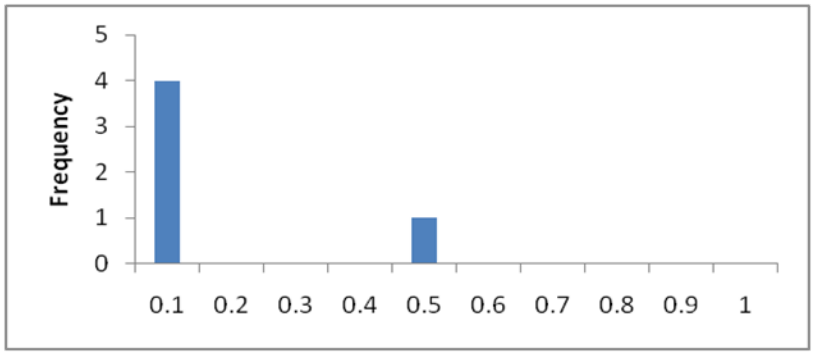


Disease

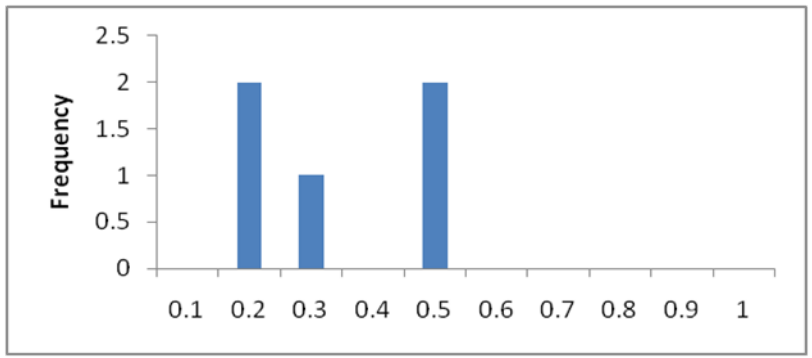
Mortality

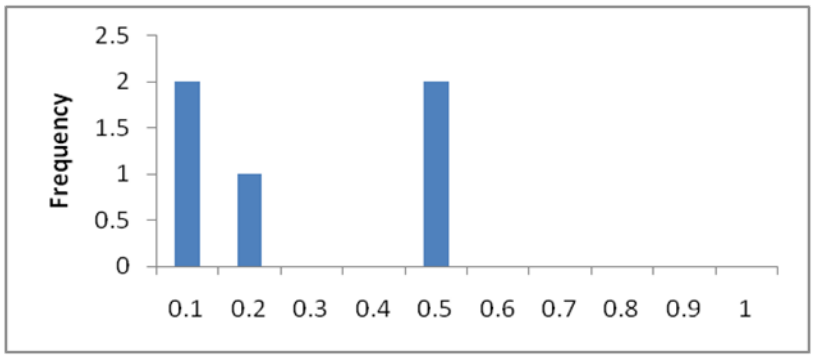
Young Viral	Disease	Mortality
Geo Mean	25%	8%
Median	40%	10%
Low	5%	1%
High	100%	50%



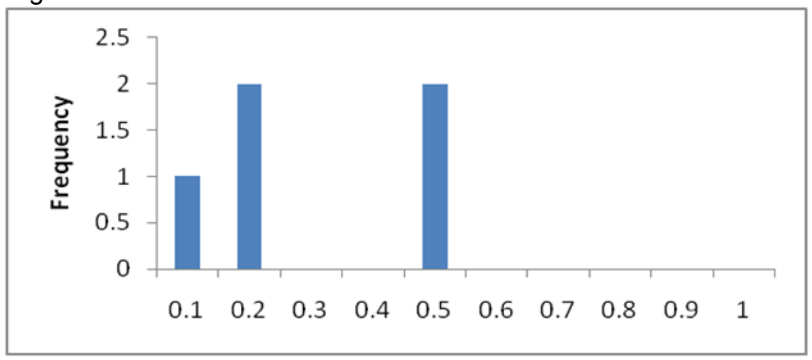


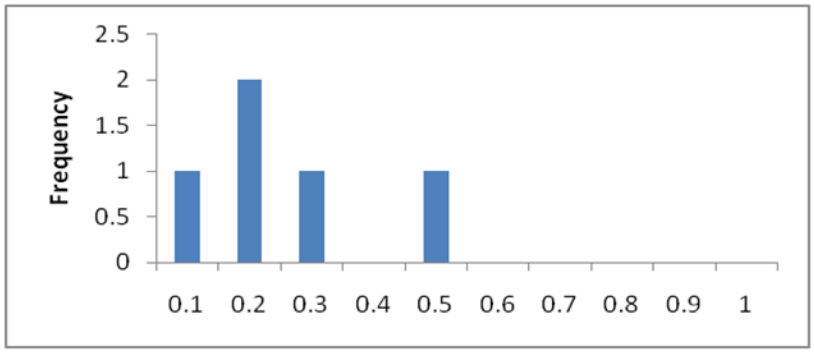
	Older	Bacterial	Disease	Mortality
Geo Mean			31%	11%
Median			30%	20%
Low			20%	1%



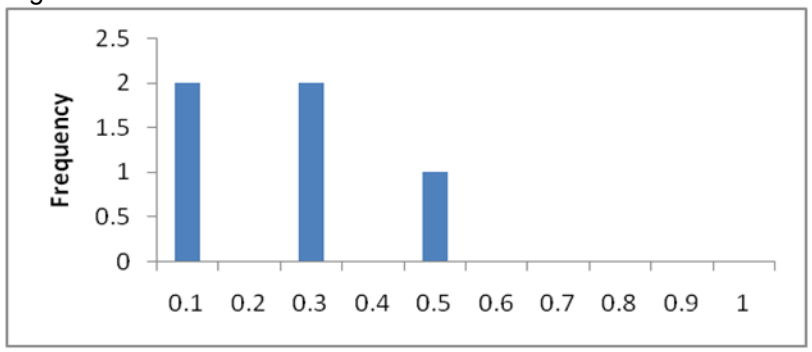


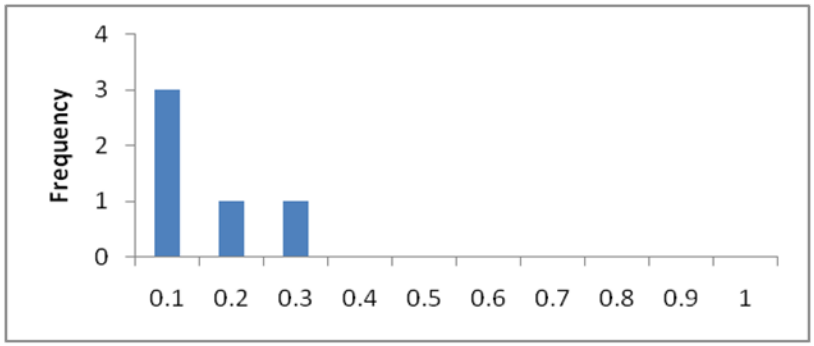
	Older	Viral	Disease	Mortality
Geo Mean			25%	14%
Median			20%	20%
Low			10%	1%
High			50%	50%



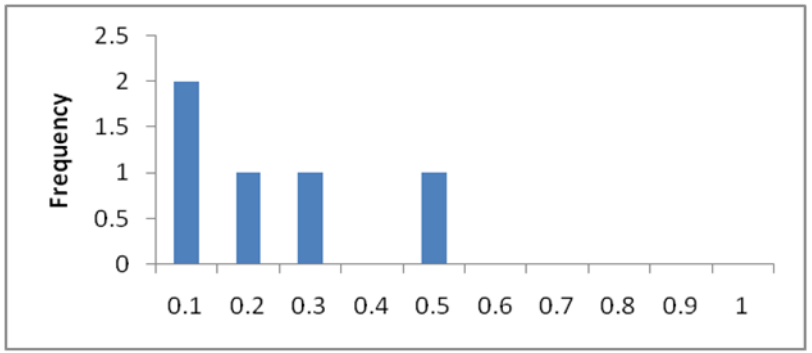


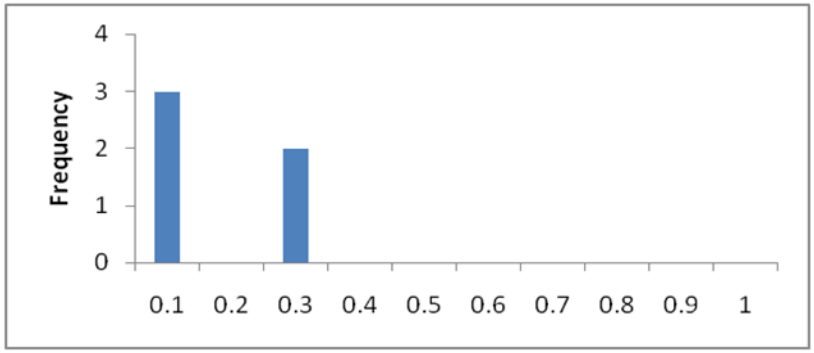
	Diabetes	Bacterial	Disease	Mortality
Geo Mean			18%	11%
Median			25%	10%
Low			5%	5%
High			50%	25%



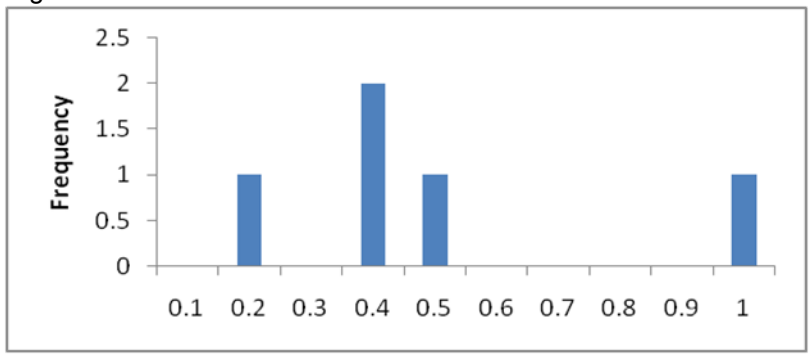


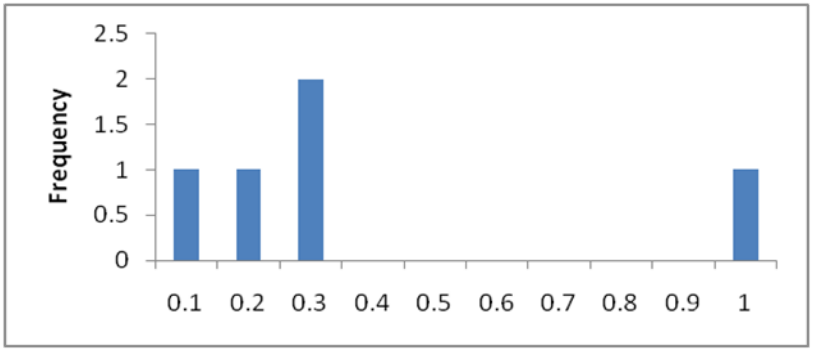
	Diabetes	Viral	Disease	Mortality
Geo Mean			17%	13%
Median			20%	10%
Low			5%	5%
High			50%	30%



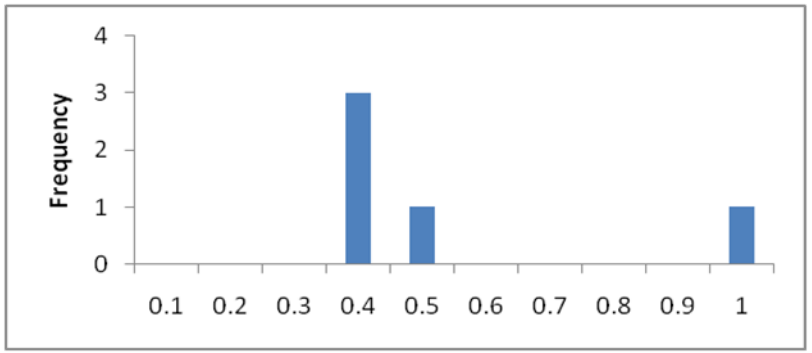


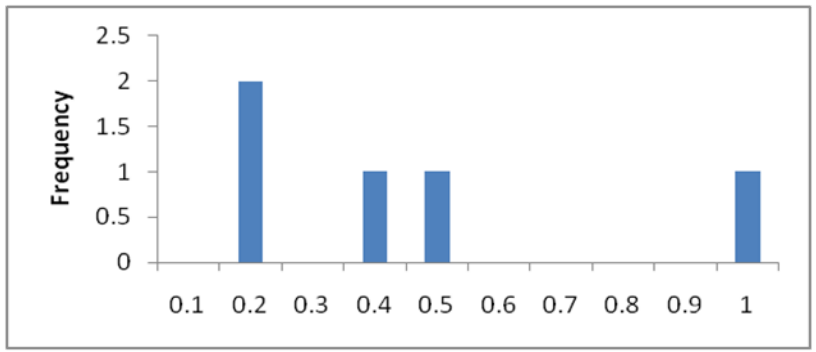
	HIV	Bacterial	Disease	Mortality
Geo Mean			44%	27%
Median			40%	25%
Low			20%	10%
High			100%	100%



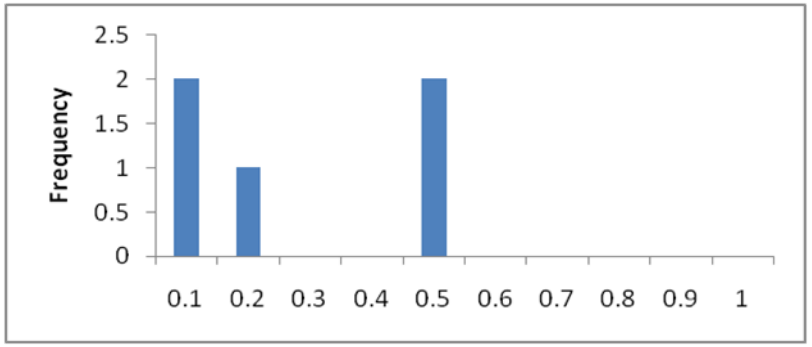


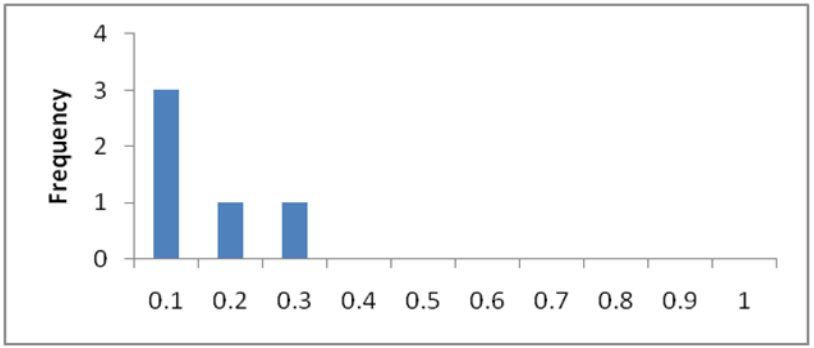
	HIV	Viral	Disease	Mortality
Geo Mean			50%	38%
Median			40%	40%
Low			40%	20%
High			100%	100%



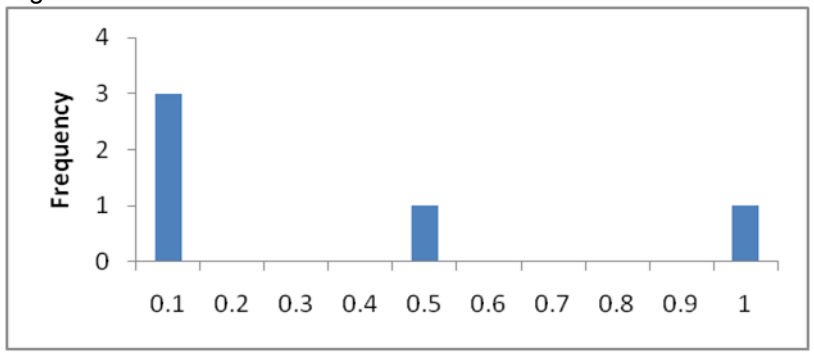


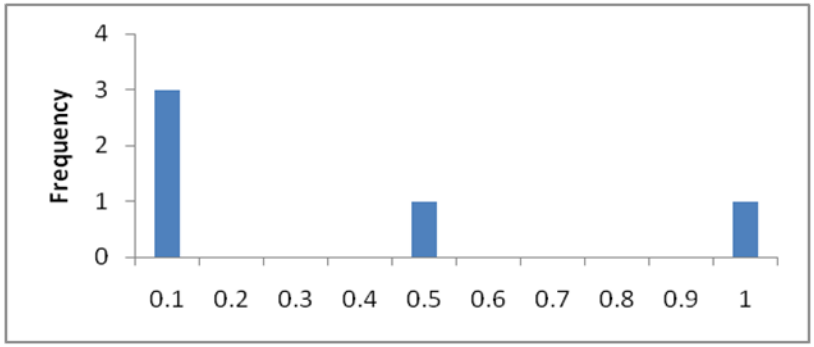
	Pregnancy	Bacterial	Disease	Mortality
Geo Mean			3%	9%
Median			20%	10%
Low			0%	1%
High			50%	25%





	Pregnancy	Viral	Disease	Mortality
Geo Mean			3%	1%
Median			10%	10%
Low			0%	0%
High			100%	25%





Attachment H-5: Face-to-Face Meeting Agenda and Results of Round 2

Expert Consultation on Infectiousness of Organisms
Studied in the NEIDL Risk Assessment

May 18, 2010

Doubletree Hotel and Executive Meeting Center
Ballroom – Section B

Teleconference Number 1-866-647-1048 Passcode 6488095

Draft Agenda

10:00 AM **Welcome and Roundtable Introductions**
Sam Bozzette, M.D., Ph.D., Consultant to NIH, Professor of Medicine and of International Relations, University of California San Diego

10:15 AM **Discussion on Dose Response**
Sam Bozzette, M.D., Ph.D.,
Wiley A. Schell, Associate Professor in Medicine, Duke University Medical Center

11:30 AM **Vote**

12:00 PM **Discussion on Transmissibility (Ro)**
Adi Gundlapalli, M.D., Ph.D., M.S., Assistant Professor, Departments of Internal Medicine, Pathology and Biomedical Informatics, University of Utah School of Medicine

12:30PM **Vote**

1:00 PM **Working Lunch: Discussion on Increased Vulnerability**
Adi Gundlapalli, M.D., Ph.D., M.S.,

1:30 PM **Vote**

1:45 PM **Discussion of Atmospheric Decay**
Adi Gundlapalli, M.D., Ph.D., M.S.,
Ken Bulmahn, Professional Engineer, Tetra Tech Risk Assessment Team Lead

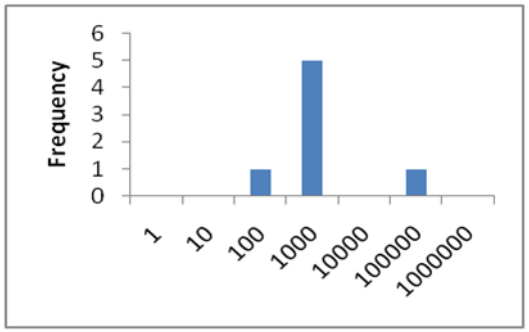
3:15 PM **Vote**

3:45 PM **Next Steps and Closing Remarks**
Sam Bozzette, M.D., Ph.D.

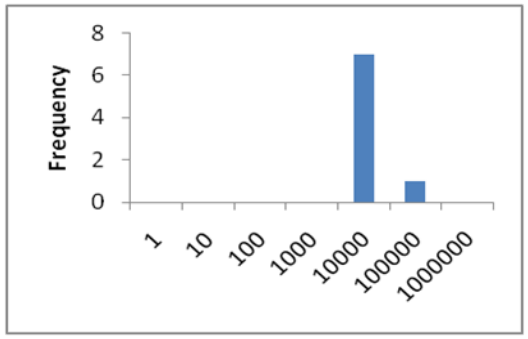
4:00 PM **Adjourn**

Round 2 Results

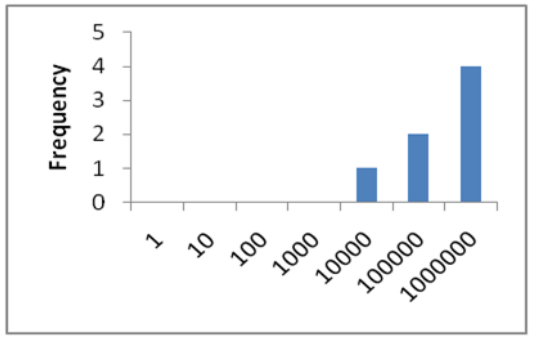
Organism		ID10	ID50	ID90
1. B. anthracis	Geo Mean	473	10,858	121,247
	Median	300	8,000	200,000
	Low	100	5,000	10,000
	High	14,800	94,300	600,000



ID10

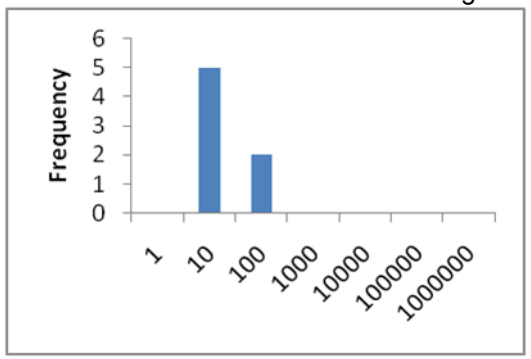


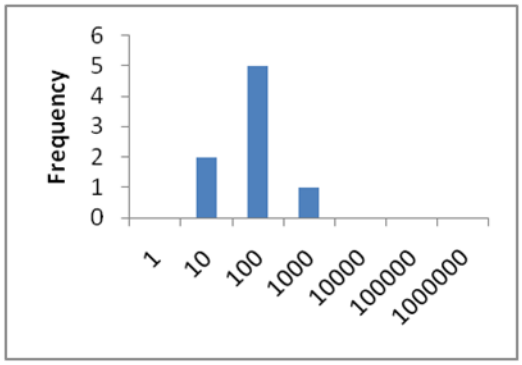
ID50

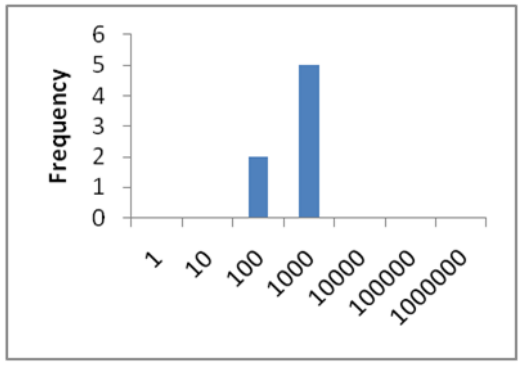


ID90

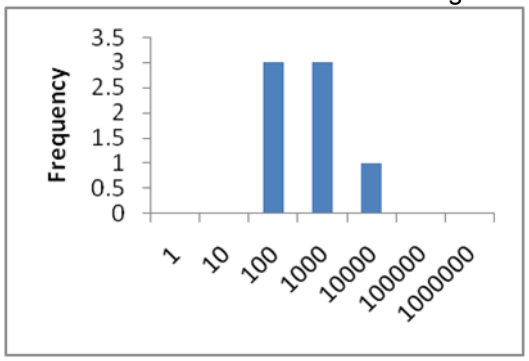
Organism		ID10	ID50	ID90
2. F. tularensis	Geo Mean	9	35	229
	Median	10	40	200
	Low	2	10	41
	High	100	500	1,000

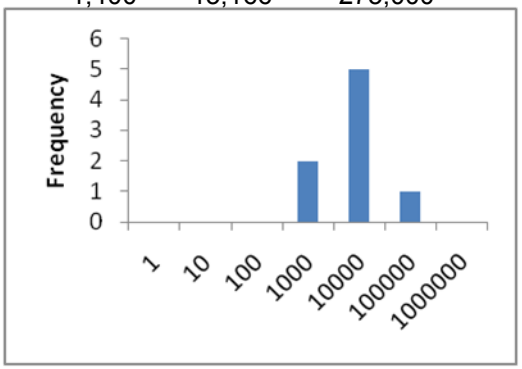


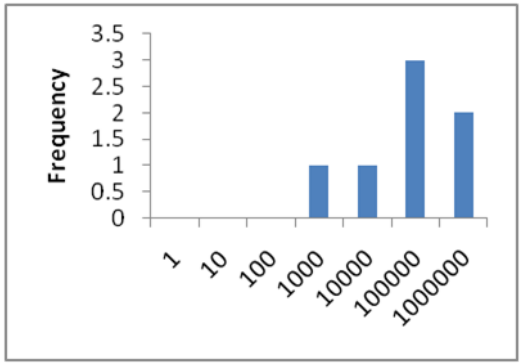




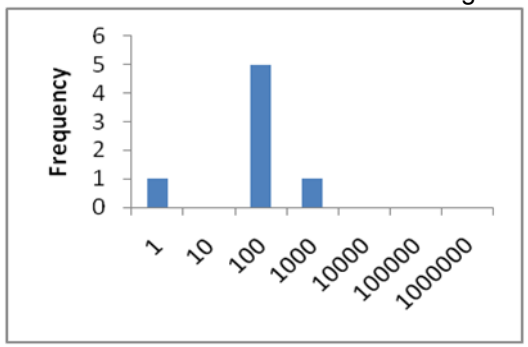
Organism		ID10	ID50	ID90
	Geo			
3. Y. pestis	Mean	255	3,408	30,535
	Median	200	5,000	30,000
	Low	100	500	1,000
	High	1,400	15,166	275,000

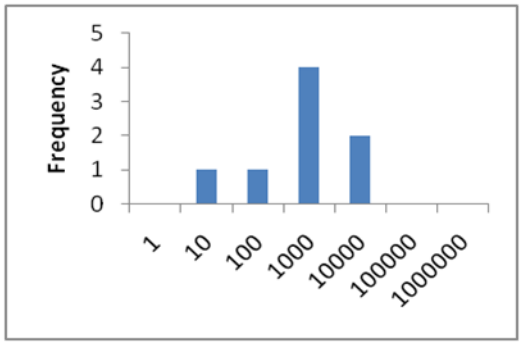


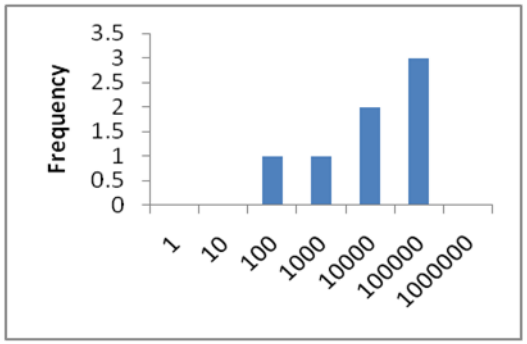




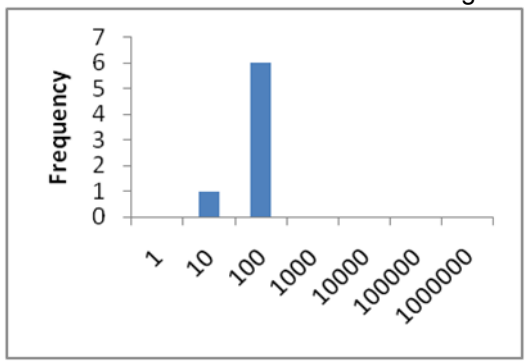
Organism		ID10	ID50	ID90
	Geo			
4. Andes hantavirus	Mean	53	402	4,906
	Median	60	375	5,000
	Low	1	10	100
	High	814	5,500	100,000

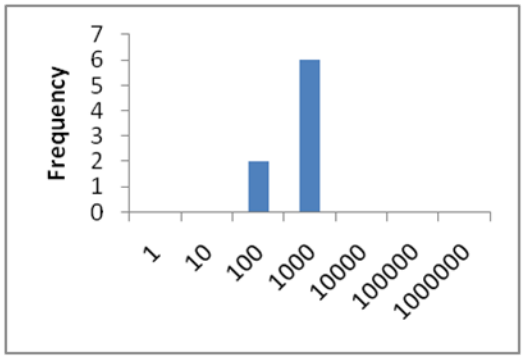


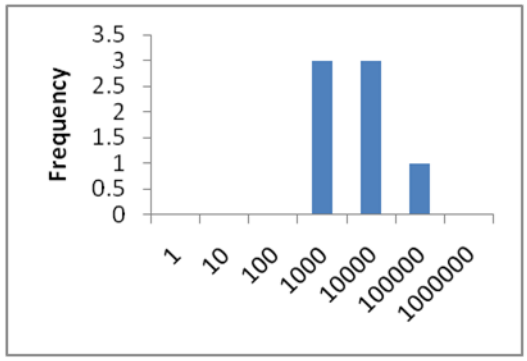




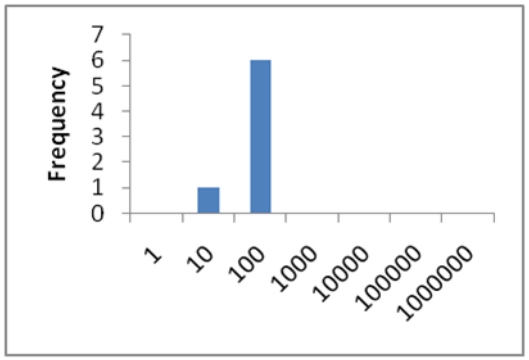
Organism		ID10	ID50	ID90
	Geo			
5. Ebola virus	Mean	38	271	3,497
	Median	30	160	2,000
	Low	10	100	400
	High	100	1,000	100,000

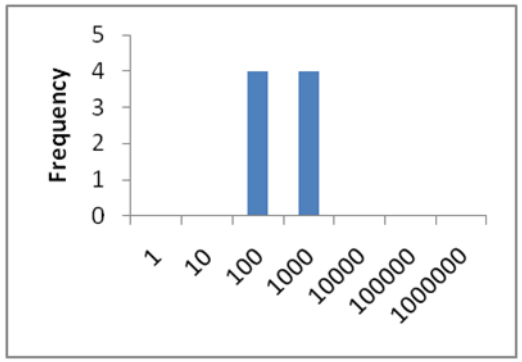


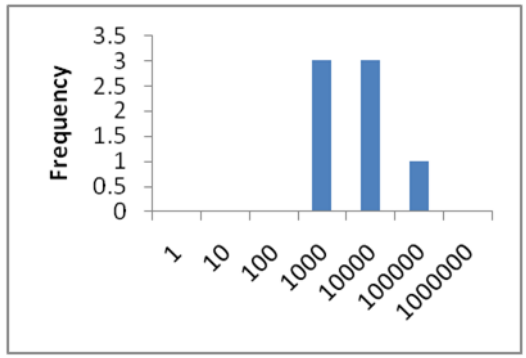




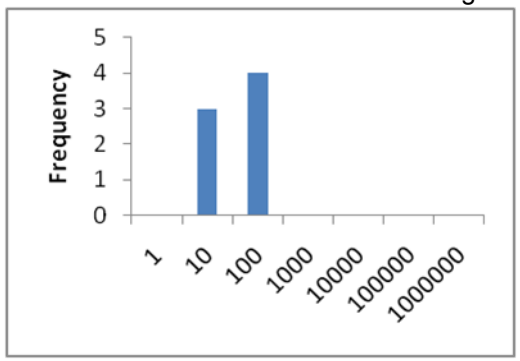
Organism		ID10	ID50	ID90
	Geo			
6. Marburg virus	Mean	28	169	2,746
	Median	20	110	2,000
	Low	10	40	400
	High	100	1,000	30,000

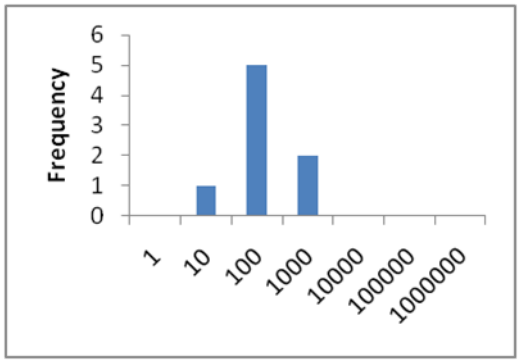


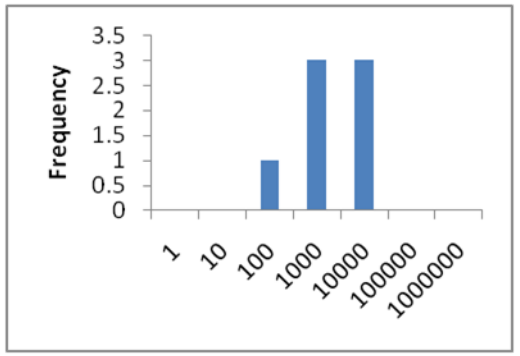




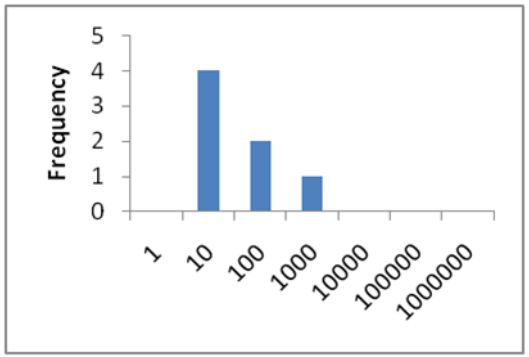
Organism		ID10	ID50	ID90
7. Junin virus	Geo Mean	17	58	1,109
	Median	20	40	1,000
	Low	2	5	49
	High	100	1,000	10,000

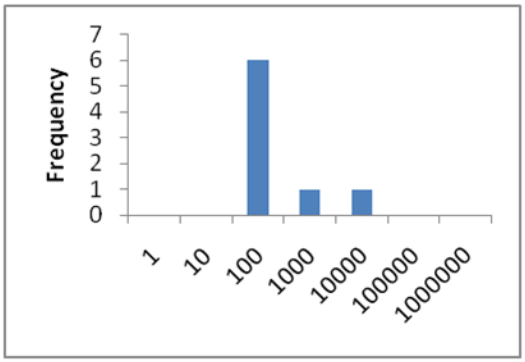


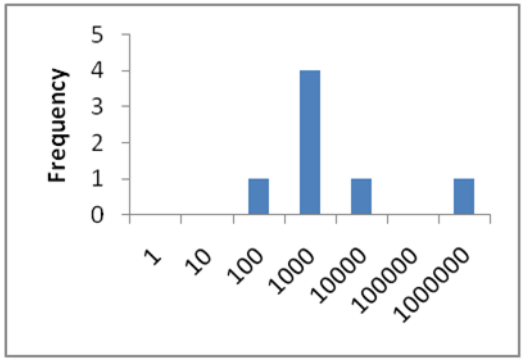




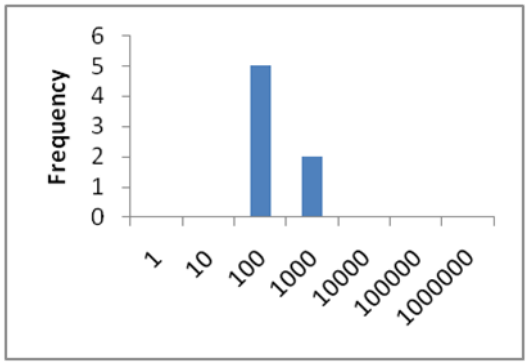
Organism		ID10	ID50	ID90
8. Lassa fever virus	Geo Mean	20	77	1,423
	Median	10	30	1,000
	Low	3	15	59
	High	500	10,000	1,000,000

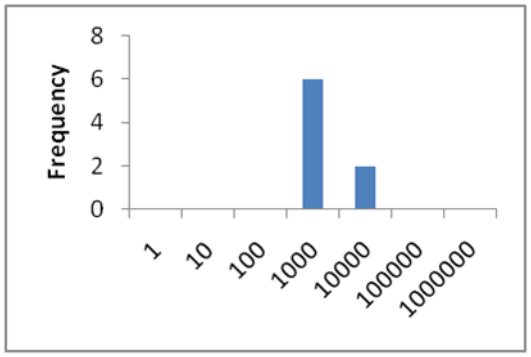


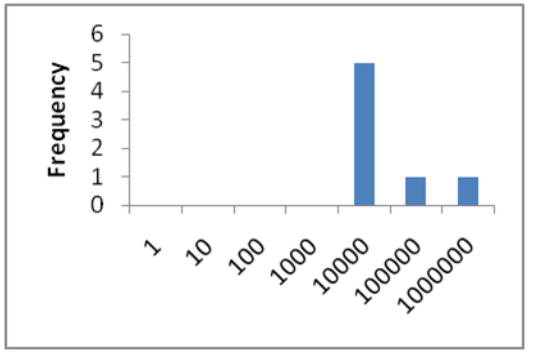




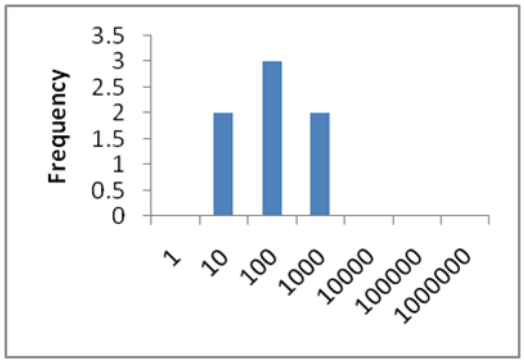
Organism		ID10	ID50	ID90
9. Nipah virus	Geo Mean	140	1,275	15,090
	Median	100	1,000	9,000
	Low	75	400	1,650
	High	1,000	10,000	1,000,000

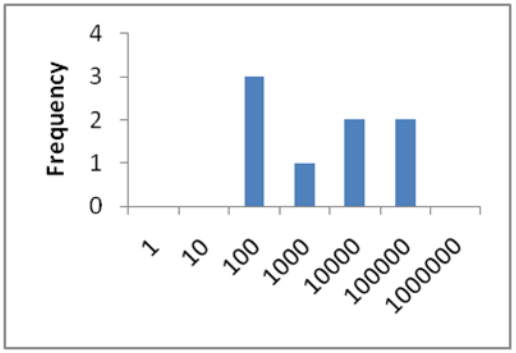


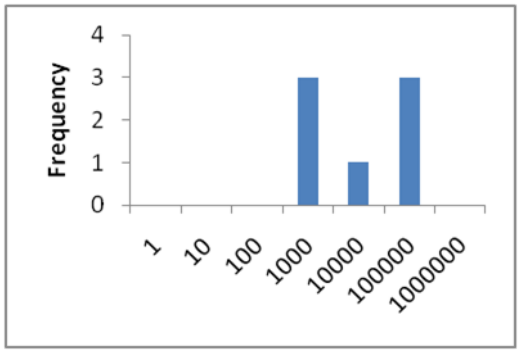




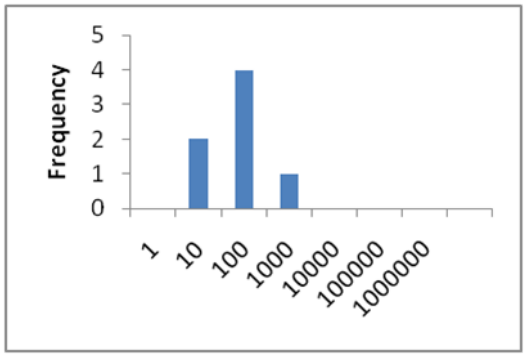
Organism		ID10	ID50	ID90
10. Rift Valley fever virus	Geo Mean	63	1,014	5,561
	Median	100	1,500	10,000
	Low	5	50	329
	High	1,000	15,000	100,000

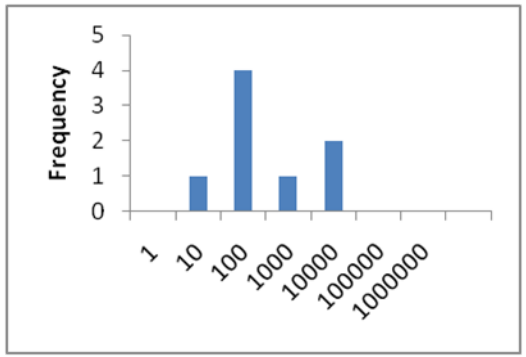


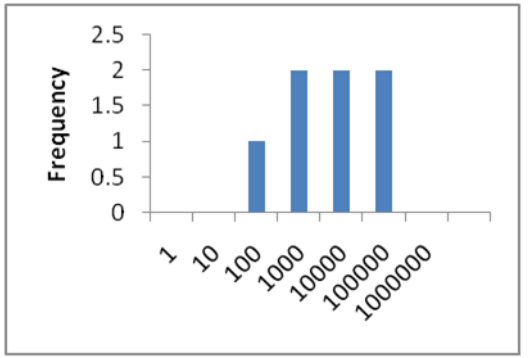




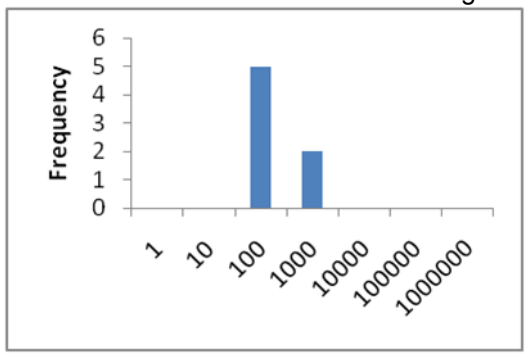
Organism		ID10	ID50	ID90
11. Russian spring-summer encephalitis virus	Geo Mean	33	222	2,737
	Median	20	71	2,000
	Low	2	10	50
	High	1,000	10,000	100,000

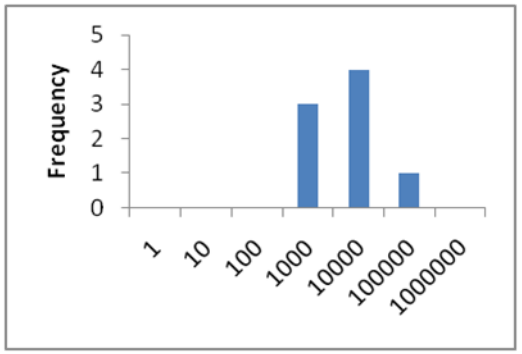


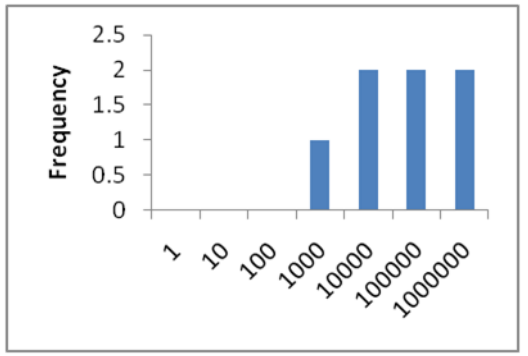




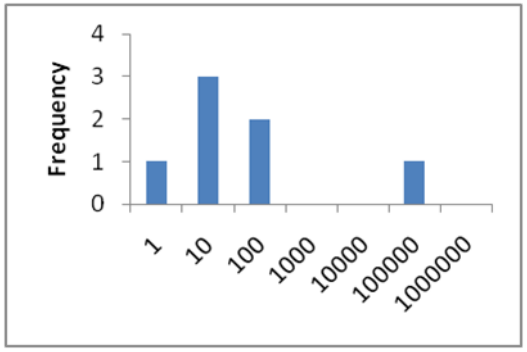
Organism		ID10	ID50	ID90
12. SARS virus	Geo Mean	119	3,013	33,320
	Median	100	6,000	40,000
	Low	43	283	950
	High	400	12,000	1,000,000

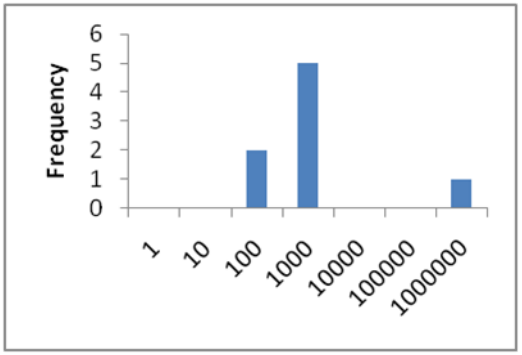


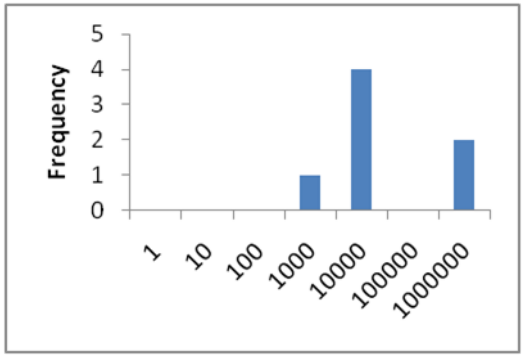




Organism		ID10	ID50	ID90
13. 1918 influenza virus	Geo Mean	39	885	24,298
	Median	10	750	10,000
	Low	1	100	1,000
	High	50,000	250,000	1,000,000

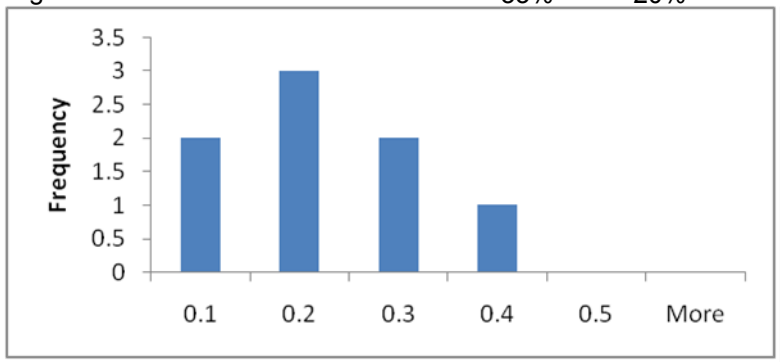




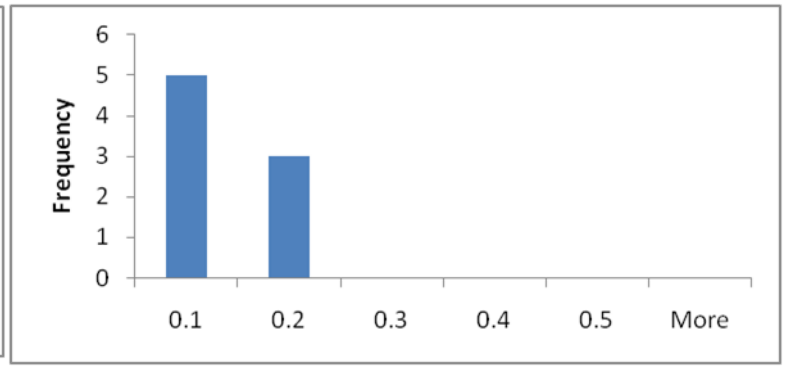


Vulnerability

Young	Bacterial	Disease	Mortality
Geo Mean		13%	8%
Median		20%	10%
Low		1%	1%
High		33%	20%

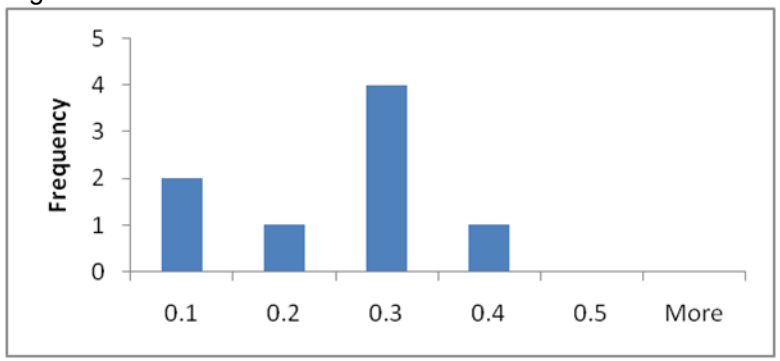


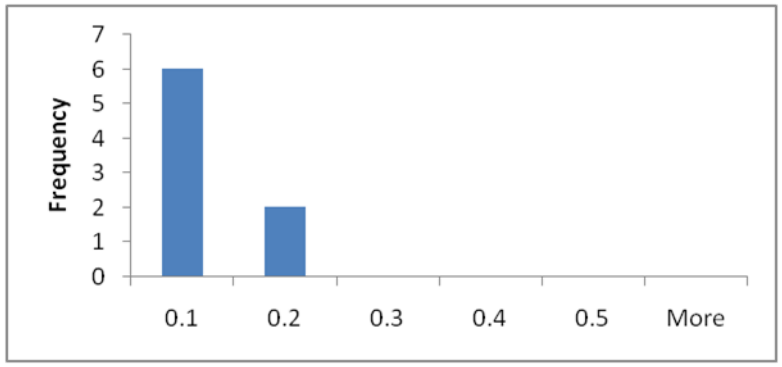
Disease



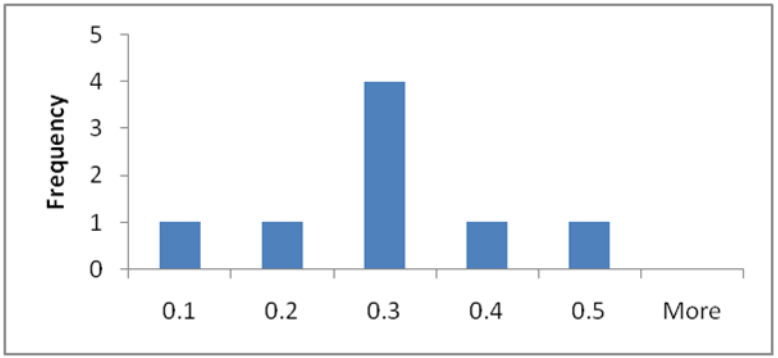
Mortality

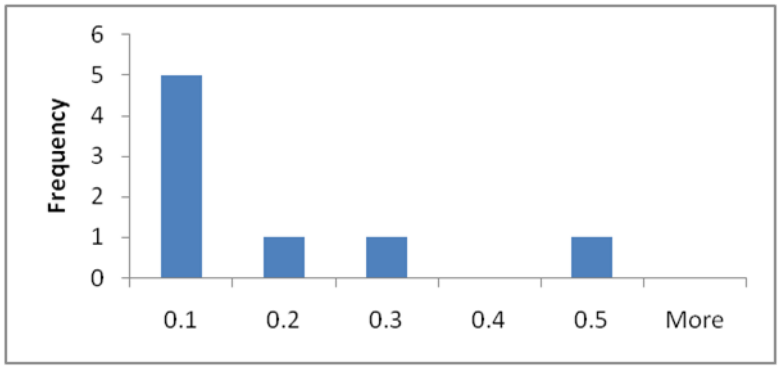
Young	Viral	Disease	Mortality
Geo Mean		11%	10%
Median		25%	10%
Low		0%	5%
High		33%	20%



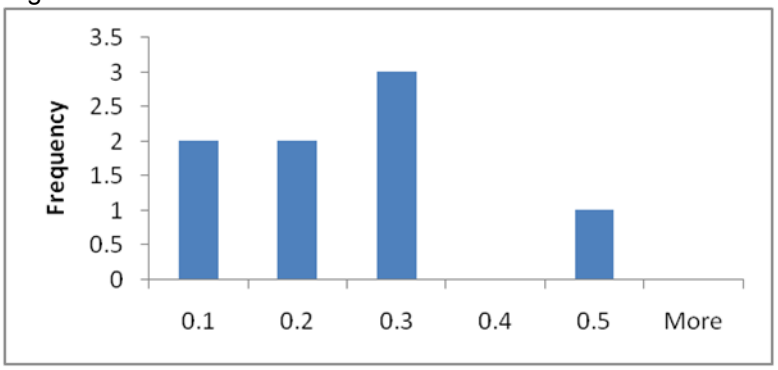


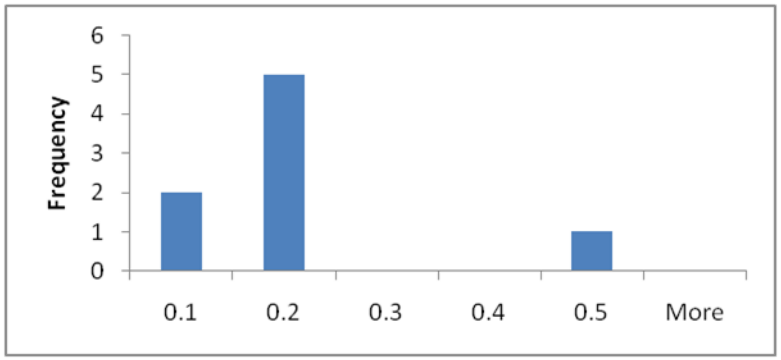
	Old	Bacterial	Disease	Mortality
Geo Mean			24%	13%
Median			30%	10%
Low			5%	5%
High			50%	50%



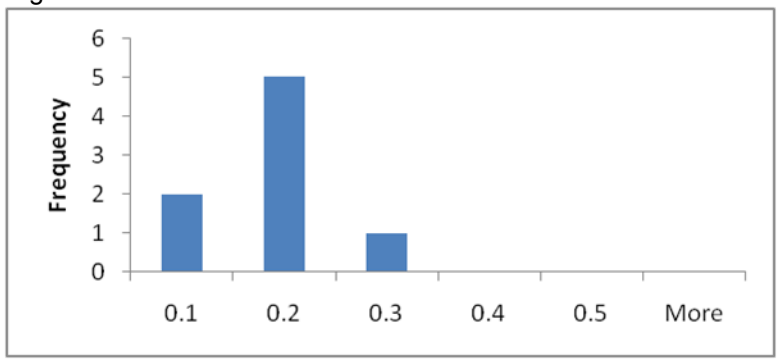


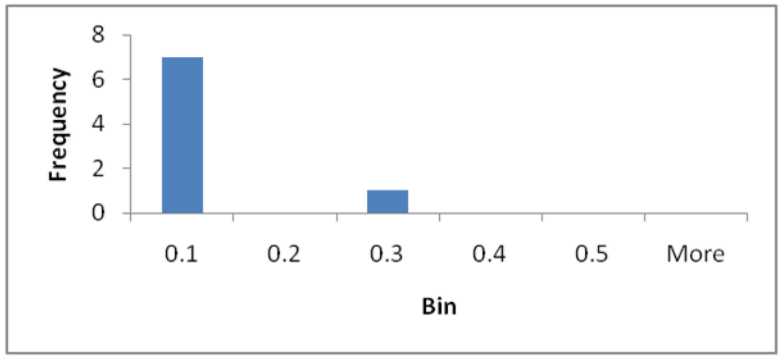
	Old	Viral	Disease	Mortality
Geo Mean			19%	14%
Median			23%	15%
Low			5%	5%
High			50%	50%



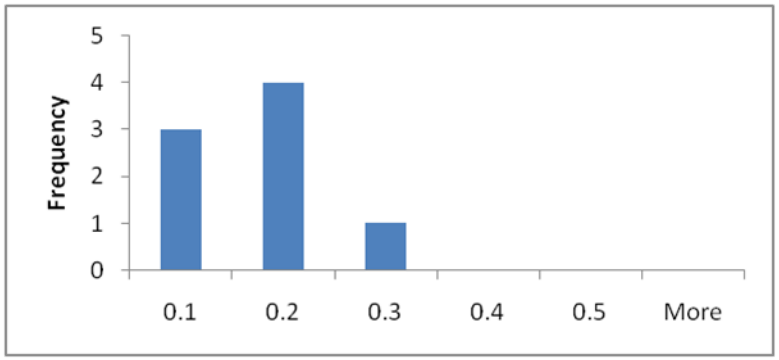


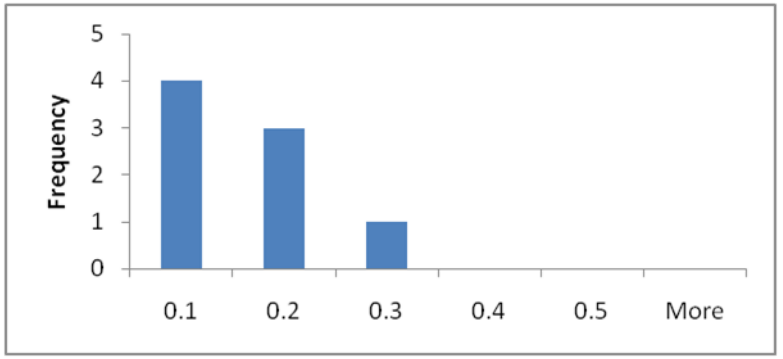
	Diabetes	Bacterial	Disease	Mortality
Geo Mean			15%	9%
Median			20%	10%
Low			5%	5%
High			25%	25%



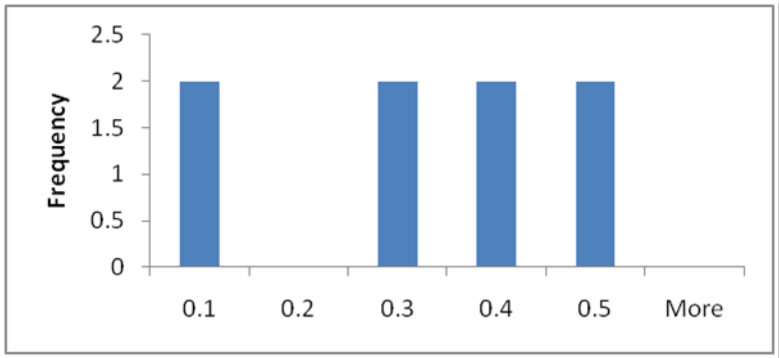


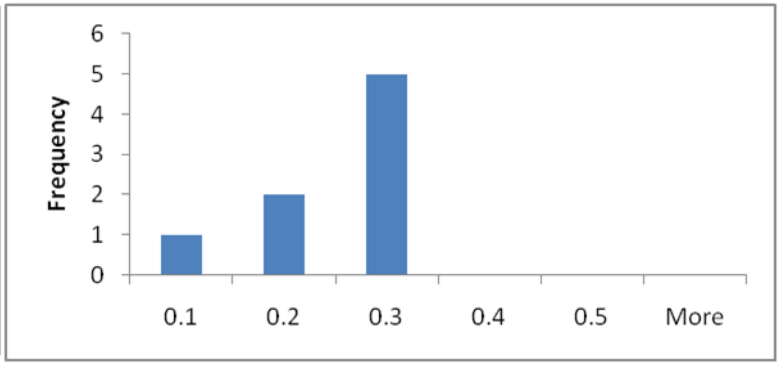
	Diabetes	Viral	Disease	Mortality
Geo Mean			13%	10%
Median			17%	11%
Low			5%	5%
High			25%	25%



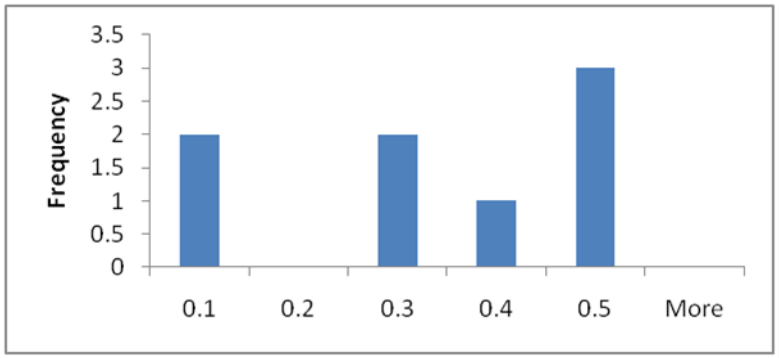


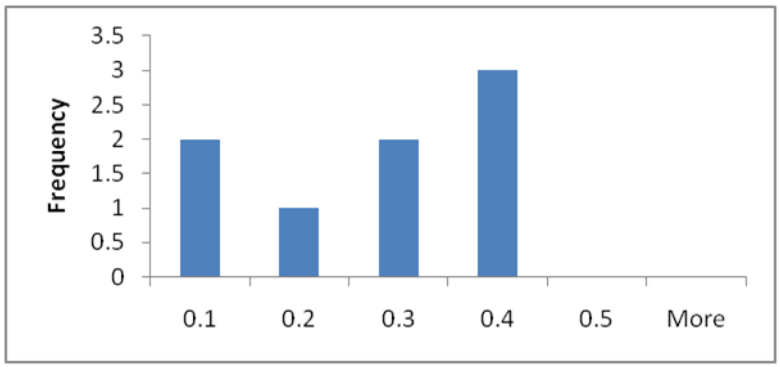
	HIV	Bacterial	Disease	Mortality
Geo Mean			27%	23%
Median			35%	25%
Low			10%	10%
High			45%	30%



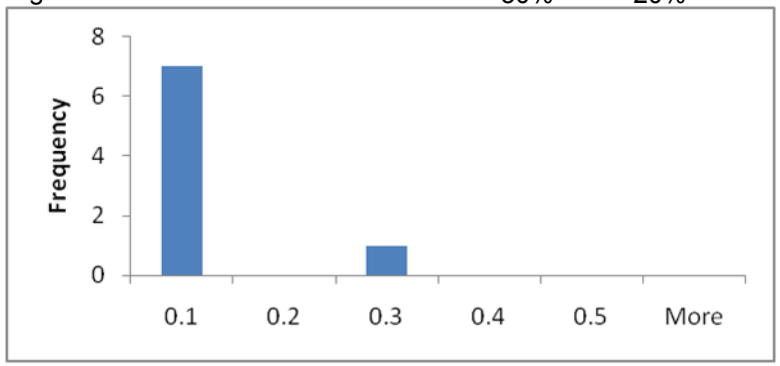


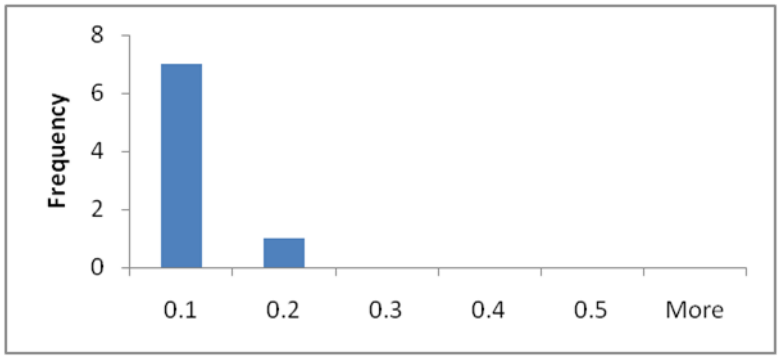
	HIV	Viral	Disease	Mortality
Geo Mean			28%	23%
Median			35%	28%
Low			10%	10%
High			50%	40%



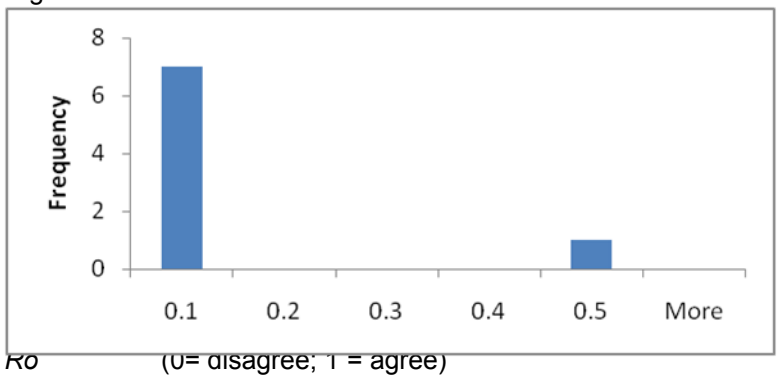


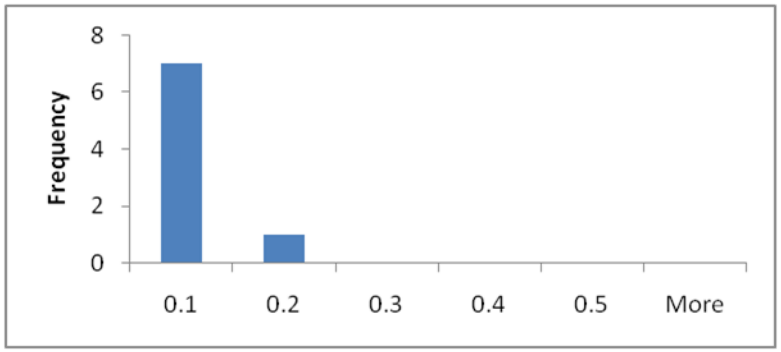
	Pregnancy	Bacterial	Disease	Mortality
Geo Mean			3%	5%
Median			5%	5%
Low			0%	1%
High			30%	20%





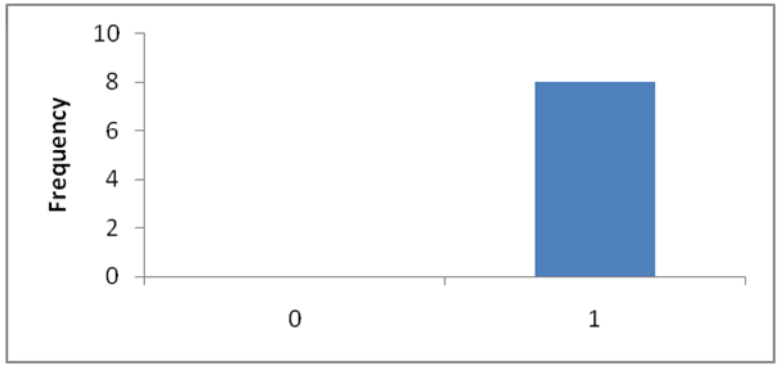
	Pregnancy	Viral	Disease	Mortality
Geo Mean			6%	3%
Median			5%	3%
Low			2%	1%
High			50%	20%



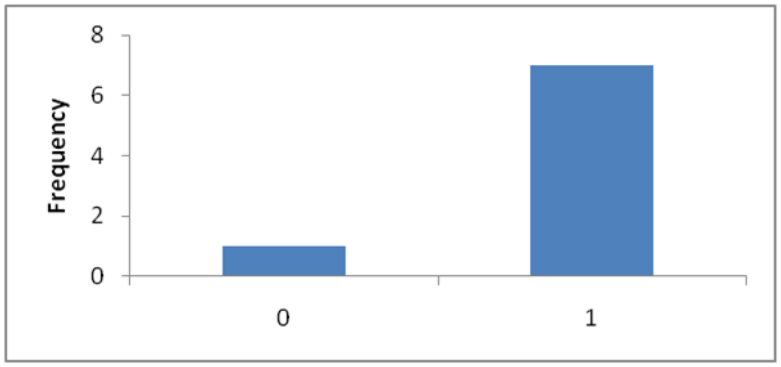


Ro (0= disagree; 1 = agree)

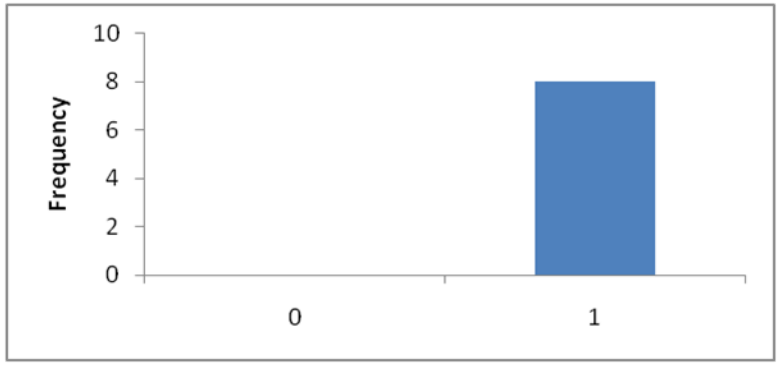
Pestis



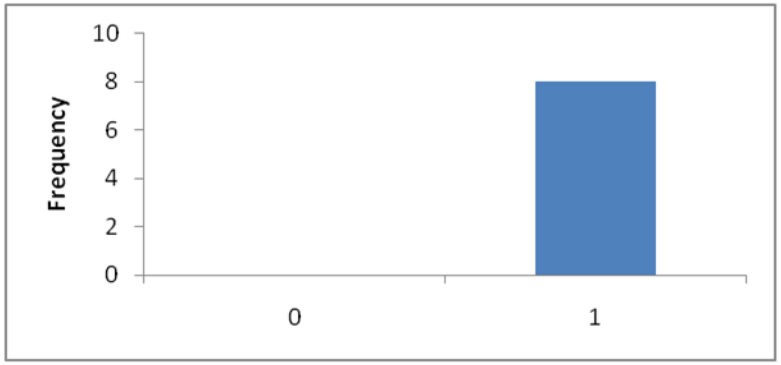
Ebola



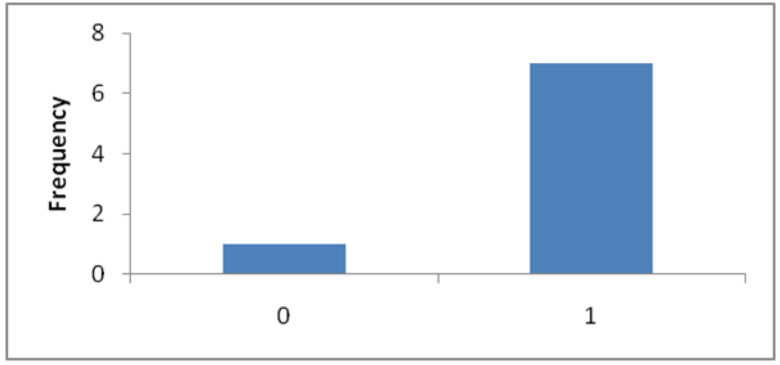
Rift



SARS

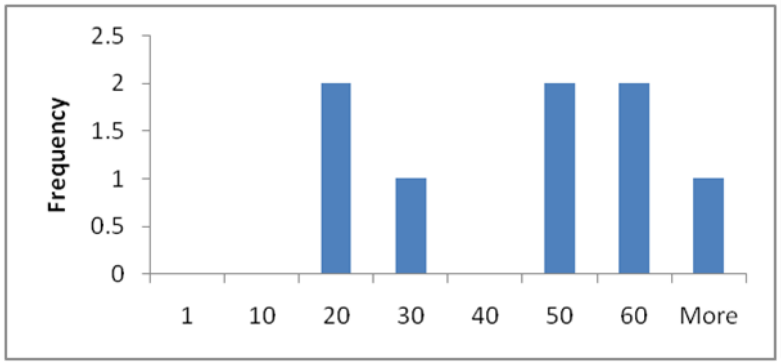


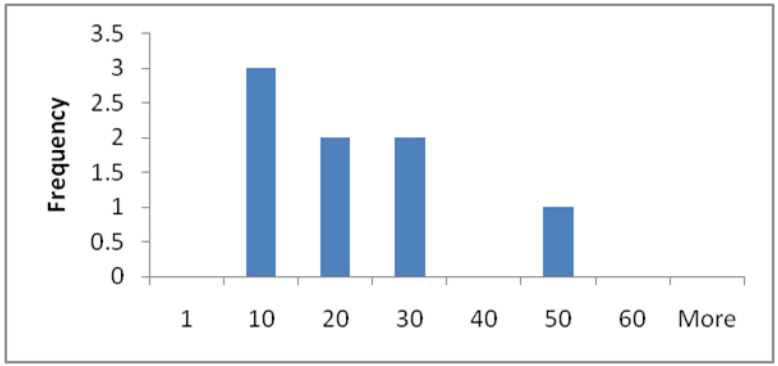
1918 Flu



		Stability	
		Night	Day
1. B. anthracis	Geo Mean	NA	NA
	Median		
	Low		
	High		

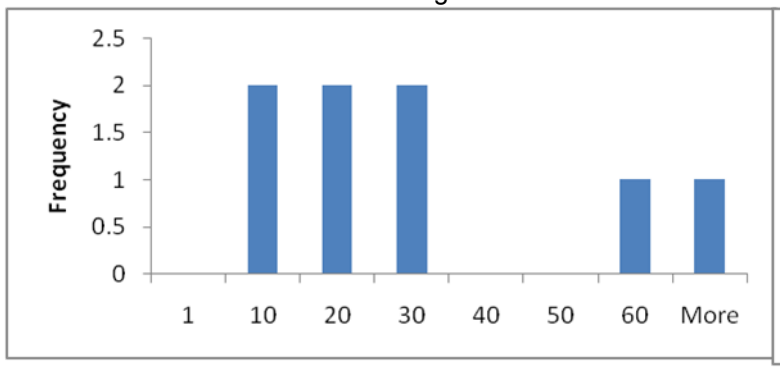
		Night	Day
2. F. tularensis	Geo Mean	42	16
	Median	48	20
	Low	20	3
	High	100	50

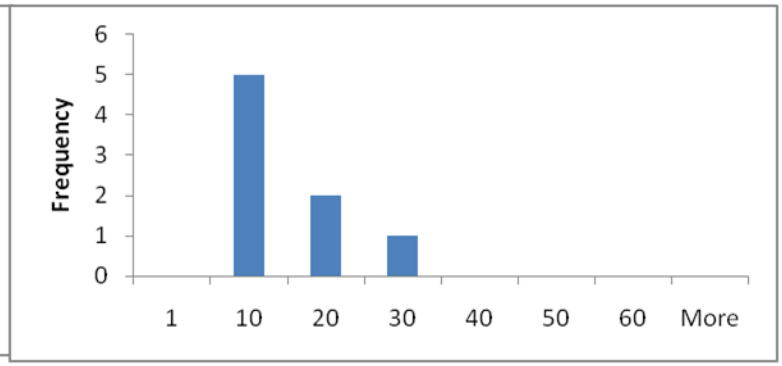




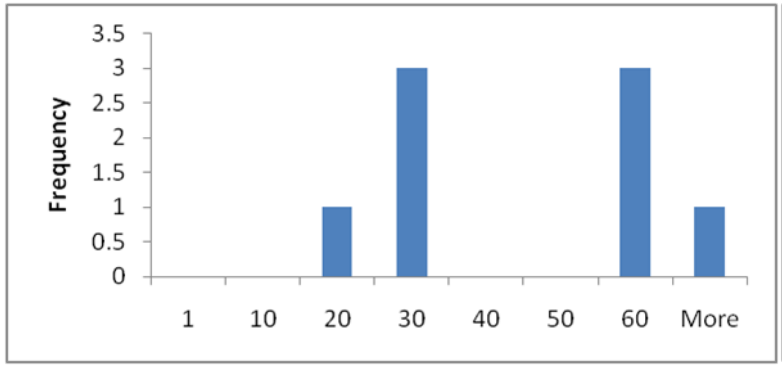
Night Day

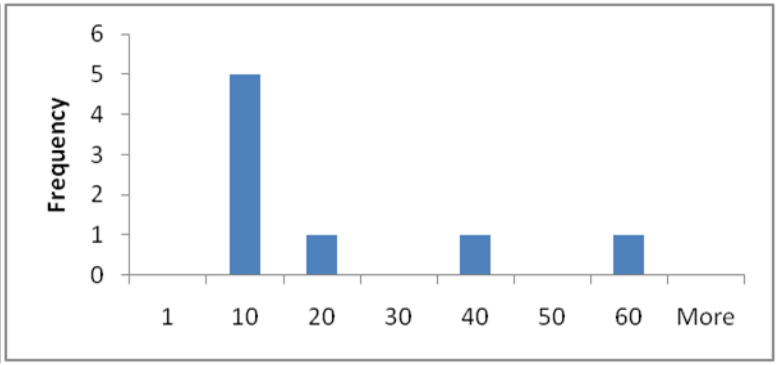
		Night	Day
3. Y. pestis	Geo Mean	25	10
	Median	25	10
	Low	8	2
	High	80	30



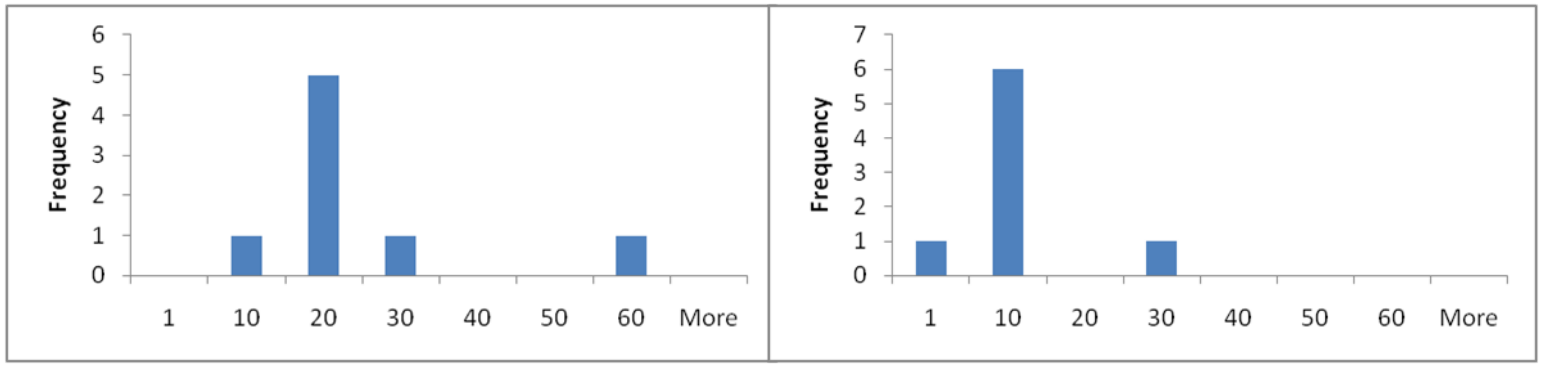


		Night	Day
4. Andes hantavirus	Geo Mean	58	16
	Median	45	10
	Low	15	10
	High	1,440	60

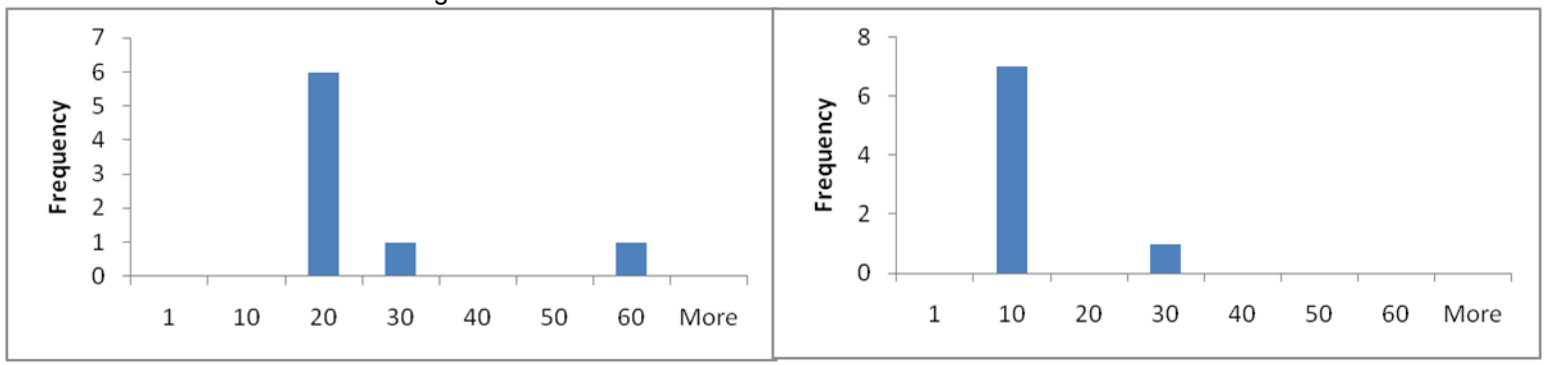




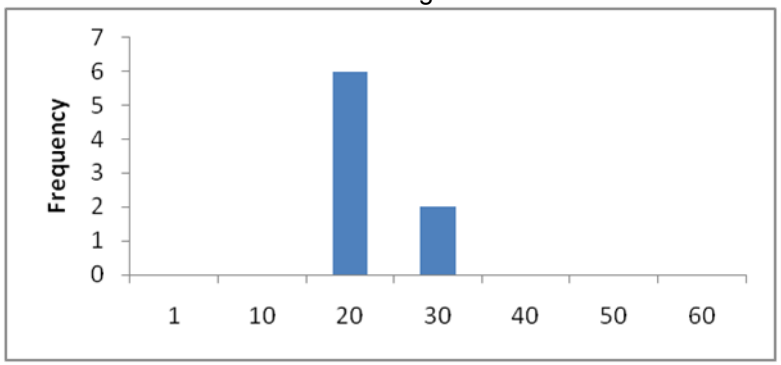
		Night	Day
5. Ebola virus	Geo		
	Mean	17	8
	Median	20	10
	Low	2	1
	High	60	30

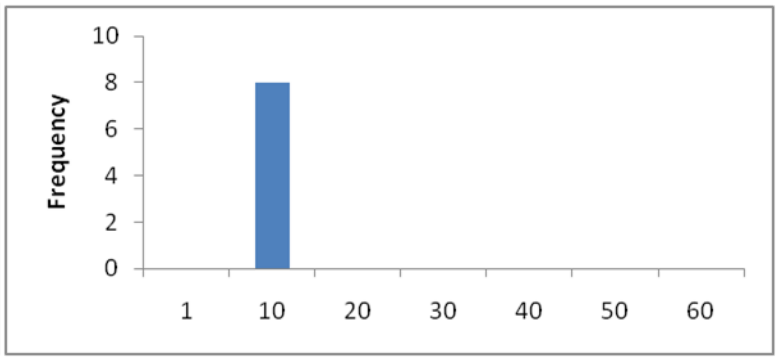


		Night	Day
6. Marburg virus	Geo		
	Mean	21	8
	Median	18	10
	Low	15	2
	High	60	30

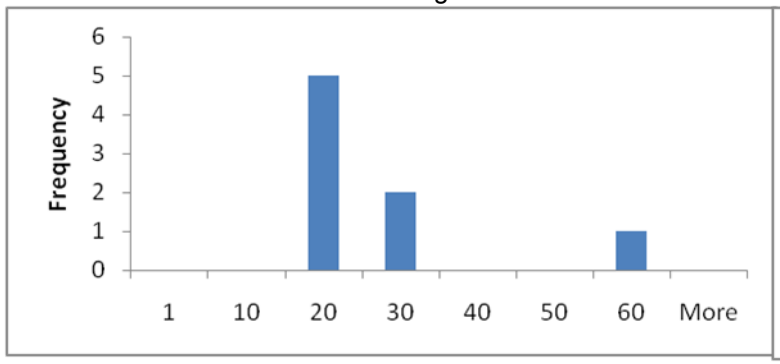


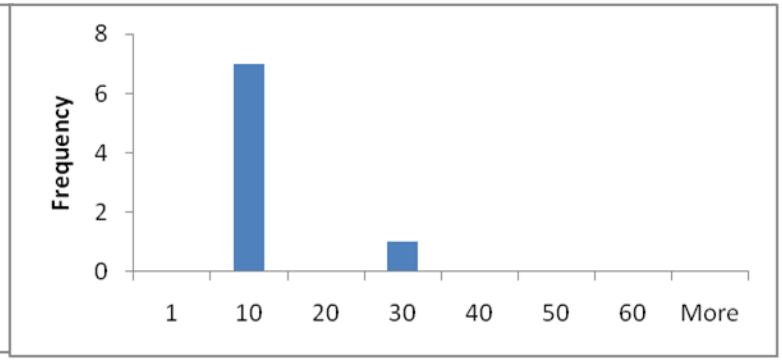
		Night	Day
7. Junin virus	Geo Mean	21	8
	Median	20	10
	Low	15	5
	High	30	10



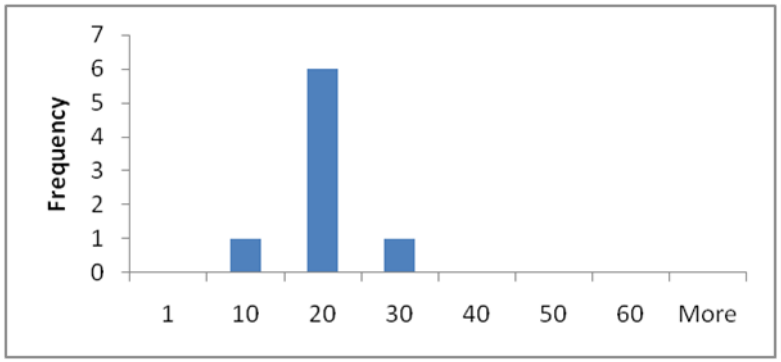


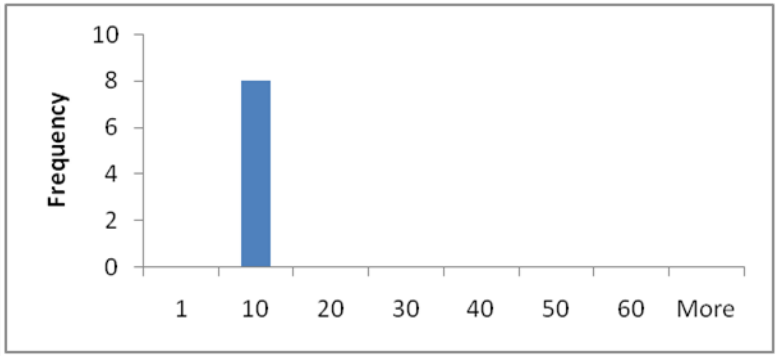
		Night	Day
8. Lassa fever virus	Geo Mean	23	10
	Median	20	10
	Low	15	5
	High	60	30



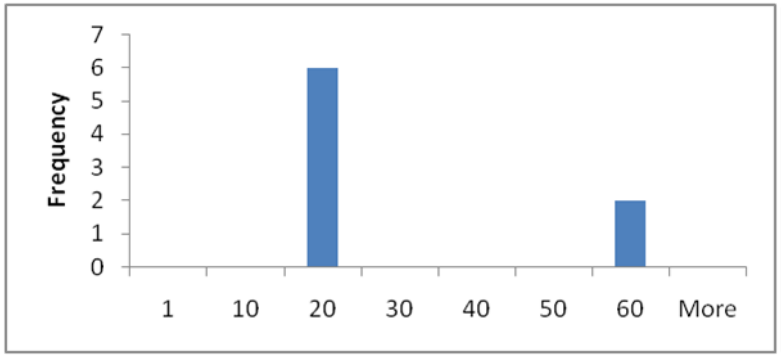


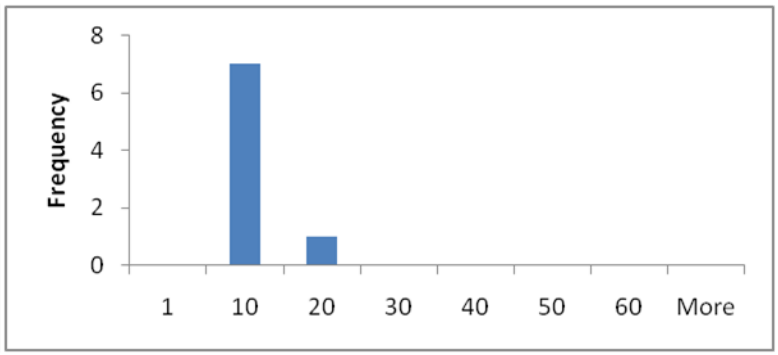
		Night	Day
9. Nipah virus	Geo Mean	16	7
	Median	20	10
	Low	5	2
	High	30	10



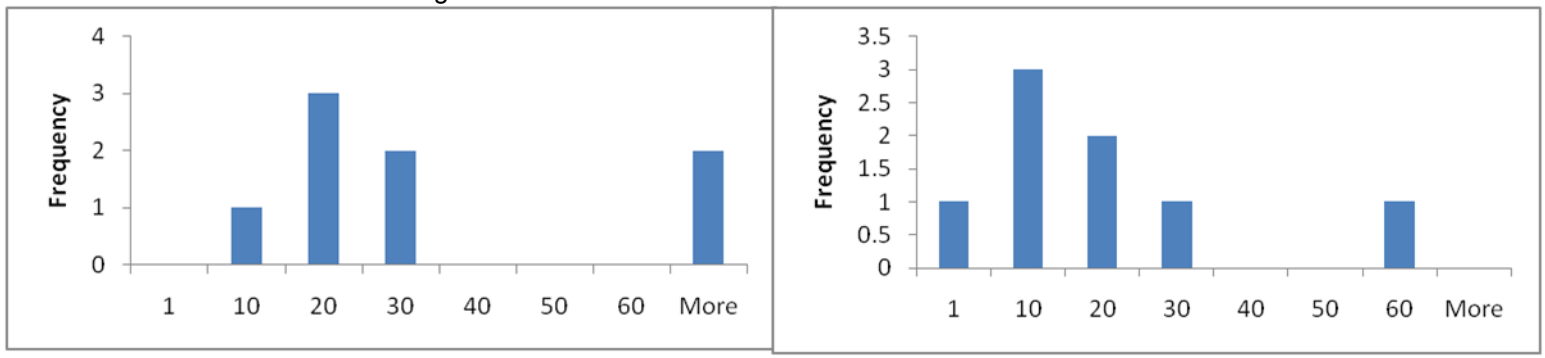


		Night	Day
10. Rift Valley fever virus	Geo Mean	25	10
	Median	20	10
	Low	15	7
	High	60	20

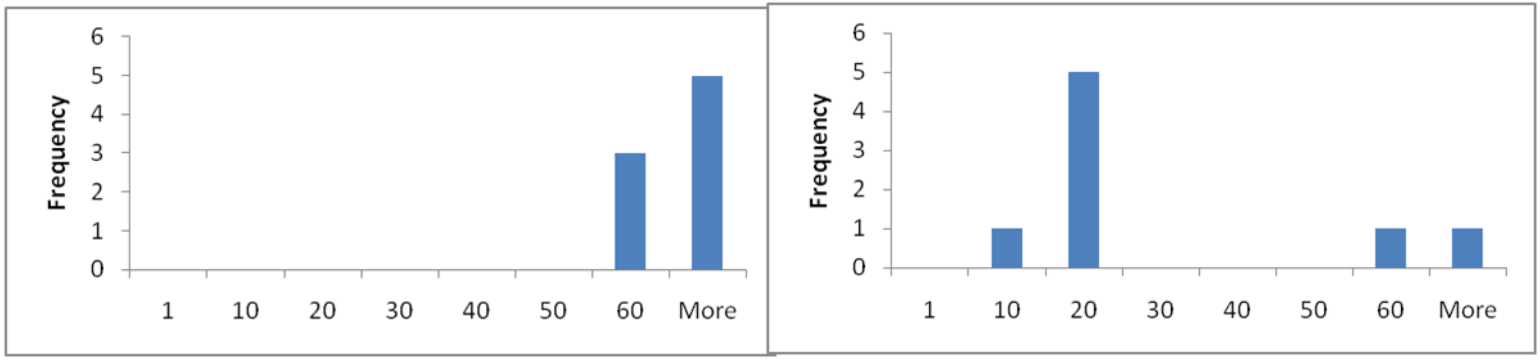




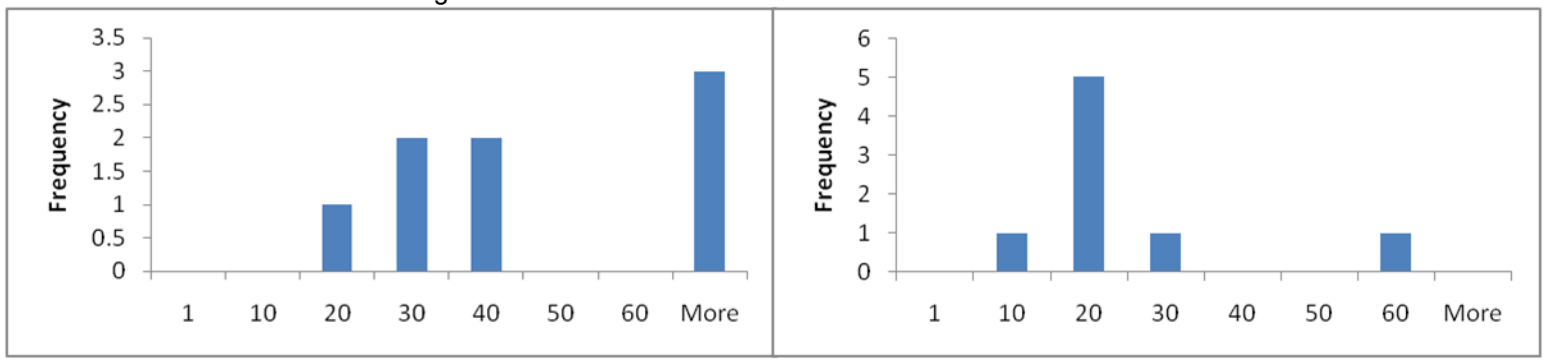
		Night	Day
11. Russian spring-summer encephalitis virus	Geo Mean	28	12
	Median	25	13
	Low	5	1
	High	120	60



		Night	Day
12. SARS virus	Geo Mean	135	35
	Median	120	20
	Low	60	10
	High	1,440	1,440



		Night	Day
13. 1918 influenza virus	Geo Mean	60	21
	Median	38	20
	Low	20	10
	High	300	60



Attachment H-6: Raw Data and Results for Round 3

Infectiousness and stability

Organism	Rater	Name	ID10	ID50	ID90	Night	Day
B. anthracis	1		1,000	5,000	50,000	Indefinite	Indefinite
	2		4,000	30,000	100,000	Indefinite	Indefinite
	3		400	10,000	200,000	Indefinite	Indefinite
	4		1,000	10,000	100,000	Indefinite	Indefinite
	5		400	10,000	100,000	Indefinite	Indefinite
	6		1,000	10,000	100,000	Indefinite	Indefinite
	7		200	8,000	10,000	Indefinite	Indefinite
	8		500	8,000	300,000	Indefinite	Indefinite
	Sum		8,500	91,000	960,000		
	Geo Mean		709	9,949	86,028		
	Median		750	10,000	100,000		
	Low		200	5,000	10,000		
	High		4,000	30,000	300,000		
F. tularensis	1		100	500	1,000	20	20
	2		2	12	41	30	3
	3		10	40	200	50	20
	4		5	10	100	45	25
	5		4	40	200	40	15
	6		4	40	400	50	20
	7		10	50	1,000	60	10
	8		20	50	200	50	25
	Sum		155	742	3,141	345	138
	Geo Mean		9	42	245	41	15
	Median		8	40	200	48	20
	Low		2	10	41	20	3
	High		100	500	1,000	60	25
Y. pestis	1		100	500	5,000	10	5
	2		700	8,000	130,000	8	2
	3		200	4,000	40,000	25	10
	4		100	1,000	1,500	30	10
	5		200	5,000	30,000	25	10
	6		400	4,000	40,000	25	10
	7		100	100,000	1,000,000	60	10
	8		250	4,000	30,000	20	15
	Sum		2,050	126,500	1,276,500	203	72
	Geo Mean		202	4,349	32,993	21	8
	Median		200	4,000	35,000	25	10
	Low		100	500	1,500	8	2
	High		700	100,000	1,000,000	60	15

Andes hantavirus	1	50	250	1,000	30	10
	2	160	1,000	3,500	60	10
	3	50	400	30,000	45	10
	4	1	10	100	30	10
	5	60	200	5,000	60	15
	6	40	400	4,000	50	18
	7	100	1,000	30,000	30	10
	8	100	1,000	10,000	60	30
	Sum	561	4,260	83,600	365	113
	Geo Mean	42	308	3,980	44	13
	Median	55	400	4,500	48	10
	Low	1	10	100	30	10
	High	160	1,000	30,000	60	30

Ebola virus	1	100	1,000	10,000	60	30
	2	19	120	400	2	1
	3	30	200	3,000	20	10
	4	100	1,000	10,000	15	5
	5	40	200	3,000	20	10
	6	30	300	3,000	10	10
	7	100	500	2,000	20	5
	8	50	200	2,000	20	10
	Sum	469	3,520	33,400	167	81
	Geo Mean	49	331	2,847	15	7
	Median	45	250	3,000	20	10
	Low	19	120	400	2	1
	High	100	1,000	10,000	60	30
Marburg virus	1	100	1,000	10,000	60	30
	2	19	120	400	15	2
	3	20	100	2,000	20	10
	4	100	1,000	10,000	15	5
	5	20	200	2,000	20	10
	6	10	100	1,000	10	10
	7	10	500	9,000	30	10
	8	20	150	2,000	15	10
	Sum	299	3,170	36,400	185	87
	Geo Mean	25	255	2,707	20	9
	Median	20	175	2,000	18	10
	Low	10	100	400	10	2
	High	100	1,000	10,000	60	30

Junin virus	1	50	400	1,000	20	10
	2	2	15	49	20	5
	3	10	50	1,000	20	10
	4	100	1,000	10,000	20	10
	5	20	40	1,000	20	10
	6	5	50	500	10	10
	7	30	100	5,000	30	10
	8	20	40	1,000	15	10
	Sum	237	1,695	19,549	155	75
	Geo Mean	17	84	1,026	19	9
	Median	20	50	1,000	20	10
	Low	2	15	49	10	5
	High	100	1,000	10,000	30	10
Lassa fever virus	1	50	300	1,000	60	30
	2	3	18	59	20	5
	3	10	50	1,000	23	10
	4	10	100	1,000	20	10
	5	20	80	1,000	20	10
	6	4	40	400	25	20
	7	300	30,000	3,000,000	30	10
	8	20	40	1,500	15	10
	Sum	417	30,628	3,005,959	213	105
	Geo Mean	17	134	1,792	24	11
	Median	15	65	1,000	22	10
	Low	3	18	59	15	5
	High	300	30,000	3,000,000	60	30

Nipah virus	1	1,000	5,000	50,000	20	5
	2	75	500	1,650	5	2
	3	100	1,000	10,000	20	10
	4	100	1,000	10,000	20	10
	5	100	1,000	10,000	15	10
	6	100	1,000	10,000	10	5
	7	1,000	30,000	500,000	20	5
	8	150	1,000	10,000	15	5
	Sum	2,625	40,500	601,650	125	52
	Geo Mean	180	1,715	15,919	14	6
	Median	100	1,000	10,000	18	5
	Low	75	500	1,650	5	2
	High	1,000	30,000	500,000	20	10
10. Rift Valley fever virus	1	1,000	5,000	50,000	20	20
	2	15	99	329	20	10
	3	100	1,500	10,000	20	10
	4	100	1,000	10,000	60	20
	5	100	1,000	10,000	25	10
	6	100	1,000	10,000	25	15
	7	10	40	300	50	10
	8	100	1,500	10,000	20	10
	Sum	1,525	11,139	100,629	240	105
	Geo Mean	79	678	5,148	27	13
	Median	100	1,000	10,000	23	10
	Low	10	40	300	20	10
	High	1,000	5,000	50,000	60	20

11. Russian spring-summer encephalitis virus	1	1,000	5,000	50,000	120	60
	2	11	71	230	100	30
	3	30	100	3,000	25	15
	4	100	1,000	10,000	20	10
	5	20	200	2,000	30	12
	6	20	200	2,000	25	10
	7	100	10,000	1,000,000	30	10
	8	20	100	2,000	20	10
	Sum	1,301	16,671	1,069,230	370	157
	Geo Mean	48	441	6,384	36	15
	Median	25	200	2,500	28	11
	Low	11	71	230	20	10
	High	1,000	10,000	1,000,000	120	60
SARS virus	1	100	1,000	10,000	200	60
	2	43	283	950	120	20
	3	100	5,000	40,000	120	20
	4	100	1,000	10,000	60	20
	5	100	8,000	40,000	140	40
	6	400	4,000	40,000	130	30
	7	1,000	3,000	300,000	60	10
	8	200	4,000	80,000	180	30
	Sum	2,043	26,283	520,950	1,010	230
	Geo Mean	156	2,197	24,861	117	25
	Median	100	3,500	40,000	125	25
	Low	43	283	950	60	10
	High	1,000	8,000	300,000	200	60

1918 influenza virus	1	50,000	250,000	1,000,000	120	20
	2	10	300	1,000,000	300	30
	3	40	1,000	20,000	40	20
	4	100	1,000	10,000	30	20
	5	400	8,000	20,000	60	20
	6	70	700	7,000	50	30
	7	10	100	3,000	30	5
	8	10	600	10,000	120	30
	Sum	50,640	261,700	2,070,000	750	175
	Geo Mean	93	1,497	30,941	68	20
	Median	55	850	15,000	55	20
	Low	10	100	3,000	30	5
	High	50,000	250,000	1,000,000	300	30

Agreement with Ro (0=disagree, 1= agree)	
	Ro
Pestis	1
	1
	1
	1
	1
	1
	0
	1
Total	7
Ebola	1
	1
	1
	1
	1
	1
	0
	1
Total	7
Rift	1
	1
	1
	1
	1
	1
	0
	1
Total	7
SARS	1
	1
	1
	1
	1
	1
	1
	1
Total	8
flu	0
	1
	1
	1
	1

	1
	1
Total	7

Vulnerability

Rater	young	Bacteria	Disease	Mortality
1			0.20	0.20
2			0.33	0.20
3			0.20	0.10
4			0.05	0.05
5			0.15	0.10
6			0.15	0.05
7			0.10	0.20
8			0.20	0.08
		Sum	1.38	0.98
		Geo Mean	0.15	0.11
		Median	0.18	0.10
		Low	0.05	0.05
		High	0.33	0.20

Rater	young	virus	Disease	Mortality
1			0.20	0.20
2			0.33	0.20
3			0.25	0.10
4			0.05	0.05
5			0.25	0.10
6			0.10	0.10
7			0.00	0.20
8			0.20	0.10
		Sum	1.38	1.05
		Geo Mean	0.09	0.12
		Median	0.20	0.10
		Low	0.00	0.05
		High	0.33	0.20

Rater	old	Bacteria	Disease	Mortality
1			0.30	0.30
2			0.50	0.50
3			0.30	0.10
4			0.05	0.05
5			0.25	0.10
6			0.20	0.10
7			0.30	0.20
8			0.25	0.10
		Sum	2.15	1.45
		Geo Mean	0.23	0.14
		Median	0.28	0.10
		Low	0.05	0.05
		High	0.50	0.50

Rater	old	Virus	Disease	Mortality
1			0.20	0.20
2			0.50	0.50
3			0.25	0.15
4			0.05	0.05
5			0.20	0.15
6			0.20	0.10
7			0.30	0.20
8			0.25	0.15
		Sum	1.95	1.50
		Geo Mean	0.21	0.16
		Median	0.23	0.15
		Low	0.05	0.05
		High	0.50	0.50

Rater	Diabetes	Bacteria	Disease	Mortality
1			0.10	0.05
2			0.25	0.25
3			0.20	0.10
4			0.05	0.05
5			0.20	0.10
6			0.15	0.10
7			0.30	0.20
8			0.20	0.10
		Sum	1.45	0.95
		Geo Mean	0.16	0.10
		Median	0.20	0.10
		Low	0.05	0.05
		High	0.30	0.25

Rater	Diabetes	Virus	Disease	Mortality
1			0.10	0.05
2			0.25	0.25
3			0.18	0.12
4			0.05	0.05
5			0.12	0.10
6			0.10	0.10
7			0.20	0.10
8			0.20	0.10
		Sum	1.20	0.87
		Geo Mean	0.13	0.10
		Median	0.15	0.10
		Low	0.05	0.05
		High	0.25	0.25

Rater	HIV	Bacteria	Disease	Mortality
1			0.40	0.20
2			0.30	0.30
3			0.10	0.25
4			0.30	0.25
5			0.30	0.25
6			0.30	0.25
7			0.20	0.10
8			0.30	0.20
		Sum	2.20	1.80
		Geo Mean	0.26	0.22
		Median	0.30	0.25
		Low	0.10	0.10
		High	0.40	0.30

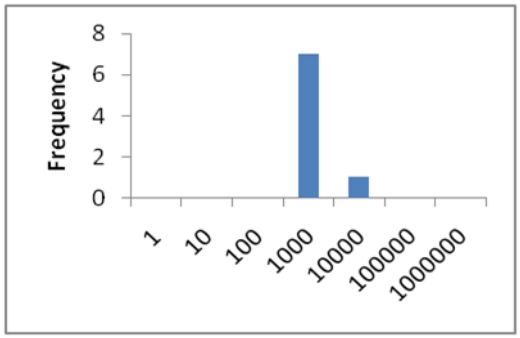
Rater	HIV	Virus	Disease	Mortality
1			0.40	0.20
2			0.30	0.30
3			0.35	0.30
4			0.10	0.10
5			0.30	0.25
6			0.30	0.25
7			0.30	0.20
8			0.30	0.25
		Sum	2.35	1.85
		Geo Mean	0.28	0.22
		Median	0.30	0.25
		Low	0.10	0.10
		High	0.40	0.30

Rater	Pregnancy	Bacteria	Disease	Mortality
1			0.10	0.10
2			0.30	0.20
3			0.00	0.05
4			0.02	0.01
5			0.05	0.05
6			0.04	0.02
7			0.10	0.10
8			0.05	0.05
		Sum	0.66	0.58
		Geo Mean	0.03	0.05
		Median	0.05	0.05
		Low	0.00	0.01
		High	0.30	0.20

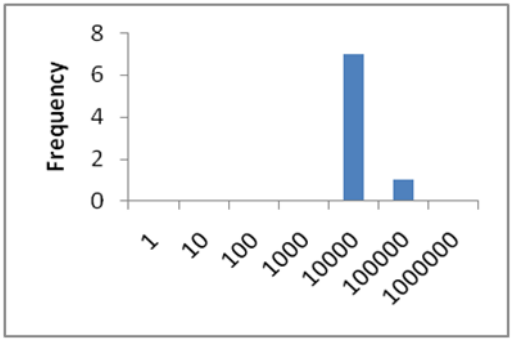
Rater	Pregnancy	Virus	Disease	Mortality
1			0.10	0.10
2			0.50	0.20
3			0.05	0.03
4			0.02	0.01
5			0.05	0.02
6			0.05	0.03
7			0.20	0.10
8			0.05	0.05
		Sum	1.02	0.54
		Geo Mean	0.08	0.05
		Median	0.05	0.04
		Low	0.02	0.01
		High	0.50	0.20

Round 3 Results
Infectivity

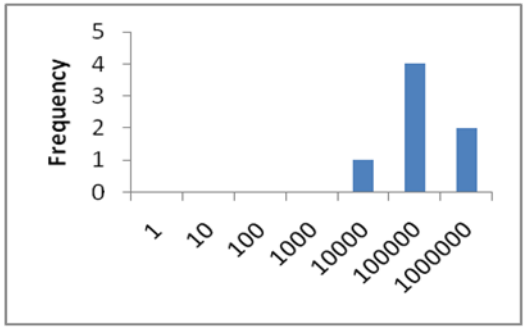
Organism		ID10	ID50	ID90
B. anthracis	Geo Mean	709	9,949	86,028
	Median	750	10,000	100,000
	Low	200	5,000	10,000
	High	4,000	30,000	300,000



ID10

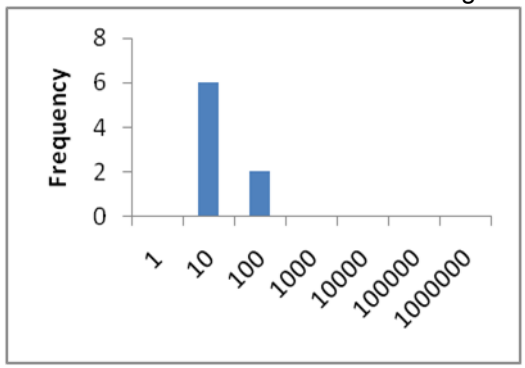


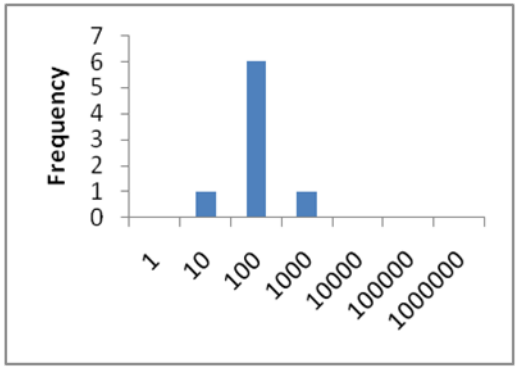
ID50

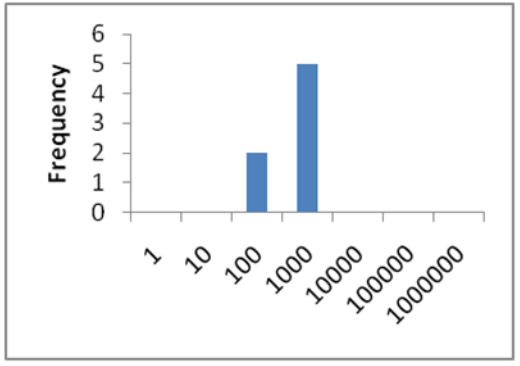


ID90

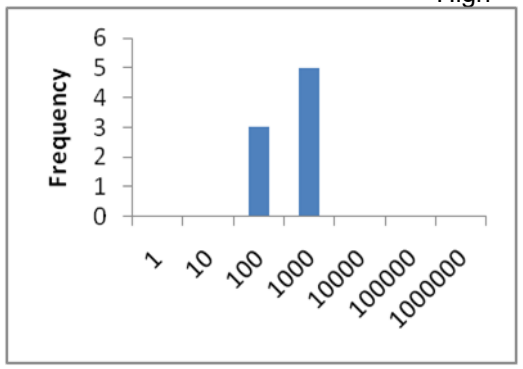
Organism		ID10	ID50	ID90
F. tularensis	Geo Mean	9	42	245
	Median	8	40	200
	Low	2	10	41
	High	100	500	1,000

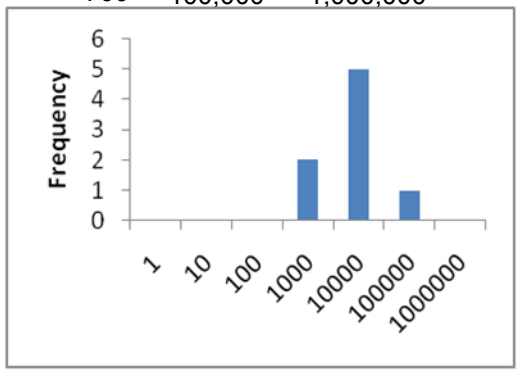


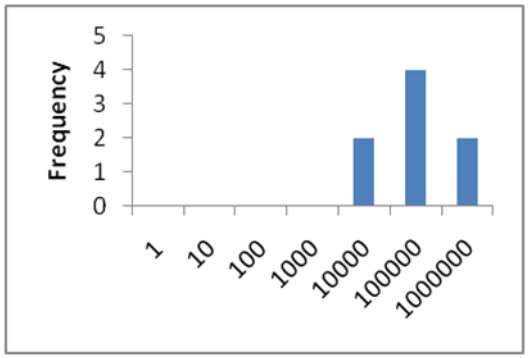




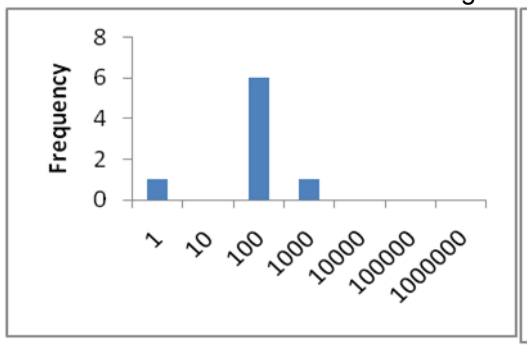
Organism		ID10	ID50	ID90
	Geo			
Y. pestis	Mean	202	4,349	32,993
	Median	200	4,000	35,000
	Low	100	500	1,500
	High	700	100,000	1,000,000

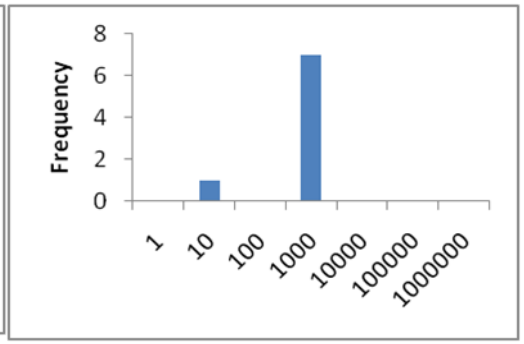


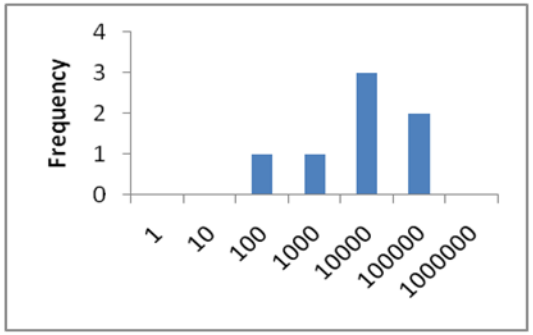




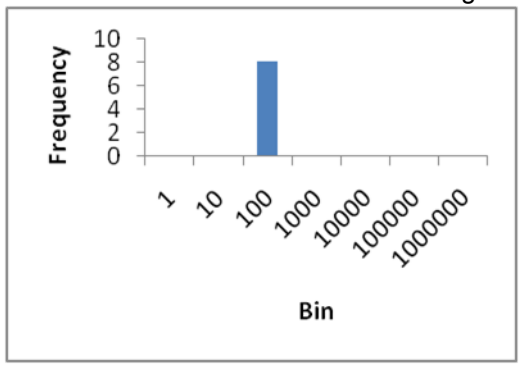
Organism		ID10	ID50	ID90
	Geo			
Andes hantavirus	Mean	42	308	3,980
	Median	55	400	4,500
	Low	1	10	100
	High	160	1,000	30,000

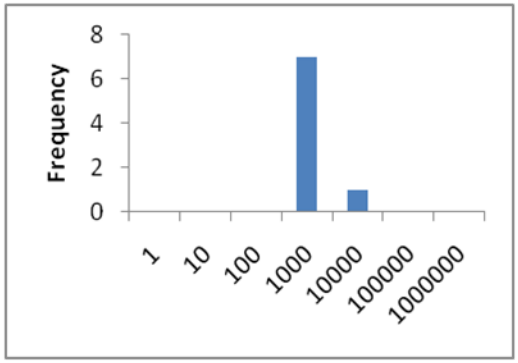


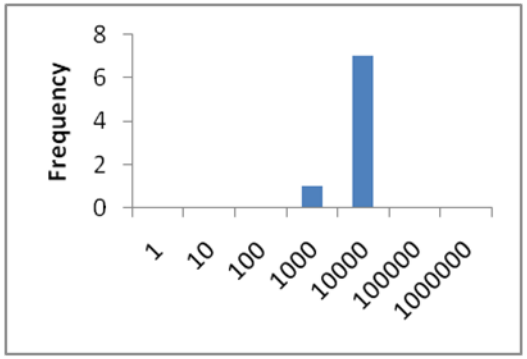




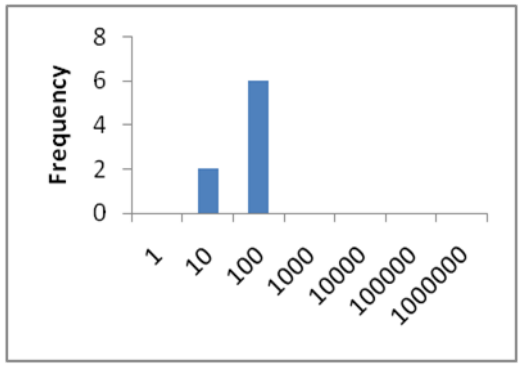
Organism		ID10	ID50	ID90
	Geo			
Ebola virus	Mean	49	331	2,847
	Median	45	250	3,000
	Low	19	120	400
	High	100	1,000	10,000

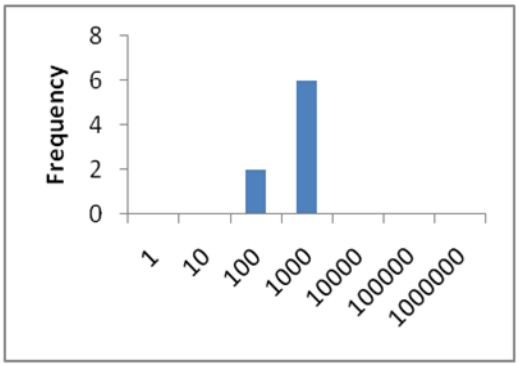


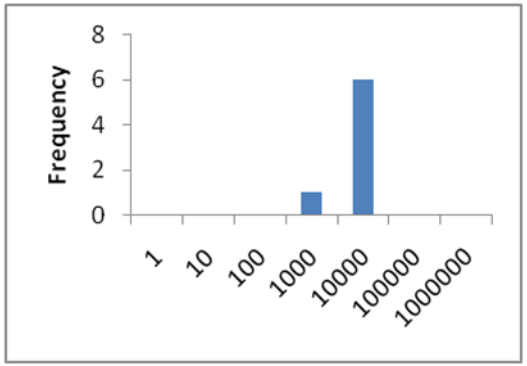




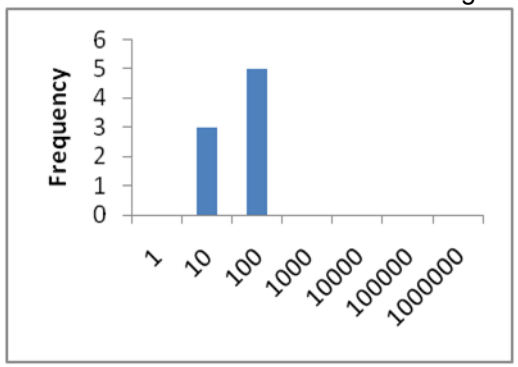
Organism		ID10	ID50	ID90
	Geo			
Marburg virus	Mean	25	255	2,707
	Median	20	175	2,000
	Low	10	100	400
	High	100	1,000	10,000

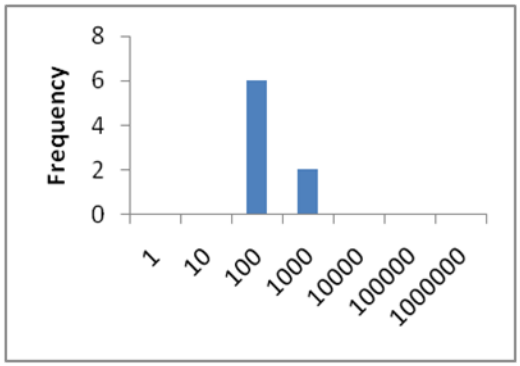


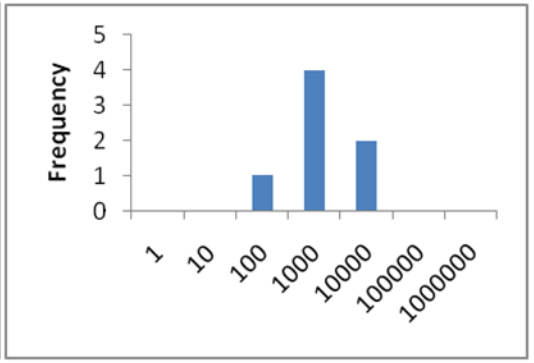




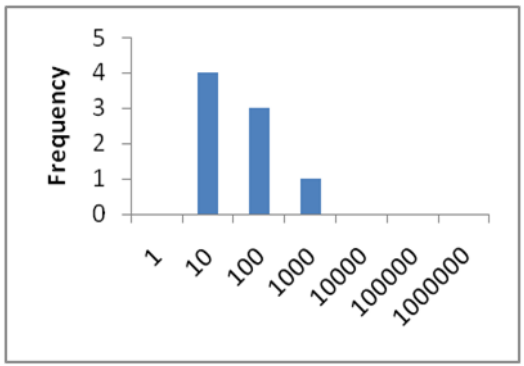
Organism		ID10	ID50	ID90
Junin virus	Geo Mean	17	84	1,026
	Median	20	50	1,000
	Low	2	15	49
	High	100	1,000	10,000

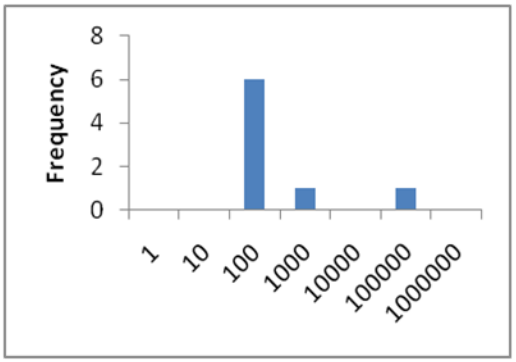


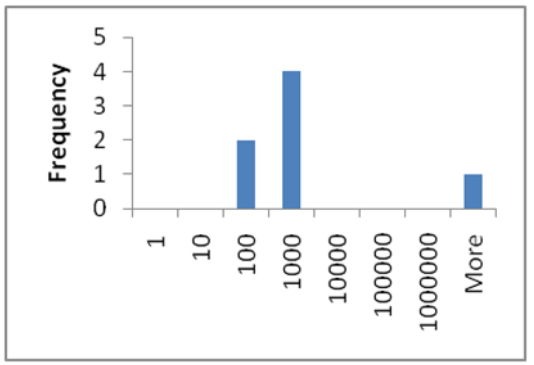




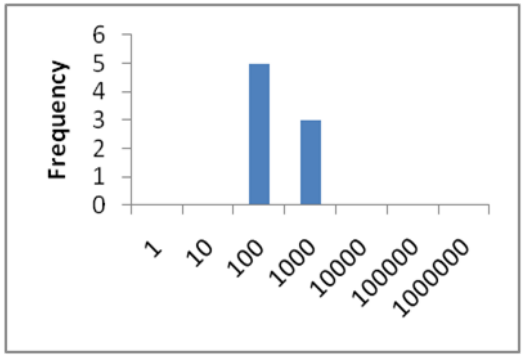
Organism		ID10	ID50	ID90
Lassa fever virus	Geo Mean	17	134	1,792
	Median	15	65	1,000
	Low	3	18	59
	High	300	30,000	3,000,000

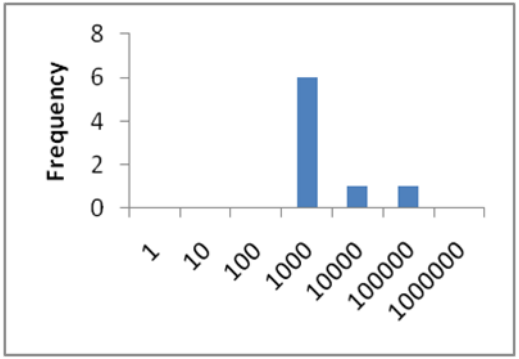


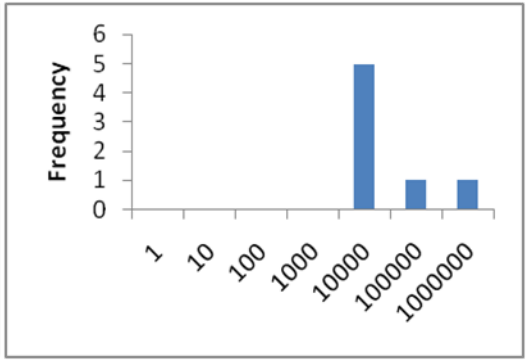




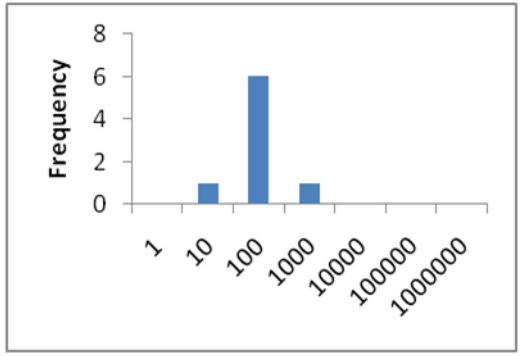
Organism		ID10	ID50	ID90
Nipah virus	Geo Mean	180	1,715	15,919
	Median	100	1,000	10,000
	Low	75	500	1,650
	High	1,000	30,000	500,000

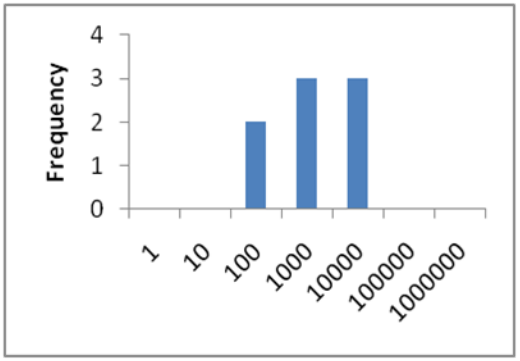


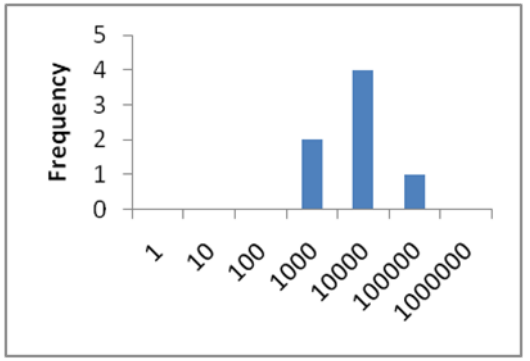




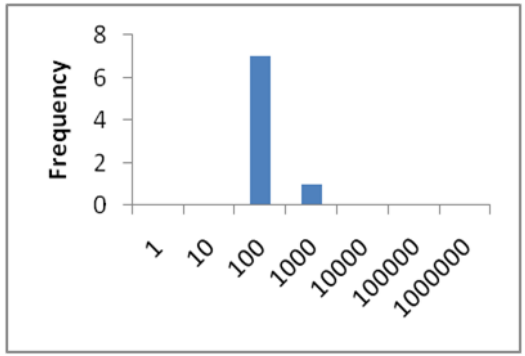
Organism		ID10	ID50	ID90
Rift Valley fever virus	Geo Mean	79	678	5,148
	Median	100	1,000	10,000
	Low	10	40	300
	High	1,000	5,000	50,000

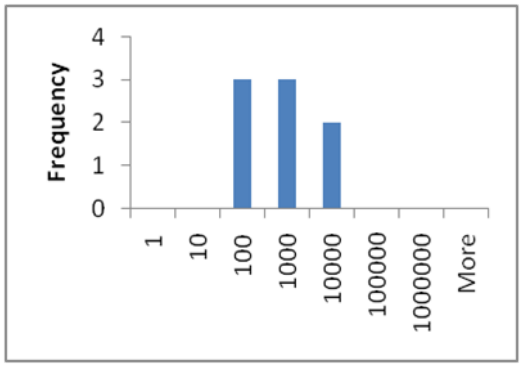


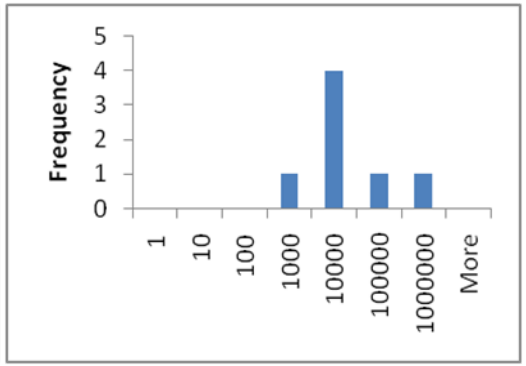




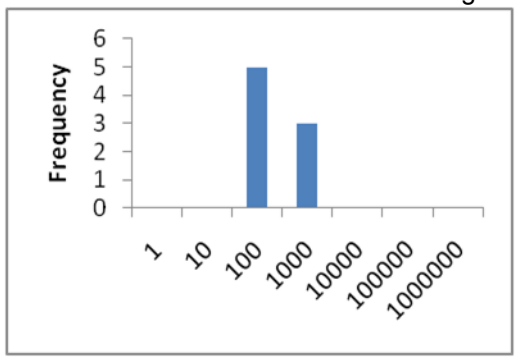
Organism		ID10	ID50	ID90
Russian spring-summer encephalitis virus	Geo Mean	48	441	6,384
	Median	25	200	2,500
	Low	11	71	230
	High	1,000	10,000	1,000,000

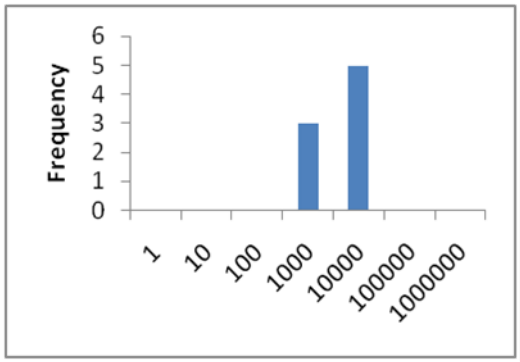


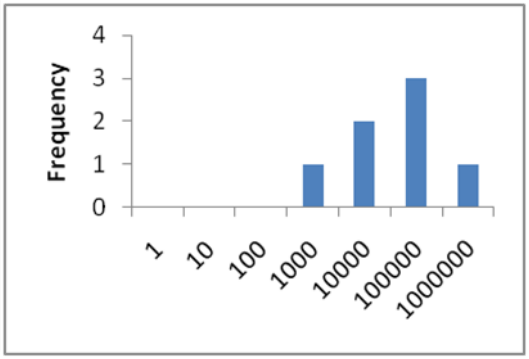




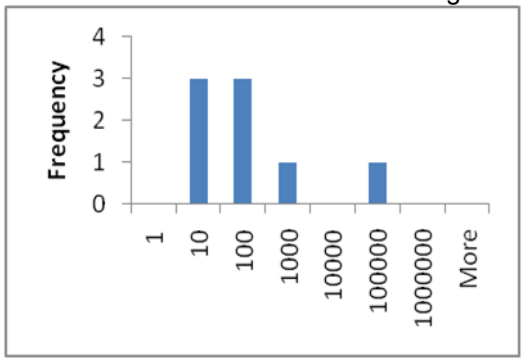
Organism		ID10	ID50	ID90
SARS virus	Geo Mean	156	2,197	24,861
	Median	100	3,500	40,000
	Low	43	283	950
	High	1,000	8,000	300,000

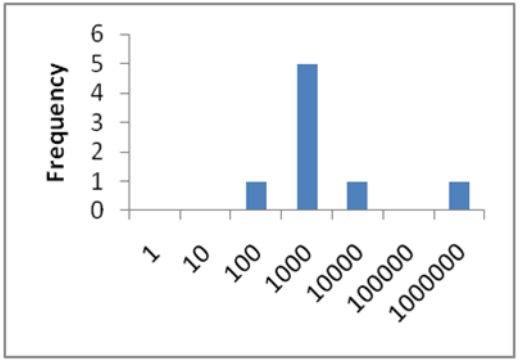


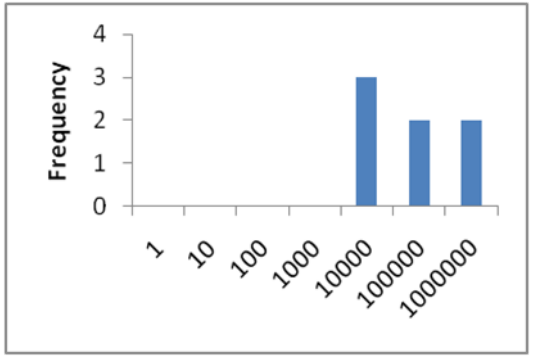




Organism		ID10	ID50	ID90
1918 influenza virus	Geo Mean	93	1,497	30,941
	Median	55	850	15,000
	Low	10	100	3,000
	High	50,000	250,000	1,000,000

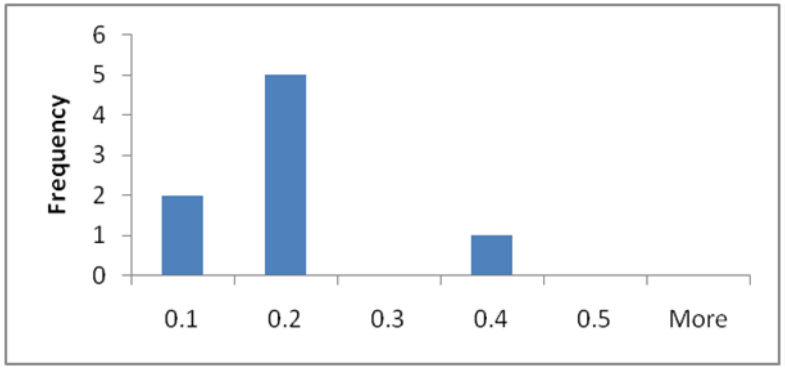




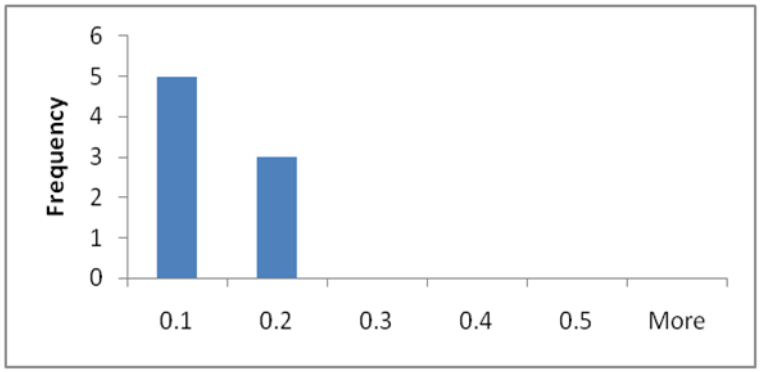


Vulnerability

	Young	Bacterial	Disease	Mortality
Geo Mean			15%	11%
Median			18%	10%
Low			5%	5%
High			33%	20%

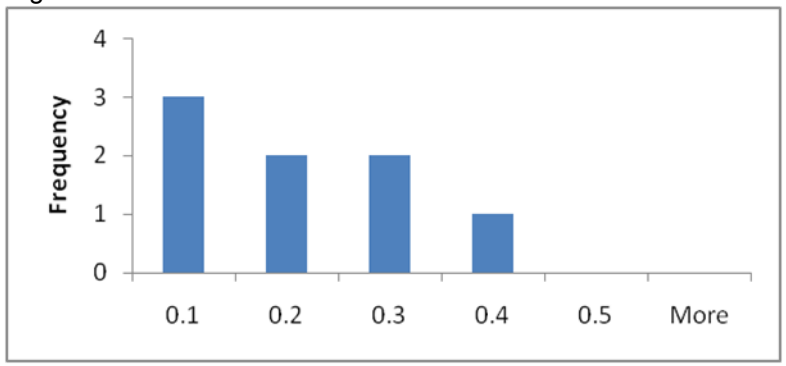


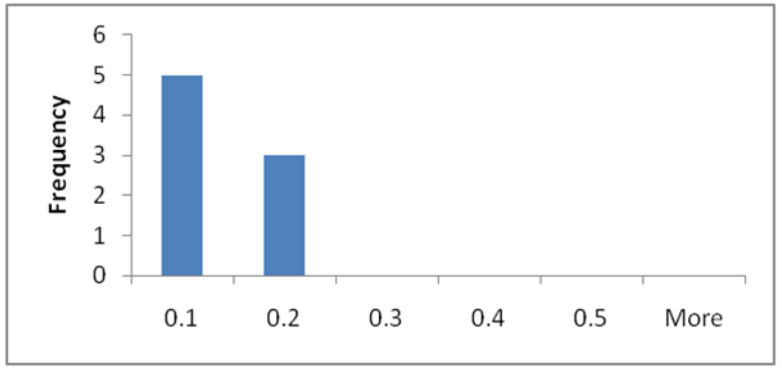
Disease



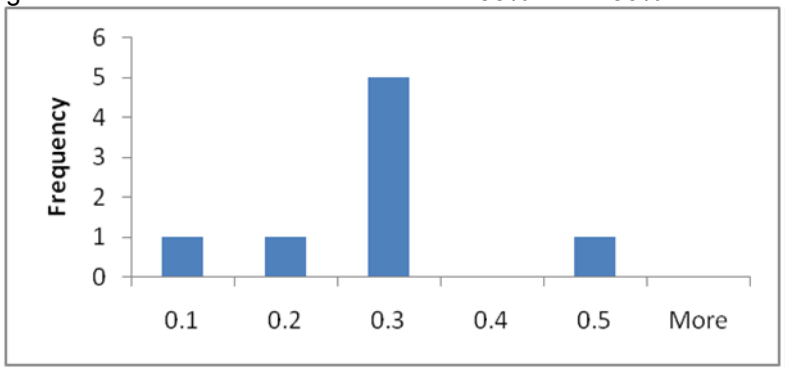
Mortality

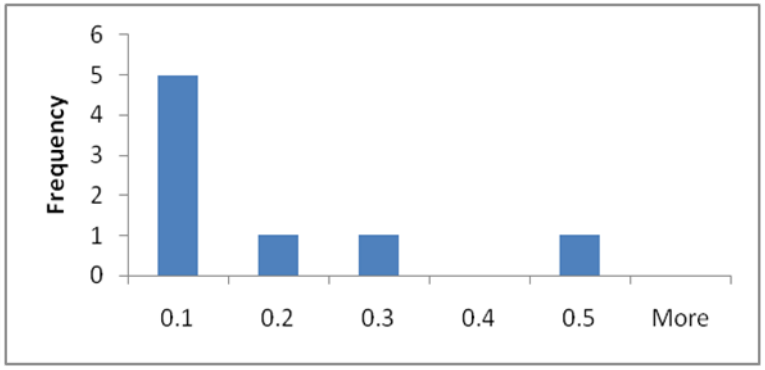
	Young	Viral	Disease	Mortality
Geo Mean			9%	12%
Median			20%	10%
Low			0%	5%
High			33%	20%



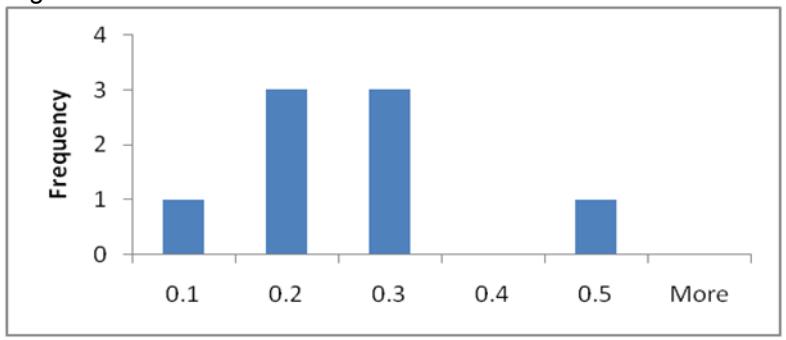


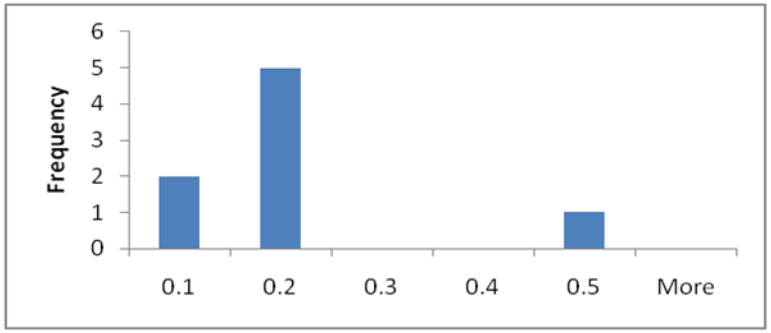
	Old	Bacterial	Disease	Mortality
Geo Mean			23%	14%
Median			28%	10%
Low			5%	5%
High			50%	50%



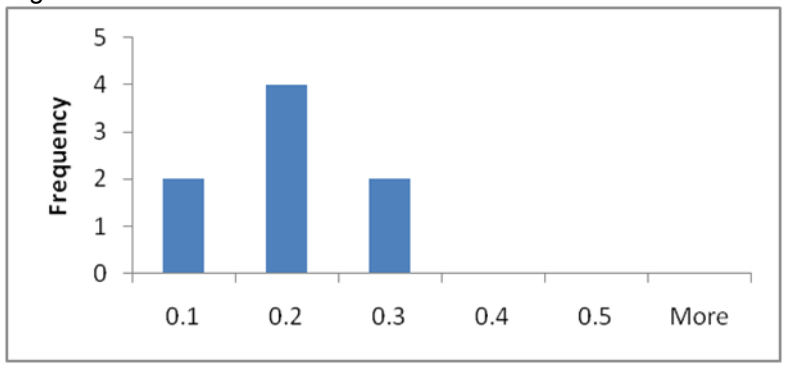


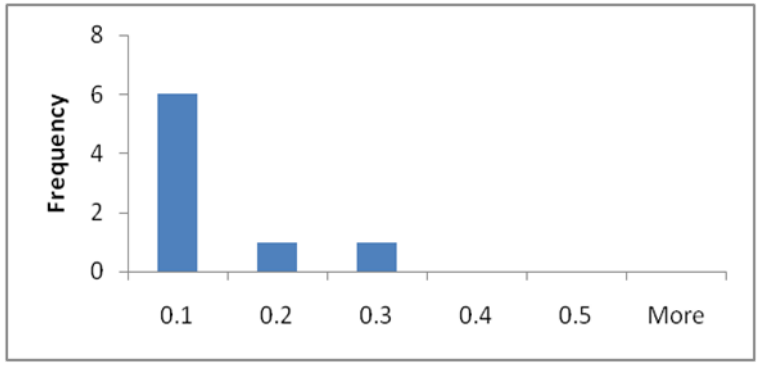
	Old	Viral	Disease	Mortality
Geo Mean			21%	16%
Median			23%	15%
Low			5%	5%
High			50%	50%



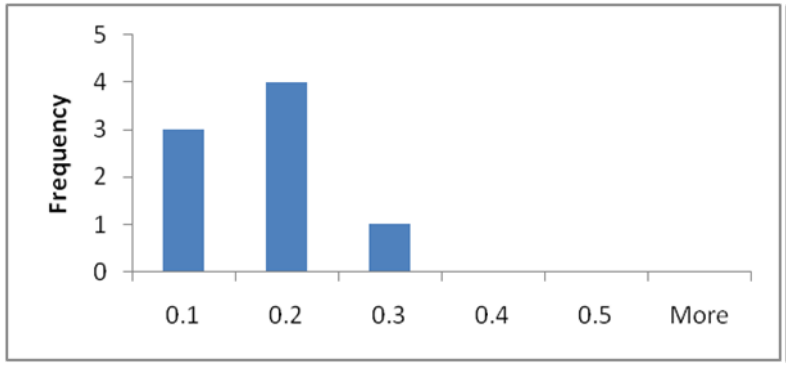


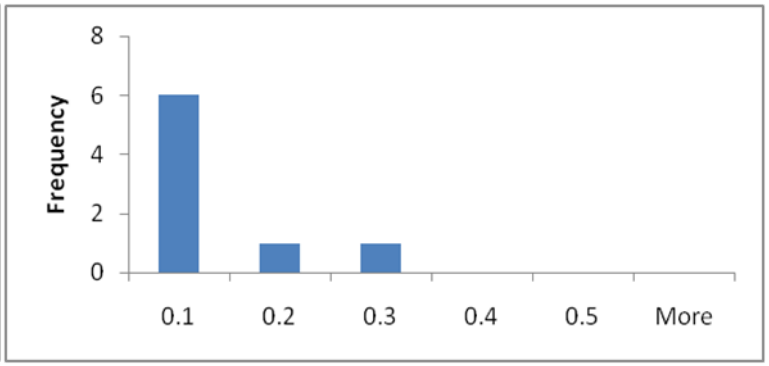
	Diabetes	Bacterial	Disease	Mortality
Geo Mean			16%	10%
Median			20%	10%
Low			5%	5%
High			30%	25%



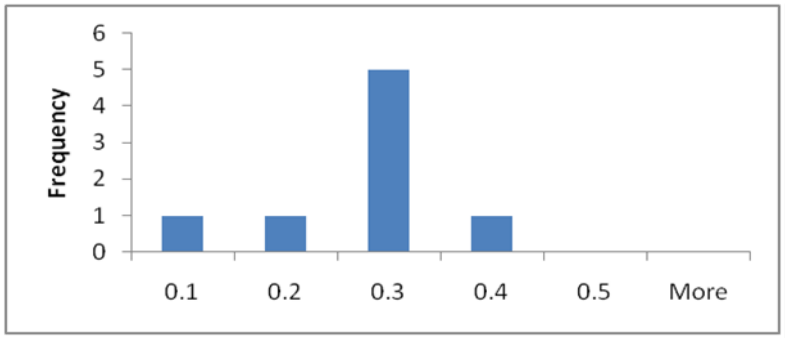


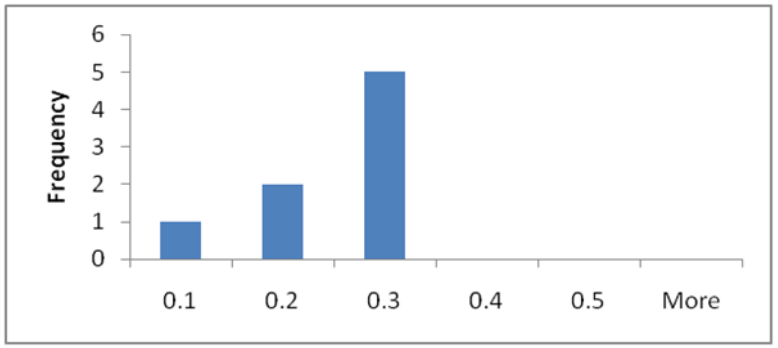
	Diabetes	Viral	Disease	Mortality
Geo Mean			13%	10%
Median			15%	10%
Low			5%	5%
High			25%	25%



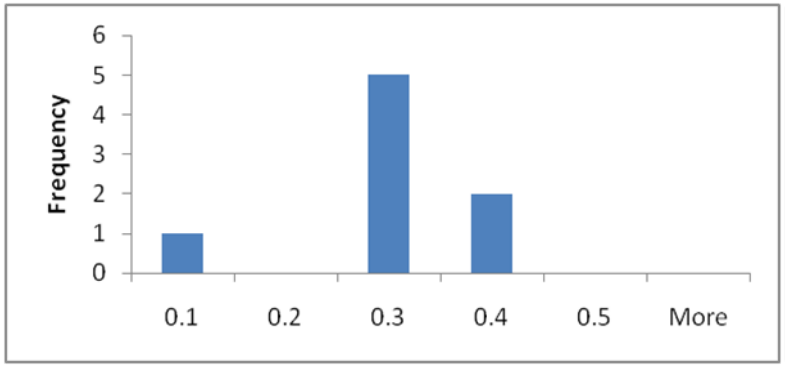


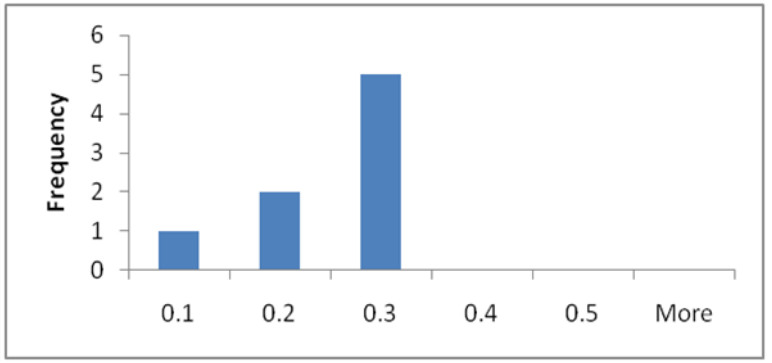
	HIV	Bacterial	Disease	Mortality
Geo Mean			26%	22%
Median			30%	25%
Low			10%	10%
High			40%	30%



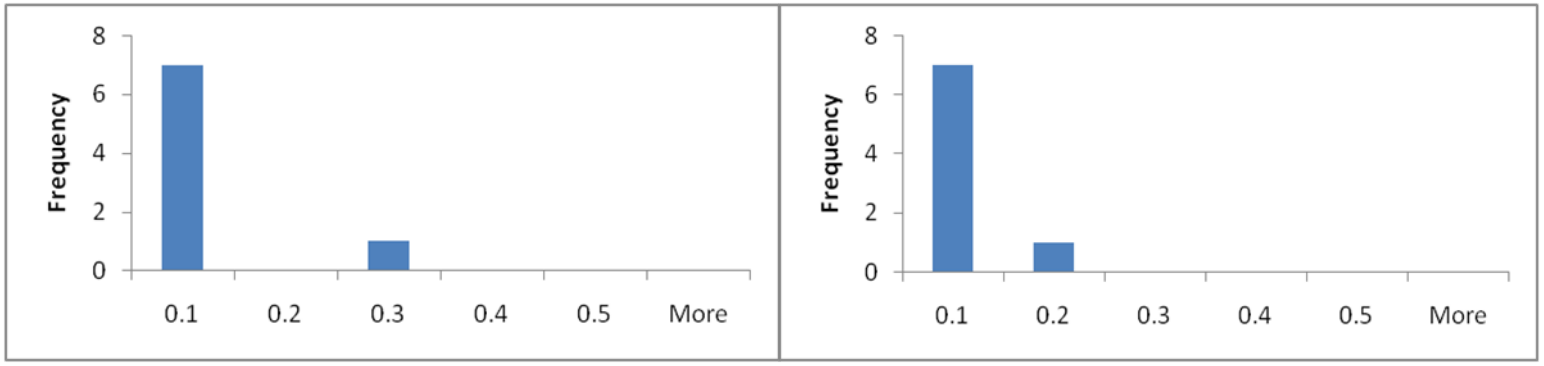


	HIV	Viral	Disease	Mortality
Geo Mean			28%	22%
Median			30%	25%
Low			10%	10%
High			40%	30%

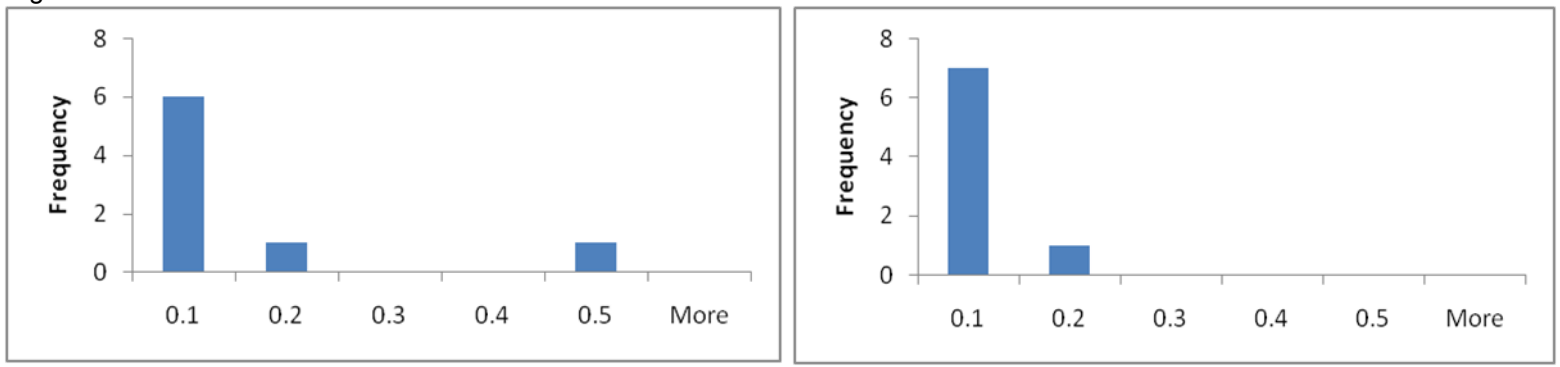




	Pregnancy	Bacterial	Disease	Mortality
Geo Mean			3%	5%
Median			5%	5%
Low			0%	1%
High			30%	20%

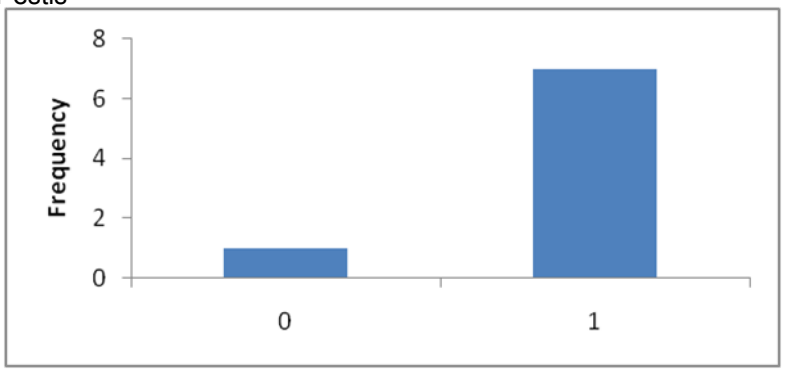


	Pregnancy	Viral	Disease	Mortality
Geo Mean			8%	5%
Median			5%	4%
Low			2%	1%
High			50%	20%

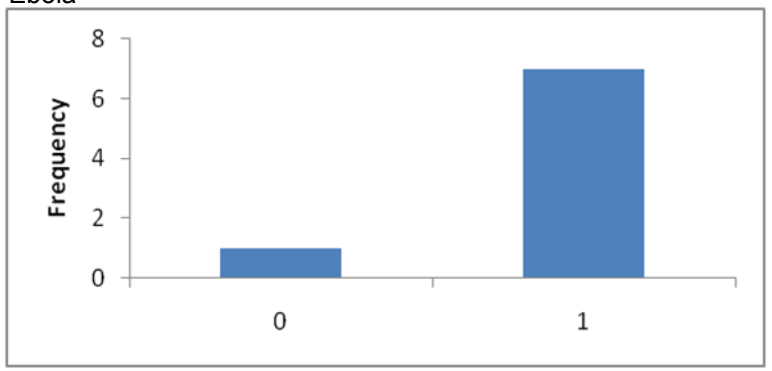


***Ro* (0= disagree, 1=agree)**

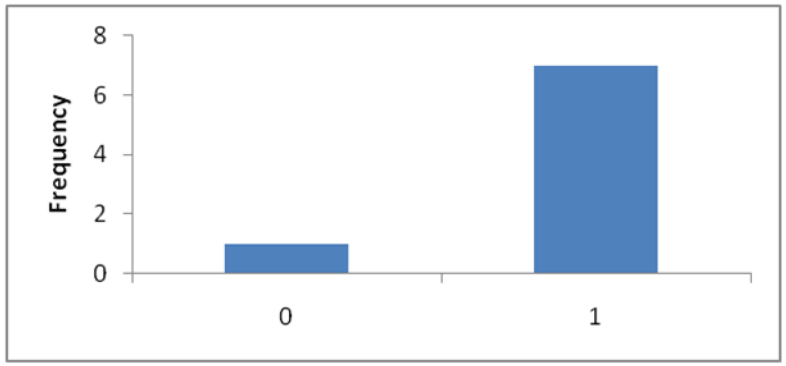
Pestis



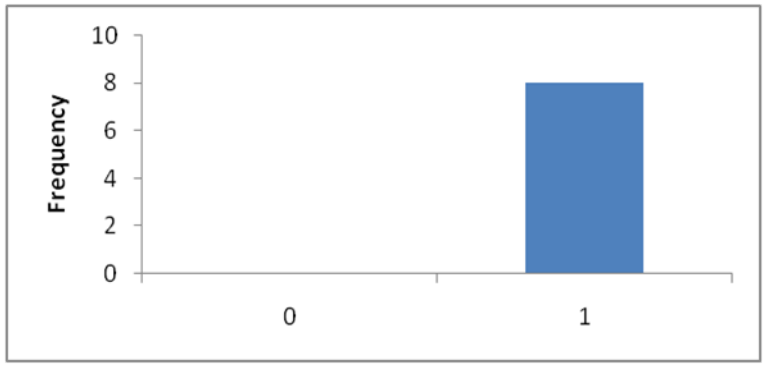
Ebola



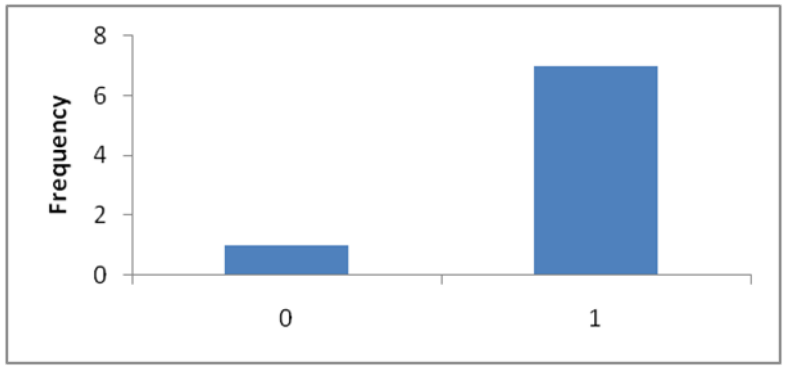
Rift



SARS



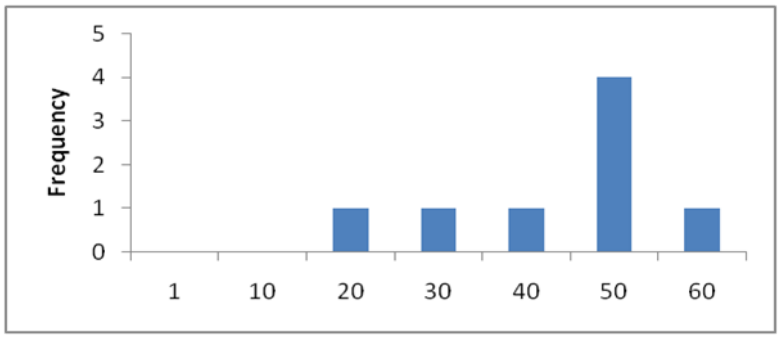
1918 Influenza



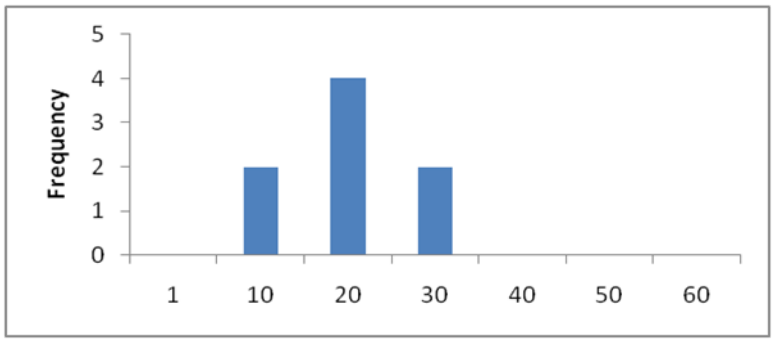
Stability

		Stability	
		Night	Day
B. anthracis	Geo Mean	NA	NA
	Median	NA	NA
	Low		
	High		

		Night	Day
F. tularensis	Geo Mean	41	15
	Median	48	20
	Low	20	3
	High	60	25

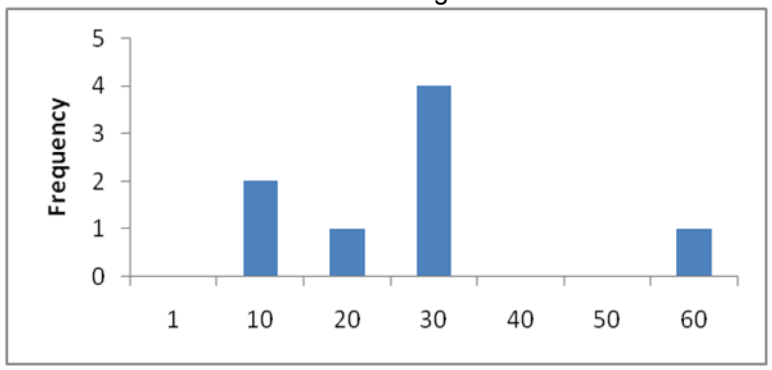


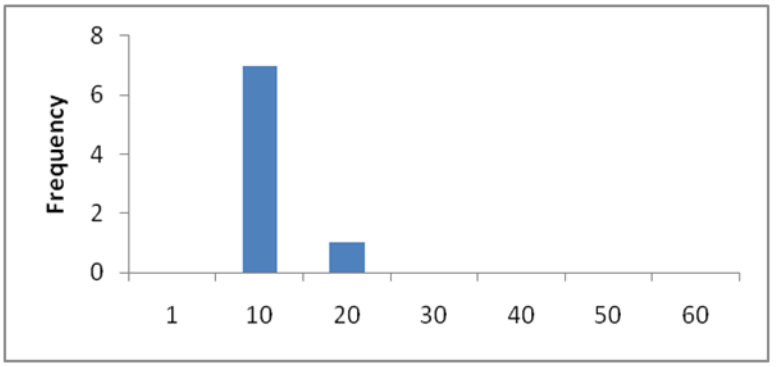
Night



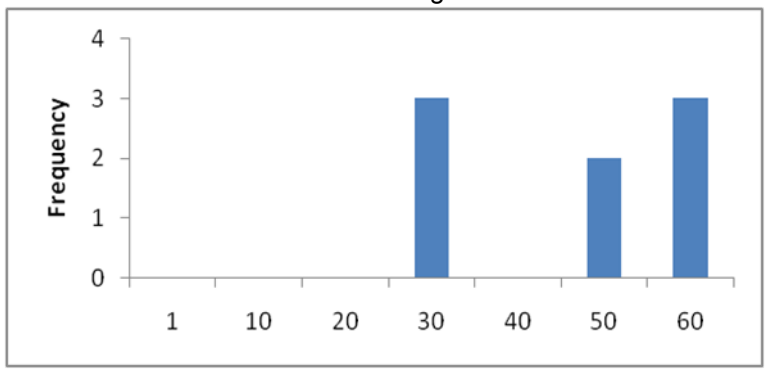
Day

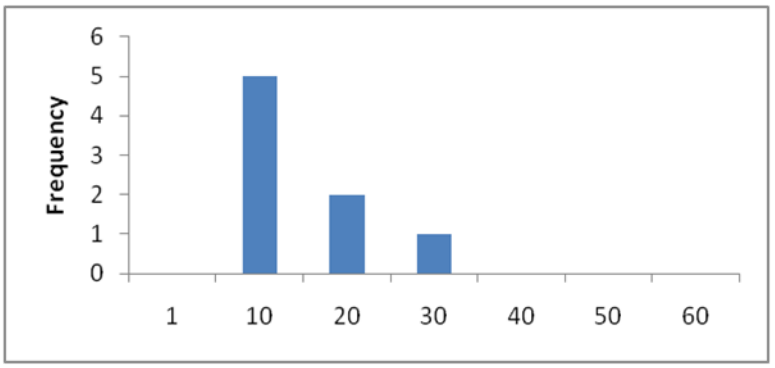
		Night	Day
Y. pestis	Geo Mean	21	8
	Median	25	10
	Low	8	2
	High	60	15



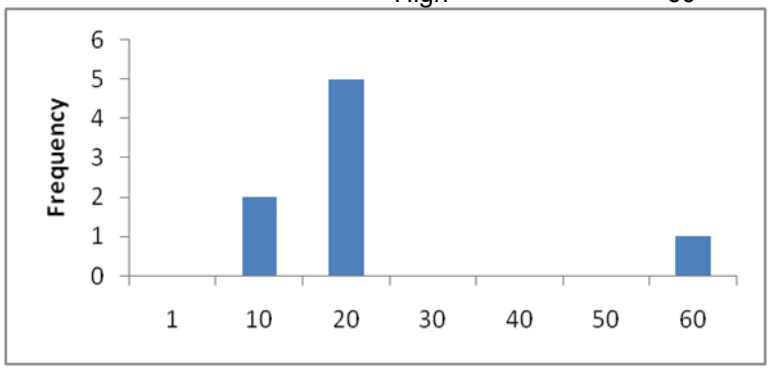


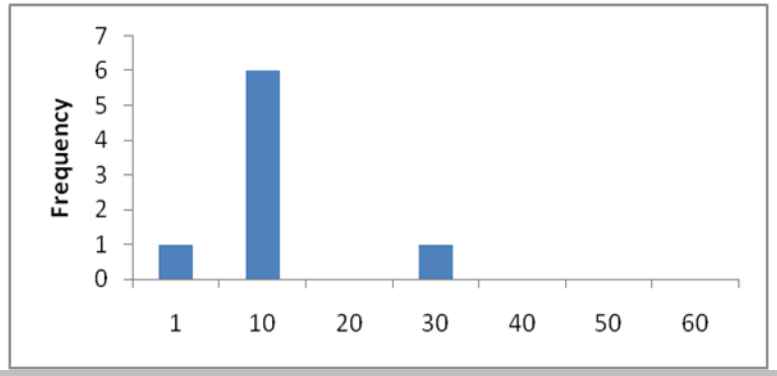
		Night	Day
Andes hantavirus	Geo Mean	44	13
	Median	48	10
	Low	30	10
	High	60	30



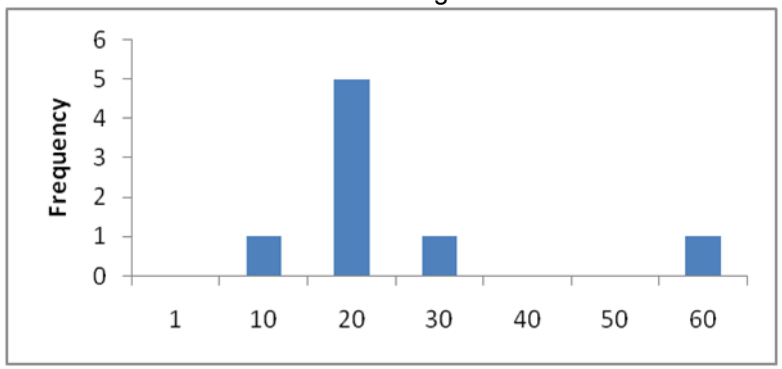


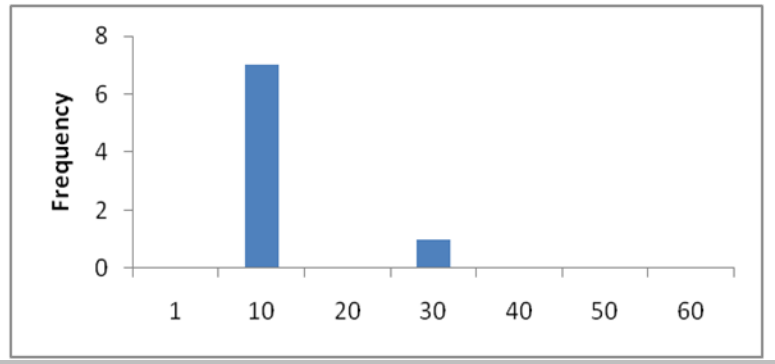
		Night	Day
Ebola virus	Geo Mean	15	7
	Median	20	10
	Low	2	1
	High	60	30



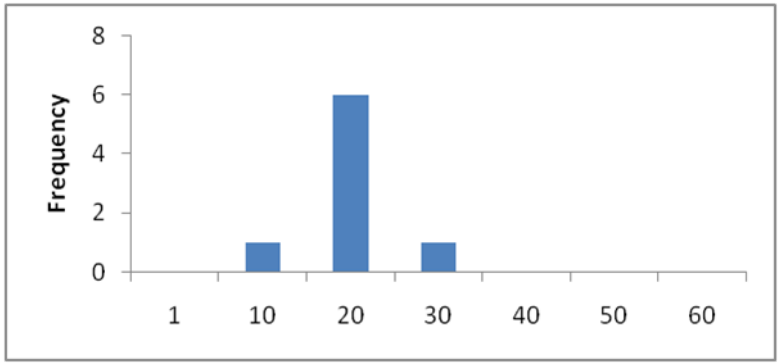


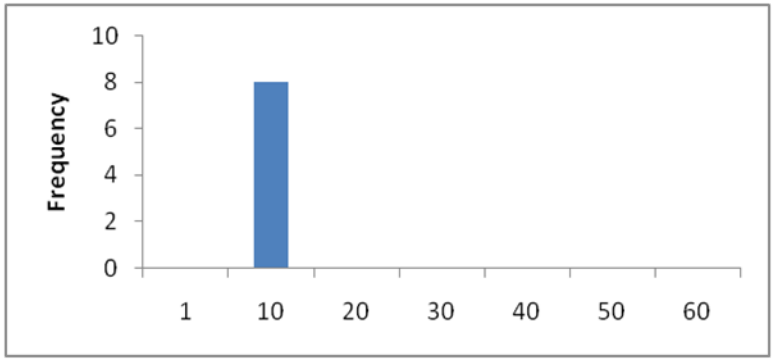
		Night	Day
Marburg virus	Geo Mean	20	9
	Median	18	10
	Low	10	2
	High	60	30



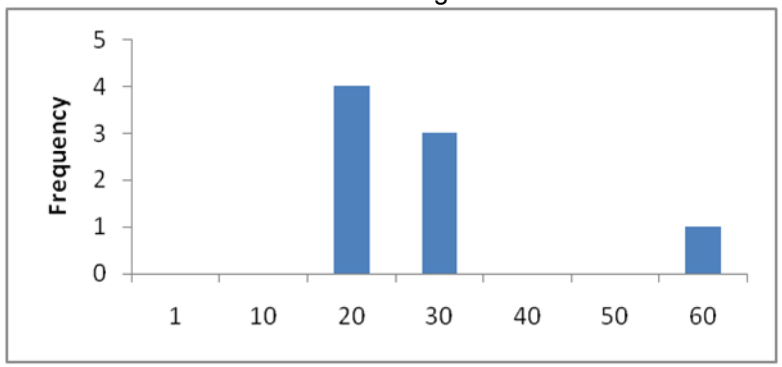


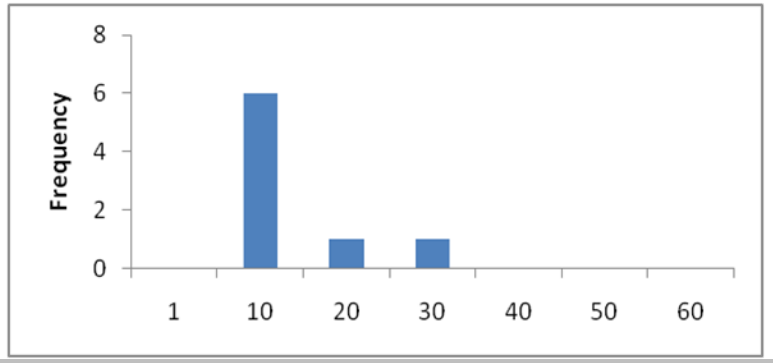
		Night	Day
Junin virus	Geo Mean	19	9
	Median	20	10
	Low	10	5
	High	30	10



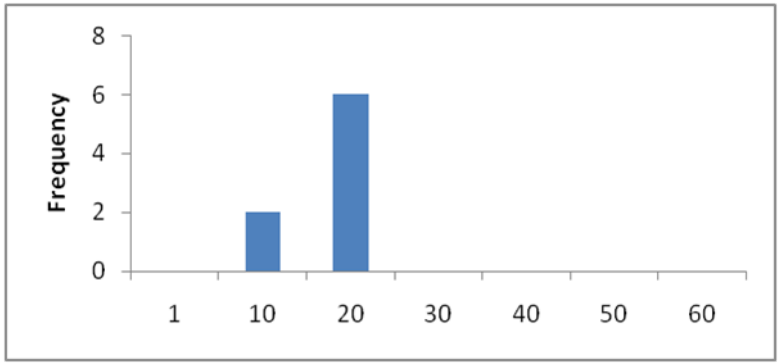


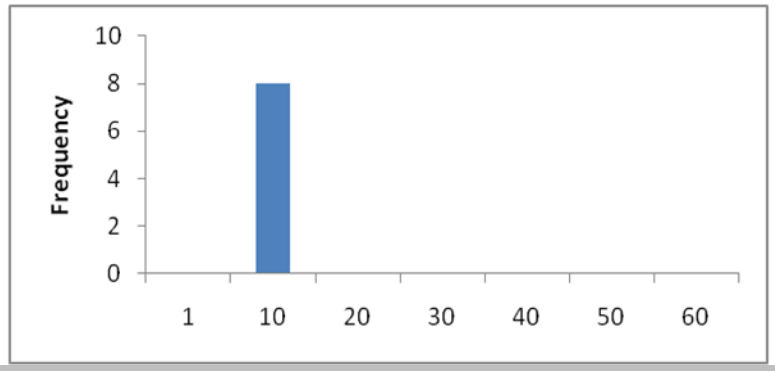
		Night	Day
Lassa fever virus	Geo Mean	24	11
	Median	22	10
	Low	15	5
	High	60	30



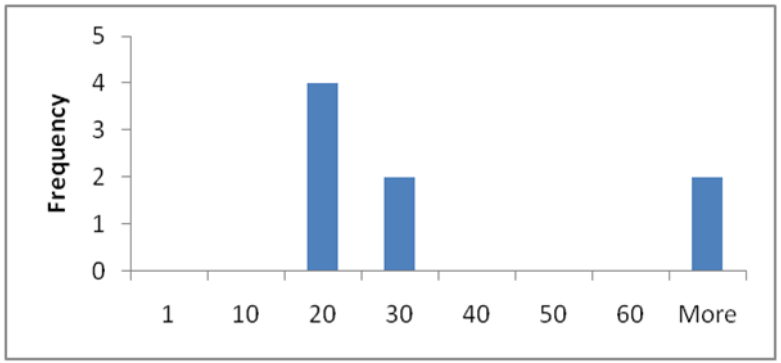


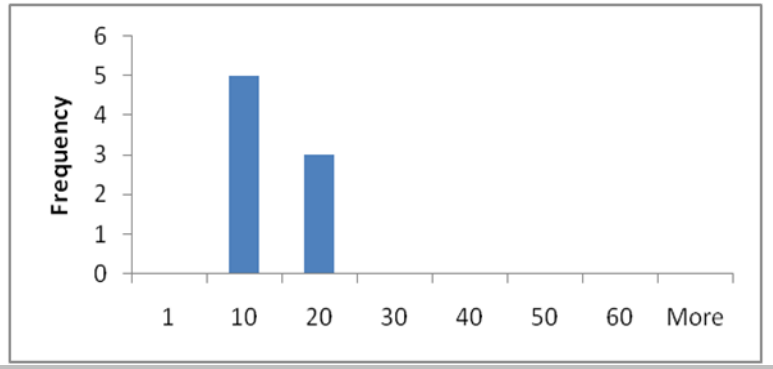
		Night	Day
Nipah virus	Geo Mean	14	6
	Median	18	5
	Low	5	2
	High	20	10



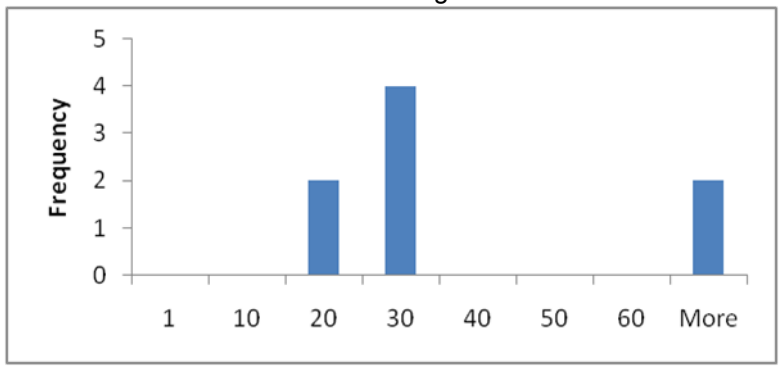


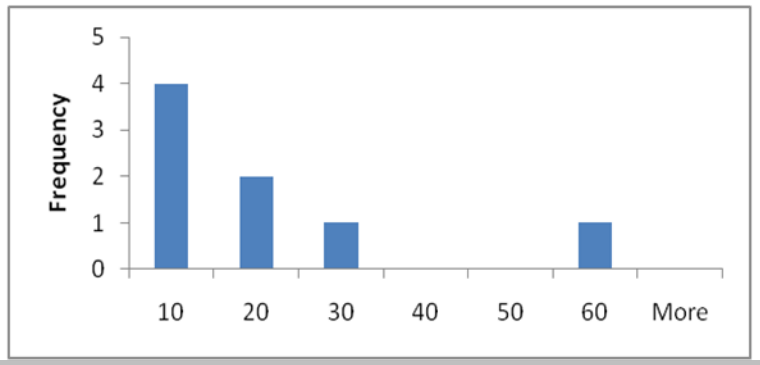
		Night	Day
Rift Valley fever virus	Geo Mean	27	13
	Median	23	10
	Low	20	10
	High	60	20



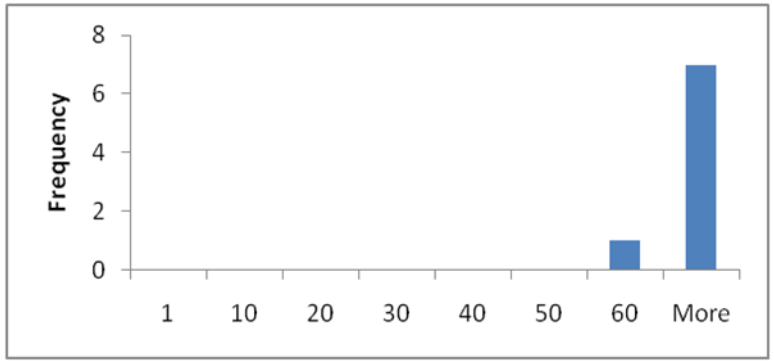


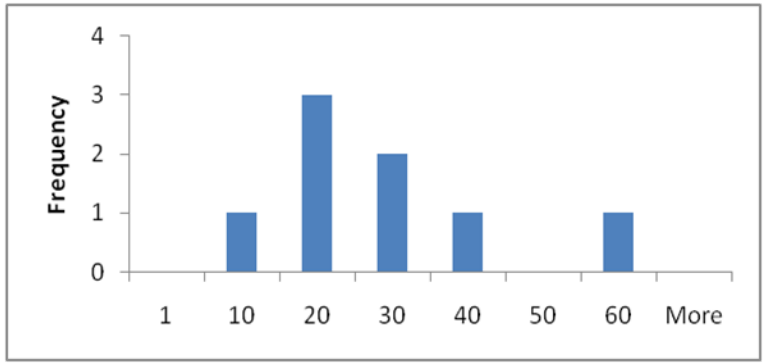
		Night	Day
Russian spring-summer encephalitis virus	Geo Mean	36	15
	Median	28	11
	Low	20	10
	High	120	60



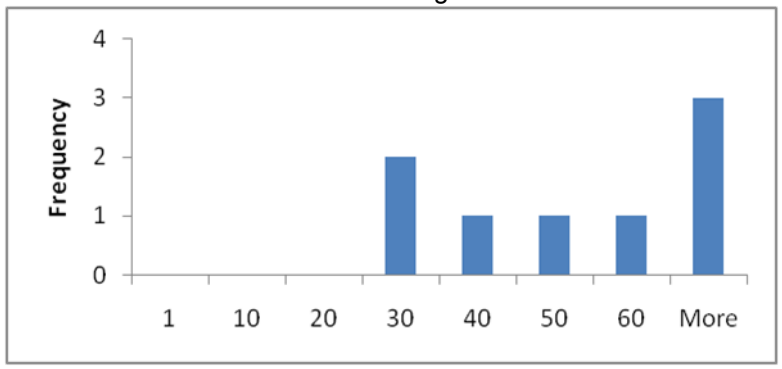


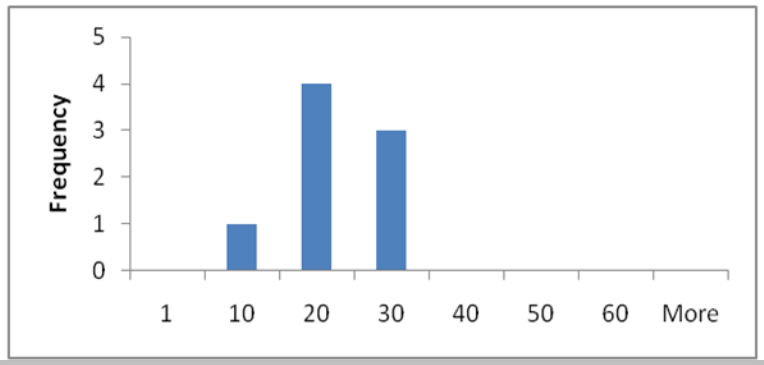
		Night	Day
SARS virus	Geo Mean	117	25
	Median	125	25
	Low	60	10
	High	200	60





		Night	Day
1918 influenza virus	Geo Mean	68	20
	Median	55	20
	Low	30	5
	High	300	30





Appendix I.

Medically Vulnerable Subpopulations

Tale of Contents

I. Medically Vulnerable Subpopulations

I.1 Introduction

The public and State and Federal Courts have expressed an interest in the demographic and health information regarding the community surrounding the NEIDL (Mahmoud 2008). To augment the environmental justice analysis presented in Chapter 10, this RA takes into account Medically Vulnerable Subpopulations (MVSP) in the communities surrounding the urban, suburban, and rural sites.

Vulnerability is a key concept in environmental justice (National Environmental Justice Advisory Council (U.S.) 2004). Vulnerability and susceptibility are often used interchangeably in the lay literature (US Environmental Protection Agency 2009). In reviewing the definitions for these terms, a suitable definition of the term *vulnerability* for this RA is provided by the NEJAC (National Environmental Justice Advisory Council (U.S.) 2004). In a report on the proposed framework for environmental justice and cumulative risk assessment, the Council addresses the concept of vulnerability: "A subpopulation is vulnerable if it is more likely to be adversely affected by a stressor than the general population." Vulnerability, in general, results from susceptibility/sensitivity, differential exposure, differential preparedness, or differential ability to recover.

The EPA, in their framework for a cumulative risk assessment, defines those that are *susceptible* as those who exhibit "greater or lesser biological response to exposure" (US Environmental Protection Agency 2003). Further, the susceptible are "those who are significantly more liable than the general population to be affected by a stressor due to life stage (e.g., children, the elderly, or pregnant women), genetic polymorphisms (e.g., the small but significant percentage of the population who have genetic susceptibilities), prior immune reactions (e.g., individuals who have been "sensitized" to a particular chemical), disease state (e.g., asthmatics), or prior damage to cells or systems (e.g., individuals with damaged ear structures due to prior exposure to toluene, making them more sensitive to damage by high noise levels)."

Extending the NEJAC and EPA framework for the purposes of this RA, the "stressors" under study are the pathogens. In general, when a pathogen comes into contact with a human (host), the outcome of that host-pathogen interaction is determined by characteristics of the pathogen and of the host (Osterholm et al. 2010). Key characteristics of the pathogen in this interaction include infectivity, pathogenicity, and virulence. Key host factors are those that influence the likelihood of infection and the occurrence and severity of disease, such as age at the time of exposure to the pathogen; co-existing chronic non-infectious

diseases; immune status at the time of infection, such as immunization status; immune deficiency, such as that due to chronic diseases, drugs, or other pathogens (e.g., HIV); gender; and genetic make-up. Important issues in the host-pathogen interaction are the route of transmission of the pathogen and the amount of pathogen to which the host is exposed.

Combining the NEJAC framework of vulnerability and the EPA definition of "susceptible" with these principles of host-pathogen interactions, this RA defines an MVSP as a group of individuals with a particular characteristic that have, in the event of exposure to a pathogen, an increased likelihood of disease from the pathogen and/or increased likelihood of death attributable to the disease.

An important point to note is that of the 13 pathogens, the entire population (general and MVSP) of the three sites could be considered susceptible to 12 of the pathogens (excluding 1918 H1N1 influenza virus). This assumption is based on the fact that none of these 12 pathogens are endemic to the area and, as such, the population would not have had an opportunity for exposure and development of immunity to those pathogens. Furthermore, even if relevant vaccines for these pathogens were available for the public, no one is likely to have been vaccinated. In the case of 1918 H1N1 influenza virus, it is likely that, apart from the children, others, especially those who have received the annual influenza vaccine or the 2009 pandemic vaccine, would have a measure of pre-existing immunity to influenza viruses. Based on the recent experience with 2009 H1N1 pandemic, this may reduce their susceptibility to the 1918 H1N1 influenza virus (Johns et al. 2010; Manicassamy et al. 2010; Medina et al. 2010). The exception is for pregnant women, who, based on observational data, appear to be more susceptible to and experience worse outcomes from pandemic influenza (Mosby et al. 2011; Rasmussen et al. 2011). So, in summary, for 12 of the pathogens, the entire population is considered susceptible. Among MVSPs, though there are no controlled studies, it is conservatively assumed that this susceptibility is further increased.

This appendix reviews the choice of specific medically vulnerable subpopulations for this RA, the methods and data sources used to derive estimates of those populations, the derivation of estimates of differential susceptibility to pathogens under study for the subpopulations, a general description of the effect of the pathogen/disease on underlying medical vulnerabilities, and the use of subpopulation data in the estimates of initial infection and secondary transmission modeling.

I.2 Choice of Medically Vulnerable Subpopulations for Risk Analysis

As noted in the definition of "susceptible" above, MVSPs are a diverse group of individuals in the community. In general, these are the very young, the very old, and those with chronic conditions such as diabetes, heart disease, respiratory conditions (e.g., asthma), and those with genetic polymorphisms/immuno-compromised states (e.g., solid-organ and bone-marrow transplants), those undergoing chemotherapy for cancer, and pregnant women. Further, the EPA considers those who are sensitized by a reaction to a chemical or stressor to be vulnerable to another stressor.

The criteria for considering a specific MVSP for this RA are: (1) the condition should be relevant in a bio-medical sense and confer increased susceptibility to a pathogen, such as those under study for this RA (bacteria and viruses); and (2) reliable estimates of the subpopulation should be available from federal, state, and local government sources that could be cited. Using these criteria, the following groups were considered:

1. Children younger than 5 years
2. Adults older than 65 years
3. Those with diabetes
4. Those with HIV/AIDS
5. Pregnant women.

Those excluded from this RA, as there are no data linking medical vulnerability in these subpopulations to a direct increase in susceptibility to pathogens (from the examples from EPA given above), are those with prior immune reactions and those with prior damage to cells or systems. Those with asthma were also excluded as there is no known direct association of asthma with increased susceptibility to infections from the pathogens considered in this RA. On the other hand, the diseases caused by the pathogens, especially those associated with respiratory disease such as influenza, may exacerbate asthma. This has been shown in children during the recent 2009 H1N1 influenza pandemic, where even secondary pneumonia has been noted (Dawood et al. 2010; Dawood et al. 2010; Dawood et al. 2011).

A further subset was excluded as there are no area-wide estimates available for these subpopulations from the urban, suburban, and rural sites: those with genetic predisposition (genetic polymorphisms) to infections; those with other immune-compromised states, such as those with solid-organ and bone-marrow transplants; and those undergoing chemotherapy for cancer. This exclusion may result in an

underestimation of the proportion of MVSPs in the populations at the three sites. However, this underestimation is considered not significant based on the small numbers of these subpopulations. For example, according to the Organ Procurement and Transplantation Network website (Health Resources and Services Administration 2011), the number of solid-organ transplants in the Commonwealth of Massachusetts averaged 761 for the years 2008-2010; for the corresponding period in the State of New Hampshire, there were an average of 55 such transplants.

I.3 Methods and Data Sources for Derivation of Estimates of MVSP

Internet searches were performed in September 2010 (and updated for census data in August 2011) on federal, state, and local government websites for reliable and reproducible estimates of MVSP in the three alternative sites considered in this RA. The three sites are: (1) Urban – BUMC BioSquare Research Park in Boston, Suffolk County, Massachusetts; (2) Suburban – Tyngsborough, Middlesex County, Massachusetts; and (3) Rural – Sargent Center in Peterborough and Hancock, Hillsborough County, New Hampshire (Table 1).

General estimates of census data were obtained from the U.S. Census with the latest estimates for towns available as 2005-2009 American Community Survey 5-Year Estimates (US Census Bureau 2010). Population estimates for the U.S., the States of Massachusetts and New Hampshire, and the City of Boston were available for 2008 from the U.S. Census Bureau. Census data for Tyngsborough Township in Middlesex County, Massachusetts, and the Townships of Peterborough and Hancock in Hillsborough County, New Hampshire, were available as estimates for the years 2005-2009. Population data for the zip code 02118 (Boston) were available only for the year 2000 from the U.S. Census Bureau.

Data on prevalence of diabetes were obtained from the CDC for national and race-based data (Centers for Disease Control 2010) and county-level data (Centers for Disease Control 2010). State-level data were obtained from the Department of Health of the Commonwealth of Massachusetts (Commonwealth of Massachusetts 2010); prevalence data of HIV/AIDS were obtained from the Department of Health of the Commonwealth of Massachusetts (Commonwealth of Massachusetts 2010; Commonwealth of Massachusetts 2010) and the State of New Hampshire (New Hampshire Department of Health and Human Services 2010).

The estimate of 1% for the subpopulation of pregnant women in any area was derived in the calculations of influenza morbidity and mortality among pregnant women by CDC authors (Jamieson et al. 2009;

Siston et al. 2010). The 1% estimates are a reasonable match with the "resident births" recorded in the areas under study for the years listed as reported by the Commonwealth of Massachusetts (Commonwealth of Massachusetts 2010) and the State of New Hampshire (New Hampshire Department of Health and Human Services 2010).

Table 1: Estimates of Medically Vulnerable Subpopulations in the Three Sites under Consideration for the NEIDL Risk Analysis

Medically Vulnerable Subpopulation (Estimates)	U.S. data 2008 estimates	MA State level data 2008 estimates	Boston City Data 2008 estimates	Zip Code 02118 (NEIDL/BU Medical Center) Census data available for 2000	Tyngs-borough, Middlesex County, MA Census data estimates for 2005-2009	New Hampshire State level data 2008 estimates	Peterborough and Hancock towns, Hillsborough County, NH Census data estimates for 2005-2009
Total Population[a]	301,237,703	6,349,097	613,086	22,173	11,594	1,235,786	7927
Children younger than 5[a] (%)	20,672,826 (6.9)	397,268 (6.3)	36,422 (5.9)	855 (3.9)	643 (5.5)	75,685 (6.1)	461 (5.8)
Adults older than 65[a] (%)	37,980,136 (12.6)	860,162 (13.5)	62,604 (10.2)	1,759 (7.9)	958 (8.2)	147,970 (12)	1721 (21.7)
Number of adults aged ≥ 20 years with diabetes (%)	17,359,000[b] (5.7)	475,000[c] (7.4)	56,404[c] (9.2)	Estimate of 12.6% from Boston City minority population estimates	864[b] (7.8)	74,000[b] (6.7)	388[b] (6.6)
With HIV infection (includes HIV/AIDS)	National prevalence rate: 447.8 per 100,000 population; Prevalence rates for blacks (1,715.1 per 100,000)	18,045[e] 28% are black Prevalence rate, overall 284.2 per 100,000 Age-adjusted rates for blacks 1,643.6 per 100,000	5,786[e]	Rates for blacks from MA data 1,643.6 per 100,000	2933 in Middlesex county	1067 in the state[d]	462 in Hillsborough county[d]
Pregnant women (calculated as 1% of the population estimate)	Estimate 1%: 3,012,377 Births 2006: 4,265,555	Estimate 1%:63491 Resident births in 2008[g]: 76,969	Estimate 1%: 6131 Number of resident births in 2008[g]: 8019	Estimate 1%:222 Resident births for this zip code not available	Estimate 1%=111 Number of resident births in 2008[7]: 116	Estimate 1%= 12358 As a comparison, births registered[f] in state of NH in 2000:12,859	Estimate 1%=79 Average births[6] for the past decade between 40-50 for Peterborough For 2000: 69

[a] U.S. Census Bureau 2010

[b] (Centers for Disease Control 2010)

[c] (Commonwealth of Massachusetts 2010)

[d] (New Hampshire Department of Health and Human Services 2010)

[e] (Commonwealth of Massachusetts 2010; Commonwealth of Massachusetts 2010)

[f] (New Hampshire Department of Health and Human Services 2010)

[g] (Commonwealth of Massachusetts 2010)

I.3.1 Differential Susceptibility to Infection among MVSP

Members of the MVSPs studied in this RA are considered to be at increased risk of some infectious diseases and also of death due to some infectious diseases. For example, children are at increased risk of respiratory infections, particularly respiratory syncytial virus and influenza (Munoz 2002); the elderly are at increased risk of some infections (Crossley et al. 1996; Liang et al. 2007); diabetics are at increased risk of some infections in general due to disturbances in several compartments of the immune system (Calvet et al. 2001; Rajagopalan 2005; Gupta et al. 2007); and those with HIV, especially advanced AIDS are at risk of many common infections due to a profound immune deficiency (Duse 1999; Gordon 2004; Corti et al. 2009).

Pregnant women are considered to be immune-suppressed and are at high risk of adverse events from seasonal and pandemic influenza (Jamieson, Honein et al. 2009; Siston, Rasmussen et al. 2010; Mosby et al. 2011; Rasmussen, Kissin et al. 2011). Seasonal-influenza related hospitalization rates from selected acute cardiopulmonary conditions were found to be nearly five-fold higher in pregnant women in the third trimester (Neuzil et al. 1998). With respect to pandemic influenza, the historical mortality rates of pregnant women have been noted to be in the range of 20–51% for the 1918 and 1957 influenza pandemics (Callaghan et al. 2010). In reviewing observations from the 2009 H1N1 influenza pandemic, pregnancy was associated with an increased risk of hospitalization, intensive care unit admission, and death (Mosby, Rasmussen et al. 2011). Pregnant women were disproportionately represented in hospitalizations and deaths; pregnancy-specific mortality rates were not provided.

The published literature often qualitatively refers to these observations and provides quantitative estimates of increase in susceptibility for small cohorts of patients; these estimates may not be generalizable to the entire population. The observation remains that members of the MVSP are at increased risk, and quantitative estimates of those increases are not generally available for infectious diseases. It should be noted that much less observational data are available for the pathogens under study in this RA. A review of the pathogens is provided in Chapter 3, and the limited references to increased susceptibility (and a few for decreased susceptibility) ascribed to these pathogens to specific MVSPs (if available) are presented in Table 2.

In the absence of quantitative estimates, the differential susceptibility of the MVSPs to infectious diseases caused by the 13 pathogens was (1) qualitatively described from a representative review of the published literature and (2) estimated from elicitation from an expert panel (Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment, Appendix H).

Qualitative Description of Differential Susceptibility among MVSPs

The evidence for differential susceptibility among MVSPs to the 13 pathogens is limited in the literature for a majority of the pathogens. The published references to increased susceptibility (and in rare cases, decreased susceptibility) are provided in Chapter 3 with the description of the pathogens. These are summarized in Table 2.

Table 2: Differential Susceptibility as Noted in the Published Literature among Members of Medically Vulnerable Subpopulations to the 13 Pathogens under Study in the RA (for details see Chapter 4). The ▲mark indicates a report of increased susceptibility to the pathogen in terms of disease and/or death; conversely, the ▼ mark indicates decreased susceptibility to the pathogen. Shaded cells indicate that there are no pertinent data available to support differential susceptibility.

	Pathogen	Age < 5 yrs	Age > 65 yrs	Diabetes	HIV/AIDS	Pregnant Women
BSL-3	*B. anthracis*					▲[1]
	F. tularensis					
	Y. pestis					
	1918 H1N1V			▲[2]		▲[3]
	SARS-CoV	▼[4]	▲[4]			
	RVFV					
	ANDV	▲[5]				
BSL-4	EBOV	▼[6]				▲[7]
	MARV	▼[8]				
	LASV					▲[9]
	JUNV					
	TBEV-FE	▲[10]	▲[10]			
	NIPV					

[1] (Kadanali et al. 2003; Jamieson et al. 2006)
[2] (Diepersloot et al. 1990; Valdez et al. 1999; 2000; Allard et al. 2010)
[3] (Jamieson, Honein et al. 2009; Labant et al. 2009; Patel et al. 2010; Louie et al. 2011; Mosby, Rasmussen et al. 2011; Rasmussen et al. 2011)
[4] (Anderson et al. 2004; Liang et al. 2004; Zhong et al. 2004; Gillim-Ross et al. 2006)
[5] (Pini et al. 1998; Ferres et al. 2004)
[6] (Dowell 1996)
[7] (Mupapa et al. 1999)
[8] (Feldmann 2006; Towner et al. 2006)
[9] (Price et al. 1988)
10 (Lindquist et al. 2008)

I.3.2 Estimates of Differential Susceptibility among MVSP to Pathogens

In the absence of quantitative data of the differential susceptibility of MVSPs to infectious diseases caused by the 13 pathogens from the published literature, estimates were generated from the expert panel elicitation conducted by the NIH.

As noted in the Final Delphi Report (Appendix H), the goal of the expert panel elicitation was to obtain estimates of the degree of increased vulnerability to infection and to severe disease that could be used as a starting point for analyses of differential health effects. The experts were instructed to provide general estimates of increased vulnerability to infections by either pathogenic bacteria or viruses (Tables 3a-b).

Table 3a: Estimates of Increased Vulnerability to Disease for MVSPs studied in this RA (Source: Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment, Appendix H)

Disease (% increase in vulnerability)		Median	Low	High	Geometric Mean
Young	Bacteria	18%	5%	33%	15%
	Viruses	20%	0%	33%	9%
Older	Bacteria	28%	5%	50%	23%
	Viruses	23%	5%	50%	21%
Diabetes	Bacteria	20%	5%	30%	16%
	Viruses	15%	5%	25%	13%
HIV	Bacteria	30%	10%	40%	26%
	Viruses	30%	10%	40%	28%
Pregnancy	Bacteria	5%	0%	30%	3%
	Viruses	5%	2%	50%	8%

Table 3b: Estimates of Increased Vulnerability to Death for MVSPs studied in this RA (Source: Expert Elicitation on Organisms Studied in the NEIDL Risk Assessment, Appendix H)

Death (% increase in vulnerability)		Median	Low	High	Geometric Mean
Young	Bacteria	10%	5%	20%	11%
	Viruses	10%	5%	20%	12%
Older	Bacteria	10%	5%	50%	14%
	Viruses	15%	5%	50%	16%
Diabetes	Bacteria	10%	5%	25%	10%
	Viruses	10%	5%	25%	10%
HIV	Bacteria	25%	10%	30%	22%
	Viruses	25%	10%	30%	22%
Pregnancy	Bacteria	5%	1%	20%	5%
	Viruses	4%	1%	20%	5%

I.3.3 Impact of Pathogen on Underlying Medical Vulnerabilities

In general, any disease condition has an effect on the human host. In the case of the 13 pathogens, the diseases caused are often aggressive and are generally expected to result in high morbidity and mortality in all populations.

Infectious diseases in general are considered to have an adverse impact on the health of the young and the elderly, thus contributing to increased morbidity and mortality. Blood sugar control in diabetic patients is disrupted in those who are experiencing active infections; thus diabetes is adversely impacted by the disease. HIV/AIDS may also be adversely impacted by another infectious disease. Pregnant women often experience pre-term birth or loss of the pregnancy when they experience a serious insult such as an infectious disease caused by a pathogen under this study. Asthma is often triggered by respiratory infections; it is plausible that a person who has been infected by one of the respiratory pathogens would have an asthma attack as a result of the infection. A recent report on the association of seasonal and pandemic influenza on children with asthma in the U.S. concludes that children with asthma experience pneumonia more frequently and are admitted to the intensive care unit more often as compared to children without asthma (Dawood, Kamimoto et al. 2011). The risk of respiratory failure and death was not increased. Children experienced exacerbations of asthma due to influenza. For all these adverse impacts on the underlying medical vulnerability, there are no specific data or methods to allow for quantifying the adverse impact.

I.3.4 Use of MVSP Data

The estimates of the MVSP among the populations at the three NEIDL sites and the estimates of increased susceptibility to disease and death caused by the pathogens are used in generating (1) estimates of initial infection after exposure to a pathogen, and (2) secondary transmission modeling of spread of disease in the community, as described below.

I.3.5 Estimates of Initial Infection

There are two categories of individuals who are affected in the scenarios of loss of biocontainment and exposure to a pathogen, namely the laboratory worker and members of the public.

I.3.6 Laboratory Worker

In general, for the loss of biocontainment scenarios analyzed for this RA, the number of laboratory workers exposed to the pathogen as a result of the release is 1 -4 workers. The laboratory workers will not

be younger than 5 years of age and are unlikely to be older than 65 years. It is likely that pregnant laboratory workers would be assigned duties other than working with BSL-3 and BSL-4 pathogens. The possibility remains that the laboratory worker could be a member of the diabetic or HIV/AIDS subpopulations. The estimates of initial infection could be adjusted based on the possibility of the laboratory worker being a member of one of the MVSPs. The challenge lies in making an assumption regarding the health status of the laboratory worker in order to estimate the probability that he/she has diabetes or HIV/AIDS. In this setting, this RA assumes that the laboratory worker is a healthy adult.

I.3.7 Members of the Public

Under release scenarios in which members of the public are directly exposed to a pathogen from a release event, it is possible that the average susceptibility profile of the local population at the three sites could result in site differences in the average probability of initial infection per person at the same level of exposure. To quantify this potential difference, populations of the five MVSPs described above are examined in this RA.

The baseline dose-response curves, derived in the dose-response appendix (Appendix J), are assumed to reflect characteristics of a heterogeneous population containing members of the five groups to some extent. The question of interest is whether the relative difference in the population profiles near the three sites has an effect on the comparative risk of initial infections occurring from scenarios in which members of the public are exposed. The following assumptions are made in order to investigate quantitatively this possibility: (1) a given dose response curve being assumed for a particular pathogen is relevant for an average U.S. population; (2) the probability that a given exposed individual is a member of an MVSP is equal to the portion of the local population that is a member of that MVSP; and (3) the average probability of infection when an individual from one of the MVSPs inhales a given number of organisms is higher than the probability for an individual who is not a member of any of the MVSPs. The increase in this probability is calculated as follows: if person A is more susceptible to infection than person B by X% (e.g., 20%), it is assumed that the probability of person A being infected at a particular dose is the same as the probability of person B being infected at a dose that is X% higher (e.g., 20% higher; for more details, see Appendices J and K).

The potential implications of these assumptions on rates of initial infections among MVSPs at the three sites are assessed in the initial infections appendix.

I.4 Secondary Transmission Modeling

Differential susceptibility also has potential implications for affecting the average number of transmissions from an infected individual interacting with a susceptible population that includes members of MVSPs. As defined in the secondary transmission chapters and appendices, the population-wide reproductive number is the average number of transmissions per infected individual. In the context of considering the effect of vulnerable portions of the susceptible sub-population, the reproductive number is considered a product of two components: (1) the average number of contacts per infected individual, and (2) the average probability of transmission per contact .

Under this framework, one can consider the portion of the average number of contacts that is likely to represent contacts with individuals in each subpopulation (based on the portion of the available contacts that are members of the subpopulation) and then how the probability of transmission per contact would change based on the differential susceptibility of that subpopulation.

The general estimates of the reproductive number that are used for this RA were based on transmission data from real outbreaks among populations that contained some portion of individuals with increased susceptibility. In this sense, the effect of differential susceptibility is already incorporated into the reproductive values being used. However, as this RA is concerned with specific populations for which data on subpopulations are available, it was determined to be appropriate to include a framework for adjusting values of the reproductive number based on how the local population characteristics may differ from a typical population.

The following assumptions are made: (1) for a given scenario, the base case value for the reproductive number is relevant for a population containing portions of each subpopulation that are in line with their overall frequency in the total U.S. population; (2) the probability that any given contact of an infectious individual is a member of a given MSVP is equal to the portion of the local population that is a member of that MVSP; (3) the average probability of transmission when an infectious individual contacts a member of an MVSP is higher that the probability resulting from contact with an individual who is not a member of the MVSPs (the percentage increase in this probability is assumed to be equal to the percentage increase of susceptibility relevant for the particular pathogen and subpopulation); (4) the MVSP identity of each simulated individual is not tracked in the secondary transmission modeling; and (5) potential differences in transmission *from* infected individuals belonging to different MVSPs are not considered.

The implications of these assumptions on quantitative estimates of site differences in transmission are assessed in the secondary transmission appendix (Appendix L).

I.5 References

Allard, R., et al. (2010). "Diabetes and the severity of pandemic influenza A (H1N1) infection." Diabetes Care **33**(7): 1491-1493.

Anderson, R. M., et al. (2004). "Epidemiology, transmission dynamics and control of SARS: the 2002-2003 epidemic." Philosophical transactions of the Royal Society of London. Series B, Biological sciences **359**(1447): 1091-1105.

Callaghan, W. M., et al. (2010). "Deaths from seasonal influenza among pregnant women in the United States, 1998-2005." Obstetrics and gynecology **115**(5): 919-923.

Calvet, H. M., et al. (2001). "Infections in diabetes." Infectious disease clinics of North America **15**(2): 407-421, viii.

Centers for Disease Control. (2010). "County Level Data for Diabetes." Retrieved 9/1/2010, 2010, from http://apps.nccd.cdc.gov/DDT_STRS2/NationalDiabetesPrevalenceEstimates.aspx

Centers for Disease Control. (2010). "National and Race-Based Data on Diabetes." Retrieved September 1, 2010, 2010, from http://apps.nccd.cdc.gov/DDTSTRS/NationalSurvData.aspx.

Centers for Disease Control and Prevention (2000). "From the Centers for Disease Control and Prevention. Influenza and pneumococcal vaccination rates among persons with diabetes mellitus--United States, 1997." JAMA : the journal of the American Medical Association **283**(1): 48-50.

Commonwealth of Massachusetts. (2010). "Massachusetts Department of Health - Diabetes." Retrieved 9/1/2010, 2010, from www.mass.gov/.../docs/.../healthymass/diabetes_recommendations.doc

Commonwealth of Massachusetts. (2010). "Massachusetts Department of Health - HIV/AIDS." Retrieved 9/1/2010, 2010, from http://www.mass.gov/?pageID=eohhs2subtopic&L=6&L0=Home&L1=Researcher&L2=Physical+Health+and+Treatment&L3=Diseases+%26+Conditions&L4=HIV%26%2347%3bAIDS&L5=Epidemiologic+Profile+Calendar+Year+2010&sid=Eeohhs2

Commonwealth of Massachusetts. (2010). "Massachusetts Department of Health - HIV/AIDS at a Glance." Retrieved 9/1/2010, 2010, from http://www.mass.gov/Eeohhs2/docs/dph/aids/2010_profiles/epidemic_glance_data.pdf

Commonwealth of Massachusetts. (2010). "Massachusetts Office of Health and Human Services - Birth Report 2008." Retrieved 9/3/2010, 2010, from http://www.mass.gov/Eeohhs2/docs/dph/research_epi/birth_report_2008.pdf

Corti, M., et al. (2009). "Respiratory infections in immunocompromised patients." Current opinion in pulmonary medicine **15**(3): 209-217.

Crossley, K. B., et al. (1996). "Infections in the elderly." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **22**(2): 209-215.

Dawood, F. S., et al. (2010). "Burden of seasonal influenza hospitalization in children, United States, 2003 to 2008." The Journal of pediatrics **157**(5): 808-814.

Dawood, F. S., et al. (2010). "Influenza-associated pneumonia in children hospitalized with laboratory-confirmed influenza, 2003-2008." The Pediatric infectious disease journal **29**(7): 585-590.

Dawood, F. S., et al. (2011). "Children With Asthma Hospitalized With Seasonal or Pandemic Influenza, 2003-2009." Pediatrics.

Diepersloot, R. J., et al. (1990). "Influenza infection and diabetes mellitus. Case for annual vaccination." Diabetes Care **13**(8): 876-882.

Dowell, S. F. (1996). "Ebola hemorrhagic fever: why were children spared?" Pediatr Infect Dis J **15**(3): 189-191.

Duse, A. G. (1999). "Nosocomial infections in HIV-infected/AIDS patients." The Journal of hospital infection **43 Suppl**: S191-201.

Feldmann, H. (2006). "Marburg hemorrhagic fever--the forgotten cousin strikes." N Engl J Med **355**(9): 866-869.

Ferres, M., et al. (2004). "Hantavirus infection in children." Current opinion in pediatrics **16**(1): 70-75.

Gillim-Ross, L., et al. (2006). "Emerging respiratory viruses: challenges and vaccine strategies." Clinical microbiology reviews **19**(4): 614-636.

Gordon, S. (2004). "Pneumococcal infections in HIV infected adults--clinical features, reasons behind the association and future hopes for prevention." Tropical doctor **34**(4): 200-203.

Gupta, S., et al. (2007). "Infections in diabetes mellitus and hyperglycemia." Infectious disease clinics of North America **21**(3): 617-638, vii.

Health Resources and Services Administration, U. S. D. o. H. H. S. (2011). "Organ Procurement and Transplantation Network " Retrieved August 30, 2011, 2011, from http://optn.transplant.hrsa.gov/latestData/stateData.asp?type=region.

Jamieson, D. J., et al. (2006). "Emerging infectious disease outbreaks: old lessons and new challenges for obstetrician-gynecologists." Am J Obstet Gynecol **194**(6): 1546-1555.

Jamieson, D. J., et al. (2009). "H1N1 2009 influenza virus infection during pregnancy in the USA." Lancet **374**(9688): 451-458.

Johns, M. C., et al. (2010). "Seasonal influenza vaccine and protection against pandemic (H1N1) 2009-associated illness among US military personnel." PLoS One **5**(5): e10722.

Kadanali, A., et al. (2003). "Anthrax during pregnancy: case reports and review." Clin Infect Dis **36**(10): 1343-1346.

Labant, A., et al. (2009). "Pandemic flu: a major concern for pregnant women." Nurs Womens Health **13**(5): 374-382.

Liang, H., et al. (2004). "Investigating public health emergency response information system initiatives in China." International journal of medical informatics **73**(9-10): 675-685.

Liang, S. Y., et al. (2007). "Infections in the elderly." Clinics in geriatric medicine **23**(2): 441-456, viii.

Lindquist, L., et al. (2008). "Tick-borne encephalitis." Lancet **371**(9627): 1861-1871.

Louie, J. K., et al. (2011). "A Review of Adult Mortality Due to 2009 Pandemic (H1N1) Influenza A in California." PLoS One **6**(4): e18221.

Mahmoud, A., D. Burke, S. Eubank, V.S. Freimuth, G Friedman-Jimenez, P. Hamburg, K.A. Holbrook, D.L. Kasper, J. Lewis, W.I. Lipkin, T.H. Murray, M.E. Northridge, J. Patterson, M. Robson, S. Stanley, W. Thomann, S. Bennett, P. Highnam, and R. Khabbaz. (2008). NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratory at Boston University Medical Center, Finding and Recommendations, Part I: Risk Assessment; Briefing of the Advisory Committee to the Director, NIH, June 6, 2008., Department of Health and Human Services.

Manicassamy, B., et al. (2010). "Protection of mice against lethal challenge with 2009 H1N1 influenza A virus by 1918-like and classical swine H1N1 based vaccines." PLoS pathogens **6**(1): e1000745.

Medina, R. A., et al. (2010). "Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus." Nature communications **1**: 28.

Mosby, L. G., et al. (2011). "2009 Pandemic influenza A (H1N1) in pregnancy: a systematic review of the literature." American journal of obstetrics and gynecology.

Mosby, L. G., et al. (2011). "2009 Pandemic influenza A (H1N1) in pregnancy: a systematic review of the literature." Am J Obstet Gynecol.

Munoz, F. M. (2002). "The impact of influenza in children." Seminars in pediatric infectious diseases **13**(2): 72-78.

Mupapa, K., et al. (1999). "Ebola hemorrhagic fever and pregnancy." J Infect Dis **179 Suppl 1**: S11-12.

National Environmental Justice Advisory Council (U.S.) (2004). Ensuring risk reduction in communities with multiple stressors : environmental justice and cumulative risks/impacts. Washington, DC?, National Environmental Justice Advisory Council.

Neuzil, K. M., et al. (1998). "Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women." American journal of epidemiology **148**(11): 1094-1102.

New Hampshire Department of Health and Human Services. (2010). "New Hampshire Department of Health - HIV/AIDS." Retrieved 9/1/2010, 2010, from http://www.dhhs.state.nh.us/NR/rdonlyres/ej3gzun7fabqzxr734kc3337p6vejm7sejtjfrizo55jqvidkidecucjrbajlnavd37ck2dubgs5bpw3yskgnkqbrlf/hiv_quarterly08.pdf

New Hampshire Department of Health and Human Services. (2010). "New Hampshire Department of Health and Human Services, Health Statistics and Data Management." Retrieved 9/3/2010, 2010, from http://www.dhhs.state.nh.us/NR/rdonlyres/eop622wjzb3pit4itenxnesusl2iswon37amcwlc5rs44ac6itcap5s4e3xijmj5tdh6wpuzkk6ln4ratib4jx7gm6a/Birth-tool-rpt.pdf

Osterholm, M. T., et al. (2010). Epidemiology of Infectious Diseases. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseaes. G. L. Mandell, J. E. Bennett and R. Dolin. Philadelphia, Churchill Livingstone. **2**.

Patel, M., et al. (2010). "Pandemic (H1N1) 2009 influenza." British journal of anaesthesia **104**(2): 128-142.

Pini, N. C., et al. (1998). "Hantavirus infection in children in Argentina." Emerging infectious diseases **4**(1): 85-87.

Price, M. E., et al. (1988). "A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy." BMJ **297**(6648): 584-587.

Rajagopalan, S. (2005). "Serious infections in elderly patients with diabetes mellitus." Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **40**(7): 990-996.

Rasmussen, S. A., et al. (2011). "Preparing for influenza after 2009 H1N1: special considerations for pregnant women and newborns." Am J Obstet Gynecol.

Rasmussen, S. A., et al. (2011). "Preparing for influenza after 2009 H1N1: special considerations for pregnant women and newborns." American journal of obstetrics and gynecology.

Siston, A. M., et al. (2010). "Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States." JAMA : the journal of the American Medical Association **303**(15): 1517-1525.

Towner, J. S., et al. (2006). "Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola." J Virol **80**(13): 6497-6516.

US Census Bureau. (2010). "US Census Data." Retrieved September 1, 2010, re-accessed August 29, 2011, 2010 and 2011, from http://factfinder.census.gov/servlet/ACSSAFFFacts?_event=&geo_id=01000US&_geoContext=01000US&_street=&_county=&_cityTown=&_state=&_zip=&_lang=en&_sse=on&ActiveGeoDi

v=&_useEV=&pctxt=fph&pgsl=010&_submenuId=factsheet_1&ds_name=null&_ci_nbr=null&qr_name=null&reg=null%3Anull&_keyword=&_industry=.

US Environmental Protection Agency (2003). Framework for Cumulative Risk Assessment. US Environmental Protection Agency. Washington DC, US Environmental Protection Agency,**:** 129.

US Environmental Protection Agency (2009). U.S. EPA. Integrated Science Assessment for Particulate Matter (Final Report). Washington DC. **EPA/600/R-08/139F**.

Valdez, R., et al. (1999). "Impact of diabetes mellitus on mortality associated with pneumonia and influenza among non-Hispanic black and white US adults." American journal of public health **89**(11): 1715-1721.

Zhong, N. S., et al. (2004). "Epidemiology of severe acute respiratory syndrome (SARS): adults and children." Paediatric respiratory reviews **5**(4): 270-274.

Appendix J.

Dose-Response Relationships

Contents

J. Dose-Response Relationships

J.1 Introduction

Dose-response assessment is used to estimate the relationship between a dose, or amount of pathogen to which a potential host is exposed, to a given response such as the establishment of infection, morbidity, or mortality. For this risk assessment (RA), dose-response assessment is an important component in converting estimates of the frequency and amount of exposure to a pathogen from the event sequence analysis to estimates of initial infection for the health effects analysis.

When a potential host is exposed to a dose containing one or more infectious microorganisms (virions or bacterial cells), there is a probability that the microorganism(s) will replicate or multiply in the host and cause an infection, possibly leading to disease. There is also a probability that the microorganisms will die off or be eliminated by the host's immune system before infection can be established. The outcome after a given exposure depends on characteristics of both the pathogen and the host. For each pathogen, this RA considers evidence for the ability of doses of various sizes to infect humans, and, because a dose of a given size might affect one human differently from another, this RA also considers the potential variability in susceptibility in heterogeneous populations.

J.2 Methodology

Dose-response is assessed by estimating a functional relationship between the amount of exposure, or dose, and the probability of that dose resulting in a response of interest in a randomly chosen individual from a given population. This section describes the general methodology for assessing the relationship between dose and response for each pathogen.

J.2.1 Responses to Exposure

An exposure can result in no detectable response if the initial organisms to which the host is exposed die off, are eliminated from the host, cannot attach to host cells because of lack of or imperfect receptor sites, or are inactivated by the host's immune system. Otherwise, the following potential responses are considered:

Infection — "Invasion and multiplication of microorganisms in body tissues" (Dorland's). A dose resulting in infection is termed an infectious dose (ID), and a dose resulting in infection of a human is termed a human infectious dose (HID). An infection might or might not result in symptomatic disease or subsequent death, but exposure to and infection by the

	microorganism may produce antibodies in the infected host that can be detected by appropriate serodiagnostic tests.
Morbidity	"The condition of being diseased or morbid" (Dorland's); for the purposes of this RA, that refers to disease attributable to infection with the pathogen.
Mortality	Death of a host; for the purposes of this RA, it refers to death attributable to the disease caused by the pathogen. A dose resulting in death is termed a lethal dose (LD).

Infection caused by a pathogen in humans is the response of interest at this stage of the RA. Infection is a prerequisite for morbidity and mortality, which are considered in a subsequent section as estimates of the percentage of infections that eventually lead to symptomatic disease and death, respectively. Infection is an important response to consider because it is also a prerequisite for potential secondary transmission. It is important to note that for certain pathogens, secondary transmission can occur in the absence of clinically evident symptoms in the primary case at the time transmission occurs.

J.2.2 Routes of Exposure

The following routes of exposure have been considered in this RA:

Inhalation	A route of exposure whereby a person inhales droplets or aerosolized particles containing one or more pathogenic microorganisms into the lungs (BMBL 2009).
Ingestion	A route of exposure whereby a person is exposed to a pathogen via ingestion of a liquid or solid contaminated with a pathogen or by contaminated hand-to-mouth exposure (BMBL 2009).
Direct contact	A route of exposure whereby a person is exposed to a pathogen directly to the skin (potentially broken), eye, or mucous membrane. (BMBL 2009)
Puncture	A route of exposure whereby microorganisms are placed below the outermost layer of the skin through a mechanical means such as a syringe needle or other sharp object (BMBL 2009).
Animal-related exposure	A route of exposure which combines animal-related elements of the other routes and that involves exposure to infectious particles from animals (e.g., mammals or arthropods) via bites, scratches, as well as airborne dispersal that could result from such sources as sneezes and saliva. Note that BMBL does not distinguish those animal-related mechanisms as a separate route, but they

are separated here because an animal has the potential to produce exposure via all routes.

Each pathogen has a natural route of infection that is dependent on the biology of the pathogen, natural reservoirs, and typical modes of transmission. In a laboratory setting such as the National Emerging Infectious Diseases Laboratories (NEIDL), some experiments would attempt to simulate the natural route of infection, but it is possible that events occurring during culturing, manipulating, transporting, and storing the pathogen could lead to potential exposures that differ from those that would occur naturally. In such circumstances, it is possible that exposure to a pathogen in a laboratory setting from a route different from its natural route of exposure could result in an infection. *For this RA, it is assumed that any route of exposure resulting from a NEIDL-related event could potentially lead to infection in the exposed individual.*

The probability of infection resulting from a given dose of a pathogen can vary according to the route of exposure. The above routes of infection are considered on a pathogen-by-pathogen basis. Generally speaking, the bulk of the dose-response assessment focuses on the inhalation route of exposure, for the following reasons. Some of the most important event sequences for this RA result in inhalational exposure, and most of the relevant animal dose-response data for many of the pathogens were derived from inhalational exposures. Other routes of exposure are considered, as appropriate, for each pathogen and compared with the estimates generated for dose-response by the inhalational route.

J.2.3 Potentially Exposed Groups

The results of each event sequence analysis are provided in terms of exposures for one or more of the following groups.

Laboratory worker	People working in the biocontainment area where the event might be initiated.
Facility worker	People working in the NEIDL but not in the biocontainment area where the event under consideration occurs. For example, they might work in other laboratories or in administrative areas.
Public	Any person outside the NEIDL-controlled perimeter, specifically referring to the population in the surrounding communities.

For the dose-response assessment, estimates of the probabilities of human infection if exposure occurs are assumed to be equal across all three groups, with the following exceptions.

- Vaccine status—As part of training and preparation for work in a high biocontainment laboratory, it is possible that laboratory workers working with certain pathogens for which a vaccine is available would have received that vaccine to prevent infection. The vaccines might or might not be available to facility workers or the general public. That possibility is considered on a pathogen-by-pathogen basis.

- Post-exposure prophylaxis—The above three groups would possibly have differential access to prophylactic regimens, if available, after being exposed. The availability and effectiveness of medication or vaccine prophylaxis are discussed on a pathogen-by-pathogen basis. Note that many of the release scenarios examined in this RA assume that the incident leading to the release is either undetected or unreported. In such situations, the issue of post-exposure prophylaxis might not be applicable.

- Population susceptibility—Because of inherent heterogeneity in the population with respect to age, immune status, and co-morbid or preexisting complicating conditions, the above three groups likely differ in average susceptibility to infection and in heterogeneity of susceptibility among group members. Those differences might or might not be uniform between members of the public near the three sites compared in this RA. In some cases, where relative susceptibility estimates for a specific population are available, adjustments to dose-response estimates are made.

J.2.4 Quantification of Exposure and Dose-Response

As described in the event sequence analyses, the manner in which exposure estimates are quantified varies by pathogen because estimates of the concentration of pathogens that would be used at the NEIDL are generated from sources that measure pathogen concentration using a variety of metrics. The literature and information available for dose-response assessment also exhibits a variety of measurement techniques used to quantify doses to which potential hosts are exposed. To translate estimates of exposure to estimates of infection probability, the units of exposure or dose must be reconciled, which sometimes requires assumptions for converting one unit of measure to another. Those assumptions are specified on a pathogen-by-pathogen basis.

J.2.5 Dose-Response Modeling

The relationship between the dose received and the probability of infection can be quantified using mathematical dose-response models. A dose-response model takes the form of a mathematical function that uses as input a quantity measuring an expected dose to which an individual is exposed and provides as output an estimate of the probability of infection that would result from that expected dose. Equivalently, the output can be considered to represent the proportion of individuals from a population estimated to become infected from the given expected dose.

Dose-response models are particularly important in estimating the probability of infection after a low initial dose. Many of the event sequences investigated in this RA lead to estimates of exposure to a small dose, sometimes on the order of a single virion or bacterial cell, and experimental data on the effects of such a low dose are scarce or non-existent. The effects of higher doses are better understood, and dose-response models are used to extrapolate information of the effects of higher doses to that of lower doses. That is, the models use information about the height and shape of the dose-response curve in regions where estimates are available, in conjunction with an assumed functional form, to then extend the curve into lower regions where estimates are needed. The assumed functional form varies from model-to-model, sometimes leading to different extrapolated estimates from the same dose-response information. For this RA, three different model forms were considered as described in the following section.

J.2.5.1 Description of Candidate Dose-Response Models

Let d be the dose received, generally quantified as the expected number of organisms with potential to infect a human in the dose. Here and throughout, it should be noted that the general term *organisms,* when referring to doses of exposure, means either bacterial cells or virions. Then, let $p(d)$ be the probability of infection if dose d is received. The mathematical function $p(d)$ describes a dose-response curve. In the literature, dose-response information is often specified as a point or points on a dose-response curve in the form ID_x, where ID stands for infectious dose and x is a number from 0 to 100 representing the percentage chance of infection. In the $p(d)$ formulation, $p(ID_x) = x/100$; for example, the ID_{50} would result in a probability of infection of 0.5.

Three different mathematical forms for the function $p(d)$ are considered, all of which are commonly used in the literature to quantify dose-response relationships for infectious pathogens. They are the exponential model, the log-probit model, and the beta Poisson model.

Exponential model

The exponential model assumes the probability of infection is given by the functional form

$$p(d) = 1 - \mathrm{e}^{-rd}. \quad (1)$$

The single parameter r is a constant defined as the probability that infection is established by a single organism. A single organism establishing infection means that the organism produces descendants in the host that survive to contribute to a sustained population in the host. In that sense, the exponential model is an example of a *single hit* model. The model assumes that multiple organisms act independently in the host. That is, the probability that any one organism in the initial dose produces descendents in an eventual infection is independent of the size of the dose. If the exact number of organisms in the dose is known, the overall probability of infection is simply the complement to the probability that none of the organisms establish infection, or $p(d) = 1 - (1 - r)^d$. The exponential model assumes that there is some uncertainty in the size of the dose. Specifically, it assumes that d is the expected value for the number of organisms in the dose, and the true value varies according to a Poisson distribution with mean d.

The exponential model is appealing because of its simplicity and its basis in clear biological assumptions. The fact that it requires only one parameter means that a single point defines the entire curve. For example, if the ID_{50} is known, then $r = (\ln 2)/\mathrm{ID}_{50}$, and the probability of infection for any dose is defined. A drawback to the exponential model is that it does not assume heterogeneity among organisms or hosts; that is, the probability r is the same for every organism in a dose and also does not change from host to host. Nonetheless, the exponential model has had some success in describing data (e.g., Haas 2002; Tamrakar 2008; Watanabe 2010; Wilkening 2006), and there is merit in choosing a relatively simple model when there is no compelling evidence that a more complicated model would be significantly more accurate.

Log-probit model

The log-probit model assumes the probability of infection is given by the functional form

$$p(d) = \Phi\left(m \ln\left(\frac{d}{\mathrm{ID}_{50}}\right)\right). \quad (2)$$

Here, ID_{50} is a constant parameter referring to the dose at which there is a 50% chance of infection (i.e., $p(\mathrm{ID}_{50}) = 0.5$), and m is a second constant parameter called the probit slope. The function Φ is the cumulative distribution function for the standard normal distribution, and ln is the natural logarithm. Traditionally, the base-10 logarithm was used in the formula, but using the natural logarithm achieves the

same purpose and results in a model that is easily transformed for purposes of curve fitting in a manner consistent with the other two models.

The model was first developed (Bliss 1934) as a convenient method for transforming experimental data into approximately linear form so that regression could more easily be done by hand. The method was popularized for use in applications to toxicology (Finney 1947) and has since become the traditional model used in toxicological risk assessment. The model is still used despite the fact that its originally espoused advantage (ease of hand calculation) is no longer relevant with the advancement of computer technology. Some authors (e.g., Tamrakar 2008) have argued that the log-probit model is not a preferred choice because it is not based on any clear assumptions about biological mechanisms for the establishment of infection. Also, when the probit slope parameter is low, the log-probit model often predicts much higher probability of infection at low doses than other models (Haas 2002), which might lead to unrealistically high estimates of risk under scenarios in which many individuals receive a small dose. Still, data on probability of infection are sparse (particularly for low doses), so researchers are sometimes unable to demonstrate conclusively that the log-probit model is a worse predictor than other models. Some have argued that the log-probit model is an appropriate model when the host population is heterogeneous (e.g., Wilkening 2006); for example, if each potential host has a tolerance (a dose that is just sufficient for establishing infection), and the variation in tolerances across the population is adequately captured by the lognormal distribution, then the log-probit model may be justified (Finney 1947).

Beta Poisson model

The beta Poisson model assumes the probability of infection is given by the functional form

$$p(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}. \quad (3)$$

Here, α and β are constant parameters. The derivation of the beta Poisson model (Furumoto 1967) begins with assumptions similar to those for the exponential model. It begins with the same *single-hit* framework, assuming that organisms act independently. Again, under those assumptions, if the exact dose d is known and if the probability r of a single organism establishing infection is constant, the probability of infection would be given as $p(d) = 1 - (1 - r)^d$. The beta Poisson model, like the exponential model, assumes that the dose d is a random Poisson-distributed variable with mean d, and additionally assumes that the probability r varies according to a beta distribution with parameters α and β. Specifically, the probability density function $f(r)$ describing the beta distribution for r is given by[1]

$$f(r) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)+\Gamma(\beta)} r^{\alpha-1}(1-r)^{\beta-1}. \quad (4)$$

[1]In equation (4), Γ refers to the Gamma function, defined $\Gamma(z) = \int_0^\infty t^{z-1}e^{-t}\,dt$

Those assumptions alone lead to a dose-response model with a complicated functional form involving the Kummer confluent hypergeometric function (see Haas 1999). The simpler beta Poisson formula above is an approximation to the exact formula, and the approximation is valid when $\beta \gg 1$ and $\beta \gg \alpha$.[2] Note that, because of that approximation, the beta Poisson model is not technically a single-hit model (Teunis 2000). It was decided that use of the simpler formula was suitable for the purposes of this RA.

The beta Poisson model is appealing in that it is a simple model based on well-defined assumptions about both the biological mechanisms of establishing infection and heterogeneity between organisms and/or hosts. There has been some success in using the model to fit experimental data (Tamrakar 2008; Huang 2010).

Some dose-response models are in the literature that are not among the three listed in this section. Many are more complicated models with several parameters that are difficult to specify accurately in the absence of a rich set of data, and, therefore, are not necessarily more accurate than a simpler model. Some have argued that threshold dose-response models should be considered in the context of risk assessment (Coleman et al. 2008). Threshold dose-response models (often referred to as *nonlinear* models) assume that there is a dose level below which the probability of infection drops non-smoothly or even drops to zero. The three models from the previous section all result in non-zero estimates of infection probability at any dose no matter how low. Threshold models are not considered for the following reasons.

- The assumption of a threshold dose may be nonconservative (i.e., could underestimate the risk) at doses below the threshold, especially when the model is applied to a population that includes individuals who might be severely immunocompromised or have a lung condition that severely hampers the ability to clear pathogens from the lungs after inhalation.
- The three models considered do allow for the possibility of very small probabilities of infection at low doses, with certain choices of parameter values. For example, the log-probit model with a high probit slope parameter results in a *threshold-like* dose-response curve that is steep, i.e., estimates a quick drop in the infection probability as the dose decreases (Wilkening 2006). If dose-response data exhibit evidence of a steep decline in infection probability as doses decrease, then the log-probit model, at least, could account for this observation.

Assumptions about the spatial distribution of organisms in a dose is another potentially important feature of dose-response models. For example, in an aerosol containing some number of organisms, some of the

[2] The symbol "≫" means "is much greater than."

aerosolized, respirable particles might contain more organisms than others because of the effect of clumping. Then, if exposure occurs among a group of individuals, some individuals could randomly inhale more organisms than others because of a heterogenous spatial distribution in the aerosol. That possibility is modeled explicitly by the exponential and beta Poisson dose-response models, which assume a Poisson-distributed number of organisms around the expected dose. It is possible that actual distributions of doses for pathogens modeled in this RA would be more highly dispersed than the Poisson distribution, leading to higher probabilities for above-average sized doses, although there is generally very little data to support using an alternate assumption. The log-probit model does not explicitly assume a given heterogeneous distribution of doses in a sample of organisms. It is assumed that any uncertainties arising from applying the log-probit model to exposure estimates for aerosols with a heterogeneous spatial distribution are small compared with the overall uncertainty of the dose-response analyses.

J.2.5.2 Evaluation of Dose-Response Models

For this RA, the three models described in the previous section were evaluated in light of the dose-response data and information available in the literature for each of the pathogens. In some cases, researchers have applied one or more of the three models to human dose-response information and/or to experimental data from exposed animals that have been proposed as suitable models for human infection. In these studies, parameters for a particular model were chosen such that the predictions of the model differ from the observations from experiments as little as possible. An appropriate model would produce a dose-response curve with both an appropriate position (for example, the numerical value of the ID_{50}) and an appropriate shape (the steepness of the curve, or how fast the probability of infection increases as the dose increases). The models found in the literature for each pathogen were evaluated, and, in some cases, models were fit to published experimental data that had not previously been fit to one or more of the models considered here. The techniques used for fitting models to published data are described in Section J.3, in conjunction with each data set to which they were applied, as the procedure can vary because of the details of each data set.

In addition, the three dose-response models were used to fit curves to the information obtained from the expert panelists who were asked via the Delphi method to provide ID estimates (Bozzette 2010). The process for fitting curves to the expert-provided values is described in the following section. In this context, the three models were evaluated and compared with each other in terms of their ability to match as closely as possible to the estimates provided by each expert for each pathogen.

J.2.5.3 Fitting Dose-Response Models to Expert-Provided Values

As part of the Delphi process, each expert provided a set of three ID's (ID_{10}, ID_{50}, and ID_{90}) corresponding to three points on a theoretical dose-response curve for each pathogen. Each set of numbers provided by the experts for each pathogen was evaluated separately against the three dose-response models. Because the three dose-response models that were used are one- or two-parameter models, it might not be possible to fit a curve exactly to a set of three points. Instead, an optimization procedure was used for each model to choose parameter values that result in a dose-response curve that comes as close as possible to fitting the three points, using criteria described as follows.

First, the models were rewritten with the probability, p, as the independent variable and the natural logarithm of the dose, $\ln(d)$, as the dependent variable:

$$\begin{aligned} &\text{Exponential model:} && \ln(d) = \ln(1/r) + \ln(-\ln(1-p)) \\ &\text{Log-probit model:} && \ln(d) = \ln(\mathrm{ID}_{50}) + \frac{1}{m}\Phi^{-1}(p) \\ &\text{Beta Poisson model:} && \ln(d) = \ln(\beta) + \ln\left[(1-p)^{-1/\alpha} - 1\right] \end{aligned} \quad (5)$$

The reason for setting p as the independent variable is that the experts were given values of p (0.1, 0.5, and 0.9) for which to predict the corresponding dose, and therefore it is natural to evaluate the models as predictors of the dose given the probability. The reason for setting the logarithm of the dose as the dependent variable (rather than the dose itself) is to avoid putting too much weight on errors at the higher probability levels, where the dose predictions can be many orders of magnitude higher than doses at lower probability.

For each set of three points, optimal values for model parameters were calculated using least-squares optimization. Specifically, the optimal parameter values are those that minimize the sum of the squared differences between the log-dose values predicted by the model and the log of the dose values predicted by the expert. Specific optimization procedures used for each of the three models are described as follows.

Exponential model fitting

To simplify the exponential model,

$$\ln(d) = \ln(1/r) + \ln(-\ln(1-p)), \quad (6)$$

the three (p, d) data pairs from each expert were transformed into (x, y) pairs according to

$$x = \ln(-\ln(1-p)) \quad \text{and} \quad y = \ln(d), \tag{7}$$

and the parameter a_1 was introduced as

$$a_1 = \ln(1/r). \tag{8}$$

Then, the exponential model has the linear form

$$y = a_1 + x, \tag{9}$$

for which the optimal value for the parameter a_1 was calculated using the `lm` (linear model) function in R (version 2.9.2) for linear least-squares fitting.

Log-probit model fitting

To simplify the log-probit model,

$$\ln(d) = \ln(\mathrm{ID}_{50}) + \frac{1}{m}\Phi^{-1}(p) \tag{10}$$

the three (p, d) data pairs from the expert were transformed into (x, y) pairs according to

$$x = \Phi^{-1}(p) \quad \text{and} \quad y = \ln(d), \tag{11}$$

and the parameters a_2 and a_3 were introduced as

$$a_2 = \ln(\mathrm{ID}_{50}) \quad \text{and} \quad a_3 = \frac{1}{m}. \tag{12}$$

Then, the log-probit model has the linear form

$$y = a_2 + a_3 x, \tag{13}$$

for which the optimal value for the parameters a_2 and a_3 were calculated using the `lm` function in R (version 2.9.2) for linear least-squares fitting.

Beta Poisson model fitting

To simplify the beta Poisson model,

$$\ln(d) = \ln(\beta) + \ln\left[(1-p)^{-1/\alpha} - 1\right], \tag{14}$$

the three (p, d) data pairs from the expert were transformed into (x, y) pairs according to

$$x = (1-p)^{-1} \quad \text{and} \quad y = \ln(d), \tag{15}$$

and the parameters a_4 and a_5 were introduced as

$$a_4 = \ln(\beta) \quad \text{and} \quad a_5 = 1/\alpha. \tag{16}$$

Then, the beta Poisson model has the nonlinear form

$$y = a_4 + \ln(x^{a_5} - 1), \tag{17}$$

for which the optimal values for the parameters a_4 and a_5 were calculated using the `nls` function in R (version 2.9.2) for nonlinear least-squares fitting, which uses the Gauss–Newton algorithm (see Fletcher 1987). For some of the data sets, the `nls` function did not find a solution because the Gauss–Newton algorithm did not converge. It was determined that in every case for which convergence did not occur, the optimal values of α and β were unbounded. That is, as α and β increase (along the curve defining the optimal relationship between α and β), the fit improves indefinitely. However, as the parameters become very large, the improvement in the fit caused by further increasing α and β becomes infinitesimal. In this case, an alternate procedure was used to determine estimates for α and β, in which the parameters were changed step-by-step until the dose-response curve at one step was the same as the curve from the previous step, within a small tolerance.

J.2.5.4 Comparison of Dose-Response Model Fits to Expert-Provided Values

The relative success of the three dose-response models in fitting each of set of three points was compared using the Bayesion information criterion (BIC) (Schwarz 1978), which is a criterion used for selecting among models with different numbers of parameters. The beta Poisson and log-probit models each have one more parameter than the exponential model, which gives them an advantage in their ability to fit the data, but additional parameters can result in overfitting. For example, many different three-parameter models could fit all three points exactly but could give very different predictions for interpolated or extrapolated points away from the data points themselves, and, therefore, use of a particular three-parameter model could be misleading. The BIC evaluates the fit of each model using the likelihood (the probability density of the data at the best-fit model parameter(s)) and also includes a penalty term that increases for a higher number of parameters.

The BIC is calculated using

$$\text{BIC} = -2\ln(L) + k\ln(n), \tag{18}$$

where L is the likelihood for the optimized model ($\ln(L)$ is calculated using the R function `logLik` on the results of the objects produced by the `lm` and `nls` functions used for curve fitting), k is the number of parameters (1 for exponential and 2 for log-probit and beta Poisson), and n is the number of data points (3 for all models). According to this criterion, the model with the lowest value of BIC is the preferred model. Note that some of the three-point sets fit perfectly to the log-probit model, and in these cases the BIC is negative infinity. For comparing models, the conservative assumption was made to eliminate a curve from consideration only if its BIC number was more than six greater than that of another curve. According to one source (Raftery 1995), a difference of more than six constitutes strong evidence that the model with the higher value can be rejected in favor of the other. The results are generated as ΔBIC values, calculated by taking the difference between the BIC value and the lowest of the three BIC values for each set of points. A ΔBIC of zero means the corresponding model scored the lowest, and a ΔBIC greater than six led to the elimination of that model from consideration.

For each pathogen, one or more dose-response curves from each expert was left in consideration after eliminating curves according to the BIC. The eliminated curves from each expert can be considered models that, when compared to the retained model(s), do not adequately represent the estimates provided by that expert. Given the importance of the choice of curve in determining risk and the fact that the experts did not reach consensus, it was decided not to choose a representative expert or a particular dose-response model for purposes of this RA. Instead, each expert's curve or set of curves was given equal weight in estimating the probability of infection. If more than one curve was left in consideration for a particular expert, each curve was given equal weight in determining the probability estimations from that expert. Because there were eight experts, if a single curve was left in consideration for an expert, that curve was weighted with 1/8 probability. If two curves were left in consideration for an expert, each of those curves was weighted with 1/16 probability. If all three curves remained for an expert, each of those curves was weighted with 1/24 probability. In that way, a distribution was defined for the probability of infection given any particular dose.

J.2.6 Synthesis of Dose-Response Information

For each pathogen, the qualitative and quantitative information was synthesized to provide a range of estimates for the probability of infection at doses of exposure that might occur as a result of NEIDL-related events. Two sets of quantitative estimates are provided for each pathogen:

1. A literature-based dose-response estimate, consisting of one of the three models described in Section J.2.5.1 along with a point estimate and uncertainty range for the parameter value(s) associated with that model.
2. A range of dose-response estimates derived from the expert-provided values, consisting of a distribution that includes all three models with fitted parameter values as described in Section J.2.5.4.

The two alternate ranges of estimates were compared, especially for low doses at which most of the exposure estimates for the event sequences occur. In some cases, the literature-based range is more conservative (estimates higher risk) than the expert-based range, and in other cases the opposite is true. All results are presented and the differences discussed in conjunction with each pathogen.

Both distributions of infection probabilities derived from the dose-response models presented in this appendix are applied to the exposure estimates in the initial infections calculation packages associated with each relevant event sequence. The uncertainty in the probability of infection described by the distributions of dose-response curves contributes to the overall uncertainty in the estimate of risk posed by each event sequence. The relative contribution of the dose-response uncertainty compared to other sources of uncertainty is evaluated in sensitivity analyses performed in conjunction with each event sequence.

J.3 Results

This section documents the dose-response assessment for each of the 13 pathogens.

J.3.1 *Bacillus anthracis*

Bacillus anthracis (*B. anthracis*) is a bacterial organism that causes anthrax and has caused infection in humans in both natural and laboratory settings, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.1.1 Routes of Exposure

Natural routes of human exposure for *B. anthracis* are cutaneous, inhalational, and gastrointestinal. From a release in or from a laboratory, possible routes of exposure could be inhalation, ingestion, direct contact, puncture, or animal-related (nonhuman primates [NHP] or rodents). The inhalational route is the focus of

the remainder of this assessment. Other routes of exposure in the laboratory and the ID for those other routes as compared to the inhalational route are addressed in the description of specific scenarios such as needlestick exposure or exposure to skin/mucous membranes.

J.3.1.2 Vaccine and Prophylaxis

There is an FDA-licensed pre-exposure vaccine for anthrax with the trade name BioThrax®, also called Anthrax Vaccine Absorbed (AVA), produced by Emergent BioSolutions, Inc., Rockville, Maryland. Centers for Disease Control and Prevention (CDC)-reported guidelines (CDC 2000, 2002) state that pre-exposure use of this vaccine should be based on quantifiable risk of exposure and recommend the vaccine for workers in settings in which repeated exposure to aerosolized *B. anthracis* spores might occur, including certain laboratory workers. Those guidelines do not recommend the vaccine for members of the general population who do not engage in work that places them at risk for repeated exposures. The vaccine would not prevent infection with *B. anthracis* cells as defined in this RA, but rather is designed to promote immune responses in the vaccinated host that effectively neutralize the toxins produced by *B. anthracis* bacteria that cause anthrax disease (Joellenbeck 2002). A precursor to the available vaccine was estimated to be 92.5 percent effective in preventing anthrax disease in a placebo-controlled field study involving at-risk mill workers (Brachman 1962).

For non-vaccinated individuals who might have been exposed to aerosolized *B. anthracis* spores, the CDC recommends the following post-exposure prophylaxis: 60 days of selected oral antibiotics in conjunction with a 3-dose regimen of BioThrax AVA vaccine, a combination that has proven effective in NHPs exposed to *B. anthracis* (CDC 2000). Antibiotics taken by exposed individuals might prevent infection if applied before inhaled spores germinate and reproduce, while the vaccine could promote an immune response to neutralize disease-causing toxins originating from active *B. anthracis* cells.

For this RA, it is assumed that most NEIDL laboratory workers assigned to work with *B. anthracis* in biosafety level (BSL)-3 labs would have received the anthrax vaccine before a potential exposure event. As noted above, the available vaccine does not prevent infection with *B. anthracis* organisms, so the dose response estimates for laboratory worker infection is assumed to be unaffected. However, vaccinated laboratory workers who become infected would have a low (less than about 10 percent based on the literature) probability of developing anthrax disease. It is also likely that recognized and reported mishaps in the laboratory involving *B. anthracis* would result in potentially exposed workers receiving the recommended prophylactic regimen, which could reduce the probability of both infection and disease. However, this RA focuses on laboratory incidents that are most likely to be undetected, in which case

prophylaxis would likely not be administered early enough to prevent infection. Those assumptions have no impact on the estimated risk posed by laboratory workers to the public, as *B. anthracis* is not transmissible person-to-person by the inhalational route, whether or not a primary case develops symptomatic disease. One possible exception to the direct person-to-person transmission of *B. anthracis* would be carriage of spores on a laboratory worker's skin or clothing that could transfer to a contact and cause skin disease (WHO 2008; Freedman 2002), but this potential scenario is not relevant to the issue of prophylaxis for inhalational anthrax.

It is possible that some members of the public in the vicinity of NEIDL would have received the anthrax vaccine because of their occupation (e.g., military personnel). However, those individuals likely make up a small percentage of the general public, so it is conservatively assumed that no members of the public would be vaccinated against anthrax before potential exposures from a NEIDL-related release. If exposures occurred in the general public, it is possible that prophylactic treatment, if administered in time, would prevent infection and/or disease that would otherwise have occurred. This possibility would be more likely for exposed individuals experiencing a long incubation period. Those issues are discussed in more detail in the sections of this RA related to potential health consequences of specific events resulting in direct public exposure.

J.3.1.3 Dose-Response Information from the Literature

This section consists of a review and discussion of selected literature and information relating to the probabilistic estimates of infection for humans exposed to a given number of *B. anthracis* spores via the inhalational route. The literature review was performed according to the methodology outlined in Chapter 3. This section concludes with a summary of the procedure for dose-response modeling of *B. anthracis* to be used in this RA, in light of the pathogen information and discussion provided here.

Human data and evidence

There are very little experimental, quantitative data for human exposures to *B. anthracis*. Data exist from human experimentation in Japan during World War II, but the experiments were done via subcutaneous and oral routes, not inhalational. The resulting data (Harris 1999) have been difficult to interpret and difficult to reconcile with epidemiological knowledge of subcutaneous infections (WHO 2008). Those data are not considered further.

Semi-quantitative data exist for exposure of non-vaccinated industrial workers handling animal products contaminated with *B. anthracis* in the early-mid 1900s. That historical evidence suggests that the

infection rate for humans exposed in this setting is very low, especially for inhalational anthrax, as most of the infections that did occur were cutaneous (WHO 2008). Workers in one mill were shown to be inhaling hundreds of spores daily with not a single infection documented (Dahlgren 1960). In a recent, detailed analysis of this study and others, the authors concluded that 600 spores or fewer would not be expected to cause disease in healthy humans and advocated the use of 600 spores as a threshold to use in risk assessments (Cohen 2007). However, it is possible that the industrial workers had developed resistance to infection from repeated low-level exposure, that there were undiagnosed cases, and/or that infections would result from low-dose exposures among members of a population with unusual susceptibility (Brachman 1966).

In another historical event, *B. anthracis* spores were accidentally released from a facility in Sverdlovsk in the former Soviet Union in 1979, causing infections in both humans and animals downwind of the facility. One study (Meselson 1994) tabulated 77 likely human cases, 66 resulting in death. Doses inhaled by the infected individuals are not known, and it is also not known how many spores were released from the facility. However, that study and others include estimates of human dose-response information using atmospheric data on the day the release likely occurred, the likely locations of the infected individuals when they were exposed, and epidemiology of the tabulated cases.

Meselson et al. (1994) calculated that the attack rate at a ceramics factory 2.8km downwind of the Sverdlovsk release was approximately 1–2 percent (18 out of about 1,500 employees infected, including 10 out of 450 employees working in a single unpartitioned building). That suggests that the average dose received at that distance would have been roughly the ID_1 or ID_2 for humans. They applied lower and upper limit ID_2 estimates of 9 spores and about 1,300 spores to calculate that the weight of material released from the facility was between a few milligrams to nearly one gram.

Wilkening et al. (2006) analyzed the Sverdlovsk case data and applied a series of theoretical dose-response models. They determined that both the spatial (distance from release) and temporal (incubation period, assumed to vary with dose) distribution of cases are consistent with dose-response curves that are less steep, i.e., curves that estimate a gradual decrease in the probability of infection as the dose decreases. They conclude that the data contraindicate dose-response functions that assume a threshold of infection, such as the extreme case of a step function, which assumes a dose below which no one is infected and above which everyone is infected and is the steepest possible curve. This conclusion that gradual curves perform better than threshold-like curves holds regardless of the exact dose numbers assumed. Under an assumption of 8,600 spores as the ID_{50}, the two best of the tested models predict ID_{10}

of 110 and 1,300 spores. Those low-dose results are dependent on the assumption of the ID_{50} value; much different values are accommodated by adjusting the number of spores released, which is a fitted parameter in the models.

Summary of human data and evidence:

- No human experimental data are available for inhalational anthrax.
- Non-vaccinated industrial workers were shown to be inhaling hundreds of spores daily with infections occurring very rarely.
- *B. anthracis* spores released from a facility in Sverdlovsk in 1979 caused dozens of human infections in the nearby community; spatial and temporal distributions of the documented cases are consistent with less steep, non-threshold-like dose-response curves.

NHP data

Glassman (1966) reports on data from unpublished work performed by Jemski in which 1,236 cynomolgus monkeys (*Macaca fascicularis*) were exposed to aerosols of *B. anthracis*. The data showed an LD_{50} of 4,130 spores (95 percent confidence interval 1,980 to 9,630) and were fit to a log-probit model resulting in a probit slope of 0.667 probits per log dose (95 percent confidence interval 0.520 to 0.818). Extrapolation under the log-probit model assumptions, with LD_{50} of 4,130 and probit slope of 0.667, results in LD_{10} of 50 spores, LD_2 of 3 spores, and LD_1 of 1 spore. The raw data from the experiments are not presented, and it cannot be determined if any of the monkeys were exposed to low doses and, if so, if any of those doses proved fatal. Furthermore, without the full data set it is not possible to evaluate whether alternate dose-response models would have fit the data better than the log-probit model, which has been outperformed by other models in fitting other data sets (Haas 2002). For example, if the exponential model had been applied with the same LD_{50} of 4,130 spores, extrapolations result in LD_{10} of 628 spores, LD_2 of 120 spores and LD_1 of 60 spores.

Brachman et al. (1966) collected data from exposures of cynomolgus monkeys (*M. fascicularis*) to contaminated air from a goat hair mill. They reported that exposure to about 1,000 *B. anthracis*-bearing particles over a 5-day period resulted in approximately 10 percent mortality, and exposure to 3,500 to 5,500 particles resulted in approximately 20–25 percent fatality. Haas (2002) analyzed the original data using a more sophisticated quantitative procedure and derived an exponential model providing a good fit to the daily risk of mortality. Applying the fitted exponential model parameter and associated 95 percent likelihood-based confidence interval gives the following results: LD_{50} of 27,000 (18,000–41,000), LD_{10} of 4100 (2,700–6,300), LD_2 of 780 (530–1,200), and LD_1 of 390 (260–600). It should be noted that, in this

case, the average daily exposures ranged from 198 to 1041 *B. anthracis*-bearing particles, so the derived LD_1 and LD_2 estimates are in the range of the data points and not extrapolations from results at much higher doses.

Druett et al. (1953) exposed rhesus monkeys (*Macaca mulatta*) to aerosols of *B. anthracis* spores resulting in a range of inhaled doses estimated between about 70,000 to 400,000 spores. They reported an estimated LD_{50} of 45,000 spores, which was derived from a log-probit regression. Haas (2002) reanalyzed the data set and determined that the log-probit model did not provide an improved fit over the exponential model, which results in the following estimates (with 95 percent likelihood-based confidence intervals): LD_{50} of 96,800 (70,700–136,000), LD_{10} of 14,700 (10,800–20,700), LD_2 of 2,820 (2,060–3,960), and LD_1 of 1,400 (1,030–1,970).

Haas (2002) synthesized and discussed the results from the above three NHP studies (Druett 1953; Brachman 1966; Glassman 1966) and determined that the exponential model can describe dose-response to inhalational anthrax in NHPs, and that low doses are predicted to produce a low, but nonzero, risk of disease. He states that other studies publishing different dose-response relationships do not provide the data necessary to evaluate the basis for the differences.

Summary of NHP data:

- A report on results from exposure of 1,236 cynomolgus monkeys gave an estimated LD_{50} of 4,130 spores and a fitted parameter for the log-probit dose-response model. The full data set is not published, so it is not possible to evaluate whether low-dose extrapolations based on those results are appropriate.
- For a different data set on exposure of cynomolgus monkeys to relatively low doses, an exponential dose-response model with an LD_{50} estimate of 27,000 spores was determined to provide an adequate fit.
- For a data set on exposure of rhesus monkeys to relatively high doses, an exponential model was selected from among alternate dose-response models, providing an LD_{50} estimate of 96,800 spores.

Table J–1a displays the above results and also compares low-dose estimates derived from the three models. The results are displayed down to $LD_{0.01}$, which represents the expected dose at which the mortality rate is estimated to be 1 in 10,000 (0.01 percent). Fractional doses (less than one) can be interpreted as an average dose over a population of potentially exposed individuals in which some

individuals would receive a dose of zero. The variation in actual doses received across individuals is modeled by the Poisson distribution. For example, an LD_1 of 0.5 would mean that if a population were exposed to an average dose of 0.5 per individual, a 1 percent mortality rate would be expected. In this example, a Poisson-distributed dose with average 0.5 means that approximately 61 percent would receive no dose, 30 percent of individuals would receive a dose of one organism, and 9 percent would receive two or more organisms.

Table J–1a. LD estimates (with 95% confidence intervals) for NHPs from *B. anthracis* dose-response models from the literature

Set	Animal	Model	LD_{50}	LD_{10}	LD_1	$LD_{0.1}$	$LD_{0.01}$
A	*Macaca fascicularis* (Glassman 1966)	LP	**4130 spores** (1980–9630)	**50** (14–110)	**1** (0.1–6)	**0.1** (0.005–0.7)	**0.01** (0.0003–0.1)
B	*Macaca fascicularis* (Brachman 1966, Haas 2002)	Exp	**27000 spores** (18000–41000)	**4100** (2700–6300)	**390** (260–600)	**38** (26–60)	**3.8** (2.6–6.0)
C	*Macaca mulatta* (Druett 1953, Haas 2002)	Exp	**96800 spores** (71000–140000)	**14700** (11000–21000)	**1400** (1000–2000)	**140** (100–200)	**14** (10–20)

Each LD_x result refers to the expected inhaled dose estimated to result in death of *x*% of exposed animals. For example, $LD_{0.01}$ refers to the dose estimated to result in death of 0.01%, or one in ten thousand animals. For the models, LP = log-probit and Exp = exponential. For data set A, the 95% confidence interval for LD_{50} was reported in the cited paper, and the intervals for LD_x, $x < 50$, were calculated using the reported best-fit LD_{50} and varying the probit slope by the interval reported in the paper. For data sets B and C, the 95% confidence intervals are likelihood-based. The model fit to Set B was chosen as the literature-based model for this RA, as described in Section J.3.1.4.

J.3.1.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published models for *B. anthracis* outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The body of human evidence with regard to inhalational anthrax suggests that humans have a moderate level of resistance to infection (WHO 2008), given the relative rarity of human cases among animal workers who likely inhaled spores repeatedly. Those cases that have occurred among humans could be the result of inhalation of an unusually high number of spores, rather than rare low-dose infections from the tail end of a probability distribution (Cohen 2007; Coleman 2008). Researchers making that argument recommend that risk assessments include discussion of the possibility that there is a threshold to human infection below which the probability of infection drops nonlinearly (Coleman et al. 2008).

However, another possible explanation for rare human cases is that some of those individuals did inhale low doses and became infected because they were unusually susceptible to disease because of an immunocompromised or pulmonary condition. About the human cases after the Sverdlovsk release, a World Health Organization anthrax report (WHO 2008) states, "many of those who succumbed had predisposing respiratory illness." The Cohen and Whalen (2007) paper, in which the authors recommend 600 spores as an appropriate threshold to human infection to use in risk assessments, states, "an exposure of 600 spores per day would not be expected to cause disease in a healthy individual who is not egregiously predisposed to anthrax or lung disease, or is immuno-compromised." Because the dose-response relationships developed for this RA are being applied to populations containing immunocompromised and potentially predisposed individuals, it was determined that applying that threshold would not be appropriate.

The study of Wilkening et al. (2006) of the Sverdlovsk data gives further support to the decision to avoid applying threshold-like dose-response relationships to a general population. They demonstrated that the distribution of cases is better explained by dose-response curves that are less steep, such as those produced by exponential models or log-probit models with a lower probit slope. Objections to the Wilkening results seem to focus criticism on the numerical results presented for potential probabilities of infection at low doses (Cohen 2007; Coleman 2008) and do not speak to the result regarding the shape of the dose-response curve, which holds regardless of the numerical ID values assumed. Given that the Sverdlovsk incident is the historical event most similar to the large-scale release scenario analyzed for this RA, it was determined that the application of dose-response curves similar in shape to those retained by Wilkening et al. (2006) would be appropriate.

In the absence of human data to further refine potential quantitative dose-response models, experimental studies involving NHPs provide the best available data from which to gain insights into potentially appropriate dose-response relationships for humans. A number of assumptions are required to apply results from NHP data to an analysis of risk to humans. The data from the studies above tabulated the response of death to generate estimates of LD or lethal dose, which is assumed to be equivalent to ID or infectious dose because the monkeys in the experiments did not receive treatment to combat infection and pulmonary anthrax is nearly always fatal if untreated. There might be species differences in susceptibility to infection between the NHPs tested and humans. In the case of the Brachman (1966) study, the cynomolgus monkeys were exposed to spores from a factory where humans worked, and some monkeys died from exposure to average daily doses less than 600 spores, which was the threshold below which humans did not become infected as determined by Cohen and Whalen (2007). Therefore, it is surmised

that applying a dose-response relationship derived from cynomolgus monkeys to humans would be conservative and would not underestimate the risk to humans. Such a model might significantly overestimate risk to humans, but the possibility that the estimates are relevant to human susceptibility cannot be fully ruled out in the absence of human data.

Log-probit regressions were traditionally performed to fit experimental monkey data to a dose-response curve (Druett 1953; Glassman 1966). These log-probit curves have been used to extrapolate probabilities of infection at doses much lower than were actually used in the experiments, often resulting in estimated probabilities of infection that are controversially high at doses of just one or a few spores. There appears to be no evidence that a monkey has ever developed infection after inhaling such a low dose. Furthermore, there is no compelling reason to place faith in extrapolations from the log-probit model, which is not based on any assumed mechanisms of infection or on any proven assumptions about the heterogeneity of susceptibility in monkeys. It appears to have been used primarily because of tradition and because it provided a convenient transformation through which regressions could easily be done by hand before the advent of statistical computing.

Haas (2002) evaluated alternatives to the log-probit model in light of the best available NHP data from the literature and concluded that the exponential model, which tends to estimate a significantly lower probability of infection at low doses than the log-probit model, performed better. For example, the log-probit model published by Glassman (1966) dramatically over-predicted the risk of mortality demonstrated by monkeys in the Brachman (1966) study, which were subjected to relatively low doses. The exponential fit to the Brachman data derived by Haas (2002) adequately described the data. Given the fact that the monkeys in the Brachman study were exposed to much lower doses than in other studies, it was determined that a model fitting these data offers the most potential insight into what the response of humans to low doses might be.

In addition to being the best-fit model to the Brachman data, the exponential model is consistent with the result from Wilkening (2006), in that the shape of the exponential curve over a range of doses is consistent with the spatial and temporal distribution of human cases observed after the Sverdlovsk release. Furthermore, the exponential model is appealing because it can be derived from assumptions about the mechanisms of the establishment of infection in the lungs, a general approach advocated by several authors (e.g., Brookmeyer 2005; Gutting 2008; Coleman et al. 2008).

The exponential model assumes that the probability p of infection given an expected inhalation of d spores is given by $p = 1 - e^{-rd}$, where r is the fitted exponential parameter equal to 2.6×10^{-5}. The statistical uncertainty with respect to this parameter value in light of the data can be assessed using different techniques. The likelihood-based confidence interval presented in Table J–1a is an estimate based on assuming that properties of the likelihood of various parameter values in light of the data are well approximated by the χ^2 distribution. An alternative approach is to use a bootstrap procedure (Haas 1999), in which randomized bootstrap replicates of the data set are created and fit to the model to create a simulated uncertainty distribution of the parameter value. This technique creates a set of parameter values that can be sampled when conducting the uncertainty analysis for the overall initial infections risk analysis. When applied to the Brachman data, the bootstrap distribution of the r value results in an estimated 95 percent confidence interval of (1.5×10^{-5} to 4.7×10^{-5}), which is slightly wider than the likelihood-based interval and therefore potentially more likely to contain the true value. For those reasons, the bootstrap distribution is applied for uncertainty analysis of this model for this RA.

At an expected dose of 600 spores, the chosen literature-based model estimates a probability of infection at about 1.5 percent (95 percent confidence range 0.9 to 2.8 percent). This estimate would appear to run counter to the conclusion by Cohen and Whalen (2007) that 600 spores can be used as threshold in risk analysis. For example, a risk assessment estimating a 1 percent infection rate for visitors to a contaminated building would likely not conclude that building is safe for the general public. However, closer examination of the authors' statements reveals that the 600 spore threshold is recommended for healthy individuals. It is not unreasonable to conservatively assume that 1 to 3 percent of a general population might be unusually predisposed or immunocompromised such that exposure to 600 spores or less could result in infection where it did not for populations of mill workers.

J.3.1.5 Dose-Response Estimates Derived from Expert Panel

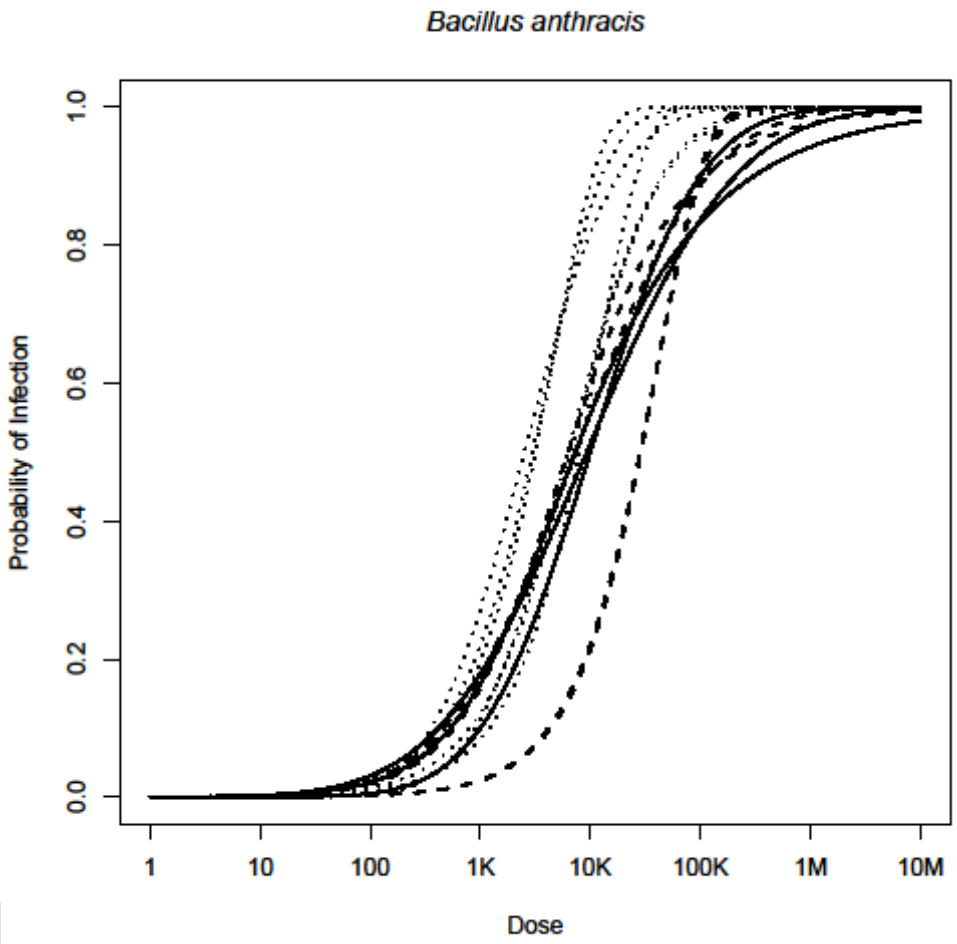
In the procedure described in Section J.2.5.4, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure are displayed in Table J–1b, including which curves were retained from each expert according to the BIC. Fourteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–1. ID estimates provided by the retained curves are shown in Table J–1c.

Table J–1b: Dose-response model fitting for *Bacillus anthracis*

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log-probit model		Beta Poisson Model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	100 5,000 50,000	8.8×10^{-5}	0.66	6,300	1.3	9.3×10^{3}	**5.2**	**0**	**1.7**
2	4,000 30,000 100,000	2.4×10^{-5}	0.80	23,000	1.1×10^{1}	4.2×10^{5}	**1.1**	8.9	**0**
3	400 10,000 200,000	5.9×10^{-5}	0.41	9,300	5.1×10^{-1}	2.3×10^{3}	18.1	**0**	10.1
4	1,000 10,000 100,000	5.5×10^{-5}	0.56	10,000	8.9×10^{-1}	8.2×10^{3}	N/A	**Exact**	N/A
5	400 10,000 100,000	7.5×10^{-5}	0.46	7,400	6.5×10^{-1}	3.3×10^{3}	8.1	**0**	**2.9**
6	1,000 10,000 100,000	5.5×10^{-5}	0.56	10,000	8.9×10^{-1}	8.2×10^{3}	N/A	**Exact**	N/A
7	200 8,000 10,000	2.2×10^{-4}	0.66	2,500	3.5	1.4×10^{4}	**0**	**1.7**	**1.0**
8	500 8,000 300,000	5.2×10^{-5}	0.40	11,000	4.6×10^{-1}	2.1×10^{3}	17.1	6.6	**0**

Fitted parameters for three dose-response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–1



Plot of all dose-response curves retained from fitting models to the information provided by the experts as part of the Delphi process, used to estimate probability of infection for each dose. Solid curves are weighted with 1/8 probability; dashed curves are weighted with 1/16 probability; dotted curves are weighted with 1/24 probability.

Table J–1c. Results for retained expert-derived *B. anthracis* dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	Exp	7,900	1,200	110	11	1.1
	LP	6,300	890	180	56	22
	BP	6,700	800	73	7.3	0.73
2	Exp	29,000	4,400	420	41	4.1
	BP	28,000	4,200	400	39	3.9
3	LP	9,300	420	33	5.2	1.1
4	LP	10,000	1,000	150	39	13
5	LP	7,400	470	49	9.5	2.4
	BP	6,300	580	51	5.1	0.51
6	LP	10,000	1,000	150	39	13
7	Exp	3,200	480	46	4.6	0.46
	LP	2,500	360	72	23	8.6
	BP	3,000	420	39	3.9	0.39
8	BP	7,300	540	46	4.5	0.45
Median (Min–Max)		**8600 org.** (2500–29000)	**690** (360–4400)	**73** (33–420)	**10** (3.9–56)	**2.4** (0.39–22)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.01}$ refers to the dose estimated to result in infection of 0.01%, or one in ten thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

Note that the estimates derived from Expert 2 are close to the estimates derived from the chosen dose-response model from the literature. That can be explained by the fact that the expert was an author of the study in which the literature-based model was found and was of the opinion that this model is applicable to humans.

J.3.1.6 Other Considerations

It is possible that different strains of *B. anthracis* would have differing levels of infectivity. It is not known which strains would be studied at the NEIDL, nor is it known which strains occurred at the animal factories referenced in Brachman et al. (1966) and Cohen and Whalen (2007), nor is it known what strain or strains were released from the facility at Sverdlovsk. Also, no particular strain or strains were specified to the expert panel members as part of the Delphi process. It is assumed that the uncertainty regarding infectivity of differing strains is captured within the overall uncertainty range for the dose-response parameters.

The units of exposure for events analyzed in this RA for *B. anthracis* are in terms of CFU (colony-forming units), which is assumed to measure the expected number of infectious units with potential to reproduce when inhaled. CFU is not a measure of the absolute number of live cells in a sample, because individual cells may aggregate or clump to form one colony. The exposures reported in Brachman et al. (1966) are in terms of *B. anthracis*-bearing particles, which were quantified using calculations based on colony counts from collected air samples, so it is appropriate to apply the curve derived from these data. The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by CFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates.

J.3.1.7 Summary of Approach

The following summarizes the two sets of dose-response estimates to be applied to exposure data for this RA. ID estimates for *B. anthracis* derived from these models are compared in Table J–1d .

- Literature-based dose-response model: the exponential model with parameter $r = 2.6 \times 10^{-5}$, as derived by Haas (2002) as a fit to low-dose NHP data reported by Brachman et al. (1966). Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (1.5×10^{-5} to 4.7×10^{-5}), for uncertainty and sensitivity analyses.
- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–1c, with the model or set of models derived from each expert weighted equally.

Table J–1d. ID estimates and associated ranges for *B. anthracis*

Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$	$ID_{0.01}$
Literature-based	**27,000 spores** (15000–47000)	**4,100** (2200–7100)	**390** (210–680)	**38** (21–68)	**3.8** (2.1–6.8)
Expert-based	**8,600 org.** (2500–29000)	**690** (360–4400)	**73** (33–420)	**10** (3.9–56)	**2.4** (0.39–22)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–1c.

For each ID point displayed in Table J–1d, the expert-based range extends lower (higher risk) than the literature-based range. Both sets of estimates are applied to the exposure estimates in the initial infection

portions of this RA to determine the implications of each estimate for the overall risk posed by *B. anthracis*.

J.3.2 *Francisella tularensis*

Francisella tularensis (*F. tularensis*) is a bacterial organism that causes tularemia and has infected humans in both natural and laboratory settings as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.2.1 Routes of Exposure

The most common natural routes of exposure and infection with *F. tularensis* for humans include animal-related exposures such as insect bites and direct contact with infected blood or tissue from animals, as well as ingestion of contaminated food or water. Infection from inhaling contaminated dust is also possible. In the laboratory, it is assumed that infection is possible through any of the routes of exposure (inhalation, ingestion, direct contact, puncture, and animal-related). This section focuses on the inhalational route of exposure.

J.3.2.2 Vaccine and Prophylaxis

As of March 2011, none of the vaccines against tularemia that have been developed are approved by the U.S. Food and Drug Administration (FDA) (CDC 2011a). For this RA, it is assumed that no laboratory worker, facility worker, or member of the general public would be vaccinated against infection with *F. tularensis*. That is a conservative assumption because it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that was previously available, a vaccine that might become FDA-approved in the future, or a vaccine that might be available before official FDA-approval, such as in the investigational new drug (IND) category. Currently, there is an IND vaccine against *F. tularensis* available for laboratory workers at any institution to request through the Special Immunizations Program at Ft. Detrick, MD (NRC 2011).

For individuals who have been exposed to aerosolized *F. tularensis* but are not yet showing symptoms, prophylactic treatment with antibiotics is recommended; for possible but unlikely exposures, increased vigilance for signs of fever and readiness to treat symptoms might be sufficient (WHO 2007). Antibiotics taken by exposed individuals may prevent infection if applied before symptoms appear. It is highly likely that recognized and reported mishaps in the laboratory involving *F. tularensis* would result in potentially exposed workers receiving the recommended prophylactic regimen, which could dramatically reduce the

probability of both infection and disease. However, this RA focuses on laboratory incidents that are most likely to be undetected, in which case prophylaxis would likely not be administered early enough to prevent infection. Those assumptions have no impact on the estimated risk posed by laboratory workers to the public, as *F. tularensis* is not transmissible person-to-person, whether or not a primary case develops symptomatic disease. If exposures occurred in the general public, it is possible that prophylactic treatment, if administered in time, would prevent infection and/or disease that would otherwise have occurred. This possibility would be more likely for exposed individuals experiencing a long incubation period. Those issues are discussed in more detail in the sections of this RA related to potential health consequences of specific events resulting in direct public exposure.

J.3.2.3 Dose-Response Information from the Literature

This section consists of a review and discussion of selected literature and information relating to the probabilistic estimates of infection for humans exposed to a given number of *F. tularensis* organisms via the inhalational route. The literature review was performed according to the methodology outlined in Chapter 3. This section concludes with a summary of the procedure for dose-response modeling of *F. tularensis* to be used in this RA, in light of the pathogen information and discussion provided here.

Human data and evidence

There is strong evidence that even small inhaled doses of *F. tularensis* can infect humans. The fact that infection of laboratory workers handling *F. tularensis* has been relatively common even in vaccinated employees, as referenced in Appendix C, suggests that this pathogen is highly infectious to humans and that relatively small inhaled doses might cause infection. Furthermore, data exist from published studies from the 1960s in which human volunteers were exposed to low inhaled doses of *F. tularensis* that resulted in subsequent infection and disease.

A study in which human volunteers were exposed to aerosolized doses (Saslaw 1961b) produced the following data: of 20 non-vaccinated men exposed, 16 (80 percent) subsequently developed disease symptoms and 4 (20 percent) did not. The raw data (Saslaw 1961b) are reproduced in Table J–2a, showing the dose inhaled by each of the 16 positive cases and 4 negative cases. Positive responses occurred after doses ranging from 10 to 52 organisms, and negative responses occurred after doses ranging from 10 to 45 organisms. Unless the measurement of the inhaled doses contained errors of a magnitude comparable to these ranges, the fact that the ranges overlap suggests that there is not a strict threshold to infection that applies to all humans.

Note that in a companion study (Saslaw 1961a), 12 non-vaccinated human volunteers were exposed to about 10 organisms of *F. tularensis* via intracutaneous inoculation on the right forearm, and all responded with local infection and eventually with mild to severe systemic disease. From that evidence, it is possible that the direct contact route of exposure results in a higher probability of infection than the inhalational route given the same dose. It is also possible that intracutaneous inoculation resulted in greater certainty that the intended and measured dose was received by each volunteer compared to the delivery of aerosols. Regardless, the inhalational route remains the focus of this dose response assessment, as inhalation of infectious particles is more likely to occur after a potential aerosol release from a NEIDL-related event. Direct contact exposure such as that which occurred in the study (Saslaw 1961a) would require organisms entering under a layer of skin of an exposed person, which would require exceptional circumstances to occur from an exposure to an aerosol. Scenarios in which individuals might be exposed to *F. tularensis* through a puncture or animal-related bite or scratch are considered separately in conjunction with the events relevant to those routes.

Table J–2a. Non-vaccinated human dose-response data (Saslaw 1961b) for *F. tularensis*

Challenge dose (organisms)	Number exposed	Number positive for disease
10	2	1
12	1	0
13	1	1
14	1	1
15	1	1
16	1	1
18	1	1
20	2	1
23	2	2
25	1	1
30	1	1
45	1	0
46	2	2
48	1	1
50	1	1
52	1	1

Tabulated from data presented in Saslaw et al. (1961b). The challenge dose was the measured number of inhaled organisms. Those showing symptoms of disease were deemed positive.

Another report (McCrumb 1961), also tabulated results for human volunteers exposed to aerosols of *F. tularensis*. All 10 non-vaccinated individuals exposed were subsequently positive for disease at doses of 20, 200, or 2000 organisms, as shown in Table J–2b.

Table J–2b. Non-vaccinated human dose-response data (McCrumb 1961) for *F. tularensis*

Challenge dose (organisms)	Number exposed	Number positive for disease
20	4	4
200	4	4
2,000	2	2

Tabulated from data presented in McCrumb (1961). The challenge dose was the measured number of inhaled organisms. Those showing symptoms of disease were deemed positive.

In a third study of inhalational *F. tularensis* exposure of human volunteers (Sawyer 1966), subjects were exposed to aerosols that had been aged for 30, 60, 120, and 180 minutes. The study found that aerosols aged for more than 60 minutes resulted in significantly decreased infectivity, except at the highest doses. Aerosols aged 30 or 60 minutes resulted in five infections out of eight exposed to a dose of 150 inhaled organisms, two out of four at 350 organisms, and four out of four at 750 organisms.

Jones et al. (2005) pooled the human volunteer dose-response data from the three studies described above (Saslaw 1961b; McCrumb 1961; Sawyer 1966) into a single data set and fit dose response models to the data. They employed a non-parametric model function and two parametric functions, one based on the Weibull distribution (a two-parameter extension to the exponential model) and one based on the lognormal distribution (equivalent to the log-probit model). The authors used the latter two models to calculate extrapolated estimates of the probability of infection at lower doses, resulting in an estimate that 20 to 40 percent of a human population would become infected after inhaling a single *F. tularensis* organism.

Animal data and evidence

A recent study (Huang 2011) analyzed data sets from the literature for the responses of animals exposed to doses of *F. tularensis* and fit the data to mathematical dose response models. The study primarily focuses on data from monkeys (*Macaca mulatta*) exposed to variously sized aerosolized particles bearing *F. tularensis* (Day 1972). The smallest particles (2.1 µm) proved the most infectious, with doses between 5 and 65 organisms resulting in death of 14 out of 24 monkeys (see Table J–2c).

Table J–2c. Monkey (*Macaca mulatta*) dose-response data (Day 1972) for *F. tularensis*

Challenge dose (organisms)	Number exposed	Number of deaths
5	6	1
11	6	3
32	6	4
65	6	6

Data from Day and Berendt (1972) and analyzed in Huang and Haas (2011). The challenge dose was the measured number of inhaled organisms on 2.1µm particles.

Huang and Haas (2011) analyzed the data in table J–2c and fit them to time-dose-response models, which are extensions to dose-response models used to estimate the probability of infection by various times after initial exposure. The best-fit model for the smallest particle-size exposure data was the exponential model incorporating the Weibull distribution for time dependence. This model reduces to the exponential model described in Section J.2.5.1 for large times after exposure. The best fit exponential model parameter (r = 0.056) results in an estimated LD_{50} of 12 organisms. The model estimates the probability of infection after an expected dose of one organism to be about 5 percent.

J.3.2.4 Literature-Based Dose Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published models for *F. tularensis* outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The human dose-response data (Saslaw 1961b; McCrumb 1961; Sawyer 1966) described in the previous section provide evidence for the infectivity of low inhaled doses of *F. tularensis* in humans. Jones et al. (2005) fit these data to dose-response models that could be used to estimate probabilities of infection at even lower doses than were used in the original experiments. However, it was determined that the models provided by Jones et al. (2005) would not be appropriate for use in this RA for the following reasons.

- The data do not appear to be extensive enough to support dose-response model fitting, in that they do not reveal a statistically significant trend for increasing probability of infection with increasing dose. Specifically, a binomial regression model fit to the data does not produce a coefficient for the dose (or the logarithm of the dose) that is statistically significantly different than zero. Also, the Cochran-Armitage test of trend (Haas 1999) demonstrates that a null hypothesis of lack of

trend cannot be rejected. Those results do not mean that a dose-response trend does not exist for humans; the data set is not large enough and/or does not consist of a wide enough range of doses to allow an underlying trend to be revealed.

- The models used in Jones et al. (2005) do not provide a statistically acceptable fit to the data according to a chi-squared test on the deviance (Haas 1999) at the given optimal parameter values.
- The Weibull and lognormal models used by Jones et al. (2005) are not based on any assumed biological mechanisms of infection or on any evidence that human susceptibility to infection varies according to those underlying distributions.

Furthermore, it was determined that the results from one of the three human volunteer studies (Sawyer 1966) are not representative of exposure scenarios to be analyzed in this RA. In that study, the aerosols to which the humans were exposed were aged for at least 30 minutes before exposure, whereas aerosols that might be released in or from the NEIDL could reach a potential host substantially faster. For aerosols aged 30 minutes, the study found that two of four humans inhaling 150 organisms and two of four humans inhaling 350 organisms were not infected. While the fact that half the humans tolerated exposure to such relatively high doses might be evidence that some humans are able to withstand a higher amount of exposure, the results might also be explained by diminished infectivity of the aerosols due to aging. Therefore, the application of these data to this RA could result in non-conservative conclusions (underestimates of the risk).

The other two data sets (Saslaw 1961b; McCrumb 1961) shown in Tables J–2a and J–2b were pooled for further analysis for this RA. The pooled data set also does not appear to be extensive enough to justify dose-response model fitting on its own. Nevertheless, the human exposure data set can be analyzed in conjunction with the NHP data and associated models. Namely, it can be tested whether or not the human data set is consistent with a given NHP data set. The Day and Berendt (1972) monkey data set for the smallest particle size and mortality response (Table J–2c) is an obvious candidate for comparison with the human data, as both the range of doses applied and the proportion of subjects responding at similar doses appear to be similar across the two data sets.

To confirm the apparent consistency between the Day and Berendt monkey data and the Saslaw et al. and McCrumb human data, two different statistical procedures were applied. First, a binomial regression was performed on the combined data set, employing coefficients for both the log-dose and a species factor

(monkey or human). The optimal coefficient for the species factor was not statistically significantly different than zero (p-value approximately 0.2), which means the species of a randomly chosen individual does not significantly influence a predictor for the probability of response at a given dose.

Second, a test was performed against a hypothesis that the two data sets come from a common dose-response model. Specifically, as the monkey data were shown to be well fit by an exponential model (Huang 2011), the hypothesis is that the human data set (for disease response) and the monkey data set (for mortality response) come from an exponential dose-response model with the same parameter value (the same value of *r* as described in Section J.2.5.1). The test is performed after fitting each of the two individual data sets and also the combined data set using the method of maximum likelihood, which chooses the optimal value of the exponential model parameter *r* that maximizes the probability of observing each data set under the model. The results are shown in Table J–2d. The optimal value of *r* for each data set is displayed along with a 95 percent confidence interval, which was generated using the bootstrapping procedure described in Haas et al. (1999). The confidence intervals for each data set overlap substantially.

To assess model fit acceptability, the optimal deviance (Haas 1999) for each model is shown and compared to the upper 5^{th} percentile of the χ^2 distribution with degrees of freedom equal to the number of distinct doses in the data set minus the number of parameters in the exponential dose-response model (1). A null hypothesis of fit acceptability cannot be rejected for any of the three data sets as each optimal deviance is less than the corresponding χ^2 statistic. Finally, a procedure was performed to determine if the data sets could be pooled, i.e., a test was performed on the hypothesis that the two data sets come from a common dose-response model. If the difference between the optimal deviance for the combined data set and the sum of the optimal deviances for the individual data sets is more than a given percentile of the χ^2 distribution with one degree of freedom, the hypothesis can be rejected (Haas 1999; Bartrand 2008; Watanabe 2010). Table J–2d shows that pooling of data set A and B can be deemed acceptable under the given statistical test.

Table J–2d. Model fitting for *F. tularensis* data sets

Data Set	Optimal *r* value (95% conf. interval)	Optimal Deviance	$\chi^2_{0.95,\,df}$	Acceptable fit?	Pooling acceptability test
A: Human (Saslaw 1961b; McCrumb 1961)	0.080 (0.040, 0.19)	$Y_A = 14$	28	YES	$Y_C - (Y_A+Y_B) = 1.6$ $\chi^2_{0.95,\,1} = 3.8$ **Acceptable to pool**
B: Monkey (Day 1972)	0.047 (0.025, 0.11)	$Y_B = 1.3$	7.8	YES	
C: Pooled A and B	0.063 (0.040, 0.11)	$Y_C = 17$	33	YES	

Data set A shown in Tables J–2a and J–2b. Data set B shown in Table J–2c. Fit deemed acceptable if optimal deviance is less than the 95th percentile χ^2 statistic with degrees of freedom (df) equal to the number of dose points minus the number of model parameters: df = (17, 3, 21) for data sets (A, B, C), respectively. Pooling of data sets A and B to form data set C deemed acceptable if the difference between the optimal deviance for data set C and the sum of the optimal deviances for data sets A and B is less than the 95th percentile χ^2 statistic with one degree of freedom (df).

Given the results of the statistical comparison between the two data sets, the pooled data set C was determined to be appropriate to use as the basis for a literature-based model to use for application to human infection for this RA. The possibility that a different dose-response model other than the exponential model provides an improved fit the pooled data set was tested. The beta-Poisson model does not decrease the deviance from Y_C (shown in Table J–2d) enough to justify the additional parameter according to the χ^2 test described in Haas (1999). Therefore, the exponential model fit to the pooled data set shown in Table J–2d is retained for use in this RA.

The ID estimates derived from the model fits to the three data sets listed in Table J–2d are displayed in Table J–2e.

Table J–2e. ID estimates from three *F. tularensis* data sets

Data Set	Model	ID_{50}	ID_{10}	ID_1
A: Human (Saslaw 1961b; McCrumb 1961)	Exp	**8.7 org.** (3.7–17)	**1.3** (0.57–2.6)	**0.13** (0.054–0.25)
B: Monkey (Day 1972)	Exp	**15 org.** (6.6–28)	**2.2** (1.0–4.2)	**0.21** (0.096–0.40)
C: Pooled A and B	Exp	**11 org.** (6.1–18)	**1.7** (0.93–2.7)	**0.16** (0.089–0.25)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. The ranges in parentheses are 95% confidence intervals based on model fits to bootstrap replicates from each data set. Exp = exponential model; org. = organisms. The model fit to data set C was chosen as the literature-based model for this RA, as described in Section J.3.2.4.

J.3.2.5 Dose-Response Estimates Derived from Expert Panel

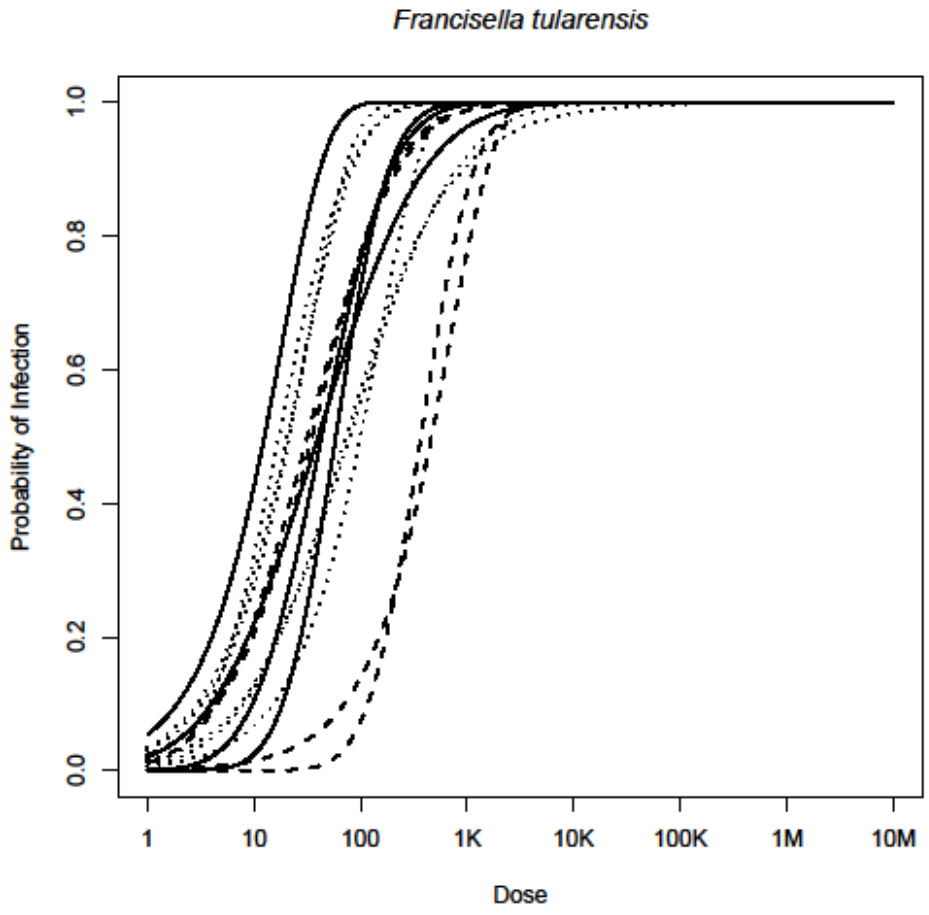
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model-fitting procedure for *F. tularensis* are displayed in Table J–2f, including which models were retained from each expert according to the BIC. Fourteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–2. ID estimates provided by the retained curves are shown in Table J–2g.

Table J–2f: Dose response model fitting for *Francisella tularensis.*

Expert	ID_{10} ID_{50} ID_{90}	Exp. Model	Log−probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	100 500 1,000	1.5×10^{-3}	1.11	370	1.0×10^{5}	6.7×10^{7}	**1.3**	**0**	2.4[a]
2	2 12 41	5.5×10^{-2}	0.85	10	1.0×10^{6}	1.8×10^{7}	**0**	8.5	1.1[a]
3	10 40 2,000	1.3×10^{-2}	0.86	43	2.2×10^{1}	1.7×10^{3}	7.4	**0**	8.5
4	5 10 100	3.2×10^{-2}	0.86	17	4.3	1.2×10^{2}	**1**	**0**	**2**
5	4 40 200	1.7×10^{-2}	0.66	32	1.6	6.6×10^{1}	6.4	**3.2**	**0**
6	4 40 400	1.4×10^{-2}	0.56	40	8.9×10^{-1}	3.3×10^{1}	N/A	**Exact**	N/A
7	10 50 1,000	7.0×10^{-3}	0.56	79	7.8×10^{-1}	5.2×10^{1}	**5.0**	**0.8**	**0**
8	20 50 200	9.4×10^{-3}	1.11	58	1.0×10^{5}	1.1×10^{7}	6.9	**0**	8

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–2



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–2g. Results for retained expert-derived *F. tularensis* dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_1
1	Exp	460	70	6.7
	LP	370	120	46
2	Exp	12	1.9	0.18
3	LP	43	9.6	2.8
4	Exp	21	3.3	0.31
	LP	17	3.8	1.1
	BP	21	2.9	0.28
5	LP	32	4.5	0.91
	BP	35	4.4	0.41
6	LP	40	4.0	0.61
7	Exp	100	15	1.4
	LP	79	7.9	1.2
	BP	73	7.4	0.66
8	LP	58	18	7.2
Weighted Average (Min–Max)		**42 org.** (12–460)	**6.0** (1.9–120)	**1.0** (0.18–46)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.01}$ refers to the dose estimated to result in infection of 0.01%, or one in ten thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

Note that the estimates derived from Expert 2 are close to the estimates derived from the dose-response derived from the literature. That can be explained by the fact that this expert was an author of one of the studies on which the literature-based model was based and was of the opinion that the associated NHP data are applicable to humans.

J.3.2.6 Other Considerations

The data reported in Day and Berendt (1972) and modeled in Huang and Haas (2011) demonstrate that the infectivity of inhaled *F. tularensis* in NHPs can be highly dependent on the size of the aerosolized particles bearing the organisms, with smaller particles resulting in higher probability of infection. The results reported in Section J.3.2.3 are for the smallest particle size tested (2.1 µm), while 7.5-µm particles produced an estimated LD_{50} roughly 44 times higher (Huang 2011). The human volunteer data referenced in Section J.3.2.3 were generated from exposures to aerosols with an average particle diameter of 0.7µ

(Saslaw 1961b). For this RA, exposure estimates are generally assumed to be relevant for particles less than 10 µm in diameter (see Chapter 4), which means that applying the results derived from exposures to only 2.1 µm or 0.7µm particles could significantly overestimate risk. However, it is possible that specific instances of aerosol releases might produce mostly small particles to which laboratory workers or members of the public could be exposed, so it was determined to be appropriate to conservatively assume that the infectivity from small particles is relevant for the events analyzed in this RA.

J.3.2.7 Summary of Approach

The following summarizes the two sets of dose-response estimates to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–2h.

- Literature-based dose-response model: The exponential model with $r = 6.3 \times 10^{-2}$. Use a distribution of r values derived from bootstrap replicates of the pooled data set, which results in a 95 percent range of (3.9×10^{-2} to 1.1×10^{-1}), for uncertainty and sensitivity analyses.
- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–2g, with the model or set of models derived from each expert weighted equally.

Table J–2h. ID estimates and associated ranges for *F. tularensis*

Model	ID_{50}	ID_{10}	ID_1
Literature-based	**11 org.** (6.1–18)	**1.7** (0.93–2.7)	**0.16** (0.089–0.25)
Expert-based	**42 org.** (12–460)	**6.0** (1.9–120)	**1.0** (0.18–46)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–2g. org. = organisms.

For each ID point displayed in Table J–2h, the literature-based range extends lower (higher risk) than the expert-based range. Both sets of estimates are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by *F. tularensis*.

J.3.3 *Yersinia pestis*

Yersinia pestis (*Y. pestis*) is a bacterial organism that causes plague and has infected humans in both natural and laboratory settings as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.3.1 Routes of Exposure

One natural route of exposure and infection in humans is animal-related through bites of infected fleas or bites or scratches from other infected animals. Those types of animal-related exposures can cause primary bubonic plague or, more rarely, primary septicemic plague. Another natural route of exposure and infection in humans is inhalation of droplets containing the bacteria, which can cause primary pneumonic plague. Inhalational exposure can occur from close contact with an animal or human having primary or secondary pneumonic plague (for references, see Chapter 3 and Appendix C).

Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP, rodent, and insect) routes. The primary focus of this section is the inhalational route, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. The inhalational route is especially important to consider for this RA because it could lead directly to the pneumonic form of plague, which in turn could lead to secondary transmission from an initially infected laboratory worker or member of the public. Infections from other routes of exposure would most likely lead to primary bubonic plague, which is not transmissible unless a severe or untreated case leads to secondary pneumonic plague. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.3.2 Vaccine and Prophylaxis

Plague vaccines have been administered to high-risk workers in the past, but as of mid-2011, none were available for civilian use in the United States (CDC 2011b). For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection with *Y. pestis*. That is a conservative assumption because it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that was previously available, that could become FDA-approved in the near future, or that could be available before official FDA-approval, such as those classified as an IND.

For individuals who have possibly been exposed to aerosolized *Y. pestis* but are not yet showing symptoms, prophylactic treatment with antibiotics is recommended. Antibiotics taken by exposed individuals could prevent infection if applied before symptoms appear. It is highly likely that recognized and reported mishaps in the laboratory involving *Y. pestis* would result in potentially exposed workers receiving the recommended prophylactic regimen, which could dramatically reduce the probability of both infection and disease. However, this RA focuses on laboratory incidents that are most likely to be undetected, in which case prophylaxis would likely not be administered early enough to prevent infection. If exposures occurred in the general public, it is possible that prophylactic treatment, if administered in time, would prevent infection and/or disease that would otherwise have occurred. That possibility would be more likely for exposed individuals experiencing a long incubation period. Those issues are discussed in more detail within the sections of this RA related to potential health consequences of specific events resulting in direct public exposure.

J.3.3.3 Dose-Response Information from the Literature

Human evidence

No direct human dose-response data for *Y. pestis* are available in the literature. The infective dose for humans exposed to *Y. pestis* aerosols has been stated to be between 100–500 organisms (Franz et al. 1997), although it is unlikely that those numbers were derived from actual human data. Epidemiological information from human outbreaks of pneumonic plague have led to estimates of a low attack rate, with approximately 8 percent of close, unprotected contacts of symptomatic primary cases becoming secondarily infected (Begier 2006; Ratsitorahina 2000). That low attack rate could be interpreted as evidence that IDs for humans via inhalation are relatively high; alternatively, the low attack rate could be explained by the possibility that the primary cases did not produce a large number of aerosolized infectious particles that could be inhaled by their close contacts during their symptomatic period.

NHP data

In one study (Speck 1957), unimmunized rhesus monkeys *(Macaca mulatta)* were exposed to aerosolized *Y. pestis* in a cloud chamber with average doses ranging from 140 to 1,500,000 inhaled organisms (Table J–3a). In a recent study (Huang 2010), those data were fit to the exponential, log-probit, and beta Poisson dose-response models and the beta Poisson model with $\alpha = 6.5 \times 10^{-1}$ and $\beta = 8.0 \times 10^{3}$ (rounded to two significant figures) provided the best fit. The best-fit model provides an estimated LD_{50} of 15,000 organisms, an LD_{10} of 1,400 organisms, and an LD_1 of 120 organisms (rounded to two significant figures). The estimated LD_1 is close to the lowest dose to which monkeys were actually exposed; however, it should be noted that all eight monkeys exposed to an average dose of 140 organisms survived.

The lowest average dose that caused death of a monkey was 580 inhaled organisms (one death out of 19 exposed). It is also noteworthy that 2 of 14 monkeys survived without treatment after exposure to the highest average dose of 1,500,000 inhaled organisms.

Table J–3a. Monkey (*Macaca mulatta*) dose-response data (Speck 1957) for *Y. pestis*

Challenge Dose (organisms)	Number exposed	Number of deaths
140	8	0
580	19	1
1,500	21	1
5,800	15	4
23,000	33	19
63,000	25	21
200,000	33	32
500,000	14	12
1,500,000	14	12

Data from Speck and Wolochow (1957) and analyzed in Huang and Haas (2011). The challenge dose was the average number of inhaled organisms in each group.

Ehrenkranz and Meyer (1955) exposed unimmunized rhesus monkeys (*Macaca mulatta*) to intratracheal doses of *Y. pestis* ranging from 10 to up to 270 million organisms (Table J–3b). Two monkeys were exposed to the lowest dose of 10 organisms and both survived, while more than half (8 out of 12) died after exposure to the next lowest dose range of 120–170 organisms. It is again noteworthy that a small fraction of monkeys survived without treatment after exposure to doses several orders of magnitude higher than doses that were lethal to other monkeys.

Table J–3b. Monkey (*Macaca mulatta*) dose-response data (Ehrenkranz 1955) for *Y. pestis*

Challenge Dose (organisms)	Number exposed	Number of deaths
10	2	0
120–270	12	8
800–2,700	18	17
9,500–12,000	18	17
120,000–270,000,000	10	9

Data from Ehrenkranz and Meyer (1955). The challenge dose was the measured number of organisms delivered via intratracheal inoculation.

It appears that dose-response model fitting to the data in Table J–3b has not been attempted, most likely because the doses to which the monkeys were exposed are not reported precisely (particularly at the highest dose range). However, it is clear that the data suggest an LD_{50} significantly lower than the LD_{50} of about 15,000 organisms suggested by the Speck and Wolochow data. Speck and Wolochow (1957) include a discussion in their report about possible reasons for the different results in the two studies, which is presented in Section J.3.3.4.

J.3.3.4 Literature-Based Dose Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published models for *Y. pestis* outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

Without human dose-response data for *Y. pestis*, experimental studies involving NHPs provide the best available data from which to gain insights into potentially appropriate dose-response relationships for humans. Results from exposure of rhesus monkeys to aerosolized *Y. pestis* (Speck 1957) appear to be the only inhalational dose-response data that have been fit to dose response models (Huang 2010), and the beta Poisson model outperformed the other candidate models, producing estimated LD values shown in Table J–3c.

Another monkey data set (Ehrenkranz 1955) has not been fit to dose-response models but appears to suggest an LD_{50} roughly two orders of magnitude lower than that estimated for the Speck and Wolochow data set (see Table J–3c). Such a significant difference can likely be explained by the fact that the two studies used different procedures for administering doses to the monkeys. In the Speck and Wolochow study, the monkeys inhaled aerosolized *Y. pestis* in a chamber, while in the Ehrenkranz and Meyer study,

the doses were delivered directly to each monkey's windpipe through intratracheal intubation. Of these two methods, inhalation of aerosolized *Y. pestis* is more consistent with types of exposure that might occur under events analyzed for this RA. Furthermore, the following passage from the discussion section of Speck and Wolochow (1957, pp. 65–67) is illuminating:

> Other methods of inducing pneumonic infections, on the other hand, involve the artifacts of anaesthesia (and its effects on pulmonary physiology), intratracheal intubation where trauma may actually lead to submucosal injection, and instillation of fluid which may for some time supply the organisms with culture medium outside the normal defense mechanisms of the host. This seems especially significant with *Past. pestis* which is much more virulent when injected into tissue, and which multiplies enormously in the alveoli in the early stages of the pneumonic infection.

Because the method of delivery used in the Ehrenkranz and Meyer study might have significantly increased the ability of the *Y. pestis* organisms to reproduce in the early stages of infection beyond what would occur in a more natural inhalation scenario, it was determined that the model fit to the Speck and Wolochow data would be more appropriate to use as the literature-based dose-response assessment for this RA.

Table J–3c. LD estimates (with 95% confidence intervals) for NHPs from *Y. pestis* dose-response models from the literature

Set	Animal	Model	LD_{50}	LD_{10}	LD_{1}	$LD_{0.1}$	$LD_{0.01}$
A	*Macaca mulatta* (Speck 1957; Huang 2010)	BP	**15,000 org.** (8,200–37,000)	**1,400** (540–4100)	**120** (45–370)	**12** (4.5–37)	**1.2** (0.45–3.7)
B	*Macaca mulatta* (Ehrenkranz 1955)	none	**<≈ 300 org.**				

Each LD_x result refers to the expected inhaled dose estimated to result in death of *x*% of exposed animals. For example, $LD_{0.01}$ refers to the dose estimated to result in death of 0.01%, or one in ten thousand animals. BP = beta Poisson; org. = organisms. For data set A, the 95% confidence intervals are based on model fits to bootstrap replicates from the data set. For data set B, the data were not fit to a model and the LD_{50} upper bound estimate is based on the result that 8 of 12 monkeys died from exposure to 120-270 organisms. The model fit to data set A was chosen as the literature-based model for this RA.

There are several possible objections to relying on the estimates for data set A in Table J–3c as estimates for probabilities of human infection:

- Speck and Wolochow (1957) acknowledge the difficulty of calculating the individual doses inhaled by each exposed monkey in their experiment and advise caution in drawing conclusions from quantitative results.
- Franz et al. (1997) state that the human *infective dose* for aerosolized *Y. pestis* is 100–500 organisms. The monkey data-based model (data set A) estimates 0.8 percent (0.3–3 percent) probability of infection at 100 organisms and 4 percent (1–10 percent) probability of infection at 500 organisms. While technically those estimates are consistent with the Franz et al. statement in that 100–500 organisms would be an ID for some humans, it is possible that Franz et al. were implying a range for the HID_{50}, in which case the monkey data-based estimates are non-conservative (estimate lower risk) by comparison. Other authors have used working estimates of about 100 organisms for the HID_{50} (e.g., Sabelnikov 2006). However, without knowing the basis for the estimates, it is impossible to judge whether they are based on relevant data or arguments that conflict with the results from data set A.
- It is not known if there are significant species differences between monkeys and humans with respect to susceptibility to infection with *Y. pestis*. The heterogeneity in susceptibility displayed by the monkeys in each cited experiment and captured by the beta Poisson dose-response model is notable; presumably a random population of humans would be relatively heterogeneous compared to a population of laboratory monkeys, so application of the beta Poisson model form is presumed to be appropriate. However, the specific probabilities of infection estimated at given doses by the model could overestimate or underestimate the risk of infection to humans. Evidence of low attack rates for close contacts of symptomatic human pneumonic plague patients (see Section J.3.3.3) might suggest that the probability of infection at low inhaled doses is relatively low for humans and consistent with the model estimates, but there are no direct data to support this.

J.3.3.5 Dose-Response Estimates Derived from Expert Panel

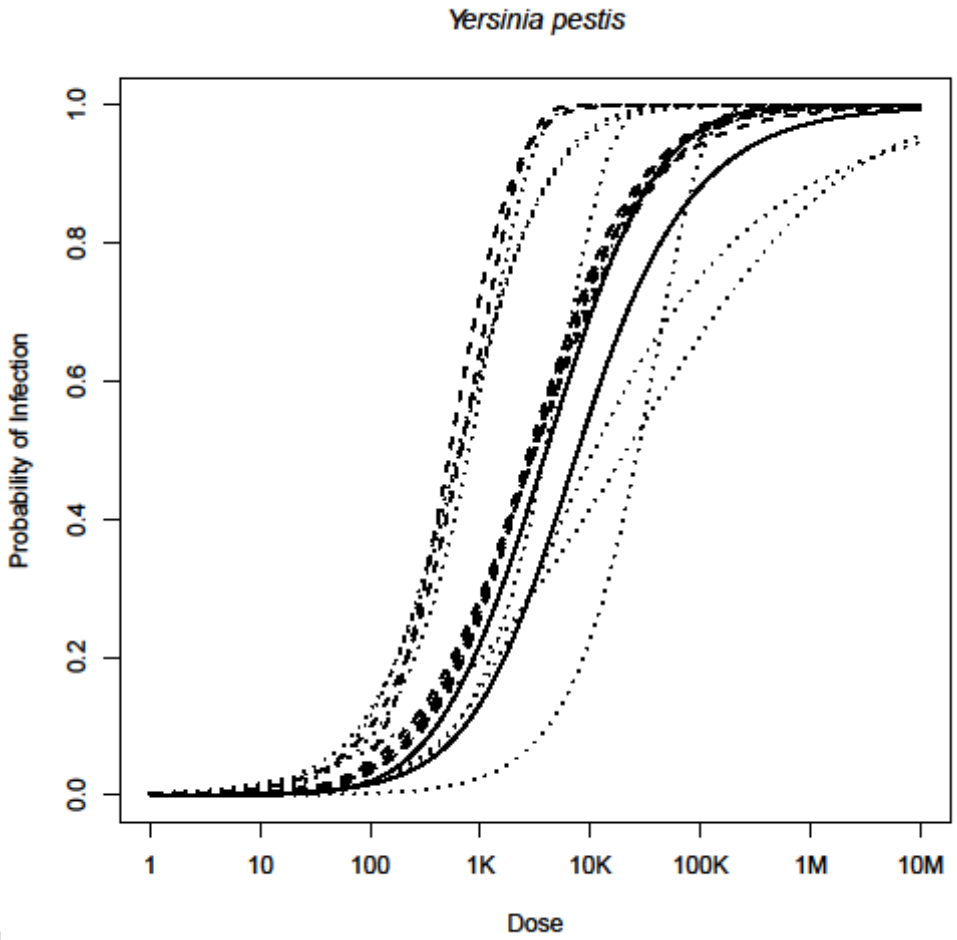
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for *Y. pestis* are displayed in Table J–3d, including which models were retained from each expert according to the BIC. Seventeen curves were retained for application in this RA, and plots of those curves are shown in Figure J–3. ID estimates provided by the retained curves are shown in Table J–3e.

Table J–3d: Dose response model fitting for *Yersinia pestis*

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log−probit model		Beta Poisson Model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	100 500 5,000	8.8×10^{-4}	0.66	630	1.3	9.3×10^{2}	**5.2**	**0**	**1.7**
2	700 8,000 130,000	6.1×10^{-5}	0.49	9,000	6.6×10^{-1}	4.2×10^{3}	20.0	6.8	**0**
3	200 4,000 40,000	1.7×10^{-4}	0.48	3,200	6.9×10^{-1}	1.6×10^{3}	9.2	**0**	**3**
4	100 1,000 1,500	1.0×10^{-3}	0.95	530	1.0×10^{5}	9.6×10^{7}	**0**	**3**	1.1[a]
5	200 5,000 30,000	1.8×10^{-4}	0.51	3,100	8.3×10^{-1}	2.2×10^{3}	**4.1**	**0**	**1**
6	400 4,000 40,000	1.4×10^{-4}	0.56	4,000	8.9×10^{-1}	3.3×10^{3}	N/A	**Exact**	N/A
7	100 100,000 1,000,000	2.6×10^{-5}	0.28	22,000	3.3×10^{-1}	1.5×10^{3}	**4.3**	**0**	**2.5**
8	250 4,000 30,000	1.8×10^{-4}	0.54	3,100	8.7×10^{-1}	2.4×10^{3}	7.1	**0**	**1.2**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–3



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–3e. Results for retained expert-derived *Y. pestis* dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	Exp	790	120	11	1.1	0.11
	LP	630	89	18	5.6	2.2
	BP	670	80	7.3	0.7	0.073
2	BP	7,700	720	64	6.3	0.63
3	LP	3,200	220	26	5.3	1.5
	BP	2,800	270	24	2.3	0.23
4	Exp	670	100	9.7	0.96	0.096
	LP	530	140	45	20	10
5	Exp	3,900	590	57	5.6	0.56
	LP	3,100	250	33	7.4	2.2
	BP	2,900	300	27	2.7	0.27
6	LP	4,000	400	61	16	5.0
7	Exp	27,000	4,100	390	39	3.9
	LP	22,000	220	5.0	0.32	0.034
	BP	11,000	580	47	4.6	0.46
8	LP	3,100	280	40	9.7	3.0
	BP	3,000	310	28	2.8	0.28
Weighted Average (Min–Max)		**3100 org.** (530–27000)	**280** (80–4100)	**37** (5.0–390)	**5.6** (0.32–39)	**0.63** (0.034–10)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.01}$ refers to the dose estimated to result in infection of 0.01%, or one in ten thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.3.6 Other Considerations

It is possible that different strains of *Y. pestis* would have differing levels of infectivity. The strain used in the study for the literature-based model was *139L* (Speck 1957). It is not known which strains would be studied at the NEIDL, and no particular strain or strains were specified to the expert panel members as part of the Delphi process. It is assumed that the uncertainty regarding infectivity of differing strains is captured within the overall uncertainty range for the dose-response parameters.

The units of exposure for events analyzed in this RA for *Y. pestis* are in terms of CFU (colony-forming units), which is assumed to measure the expected number of infectious units with potential to reproduce when inhaled. CFU is not a measure of the absolute number of live cells in a sample, because individual cells may aggregate or clump to form one colony. This potential heterogeneous distribution of cells among airborne particles in a dose is accounted for by the beta Poisson dose-response model, which

assumes a Poisson-distributed number of organisms around the expected dose (see Section J.2.5.1). The exposures reported in Speck and Wolochow (1957) are in terms of *Y. pestis* organisms, which were quantified using calculations based on colony counts from collected air samples, so it is appropriate to apply the curve derived from these data.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by CFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates.

J.3.3.7 Summary of Approach

The following summarizes the two sets of dose-response estimates to be applied to *Y. pestis* exposure data for this RA. ID estimates derived from these models are compared in Table J–3f.

- Literature-based dose-response model: The beta Poisson dose-response model with $\alpha = 6.5 \times 10^{-1}$ and $\beta = 8.0 \times 10^{3}$. Use a distribution of (α, β) pairs derived from bootstrap replicates of the pooled data set, for uncertainty and sensitivity analyses.
- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–3e, with the model or set of models derived from each expert weighted equally.

Table J–3f. ID estimates and associated ranges for *Y. pestis*

Model	LD_{50}	LD_{10}	LD_{1}	$LD_{0.1}$	$LD_{0.01}$
Literature-based	**15,000 org.** (8200–36000)	**1,400** (530–4000)	**120** (44–370)	**12** (4.4–37)	**1.2** (0.44–3.7)
Expert-based	**3,100 org.** (530–27000)	**280** (80–4100)	**37** (5.0–390)	**5.6** (0.32–39)	**0.63** (0.034–10)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–3e. org. = organisms.

For each ID point displayed in Table J–3f, the expert-based range extends lower (higher risk) than the literature-based range. Both sets of estimates are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by *Y. pestis*.

J.3.4 1918 H1N1 Influenza Virus

1918 H1N1 influenza virus (1918 H1N1V) is a strain of influenza A virus that caused a worldwide human influenza pandemic in 1918–1919, as described in Chapter 3 and Appendix C. Descendent lineages of 1918 H1N1V persist naturally in the human population today but differ in pathogenicity from the 1918 parent virus (Taubenberger 2006). 1918 H1N1V was reconstructed in 2005 (Tumpey 2004) and has since been studied in laboratories. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.4.1 Routes of Exposure

The primary natural routes of exposure and infection for humans to influenza viruses, including 1918 H1N1V, are assumed to be inhalation of aerosols or droplets and direct contact through handling of contaminated fomites. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational and direct contact (intranasal) routes are the primary focus of the dose-response assessment in this section. The ingestion route is assumed to require an equal or higher dose to achieve the same probability of infection as compared to the inhalational route. The puncture and animal-related routes are discussed separately in conjunction with the event sequences for which those routes are relevant.

J.3.4.2 Vaccine and Prophylaxis

No vaccine is available that is designed to protect specifically against 1918 H1N1V. Seasonal influenza vaccines are licensed by FDA and are available to laboratory workers and the general public on a yearly basis. The seasonal vaccine is updated each year to protect against strains predicted to be most common in the upcoming flu season, normally including a strain of H1N1 virus. The efficacy of any particular seasonal vaccine against 1918 H1N1V for humans is not known; however, there is evidence that individuals who had received previous seasonal vaccines were moderately protected against the 2009 H1N1 pandemic influenza virus (Johns 2010). In addition, the 2009 H1N1 pandemic influenza vaccine, which was included in the most recent 2010–2011 seasonal vaccine, was shown to be effective in protecting mice against 1918 H1N1V (Medina 2010). It is also to be noted that older individuals who had experienced prior influenza pandemics were at least partially protected from the 2009 H1N1 strain (Ikonen 2010).

Given the evidence above, it is possible that any laboratory worker or member of the general public who had received previous H1N1 vaccines or had recovered from an H1N1 virus infection would be at least

partially protected from infection after exposure to 1918 H1N1V. It is noted in Section J.3.4.3 that the human dose-response data for influenza viruses are generally limited to individuals who were shown to lack protective antibodies to the given strain before experimental exposure, which may contribute to overestimating the probability of infection for a general population that likely includes individuals with some level of protection. This issue is discussed further in the summary Section J.3.4.7. It is assumed the experts who provided ID estimates for 1918 H1N1V (Section J.3.4.5) took into account their estimates for portions of the population who may be partially protected, and no adjustments to the models derived from their estimates are made. For laboratory acquired infection LAI scenarios, it is conservatively assumed that laboratory workers would be protected due to past infections or immunizations with the same probability as a random member of the general public. This assumption is conservative because it is likely that a worker assigned to handle 1918 H1N1V would be especially encouraged to receive all available influenza vaccinations.

Chemoprophylaxis with antiviral agents has been shown to be effective in preventing influenza illness among individuals who were likely exposed and is recommended for individuals at high risk for complications who have not been immunized and are likely to be exposed (CDC 2011c). It is likely that recognized and reported mishaps in the laboratory involving 1918 H1N1V would result in potentially exposed workers receiving antiviral medication, which may or may not be effective in preventing infection or disease. However, this RA focuses on laboratory incidents that are most likely to be undetected, in which case prophylaxis would likely not be administered early enough to prevent infection. If exposures occurred in the general public, it is possible that prophylactic treatment, if administered in time, would prevent infection and/or disease that would otherwise have occurred. This possibility might be more likely for exposed individuals experiencing a long incubation period. These issues are discussed in more detail within the sections of this RA related to potential health consequences of specific events resulting in direct public exposure.

J.3.4.3 Dose-Response Information from the Literature

There are no direct human dose-response data for 1918 H1N1V. There are numerous data sets from experimental human exposures to other strains of influenza virus, including H1N1 strains. Carrat et al. (2008) performed an extensive literature search of human volunteer studies on influenza and reviewed results reported in 71 papers, which were published between 1965 and 2005. They selected for statistical analysis only those subgroups of volunteers who had been tested for antibodies and met criteria for being considered unprotected from infection before exposure. The resulting data set represents 61 subgroups

consisting of 1009 participants challenged with given doses of influenza A/H1N1, A/H3N2, A/H2N2, or influenza B.

The dose-response data for human infection (Carrat 2008) for influenza A/H1N1, the strains that are presumably closest to 1918 H1N1V, are compiled in Table J–4a.

Table J–4a. Human volunteer dose-response data (Carrat 2008) for influenza A/H1N1

Challenge dose (log_{10} $TCID_{50}$)	Number exposed	Number infected	Percent infected
4	21	17	81.0%
4.5	9	8	88.9%
5	103	91	88.3%
6	79	66	83.5%
6.4	28	26	92.9%
6.7	22	20	90.9%
7	207	198	95.7%

Data from Carrat et al. (2008); see references therein for source studies. The challenge doses listed are in units of base-10 logarithm (log_{10}) of the median tissue culture infective dose ($TCID_{50}$). The exposure route for all groups contained in this table was intranasal.

The lowest dose listed in Table J–4a is 10,000 $TCID_{50}$ (median tissue culture infective dose[3]), so these data provide only indirect information on the likely response of humans to exposure at much lower doses, for example, doses on the order of one $TCID_{50}$. The Carrat et al. (2008) literature review found only one study in which humans were exposed to very low doses (Alford 1966) via the inhalational route. The strain of influenza used in this study was influenza A/Bethesda/10/63 (H2N2). In the study, eleven volunteers who were previously unprotected from the virus (according to the protection criteria used by Carrat et al.) inhaled 1, 2, or 5 $TCID_{50}$ of the virus and six were subsequently infected. See Table J–4b for details.

[3] In the terminology used for this RA, $TCID_{50}$ is termed $CCID_{50}$ (median cell culture infectious dose). In Section 3.4.3, the units as reported by the cited authors are retained. In future sections where the data are used for analysis, the term $CCID_{50}$ is used to maintain consistency with the rest of the RA.

Table J–4b. Unprotected human volunteer dose-response data (Alford 1966) for influenza A/H2N2

Challenge dose ($TCID_{50}$)	Number exposed	Number infected
1	1	1
2	4	1
5	6	4

$TCID_{50}$ = median tissue culture infective dose

J.3.4.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data and evidence for 1918 H1N1V and other strains of influenza virus outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The data in Table J–4a were tested for dose-response behavior (i.e., increasing probability of infection with increasing dose). The Cochran-Armitage test of trend concludes that a null hypothesis of lack of trend can be rejected ($p < 0.002$), which means that it is appropriate to attempt dose-response model fitting to these data (Haas 1999). The exponential, log-probit, and beta Poisson models were fit to the data using the method of maximum likelihood, which chooses optimal values that maximizes the probability of observing each data set under the model. The results are shown in Table J–4c. To assess model fit acceptability, the optimal deviance (Haas 1999) for each model is shown and compared to the upper 5th percentile of the χ^2 distribution with degrees of freedom equal to the number of distinct doses in the data set minus the number of parameters in the dose-response model. A null hypothesis of fit acceptability can be rejected for the exponential model as the optimal deviance is greater than the corresponding χ^2 statistic. Both the log-probit model and the beta Poisson model provide much improved fits over the exponential model and both model fits are deemed acceptable according to this statistical test. The log-probit model produces a fit with slightly lower deviance than the beta Poisson model.

Table J–4c. Model fitting for human volunteer influenza A/H1N1 data set

Model	Optimal parameter values	Optimal deviance	$\chi^2_{0.95, df}$	Acceptable fit?	ID_{50}	ID_{10}	ID_1
Exp	$r = 1.2 \times 10^{-6}$	663	12.6	NO	-	-	-
LP	$m = 0.10$ $ID_{50} = 2.8$	5.9	11.1	YES	2.8 $CCID_{50}$	1.2×10^{-5}	5.2×10^{-10}
BP	$\alpha = 0.17$ $\beta = 0.89$	6.2	11.1	YES	49 $CCID_{50}$	0.75	0.054

$CCID_{50}$ = median cell culture infective dose; Exp = exponential; LP = log-probit; BP = Beta Poisson; df = degrees of freedom

The results in the last three columns of Table J–4c demonstrate that the choice of model has large implications for the estimated probability of infection at low doses. For example, the log-probit model produces an ID_1 eight orders of magnitude lower than the ID_1 produced by the beta-Poisson model. The low-dose experimental results shown in Table J–4b provide some insight into what might be a reasonable value for the ID_{50}, as six of eleven (54.5 percent) of unprotected humans were infected after inhalation of 1–5 $CCID_{50}$ of influenza virus. These data suggest that the log-probit fit to the H1N1 data, which produces an estimated ID_{50} of 2.8 $CCID_{50}$, might be an appropriate model. However, the extremely low values for the ID_{10} and ID_1 estimates of this log-probit model are not directly supported by any data. Furthermore, and perhaps most importantly, an uncertainty analysis of the dose-response model parameters using a bootstrap resampling (Haas 1999) of the data in Table J–4a, reveals that extrapolated estimates are extremely sensitive to perturbations of the data, such that the uncertainty ranges for low-dose estimates would traverse several orders of magnitude. Given these difficulties, it was determined that the data in the literature do not support quantitative dose-response estimates for 1918 H1N1V that would provide useful insight for this RA.

J.3.4.5 Dose-Response Estimates Derived from Expert Panel

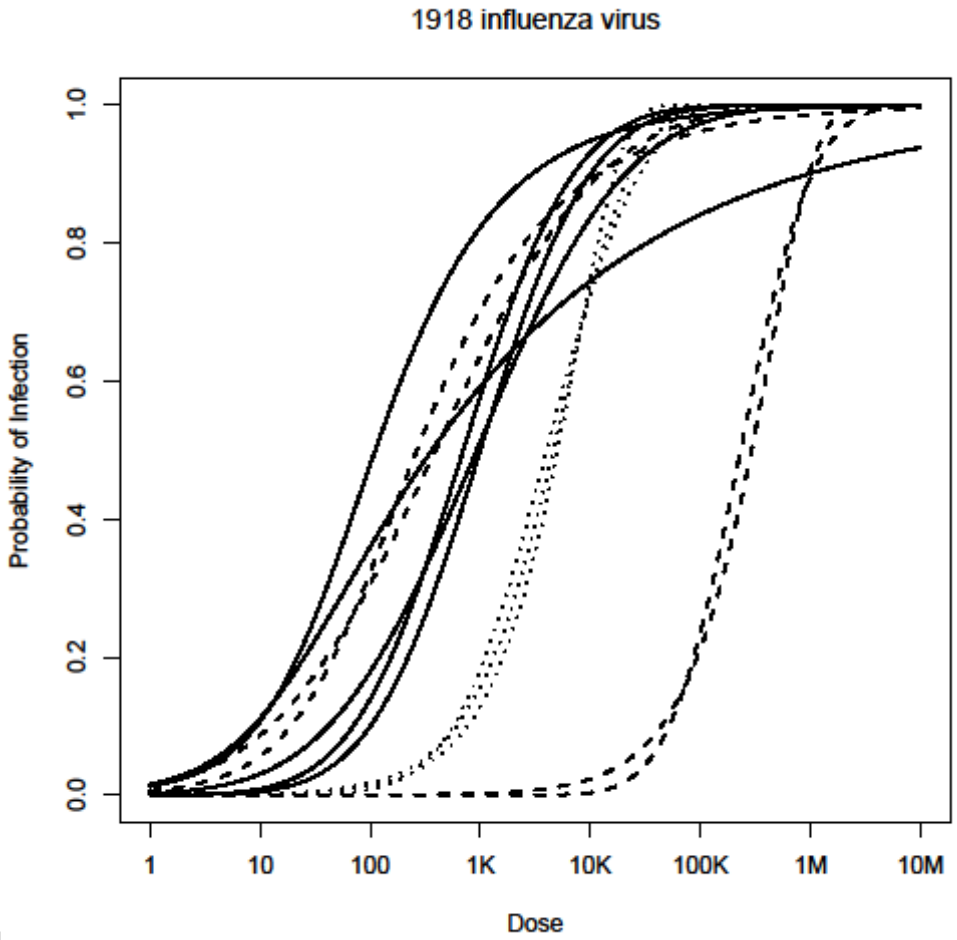
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for 1918 H1N1V are displayed in Table J–4d, including which models were retained from each expert according to the BIC. Twelve curves were retained for application in this RA, and plots of those curves are shown in Figure J–4. ID estimates provided by the retained curves are shown in Table J–4e.

Table J–4d: Dose response model fitting for 1918 H1N1 influenza virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log–probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	50,000 250,000 1,000,000	2.4×10^{-6}	0.86	230,000	1.0×10^{5}	4.2×10^{10}	**3.6**	**0**	4.7[a]
2	10 300 1,000,000	3.8×10^{-4}	0.22	1,400	2.1×10^{-1}	1.3×10^{1}	18.4	12.3	**0**
3	40 1,000 20,000	5.9×10^{-4}	0.41	930	5.1×10^{-1}	2.3×10^{2}	18.1	**0**	10.1
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	400 8,000 20,000	1.4×10^{-4}	0.66	4,000	2.2	1.3×10^{4}	**0**	**1.3**	**0.2**
6	70 700 7,000	7.9×10^{-4}	0.56	700	8.9×10^{-1}	5.7×10^{2}	N/A	**Exact**	N/A
7	10 100 3,000	3.8×10^{-3}	0.45	140	5.4×10^{-1}	4.3×10^{1}	15.0	7.2	**0**
8	10 600 10,000	1.4×10^{-3}	0.37	390	4.5×10^{-1}	7.3×10^{1}	8.8	**0**	**4.2**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–4



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–4e. Results for retained expert-derived 1918 H1N1V dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	Exp	290000	44000	4200	420	42
	LP	230000	52000	15000	6300	3000
2	BP	360	8.6	0.64	0.063	0.0063
3	LP	930	42	3.3	0.52	0.11
4	LP	1000	100	15	3.9	1.3
5	Exp	5000	760	73	7.3	0.72
	LP	4000	570	110	36	14
	BP	4600	610	57	5.7	0.56
6	LP	700	70	11	2.7	0.88
7	BP	110	9.2	0.80	0.079	0.0079
8	LP	390	12	0.74	0.095	0.017
	BP	260	19	1.6	0.16	0.016
Median (Min–Max)		**700 org.** (110–290000)	**42** (8.6–52000)	**3.3** (0.64–15000)	**0.52** (0.063–6300)	**0.11** (0.0063–3000)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.01}$ refers to the dose estimated to result in infection of 0.01%, or one in ten thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.4.6 Other Considerations

The units of exposure for events analyzed in this RA for 1918 H1N1V are in terms of PFU (plaque forming units), which is assumed to measure the expected number of infectious units with potential to reproduce when inhaled. PFU is not a measure of the absolute number of virions in a sample, because individual virions may aggregate or clump to form one plaque and because the plaque assay may not be entirely sensitive to detecting all virions that would have the ability to infect cells in a different medium or host.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates. It is possible that this assumption is non-conservative if the plaque assay used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. The potential magnitude of non-conservatism in assuming that *organisms* (as envisioned by the experts) are adequately measured by PFU is unknown.

J.3.4.7 Summary of Approach

The following summarizes the dose-response estimates for 1918 H1N1V to be applied to exposure data for this RA.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–4e, with the model or set of models derived from each expert weighted equally.

While it was determined that the available published experimental data did not support a literature-based dose-response model, it can be assessed whether the information that does exist in the literature is adequately represented within the range of expert-derived models. First, the H1N1 human volunteer data in Table J–4a suggests that doses ranging from ten thousand to one million $CCID_{50}$ result in approximately 80 to 95 percent infection probability. The expert estimates of the ID_{90} range from three thousand to one million organisms (assumed to be equivalent to PFU). Titers of virus in units of $CCID_{50}$ are generally higher than in units of PFU. Assuming the difference in virus quantities as measured by PFU and $CCID_{50}$ is not more than about a factor of ten, the ranges of ID_{90} estimates from the expert-derived models are not inconsistent with what was seen in the data. Second, the low-dose inhalational data shown in Table J–4b reveal that roughly 50 percent of unprotected humans were infected after inhaling doses of 1–5 $CCID_{50}$. The lowest ID_{50} estimated by the experts was 100 organisms (PFU), which appears to suggest that the Table J–4b data might support higher infectivity than is represented by the expert range of estimates, especially considering that the infective doses in the volunteer experiment were likely even lower in units of PFU. However, the data in Table J–4b were restricted to cases where the human volunteers showed no or very little previous protection from the virus strain. As discussed in Section J.3.4.2, it is likely that some percentage of laboratory workers and the general public would be protected from infection with 1918 H1N1V. Therefore, it is reasonable that the expert estimates suggest a lower overall probability of infection than do data that were restricted to unprotected humans.

J.3.5 SARS-Associated Coronavirus

SARS-associated coronavirus (SARS-CoV) causes severe acute respiratory syndrome (SARS), a highly infectious zoonotic disease of humans, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.5.1 Routes of Exposure

The primary natural routes of exposure and infection for humans are assumed to be inhalation of aerosolized droplets and intranasal exposure after touching contaminated fomites or hands of a primary case. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational and direct contact (intranasal) routes are the primary focus of the dose-response assessment in this section, and it is assumed that the dose-response relationship is similar for both routes. The ingestion route is assumed to require an equal or higher dose to achieve the same probability of infection as compared to the inhalational route. The puncture and animal-related routes are discussed separately in conjunction with the event sequences for which those routes are relevant.

J.3.5.2 Vaccine and Prophylaxis

There are currently no vaccines and no approved or validated post-exposure prophylactic regimens available for SARS-CoV.

J.3.5.3 Dose-Response Information from the Literature

There are no direct human dose-response data for SARS-CoV. The virus is highly transmissible person-to-person, and there were documented cases of airborne transmission across floors of buildings in hospitals and in an apartment complex as well as on airplanes (see Chapter 3 and Appendix C for details and references). This evidence of transmissibility might suggest that the IDs for humans are relatively low. Alternatively, the frequent occurrence of transmissions could have occurred because primary cases shed a large number of pathogens resulting in very high doses received by secondary cases. There is no information on the likely doses received in the known cases of LAI.

There was one dose-response modeling study of SARS-CoV found in the literature (Watanabe 2010), which consisted of an analysis of multiple data sets generated by others from experiments on mice. One data set (De Albuquerque 2006) was generated from intranasal inoculation of A/J mice with murine hepatitis virus (MHV-1), a virus that may be in the same genome-sequence-based grouping of coronaviruses as SARS-CoV (Watanabe 2010) and that produced pulmonary pathological features of SARS in the infected mice (De Albuquerque 2006). Mice were exposed to a wide range of doses, including one group exposed to a low dose of 5 PFU. Another data set (DeDiego 2008) was generated from intranasal inoculation with recombinant SARS-CoV of transgenic mice developed to express the human receptor for SARS-CoV (McCray 2007). The mice were exposed to a range of doses as low as 240 PFU. In both data sets, the response recorded in the exposed mice was death.

The exponential model and the beta Poisson model were the candidate dose-response models employed in Watanabe et al. (2010). The exponential model provided statistically significant fits to both data sets, and the beta Poisson model either could not be applied or did not provide a statistically significant improvement over the exponential model. Despite the fact that the two data sets were derived for different viruses on different strains of mice, the fitted exponential parameter was similar across the two data sets, and the authors demonstrated through a statistical procedure that the two data sets could be pooled for purposes of fitting a single exponential model to the combined data. They recommended the exponential fit to this pooled data set ($k = 4.1 \times 10^2$, where k is the reciprocal of the parameter r described in section J.2.5.1) as a dose-response model for SARS-CoV with potential relevance for the endpoint of human infection. This model results in an estimated ID_{50} of 280 PFU (95 percent confidence interval 130 to 530 PFU), ID_{10} of 43 PFU (95 percent confidence interval 20 to 81 PFU), and ID_1 of 4 PFU (95 percent confidence interval not reported, but estimated 2 to 8 PFU).

Watanabe et al. (2010) also summarized and fit models when possible to thirteen other data sets from six other papers in the literature involving viruses related to SARS-CoV. One of those papers (Bradburne, 1967), studied the effects of exposing human volunteers via intranasal inoculation to human coronavirus 229E (HCoV-229E), which causes common cold symptoms in humans. In this study, the doses of exposure were recorded in units of TCD_{50} (median tissue culture dose)[4], and the response to exposure recorded was the presence or absence of cold symptoms, which is assumed to indicate the presence or absence of infection. The exponential model was shown to provide a statistically significant fit to this data, and the beta Poisson model did not provide a statistically significant improvement over the exponential model. The fitted exponential parameter results in estimates of the ID_{50} at 13 TCD_{50} and ID_{10} at 2.0 TCD_{50}. To compare these results to those obtained from the pooled data set derived from the De Albuquerque and DeDiego mouse experiments, Watanabe et al. make note of a report (Schmidt, 1979) that the 50 percent endpoint assay used to calculate TCD_{50} is 10 to 30 times less sensitive than the plaque assay used to calculate PFU for HCoV-229E. If one assumes a conversion factor of 10 to 30 in converting the ID_{50} and ID_{10} results from units of TCD_{50} to PFU, the model estimates based on the Bradburne human data fall into the 95 percent confidence interval ranges for the estimates based on the pooled mouse data, suggesting that the results are consistent.

[4] In the terminology used in this RA, TCD_{50} is termed $CCID_{50}$ (median cell culture infectious dose), which is used elsewhere in this RA. Here, the units as reported by the cited authors are retained.

Ten other animal data sets for SARS-CoV-related coronaviruses examined by Watanabe et al. were amenable to dose-response model fitting, and the exponential model provided a statistically significant fit to all but two: 8 week old rats exposed to porcine hemagglutinating encephalomyelitis virus 67-N (HEV-67N), (Hirano 2001), and chicks exposed to avian infectious bronchitis virus (Uenaka 1998). The beta Poisson model provided a statistically significant improvement in fit over the exponential model for these two data sets only. Two of the ten data sets resulted in a best fit model that estimates IDs lower than those derived from the De Albuquerque / DeDiego pooled data. Both of those two data sets (Hirano 2001, Hirano 2004) were for young (1 week old) rodents exposed to HEV-67N, and in both studies older rodents were shown to have susceptibility more in line with the other data sets or lower. This age-dependent pattern of susceptibility is not consistent with the observed pattern of SARS-CoV cases in humans, which suggested a lower susceptibility in younger individuals and higher mortality rate in older individuals (see Chapter 3 and Appendix C).

Table J–5a. Estimates of LD for mice and ID for humans (with 95% confidence intervals) from coronavirus dose-response models from the literature

Data Set	Model	L/ID_{50}	L/ID_{10}	L/ID_{1}	$L/ID_{0.1}$
A: tgMice/rSARS-CoV (De Albuquerque 2006, Watanabe 2010)	Exp	**230 PFU** (0–630)	**35** (0–96)	**3.4** (0–9.2)	**0.34** (0–0.92)
B: Mice / MHV-1 (DeDiego 2008, Watanabe 2010)	Exp	**320 PFU** (110–1100)	**50** (16–160)	**4.7** (1.5–15)	**0.47** (0.15–1.5)
C: Pooled A and B (Watanabe 2010)	Exp	**280 PFU** (110–580)	**43** (17–89)	**4.1** (1.6–8.5)	**0.41** (0.16–0.85)
D: Humans / HCoV-229E (Bradburne 1967, Watanabe 2010)	Exp	**13 TCD_{50}** (5.7–28)	**1.9** (0.87–4.3)	**0.19** (0.083–0.41)	**0.018** (0.0083–0.041)

Each L/ID_x result refers to the expected inhaled dose estimated to result in death/infection of *x*% of exposed individuals. 95% confidence intervals based on model fits to bootstrap replicates from each data set; lower limit of zero for data set A means that more than 2.5% of replicates included no negative cases. Abbreviations: tgMice = transgenic mice; rSARS-CoV = recombinant SARS-CoV; MHV-1 = murine hepatitis virus; HCoV = human coronavirus; Exp = exponential; PFU = plaque forming unit; TCD_{50} = median tissue culture infective dose. As described in the text, for data set D, a conversion factor of 10-30 may be appropriate for converting the given TCD_{50} values into units of PFU. The model fit to data set C was chosen as the literature-based model for this RA, as described in Section J.3.5.4.

J.3.5.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published models for SARS-CoV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The Watanabe et al. (2010) study is quite recent and seems to have exhaustively covered the animal models that are the most relevant to human infection with SARS-CoV and for which published data amenable to quantitative dose-response assessment exist. The data examined were from experiments using related viruses and not SARS-CoV specifically; efforts to expose rodents and NHPs to SARS-CoV itself have revealed that those species do not succumb to disease as humans do even at very high doses, so those experiments have not shed light on human dose-response. The viruses used in the experiments examined by Watanabe et al. produced responses in the animals similar to the human response to SARS-CoV and, therefore, may be more relevant.

The pooled mouse data set identified in the Watanabe et al. study appears to be the most relevant animal model for human infection, and the best fit exponential model for these data is applied to the exposure data for this RA. There are potential objections to applying a model derived from mouse data to humans. However, the fact that data from human exposure to a related coronavirus are consistent with the mouse data is evidence that the species differences in susceptibility to the respective viruses may not be large. Nevertheless, given that SARS-CoV appears to be unique in its virulence and pathogenicity to humans, the assumption that human dose-response is adequately assessed by this model must be applied cautiously.

J.3.5.5 Dose-Response Estimates Derived from Expert Panel

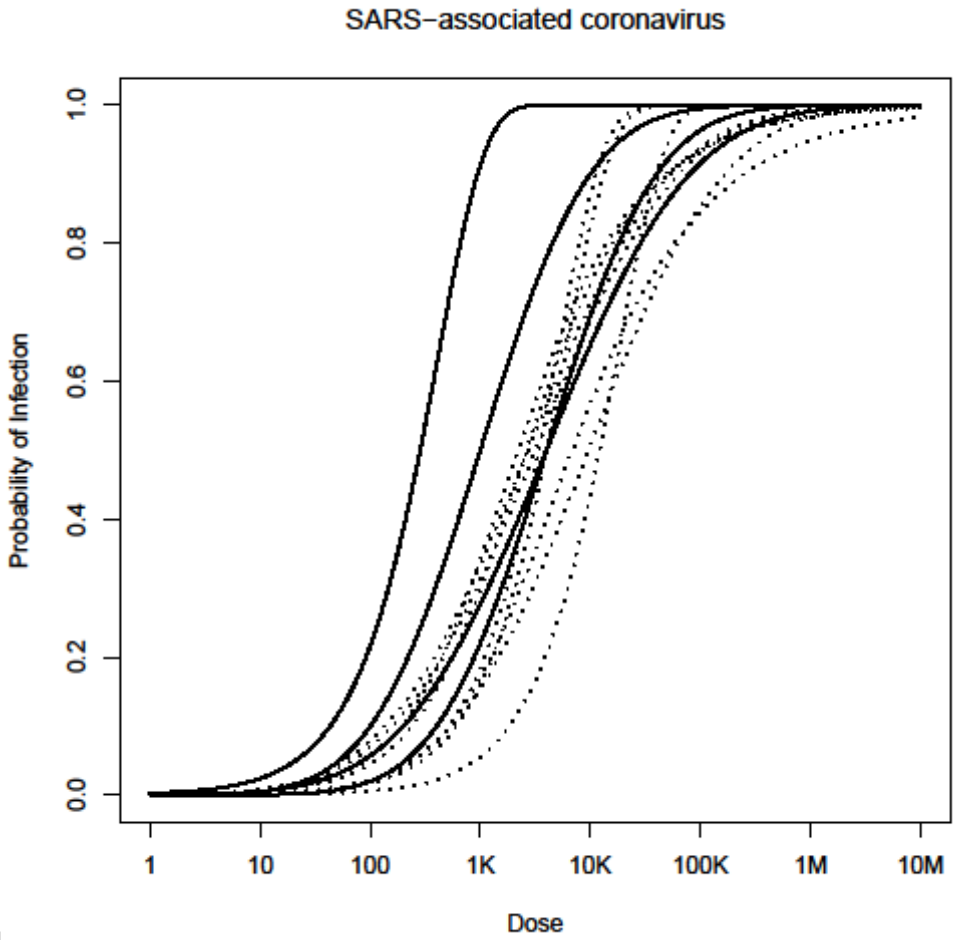
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for SARS-CoV are displayed in Table J–5b, including which models were retained from each expert according to the BIC. Fourteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–5. ID estimates provided by the retained curves are shown in Table J–5c.

Table J–5b: Dose-response model fitting for SARS-associated coronavirus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log–probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
2	43 283 950	2.4×10^{-3}	0.83	230	1.0×10^{2}	4.1×10^{4}	**0**	21.8	0.5[a]
3	100 5,000 40,000	2.0×10^{-4}	0.43	2,700	5.9×10^{-1}	9.8×10^{2}	**5.1**	**0**	**2.1**
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	100 8,000 40,000	1.7×10^{-4}	0.43	3,200	6.3×10^{-1}	1.3×10^{3}	**3**	**0**	**1.4**
6	400 4,000 40,000	1.4×10^{-4}	0.56	4,000	8.9×10^{-1}	3.3×10^{3}	N/A	**Exact**	N/A
7	1,000 3,000 300,000	5.7×10^{-5}	0.45	9,700	4.8×10^{-1}	2.1×10^{3}	**4.1**	**1.8**	**0**
8	200 4,000 80,000	1.4×10^{-4}	0.43	4,000	5.3×10^{-1}	1.1×10^{3}	N/A	**Exact**	N/A

Fitted parameters for three dose-response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–5



Plot of all dose-response curves retained from fitting models to the information provided by the experts as part of the Delphi process, used to estimate probability of infection for each dose. Solid curves are weighted with 1/8 probability; dashed curves are weighted with 1/16 probability; dotted curves are weighted with 1/24 probability.

Table J–5c. Results for retained expert-derived SARS-CoV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	LP	1000	100	15	3.9	1.3
2	Exp	280	43	4.1	0.41	0.041
3	Exp	3400	520	49	4.9	0.49
	LP	2700	140	12	2.0	0.46
	BP	2200	190	17	1.7	0.17
4	LP	1000	100	15	3.9	1.3
5	Exp	4000	600	58	5.8	0.58
	LP	3200	160	14	2.3	0.53
	BP	2600	240	21	2.1	0.21
6	LP	4000	400	61	16	5.0
7	Exp	12000	1800	180	18	1.7
	LP	9700	560	55	10	2.5
	BP	6900	520	45	4.4	0.44
8	LP	4000	200	17	2.9	0.67
Median (Min–Max)		**2900 org.** (280–12000)	**200** (43–1800)	**17** (4.1–180)	**3.9** (0.41–18)	**0.67** (0.041–5.0)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.01}$ refers to the dose estimated to result in infection of 0.01%, or one in ten thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

It is noted that the estimates derived from Expert 2 are the same as the estimates derived from the chosen dose-response model from the literature. This can be explained by the fact that this expert was an author of the study in which the literature-based model was found and clearly was of the opinion that this model is applicable to humans.

J.3.5.6 Other Considerations

The units of exposure for events analyzed in this RA for SARS-CoV are in terms of PFU (plaque forming units), which is assumed to measure the expected number of infectious units with potential to reproduce when inhaled. PFU is not a measure of the absolute number of virions in a sample, because individual virions may aggregate or clump to form one plaque and because the plaque assay may not be entirely sensitive to detecting all virions that would have the ability to infect cells in a different medium or host. The exposures in the mouse experiments that form the basis for the literature-based dose-response estimates are in terms of PFU, so it is appropriate to apply the curves derived from these data.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates. It is possible that this assumption is non-conservative if the plaque assay used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. Some authors have reported counts of viral genomes in samples of SARS-CoV virus that were also assayed for plaques, resulting in estimates of about 300 genome equivalents per PFU (Sampath 2005), 360 viral genomes per PFU (Vicenzi 2004), and 1200-1600 genomic equivalents per PFU (Houng 2004). These data are of limited use, however, as it is likely that many or perhaps most viral genomes did not become packaged into fully formed, viable virions that could possibly infect cells in a potential host. Therefore, the potential magnitude of non-conservatism in assuming that *organisms* (as envisioned by any particular expert) are adequately measured by PFU is unknown.

J.3.5.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for SARS-CoV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–5d.

- Literature-based dose-response model: The exponential model with $r = 2.5 \times 10^{-3}$, derived from the pooled data set C described in Table J–5a. Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (1.2×10^{-3} to 6.8×10^{-3}), for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–5c, with the model or set of models derived from each expert weighted equally.

Table J–5d. ID estimates and associated ranges for SARS-CoV.

Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
Literature-based	**280 PFU** (120–580)	**43** (18–88)	**4.1** (1.7–8.4)	**0.41** (0.17–0.84)	**0.041** (0.017–0.084)
Expert-based	**2900 org.** (280–12000)	**200** (43–1800)	**17** (4.1–180)	**3.9** (0.41–18)	**0.67** (0.041–5.0)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–5c. Abbreviations: PFU = plaque forming unit; org. = organisms.

For each ID point displayed in Table J–5d, the literature-based range extends lower (higher risk) than the expert-based range. It is assumed that dose units of PFU and organisms (as conceived by the experts) are equivalent, although this assumption may be non-conservative for the expert-based numbers as described in Section J.3.5.6. The literature-based results, being more conservative than the expert-based results by roughly a factor of ten, can in part serve to assess the implications of the possibility that the expert-based results are non-conservative by a similar factor. Both sets of estimates shown in Table J–5d are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by SARS-CoV.

J.3.6 Rift Valley Fever Virus

Rift Valley fever virus (RVFV) causes Rift Valley fever, a highly infectious zoonotic disease of animals and humans, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.6.1 Routes of Exposure

The primary natural routes of exposure and infection for humans are assumed to be animal-related, through bites from infected mosquitoes, direct contact from infected livestock or contaminated fomites, or inhalation of aerosols from contaminated blood and bodily fluids from infected animals. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (insect, NHP, and rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. The direct contact and ingestion routes are

assumed to require an equal or higher dose to achieve the same probability of infection as compared to the inhalational route. The puncture and animal-related routes are discussed separately in conjunction with the event sequences for which those routes are relevant.

J.3.6.2 Vaccine and Prophylaxis

An attenuated vaccine for RVFV currently is available under an FDA IND authority for use in at-risk military and laboratory personnel from the Special Immunizations Program at the US Army Medical Research Institute of Infectious Diseases, Department of Defense, Fort Detrick, MD (NRC 2011). Research continues on an inactivated formalinized cell culture origin human vaccine (Meegan 1989) and a reverse genetics-generated recombinant RVFV vaccine candidate containing precise deletions of complete virus genes with known roles in virulence; both show promise for humans (Bird 2008). Attenuated and formalin-inactivated vaccines have been used for ruminant livestock, although the attenuated vaccine is abortigenic in pregnant ewes (Meegan 1989).

For this RA, it is assumed that most NEIDL laboratory workers assigned to work with RVFV in BSL-3 labs would have received RVFV vaccine available from the Special Immunizations Program, described above, before a potential exposure event. This assumption has no impact on the estimated risk posed by laboratory workers to the public, as RVFV is not transmissible person-to-person. It is assumed that no member of the public would be vaccinated against RVFV in the case of direct exposure from a NEIDL-related release event.

There is no established course of prophylaxis or specific treatment for individuals exposed to or infected with RVFV (Sidwell 2003, Bird 2009). Treatment of exposed humans with immune plasma and ribavirin has been recommended (Swanepoel 2009).

J.3.6.3 Dose-Response Information from the Literature

There are no human dose-response data available for RVFV. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing RVFV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on RVFV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

A dose-response data set was reported in a study in which ICR mice were exposed to various doses of RVFV via the inhalational route and observed for mortality response (Brown 1981). Groups of mice were

exposed to doses as low as 3.2 PFU of four different strains of RVFV. The data are reproduced in Table J–6a.

Table J–6a. Mice dose-response data (Brown 1981) for RVFV

Strain	Challenge Dose (PFU)	Number exposed	Number of deaths
ZH-501	4.0	40	3
	50	43	13
	500	40	26
	6300	40	39
	50,000	37	37
Entebbe	7.9	20	0
	79	20	12
	790	20	19
	6300	20	20
	63,000	20	20
SA-51	3.2	20	0
	40	18	2
	500	20	10
	6300	20	20
	63,000	20	20
SA-75	3.2	20	0
	32	20	4
	160	20	16
	1300	20	20
	20,000	20	20

Data from Brown et al. (1981). The challenge dose was the number of inhaled PFU in each group.

Brown et al. fit the log-probit model to the data in Table J–6a from each strain separately, from which LD_{50} estimates ranging from about 80 PFU to about 400 PFU were generated. They concluded that there were statistical differences in infectivity between strains but that the differences were not practically significant. It is noted that the lowest dose that caused death was 4.0 PFU (4 dead out of 40 exposed to the ZH-501 strain).

Keefer et al. (1972) exposed young dogs and cats to a wide range of inhalational doses of RVFV, measured in units of median mouse intracerebral lethal dose ($MICLD_{50}$), and observed infections at all doses. Infection was indicated by the presence of neutralizing antibodies after exposure. The data from this study are presented in Table J–6b.

Table J–6b. Young dog and cat dose-response data (Keefer 1972) for RVFV (Van Wyk strain)

Animal	Challenge Dose ($MICLD_{50}$)	Number exposed	Number infected
Young Dog	17–36	8	6
	945–1550	4	4
	36000–51400	4	4
Young Cat	5–7	4	2
	27–36	4	4
	1065–1725	4	4

Data from Keefer et al. (1972). The challenge dose was the number of inhaled $MICLD_{50}$ in each group. Infection was indicated by the presence of neutralizing antibodies.

Keefer et al. used the data in Table J–6b to estimate an ID_{50} for young dogs of approximately 25 $MICLD_{50}$ and an ID_{50} for young cats of about 5–7 $MICLD_{50}$.

Miller et al. (1963) exposed hamsters and rhesus monkeys (*Macaca mulatta*) to inhalational doses of RVFV, measured in units of median mouse intraperitoneal lethal dose ($MIPLD_{50}$). The authors assumed that infection was indicated by mortality in hamsters, and they observed deaths at all doses, including 6 deaths out of 24 exposed to the lowest dose of 0.2 $MIPLD_{50}$. Infection in rhesus monkeys was observed in all sixteen monkeys exposed to doses ranging from 76 to 2820 $MIPLD_{50}$, as indicated by post-exposure detection of viremia and neutralizing antibodies. The data from this study are presented in Table J–6c.

Table J–6c. Hamster and rhesus monkey inhalational dose-response data (Miller 1963) for RVFV (pantropic strain)

Animal	Challenge Dose ($MIPLD_{50}$)	Number exposed	Number infected
Hamster	0.2	24	6
	0.7	24	14
	2	24	20
	14	24	23
Rhesus Monkey	76	4	4
	145	4	4
	275	4	4
	2820	4	4

Data from Miller et al. (1963). The challenge dose was the number of inhaled $MIPLD_{50}$ in each group. The endpoints to indicate infection were death for hamsters and viremia and measured levels of neutralizing antibodies for monkeys.

Miller et al. fit the log-probit model to the hamster data in Table J–6c, from which they reported an estimated an LD_{50} for hamsters of 0.525 $MIPLD_{50}$ (95 percent confidence interval 0.334–0.824).

Two studies (Easterday 1962, Easterday 1963) reported dose-response data on lambs, mice, and hamsters exposed to aerosols of the van Wyk strain of RVFV. The data are shown in Table J–6d.

Table J–6d. Animal inhalational dose-response data for RVFV (van Wyk strain)

Animal	Challenge Dose ($MIPLD_{50}$)	Number exposed	Number infected
Lamb (Easterday 1962)	0.9	1	0
	1.0	1	1
	9.0	2	2
	67.0	1	1
	78.0	1	1
Mouse (Easterday 1963)	0.2	20	0
	0.6	20	0
	1.9	20	0
	2.4	20	0
	3.7	19	2
	12.0	20	6
	24.0	20	8
	188.0	20	16
	937.0	20	16
Hamster (Easterday 1963)	0.14	9	0
	0.7	6	0
	2.7	9	1
	8.6	10	2
	17.25	10	6
	55.0	9	7
	860.0	10	10
	4300.0	9	8

The challenge dose was the number of inhaled $MIPLD_{50}$ (median mouse intraperitoneal lethal dose) in each group. The endpoints to indicate infection were death for hamsters and mice and detection of viremia for lambs.

A review paper (Easterday 1965) reports that the author also infected two species of NHPs (*Macaca mulatta* and *Macaca fascicularis*) with aerosols of RVFV, although the data appear not to have been published in a peer-reviewed journal. The author states, "the amount of virus necessary to infect by the respiratory route (aerosol) was calculated to be less than 1.0 $MIPLD_{50}$."

J.3.6.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published models for RVFV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

Section J.3.6.3 contains several animal dose-response data sets from which dose-response models can be derived and compared. Brown et al. fit the log-probit model to their mouse data (Table J–6a), but they did not statistically assess the adequacy of the model fit nor did they attempt fitting of alternate models. For this RA, the exponential, log-probit, and beta Poisson models were fit to the data in Table J–6a to compare the fits and assess the implications of different model assumptions on low-dose estimates derived from the models. The results of this procedure are shown in Table J–6e.

Table J–6e. Model fitting for inhalational RVFV mice dose-response data sets (Brown 1981)

Strain	Model	Optimal param. values	Optimal deviance	$\chi^2_{0.95,\,df}$	Accept-able fit?	LD_{50} (PFU)	LD_{10}	LD_1	$LD_{0.1}$	$LD_{0.01}$
ZH-501	Exp	r = 0.0020	39	9.5	NO	-	-	-	-	-
	LP	m = 0.45 LD_{50} = 150	1.8	7.8	YES	150	8.4	0.81	0.15	0.036
	BP	α = 0.69 β = 78	5.0	7.8	YES	140	13	1.1	0.11	0.011
Entebbe	Exp	r = 0.0063	7.8	9.5	YES	110	17	1.6	0.16	0.016
	LP	m = 0.85 LD_{50} = 75	2.2	7.8	YES	75	17	4.9	2.0	0.95
	BP	α = 1.9 β = 180	4.1	7.8	YES	76	9.9	0.92	0.092	0.0091
SA-51	Exp	r = 0.0015	1.0	9.5	YES	460	70	6.6	0.66	0.066
	LP	m = 0.67 LD_{50} = 360	2.2	7.8	YES	360	53	11	3.5	1.4
	BP	α = 12 β = 7400	0.98	7.8	YES	450	67	6.4	0.64	0.064
SA-75	Exp	r = 0.0089	1.6	9.5	YES	78	12	1.1	0.11	0.011
	LP	m = 1.1 LD_{50} = 71	0.067	7.8	YES	71	21	8.0	3.9	2.2
	BP	α = 7.8M β = 880M	1.6	7.8	YES	78	12	1.1	0.11	0.011

Dose-response model fitting to the inhalational mouse data (Brown 1981) from Table J–6a. Exp = exponential, LP = log-probit, BP = beta Poisson. Model parameters are defined in Section J.2.5.1. Fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to 4 for the exponential model and 3 for the log-probit and beta Poisson models. Each ID_x result refers to the expected inhaled dose estimated to result in death/infection of x% of exposed individuals under each model. All ID estimates in units of PFU (plaque forming units).

All three models provide acceptable fits to the data from each of the four strains, except for the exponential model to the ZH-501 strain. The log-probit model produces the lowest deviance for the ZH-501 and Entebbe strains, although the beta Poisson models also provide acceptable fits to these strains, and the difference in optimal deviance is not enough to warrant a definitive choice of the log-probit model as the preferred model. The exponential model would be the preferred choice for the SA-51 and SA-75 strains, as the other models do not lower the optimal deviance enough to warrant the additional parameter used by the log-probit or beta Poisson models (Haas 1999).

The right-most columns of Table J–6e illustrate the effects of assuming different models on the estimates for low-dose infectivity of RVFV. The beta Poisson model or the exponential model (when it is acceptable) are more conservative (estimate higher infectivity) at low doses than the log-probit model. Given that the log-probit model is not definitively preferred over the other models for any strain according the given statistical assessments, the higher-risk implications of the other models cannot be ignored and, therefore, the log-probit model is not chosen as the literature-based model to represent this data for this RA.

The beta Poisson model provides an acceptable fit for all four strains and provides relatively conservative infectivity estimates at low doses. Therefore, the beta Poisson model is carried forward for consideration as the literature-based model for this RA. Table J–6f displays the estimates derived from each of the four beta Poisson models along with 95 percent confidence intervals calculated from bootstrap resampling of each data set (Haas 1999). While the estimates appear similar across the different strains, the data sets cannot be pooled to form a single data set according to the statistical test described in Section J.3.2.4. It is not known which strains of RVFV would be more or less likely to be studied at NEIDL. Therefore, the four different models are retained separately and given equal weight in forming an overall model for further consideration. The *combined* row in Table J–6f represents an equal weighting of the four individual strain models, with the 95 percent confidence interval derived from random sampling from the bootstrapped estimates from all four sets.

Table J–6f. Model fitting for inhalational RVFV mice dose-response data sets (Brown 1981)

Strain	Model	LD_{50}	LD_{10}	LD_{1}	$LD_{0.1}$	$LD_{0.01}$
ZH-501	BP	**140 PFU** (60–250)	**13** (5.0–30)	**1.1** (0.43–2.8)	**0.11** (0.042–0.28)	**0.011** (0.0042–0.028)
Entebbe	BP	**76 PFU** (37–180)	**9.9** (4.5–27)	**0.92** (0.41–2.5)	**0.092** (0.040–0.25)	**0.0091** (0.0040–0.025)
SA-51	BP	**450 PFU** (220–840)	**67** (30–130)	**6.4** (2.8–12)	**0.64** (0.28–1.2)	**0.064** (0.028–0.12)
SA-75	BP	**78 PFU** (48–130)	**12** (6.6–20)	**1.1** (0.61–2.0)	**0.11** (0.061–0.19)	**0.011** (0.0061–0.019)
Combined	BP	**186 PFU** (47–650)	**25** (5.4–99)	**2.4** (0.48–9.4)	**0.24** (0.047–0.94)	**0.024** (0.0047–0.094)

Each LD_x result refers to the expected inhaled dose, in plaque-forming units (PFU), estimated to result in death of *x*% of exposed individuals. 95% confidence intervals based on model fits to bootstrap replicates from each data set. Abbreviations: BP = Beta Poisson. Units of dose are PFU.

Keefer et al. (1972) provided ID_{50} estimates based on their young dog and cat data (Table J–6b), but did not specify what quantitative procedure or assumed dose-response model, if any, was used to derive their estimates. For this RA, the exponential model was fit to the data in Table J–6b, using the midpoint of the given dose range for each dose group. The beta-Poisson and log-probit models are not appropriate models to fit to these data because they are two-parameter models and, for each animal, there was only one dose level at which an infection rate less than 100 percent was observed. Regardless, the exponential model fit results in a very low optimal deviance, which could not be improved enough to justify the use of a model with additional parameters. In addition, it was tested whether the results from the two data sets were consistent enough to be pooled, under the same statistical test described in Section J.3.2.4. As shown in Table J–6g, it was determined that pooling the two data sets was acceptable, and the model fit to this pooled data set is carried forward as a candidate model for this RA.

Table J–6g. Model fitting for inhalational RVFV dose-response data sets (Keefer 1972)

Data Set	Model	Optimal param. values	Optimal deviance	$\chi^2_{0.95, df}$	Accept-able fit?	Pooling acceptability test
A: Young Dog	Exp	$r = 0.052$	$Y_A = 0$	6.0	YES	$Y_C - (Y_A+Y_B) = 1.6$
B: Young Cat	Exp	$r = 0.13$	$Y_B = 0.16$	6.0	YES	$\chi^2_{0.95, 1} = 3.8$
C: Pooled A and B	Exp	$r = 0.072$	$Y_C = 1.8$	7.8	YES	**Acceptable to pool**

Dose-response model fitting to the inhalational animal data (Keefer 1972) from Table J–6b. Exp = exponential. Model parameter *r* is defined in Section J.2.5.1. Fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to (2, 2, 3) for data sets (A, B, C), respectively. Optimal deviance for the exponential fit to the young dog data is not exactly zero but is less than the degree of computer precision used.

Miller et al. (1963) generated dose-response data sets for inhalational exposure of hamsters and rhesus monkeys (Table J–6c), and they fit the log-probit model to the hamster data set. It is not possible to find an optimal dose-response model for the monkey data set, because every monkey became infected at every dose level. However, the data do provide some insight into probable upper bounds for ID levels for rhesus monkeys. For example, the exponential dose-response model can be evaluated in terms of how low the model parameter *r* can be while still producing a deviance low enough to be acceptable under statistical criteria in light of the data. The results of the exercise are shown in Table J–6h. For the hamster data set, Miller et al. did not test alternate dose response models, so the exponential, log-probit, and beta Poisson models were fit to the data as alternate candidate models for this RA. The results in Table J–6h show that log-probit and beta Poisson models provide acceptable fits to the data set, with the beta Poisson model producing a slightly lower optimal deviance and more conservative infectivity estimates at very low doses. Therefore, the beta Poisson model fit is carried forward for further consideration.

Table J–6h. Model fitting for inhalational RVFV animal dose-response data set (Miller 1963)

Animal	Model	Optimal or bound param. values	Deviance	$\chi^2_{0.95,df}$	Accept-able fit?	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
Monkey	Exp	$r > 0.01$	< 7.8	7.8	-	< 70	< 10	< 1	< 0.1
Hamster	Exp	$r = 0.75$	18	7.8	NO	-	-	-	-
	LP	$m = 0.69$ $LD_{50} = 0.52$	0.69	6.0	YES	0.52	0.064	0.011	0.0033
	BP	$\alpha = 1.1$ $\beta = 0.57$	0.33	6.0	YES	0.52	0.059	0.0054	0.00053

Dose-response modeling for the inhalational animal data (Miller 1963) from Table J–6c. Exp = exponential, LP = log-probit, BP = beta Poisson. Model parameters are defined in Section J.2.5.1. For the monkey data, the lower bound *r* value produces deviance equal to 95th percentile of the chi-squared distribution with df (degrees of freedom) equal to 3, the number of distinct doses in the data set (4) minus the number of model parameters (1). For the hamster data, fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to 3 for the exponential model and 2 for the log-probit and beta Poisson models. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals under each model. Units of dose are $MIPLD_{50}$.

Easterday et al. (1962) and Easterday and Murphy (1963), generated dose response data sets for inhalational exposure of lambs, mice, and hamsters (Table J–6d), but it appears they did not attempt dose-response model fitting to the data. The data for lambs are not extensive enough to justify dose-response model fitting, as they do not reveal a statistically significant trend for increasing infection rate for increasing dose. However, the data do provide some insight into probable upper bounds for ID levels for lambs. For example, the exponential dose-response model can be evaluated in terms of how low the model parameter *r* can be while still producing a deviance low enough to be acceptable under statistical criteria in light of the data. The results of the exercise are shown in Table J–6i. The data for mice and hamsters were appropriate for fitting to the exponential, log-probit, and beta Poisson models, with results also shown in Table J–6i.

Table J–6i. Model fitting for inhalational RVFV animal dose-response data sets (Easterday 1962, Easterday 1963)

Animal	Model	Optimal or bounded param. values	Deviance	$\chi^2_{0.95,\,df}$	Accept-able fit?	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
Lamb	Exp	$r > 0.6$	< 9.5	9.5	-	< 10	< 2	< 0.2	< 0.02
Mouse	Exp	$r = 0.005$	48	14	NO	-	-	-	-
	LP	$m = 0.47$ $ID_{50} = 58$	6.7	13	YES	58	3.7	0.39	0.076
	BP	$\alpha = 0.48$ $\beta = 15$	5.1	13	YES	47	3.6	0.31	0.031
Hamster	Exp	$r = 0.034$	65	14	NO	-	-	-	-
	LP	$m = 0.44$ $ID_{50} = 25$	7.8	13	YES	25	1.4	0.14	0.025
	BP	$\alpha = 0.61$ $\beta = 9.6$	5.5	13	YES	20	1.8	0.16	0.016

Dose-response model fitting to inhalational animal data (Easterday 1962, Easterday 1963) from Table J–6d. Exp = exponential, LP = log-probit, BP = beta Poisson. Model parameters are defined in Section J.2.5.1. For lambs, the lower bound *r* value produces deviance equal to 95th percentile of the chi-squared distribution with df (degrees of freedom) equal to 4, the number of distinct doses in the data set (5) minus the number of model parameters (1). For mice and hamsters, fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to the number of distinct doses in the data set minus the number of parameters for the fitted model. Each ID_x result refers to the expected inhaled dose estimated to result in death/infection of *x*% of exposed individuals under each model. Units of dose are $MIPLD_{50}$.

The lamb-based results in Table J–6i are intended to be guidelines for upper bounds of ID estimates. For example, if the true ID_{50} for lambs is greater than about 10 $MIPLD_{50}$ and if the exponential model is appropriate, then it would be unlikely (less than about 5 percent chance) that the data in Table J–6d would be observed. These results give no information on lower bounds for IDs for lambs. For the mouse and hamster data, the exponential model does not provide an acceptable fit under the given statistical criteria. The log-probit and beta Poisson models provide acceptable fits and lead to similar ID estimates at the levels shown in the table. Because the beta Poisson model produces a slight lower deviance and slightly more conservative estimates at the $ID_{0.1}$ level and lower, the beta Poisson model fits to the mouse and hamster data are carried forward for further consideration.

The candidate literature-based models, as well as information from some animal data that could not be fit to models, are compared in Table J–6j.

Table J–6j. Candidate models and information derived from inhalational RVFV animal dose-response data sets

Animal (Ref)	Model	I/LD_{50}	I/LD_{10}	I/LD_{1}	$I/LD_{0.1}$
Mouse (Brown 1981)	BP	**186 PFU** (47–650)	**25** (5.4–99)	**2.4** (0.48–9.4)	**0.24** (0.047–0.94)
Mouse (Easterday 1963)	BP	**47 $MIPLD_{50}$** (28–120)	**3.5** (1.8–9.9)	**0.31** (0.15–0.88)	**0.030** (0.014–0.087)
Young Dog & Cat (Keefer 1972)	Exp	**9.7 $MICLD_{50}$** (2.9–18)	**1.5** (0.44–2.8)	**0.14** (0.042–0.27)	**0.014** (0.0042–0.026)
Hamster (Miller 1963)	BP	**0.52 $MIPLD_{50}$** (0.29–0.91)	**0.059** (0.026–0.13)	**0.0054** (0.0023–0.012)	**0.00053** (0.00023–0.0012)
Hamster (Easterday 1963)	BP	**20 $MIPLD_{50}$** (8.8–75)	**1.8** (0.56–7.0)	**0.16** (0.044–0.66)	**0.016** (0.0043–0.065)
Lamb (Easterday 1962)	Exp	**< 10 $MIPLD_{50}$**	**< 2**	**< 0.2**	**< 0.02**
Rhesus Monkey (Miller 1963)	Exp	**< 70 $MIPLD_{50}$**	**< 10**	**< 1**	**< 0.1**
Rhesus Monkey (Easterday 1965)	None	**< 1.0 $MIPLD_{50}$**	-	-	-
Cynomolgus Monkey (Easterday 1965)	None	**< 1.0 $MIPLD_{50}$**	-	-	-

Each I/LD_x result refers to the expected inhaled dose estimated to result in infection/death of *x*% of exposed individuals. 95% confidence intervals based on model fits to bootstrap replicates from each data set.
Abbreviations: BP = beta Poisson, Exp = exponential, PFU = plaque-forming units, $MICLD_{50}$ = median mouse intracerebral lethal dose, $MIPLD_{50}$ = median mouse intraperitoneal lethal dose.

Mouse-based models: It appears from the results in Table J–6j that mice can be considered less susceptible to infection with RVFV via aerosols than other animals, with the possible exception of hamsters, for which results were similar in the same study (Easterday 1963). To attempt to reconcile the different units of dose (PFU) used by Brown et al., it is noted that titers of RVFV stock found in the literature were generally smaller in units of PFU than they were in units of median mouse lethal dose (e.g., Klein 1970, Klein 1971). This relationship would imply that the ID values in units of PFU would be even higher in units of $MIPLD_{50}$ or $MICLD_{50}$ which strengthens support for the notion that mice are less susceptible to infection from aerosols than other animals. Given that it appears that mice are significantly less susceptible to aerosols of RVFV than are larger mammals with physiologies more similar to humans,

it was determined that applying a mouse-based dose-response model would not be appropriate for this RA.

Hamster-based models: The two hamster-based models shown in Table J–6j give significantly different ID estimates from each other. It is not clear what caused the difference, as the two papers describe the use of similar RVFV strain preparations and similar animals used in the studies. The different estimates could have resulted from different procedures used to estimate the amount of virus inhaled by the hamsters; both sets of authors acknowledged the difficulty of measuring the low doses applied. Given the inconsistency in these results, it was determined that a hamster-based dose-response model would not be appropriate to apply for this RA.

Young dog and cat-based model: The model based on young dog and cat data (Keefer 1972) expresses the dose in units of $MICLD_{50}$, which is similar to $MIPLD_{50}$ except the route of exposure is intracerebral vs. intraperitoneal. One study (Boyle 1965) tested the same RVFV suspension on mice by both routes of exposure and obtained titer concentrations that were roughly 3-fold less by $MIPLD_{50}$ than by $MICLD_{50}$. Assuming that a similar relationship applies to the samples used by Keefer et al. and the Easterday studies, the ID estimates for young dogs and cats could be reduced by a factor of three to compare to the Easterday-derived estimates. That suggests that the young dog- and cat-based estimates could be consistent with the evidence shown for lambs and monkeys, and, therefore might be an appropriate set of estimates to apply for this RA. Furthermore, exposure estimates for this RA are given in units of $MICLD_{50}$, so the young dog- and cat-based model can be applied directly to the exposure estimates without adding another layer of uncertainty in converting to different units.

Goat and monkey evidence: The goat and monkey data suggest that these animals might be as, or perhaps more, susceptible to infection from aerosols of RVFV as young dogs and cats. However, the data for these animals are not available or not extensive enough to perform model fitting or a proper statistical comparison.

In light of the points in the above discussion, it was determined that the young dog- and cat-based model would be most appropriate to serve as the literature-based model for this RA. It is not known how the susceptibility of humans to RVFV inhalation would compare to any of the animals from which the estimates in Table J–6j were derived, so this model is applied with caution.

J.3.6.5 Dose-Response Estimates Derived from Expert Panel

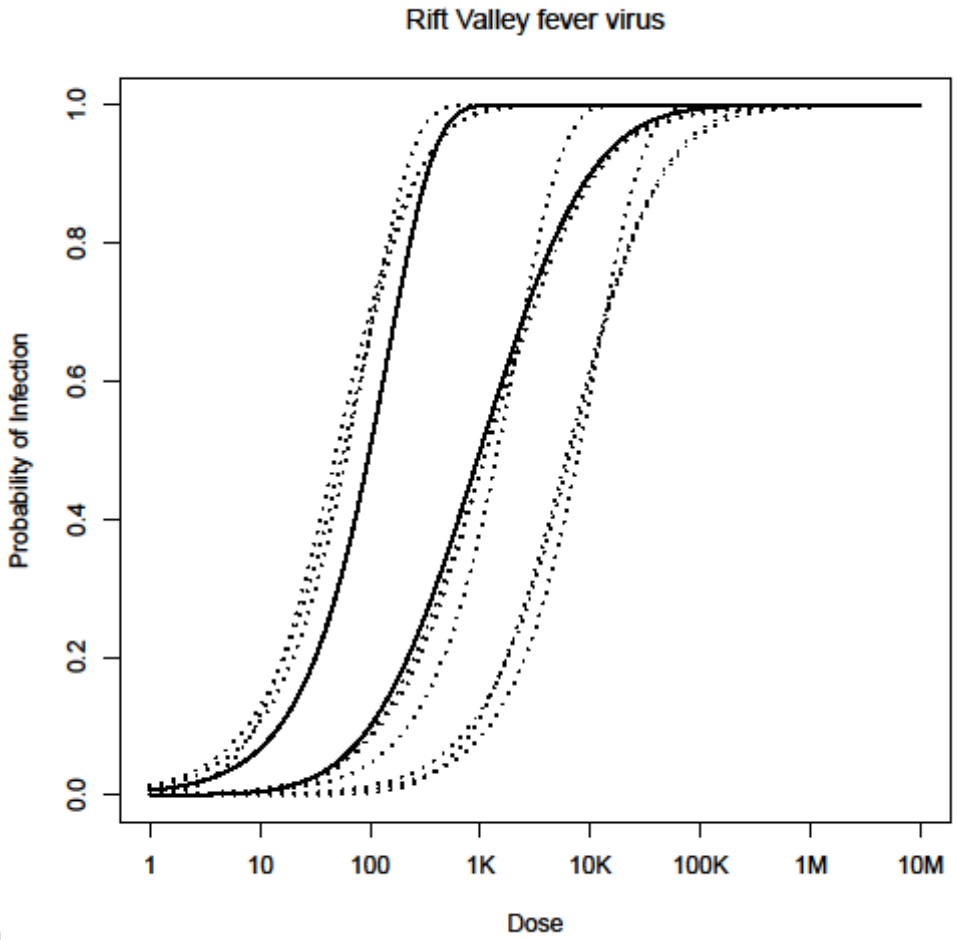
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for RVFV are displayed in Table J–6k, including which models were retained from each expert according to the BIC. Sixteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–6. ID estimates provided by the retained curves are shown in Table J–6l.

Table J–6k: Dose response model fitting for Rift Valley fever virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log−probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	1,000 5,000 50,000	8.8×10^{-5}	0.66	6,300	1.3	9.3×10^{3}	**5.2**	**0**	**1.7**
2	15 99 329	7.0×10^{-3}	0.83	79	1.0×10^{3}	1.4×10^{5}	**0**	28.7	1.1[a]
3	100 1,500 10,000	4.8×10^{-4}	0.56	1,100	9.7×10^{-1}	1.1×10^{3}	**6**	**0**	**0.5**
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
6	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
7	10 40 300	1.1×10^{-2}	0.75	49	2.4	1.7×10^{2}	**3.8**	**0**	**3.4**
8	100 1,500 10,000	4.8×10^{-4}	0.56	1,100	9.7×10^{-1}	1.1×10^{3}	**6**	**0**	**0.5**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–6



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–6l. Results for retained expert-derived RVFV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$	$ID_{0.01}$
1	Exp	7900	1200	110	11	1.1
	LP	6300	890	180	56	22
	BP	6700	800	73	7.3	0.73
2	Exp	99	15	1.4	0.14	0.014
3	Exp	1400	220	21	2.1	0.21
	LP	1100	110	18	4.4	1.4
	BP	1100	120	11	1.1	0.11
4	LP	1000	100	15	3.9	1.3
5	LP	1000	100	15	3.9	1.3
6	LP	1000	100	15	3.9	1.3
7	Exp	62	9.4	0.90	0.089	0.0089
	LP	49	9.0	2.3	0.82	0.35
	BP	57	7.6	0.71	0.071	0.0071
8	Exp	1400	220	21	2.1	0.21
	LP	1100	110	18	4.4	1.4
	BP	1100	120	11	1.1	0.11
Median (Min–Max)		**1000 org.** (49–7900)	**100** (7.6–1200)	**15** (0.71–180)	**3.9** (0.071–56)	**1.2** (0.0071–22)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.01}$ refers to the dose estimated to result in infection of 0.01%, or one in ten thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.6.6 Other Considerations

The units of exposure for events analyzed in this RA for RVFV are in terms of $CCID_{50}$ (median cell culture infective dose) and $MICLD_{50}$ (median mouse intracerebral lethal dose). Alternate exposure estimates are also given in plaque-forming units (PFU). The exposures in the young dog and cat experiments that form the basis for the literature-based dose-response estimates are in terms of $MICLD_{50}$, so it is appropriate to apply the literature-based dose response estimates to the exposure estimates.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU, and that it is appropriate to apply the curves derived from their estimates to the PFU exposure estimates. It is possible that this assumption is non-conservative if the plaque assay used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. One study reported a count of 1,000–3,000 virus subunits per PFU of RVFV (Garcia 2001), and a recent

study has reported counts of viral genomes as well as virus particles in samples of RVFV that were also assayed for FFU, resulting in estimates of about 290,000 viral genomes per FFU and about 2,500 virus particles per FFU (Weidmann 2011). It is clear from these data that the number of viral genomes in a sample of RVFV is not an appropriate estimate for the number of organisms, because there were 119 times as many genomes as there were particles available to contain genomes and form complete virions. The number of virus particles observed might be a more accurate estimate for the number of organisms, but it is also possible that many of those particles did not represent replication-competent virions with the ability to infect host cells, which is supported by the fact that roughly 2,500 particles were measured for each focus-forming unit in the cell culture. However it is possible that some portion of the particles that were not infective in the cell culture would be infective to cells in a live host. Therefore, these data suggest that the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU might be non-conservative.

J.3.6.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for RVFV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–6m.

- Literature-based dose-response model: the exponential model with $r = 0.072$, derived by fitting the model to consolidated young dog and cat inhalational dose-response data (Table J–6b). Use a distribution of r values derived from bootstrap replicates of the data set for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–6k, with the model or set of models derived from each expert weighted equally.

Table J–6m. ID estimates and associated ranges for RVFV.

Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
Literature-based	**9.7 $MICLD_{50}$** (2.9–18)	**1.5** (0.44–2.8)	**0.14** (0.042–0.27)	**0.014** (0.0042–0.026)
Expert-based	**1000 org.** (49–7900)	**100** (7.6–1200)	**15** (0.71–180)	**3.9** (0.071–56)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–6l. Abbreviations: PFU = plaque forming unit; org. = organisms.

For each ID point displayed in Table J–6m, the literature-based range extends lower (higher risk) than the expert-based range. It is assumed that dose units of PFU and organisms (as conceived by the experts) are equivalent, although this assumption may be non-conservative for the expert-based numbers as described in Section J.3.6.6. The literature-based results, being more conservative than the expert-based results, can in part serve to assess the implications of the possibility that the expert-based results are non-conservative by a similar factor. Both sets of estimates shown in Table J–6m are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by RVFV. As noted in Section J.3.6.6, alternate exposure estimates are given in units of $MICLD_{50}$ and also in units of PFU.

J.3.7 Andes Virus

Andes virus (ANDV) is a hantavirus that causes hantavirus pulmonary syndrome (HPS), a highly infectious, zoonotic, rodent-transmitted disease that has infected humans, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.7.1 Routes of Exposure

For ANDV, the primary natural routes of exposure and infection for humans are assumed to be through inhalation of aerosolized excretions from infected rodents, specifically the long-tailed pygmy rice rat, or direct contact with rodent excretions or contaminated fomites. Ingestion of particles contaminated with rodent excretions or bites from infected rodents might also cause infection. Bodily fluids of infected humans may cause infection in contacts as well. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and

rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.7.2 Vaccine and Prophylaxis

No vaccines are available for ANDV, although experimental vaccines might be efficacious in animal models as described in Chapter 3. For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with ANDV. That is a conservative assumption because it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that could become FDA-approved or that could be available before official FDA-approval, such as those classified as an IND.

No prophylactic treatments have been shown to protect against ANDV infection after exposure, nor do any specific treatments or cures exist for HPS.

J.3.7.3 Dose-Response Information from the Literature

There are no human dose-response data available for ANDV. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing ANDV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on ANDV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

Hooper et al. (2008) exposed groups of Syrian hamsters to different amounts of ANDV through different routes of exposure, including the intranasal route, which serves as a model of exposure to respiratory mucosa that would occur upon inhalation. Syrian hamsters had previously been shown to be an animal model that closely mimics human HPS (Hooper 2001). The dose-response data from intranasal exposure of the hamsters are shown in Table J–7a. The authors reported an LD_{50} of 95 PFU and an ID_{50} of 48 PFU, which appear to have been calculated under the assumptions of the log-probit model.

Table J–7a. Hamster intranasal dose-response data (Hooper 2008) for ANDV.

Challenge Dose (PFU)	Number exposed	Number infected
2	8	0
20	8	2
200	8	7
2000	8	8
20000	8	8

Data from Hooper et al. (2008). Infected animals included animals that died and animals that survived for 35 days but were seropositive for antinucleocapsid antibodies at day 35.

J.3.7.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published model for ANDV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The Syrian hamster data from Hooper et al. (2008) appears to be the only intranasal or inhalational dose-response animal data available in the literature. The hamster data with infection as the response is amenable to dose-response model fitting. Hooper et al. (2008) reported median lethal and ID estimates that appear to have been derived from a log-probit model, but they did not assess the goodness of fit for this model nor did they report attempts to fit alternate models. For this RA, the exponential, log-probit, and beta Poisson models were fit to the data, and the model-fitting results are presented in Table J–7b.

Table J–7b. Model fitting for intranasal ANDV hamster dose-response data set (Hooper 2008)

Model	Optimal param. values	Optimal deviance	$\chi^2_{0.95,\,df}$	Accept-able fit?	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$	$ID_{0.01}$
Exp	$r = 0.011$	0.50	9.5	YES	63 PFU	9.6	0.91	0.091	0.0091
LP	$m = 0.84$ $ID_{50} = 48$	0.097	7.8	YES	48 PFU	10	2.9	1.2	0.55
BP	$\alpha = 5.3$ $\beta = 410$	0.45	7.8	YES	57 PFU	8.3	0.78	0.078	0.0078

Dose-response modeling for the intranasal hamster data (Hooper 2008) from Table J–7a. Exp = exponential, LP = log-probit, BP = beta Poisson, PFU = plaque forming units. Model parameters are defined in Section J.2.5.1. Fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to 4 for the exponential model and 3 for the log-probit and beta Poisson models. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals under each model.

All three models provide acceptable fits to the data. The exponential model is the preferred choice of the three, as the log-probit model and beta Poisson models do not lower the optimal deviance enough to justify the use of an extra parameter (Haas 1999). Therefore, the exponential model is used as the literature-based dose-response estimate for this RA.

J.3.7.5 Dose-Response Estimates Derived from Expert Panel

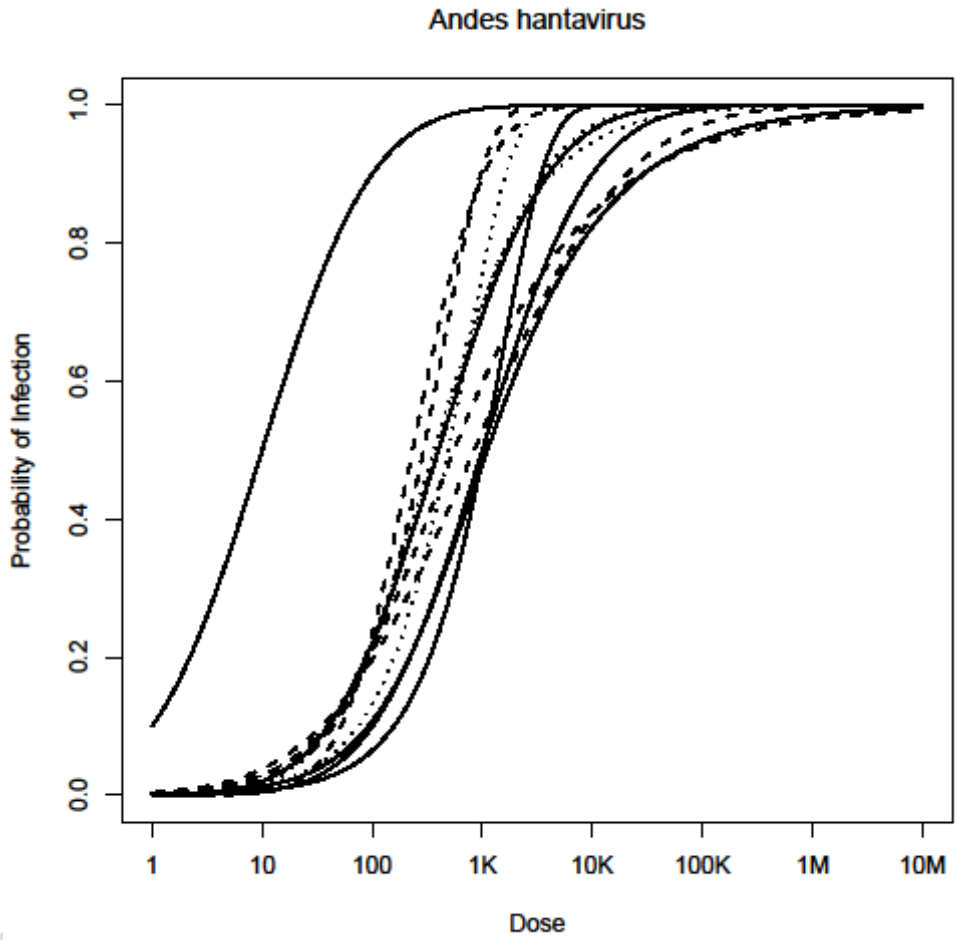
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for ANDV are displayed in Table J–7c, including which models were retained from each expert according to the BIC. Twelve curves were retained for application in this RA, and plots of those curves are shown in Figure J–7. ID estimates provided by the retained curves are shown in Table J–7d.

Table J–7c: Dose response model fitting for Andes virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log–probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	50 250 1,000	2.4×10^{-3}	0.86	230	1.0×10^{5}	4.2×10^{7}	**3.6**	**0**	4.7[a]
2	160 1,000 3,500	6.7×10^{-4}	0.83	820	1.0×10^{2}	1.5×10^{5}	**0**	11.4	1.1[a]
3	50 400 30,000	6.5×10^{-4}	0.40	840	4.3×10^{-1}	1.4×10^{2}	9.9	**4.7**	**0**
4	1 10 100	5.5×10^{-2}	0.56	10	8.9×10^{-1}	8.2	N/A	**Exact**	N/A
5	60 200 5,000	1.4×10^{-3}	0.58	390	8.1×10^{-1}	2.7×10^{2}	**2.7**	**0.3**	**0**
6	40 400 4,000	1.4×10^{-3}	0.56	400	8.9×10^{-1}	3.3×10^{2}	N/A	**Exact**	N/A
7	100 1,000 30,000	3.8×10^{-4}	0.45	1,400	5.4×10^{-1}	4.3×10^{2}	15	7.2	**0**
8	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–7



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–7d. Results for retained expert-derived ANDV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	Exp	290	44	4.2	0.42	0.042
	LP	230	52	15	6.3	3.0
2	Exp	1000	160	15	1.5	0.15
3	LP	840	34	2.5	0.38	0.079
	BP	550	39	3.3	0.32	0.032
4	LP	10	1.0	0.15	0.039	0.013
5	Exp	490	75	7.1	0.71	0.071
	LP	390	43	7.1	1.9	0.64
	BP	370	37	3.4	0.33	0.033
6	LP	400	40	6.1	1.6	0.50
7	BP	1100	92	8.0	0.79	0.079
8	LP	1000	100	15	3.9	1.3
Median (Min–Max)		**520 org.** (10–1100)	**44** (1.0–160)	**7.1** (0.15–15)	**0.79** (0.039–6.3)	**0.079** (0.013–3.0)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.7.6 Other Considerations

The units of exposure for events analyzed in this RA for ANDV are in terms of $CCID_{50}$ (median cell culture infective dose). The exposures in the hamster experiments that form the basis for the literature-based dose-response estimates are in terms of PFU. It is likely that the exposure estimates would be lower in terms of PFU, because the estimated inventory concentration (1×10^6 $CCID_{50}$ / ml), which is proportional to the exposure estimates, was chosen in those units because titers in other units, including PFU, were generally found to be lower in the literature. However, there is at least one example of up to 10^6 PFU / mL being achieved in the laboratory for ANDV (Wahl-Jensen 2007). Therefore, it is conservatively assumed that it is appropriate to apply the dose-response estimates in terms of PFU to the exposure estimates in units of $CCID_{50}$.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU. It is possible that this assumption is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. There is

little information in the literature on the sensitivity of ANDV assays in detecting virions. Therefore, the potential magnitude of non-conservatism resulting from the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU is unknown.

J.3.7.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for ANDV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–7e.

- Literature-based dose-response model: the exponential model with $r = 0.011$, derived by fitting the model to Syrian hamster intranasal dose-response data (Hooper 2008). Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (0.0051 to 0.029), for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–7d, with the model or set of models derived from each expert weighted equally.

Table J–7e. ID estimates and associated ranges for ANDV.

Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
Literature-based	**63 PFU** (24–140)	**9.6** (3.6–21)	**0.91** (0.35–2.0)	**0.091** (0.034–0.20)	**0.0091** (0.0034–0.020)
Expert-based	**520 org.** (10–1100)	**44** (1.0–160)	**7.1** (0.15–15)	**0.79** (0.039–6.3)	**0.079** (0.013–3.0)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–7d. Abbreviations: PFU = plaque forming unit; org. = organisms.

Both sets of estimates shown in Table J–7e are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by ANDV.

J.3.8 Ebola Virus

Ebola virus (EBOV) causes Ebola hemorrhagic fever (EHF), a highly infectious, zoonotic disease that has infected humans in both natural and laboratory settings, as described in Chapter 3 and Appendix C. This

section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.8.1 Routes of Exposure

For EBOV, the primary natural routes of exposure and infection for humans are assumed to be through inhalation of aerosolized particles from contaminated bodily fluids of infected animals or humans, or through ingestion or direction contact with such fluids. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.8.2 Vaccine and Prophylaxis

There are currently no vaccines available for EBOV, although candidate vaccines are in development that have been shown to be successful in protecting NHPs from disease after inhalational exposure (Geisbert 2008, Pratt 2010). For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with EBOV. This is a conservative assumption, as it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that may become FDA-approved or that may be available before official FDA-approval, such as those classified as an IND.

There are no prophylactic treatments that have been shown to protect against EBOV infection after exposure, nor are there any validated specific treatments for disease after symptoms appear.

J.3.8.3 Dose-Response Information from the Literature

There are no human dose-response data available for EBOV. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing EBOV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on EBOV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

Unimmunized NHPs (*Macaca mulatta* or *Macaca fascicularis*) have been infected after inhalational exposure to EBOV in a number of studies. Johnson et al. (1995) exposed two monkeys to an inhalational

dose of 400 PFU and two monkeys to a dose of 50,000 PFU and all four monkeys subsequently died of disease. Geisbert et al. (2008), in a vaccine study, exposed three unprotected control group monkeys to target inhalational doses of 1000 PFU and all three monkeys subsequently died of disease. Pratt et al. (2010), in another vaccine study, exposed eight unprotected control group monkeys to doses as low as 100 PFU to as high as 1200 PFU, and all eight subsequently died of disease. These authors also make reference to *historical controls,* apparently unpublished data to which the authors had access, consisting of an additional six unprotected monkeys that died after exposure of approximately 20 PFU to as high as 500 PFU. The available unimmunized NHP data from the above three studies are summarized in Table J–8a.

Table J–8a. NHP inhalational dose-response data for EBOV

Challenge Dose (PFU)	Number exposed	Number died	Strain	Reference
20	3	3	Sudan	Pratt 2010 (historical controls)
100–500	3	3	Sudan	Pratt 2010 (historical controls)
100–500	2	2	Sudan	Pratt 2010
400	2	2	Zaire	Johnson 1995
500	1	1	Zaire	Pratt 2010
800–1200	3	3	Zaire	Pratt 2010
900–1000	1	1	Zaire	Pratt 2010
1000	3	3	Zaire	Geisbert 2008
1100	1	1	Zaire	Pratt 2010
50,000	2	2	Zaire	Johnson 1995

Data from unprotected (unimmunized) monkeys that inhaled the given dose in plaque-forming units (PFU).

As shown in Table J–8a, these studies did not observe any unprotected NHPs surviving exposure, which suggests that doses lower than 20 PFU might also be highly infective to monkeys. Another study (P'iankov 1995) did observe dose-dependent survivorship in rhesus macaques (*Macaca mulatta*), with nine out of sixteen monkeys surviving after inhalational exposure of EBOV. The survivors occurred at the lower doses, which were quantified in units of median mouse intracerebral lethal dose ($MICLD_{50}$). The data from this study are shown in Table J–8b.

Table J–8b. NHP inhalational dose-response data for EBOV (P'iankov 1995)

Challenge Dose ($MICLD_{50}$)	Number exposed	Number died
2.9	4	0
3.5	4	1
16.2	4	2
214	4	4
3550	4	4

Data from P'iankov et al. (1995). Dose amounts were quantified in units of median mouse intracerebral lethal dose ($MICLD_{50}$) using suckling mice.

Based on the data in Table J–8b, P'iankov et al. reported an estimated LD_{50} range of 5–20 $MICLD_{50}$.

J.3.8.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data and evidence for EBOV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The NHP dose-response data in Table J–8a, with doses quantified in terms of PFU, are not amenable to dose-response model fitting because a 100 percent death rate was observed within every dose group. The P'iankov et al. (1995) data in Table J–8b are amenable to model fitting, but the doses are expressed in different units ($MICLD_{50}$). A report in the literature (Moe 1981) related units of PFU, $MICLD_{50}$ and $CCID_{50}$ by performing titration of the Zaire strain of EBOV by all three methods. They found a PFU/$MICLD_{50}$ ratio of 2.5/9.0. Therefore, this factor was used to convert units of $MICLD_{50}$ to units of PFU, and the data from Tables J–8a and J–8b were combined to form a single data set for analysis in this RA, shown in Table J–8c. Because the degree of precision for some dose levels was reported to only one significant digit, dose levels were rounded and consolidated for the data set. Also, the dose groups in Table J–8a reported as ranges were averaged over the endpoints of each range. Analysis showed that these consolidation and averaging procedures did not alter the model fitting results to one significant digit, well within the 95 percent confidence range.

Table J–8c. Consolidated NHP inhalational dose-response data for EBOV

Challenge Dose (PFU)	Number exposed	Number died	Reference
0.8	4	0	P'iankov 1995
1	4	1	P'iankov 1995
5	4	2	P'iankov 1995
20	3	3	Pratt 2010
60	4	4	P'iankov 1995
300	5	5	Pratt 2010
400	2	2	Johnson 1995
500	1	1	Pratt 2010
1000	12	12	Geisbert 2008, Pratt 2010, P'iankov 1995
50,000	2	2	Johnson 1995

Data from unprotected (unimmunized) monkeys that inhaled the given dose, rounded to one significant figure. Data from P'iankov et al. (1995) were converted from units of $MICLD_{50}$ to PFU assuming 2.5 PFU = 9.0 $MICLD_{50}$ as explained in the text.

The combined data in Table J–8c were fit to the exponential model, which produced an acceptable fit and an optimal deviance low enough that the beta Poisson model could not possibly improve the fit enough to justify the additional parameter (Haas 1999). The model fitting statistics and ID estimates derived from the model are shown in Table J–8d.

Table J–8d. Model fitting for inhalational NHP dose-response data set

Model	Optimal param. value	Optimal deviance	$\chi^2_{0.95,\ df=9}$	LD_{50}	LD_{10}	LD_1
Exp	$r = 0.2$	1.6	17	**4 PFU** (1–10)	**0.7** (0.2–2)	**0.06** (0.02–0.2)

Dose-response model fitting to the NHP data from Table J–8c. Exp = exponential. Model parameter is defined in Section J.2.5.1. Fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to 9, the number of distinct doses minus the number of parameters. Each LD_x result refers to the expected inhaled dose estimated to result in death of *x*% of exposed individuals under the model. Units of dose are plaque-forming units (PFU). Intervals under the LD estimates are 95% confidence intervals based on model fits to bootstrap replicates from the data set.

The exponential model described in Table J–8d serves as the literature-based dose-response model for this RA, under the assumption that a lethal dose for NHPs under laboratory conditions adequately represents an ID for humans potentially exposed to aerosols from a laboratory release. It is possible that the very low inhalational IDs observed among monkeys and estimated by the model overestimate the probability of infection for humans at low doses, especially in light of the fact that aerosol infection and airborne

transmission appears to have occurred infrequently during human outbreaks of EHF (Geisbert 2004). However, there are alternate explanations for infrequent occurrence of airborne transmission. It is possible that EBOV does not survive well in respirable particles outside laboratory scenarios or that humans infected with EBOV do not shed infectious particles that are easily inhaled (Leffel 2004). Therefore, the possibility that humans would be as susceptible to infection as NHPs after inhaling aerosolized EBOV released from a laboratory cannot be ruled out.

J.3.8.5 Dose-Response Estimates Derived from Expert Panel

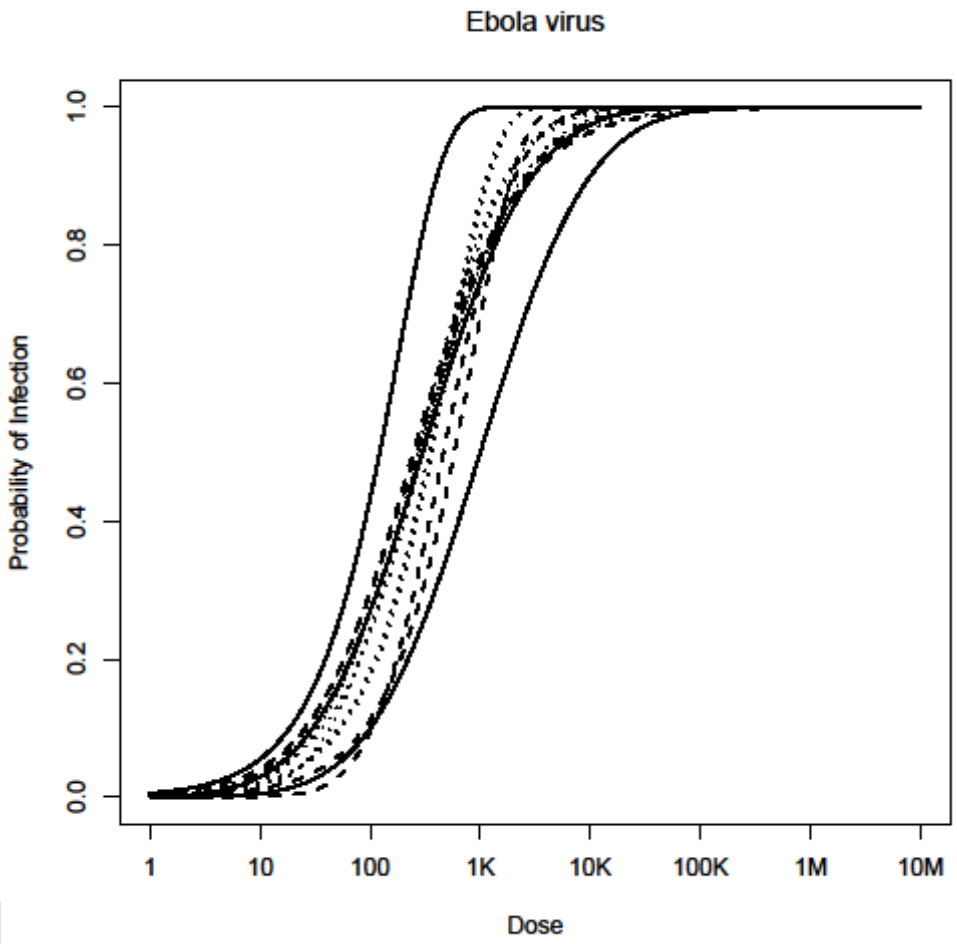
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for EBOV are displayed in Table J–8e, including which models were retained from each expert according to the BIC. Fourteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–8. ID estimates provided by the retained curves are shown in Table J–8f.

Table J–8e: Dose response model fitting for Ebola virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log–probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
2	19 120 400	5.7×10^{-3}	0.84	97	1.0×10^{6}	1.8×10^{8}	**0**	13.7	1.1[a]
3	30 200 3,000	2.1×10^{-3}	0.56	260	8.2×10^{-1}	1.9×10^{2}	8.1	**1.2**	**0**
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	40 200 3,000	1.9×10^{-3}	0.59	290	9.3×10^{-1}	2.5×10^{2}	**4.5**	**0.1**	**0**
6	30 300 3,000	1.8×10^{-3}	0.56	300	8.9×10^{-1}	2.5×10^{2}	N/A	**Exact**	N/A
7	100 500 2,000	1.2×10^{-3}	0.86	460	1.0×10^{5}	8.4×10^{7}	**3.6**	**0**	4.7[a]
8	50 200 2,000	2.0×10^{-3}	0.69	270	1.5	5.1×10^{2}	**3.3**	**0**	**1.8**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–8



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–8f. Results for retained expert-derived EBOV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	LP	1000	100	15	3.9	1.3
2	Exp	120	19	1.8	0.18	0.018
3	LP	260	26	4.0	1.0	0.33
	BP	250	25	2.3	0.23	0.023
4	LP	1000	100	15	3.9	1.3
5	Exp	360	55	5.3	0.52	0.052
	LP	290	33	5.7	1.6	0.55
	BP	280	31	2.8	0.27	0.027
6	LP	300	30	4.6	1.2	0.38
7	Exp	580	89	8.5	0.84	0.084
	LP	460	100	31	13	6.0
8	Exp	340	52	4.9	0.49	0.049
	LP	270	43	9.5	3.2	1.3
	BP	300	37	3.4	0.34	0.034
Median (Min–Max)		**300 org.** (120–1000)	**40** (19–100)	**5.1** (1.8–31)	**1.2** (0.18–13)	**0.38** (0.018–6.0)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.8.6 Other Considerations

The units of exposure for events analyzed in this RA for EBOV are in terms of $CCID_{50}$ (median cell culture infective dose). For the literature-based dose-response model for this RA, the dose amounts are expressed in plaque-forming units (PFU), so potential differences between amounts of exposure measured in $CCID_{50}$ vs. PFU must be addressed. A study in the literature (Moe 1981) compared titration amounts from the same sample of Ebola virus in both PFU and $CCID_{50}$, and found a PFU/$CCID_{50}$ ratio of 1/12. Therefore, in the initial infections analyses for release events in this RA, the exposure estimates in $CCID_{50}$ are scaled by a factor of 1/12 before being applied to the literature-based dose response model described in Section J.3.8.4.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU. Therefore, the exposure estimates are scaled by a factor of 1/12, as described in the previous paragraph, before being applied to the expert-derived dose-response estimates as well. It is possible that this

assumption is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. For EBOV, many studies suggest that there could be multiple virions in sample containing 1 PFU, including experiments in which less than one PFU of virus have been shown to infect animals. Electron microscopy and PCR studies have identified 1000–10,000 EBOV genomes per PFU (Towner 2004, Trombley 2010), although many of those genomes are likely not incorporated into viral particles, and therefore are regarded as posing no threat to a potential host, as evidenced by a finding of 74–336 genomes per available shell in a sample of EBOV (Weidmann 2011). Furthermore, it is likely that even many virions with an incorporated genome may be otherwise defective or lack the ability to infect host cells. Therefore, the potential magnitude of non-conservatism resulting from the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU is unknown.

J.3.8.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for EBOV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–8g.

- Literature-based dose-response model: the exponential model with $r = 0.2$, derived by fitting the model to consolidated NHP inhalational dose-response data (Table J–8c). Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (0.07 to 0.5), for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–8f, with the model or set of models derived from each expert weighted equally.

Table J–8g. ID estimates and associated ranges for EBOV.

Model	ID_{50}	ID_{10}	ID_1
Literature-based	**4 PFU** (1–10)	**0.7** (0.2–2)	**0.06** (0.02–0.2)
Expert-based	**300 org.** (120–1000)	**40** (19–100)	**5.1** (1.8–31)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–8f. Abbreviations: PFU = plaque forming unit; org. = organisms.

It is assumed that dose units of PFU and organisms (as conceived by the experts) are equivalent, although this assumption may be non-conservative for the expert-based numbers as described in Section J.3.8.6. The literature-based results, being more conservative than the expert-based results by roughly a factor of 100, can in part serve to assess the implications of the possibility that the expert-based results are non-conservative by a similar factor. Both sets of estimates shown in Table J–8g are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by EBOV. As described in Section J.3.8.6, the exposure estimates from the event sequence analyses are in units of $CCID_{50}$, which are first converted to units of PFU before the dose response models described here are applied.

J.3.9 Marburg Virus

Marburg virus (MARV) causes Marburg hemorrhagic fever (MHF), a highly infectious, zoonotic disease that has infected humans in both natural and laboratory settings, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.9.1 Routes of Exposure

For MARV, the primary natural routes of exposure and infection for humans are assumed to be through inhalation of aerosolized particles from contaminated bodily fluids of infected animals or humans, or through ingestion or direction contact with such fluids. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section,

in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.9.2 Vaccine and Prophylaxis

There are currently no vaccines available for MARV, although candidate vaccines are in development that have been shown to be successful in protecting NHPs from disease after inhalational exposure (Geisbert 2008). For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with MARV. This is a conservative assumption, as it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that may become FDA-approved or that may be available before official FDA-approval, such as those classified as an IND.

There are no prophylactic treatments that have been shown to protect against MARV infection after exposure, nor are there any validated specific treatments for disease after symptoms appear.

J.3.9.3 Dose-Response Information from the Literature

There are no human dose-response data available for MARV. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing MARV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on MARV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

Unimmunized NHPs (*Macaca mulatta* or *Macaca fascicularis*) have been infected after inhalational exposure to EBOV in a number of studies. Geisbert et al. (2008), in a vaccine study, exposed two unprotected control group monkeys to target inhalational doses of 1000 PFU and both monkeys subsequently died of disease. Alves et al. (2010), in another vaccine study, exposed six unprotected control group monkeys to doses ranging from 2 PFU to 705 PFU, and all six subsequently died of disease. The available unimmunized NHP data from the above two studies are summarized in Table J–9a.

Table J–9a. NHP inhalational dose-response data for MARV

Challenge Dose (PFU)	Number exposed	Number died	Strain	Reference
2	1	1	Angola	Alves 2010
11	1	1	Angola	Alves 2010
14	1	1	Angola	Alves 2010
99	1	1	Angola	Alves 2010
339	1	1	Angola	Alves 2010
705	1	1	Angola	Alves 2010
1000	2	2	Musoke	Geisbert 2008

Data from unprotected (unimmunized) monkeys that inhaled the given dose of MARV

As shown in Table J–9a, these studies did not observe any unprotected NHPs surviving exposure, which suggests that doses lower than 2 PFU might also be infective to monkeys. Another study (Bazjutin 1992) did observe dose-dependent survivorship in green monkeys (*Chlorocebus sabaeus*), with four out of ten monkeys surviving after inhalational exposure to very low doses of MARV. It appears that the four survivors were actually infected, based on titers of virus found in their blood, but eventually recovered. The doses were quantified in units of median guinea pig intraperitoneal lethal dose ($GPIPLD_{50}$). The data from this study are shown in Table J–9b.

Table J–9b. NHP inhalational dose-response data for MARV (Bazjutin 1992)

Challenge Dose ($GPIPLD_{50}$)	Number exposed	Number died
0.003–0.1	10	6
5	5	5

Data from Bazjutin et al. (1992). Dose amounts were quantified in units of median guinea pig intraperitoneal lethal dose ($GPIPLD_{50}$).

J.3.9.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data and evidence for MARV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The NHP dose-response data in Table J–9a, with doses quantified in terms of PFU, are not amenable to dose-response model fitting because a 100 percent death rate was observed at every dose. The Bazjutin et al. (1992) data in Table J–9b observed four survivors exposed to low doses. However, there are a number of issues that make quantitative dose-response modeling difficult with these data.

- The relationship between $GPIPLD_{50}$ and PFU or other common measures is unclear. Hevey et al. (2002) report a PFU/guinea pig LD_{50} ratio of about 0.5, but it's unclear if the data or methodology used to derive that relationship is consistent with what was done in Bazjutin et al.

- The most potentially important information for quantitative risk assessment at low doses lies in the lowest dose group, but the dose range for this group covers almost two orders of magnitude (0.003–0.1 $GPIPLD_{50}$) and it is not specified which doses within that range were inhaled by the survivors.

Based on the above difficulties, it was determined that a quantitative dose-response model based on the data in Tables J–9a and J–9b would not be appropriate. Nevertheless, it is clear that the data imply that low doses on the order of 1 PFU and potentially lower can be lethal to monkeys when inhaled.

Ebola virus (EBOV) is closely related to MARV, and a literature-based dose response curve for EBOV was described in Section J.3.8.4. The estimates derived from that model are consistent with the MARV dose-response data in Table J–9a (the deviance produced by the model applied to the data is less than the 95^{th} percentile of the corresponding chi-squared distribution). In the absence of solid quantitative data for MARV with which to parameterize the low-dose region of a dose-response curve, and given the numerous similarities between EBOV and MARV, it was decided to apply the EBOV literature-based dose-response curve to MARV for this RA.

It is possible that the very low inhalational IDs observed among monkeys and estimated by the model overestimate the probability of infection for humans at low doses, especially in light of the fact that aerosol infection and airborne transmission appears to have occurred infrequently during human outbreaks of MHF (Geisbert 2004). However, there are alternate explanations for infrequent occurrence of airborne transmission. It is possible that MARV does not survive well in respirable particles outside laboratory scenarios or that humans infected with MARV do not shed infectious particles that are easily inhaled (Leffel 2004). Therefore, the possibility that humans would be as susceptible to infection as NHPs after inhaling aerosolized MARV released from a laboratory cannot be ruled out.

J.3.9.5 Dose-Response Estimates Derived from Expert Panel

In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for MARV are

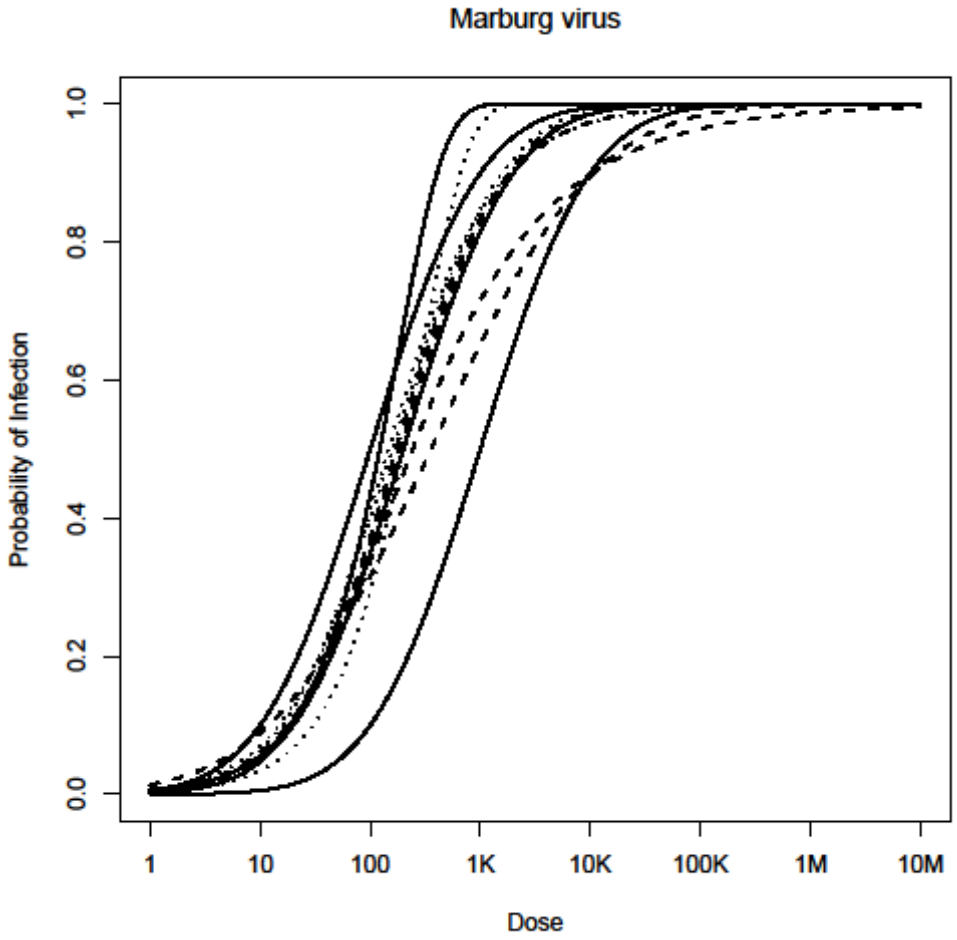
displayed in Table J–9c, including which models were retained from each expert according to the BIC. Twelve curves were retained for application in this RA, and plots of those curves are shown in Figure J–9. ID estimates provided by the retained curves are shown in Table J–9d.

Table J–9c: Dose-response model fitting for Marburg virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log–probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
2	19 120 400	5.7×10^{-3}	0.84	97	1.0×10^{6}	1.8×10^{8}	**0**	13.7	1.1[a]
3	20 100 2,000	3.5×10^{-3}	0.56	160	7.8×10^{-1}	1.0×10^{2}	**5.0**	**0.8**	**0**
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	20 200 2,000	2.8×10^{-3}	0.56	200	8.9×10^{-1}	1.6×10^{2}	N/A	**Exact**	N/A
6	10 100 1,000	5.5×10^{-3}	0.56	100	8.9×10^{-1}	8.2×10^{1}	N/A	**Exact**	N/A
7	10 500 9,000	1.6×10^{-3}	0.38	360	4.6×10^{-1}	6.8×10^{1}	10.0	**0**	**4.8**
8	20 150 2,000	3.0×10^{-3}	0.56	180	8.4×10^{-1}	1.3×10^{2}	10.3	**1.7**	**0**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–9



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–9d. Results for retained expert-derived MARV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	LP	1000	100	15	3.9	1.3
2	Exp	120	19	1.8	0.18	0.018
3	Exp	260	26	4.0	1.0	0.33
3	LP	250	25	2.3	0.23	0.023
3	BP	1000	100	15	3.9	1.3
4	LP	360	55	5.3	0.52	0.052
5	LP	290	33	5.7	1.6	0.55
6	LP	280	31	2.8	0.27	0.027
7	LP	300	30	4.6	1.2	0.38
7	BP	580	89	8.5	0.84	0.084
8	LP	460	100	31	13	6.0
8	BP	340	52	4.9	0.49	0.049
Median (Min–Max)		**200 org.** (100–1000)	**19** (10–100)	**2.4** (0.74–15)	**0.39** (0.098–3.9)	**0.13** (0.013–1.3)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.9.6 Other Considerations

The units of exposure for events analyzed in this RA for MARV are in terms of $CCID_{50}$ (median cell culture infective dose). For the literature-based dose-response model for this RA, the dose amounts are expressed in plaque-forming units (PFU), so potential differences between amounts of exposure measured in $CCID_{50}$ vs. PFU must be addressed. No study was found in the literature comparing titration amount from the same MARV sample using both types of assays. In the absence of this information, it was assumed that same relationship found for EBOV ($PFU/CCID_{50}$ ratio of 1/12, described in Section J.3.8.6) also applies for MARV. In the initial infections analyses for release events in this RA, the exposure estimates in $CCID_{50}$ are scaled by a factor of 1/12 before being applied to the literature-based dose response model described in Section J.3.9.4.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU. Therefore, the exposure estimates are scaled by a factor of 1/12, as described in the previous paragraph, before being applied to the expert-derived dose-response estimates as well. It is possible that this

assumption is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. For MARV, a PCR study found as low as 0.1 PFU/PCR (Trombley 2010). Another study found that samples of MARV contained approximately 3–5 times more particle shells than genomes, suggesting that genomes identified via PCR could all potentially be packaged into a viral particle. However, it is likely that many virions, even with an incorporated genome, may be otherwise defective or lack the ability to infect host cells. Therefore, the potential magnitude of non-conservatism resulting from the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU is unknown but likely less than a factor of 10.

J.3.9.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for MARV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–9e.

- Literature-based dose-response model: the exponential model with $r = 0.2$, derived for the closely related Ebola virus based on NHP inhalational dose-response data (Table J–8c). Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (0.07 to 0.5), for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–9d, with the model or set of models derived from each expert weighted equally.

Table J–9e. ID estimates and associated ranges for MARV.

Model	ID_{50}	ID_{10}	ID_{1}
Literature-based	**4 PFU** (1–10)	**0.7** (0.2–2)	**0.06** (0.02–0.2)
Expert-based	**200 org.** (100–1000)	**19** (10–100)	**2.4** (0.74–15)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–9d. Abbreviations: PFU = plaque forming unit; org. = organisms.

It is assumed that dose units of PFU and organisms (as conceived by the experts) are equivalent, although this assumption may be non-conservative for the expert-based numbers as described in Section J.3.8.6. The literature-based results, being more conservative than the expert-based results by roughly a factor of 50, can in part serve to assess the implications of the possibility that the expert-based results are non-conservative by a similar factor. Both sets of estimates shown in Table J–9e are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by MARV. As described in Section J.3.9.6, the exposure estimates from the event sequence analyses are in units of $CCID_{50}$, which are first converted to units of PFU before the dose response models described here are applied.

J.3.10 Lassa Virus

Lassa virus (LASV) causes Lassa fever, a highly infectious, zoonotic, rodent-transmitted disease that has infected humans in both natural and laboratory settings, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.10.1 Routes of Exposure

For LASV, the primary natural routes of exposure and infection for humans are assumed to be through direct contact with or ingestion of excretions from infected rodents. Inhalation of particles contaminated with rodent excretions might also cause infection. Bodily fluids of infected humans may cause infection in contacts as well. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.10.2 Vaccine and Prophylaxis

There are currently no vaccines available for LASV, although at least one vaccine is in development that has been shown to be successful in protecting NHPs from disease (Geisbert 2005). For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with LASV. This is a conservative assumption, as it is possible that some individuals (especially laboratory workers) would be partially protected from an

administered vaccine that may become FDA-approved or that may be available before official FDA-approval, such as those classified as an IND.

There are no prophylactic treatments that have been shown to protect against LASV infection after exposure. The antiviral drug ribavirin has been shown to be an effective treatment for Lassa fever if administered soon after symptoms appear (see Chapter 3 and Appendix C for references).

J.3.10.3 Dose-Response Information from the Literature

There are no human dose-response data available for LASV. Epidemiological evidence suggests that humans have become infected through inhaling aerosolized particles containing LASV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on LASV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

There are data demonstrating that moderate to high doses of LASV are highly infectious in NHPs via the inhalational route of exposure. Stephenson et al. (1984) exposed nine cynomolgus monkeys (*Macaca fascicularis*) to inhaled doses ranging from 468 to 24500 PFU and all nine died from subsequent disease. Given the absence of negative cases (i.e., survivors) in this data set, it is not possible to derive a dose-response model from these data that would facilitate estimates of the probability of infection at lower doses, although the data suggest that the LD_{50} for cynomolgus monkeys would be below the given range of doses.

Stephenson et al. (1984) also exposed outbred guinea pigs to aerosols of LASV at lower doses (see Table J–10a). These data were fit to dose-response models by Tamrakar and Haas (2008), who tested the exponential, beta Poisson, and log-probit models and accepted the exponential model as the best fit. Their best-fit model estimates an ID_{50} of 18 PFU (95 percent confidence interval 6.7–40), an ID_{10} of 2.7 PFU (1.0–6.0), and an ID_1 of 0.26 PFU (0.098–0.58).

Table J–10a. Guinea pig inhalational dose-response data (Stephenson 1984) for LASV.

Challenge Dose (PFU)	Number exposed	Number infected
5	8	1
48	8	7
724	8	8
5370	8	8

Data from Stephenson et al. (1984) and analyzed in Tamrakar and Haas (2008). Infected animals included animals that died and animals that survived but were seropositive for humoral antibodies.

J.3.10.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data, evidence, and published model for LASV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

Tamrakar and Haas (2008) found the monkey and guinea pig data from Stephenson at al. (1984) to be the only inhalational dose-response animal data available in the literature. The guinea pig data with infection as the response was amenable to dose-response model fitting, and the best fit (exponential) model to this data is the literature-based dose response model to be used for this RA. IDs estimated by the model at varying probabilities are shown in Table J–10b.

Table J–10b. ID estimates (with 95% confidence intervals) for animals from LASV inhalational dose-response models from the literature

Set	Animal	Model	ID_{50}	ID_{10}	ID_1
A	Guinea pig (Stephenson 1984, Tamrakar 2008)	Exp	**18 PFU** (6.7–40)	**2.7 PFU** (1.0–6.0)	**0.26 PFU** (0.098–0.58)
B	*Macaca fascicularis* (Stephenson 1984)	none	**< ≈468 PFU**		

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed animals. For example, ID_1 refers to the dose estimated to result in death of 1%, or one in one hundred animals. For the models, Exp = exponential. For data set A, the 95% confidence intervals are based on model fits to bootstrap replicates from the data set. For data set B, the data were not fit to a model and the ID_{50} upper bound estimate is based on the result that 9 of 9 monkeys died from exposure to 468-24500 PFU. The model fit to data set A was chosen as the literature-based model for this RA.

This model is not inconsistent with the monkey data, as the model estimates less than 10^{-8} probability of avoiding infection at the dose range to which all nine monkeys succumbed. However, it is unknown whether monkeys or humans would exhibit similar responses to the guinea pigs at low doses. Therefore, the estimates derived from this model are to be applied cautiously.

J.3.10.5 Dose-response Estimates Derived from Expert Panel

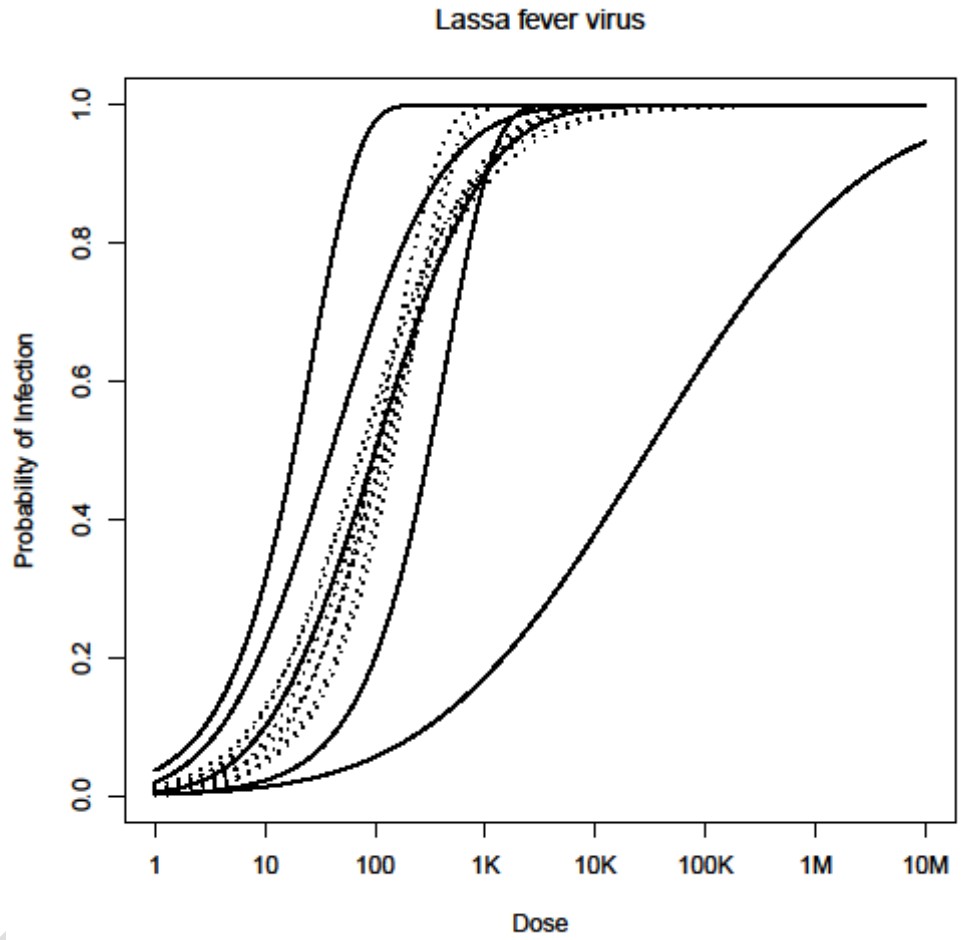
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for LASV are displayed in Table J–10c, including which models were retained from each expert according to the BIC. Fourteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–10. ID estimates provided by the retained curves are shown in Table J–10d.

Table J–10c: Dose response model fitting for Lassa virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log−probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	50 300 1,000	2.2×10^{-3}	0.86	250	1.0×10^{6}	4.5×10^{8}	**0**	8.2	1.1[a]
2	3 18 59	3.8×10^{-2}	0.86	15	1.0×10^{6}	2.7×10^{7}	**0**	7.8	1.1[a]
3	10 50 1,000	7.0×10^{-3}	0.56	79	7.8×10^{-1}	5.2×10^{1}	**5.0**	**0.8**	**0**
4	10 100 1,000	5.5×10^{-3}	0.56	100	8.9×10^{-1}	8.2×10^{1}	N/A	**Exact**	N/A
5	20 80 1,000	4.7×10^{-3}	0.66	120	1.2	1.6×10^{2}	**3.2**	**0**	**1**
6	4 40 400	1.4×10^{-2}	0.56	40	8.9×10^{-1}	3.3×10^{1}	N/A	**Exact**	N/A
7	300 30,000 3,000,000	1.8×10^{-5}	0.28	30,000	2.9×10^{-1}	1.3×10^{3}	N/A	**Exact**	N/A
8	20 40 1,500	5.2×10^{-3}	0.59	110	7.8×10^{-1}	6.9×10^{1}	**1.3**	**0.2**	**0**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–10



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–10d. Results for retained expert-derived LASV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
1	LP	310	47	4.5	0.45
2	Exp	18	2.8	0.27	0.027
3	Exp	100	15	1.4	0.14
	LP	79	7.9	1.2	0.31
	BP	73	7.4	0.66	0.066
4	LP	100	10	1.5	0.39
5	Exp	150	22	2.1	0.21
	LP	120	17	3.4	1.0
	BP	120	14	1.3	0.13
6	LP	40	4.0	0.61	0.16
7	LP	30000	300	7.0	0.45
8	Exp	130	20	1.9	0.19
	LP	110	12	2.1	0.58
	BP	98	9.9	0.89	0.088
Median (Min–Max)		**100 org.** (18–30000)	**11** (2.8–300)	**1.5** (0.27–7.0)	**0.26** (0.027–1.0)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

It is noted that the estimates derived from Expert 2 are very close to the estimates derived from the chosen dose-response model from the literature. This can be explained by the fact that this expert was an author of the study in which the literature-based model was found and clearly was of the opinion that this model is applicable to humans.

J.3.10.6 Other Considerations

The units of exposure for events analyzed in this RA for LASV are in terms of $CCID_{50}$ (median cell culture infective dose), FFU (fluorescent focus units), and PFU (plaque forming units). It is assumed that the estimated inventory concentration of LASV that would be used at the NEIDL is an appropriate estimate under all three units of measure. These units do not measure the absolute number of virions in a sample, because individual virions may aggregate or clump to infect a cell or to form one plaque or focus unit and because a given assay may not be entirely sensitive to detecting all virions that would have the ability to infect cells in a different medium or host. The exposures in the guinea pig experiments that form

the basis for the literature-based dose-response estimates are in terms of PFU, so it is appropriate to apply the curves derived from these data to the exposure estimates.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU or FFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates. It is possible that this assumption is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. A recent study has reported counts of viral genomes as well as virus particles in samples of LASV that were also assayed for FFU, resulting in estimates of about 2200 viral genomes per FFU and about 100 virus particles per FFU (Weidmann 2011). It is clear from these data that the number of viral genomes in a sample of LASV is not an appropriate estimate for the number of organisms, because there were 22 times as many genomes as there were particles available to contain genomes and form complete virions. The number of virus particles observed might be a more accurate estimate for the number of organisms, but it is also possible that many of those particles did not represent replication competent virions with the ability to infect host cells, which is supported by the fact that roughly 99 percent of those particles did not form FFU in the cell culture. However, it is possible that some portion of the particles that were not infective in the cell culture would be infective to cells in a live host. Therefore, these data suggest that the assumption that numbers of organisms as envisioned by the experts are adequately measured by FFU might be non-conservative by up to a factor of 100.

J.3.10.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for LASV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–10e.

- Literature-based dose-response model: the exponential model with $r = 0.039$, derived by Tamrakar and Haas (2008) for guinea pig inhalational dose-response data (Stephenson 1984). Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (0.017 to 0.10), for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–10d, with the model or set of models derived from each expert weighted equally.

Table J–10e. ID estimates and associated ranges for LASV.

Model	ID_{50}	ID_{10}	ID_1
Literature-based	**18 PFU** (6.7–40)	**2.7** (1.0–6.1)	**0.26** (0.098–0.58)
Expert-based	**100 org.** (18–30000)	**11** (2.8–300)	**1.5** (0.27–7.0)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–10d. Abbreviations: PFU = plaque forming unit; org. = organisms.

For each ID point displayed in Table J–10e, the literature-based range extends lower (higher risk) than the expert-based range. It is assumed that dose units of PFU and organisms (as conceived by the experts) are equivalent, although this assumption may be non-conservative for the expert-based numbers as described in Section J.3.10.6. The literature-based results, being more conservative than the expert-based results, can in part serve to assess the implications of the possibility that the expert-based results are non-conservative by a similar factor. Both sets of estimates shown in Table J–10e are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by LASV.

J.3.11 Junin Virus

Junin virus (JUNV) causes Argentine hemorrhagic fever (AHF), a highly infectious, zoonotic disease that has infected humans in both natural and laboratory settings, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.11.1 Routes of Exposure

For JUNV, the primary natural route of exposure and infection for humans is assumed to be through inhalation of aerosolized virus from excretions from infected rodents. Direct contact with or ingestion of contaminated fluids or fomites or bites from infected rodents may also cause infection. Bodily fluids of infected humans may cause infection in contacts as well. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or

outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.11.2 Vaccine and Prophylaxis

A live attenuated vaccine, Candid-1, has been effective in decreasing incidence of AHF in Argentina (see Chapter 3 for references). However, this vaccine is not FDA-approved for use in the United States. For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with JUNV. This is a conservative assumption, as it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that may become FDA-approved or that may be available before official FDA-approval, such as those classified as an IND.

The Candid-1 vaccine is used for prophylaxis in Argentina but is unavailable in the United States. Specific treatment measures such as immune plasma and ribavirin have been effective in reducing the risk of mortality for AHF patients (See Chapter 3 for references).

J.3.11.3 Dose-Response Information from the Literature

There are no human dose-response data available for JUNV. Epidemiological evidence suggests that humans may become infected through inhaling aerosolized particles containing JUNV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on NIPV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

A NHP, the rhesus macaque (*Macaca mulatta*), was shown to be susceptible to infection with JUNV after inhalational exposure and developed disease symptoms similar to those observed in humans (Kenyon 1992). Six monkeys were exposed to inhalational doses ranging from 32 PFU (plaque forming units) to 20,000 PFU. One monkey exposed to a high dose died before disease symptoms developed from a probable complication due to anesthesia. All the remaining five monkeys experienced disease symptoms and subsequently died. The doses inhaled by these five monkeys are shown in Table J–11a.

Table J–11a. *Macaca mulatta* inhalational dose-response data (Kenyon 1992) for JUNV.

Challenge Dose (PFU)	Number exposed	Number infected and died
32	1	1
50	1	1
79	1	1
20,000	2	2

Data from Kenyon et al. (1992). PFU = plaque forming units.

Guinea pigs have also been used extensively as an animal model for human AHF, but no studies were found in which guinea pigs were exposed to low doses (comparable to Table J–11a) via the inhalational route. One study (Parodi 1958), found a 100 percent infection rate among guinea pigs exposed intranasally to doses of a virulent strain of JUNV of 3200 $CCID_{50}$ and 32,000 $CCID_{50}$.

J.3.11.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data and evidence for JUNV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The NHP (*Macaca mulatta*) inhalational dose response data (Kenyon 1992) shown in Table J–11a provide the most potentially relevant information for infectivity of JUNV via the inhalational route at low doses. The data are not amenable to dose-response model fitting, as the infection rate was 100 percent across all doses. However, the data do provide some insight into probable upper bounds for ID levels for *Macaca mulatta*. For example, the exponential dose-response model can be evaluated in terms of how low the model parameter *r* can be while still producing a deviance low enough to be acceptable under statistical criteria in light of the data. Table J–11b shows the result of this exercise.

Table J–11b. Exponential dose-response model evaluation and upper bound ID estimates in light of NHP inhalational dose-response data (Kenyon 1992).

Animal	Model	Parameter value lower bound	Deviance	ID_{50} upper bound	ID_{10} upper bound	ID_1 upper bound
Macaca mulatta (Kenyon 1992)	Exp	$r > 0.0064$	$< \chi^2_{0.95,\,3} = 7.8$	**< 110 PFU**	**< 16 PFU**	**< 1.6 PFU**

Exponential model evaluation in light NHP exposure data (Kenyon 1992) shown in Table J–11a. Lower bound *r* value produces deviance equal to 95th percentile of the chi-squared distribution with 3 degrees of freedom, the number of distinct doses in the data set (4) minus the number of model parameters (1). Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed animals. For example, ID_1 refers to the dose estimated to result in death of 1%, or one in one hundred animals. PFU = plaque forming units.

The results in Table J–11b are intended to be guidelines for upper bounds of ID estimates. For example, if the true ID_{50} for *Macaca mulatta* were greater than 110 PFU and if the exponential model is appropriate, then it would be unlikely (less than about 5 percent chance) that the data in Table J–11a would be observed. These results give no information on lower bounds for IDs. In the absence of further data, it is not possible to estimate how low the ID_{50} or other ID levels might be. Therefore, it was determined that a literature-based dose-response estimate would not be applied for this RA. However, the results in Table J–11b are used to assess the expert-derived estimates in Section J.3.11.7.

J.3.11.5 Dose-Response Estimates Derived from Expert Panel

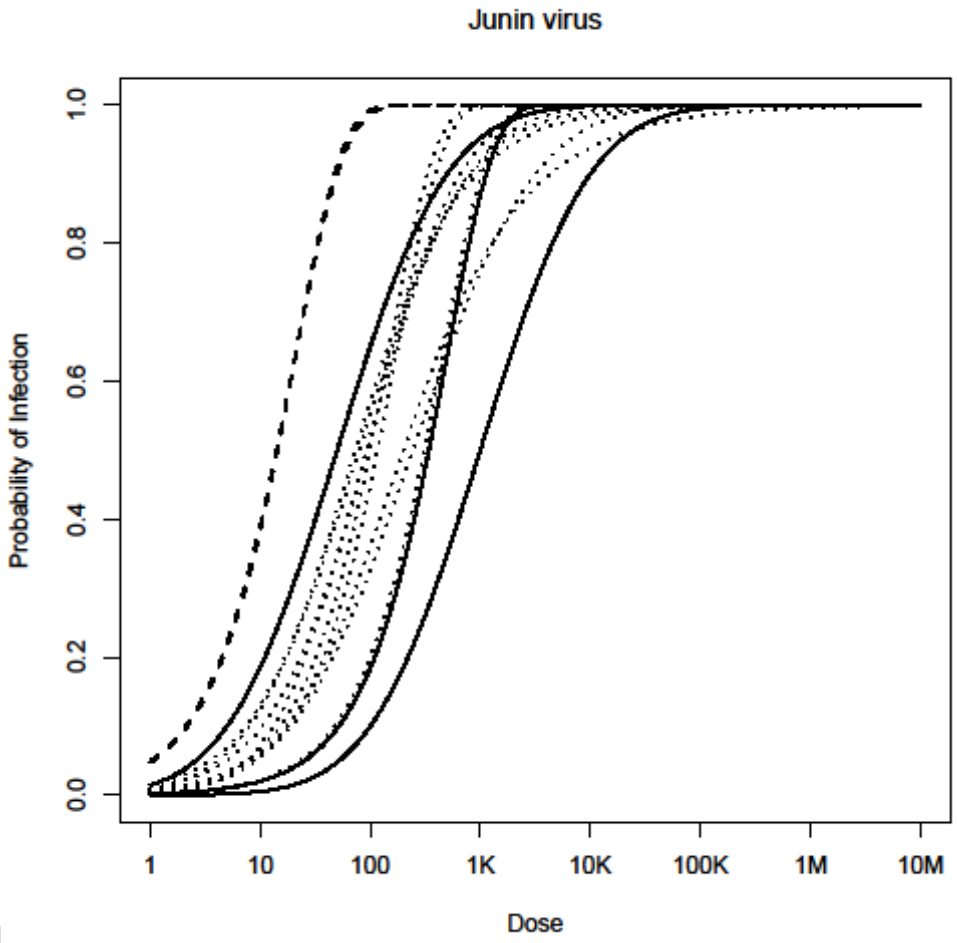
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for JUNV are displayed in Table J–11c, including which models were retained from each expert according to the BIC. Seventeen curves were retained for application in this RA, and plots of those curves are shown in Figure J–11. ID estimates provided by the retained curves are shown in Table J–11d.

Table J–11c: Dose response model fitting for Junin virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log–probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	50 400 1,000	2.0×10^{-3}	0.86	270	1.0×10^{6}	4.9×10^{8}	**0**	6.1	1.1[a]
2	2 15 49	4.9×10^{-2}	0.80	11	1.3×10^{1}	2.6×10^{2}	**0.3**	8.7	**0**
3	10 50 1,000	7.0×10^{-3}	0.56	79	7.8×10^{-1}	5.2×10^{1}	**5.0**	**0.8**	**0**
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	20 40 1,000	5.9×10^{-3}	0.66	93	1.0	9.4×10^{1}	**0.9**	**0**	**0.3**
6	5 50 500	1.1×10^{-2}	0.56	50	8.9×10^{-1}	4.1×10^{1}	N/A	**Exact**	N/A
7	30 100 5,000	2.2×10^{-3}	0.50	250	5.9×10^{-1}	8.9×10^{1}	**3.7**	**1.3**	**0**
8	20 40 1,000	5.9×10^{-3}	0.66	93	1.0	9.4×10^{1}	**0.9**	**0**	**0.3**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–11



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–11d. Results for retained expert-derived JUNV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	Exp	340	52	4.9	0.49	0.049
2	Exp	14	2.2	0.21	0.021	0.0021
	BP	14	2.1	0.20	0.020	0.0020
3	Exp	100	15	1.4	0.14	0.014
	LP	79	7.9	1.2	0.31	0.099
	BP	73	7.4	0.66	0.066	0.0066
4	LP	1000	100	15	3.9	1.3
5	Exp	120	18	1.7	0.17	0.017
	LP	93	13	2.7	0.83	0.32
	BP	93	10	0.94	0.093	0.0093
6	LP	50	5.0	0.77	0.19	0.0627
7	Exp	310	47	4.5	0.45	0.0447
	LP	250	19	2.4	0.52	0.15
	BP	200	17	1.5	0.15	0.015
8	Exp	120	18	1.7	0.17	0.017
	LP	93	13	2.7	0.83	0.32
	BP	93	10	0.94	0.093	0.0093
Median (Min–Max)		**96 org.** (14–1000)	**14** (2.1–100)	**1.6** (0.20–15)	**0.19** (0.020–3.9)	**0.049** (0.0020–1.3)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.11.6 Other Considerations

The units of exposure for events analyzed in this RA for JUNV are in terms of PFU (plaque forming units). These units do not measure the absolute number of active virions in a sample, because individual virions may aggregate or clump to infect a cell or to form one plaque and because a given assay may not be entirely sensitive to detecting all virions that would have the ability to infect cells in a different medium or host. The exposures in the NHP experiment that was assessed in Section J.3.11.3 were measured in units of PFU, so it is appropriate to evaluate information derived from these data for the exposure estimates.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates. It is

possible that this assumption is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. One study in the literature performed real-time PCR and reported that as little as 0.0001 PFU can be detected by PCR. These data are of limited use because many of those genomes are likely not incorporated into viral particles, and therefore are regarded as posing no threat to a potential host. Furthermore, it is likely that even many virions with an incorporated genome may be otherwise defective or lack the ability to infect host cells. Therefore, the potential magnitude of non-conservatism resulting from the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU is unknown.

J.3.11.7 Summary of Approach

The following summarizes the dose-response estimates for JUNV derived in this section. ID estimates derived from these models are compared in Table J–11e.

- Literature-based dose-response information: the exponential model was evaluated in light of NHP (*Macaca mulatta*) dose-response data and it was determined that parameter values for $r > 0.0064$, would not be inconsistent with the data. It was not possible to derive a best-fit curve from the data.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–11d, with the model or set of models derived from each expert weighted equally.

Table J–11e. ID estimates and associated ranges for JUNV.

Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
Literature-based	**< 110 PFU**	**< 16**	**< 1.6**	**< 0.16**
Expert-based	**96 org.** (14–1000)	**14** (2.1–100)	**1.6** (0.20–15)	**0.19** (0.020–3.9)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based values are upper bounds listed here for comparison purposes. The expert-based ranges are the minimum and maximum values from Table J–11d. Abbreviations: PFU = plaque forming units; org. = organisms.

The expert-based estimates shown in Table J–11e are applied to the exposure estimates in the initial infection portions of this RA to determine the implications for the overall risk posed by JUNV. The

median point estimates (in bold) are close to the literature-based upper bounds estimated from NHP data, which means that about half the weight of the expert based range is above that estimated upper bound. Those estimates above the median are not necessarily inaccurate, as it is possible that humans are not as susceptible to infection from JUNV aerosols as are *Macaca mulatta*. The part of the distribution that lies below the literature-based upper bound can serve to represent the possibility that human susceptibility to JUNV is consistent with what was observed among NHPs.

J.3.12 Tick-Borne Encephalitis Virus, Far Eastern Subtype

(Formerly called Tick-borne encephalitis complex (Russian spring summer encephalitis virus))

The Far Eastern sub-type of tick-borne encephalitis virus (TBEV-FE) is a highly infectious, tick-borne virus that has infected humans in both natural and laboratory settings, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.12.1 Routes of Exposure

For TBEV-FE, the primary natural route of exposure and infection for humans is assumed to be through bites from infected ticks. Infection has also been linked to ingestion of contaminated animal products. Infection from inhalational exposure has also occurred in laboratory settings. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP, rodent, and tick) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.12.2 Vaccine and Prophylaxis

A vaccine is available in some countries where the disease is endemic, but it is not currently available in the United States. For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with TBEV-FE. This is a conservative assumption, as it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that may become FDA-approved or that may be available before official FDA-approval, such as those classified as an IND, in the future.

It is also assumed that no prophylactic treatment would be available to prevent infection in exposed individuals. There is no specific drug therapy that has been shown to be effective against TBE disease after infection occurs in humans.

J.3.12.3 Dose-Response Information from the Literature

There are no human dose-response data available for TBEV-FE. Evidence from LAI suggests that humans may have become infected through inhaling aerosolized particles containing TBEV of various sub-types, but the amount of virus inhaled by humans who became infected is not known.

As described in Chapter 3 and Appendix C, experiments on laboratory animal infection with TBEV have been generally ineffective in reproducing aspects of human disease. The example in the literature that might be most relevant to inhalational exposure of humans to TBEV-FE is a study (Hambleton 1983) in which rhesus monkeys (*Macaca mulatta*) were exposed via the intranasal route to TBEV-CE (Central European sub-type) and exhibited symptoms similar to those seen in humans. Ten monkeys were exposed intranasally to very high doses ranging from 300 million to 800 million PFU. Of these ten, six experienced severe clinical symptoms leading to death, two appeared to have sub-clinical infection, and two were sacrificed early. An additional five animals were exposed to similarly high doses via the intravenous route, presumably closer to the natural route of infection in humans (tick bite), but none of these animals showed clinical symptoms of disease.

J.3.12.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data and evidence for TBEV-FE outlined in the previous section and discusses why a literature-based dose-response estimate is not used for this RA.

Dose-response literature is extremely sparse for TBEV-FE, especially for inhalational exposure. The one animal study described in Section J.3.12.3 (Hambleton 1983) is of very limited use. First, the susceptibility of rhesus monkeys to TBEV appears to be of a different nature than that of humans, as monkeys that were exposed to high doses via the route closest to the natural route of infection in humans did not develop disease. Second, the doses applied were extremely high and not described in enough detail on which to base a dose-response model, which would have to extrapolate down many orders of magnitude to shed any light on what the probability of infection might be at exposure levels relevant for this RA.

It is noted that laboratory infections of humans did occur via the inhalational route in the past, which suggests that humans may be susceptible to relatively low doses by that route. However, it is impossible

to derive quantitative estimates from this information. Therefore, a literature-based dose response model is not applied to TBEV-FE exposure estimates for this RA.

J.3.12.5 Dose-response Estimates Derived from Expert Panel

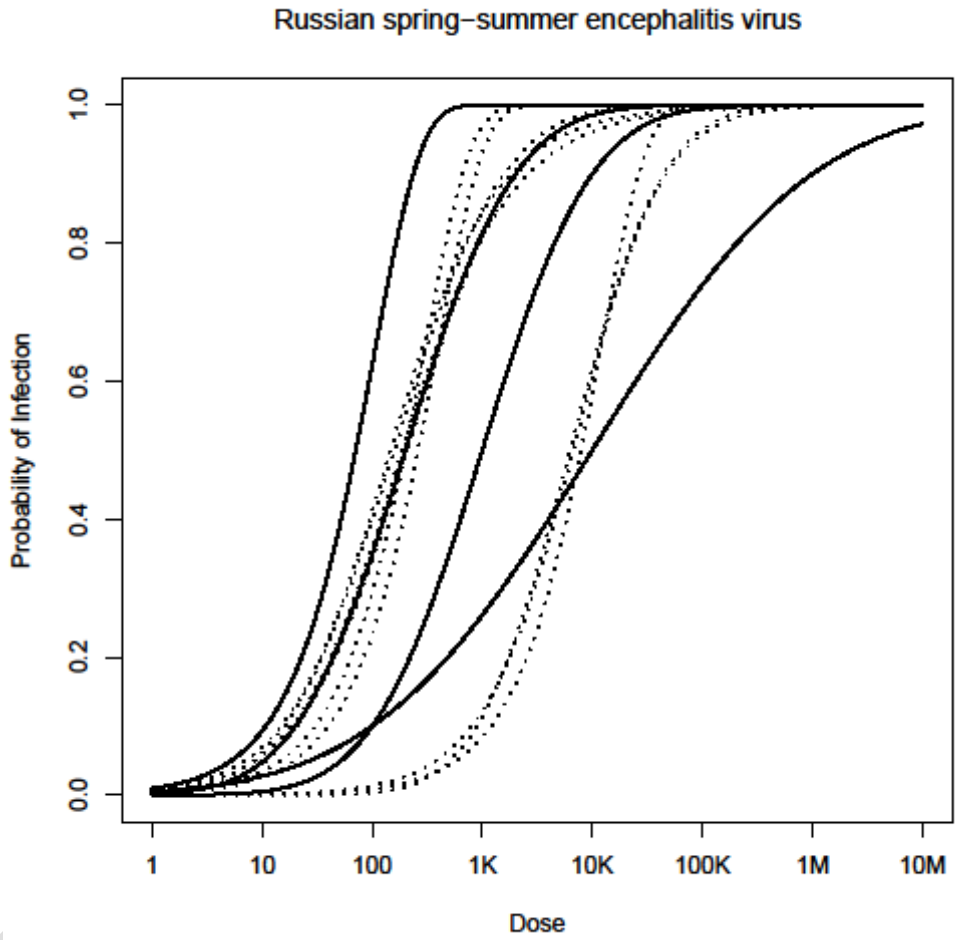
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for TBEV-FE are displayed in Table J–12a, including which models were retained from each expert according to the BIC. Fourteen curves were retained for application in this RA, and plots of those curves are shown in Figure J–12. ID estimates provided by the retained curves are shown in Table J–12b.

Table J–12a: Dose response model fitting for TBEV-FE

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log−probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	1,000 5,000 50,000	8.8×10^{-5}	0.66	6,300	1.3	9.3×10^{3}	**5.2**	**0**	**1.7**
2	11 71 230	9.8×10^{-3}	0.84	56	1.0×10^{7}	1.0×10^{9}	**0**	14.3	1.1[a]
3	30 100 3,000	2.7×10^{-3}	0.56	210	7.3×10^{-1}	1.2×10^{2}	**3**	**0.6**	**0**
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	20 200 2,000	2.8×10^{-3}	0.56	200	8.9×10^{-1}	1.6×10^{2}	N/A	**Exact**	N/A
6	20 200 2,000	2.8×10^{-3}	0.56	200	8.9×10^{-1}	1.6×10^{2}	N/A	**Exact**	N/A
7	100 10,000 1,000,000	5.5×10^{-5}	0.28	10,000	2.9×10^{-1}	4.4×10^{2}	N/A	**Exact**	N/A
8	20 100 2,000	3.5×10^{-3}	0.56	160	7.8×10^{-1}	1.0×10^{2}	**5**	**0.8**	**0**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–12



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–12b. Results for retained expert-derived TBEV-FE dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_{1}	$ID_{0.1}$	$ID_{0.01}$
1	Exp	7900	1200	110	11	1.1
	LP	6300	890	180	56	22
	BP	6700	800	73	7.3	0.73
2	Exp	71	11	1.0	0.10	0.010
3	Exp	260	40	3.8	0.38	0.038
	LP	210	21	3.2	0.81	0.26
	BP	190	18	1.6	0.16	0.016
4	LP	1000	100	15	3.9	1.3
5	LP	200	20	3.1	0.78	0.25
6	LP	200	20	3.1	0.78	0.25
7	LP	10000	100	2.3	0.15	0.016
8	Exp	200	30	2.9	0.29	0.029
	LP	160	16	2.4	0.62	0.20
	BP	150	15	1.3	0.13	0.013
Median (Min–Max)		**200 org.** (71–10000)	**20** (10–1200)	**3.1** (1.0–180)	**0.78** (0.10–56)	**0.25** (0.010–22)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. Abbreviations: org. = organisms, Exp = exponential, LP = log-probit and BP = beta Poisson.

J.3.12.6 Other Considerations

The units of exposure for events analyzed in this RA for TBEV-FE are in terms of MID_{50} (median mouse infective dose). The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by plaque-forming units (PFU). It is likely that the exposure estimates would be lower in terms of PFU, because the estimated inventory concentration (1×10^8 MID_{50} / ml), which is proportional to the exposure estimates, was chosen in those units because titers in other units, including PFU, were generally found to be lower in the literature. However, there is at least one example of up to 10^8 PFU / mL being achieved for TBEV in tick cell lines (Ruzek 2008). Therefore, it is conservatively assumed that it is appropriate to apply the expert-derived dose-response estimates to the exposure estimates in units of MID_{50}.

It is possible that the assumption that the expert ID estimates are representative of PFU is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with

potential to infect a human as envisioned by the experts. No studies were found in the literature that attempted to quantify the number of virions represented by one PFU or one MID_{50}. Therefore, the potential magnitude of non-conservatism resulting from the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU is unknown.

J.3.12.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for TBEV-FE to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–12c.

- Literature-based dose-response model: none
- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–12b, with the model or set of models derived from each expert weighted equally.

Table J–12c. ID estimates and associated ranges for TBEV-FE.

Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$	$ID_{0.01}$
Expert-based	**200 org.** (71–10000)	**20** (10–1200)	**3.1** (1.0–180)	**0.78** (0.10–56)	**0.25** (0.010–22)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–12b. Abbreviations: org. = organisms.

The estimates shown in Table J–12c are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by TBEV-FE. The literature on TBEV dose-response is not extensive enough to make an evaluation of these ID estimates. However, the fact that LAI have occurred via exposure to aerosols imply that relatively low doses might be able to cause infection in humans, which appears to be adequately captured by the range of estimates shown here.

J.3.13 Nipah Virus

Nipah virus (NIPV) is a highly infectious, zoonotic virus that has infected humans, causing encephalitis and/or respiratory disease, as described in Chapter 3 and Appendix C. This section synthesizes the available dose-response information and derives a range of dose-response estimates to be applied to the exposure results from each of the event sequence analyses.

J.3.13.1 Routes of Exposure

For NIPV, the primary natural routes of exposure and infection for humans are assumed to be through direct contact with or ingestion or inhalation of excretions from infected animals. Bodily fluids of infected humans may cause infection in contacts as well. Infections resulting from laboratory activities are assumed to be possible via inhalation, ingestion, direct contact, puncture, and animal-related (NHP and rodent) routes. The inhalational route is the primary focus of the dose-response assessment in this section, in support of the events analyzed in this RA that lead to aerosol releases in the laboratory or outside the NEIDL. Non-inhalational routes of exposure and their potential consequences are considered separately and as needed in conjunction with relevant events.

J.3.13.2 Vaccine and Prophylaxis

There are currently no vaccines available for NIPV. Animal models on which vaccines may be tested are currently in early stages of development (Bossart 2009, Geisbert 2010). For this RA, it is assumed that no potentially exposed laboratory worker, facility worker, or member of the general public would be vaccinated against infection or disease with NIPV. This is a conservative assumption, as it is possible that some individuals (especially laboratory workers) would be partially protected from an administered vaccine that may become FDA-approved or that may be available before official FDA-approval, such as those classified as an IND, in the future.

There are no prophylactic treatments that have been shown to protect against NIPV infection in humans after exposure. A neutralizing human monoclonal antibody was shown to protect ferrets from disease after exposure to otherwise lethal doses (Bossart 2009). It is conservatively assumed that no such treatments would be available to exposed laboratory workers or members of the public.

J.3.13.3 Dose-Response Information from the Literature

There are no human dose-response data available for NIPV. Epidemiological evidence suggests that humans may have become infected through inhaling aerosolized particles containing NIPV, but the amount of virus inhaled by humans who became infected is not known. In the absence of human data, studies on NIPV infectivity in animals provide the best-available information through which dose-response information potentially relevant for humans might be obtained.

As described in Chapter 3 and Appendix C, several animal species have been tested in laboratory settings for susceptibility to NIPV. The two most promising animal models for representing human infection and disease are the ferret (Bossart 2009) and the African green monkey (Geisbert 2010).

Bossart et al. (2009) exposed ferrets to different doses of NIPV oral-nasally and, among unprotected animals, observed the data shown in Table J–13a. The authors stated an ID_{50} estimate of 500 $CCID_{50}$ based on one out of two animals becoming infected at that dose.

Table J–13a. Ferret oral-nasal dose-response data (Bossart 2009) for NIPV.

Challenge Dose ($CCID_{50}$)	Number exposed	Number infected
50	2	0
500	2	1
5000	4	4
50,000	2	2

Data from Bossart et al. (2009). Dose units were reported in median tissue culture infectious dose ($TCID_{50}$), which is referred to as $CCID_{50}$ in this RA. Infected animals developed disease and were euthanized. Other animals exhibited no symptoms, did not shed detectable virus, and did not seroconvert.

Geisbert et al. (2010) exposed eight African green monkeys to doses ranging from 2500 plaque-forming units (PFU) to 1,300,000 PFU administered intratracheally or intratracheally and orally. All eight animals were infected and became clinically ill. All animals succumbed to disease or were euthanized except for one animal, exposed to 7000 PFU intratracheally and orally, which experienced severe clinical illness but eventually recovered after long convalescence.

J.3.13.4 Literature-Based Dose-Response Estimate

This section consists of a discussion of the dose-response data and evidence for NIPV outlined in the previous section and provides the literature-based dose-response estimate to be used for this RA.

The dose-response data in Table J–13a for ferrets, one of two animals proposed as the best available platforms for evaluation of potential immunization and treatment of humans (Bossart 2009, Geisbert 2010), is amenable to dose-response model fitting. The exponential model was fit to the data and the best-fit parameter results in very low optimal deviance. The model fitting result and IDs estimated by the model at varying probabilities are shown in Table J–13b.

Table J–13b. Model fitting results and ID estimates (with 95% confidence intervals) for ferrets from NIPV oral-nasal dose-response data (Bossart 2009)

Model	Optimal param. value	Optimal deviance	$\chi^2_{0.95,\ df=3}$	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
Exp	$r = 0.001$	0.3	7.8	**500 $CCID_{50}$** (100–1000)	**80** (20–200)	**8** (2–20)	**0.8** (0.2–2)

Dose-response model fitting to the NHP data from Table J–13a. Exp = exponential. Model parameter is defined in Section J.2.5.1. Fit acceptability was confirmed if the deviance at the optimal parameter values was less than the corresponding 95th percentile chi-squared statistic with degrees of freedom (df) equal to 3, the number of distinct doses minus the number of parameters. Each ID_x result refers to the expected oral-nasal dose estimated to result in death of *x*% of exposed individuals under the model. Estimates are rounded to one significant figure. Units of dose are median cell culture infectious dose ($CCID_{50}$). Intervals under the LD estimates are 95% confidence intervals based on model fits to bootstrap replicates from the data set.

The dose-response data from African green monkeys (Geisbert 2010) are not amenable to dose-response model fitting because 100 percent of animals exposed were infected. The dose range administered to the monkeys in this study was 2500–1,300,000 PFU. Assuming that those doses would have been roughly equal or higher in units of $CCID_{50}$, these results are consistent with the exponential dose response model in Table J–13b, which estimates a greater than 95 percent probability of infection at a dose of 2500 $CCID_{50}$. The doses administered to the monkeys appear to have been too high to shed any additional light on the low-dose infectivity of NIPV.

Given that the ferrets in the Bossart et al. (2009) study were exposed oral-nasally, which is a reasonable surrogate for inhalational exposure that might occur as a result of an aerosol release from a laboratory, that ferrets respond to NIPV infection quite similarly to humans, and that the data cover the low-dose end of a potential dose-response curve, the ferret data-based model in Table J–13b is retained as the literature-based model to be applied to exposure estimates for this RA.

J.3.13.5 Dose-Response Estimates Derived from Expert Panel

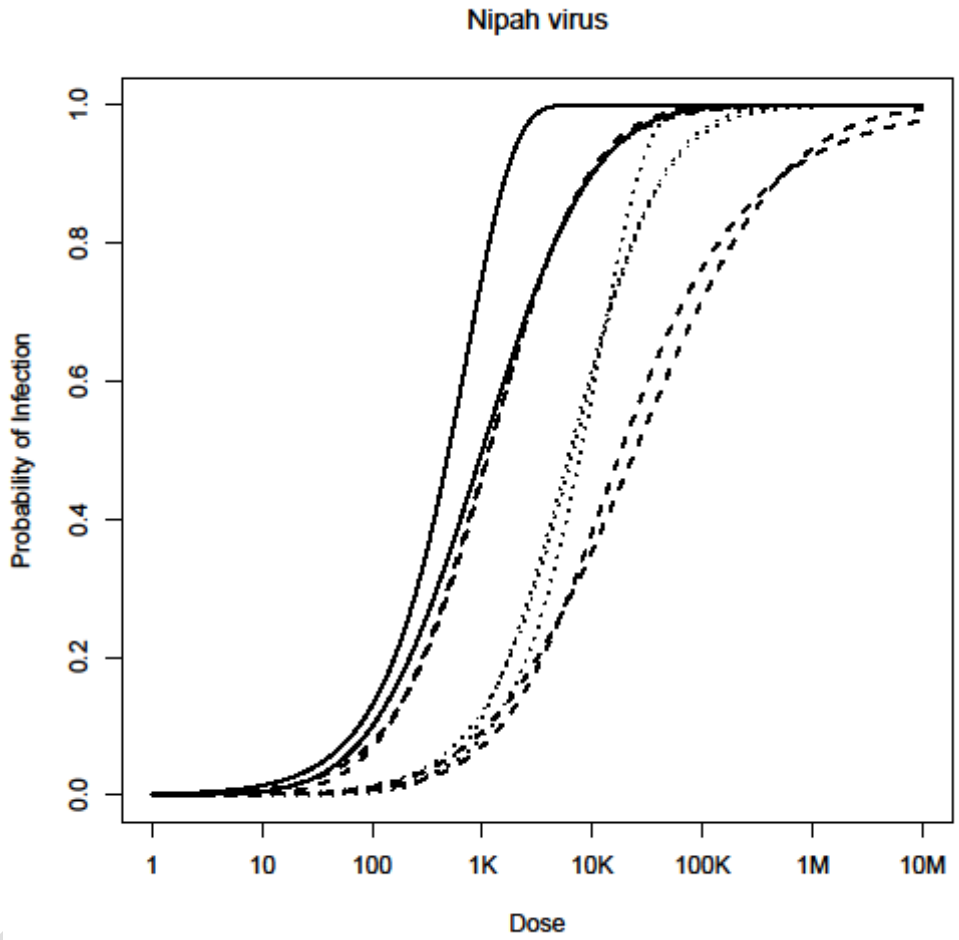
In the procedure described previously, dose-response curves were fit to the ID_{10}, ID_{50}, and ID_{90} estimates provided by each expert on the Delphi panel. The results of this model fitting procedure for NIPV are displayed in Table J–13c, including which models were retained from each expert according to the BIC. Twelve curves were retained for application in this RA, and plots of those curves are shown in Figure J–13. ID estimates provided by the retained curves are shown in Table J–13d.

Table J–13c: Dose response model fitting for Nipah virus

Expert	ID_{10} ID_{50} ID_{90}	Exp. model	Log−probit model		Beta Poisson model		ΔBIC^{Exp}	ΔBIC^{Lp}	ΔBIC^{BP}
		r	m	ID_{50}	α	β			
1	1,000 5,000 50,000	8.8×10^{-5}	0.66	6,300	1.3	9.3×10^{3}	**5.2**	**0**	**1.7**
2	75 500 1,650	1.4×10^{-3}	0.83	400	1.0×10^{3}	7.2×10^{5}	**0**	21.6	1.1[a]
3	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
4	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
5	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
6	100 1,000 10,000	5.5×10^{-4}	0.56	1,000	8.9×10^{-1}	8.2×10^{2}	N/A	**Exact**	N/A
7	1,000 30,000 500,000	2.2×10^{-5}	0.41	25,000	5.2×10^{-1}	6.5×10^{3}	12.2	**0**	**5.8**
8	150 1,000 10,000	4.8×10^{-4}	0.61	1,100	1.1	1.3×10^{3}	9.1	**0**	**1.1**

Fitted parameters for three dose response models to each set of three data points from each expert panelist. Optimal parameter values were rounded to two significant figures. ΔBIC is the Bayesian Information Criterion value relative to the lowest value in that row, where a lower value indicates that the model better represents the available information. Bolded values in the BIC columns indicate that the model for that column was kept in consideration for representing the data provided by the expert panelist in that row, and grey values indicate that the model was eliminated from consideration, generally because its value was more than six greater than the lowest BIC value in that row. [a] indicates that the Beta Poisson model fit produced a curve virtually identical to the exponential model, so it was redundant to keep it in consideration. *Exact* entries indicate that the Log-probit model fit the expert panelist values in that row exactly (which results in a BIC value of negative infinity), so the other two models in that row are not applied (N/A). Abbreviations: Exp = Exponential model; Lp = Log-probit model; BP = Beta Poisson model.

Figure J–13



Plot of all retained dose response curves used to estimate probability of infection for each dose. Solid curves were weighted with 1/8 probability; dashed curves were weighted with 1/16 probability; dotted curves were weighted with 1/24 probability.

Table J–13d. Results for retained expert-derived NIPV dose-response models

Expert	Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$	$ID_{0.01}$
1	1	7900	1200	110	11	1.1
	2	6300	890	180	56	22
	3	6700	800	73	7.3	0.73
2	1	500	75	7.2	0.72	0.072
3	2	1000	100	15	3.9	1.3
4	2	1000	100	15	3.9	1.3
5	2	1000	100	15	3.9	1.3
6	2	1000	100	15	3.9	1.3
7	2	25000	1100	88	14	3.0
	3	18000	1500	130	13	1.3
8	2	1100	140	25	7.2	2.6
	3	1200	130	12	1.2	0.12
Median (Min–Max)		**1000 org.** (500–25000)	**100** (75–1400)	**15** (7.2–180)	**3.9** (0.72–56)	**1.3** (0.072–22)

Results are listed for the models that were retained after applying the Bayesian information criterion for model comparison. Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed individuals. For example, $ID_{0.1}$ refers to the dose estimated to result in infection of 0.1%, or one in one thousand individuals. The medians were calculated after weighing each of the eight experts equally and weighing each model equally within each expert if more than one was retained. For the models, Exp = exponential, LP = log-probit and BP = beta Poisson.

It is noted that the estimates derived from Expert 2 are very close to the estimates derived from the chosen dose-response model from the literature. While no published NIPV dose-response models by this expert or any other was found in the literature, it is presumed that the expert independently carried out an analysis of the Bossart et al. (2009) data set in a similar fashion to what has been presented here.

J.3.13.6 Other Considerations

The units of exposure for events analyzed in this RA for NIPV are in terms of PFU (plaque forming units) or $CCID_{50}$ (median cell culture infective dose). It is assumed that the estimated inventory concentration of NIPV that would be used at the NEIDL is an appropriate estimate under both units of measure. These units do not measure the absolute number of virions in a sample, because individual virions may aggregate or clump to infect a cell or to form one plaque and because a given assay may not be entirely sensitive to detecting all virions that would have the ability to infect cells in a different medium or host. The exposures in the ferret experiments that form the basis for the literature-based dose-response estimates were measured in units of $CCID_{50}$, so it is appropriate to apply the curves derived from these data to the exposure estimates.

The expert panelists were asked to provide their ID estimates in terms of number of organisms. It is assumed that the expert values represent numbers of potentially infectious units, as estimated by PFU, and that it is appropriate to apply the curves derived from their estimates to the exposure estimates. It is possible that this assumption is non-conservative if the assays used to derive the estimated inventories did not count all the organisms with potential to infect a human as envisioned by the experts. Studies in the literature have reported viral genome copies : PFU ratios in samples of NIPV ranging from as low as 20:1 to as high as 760,000:1, and several results in between (Guillaume 2004, Weingartl 2005, Chang 2006). Aside from the large uncertainty and/or variability implied by these data, they are of limited use because many of those genomes are likely not incorporated into viral particles, and therefore are regarded as posing no threat to a potential host. Furthermore, it is likely that even many virions with an incorporated genome may be otherwise defective or lack the ability to infect host cells. Therefore, the potential magnitude of non-conservatism resulting from the assumption that numbers of organisms as envisioned by any particular expert are adequately measured by PFU is unknown.

J.3.13.7 Summary of Approach

The following summarizes the two sets of dose-response estimates for NIPV to be applied to exposure data for this RA. ID estimates derived from these models are compared in Table J–13e.

- Literature-based dose-response model: the exponential model with $r = 0.001$, derived for ferret oral-nasal dose-response data (Bossart 2009). Use a distribution of r values derived from bootstrap replicates of the data set, which results in a 95 percent range of (0.0006 to 0.01), for uncertainty and sensitivity analyses.

- Range of dose-response models derived from the expert-provided values: the distribution of estimates shown in Table J–13d, with the model or set of models derived from each expert weighted equally.

Table J–13e. ID estimates and associated ranges for NIPV.

Model	ID_{50}	ID_{10}	ID_1	$ID_{0.1}$
Literature-based	**500 $CCID_{50}$** (100–1000)	**80** (20–200)	**8** (2–20)	**0.8** (0.2–2)
Expert-based	**1000 org.** (500–25000)	**100** (75–1400)	**15** (7.2–180)	**3.9** (0.72–56)

Each ID_x result refers to the expected inhaled dose estimated to result in infection of *x*% of exposed humans. The literature-based ranges are the 95% intervals derived from the bootstrap parameter distribution; the actual range of values applied to the RA may be wider. The expert-based ranges are the minimum and maximum values from Table J–13d. Abbreviations: $CCID_{50}$ = median cell culture infective dose; org. = organisms.

Both sets of estimates shown in Table J–13e are applied to the exposure estimates in the initial infection portions of this RA to determine the implications of each estimate for the overall risk posed by NIPV.

J.4 References

Alford 1966 — Alford, R.H., Kasel, J.A., Gerone, P.J. and Knight, V. Human influenza resulting from aerosol inhalation. *Proceedings of the Society for Experimental Biology and Medicine*, 122(3):800-804, 1966.

Alves 2010 — Alves, D.A., Glynn, A.R., Steele, K.E., Lackemeyer, M.G., Garza, N.L., Buck, J.G., Mech, C. and Reed, D.S. Aerosol exposure to the Angola strain of Marburg virus causes lethal viral hemorrhagic fever in cynomolgus macaques. *Veterinary Pathology*, 47(5):831–851, 2010.

Bartrand 2008 — Bartrand, T.A., Weir, M.H., and Haas, C.N. Dose-response models for inhalation of Bacillus anthracis spores: Interspecies comparisons. *Risk Analysis*, 28(4):1115–1124, 2008.

Bazhutin 1992 — Bazhutin, N.B., Belanov, E.F., Spiridonov, V.A., Voitenko, A.V., Krivenchuk, N.A., Krotov, S.A., Omel'chenko, N.I., Tereshchenko, A.Yu. and Komichev, V.V. [The effect of the methods for producing an experimental Marburg virus infection on the characteristics of the course of the disease in green monkeys]. *Vopr Virusol*, 37(3):153–156, 1992.

Begier 2006 — Begier, E.M., Asiki, G., Anywaine, Z., Yockey, B., Schriefer, M.E., Aleti, P., Ogen-Odoi, A., Staples, J.E., Sexton, C., Bearden, S.W. and Kool, J.L. Pneumonic plague cluster, Uganda, 2004. *Emerging Infectious Diseases*, 12(3):460–467, 2006.

Bird 2008 — Bird, B. H., Albarino, C.G. et al. Rift Valley fever virus lacking the NSs and NSm genes is highly attenuated, confers protective immunity from virulent virus challenge, and allows for differential identification of infected and vaccinated animals. *Journal of Virology*, 82(6):2681–2691, 2008.

Bird 2009 — Bird, B. H., Ksiazek, T.G., et al. Rift Valley fever virus. *J Am Vet Med Assoc* 234(7):883–893, 2009.

Bliss 1934 — Bliss, C.I. The method of probits. *Science*, 79:38–39, 1934.

BMBL 2009 — U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (5th ed.), Washington, D.C.: U.S. Government Printing Office, 2009.

Bossart 2009 — Bossart, K.N., Zhu, Z., Middleton, D., Klippel, J., Crameri, G., Bingham, J., McEachern, J.A., Green, D., Hancock, T.J., Chan, Y., Hickey, A.C., Dimitrov, D.S., Wang, L. and Broder, C.C. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLOS Pathogens*, 5(10):e1000642, 2009.

Boyle 1965 — Boyle, J.J. Titration of Rift Valley fever virus in hamster kidney cells in the absence of serum. *American Journal of Veterinary Research*, 26:190–191, 1965.

Brachman 1962 — Brachman, P.S., Gold, H., Plotkin, S.A., Fekety, F.R., Werrin, M. and Ingraham, N.R. Field evaluation of a human anthrax vaccine. *American Journal of Public Health*, 52(4):632–645, 1962.

Brachman 1966 — Brachman, P.S., Kaufmann, A.F. and Dalldorf, F.G. Industrial inhalational anthrax. *Bacteriological Reviews*, 30(3):646–657, 1966.

Bradburne 1967 — Bradburne, A.F., Bynoe, M.L. and Tyrrell, D.A.J. Effects of a "new" human respiratory virus in volunteers. *British Medical Journal*, 3:767–769, 1967.

Bray 1998 — Bray, M., Davis, K., Geisbert, T., Schmaljohn, C. and Huggins, J. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. *The Journal of Infectious Diseases*, 178:651–661, 1998.

Brookmeyer 2005 — Brookmeyer, R., Johnson, E. and Barry, S. Modeling the incubation period of anthrax. *Statistics in Medicine*, 24:531–542, 2005.

Brown 1981 — Brown, J.L., Dominik, J.W. and Morrissey, R.L. Respiratory infectivity of a recently isolated Egyptian strain of Rift Valley fever virus. *Infection and Immunity*, 33(3):848–853, 1981.

Carrat 2008 — Carrat, F., Vergu, E., Ferguson, N.M., Lemaitre, M., Cauchemez, S., Leach, S. and Valleron, A. Time lines of infection and disease in human influenza: A review of volunteer challenge studies. *American Journal of Epidemiology*, 167(7):775–785, 2008.

Chang 2006 — Chang, L.Y., Ali, A.R., Hassan, S.S. and AbuBakar, S. Quantitative estimation of Nipah virus replication kinetics in vitro. *Virology Journal*, 3:47, 2006.

CDC 2000 — Centers for Disease Control and Prevention. Use of anthrax vaccine in the United States: Recommendations of the Advisory Committee on Immunization Practices. *MMWR*, 49:1–20, 2000.

CDC 2002 — Centers for Disease Control and Prevention. Notice to readers: Use of anthrax vaccine in response to terrorism: supplemental recommendations of the Advisory Committee on Immunization Practices. *MMWR*, 51:1024–1026, 2002.

CDC 2011a — Centers for Disease Control and Prevention. *CDC – Prevention – Tularemia*. http://www.cdc.gov/tularemia/prevention, accessed March 21, 2011.

CDC 2011b — Centers for Disease Control and Prevention. *Plague Information - CDC Division of Vector-Borne Infectious Diseases (DVBID)*. http://www.cdc.gov/ncidod/dvbid/plague/info.htm, accessed March 22, 2011.

CDC 2011c — Centers for Disease Control and Prevention. Antiviral agents for the treatment and chemoprophylaxis of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Report*, 60(RR01):1–24, 2011.

Coleman 2008 — Coleman, M.E., Thran, B., Morse, S.S., Hugh-Jones, M. and Massulik, S. Inhalation anthrax: dose response and risk analysis. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*, 6(2):147–159, 2008.

Cohen 2007 — Cohen, M.L., and Whalen, T. Implications of low level human exposure to respirable *B. anthracis*. *Applied Biosafety*, 12(2):109–115, 2007.

Dahlgren 1960 — Dahlgren, C.M., Buchanan, L.M. and Decker, H.M. *Bacillus anthracis* aerosols in goat hair processing mills. *American Journal of Hygiene*, 72:24–31, 1960.

Day 1972 — Day, W.C. and Berendt, R.F. Experimental tularemia in *Macaca mulatta*: relationship of aerosol particle size to the infectivity of airborne *Pasteurella tularensis*. *Infection and Immunity*, 5:77–82, 1972.

De Albuquerque 2006 — De Albuquerque, N., Baig, E., Xuezhong, M., Shalev, I., Phillips, M.J., Habal, M., Leibowitz, J., McGilvray, I., Butany, J., Fish, E. and Levy, G. Murine hepatitis virus strain 1 as a model for severe acute respiratory distress syndrome (SARS). In Perlman, S. and Holmes, K.V., eds. *The Nidoviruses: Toward Control of SARS and Other Nidovirus Diseases*. New York: Springer, 2006.

DeDiego 2008 — DeDiego, M.L., Pewe, L., Alvarez, E., Rejas, M.T., Perlman, S. and Enjuanes, L. Pathogenicity of severe acute respiratory coronavirus deletion mutants in hACE-2 transgenic mice. *Virology*, 376:379–389, 2008.

Dorland's — *Dorland's Medical Dictionary for Health Consumers*. Philadelphia: Saunders Press (Elsevier), 2007.

Druett 1953 — Druett, H.A., Henderson, D.W., Packman, L. and Peacock, S. Studies on respiratory infection. I. The influence of particle size on respiratory infection with anthrax spores. *Journal of Hygiene*, 51:159–371, 1953.

Easterday 1962 — Easterday, B.C., Murphy, L.C. and Bennett, D.G. Experimental Rift Valley fever in lambs and sheep. *American Journal of Veterinary Research*, 23:1231–1240, 1962.

Easterday 1963 — Easterday, B.C. and Murphy, L.C. Studies on Rift Valley fever in laboratory animals. *The Cornell Veterinarian*, 53:423–433, 1963.

Easterday 1965 — Easterday, B.C. Rift Valley fever. *Advances in Veterinary Science*, 10:65–127, 1965.

Ehrenkranz 1955 — Ehrenkranz, N.J. and Meyer, K.F. Studies on immunization against plague: VIII. Study of three immunizing preparations in protecting primates against pneumonic plague. *Journal of Infectious Diseases*, 96(2): 138–144, 1955.

Finney 1947 — Finney, D.J. *Probit Analysis*. Cambridge, UK: Cambridge University Press, 1947.

Fletcher 1987 — Fletcher, R. *Practical Methods of Optimization* (2nd ed.). New York: John Wiley & Sons, 1987.

Franz 1997 — Franz, D.R., Jahrling, P.B., Friedlander, A.M., McClain, D.J., Hoover, D.L., Bryne, W.R., Pavlin, J.A., Christopher, G.W. and Eitzen, E.M. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*, 278(5):399–411, 1997.

Freedman 2002 — Freedman, A., Afonja, O., Chang, M.W., Mostashari, F., Blaser, M., Perez-Perez, G., Lazarus, H., Schacht, R., Guttenberg, J., Traister, M. and Borkowsky, W. Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old infant. *JAMA*, 287(7):869–874, 2002.

Furumoto 1967 — Furumoto, W.A. and Mickey, R. A mathematical model for the infectivity-dilution curve of tobacco mosaic virus: theoretical considerations. *Virology*, 32:216–223, 1967.

Garcia 2001 — Garcia, S., Crance, J.M., Billecocq, A., Peinnequin, A., Jouan, A., Bouloy, M. and Garin, D. Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. *Journal of Clinical Microbiology*, 39(12):4456–4461, 2001.

Geisbert 2004 — Geisbert, T.W. and Jahrling, P.B. Exotic emerging viral diseases: progress and challenges. *Nature Medicine*, 10(12 Suppl):S110-121, 2004.

Geisbert 2005 — Geisbert, T.W., Jones, S., Fritz, E.A., Shurtleff, A.C., Geisbert, J.B., Liebscher, R., Grolla, A., Stroher, U., Fernando, L., Daddario, K.M., Guttieri, M.C., Mothe, B.R., Larsen, T., Hensley, L.E., Jahrling, P.B. and Feldmann, H. Development of a new vaccine for the prevention of Lassa fever. *PLOS Medicine*, 2(6):537–545, 2005.

Geisbert 2008 — Geisbert, T.W., Daddario-DiCaprio, K.M., Geisbert, J.B., Reed, D.S., Feldmann, F., Grolla, A., Stroher, U., Fritz, E.A., Hensley, L.E., Jones, S.M. and Feldmann, H. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine*, 26(52):6894–6900, 2008.

Geisbert 2010 — Geisbert, T.W., Daddario-DiCaprio, K.M., Hickey, A.C., Smith, M.A., Chan, Y., Wang, L., Mattapallil, J.J., Geisbert, J.B., Bossart, K.N. and Broder, C.C. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLOS One*, 5(5):e10690, 2010.

Glassman 1966 — Glassman, H.N. Discussion. *Bacteriological Reviews*, 30(3):657–659, 1966.

Guillaume 2004 — Guillaume, V., Lefeuvre, A., Faure, C., Marianneau, P., Buckland, R., Lam, S.K., Wild, T.F. and Deubel, V. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). *Journal of Virological Methods*, 120(2):229-237, 2004.

Gutting 2008 — Gutting, B.W., Channel, S.R., Berger, A.E., Gearhart, J.M., Andrews, G.A., Sherwood, R.L. and Nichols, T.L. Mathematically modeling inhalational anthrax. *Microbe*, 3(2):78–85, 2008.

Haas 1999 — Haas, C.N., Rose, J.B. and Gerba, C.P. *Quantitative Microbial Risk Assessment*. New York: John Wiley and Sons, 1999.

Haas 2002 — Haas, C.N. On the risk of mortality to primates exposed to anthrax spores. *Risk Analysis*, 22:189– 193, 2002.

Hambleton 1983 — Hambleton, P., Stephenson, J.R., Baskerville, A. and Wiblin, C.N. Pathogenesis and immune response of vaccinated and unvaccinated rhesus monkeys to tick-borne encephalitis virus. *Infection and Immunity*, 40(3):995–1003, 1983.

Harris 1999 — Harris, S. The Japanese biological warfare programme: an overview. In: Geissler, E. and van Courtland Moon, J.E., eds. *Biological and toxin weapons: research, development and use from the Middle Ages to 1945*. SIPRI Chemical and Biological Warfare Studies, 18:127–152. Oxford: Oxford University Press 1999.

Hevey 2002 — Hevey, M., Negley, D., VanderZanden, L., Tammariello, R.F., Geisbert, J., Schmaljohn, C., Smith, J.F., Jahrling, P.B. and Schmaljohn, A.L. Marburg virus vaccines: comparing classical and new approaches. *Vaccine*, 20:586–593, 2002.

Hirano 2001 — Hirano, N., Haga, S., Sada, Y. and Tohyama, K. Susceptibility of rats of different ages to inoculation with swine haemagglutinating encephalomyelitis virus (a coronavirus) by various routes. *Journal of Comparative Pathology*, 125:8–14, 2001.

Hirano 2004 — Hirano, N., Nomura, R., Tawara, T. and Tohyama, K. Neurotropism of swine haemagglutinating encephalomyelitis virus (coronavirus) in mice depending on host age and route of infection. *Journal of Comparative Pathology*, 130:58–65, 2004.

Hooper 2001 — Hooper, J.W., Larsen, T., Custer, D.M. and Schmaljohn, C.S. A lethal disease model for hantavirus pulmonary syndrome. *Virology*, 289:6–14, 2001.

Hooper 2008 — Hooper, J.W., Ferro, A.M. and Wahl-Jensen, V. Immune serum produced by DNA vaccination protects hamsters against lethal respiratory challenge with Andes virus. *Journal of Virology*, 82(3):1332–1338, 2008.

Houng 2004 — Houng, H. S., D. Norwood, G. V. Ludwig, W. Sun, M. Lin, and D. W. Vaughn. Development and evaluation of an efficient 3'-noncoding region based SARS coronavirus (SARS-CoV) RT-PCR assay for detection of SARS-CoV infections. *J Virol Methods* 120 (1):33-40, 2004.

Huang 2010 — Huang, Y., Hong, T., Bartrand, T.A., Gurian, P.L., Haas, C.N., Liu, R. and Tamrakar, S.B. How sensitive is safe? Risk-based targets for ambient monitoring of pathogens. *IEEE Sensors Journal*, 10(3):668–673, 2010.

Huang 2011 — Huang, Y. and Haas, C.N. Quantification of the relationship between bacterial kinetics and host response for monkeys exposed to aerosolized *Francisella tularensis*. *Applied and Environmental Microbiology*, 77(2): 485–490, 2011.

Ikonen 2010 — Ikonen N, Strengell M, Kinnunen L, Österlund P, Pirhonen J, Broman M, Davidkin I, Ziegler T, Julkunen I. High frequency of cross-reacting antibodies against 2009 pandemic influenza A(H1N1) virus among the elderly in Finland. *Euro Surveill*, 15(5):pii=19478, 2010.

Joellenbeck 2002 — Joellenbeck, L., Zwanziger, L.L., Durch, J.S., Strom, B.L., eds. *The anthrax vaccine: Is it safe? Does it work?* Washington, D.C.: National Academy Press, 2002.

Johns 2010 — Johns MC, Eick AA, Blazes DL, Lee S-e, Perdue CL, et al. Seasonal influenza vaccine and protection against pandemic (H1N1) 2009-associated illness among US Military Personnel. *PLoS ONE*, 5(5), 2010.

Johnson 1995 — Johnson, E., Jaax, N., White, J. and Jahrling, P. Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *International Journal of Experimental Pathology*, 76:227–236, 1995.

Jones 2005 — Jones, R.M., Nicas, M., Hubbard, A., Sylvester, M.D., and Reingold, A. The infectious dose of *Francisella tularensis* (tularemia). *Applied Biosafety*, 10(4):227–239, 2005.

Keefer 1972 — Keefer, G.V., Zebarth, G.L. and Allen, W.P. Susceptibility of dogs and cats to Rift Valley fever by inhalation or ingestion of virus. *The Journal of Infectious Diseases*, 125(3):307–309, 1972.

Kenyon 1992 — Kenyon, R.H., McKee, Jr., K.T., Zack, P.M., Rippy, M.K., Vogel, A.P., York, C., Meegan, J., Crabbs, C. and Peters, C.J. Aerosol infection of rhesus macaques with Junin virus. *Intervirology*, 33:23–31, 1992.

Klein 1970 — Klein, F., Mahlandt, B.G., Eyler, S.L. and Lincoln, R.E. Relationship between plaque assay and mouse assay for titrating Rift Valley fever virus. *Proceedings of the Society for Experimental Biology and Medicine*, 134(4):909–914, 1970.

Klein 1971 — Klein, F., Jones, Jr., W.I., Mahlandt, B.G. and Lincoln, R.E. Growth of pathogenic virus in a large-scale tissue culture system. *Applied Microbiology*, 21(2):265–271, 1971.

Leffel 2004 — Leffel, E.K. and Reed, D.S. Marburg and Ebola viruses as aerosol threats. *Biosecurity and Bioterrorism*, 2(3):186–191, 2004.

McCray 2007 — McCray Jr., P.B., Pewe, L., Wohlford-Lenane, C., Hickey, M., Manzel, L., Shi, L., Netland, J., Jia, H.P., Halabi, C., Sigmund, C.D., Meyerholz, D.K., Kirby, P., Look, D.C. and Perlman, S. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *Journal of Virology*, 81(2):813–821, 2007.

McCrumb 1961 — McCrumb, Jr, F.R. Aerosol infection of man with *Pasteurella tularensis*. *Bacteriological Review*, 25(3):262–267, 1961.

McKay 1979 — McKay, M.D., Beckman, R.J. and Conover, W.J. A comparison of three methods for selecting values of input variables in the analysis of output from a computer code. *Technometrics*, 21:239–245, 1979.

Medina 2010 — Medina, R.A, Manicassamy, B., Stertz, S., Seibert, C.W., Hai, R., Belshe, R.B., Frey, S.E., Basler, C.F., Palese, P. and Garcia-Sastre, A. Pandemic 2009 H1N1 vaccine protects against 1918 Spanish influenza virus. *Nature Communications*, 1:28, 2010.

Meegan 1989 — Meegan, J. M. and Bailey, C.L. Rift Valley Fever. In: Monath, T.P. *The Arboviruses: Epidemiology and Ecology*, 4:51–76. Boca Raton: CRC Press, 1989.

Meselson 1994 — Meselson, M., Guillemin, J., Hugh-Jones, M., Langmuir, A., Popova, I., Shelokov, A. and Yampolskaya, O. The Sverdlovsk anthrax outbreak of 1979. *Science*, 266(5188):1202–1208, 1994.

Miller 1963 — Miller, W.S., Demchak, P., Rosenberger, C.R., Dominik, J.W. and Bradshaw, J.L. Stability and infectivity of airborne yellow fever and Rift Valley fever viruses. *American Journal of Hygiene*, 77:114–121, 1963.

Moe 1981 — Moe, J.B., Lambert, R.D. and Lupton, H.W. Plaque assay for Ebola virus. *Journal of Clinical Microbiology*, 13(4):791–793, 1981.

NRC 2011 — Committee on Special Immunizations Program for Laboratory Personnel Engaged in Research on Countermeasures for Select Agents, National Research Council. *Protecting the Frontline in Biodefense Research: The Special Immunizations Program*. Washington, D.C.: The National Academies Press, 2011.

Parodi 1958 — Parodi, A.S., Greenway, D.J., Rugiero, H.R., Frigerio, M., De La Barrera, J.M., Mettler, N., Garzon, F., Boxaca, M., Guerrero, L., and Nota, N. [Concerning the epidemic outbreak in Junin]. *El Dia Medico*, 30(62):2300–2301, 1958.

P'iankov 1995 — P'iankov, O.V., Sergeev, A.N., P'iankova, O.G. and Chepurnov, A.A. [Experimental Ebola fever in *Macaca mulatta*]. *Vopr Virusol*, 40(3):113-115, 1995.

Pratt 2010 — Pratt, W.D., Wang, D., Nichols, D.K., Luo, M., Woraratanadharm, J., Dye, J.M., Holman, D.H. and Dong, J.Y. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clinical and Vaccine Immunology*, 17(4):572–581, 2010.

Raftery 1995 — Raftery, A.E. Bayesian Model Selection in Social Research. *Sociological Methodology*, 25:111–163 , 1995.

Ratsitorahina 2000 — Ratsitorahina, M., Rabarijaona, L., Chanteau, P. and Boisier, P. Seroepidemiology of human plague in the Madagascar highlands. *Tropical Medicine & International Health*, 5(2):94–98, 2000.

Ruzek 2008 — Ruzek, D., Bell-Sakyi, L., et al. Growth of tick-borne encephalitis virus (European subtype) in cell lines from vector and non-vector ticks. *Virus Res* 137(1):142–146, 2008.

Ryabchikova 1993 — Ryabchikova, S. Baranova, S., Tkachev, V. and Grazhdantseva, A. Morphological changes in Ebola virus infection in guinea pigs. *Vopr Virusol*, 38:176-179, 1993.

Sabelnikov 2006 — Sabelnikov, A., Zhukov, V. and Kempf, R. Probability of real-time detection versus probability of infection for aerosolized biowarfare agents: A model study. *Biosensors & Bioelectronics*, 21:2070–2077, 2006.

Sampath 2005 — Sampath, R., S. A. Hofstadler, L. B. Blyn, M. W. Eshoo, T. A. Hall, C. Massire, H. M. Levene, J. C. Hannis, P. M. Harrell, B. Neuman, M. J. Buchmeier, Y. Jiang, R. Ranken, J. J. Drader, V. Samant, R. H. Griffey, J. A. McNeil, S. T. Crooke, and D. J. Ecker. Rapid identification of emerging pathogens: coronavirus. *Emerg Infect Dis* 11 (3):373-9, 2005.

Saslaw 1961a — Saslaw, S., Eigelsbach, H.T., Wilson, H.E., Prior, J.A. and Carhart, S. Tularemia vaccine study. I. Intracutaneous challenge. *Archives of Internal Medicine*, 107(5):689–701, 1961.

Saslaw 1961b — Saslaw, S., Eigelsbach, H.T., Prior, J.A., Wilson, H.E. and Carhart, S. Tularemia vaccine study. II. Respiratory challenge. *Archives of Internal Medicine*, 107(5):702–714, 1961.

Sawyer 1966 — Sawyer, W.D., Jemski, J.V., Hogge, Jr., A.L., Eigelsbach, H.T., Wolfe, E.K., Dangerfield, H.G., Gochenour, Jr., W.S. and Crozier, D. Effect of aerosol age on the infectivity of airborne *Pasteurella tularensis* for *Macaca mulatta* and man. Journal of Bacteriology, 91(6):2180–2184, 1966.

Schmidt 1979 — Schmidt, O.W., Cooney, M.K., Kenny, G.E. Plaque assay and improved yield of human coronaviruses in a human rhabdomyosarcoma cell line. *Journal of Microbiology*, 9(6):722–728, 1979.

Schwarz 1978 — Schwarz, G. Estimating the dimension of a model. *The Annals of Statistics*, 6:461–464, 1978.

Sidwell 2003 — Sidwell, R.W. and Smee, D.F. Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control. *Antiviral Research*, 57(1–2):101–111, 2003.

Speck 1957 — Speck, R.S. and Wolochow, H. Studies on the experimental epidemiology of respiratory infections. VIII. Pneumonic plague in macacus rhesus. *Journal of Infectious Diseases*, 100:58–69, 1957.

Stephenson 1984 — Stephenson, E.H., Larson, E.W. and Dominik, J.W. Effect of environmental factors on aerosol-induced Lassa virus infection. *Journal of Medical Virology*, 14:295–303, 1984.

Swanepoel 1994 — Swanepoel, R. and Coetzer, J.A.W. Rift Valley Fever. In: Coetzer, J.A.W. and Tustin, R.C. *Infectious Diseases of Livestock with Special Reference to Southern Africa*, 1:688–717. New York: Oxford University Press, 1994.

Tamrakar 2008 — Tamrakar, S.B. and Haas, C.N. Dose-response model for Lassa virus. *Human and Ecological Risk Assessment*, 14:742–752, 2008.

Taubenberger 2006 — Taubenberger, J. K. and Morens, D. M. 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis* 12(1):15-22, 2006.

Teunis 2000 — Teunis, P.F.M. and Havelaar, A.H. The Beta Poisson dose-response model is not a single-hit model. *Risk Analysis*, 20:513–520, 2000.

Towner 2004 — Towner, J. S., P. E. Rollin, D. G. Bausch, A. Sanchez, S. M. Crary, M. Vincent, W. F. Lee, C. F. Spiropoulou, T. G. Ksiazek, M. Lukwiya, F. Kaducu, R. Downing, and S. T. Nichol. Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *Journal of Virology*,78(8):4330–4341, 2004.

Trombley 2010 — Trombley, A. R., L. Wachter, J. Garrison, V. A. Buckley-Beason, J. Jahrling, L. E. Hensley, R. J. Schoepp, D. A. Norwood, A. Goba, J. N. Fair, and D. A. Kulesh. Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and New World hantaviruses. *Am J Trop Med Hyg* 82(5):954–960, 2010.

Tumpey 2004 — Tumpey, T. M., Garcia-Sastre, A., Taubenberger, J.K., Palese, P., Swayne, D.E. and Basler, C.F. Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. *Proc Natl Acad Sci U S A,* 101(9):3166-71, 2004.

Uenaka 1998 — Uenaka, T., Kishimoto, I., Uemura, T., Ito, T., Umemura, T. and Otsuki, K. Cloacal inoculation with the Connecticut strain of avian infectious bronchitis virus: An attempt to produce nephropathogenic virus by in vivo passage using cloacal inoculation. *Journal of Veterinary Medical Science*, 60(4):495–502, 1998.

Vicenzi 2004 — Vicenzi, E., F. Canducci, D. Pinna, N. Mancini, S. Carletti, A. Lazzarin, C. Bordignon, G. Poli, and M. Clementi. Coronaviridae and SARS-associated coronavirus strain HSR1. *Emerg Infect Dis* 10 (3):413-8, 2004.

Watanabe 2010	Watanabe, T., Bartrand, T.A., Weir, M.H., Omura, T. and Haas, C.N. Development of a dose-response model for SARS coronavirus. *Risk Analysis*, 30:1129–1138, 2010.
Weidmann 2011	Weidmann, M., Sall, A.A., Manuguerra, J., Koivogui, L., Adjami, A., Traore, F.F., Hedlund, K, Lindegren, G. and Mirazimi, A. Quantitative analysis of particles, genomes and infectious particles in supernatants of haemorrhagic fever virus cell cultures. *Virology Journal*, 8:81, 2001
Weingartl 2005	Weingartl, H., Czub, S., Copps, J., Berhane, Y., Middleton, D., Marszal, P., Gren, J., Smith, G., Ganske, S., Manning, L. and Czub, M. Invasion of the central nervous system in a porcine host by Nipah virus. *Journal of Virology*, 79(12):7528–7534, 2005.
Wahl-Jensen 2007	Wahl-Jensen, V., Chapman, J. et al. Temporal analysis of Andes virus and Sin Nombre virus infections of Syrian hamsters. *Journal of Virology,* 81(14):7449–7462, 2007.
WHO 2007	World Health Organization. *WHO Guidelines on Tularemia*. Geneva: WHO Press, 2007.
WHO 2008	World Health Organization. *Anthrax in humans and animals* (4th ed.). Geneva: WHO Press, 2008.
Wilkening 2006	Wilkening, D.A. Sverdlovsk revisited: modeling human inhalation anthrax. *Proceedings of the National Academy of Sciences of the USA*, 103:7589–7594, 2006.

Appendix K:
Initial Infection

Table of Contents

K. Initial Infection

K.1 Introduction

Initial infections analyses for this risk assessment (RA) consist of qualitative and quantitative estimates of potential infection and health effects in those exposed to a pathogen as a direct result of each of the events carried forward from the event sequence analyses. A distinction is made with regard to *initial* infection, addressed in this appendix as that occurring after direct exposure to a pathogen as a result of a release, and *secondary* infection that results from exposure to an already infected individual. Secondary infections are addressed in Appendix L.

The dose-response analysis (Appendix J) provides estimates for the probability of infection resulting from inhalational exposure to different amounts (doses) of each pathogen. This appendix links these dose-response estimates with the exposure estimates from the event sequence analyses to provide estimates for the frequency with which initial infections might occur as a result of NEIDL-related events. This appendix also discusses potential health effects, morbidity, and mortality that might result in those becoming infected.

The initiating event for most exposures considered in this RA and subsequent initial infections is an event involving a laboratory accident, which has the potential to result in a laboratory-associated infection (LAI). A special case of initial infection is where the individual acquires the pathogen directly from an infected animal or arthropod that was inadvertently released from the laboratory/facility. Yet another special case of initial infections is where there is a large scale disaster such as an earthquake resulting in direct exposure of pathogen to members of the public.

K.2 Methodology

The estimation of initial infections is based on the following:

i. Outputs of the event sequence analyses that provide an estimate or range of
   a. The frequency categories of the incidents
   b. The number of individuals exposed in the three categories—laboratory workers, facility workers, and members of the public
   c. The route of exposure
   d. The amount of exposure (dose) per exposed individual

ii. Dose-response curves for each pathogen based on estimates of human infectious doses (HID) for the 13 pathogens (see Appendix J)
   a. Literature-based estimates derived from published models and from models fit to human and/or animal data for the RA
   b. Expert-based estimates of HID from expert opinion using a modified Delphi method

iii. Mitigation strategies available for each pathogen
   a. Vaccine status of the exposed individual, if there is a vaccine available for the particular pathogen
   b. Availability of post-exposure prophylaxis to the exposed individual; these are usually medications, though occasionally vaccines may be administered post-exposure

iv. Population susceptibility to infection
   a. Estimates of the portion of the population exposed from each event sequence belonging to medically vulnerable sub-populations (MVSP)
   b. Estimates of the increased susceptibility to infection of MVSP members as compared to healthy individuals

v. Health consequences to the initially infected individual
   a. Estimates of morbidity in those becoming infected and experiencing subsequent disease symptoms
   b. Estimates of mortality resulting from infection and disease

The outputs of the initial infections calculations are in the form of probabilistic estimates of the number of initial infections for each pathogen that might occur from each event sequence. These estimates are combined with the estimated frequency of occurrence of each incident to derive estimated frequencies of the occurrence of initial infections and associated consequences. These estimates are subsequently extended to potential further consequences due to secondary transmission in Appendix L.

When developing the calculations, it is important to distinguish *uncertainty* and *variability*. Uncertainty refers to the lack of knowledge of the true value of parameters. Variability refers to the effect of chance and inherent unpredictability of the way events might occur (stochasticity). In the estimation of initial infections, the effect of uncertainty for some key unknown values was assessed by systematically

comparing results under different assumptions for their values, while the effect of variability was assessed by performing probabilistic calculations under each tested scenario.

K.2.1 Needlestick

This section describes the methodology for generating estimates for initial infections resulting from Needlestick events described in Chapter 4 and Appendix F.

Chapter 4 described four distinct sub-events, depending on the setting of the event (BSL-3 or BSL-4 laboratories) and whether or not the event is detected and reported. The initial infections analyses are primarily concerned with consequences from potential LAI's without prompt detection and reporting because those events pose potential risk to the public through secondary transmission. Therefore, the events that assume prompt detection and reporting are not carried forward for detailed analysis in this appendix. The frequency categories assigned to the other two sub-events are listed in Table K–1. An explanation of how the category was determined for each event is provided in Chapter 4.

Table K–1. Frequency categories for needlestick events.

Event	Frequency category
Needlestick in BSL-3 laboratory *without* prompt detection and reporting	B < 1 per 100 years > 1 per 10,000 years
Needlestick in BSL-4 laboratory *without* prompt detection and reporting	B < 1 per 100 years > 1 per 10,000 years

As described in Chapter 4 and further detailed in Appendix F, it is conservatively assumed that every needlestick occurring under each scenario in Table K–1 would deliver a sufficiently high dose to cause an infection in the laboratory worker. Therefore, no further analysis on the likelihood of initial infections for needlestick events is provided in this appendix. The frequency categories assigned to each sub-event are carried forward and assumed as the frequency categories for one initial infection occurring in a laboratory worker. The frequency estimates for events without prompt detection and reporting also serve as estimates for the frequency with which a needlestick-infected laboratory worker would leave the facility and interact with contacts. Potential consequences following this scenario are discussed in Chapter 9 and Appendix L.

The results section for Needlestick events (Section K.3.1 below) includes descriptions of potential health consequences for a laboratory worker infected via needlestick for each pathogen. Section K.3.1 also includes estimates for the frequency of mortalities among laboratory workers infected via needlestick without prompt detection and reporting.

K.2.2 BSL-3 Centrifuge Infectious Aerosol Release

This section describes the methodology for generating estimates for initial infections resulting from *BSL-3 Centrifuge Infectious Aerosol Release* events described in Chapter 4 and Appendix F.

This event involves only those pathogens that may be studied under BSL-3 laboratory conditions (i.e., 7 of 13 pathogens). Centrifuge release scenarios involving BSL-4 pathogens are not carried forward to the initial infection analyses because the analysis described in Chapter 4 and Appendix F determined that it would not be credible for an aerosol exposure to go undetected in a BSL-4 laboratory. For the BSL-3 release scenario analyzed in this section, all potentially exposed individuals are laboratory workers. This section does not discuss risk to the public as a result of a centrifuge release. The potential consequences of laboratory workers leaving the facility after becoming infected and interacting with contacts in the general public are discussed in Chapter 9 and Appendix L.

The potential route of exposure for laboratory workers during or after a centrifuge release is assumed to be through direct contact, ingestion, or inhalation. Therefore, the dose-response information generated in Appendix J, which focuses primarily on inhalational exposure, can be applied. The centrifuge release information provided in Appendix F and the dose-response information provided in Appendix J are synthesized in Section K.3.2 of this appendix for each of the seven BSL-3 pathogens.

The remainder of this section describes the methodology for generating quantitative estimates of the frequency of centrifuge release events that lead to one or more initial infections from each pathogen, as well as the uncertainty in the estimations and the sensitivity of the estimations to uncertainties in the input values. *As this RA specifically considers centrifuge release scenarios that are undetected or unreported,* the initial infections results can also serve as estimates for the frequency of infected laboratory workers leaving the facility after a centrifuge incident, with subsequent potential to transmit to the public.

K.2.2.1 Overall assumptions

The following assumptions were made in the calculations, though deviations from these assumptions were not tested.

- The true frequency of centrifuge release is constant over time and is within the range specified in Chapter 4. Note that assuming a constant frequency does not mean that incidents are assumed to happen at regularly spaced intervals.
- The number of workers potentially exposed after an incident is variable but always within the range 1–4.
- The expected dose received by each worker is variable but always within the ranges specified in Chapter 4.
- The dose received by one worker is independent of the dose received by another worker. This assumption might be violated because the dose is affected by variability in the incident itself, which would have a common effect on all workers in the room. However, it is reasonable to assume that much of the variability in dose received depends on, for example, where the worker is positioned in the room relative to the centrifuge and air flow or the worker's personal protective equipment and actions.
- A true dose-response curve exists for each pathogen, is applicable to each potentially exposed person, and is within the range of dose-response curves derived in Appendix J.

K.2.2.2 Input values

This section specifies the values, distributions, and functions that are used in the calculations. There are two types of calculations presented for each pathogen—a *central estimate example* and *uncertainty results*.

For the central estimate example, a single value, distribution, or function is selected for each of the inputs. It is important to note that the results calculated from these central estimate inputs are not intended to be used as if they are certain predictions of what the frequency of infections would be at the NEIDL. This is because many of the inputs to the calculations are associated with rather wide uncertainty ranges. Instead, these results serve to illustrate the steps of the calculations and to provide point estimates near the center of the uncertainty range. For the uncertainty results, each input is assigned a range of values, distributions, or functions, and various sets of inputs are drawn randomly to generate a range of outputs.

The central estimate inputs and uncertainty ranges are specified as follows.

Number of lab workers potentially exposed (*w*). The number of workers *w* present in the biocontainment area of a potential centrifuge release is assumed to fall in the range 1–4, as described in Chapter 4. Assumptions regarding this input are important in calculations of the likelihood of one or more more initial infections occurring.

- **Central estimate example: $w = 2$**
  For the central estimatecentral estimate, it is assumed that there would be exactly two workers present in the biocontainment area of a centrifuge during a potential release, with no variability.

- **Uncertainty range:**
  In reality, there is variability in the exact number of people within the range 1–4 who would potentially be exposed by any given centrifuge release. This means, for example, that at some times there could be as many as four workers in the room where a release occurs, while at other times there could be one, two, or three. This variability is described quantitatively through the use of a probability distribution; that is, the probability that the number of workers in the room will be 1, 2, 3, or 4 at any given time. Specifically, we define a probability mass function $f(w)$, which is the probability that the number of workers in the room is *w*. Multiple distributions were tested in the analysis. A realistic distribution could be skewed toward the lower or higher end of the range. Three distributions, f_1, f_2, and f_3 shown in Table K–2, were used to generate the uncertainty results. The distribution equivalent to the central estimate example is also included in that table for reference.

Table K–2: Candidate distributions for the number of workers in a room during a potential centrifuge release.

Distribution	Prob ($w = 1$)	Prob ($w = 2$)	Prob($w = 3$)	Prob($w = 4$)	Average w
Central estimate example	0	1	0	0	2
$f_1(w)$	0.5	0.3	0.2	0	1.7
$f_2(w)$	0.25	0.25	0.25	0.25	2.5
$f_3(w)$	0	0.2	0.3	0.5	3.3

The central estimate example row in Table K–2 demonstrates the assumption that there will always be exactly two laboratory workers potentially exposed during a release (no variability). The other three distributions in Table K–2 assume variability in the number of laboratory workers. For example, distribution f_3 assumes that there is no chance that only one laboratory worker would be in the room during a release, a 20% chance that there would be two laboratory workers, a 30% chance that there would be three laboratory workers, and a 50% chance that there would be four laboratory workers. These probabilities lead to an estimated average of 3.3 laboratory workers in the room at any given time. For the uncertainty analysis, it is assumed that the true distitribution of the number of laboratory workers is equally likely to be f_1, f_2, or f_3.

Amount of exposure per worker (d). The amount of exposure is the expected dose inhaled by a laboratory worker, which is denoted d. The value of d for each laboratory worker is assumed to fall in the ranges listed in Chapter 4 for each pathogen. All the ranges are given from $d = 0$ to $d = d_{max}$, where d_{max} depends on the pathogen and whether or not the laboratory worker has full or partial respiratory protection. Assumptions about the likelihood of inhaling various doses within the range are important in determining the probability that a worker becomes infected.

- **Central estimate example:**

  The central estimate assumes that there is variability in the expected dose within the range 0 to d_{max} that a worker would receive during any given incident. By random chance, one laboratory worker might inhale a dose close to d_{max}, another might inhale a dose close to zero, and a third might inhale a dose in between. The central estimate example employs a simple probability

distribution to characterize this variability. Specifically, it is assumed that for each laboratory worker, there is a 20% probability that the dose d is 0, a 20% probability that d is one-fourth of d_{max}, a 20% probability that d is one-half of d_{max}, a 20% probability that d is three-fourths of d_{max}, and a 20% probability that the dose is d_{max}.

- **Uncertainty range**

  To describe the different dose distributions tested in the uncertainty analysis, the following notation is introduced. The probability mass function $g(d)$ is the probability that the expected dose received is d. Multiple distributions were tested as part of the uncertainty analysis. A realistic distribution could be skewed toward either end of the range. The three alternative distributions described below were tested.

  First, each dose range is split into $N = 1000$ evenly-spaced doses d_i from $d_1 = 0$ to $d_{1000} = d_{max}$ (note that for the central estimate $N = 5$ was used). Non-integer values of d are acceptable as the input to the dose-response models is the *expected* dose, while the dose-response equations calculate the average probability of infection over possible integer numbers of dose units around the expected value. Next, the value M is defined as $M = N(N-1)/2$. Using these definitions, the three distibutions are as follows:

$$g_1(d_i) = (N - i)/M$$

$$g_2(d_i) = 1/N$$

$$g_3(d_i) = (i - 1)/M$$

The g_1 distribution is a discretized triangular distribution that assigns higher probability to the received dose being in the lower part of the dose range. The g_2 distribution is a uniform distribution over the range 0 to d_{max} , inclusive. The g_3 distribution is a discretized triangular distribution that assigns higher probability to the received dose being in the higher part of the dose range. All three distributions are weighted equally in the uncertainty analysis.

It is unlikely that the true distribution of doses received over many releases would resemble the form of any of these three examples. However, the three distributions are useful test cases for the

range of possibilities for distributions that are skewed toward either end of the dose range and especially for testing the sensitivity of the outcome to uncertainty in the dose distribution. Note that because the upper limit of each dose range was selected as a conservative upper bound, the g_1 distribution, which places the lowest weight on higher doses, is presumed to be closest to what the true distribution would be over many incidents. Because g_2 and g_3 are given equal weight to g_1, the uncertainty analsysis places a conservative amount of weight on dose distributions that most likely overestimate the average exposure level.

Probability of partial respiratory protection (p_f). It was assumed, and as stated in Appendix F, that there is a 1% chance that one out of four workers would have partial rather than full respiratory protection. This would resultin a two order of magnitude increase in exposure should a centrifuge release occur.

- **Central estimate example: $p_f = 0.0025$**
  The assumption from Appendix F is carried forward to the calculations used in this chapter, where it is assumed that there is a 0.25% chance that any given worker will have partial rather than full respiratory protection during a centrifuge release (resulting in about a 1% chance that one out of four will have partial protection). Therefore, it is assumed that the probability p_f = 0.0025 that an exposed worker will receive a dose in the range estimated for the *BSL-3 Centrifuge Infectious Aerosol Release with Partial Respiratory Protection* event and a probability $1 - p_f$ = 0.9975 that an exposed worker will receive a dose in the range estimated for the *BSL-3 Centrifuge Infectious Aerosol Release with Full Respiratory Protection* event.

- **Uncertainty analysis**
  Variations from the central estimate example assumption were not tested in the uncertainty analysis. The assumption in Appendix F from which the value of p_f was derived was selected conservatively, which means that the *true* value of p_f is presumed to be lower than 0.0025. Therefore, if lower values of p_f were applied, the calculations would result in lower risk than the results presented here.

Probability of infection given the expected dose, or dose-response curve ($p(d)$). As described in Appendix J, a dose-response curve $p(d)$ describes the probability p of infection given an expected dose d

inhaled by a worker. For most pathogens, both a literature-based curve and an expert-based curve were generated, along with uncertainty ranges for each.

- **Central estimate example**

  The best fit literature-based dose-response curve was used in the central estimate example for each pathogen. For one BSL-3 pathogen (1918 H1N1 influenza virus), a literature-based curve was not derived. In this case, a middle-range curve derived from the set of estimates from one expert was employed for the central estimate example. Table K–3 shows the model used for each of the seven BSL-3 pathogens. Full descriptions of the models are provided in Appendix J.

Table K–3. Central estimate example dose-response curves for each BSL-3 pathogen.

Pathogen	Model	Parameter value(s)	Units of dose	Source
B. anthracis	Exponential	$r = 2.6 \times 10^{-5}$	CFU[a]	Literature-based model
F. tularensis	Exponential	$r = 0.063$	CFU	Literature-based model
Y. pestis	Beta Poisson	$\alpha = 0.065$ $\beta = 8000$	CFU	Literature-based model
1918 H1N1V	Log-Probit	$m = 0.41$ ID_{50}[b] $= 930$	PFU[c]	Expert-based model[d]
SARS-CoV	Exponential	$r = 0.0025$	PFU	Literature-based model
RVFV	Exponential	$r = 0.072$	$MICLD_{50}$[e]	Literature-based model
ANDV	Exponential	$r = 0.011$	$CCID_{50}$[f]	Literature-based model

[a] Colony forming units
[b] Infectious dose to 50% of exposed individuals
[c] Plaque forming units
[d] The log-probit model with parameters fit to the ID estimates of Expert 3 from the modified Delphi process. This model was selected for use because it leads to ID estimates that equal the median of the range of expert-derived estimates for the ID_{10} and lower in Appendix J, Table K–4e. An expert-based model example was selected because no literature-based model was derived for 1918 H1N1V.
[e] Mouse intracerebral lethal dose 50% (the dose that kills 50% of mice following intracerebral inoculation)
[f] Cell culture median infectious dose

- **Uncertainty range**

  Both the literature-based range and the expert-based range were tested separately as part of the uncertainty analysis for each pathogen. For literature-based dose-response estimates, a range of parameter values, derived from bootstrap resampling of the data set from which the dose-response

model was derived, was used for the uncertainty analysis. For expert-based dose-response estimates, the full distribution of expert-derived curves was applied for the uncertainty analysis. The method for deriving each of these distributions is described in Appendix J.

Frequency of a centrifuge release ($\lambda_{release}$). The expected frequency of the occurrence of a centrifuge release (average number of releases per time) is denoted $\lambda_{release}$. The frequency range assigned to a centrifuge release in Chapter 4 is comprised of those values in the A frequency category, as follows:

$$1 \text{ per } 100 \text{ years} < \lambda_{release} < 1 \text{ per year}$$

- **Central estimate example**: $\lambda_{release}$ = 1 per 50 years
  The central estimate frequency was selected such that the average return period is half of the maximum return period of the range (half of 100 years).

- **Uncertainty range**
  Individual frequencies from throughout the range given in Chapter 4 were tested as part of the uncertainty analysis. Specifically, for each calculation, a return period for a release was drawn from a uniform distribution over the given range. The incident frequency was then calculated by taking the inverse of the return period.

K.2.2.3 Calculations

The following steps describe the calculations performed that lead to the results for each combination of test cases. Step-by-step examples of these calculations for the central estimate example inputs are shown in conjunction with each pathogen in the results section.

- **Expected probability of infection per worker (q)**
  Let the selected dose-response curve be $p(d)$. Let $g(d)$ be the probability mass function of the expected dose received per worker (i.e., the probability that the expected dose is d). Each dose range was split into N evenly-spaced doses d_i from $d_1 = 0$ to $d_N = d_{\max}$. The equation for q is then

$$q = \sum_{i=1}^{N} p(d_i) g(d_i).$$

  As described in Section K.2.2.2 above, the value $N = 5$ is used for the central estimate example while $N = 1000$ for the uncertainty analyis. Note that there are two values of q for each

pathogen—one pertaining to the dose range for exposure with full respiratory protection and one pertaining to the dose range for exposure with partial respiratory pathogen.

- **Probability of *x* initial infections per incident (μ_x)**

  Let q_1 and q_2 be the expected probability of infection for an exposed worker with full respiratory protection and with partial respiratory protection, respectively. Then the expected probability for a worker to both have full respiratory protection and be infected is $p_1 = (1 - p_f)q_1$; the expected probability for a worker to both have partial respiratory infection and be infected is $p_2 = p_f q_2$;and the overall expected probability of infection for a particular worker is $p_T = p_1 + p_2$. Let $f(w)$ be the probability mass function of the number of workers in the room at the time of the incident. The equations for μ_x are provided below. Note that the equations are written for p_T, which is replaced by p_1 and p_2 for calculating the μ_x values specific to *with full respiratory protection* and *with partial respiratory protection* infections.

$$\mu_0 = (1 - p_T)^2 f(2) + (1 - p_T)^3 f(3) + (1 - p_T)^4 f(4)$$
$$\mu_1 = 2p_T(1 - p_T) f(2) + 3p_T(1 - p_T)^2 f(3) + 4p_T(1 - p_T)^3 f(4)$$
$$\mu_2 = (p_T)^2 f(2) + 3(p_T)^2(1 - p_T) f(3) + 6(p_T)^2(1 - p_T)^2 f(4)$$
$$\mu_3 = (p_T)^3 f(3) + 4(p_T)^3(1 - p_T) f(4)$$
$$\mu_4 = (p_T)^4 f(4)$$

- **Frequency of an *x*-infection event per year (λ_x)**

  Let λ_{release} be the estimated frequency of a centrifuge release. To obtain the frequency of an event resulting in *x* infections, the release frequency is multiplied by the probability of *x* initial infections per incident, μ_x

$$\lambda_x = \mu_x \lambda_{\text{release}}$$

K.2.2.4 Uncertainty analysis

All the uncertainties described above contribute to the overall uncertainty in estimating the frequency of initial infections. In order to efficiently evaluate the effects of these compounding uncertainties, random combinations of the above values and distributions were generated using Latin hypercube sampling, which ensures that all the sets of input data are representative of the full variability of the input values (McKay et al. 1979; Blower and Dowlatabadi 1994). For each combination of input data (i.e., distribution of the number of workers, distribution of the dose received by each worker, dose-response curve, and

incident frequency), the calculations described above were performed to estimate the frequency of initial infections for each pathogen, taking into account the variabilities described by the distributions.

A total of 10,000 input combinations were generated for each pathogen, and each combination results in a different set of frequencies λ_x (frequency of a centrifuge release resulting in *x* initial infections). These values were re-organized to compute estimated frequencies of releases leading to *x or more* initial infections. Results were tallied according to how many of the 10,000 output values fell into each of the frequency categories described in Chapter 4.

K.2.2.5 Sensitivity analysis

Sensitivity analysis is perfomed using partial rank correlation (PRC), a nonparametric technique for statistically evaluating the independent effects of each input parameter on the outcome value (Iman and Conover 1980). The technique is intended for assessing input variables that have a monotonic correlation with the output variable; that is, as the input parameter increases across its range of values while other parameters are held constant, the output variable should tend to increase or decrease across the entire range with no reversal in trend. Each uncertain input parameter is assigned a partial rank correlation coefficient (PRCC) that quantifies degree of monotonicity, or the extent to which an increase in the input variable causes an increase (positive PRCC) or decrease (negative PRCC) in the output variable. PRCC values for different input parametes can be compared to determine the relative contribution to the uncertainty in the output value from the uncertainty in each input parameter value.

For the centrifuge events, PRCC values were calculated for four input values, the distribution for the number of exposed workers (range 1 to 3); the distribution for the amount of exposure (range 1 to 3); the index of the dose-response curve, where the index is the rank-order according to the average probability of infection it predicts; and the frequency of the centrifuge release (range 10^{-2} to 1 per year). Each of the four values are positively correlated with the ouput value of the rate of infections per time. The PRCC values were calculated against the output variable to evaluate the relative importance of the uncertainty in the four values in contributing to the uncertainty in the infection rate. The PRCC calculations were performed using the R function pcc.

K.2.3 Earthquake Release

This section describes the methodology for generating estimates for initial infections resulting from earthquake release events described in Chapter 4 and Appendix F.

This event is relevant to all 13 pathogens analyzed in this RA. All potentially exposed individuals are assumed to be members of the public. This section discusses only initial infections; that is, members of the public directly exposed to an aerosol released from NEIDL. The secondary transmission sections of this RA discuss the potential consequences of initially infected individuals interacting with public contacts.

The potential route of exposure for members of the public from this event is assumed to be through inhalation. Therefore, the dose-response information generated in Appendix J, which focuses primarily on inhalational exposure, can be applied. The earthquake release information provided in Appendix F and the dose-response information provided in Appendix J are sythesized in Section K.3.4 of this appendix for each of the 13 pathogens.

The remainder of this section describes the methodology for generating quantitative estimates of the frequency of earthquake release events that lead to one or more initial infections or mortalities from each pathogen, as well as the uncertainty in the estimations and the sensitivity of the estimations to uncertainties in the input values. The initial infections results subsequently serve as inputs to secondary transmission analyses.

K.2.3.1 Overall assumptions

The following assumptions were made in the calculations, and deviations from these assumptions were not tested:

- The true frequency of an earthquake release is constant over time and is within the range specified in Chapter 4. Note that assuming a constant frequency does not mean that incidents are assumed to happen at regularly spaced intervals.
- A true dose-response curve exists for each pathogen, is applicable to each potentially exposed person, and is within the range of dose-response curves derived in Appendix J.

K.2.3.2 Input values

This section specifies the values, distributions, and functions that are used in the calculations. There are two types of calculations presented for each pathogen—a *central estimate example* and *uncertainty results*.

For the central estimate example, a single value, distribution, or function is selected for each of the inputs. It is important to note that the results calculated from these central estimate inputs are not intended to be used as if they are certain predictions of what the frequency of infections would be at the NEIDL, as many of the inputs to the calculations are associated with rather wide uncertainty ranges. Instead, these results serve to illustrate the steps of the calculations and to provide point estimates near the center of the uncertainty range.

For the uncertainty results, certain inputs are assigned a range of values, distributions, or functions, and various sets of inputs are drawn randomly to generate a range of outputs. The process for generating sets of inputs is described in more detail in Section K.2.3.4 below.

The central estimate inputs and uncertainty ranges are specified as follows.

Number of people potentially exposed in each annular ring (N_r). Appendix F provides average population estimates for the potentially exposed population in each annular ring at various radii out from the release site, for all three sites. For a particular site, the population in annular ring r (for r = 1–10, extending out from the release point) is denoted N_r. Assumptions about this input are important in calculations of the likelihood of one or more more initial infections occurring.

- **Central estimate example:**
  For the central estimate example, the population numbers N_r from Appendix F are applied as the expected number of people potentially exposed in each annular ring. It is assumed that there is variability in each of these numbers that is described by the Poisson distribution with mean N_r. Note that this variability distribution is not intended to reflect the true population variability at each site, which in reality varies across both time and space and is likely to be quite complicated. The Poisson distribution is used to handle fractional average population estimates so that the probabilistic outputs required for the results can be calculated.

- **Uncertainty range:**
  Variations from the central estimate example assumption were not tested in the uncertainty analysis. It is presumed that the census data and other sources of population estimates used to derive values of N_r are reasonably accurate, especially when compared to other uncertain inputs. More importantly, the central estimate is a conservative estimate of the average number of people

at risk of exposure because it assumes that each person in a particular sector of the annular ring is exposed to an average dose from the centerline of the aerosol plume. In reality, only a fraction of the population in a given annular ring would receive that level of exposure. Therefore, it is presumed that N_r is more likely to overestimate than underestimate the *true* average population at risk in each annular ring.

Amount of exposure per person in each annular ring (d_r). The amount of exposure is the expected dose inhaled by a person in annular ring r, which is denoted d_r. Exposure amounts at various radii from the release point along the centerline of the plume are provided in Appendix F.

- **Central estimate example:**
  For the central estimate example, the centerline exposure estimates d_r from Appendix F at the radial midpoint of each annular ring are applied as the expected dose inhaled by each person in the annular ring, with no variability.

- **Uncertainty range**
  Two different sets of exposure estimates were provided in Appendix F, called beyond design basis (BDB) release and maximum reasonably foreseeable (MRF) release. Central estimate results are presented for each case in this Appendix, which give a rough measure of an uncertainty range for probable outcomes. Additional variations from each central estimate example were not tested in the uncertainty analysis. For the MRF release scenario, the values d_r arise from a large number of conservative assumptions, as discussed in Appendix F. Therefore, the results under the MRF assumption are presumed to represent the maximum results across an uncertainty range.

Probability of infection given the expected dose, or dose-response curve ($p(d)$). As described in Appendix J, a dose-response curve $p(d)$ describes the probability p of infection given an expected dose d inhaled by a worker. For most pathogens, both a literature-based curve and an expert-based curve were generated, along with uncertainty ranges for each.

- **Central estimate example**
  The best fit literature-based dose-response curve was used in the central estimate example for each pathogen. For three pathogens (1918 H1N1 influenza virus; Junin virus; and tick-borne encephalitis virus, Far Eastern subtype), a literature-based curve was not derived. In these cases, a middle-range curve derived from the set of estimates from one expert was employed for the

central estimate example. The models selected for the BSL-3 pathogens were the same ones selected for the central estimate examples of the BSL-3 centrifuge infectious aerosol release (see Table K–3). Table K–4 below summarizes the models selected for the BSL-4 pathogens.

Table K–4. Central estimate example dose-response curves for each BSL-4 pathogen.

Pathogen	Model	Parameter value(s)	Units of dose	Source
EBOV	Exponential	$r = 0.16$	PFU[a]	Literature-based model
MARV	Exponential	$r = 0.16$	PFU[a]	Literature-based model
LASV	Exponential	$r = 0.039$	PFU	Literature-based model
JUNV	Exponential	$r = 0.0070$	PFU	Expert-based model[b]
TBEV-FE	Log-Probit	$m = 0.56$ $ID_{50} = 200$	PFU	Expert-based model[c]
NIPV	Exponential	$r = 0.0013$	$CCID_{50}$	Literature-based model

[a] The units of the earthquake release exposure estimates for EBOV and MARV are $CCID_{50}$, and these numbers are converted to PFU using a factor of 1/12 before the given dose-response model is applied, as described in Appendix J.
[b] For JUNV, the exponential fit to the ID estimates of Expert 3 was applied because the derived estimates most closely tracked median expert estimates, or were slightly lower than the median at the lowest ID levels (see Appendix J, Table J–11d).
[c] For TBEV-FE, the log-probit fit to the ID estimates of Experts 5 and 6 was applied because the derived estimates were equal to the median expert estimates at every ID point, as shown in Appendix J, Table J–12b.

- **Uncertainty range**

  Both the literature-based range and the expert-based range were tested separately as part of the uncertainty analysis for each pathogen. For literature-based dose-response estimates, a range of parameter values, derived from bootstrap resampling of the data set from which the dose-response model was derived, was used for the uncertainty analysis. For expert-based dose-response estimates, the full distribution of expert-derived curves was applied for the uncertainty analysis. The method for deriving each of these distributions is further described in Appendix J.

Case fatality rate (*m*). The case fatalilty rate, *m* (for mortality), is the probability that a person infected with the pathogen would subsequently die from illness caused by the infection. Case fatality rates observed and estimated for each pathogen are reviewed in Chapter 3 and Appendix C.

- **Central estimate example**

  The assumed case fatality rate for each pathogen is listed in Table K–4.

Table K–4. Central estimate example case fatality rate for each BSL-4 pathogen.

Pathogen	Case fatality rate (*m*)	Pathogen	Case fatality rate (*m*)
B. anthracis	45%	EBOV	90%
F. tularensis	2%	MARV	100%
Y. pestis	15%	LASV	2%
1918 H1N1V	2.5%	JUNV	1%
SARS-CoV	10%	TBEV-FE	40%
RVFV	2%	NIPV	70%
ANDV	50%		

- **Uncertainty range**

  No alternate case fatality rate estimates were tested. For pathogens for which a range of case fatality rates were found in the literature, the upper end of the range was applied in the central estimate example; thus, those values might overestimate but are unlikely to underestimate the average case fatality rate in the event of a release leading to infections.

Frequency of an earthquake release ($\lambda_{release}$). The expected frequency of the occurrence of an earthquake release (average number of releases per time) is denoted $\lambda_{release}$. The frequency range assigned to an earthquake release consists of those values in the C frequency category, as follows:

$$1 \text{ in } 1 \text{ million years} < \lambda_{release} < 1 \text{ in } 10{,}000 \text{ years}$$

- **Central estimate example**: $\lambda_{release}$ = 1 in 100,000 years

  The central estimate frequency was selected such that the average return period is halfway between the boundaries of the frequency category on a logarithmic scale.

- **Uncertainty range**

  Individual frequencies from throughout the range given in Chapter 4 were tested as part of the uncertainty analysis. Specifically, for each calculation, a return period for the incident was drawn from a uniform distribution on a logarithmic scale; that is, a number *x* was drawn from a uniform distribution on the range 4 to 6, and then $\lambda_{release}$ was calculated as $1/10^x$. This choice of distribution puts a conservative amount of weight on higher frequencies within the range.

K.2.3.3 Calculations

The following steps describe the calculations performed that lead to the results for each combination of test cases. Step-by-step examples of these calculations for the central estimate example inputs are shown in conjunction with each pathogen in the results section.

- **Expected probability of infection per person in each annular ring ($p(d_r)$)**

  Let d_r be the expected dose assumed for each person in annular ring *r*, and let $p(d)$ be the selected dose-response curve. Then the expected probability of infection per person in annular ring *r* is $p(d_r)$. The formula is applied to each annular ring separately.

- **Probability of x initial infections in annular ring *r* per release (μ_x^r)**

  Under the assumption that the number of people potentially exposed in each annular ring is Poisson distributed with mean N_r, the following formulas are applied for the probability of zero, one, or two initial infections in annular ring *r*:

$$\mu_0^r = \mathrm{e}^{-p(d_r)N_r}$$

$$\mu_1^r = p(d_r)N_s\mathrm{e}^{-p(d_r)N_r}$$

$$\mu_2^r = \frac{1}{2}[p(d_r)N_r]^2\ \mathrm{e}^{-p(d_r)N_r}$$

- **Probability of *x* deaths in annular ring *r* per release (δ_x^r)**

  The assumed case fatality rate *m* is multiplied by the expected, per-person probability of infection $p(d_r)$ to estimate the per-person probability of death in each annular ring. This product is then applied to the same formulas provided in the previous bullet to get the probability of zero, one, or two deaths in each annular ring *r*:

$$\delta_0^r = \mathrm{e}^{-p(d_r)mN_r}$$

$$\delta_1^r = p(d_r)N_s\mathrm{e}^{-p(d_r)mN_r}$$

$$\delta_2^r = \frac{1}{2}[p(d_r)N_r]^2 \, \mathrm{e}^{-p(d_r)mN_r}$$

- **Probability of *x* or more total initial infections, per release ($\mu_{\geq x}$)**

  The μ_x^r values across all annular rings were used to calculate $\mu_{\geq x}$, the probability that a release results in *x* or more total infections (across all 10 annular rings). The formulas are as follows:

$$\mu_{\geq 1} = 1 - \prod_{r=1}^{10} \mu_0^r$$

$$\mu_{\geq 2} = \mu_{\geq 1} - \sum_{r_1=1}^{10} \left(\mu_1^{r_1} \prod_{r_2 \neq r_1} \mu_0^{r_2} \right)$$

$$\mu_{\geq 3} = \mu_{\geq 2} - \sum_{s_1=1}^{10} \left(\mu_2^{r_1} \prod_{r_2 \neq r_1} \mu_0^{r_2} \right) - \sum_{r_1=1}^{9} \left(\sum_{r_2=r_1+1}^{10} \left(\mu_1^{r_1} \mu_1^{r_2} \prod_{\substack{r_3 \neq r_1 \\ r_3 \neq r_2}} \mu_0^{r_3} \right) \right)$$

  These formulas are sufficient for most pathogens and scenarios, as the estimated probability of three or more infections is small enough that calculating probabilites of a higher number of infections would not add useful information. For any scenario in which the probability of more than three infections is potentially significant, probabilities of higher numbers of initial infections are estimated through simulations because the exact formulas become unwieldy for higher values of *x*. For the simulations, 100,000 random population values are drawn for each annular ring from a Poisson distribution with mean N_r, and using each of those values along with the probability $p(d_r)$, a random number of initial infections is drawn from a binomial distribution. Next, the simulated numbers of infections are totaled and tabulated to estimate the values of $\mu_{\geq x}$ for selected values of *x*.

- Probability of *x* or more total deaths, per release ($\delta_{\geq x}$). The same formulas in the previous bullet point apply, replacing μ with δ.

- Frequency of an *x*-or-more-infection or *x*-or-more-death event per year ($\lambda_{\geq x}$). Let λ_{release} be the estimated frequency of an earthquake release event. To obtain the frequency of an event resulting in *x* or more initial infections or deaths, the release frequency is multiplied by the probability of *x* or more initial infections per release, $\mu_{\geq x}$, or the probability of *x* or more deaths per release, $\delta_{\geq x}$

$$\lambda_{\geq x} = \mu_{\geq x}\lambda_{\text{release}} \text{ (for infections)}$$
$$\lambda_{\geq x} = \delta_{\geq x}\lambda_{\text{release}} \text{ (for deaths)}$$

Example Calculations

The following example serves to illustrate the steps of the calculations described above. The input values are taken from the following scenario: maximum reasonably foreseeable release of *B. anthracis* at the urban site. Central estimate assumptions for each of the input values have been described previously. Step-by-step calculations and results under these input values are summarized as follows. See Section K.3.3 for full results from all pathogens at each site and under each release scenario.

Table K–5. Central estimate, annular ring-specific calculations for urban site earthquake MRF release of *B. anthracis*

Annular ring (r)	Dose (CFU) per person (d_r)	Infection probability per person ($p(d_r)$)	Potentially exposed population (N_r)	Conditional Probability of x initial infections, given a MRF release (μ_x^r)
1	2.9×10^{-2}	7.5×10^{-7}	31	$x = 0$: 0.999977 $x = 1$: 2.3×10^{-5} $x = 2$: 2.7×10^{-10}
2	3.1×10^{-3}	8.1×10^{-8}	108	$x = 0$: 0.9999913 $x = 1$: 8.7×10^{-6} $x = 2$: 3.8×10^{-11}
3	1.1×10^{-3}	2.9×10^{-8}	196	$x = 0$: 0.9999944 $x = 1$: 5.6×10^{-6} $x = 2$: 1.6×10^{-11}
4	6.2×10^{-4}	1.6×10^{-8}	372	$x = 0$: 0.9999940 $x = 1$: 6.0×10^{-6} $x = 2$: 1.8×10^{-11}
5	4.1×10^{-4}	1.1×10^{-8}	376	$x = 0$: 0.9999960 $x = 1$: 4.0×10^{-6} $x = 2$: 8.1×10^{-12}
6	3.0×10^{-4}	7.8×10^{-9}	178	$x = 0$: 0.9999986 $x = 1$: 1.4×10^{-6} $x = 2$: 9.5×10^{-13}
7	2.3×10^{-4}	5.9×10^{-9}	165	$x = 0$: 0.9999990 $x = 1$: 9.8×10^{-7} $x = 2$: 4.8×10^{-13}
8	1.8×10^{-4}	4.7×10^{-9}	250	$x = 0$: 0.9999988 $x = 1$: 1.2×10^{-6} $x = 2$: 7.0×10^{-13}
9	1.5×10^{-4}	3.9×10^{-9}	310	$x = 0$: 0.9999988 $x = 1$: 1.2×10^{-6} $x = 2$: 7.2×10^{-13}
10	1.2×10^{-4}	3.2×10^{-9}	215	$x = 0$: 0.9999993 $x = 1$: 7.0×10^{-7} $x = 2$: 2.4×10^{-13}

The results in Table K–5 provide information about what the example dose-response function $p(d)$ estimates for the probability that infections would occur among people in each annular ring. For example, in $r = 1$ (between 30 and 100 meters [m] from the release source), assuming the average dose inhaled per person is 0.029 CFU, the dose-response model estimates a 7.5×10^{-7} probability of infection per person.

Next, using this probability and the estimate of 31 people potentially exposed in the annular ring on average, the formulas given in Section K.2.2.3 lead to the estimated probabilities in the third column. For example, the probability that 1 infection occurs out of an average of 31 people in annular ring 1 is estimated to be 2.3×10^{-5}, or about a 1 in 40,000 chance. The probability of two infections occurring in annular ring 1 is estimated to be 2.7×10^{-10}, or about a 1 in 4 billion chance.

The calculations in Table K–5 are repeated for case fatality rates. Each $p(d_r)$ value in the third column is multiplied by the assumed case fatality rate for *B. anthracis* infection ($m = 0.45$), and each of these products is used to calculate δ_x^r using the formulas supplied in this section.

Table K–6. Central estimate overall calculations for urban site earthquake MRF release of *B. anthracis*

Number of initial infections overall (x)	Probability x or more initial infections overall ($\mu_{\geq x}$)	Frequency of release leading to x or more initial infections ($\lambda_{\geq x}$)
1 or more	5.3×10^{-5}	5.3×10^{-10}/year = 1 in > 10 million years
2 or more	1.4×10^{-9}	1.4×10^{-14}/year = 1 in > 10 million years
3 or more	2.5×10^{-14}	2.5×10^{-19}/year = 1 in > 10 million years

The probability of x or more initial infections overall is calculated based on the annular ring-specific probabilities from Table K–5 and the formulas provided in this section. For example, the probability of one or more infections is one minus the probability that there are no infections in any of the 10 annular rings, which is the product of the ten $x = 0$ probabilities in the last column of Table K–5. Next, the frequency of a release leading to x or more infections is calculated by multiplying $\mu_{\geq x}$ by 10^{-5}/year, which is the assumed frequency of an earthquake release for the central estimate example.

These calculations are repeated in the same fashion for the frequency of an earthquake release leading to x or more deaths.

K.2.3.4 Uncertainty analysis

For each combination of input data (dose-response curve and earthquake release frequency), the calculations described above were performed to estimate the frequency of initial infections for each

pathogen. The combinations of inputs were generated using the Latin hypercube sampling scheme described in Section K.2.2.4 above.

A total of 10,000 input combinations were generated for each pathogen, and each unique combination results in a set of frequencies $\lambda_{\geq x}$ (frequency of an earthquake release resulting in x or more initial infections). Results were tallied according to how many of the 10,000 output values fell into each of the frequency categories described in Chapter 4.

K.2.4 Medically Vulnerable Sub Populations

The methodology for the initial infections analyses described in the sections above uses an assumption that the dose-response and case fatality estimates are applicable to all potentially exposed populations. This assumption might be violated for a population that has a different profile of vulnerability to disease and/or death than did the populations on which the dose-response and case fatality estimates were based. For this RA, the issue of population vulnerability is investigated by evaluating populations at the three sites for the presence of five medically vulnerable sub populations (MVSP), and as discussed in Appendix I for children under 5, adults over 65, people with diabetes, people with HIV/AIDS, and pregnant women.

The dose-response models applied in the previous sections of this appendix are assumed to be applicable to human populations containing MVSP to some extent. The derivation and justification of the models, discussed in detail in Appendix J, included consideration of the possibility that some potentially exposed individuals might be more susceptible to infection at a given dose than others. Although the literature-based models were mostly derived from experimental human and/or animal data in which exposed groups might have been more homogeneous than a general human population, the justification for applying non-threshold models to these data was partly based on the fact that the models would be applied to human populations in which some individuals might be especially susceptible to low doses because of immune status or some previous or concurrent health condition. The expert-based dose-response models were based on the outcome of a modified Delphi process in which it was not specified to the experts that their estimates should be based on healthy individuals only; presumably, the experts took into consideration the susceptibility profile of a typical human population in arriving at their infectious dose estimates. For case fatality estimates, the baseline rates assumed for each pathogen are based on data observed in human outbreaks that occurred among populations containing many, if not all, of the MVSP considered in this RA.

Despite that the dose-response and case fatality estimates are already assumed to encompass MVSP, it is worth considering whether particular potentially exposed populations considered for this RA contain an atypical proportion of individuals belonging to certain MVSP, and whether this proportion of MVSP is sufficiently different from the norm to warrant adjusting the baseline initial infections and case fatality estimates.

The quantitative analysis described in the following sub-sections is only applied to the earthquake release scenarios in which the potentially exposed group (members of the public surrounding NEIDL) might contain MVSP of higher or lower proportion than a typical human population. As discussed in Appendix I, the population of laboratory workers, who are the potentially exposed group for the needlestick and centrifuge events, might include proportions of some, but not all, of the five MVSP. It is surmised that the population of laboratory workers assigned to work with BSL-3 or BSL-4 pathogens would have a lower proportion of each MVSP, and of unhealthy or immunocompromised individuals in general, than the proportions occurring in the overall population. Therefore, it is acknowledged that the dose-response models applied in the centrifuge initial infections analysis may overestimate the risk to laboratory workers in this regard, though no attempt is made to quantify the potential degree of overestimation. The needlestick analysis included the assumption that every exposure would lead to an infection, regardless of whether the exposed worker is a member of an MVSP.

K.2.4.1 Overall assumptions

The following assumptions are made in order to quantitatively investigate possible effects of MVSP on the initial infections results for the earthquake release scenarios:

- A given dose-response curve being assumed for a particular pathogen is relevant for a population comprised of MVSP at proportions in line with the overall U.S. population.
- The probability that a given exposed individual is a member of a MVSP is equal to the portion of the local population that is assumed to belong to that MVSP.
- No individuals are members of more than one of the five MVSP. It is also assumed that accounting for individuals belonging to multiple MVSP would not change the results significantly.
- The portion of a population that is not a member of any of the five designatd MVSP has average susceptibility status in line with *healthy adults* for purposes of comparatively quantifying estimates of the increased susceptiblity of MVSP. This is a necessary assumption for conducting

the calculations to investigate the potential effects of the presence of the five MVSP; it is acknowledged that the five MVSP do not encompass all individuals who might have increased susceptibility to disease.

- If person A is *x*% more susceptible to disease than person B, that is assumed to mean that the probability of person A being infected at a given expected dose *d* is the same as the probability of person B being infected at an expected dose *x*% higher than *d*.

K.2.4.2 Input values

This section specifies the values that are used in the calculations. For this part of the analysis, only single point estimates are applied for each input. While uncertainties do exist for these inputs, the given values are sufficient for the purpose of this exercise, which is to explore the potential role that MVSP proportions might play at each site in affecting the initial infections estimates. Some qualitative discussion of the effects of input uncertainties is included in the results section.

First, the index *k* is defined, which represents an index for the five MVSP described in Appendix I, as follows:

- $k = 1$ for children under 5 years of age
- $k = 2$ for adults over 65
- $k = 3$ for people with diabetes
- $k = 4$ for people with HIV/AIDS
- $k = 5$ for pregnant women

Given this index, the following inputs are defined and specified.

Proportion of U.S. and local populations belonging to MVSP *k* (x_k, y_k). Appendix I provides estimates for proportions of each MVSP according to U.S. data and data and estimates from areas near the three sites. The assumed U.S. proportions are termed x_k, for $k = 1$ to 5, and the values are taken directly from Table 1 in Appendix I. The assumed urban resident proportions are taken from the column in Table 1 of Appendix I that estimates the proportion of each MVSP residing in zip code 02118, which covers the area around the NEIDL site for which exposure estimates are provided for the earthquake release events. For the non-resident portion of the urban population estimates, the Massachusetts statewide MVSP estimates were assumed, based on the assumption that daytime students, workers, and other vistors and passersby in

the vicinity of the NEIDL location typically reside in a wide area around the city. For certain MVSP, the non-resident MVSP estimates are conservatively high; for example, there would be no children under 5 years of age among BU students, faculty, and employees at BUMC, which comprise a large portion of the non-resident population estimates. Therefore, the estimated urban non-resident proportion of children under 5 years of age and, perhaps to a lesser extent, adults over 65 are conservatively high. The suburban site estimates were taken from the Tyngsborough, MA data listed in Table 1 of Appendix I. The rural site estimates were calculated by combining the data from Peterborough, NH and Hancock, NH listed in Table 1 of Appendix I. Table K–7 specifies the values applied in this analysis

Table K–7: Inputs for MVSP population values[a]

MVSP (k)	Proportion of MVSP in U.S. population (x_k)	Proportion of MVSP in local population (y_k)			
		Urban resident	Urban non-resident	Suburban	Rural
1: Children under 5	0.069	0.039	0.063	0.055	0.058
2: Adults over 65	0.126	0.079	0.135	0.082	0.217
3: People with diabetes	0.057	0.126	0.074	0.078	0.066
4: People with HIV/AIDS	0.0045	0.016	0.0028	0.0020	0.0011
5: Pregnant Women	0.01	0.01	0.01	0.01	0.01

[a] See Appendix I for sources of these estimates

Increased susceptibility of MVSP *k* to pathogens (q_k). Appendix I includes a discussion of evidence for increased susceptibility to the 13 pathogens of members of the 5 MVSP. The quantitative estimates from experts on the Delphi panel, which were estimates of percentage increase in susceptibility to disease and mortality for viruses and bacteria of each MVSP compared to healty adults (denoted q_k), are also listed in Appendix I. For this analysis, the maximum estimate from each set of expert estimates was applied to reduced the possibility of producing non-conservative results. These values are listed in Table K–8.

Table K–8: Inputs for MVSP relative susceptibility values[a]

MVSP (k)	Increased susceptibility compared to healthy adult (q_k)			
	to disease from bacteria	to mortality from bacteria	to disease from viruses	to mortality from viruses
1: Children under five	0.33	0.2	0.33	0.2
2: Adults over 65	0.5	0.5	0.5	0.5
3: People with diabetes	0.3	0.25	0.25	0.25
4: People with HIV/AIDS	0.4	0.3	0.4	0.3
5: Pregnant Women	0.3	0.2	0.5	0.2

[a] See Appendix I for sources of these estimates. The q_k numbers are the maximum values from the expert estimates.

K.2.4.3 Calculations

The following steps describe the calculations performed that lead to the results presented in Section K.3.5.

Probability of infection or death for MVSP *k* relative to a healthy adult

The following terms are defined:

- $p_h(d)$ is the infection probability of a healthy adult inhaling a dose with expected number of organisms d.
- $p_k(d)$ is the infection probability of a person belonging to MVSP k inhaling a dose with an expected number of organisms d.

Next, the relationship between p_h and q_k under the assumptions listed in Section K.2.4.1 above is expressed mathematically as

$$p_k(d) = p_h((1 + q_k)d).$$

Because the three dose-response forms used in this risk assessment are non-linear, the amount that p changes for a given value of q will be different depending on which particular model is being assumed. Under the stated assumptions, formulas under each model for calcuating p_k for given values of p_h and q_k at the same exposure are expressed below.

Exponential model: $p_k = 1 - (1 - p_h)^{1+q_k}$

Log-probit model: $p_k = \Phi[\Phi^{-1}(p_h) + m\ln(1 + q_k)]$

Beta Poisson model: $p_k = 1 - \left[1 + \left((1 - p_h)^{-1/\alpha} - 1\right)(1 + q_k)\right]^{-\alpha}$

These formulas are also applied for estimates of increased susceptibility to mortality, wherein *p* stands for the probability of death given infection.

Probability of infection or death for a healthy adult

The value of p_h for a pathogen at a given dose or for mortality is not obvious because the baseline dose-response curves and case fatality rates are assumed to be applicable for a heterogeneous population containing both healthy and unhealthy individuals, as discussed above. To make use of the above formulas, p_h must first be calculated, which is possible under the stated assumptions, and as described below.

The following additional term is defined as follows:

- p is the average probability of either infection at a given dose or mortality (assumed to be applicable to a general U.S. population).

Next the value of p can be expressed in terms of x_k, p_h, and q_k. First, p is broken down into its components, the probabilities assigned to each of the six groups (five MVSP plus everyone else) multiplied by the portion of the U.S. population in each group, as follows:

$$p = \sum_{k=1}^{5} p_k x_k + p_h \left(1 - \sum_{k=1}^{5} x_k\right)$$

Here, p_k depends on p_h and q_k and is defined according to which dose-response model is being assumed, as described above. For example, if the exponential model is being assumed, the equation becomes

$$p = \sum_{k=1}^{5} [1 - (1 - p_h)^{1+q_k}] x_k + p_h \left(1 - \sum_{k=1}^{5} x_k\right)$$

In the above equation, the value of p is given, as are all the parameters on the right-hand side, except p_h. The value of p_h is determined for specific examples using a numerical root finder.

Adjusted average probability of infection or death at a given site

Using the values of p_h and p_k, for k = 1 to 5, as derived above for a particular dose or for mortality, the average probability of infection or death at a given site is calculated using the MVSP population values y_k for that site

$$p = \sum_{k=1}^{5} p_k y_k + p_h \left(1 - \sum_{k=1}^{5} y_k\right)$$

Adjusted probability of *x* or more infections or deaths resulting from earthquake release

The adjusted probabilities p calculated using the preceding equation are applied to the earthquake release calculations described in Section K.2.3.3. The results are then compared to the baseline results for representative pathogens and scenarios.

Probability of *x* or more infections or deaths among each individual MVSP resulting from earthquake release

For each MVSP k, the potentially exposed population estimates from the earthquake analysis at each site are multiplied by the y_k values for that site to estimate the potentially exposed MVSP. Next, the corresponding p_k values, calculated as described above, are applied in conjunction with these population estimates to the earthquake release calculations described in Section K.2.3.3. This process is repeated using the values x_k instead of y_k, for the purpose of comparing what the estimated risk to the MVSP population at each site would be if the population proportions were consistent with U.S. averages.

Example calculations

As an example, the probability of infection with *F. tularensis* at an expected inhaled dose of 1 CFU is considered. The literature-based dose-response model derived in Appendix J (an exponential model) produces an estimate for the probability of infection of about 6.1% at this dose. Again, this is assumed to be an average probability of infection across a population containing MVSP that is similar to U.S. averages.

The input values used in the first part of the calculations are the x_k values, representing the proportion of each MVSP according to U.S. averages and shown in Table K–7, and the q_k values for susceptibility to disease from bacteria, shown in Table K–8. These values are applied to the equations described above for calculating p_h; the probability of infection for healthy adults, which can then be used to calculate p_k for k = 1 to 5; and the probabilities of infection for individuals in each MVSP. The results of these calculations are shown in Table K–9.

Table K–9: Example results for probability of infection for population groups.[a]

Population	Probability of infection (p) from exposure to 1 CFU of *F. tularensis*
Overall population	$p = 0.061$
Healthy adult	$p_h = 0.055$
Children under 5	$p_1 = 0.073$
Adults over 65	$p_2 = 0.082$
People with diabetes	$p_3 = 0.071$
People with HIV/AIDS	$p_4 = 0.077$
Pregnant Women	$p_5 = 0.071$

[a] The overall population probability was calculated directly from the literature-based dose-response model for *F. tularensis*. The healthy adult probability is applied to the portion of the overall population that does not belong to any of the five MVSP.

These results show that the given assumptions lead to an estimate for the probability of infection at 1 CFU exposure to *F. tularensis* for healthy adults to be 5.5%, and the probability for members of MVSP range from 7.1% to 8.2%. The weighted average of these probabilities over each sub-group is the original 6.1% probability assumed for the overall population. Similar calculations are applied for the probability of infection at each level of exposure relevant for the earthquake release scenarios for each pathogen and also for case fatality rates.

Results such as those in Table K–9, along with the site MVSP estimates y_k listed in Table K–7, form the basis for calculating the site-adjusted and MVSP-specific earthquake release results presented in Section K.3.5 below.

K.3 Results

K.3.1 Needlestick

The health consequences resulting from exposure to a pathogen via needlestick are dependent on several pathogen characteristics and host factors. This RA assumes that the infectious dose of the pathogen delivered via needlestick is sufficient to cause disease (see Chapter 4, Section 4.2.3.2); therefore, no probabilistic calculations for initial infections are performed in this Appendix. Further, this RA assumes that the pathogen is viable in the needle at the time of the needlestick. An important characteristic is the pathogen's ability to cause infection by this route. As with all infections, the health consequences are also dependent on the immune status and general health of the affected person (host). For this RA, for needlestick injuries involving laboratory workers, the assumption is that the laboratory worker is a healthy young adult. For needlestick events that are not promptly detected and reported, the health consequences are also based on the assumption that post-exposure prophylaxis (if available), quarantine, and supportive measures are not instituted until the laboratory worker exhibits symptoms and seeks medical attention.

Of the BSL-3 pathogens, and based on the biology of the pathogen and routes of transmission of the disease noted in natural, accidental, and laboratory settings (as described in detail in Chapter 4 and Appendix C), it is assumed that *B. anthracis*, *F. tularensis*, *Y. pestis,* RVFV, and ANDV would cause an illness similar to the natural disease if introduced into the host by the percutaneous route (i.e., needlesticks). This represents a conservative approach as the exact course and extent of disease resulting from a needlestick is not known for these pathogens. Pandemic influenza viruses have been detected in the blood (viremia), whereas seasonal influenza viruses have a less prominent viremia (Likos et al. 2007; Stramer et al. 2009). This viremia is noted to be secondary to the illness and the primary pathology involving influenza is in the respiratory tract with the specific tropism of the virus to that area. Thus, if 1918 H1N1V were to be introduced into the host via the percutaneous route, it is considered likely that viremia and some systemic illness would occur. Although it is considered unlikely that the host will develop the classical influenza disease involving the respiratory tract, this outcome cannot be ruled out. A similar situation is considered for SARS-CoV.

Of the BSL-4 pathogens, EBOV, MARV, LASV, JUNV, and TBEV-FE are considered capable of causing illness similar to the natural disease by the percutaneous route. Based on the biology and routes of transmission of NIPV, it is considered unlikely that pneumonia or encephalitis would occur as seen in the

natural outbreaks if the virus is transmitted to the host via the percutaneous route. However, as the course and extent of disease resulting from a needlestick is not known, this also cannot be ruled out.

Table K–10 summarizes the potential health consequences in the laboratory worker and subsequent risk to the public. In the setting of illness caused by needlestick, the presentation and disease course might be different from that of the natural disease—especially for pathogens that are not normally known to cause disease by direct introduction into the host through the skin such as 1918 H1N1V, SARS-CoV, and NIPV. However, in the absence of data to suggest otherwise, this RA makes the conservative assumption that the illness caused by needlestick would mimic the natural disease with respect to the course, the organ systems affected, the morbidity and mortality caused, and potential for secondary transmission.

The issues of pre-existing immunity and potentially available vaccines for each pathogen are further discussed in Appendix J. Full descriptions of the clinical diseases caused by the pathogens are provided in Chapter 3 and Appendix C. The case fatality estimates provided here are summarized in Chapter 3, Appendix C and (Mahmoud et al. 2008) and are assumed to apply to heterogeneous populations containing individuals from medically vulnerable groups. The case fatality estimates are based on observations from natural outbreaks and as such represent mortality from the natural disease. In the setting of illness caused by needlestick, the presentation and disease course can be different from that of the natural disease; thus, the case fatality estimates from natural disease outbreaks may not necessarily apply. Applying these case fatality estimates to laboratory workers may be considered conservative, as it is presumed that laboratory workers are healthy adults as discussed further in Appendix I. Potential risk to the public from laboratory workers infected with transmissible pathogens is further discussed in Chapter 9 and Appendix L.

1 **Table K–10. Summary of Health Consequences in Laboratory Worker from Needlestick**

Pathogen	Pre-existing immunity to pathogen in laboratory worker	Resulting illness via needlestick route	Case fatality estimates from infection	Potential for secondary transmission to members of the public if initial infection is undetected and/or unreported
Biosafety Level 3 Pathogens				
B. anthracis	Possible, as vaccine is available	Bacteremia and sepsis with *B anthracis*	45%	No
F. tularensis	No	Bacteremia, sepsis and pneumonic form of tularemia	< 2%	No
Y. pestis	No	Bacteremia, primary septicemic plague and pneumonic plague	15%	Yes, if pneumonic plague is present
1918 H1N1V	Possible, due to cross-protection from past influenza vaccines or infections	Influenza	2.5%	Yes, if respiratory symptoms are present
SARS-CoV	No	SARS	10%	Yes, if respiratory symptoms are present
RVFV	Possible, as vaccine is available	Rift Valley fever	0.5–2%	No
ANDV	No	Viremia and Hantavirus pulmonary syndrome	50%	Yes, if respiratory symptoms are present
Biosafety Level 4 Pathogens				
EBOV	No	Ebola hemorrhagic fever	40–90%	Yes
MARV	No	Hemorrhagic fever	100%	Yes
LASV	No	Lassa fever (hemorrhagic fever)	1–2%	Yes
JUNV	No	Argentine hemorrhagic fever	< 1%	Yes
TBEV-FE	No	Encephalitis	20–40%	No
NIPV	No	Viremia, encephalitis and/or pneumonia	40–70%	Yes, if respiratory symptoms are present

The case fatality estimates provided in Table K–10 can be combined with the frequency category for a needlestick incident to estimate the frequency with which deaths would occur among laboratory workers due to infections via needlestick. The death rates are presumably most applicable to needlestick events without prompt detection and reporting, in which treatment of the infection would likely not begin until after symptoms appear, and which is likely most often the case during natural disease outbreaks from which the case fatality rate estimates were derived. The frequency of needlestick events without prompt detection and reporting was assigned to the frequency category B (0.01 to 0.0001 per year). Frequencies within the category range were multiplied with case fatality rates, with the results shown in Table K–11.

Table K–11. Frequency of mortality among laboratory workers for needlestick event without prompt detection and reporting.

Pathogen	Assumed case fatality rate	Frequency range of laboratory worker mortalities	Frequency category
BSL-3 Pathogens			
B. anthracis	0.45	0.0045 to 0.000045/yr ≈ 1 per 200 to 20,000 yrs	B or C
F. tularensis	0.02	0.0002 to 0.000002/yr ≈ 1 per 5,000 to 500,000 yrs	B or C
Y. pestis	0.15	0.0015 to 0.000015/yr ≈ 1 per 700 to 70,000 yrs	B or C
1918 H1N1V	0.025	0.00025 to 0.0000025/yr ≈ 1 per 4,000 to 400,000 yrs	B or C
SARS-CoV	0.1	0.001 to 0.00001/yr ≈ 1 per 1,000 to 100,000 yrs	B or C
RVFV	0.02	0.0002 to 0.000002/yr ≈ 1 per 5,000 to 500,000 yrs	B or C
ANDV	0.5	0.005 to 0.00005/yr ≈ 1 per 200 to 20,000 yrs	B or C
BSL-4 Pathogens			
EBOV	0.9	0.009 to 0.00009/yr ≈ 1 per 100 to 10,000 yrs	B
MARV	1	0.01 to 0.0001/yr ≈ 1 per 100 to 10,000 yrs	B
LASV	0.02	0.0002 to 0.000002/yr ≈ 1 per 5,000 to 500,000 yrs	B or C

Pathogen	Assumed case fatality rate	Frequency range of laboratory worker mortalities	Frequency category
JUNV	0.01	0.0001 to 0.000001/yr ≈ 1 per 10,000 to 1 million yrs	C
TBEV-FE	0.4	0.004 to 0.00004/yr ≈ 1 per 200 to 20,000 yrs	B or C
NIPV	0.7	0.007 to 0.00007/yr ≈ 1 per 100 to 10,000 yrs	B

A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years; C = 1 in 10,000 to 1 million years; D = 1 in > 1 million years

For the needlestick event *with* prompt detection and reporting, the case fatality rate estimates for some pathogens would likely be lower than those listed in the second column of Table K–1b. This is because a laboratory worker promptly reporting a potential infection would receive the best available care in a timely manner. However, for some pathogens, even the best available care provided immediately might not be effective in significantly reducing the likelihood of morbidity or mortality. Given that needlesticks with prompt detection and reporting are assumed to occur in frequency category A, it possible that deaths due to this event would also occur in frequency category A—especially for pathogens with a very high average case fatality rate such as EBOV, MARV, and NIPV. Beyond the analysis described above, quantitative estimates were not attempted, due to a paucity of available data on the ability of prompt care to prevent death from infection with these pathogens.

K.3.2 BSL-3 Centrifuge Aerosol Release

This section is organized by pathogen. For each pathogen, the results from Chapter 4 and Appendix F detailing the range of doses estimated for each lab worker are synthesized with the dose-response information from Appendix J. Next, the results from the calculations, uncertainty analysis, and sensitivity analysis described in Sections K.2.2.3 to K.2.2.5 are provided.

K.3.2.1 *Bacillus anthracis*

This section discusses the potential for infection with *B. anthracis* among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of *B. anthracis* was 0–2 CFU for laboratory workers with full respiratory protection, and 0–200 CFU for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of *B. anthracis* in either of these dose ranges. The lowest dose to which nonhuman primates have succumbed in published

experimental studies was about 200 CFU, which is also the maximum dose estimated for partial respiratory protection. However, there is also no direct evidence that infection from inhaling doses lower than 200 CFU or even 2 CFU is impossible, especially in a heterogeneous population containing individuals who have respiratory health problems or are immunocompromised. The dose-response models fit to the available data and to the expert estimates reflect the possibility that low doses result in a non-zero probability of infection.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the assumed values and distributions are shown in Tables K–12, K–13, and K–14.

Table K–12. Central estimate calculations for centrifuge release of *B. anthracis*, part I

Output	Respiratory protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(0.5) = 1.3 \times 10^{-5}$ $p(1) = 2.6 \times 10^{-5}$ $p(1.5) = 3.9 \times 10^{-5}$ $p(2) = 5.2 \times 10^{-5}$ $q_1 = [\, p(0) + p(0.5) + p(1) + p(1.5) + p(2) \,]/5$ $\mathbf{q_1 = 2.6 \times 10^{-5} \approx 0.003\%}$
	Partial	$p(0) = 0$ $p(50) = 1.3 \times 10^{-3}$ $p(100) = 2.6 \times 10^{-3}$ $p(150) = 3.9 \times 10^{-3}$ $p(200) = 5.2 \times 10^{-3}$ $q_2 = [\, p(0) + p(50) + p(100) + p(150) + p(200) \,]/5$ $\mathbf{q_2 = 2.6 \times 10^{-3} \approx 0.3\%}$

The results in Table K–12 provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. The five doses shown in each case are the five evenly spaced values across the ranges 0–2 and 0–200 CFU as described previously in the methodology sections. For workers with full respiratory protection, the function predicts up to 5.2×10^{-5} probability of infection at the maximum dose $d = 2$. The average probability of infection across the five

doses shown calculates to 2.6×10^{-5}, or approximately a 3-in-100,000 chance of infection per worker. For workers with partial respiratory protection, the function predicts up to 5.2×10^{-3} probability of infection at the maximum dose $d = 200$. The average probability of infection across the five doses shown calculates to 2.6×10^{-3}, or an approximately 3-in-1,000 chance of infection per worker. While these probabilities are small, they are potentially significant depending on the number of workers exposed and the frequency with which releases occur. These are incorporated in the next parts of the calculations.

Table K–13. Central estimate calculations for centrifuge release of *B. anthracis*, part II.

Output	Respiratory protection	Calculation
Probability of x initial infections per release (μ_x).	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ $\boldsymbol{\mu_1 = 5.2 \times 10^{-5} \approx 0.005\%}$ $\boldsymbol{\mu_2 = 6.8 \times 10^{-10} \approx 0.00000007\%}$
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ $\boldsymbol{\mu_1 = 1.3 \times 10^{-5} \approx 0.001\%}$ $\boldsymbol{\mu_2 = 4.2 \times 10^{-11} \approx 0.000000004\%}$
	Full or Partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ $\boldsymbol{\mu_1 = 6.5 \times 10^{-5} \approx 0.007\%}$ $\boldsymbol{\mu_2 = 1.1 \times 10^{-9} \approx 0.0000001\%}$

The calculations in Table K–13 above continue where the calculations from the previous table (K–12) left off, and include the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, that one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability p_1 of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection (assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–12). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the

probability that one becomes infected with respiratory protection and the other does not, and multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection (assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–12).

The final set of calculations in Table K–13 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here, the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–13 show that under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. Although this result may seems counterintuitive (i.e., because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection), it is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, and the rarity of occurrence of these higher-level exposures results in the lower derived probabilities of experiencing one or two infections.

Overall, the results show that under the central estimate example input values, the probability of an infection resulting from a centrifuge release of *B. anthracis* is estimated at about 6.5×10^{-5}, which is about a 1-in-15,000 chance. This is a small chance, but not necessarily negligible depending on how often centrifuge releases might occur, which is incorporated in the next set of calculations. The central estimate results above also show that the probability of *two* workers becoming infected by a single centrifuge release is vanishingly small.

Table K–14. Central estimate calculations for centrifuge release of *B. anthracis*, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 1.0 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.4 \times 10^{-11}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 1 million years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 2.6 \times 10^{-7}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 8.5 \times 10^{-13}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 4 million years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Full or Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 1.3 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 2.1 \times 10^{-11}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 800,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**

The calculations in Table K–14 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–13) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–14 gives the estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event would be placed in the C frequency category (between 1 in 10,000 years and 1 in one million years). Events leading to two-or-more initial infections would be placed in the D frequency category (less than 1 in one million years).

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–15, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–15: Uncertainty results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for *B. anthracis*.[a]

Dose-response estimate	Number of initial infections	A 1 in 1 to 100 yrs	B 1 in 100 to 10,000 yrs	C 1 in 10,000 to 1 million yrs	D 1 in > 1 million yrs
Literature-based	1 or more	0	20 (< 1%)	7606 (76%)	2374 (24%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	217 (2%)	7484 (75%)	2299 (23%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations which resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results may differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–15 show that most input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within the frequency category C. A small percentage of estimates fall in the frequency category B, and the rest in the frequency category D. The distribution of estimates using the expert-based dose-response curves is similar to the distribution resulting from the literature-based curves, but with slightly more weight in the category B. From these results, it is concluded that a realistic average return period for a centrifuge release resulting in at least one *B. anthracis* infection among laboratory workers is presumed to be greater than 10,000 years.

Sensitivity Analysis

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–16. The output variable selected for sensitivity evaluation was the frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–16: Sensitivity results – PRCC. The effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection)

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Literature-based	0.74	0.75	0.75	0.95
Expert-based	0.50	0.58	0.94	0.86

Because each PRCC in Table K–16 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–15. For the estimates using the literature-based dose-response curve, the PRCC for the release frequency input is the highest, which means that reducing the uncertainty in estimating the frequency with which releases occur from centrifuges in BSL-3 laboratories would have the greatest effect on reducing the uncertainty in the results. For the estimates using the expert-based dose-response curve, the PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with *B. anthracis* would have the greatest impact in reducing the uncertainty in those results.

K.3.2.2 *Francisella tularensis*

This section discusses the potential for infection with *F. tularensis* among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of *F. tularensis* was 0–2 CFU for laboratory workers with full respiratory protection and 0–200 CFU for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there exist published data from studies on human volunteers who inhaled doses of *F. tularensis* measured as low as 10 CFU and became infected. There is no direct evidence of humans being infected from inhaling doses smaller than 2 CFU, though the high rate of infection observed in the range of about 10 CFU to 50 CFU suggests that infection at even lower doses might be possible—a notion that is reflected in the dose-response models derived in Appendix J. For the partial-protection 0–200 CFU dose range estimate, the human data (and the models derived from them) strongly suggest a high likelihood of infection at inhaled doses in the higher parts of the range.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the assumed values and distributions are shown in Tables K–17, K–18, and K–19.

Table K–17. Central estimate calculations for centrifuge release of *F. tularensis*, part I

Output	Respiratory protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(0.5) = 0.031$ $p(1) = 0.061$ $p(1.5) = 0.090$ $p(2) = 0.12$ $q_1 = [\, p(0) + p(0.5) + p(1) + p(1.5) + p(2) \,]/5$ $\boldsymbol{q_1 = 0.060 \approx 6\%}$
	Partial	$p(0) = 0$ $p(50) = 0.96$ $p(100) = 0.998$ $p(150) = 0.99992$ $p(200) = 0.999997$ $q_2 = [\, p(0) + p(50) + p(100) + p(150) + p(200) \,]/5$ $\boldsymbol{q_2 = 0.79 \approx 80\%}$

Table K–18. Central estimate calculations for centrifuge release of *F. tularensis*, part II.

Output	Respiratory Protection	Calculation
Probability of x initial infections per release (μ_x).	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ $\boldsymbol{\mu_1 = 0.11 \approx 10\%}$ $\boldsymbol{\mu_2 = 0.0036 \approx 0.4\%}$
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ $\boldsymbol{\mu_1 = 0.0039 \approx 0.4\%}$ $\boldsymbol{\mu_2 = 3.9 \times 10^{-6} \approx 0.0004\%}$
	Full or partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ $\boldsymbol{\mu_1 = 0.12 \approx 10\%}$ $\boldsymbol{\mu_2 = 0.0038 \approx 0.4\%}$

The results in Table K–17 above provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. For workers with full respiratory protection, the function predicts up to 0.12 probability of infection at the maximum dose $d = 2$. The average probability of infection across the five doses shown calculates to 0.060, or an approximately 1-in-20 chance of infection per worker. For workers with partial respiratory protection, the function estimates greater than 95% probability of infection at the doses $d = 50$ or higher, with near certainty of infection at the maximum estimated dose $d = 200$. The average probability of infection across the five doses shown calculates to 0.79, or roughly 80% chance of infection per worker.

The calculations in Table K–18 above continue where the calculations from the previous table left off, now including the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, that one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability (p_1) of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection

(assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–17). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the probability that one becomes infected with respiratory protection and the other does not, multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection (assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–17).

The final set of calculations in Table K–18 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–18 show that, under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. This result may seem counterintuitive because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection. However, this is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, and the rarity of occurrence of these higher-level exposures results in the lower derived probabilities experiencing one or two infections.
Overall, the results show that, under the central estimate example input values, the probability of one out of two workers being infected after exposure resulting from a centrifuge release of *F. tularensis* is estimated at about 0.12, which is about a 1-in-9 chance. The probability of both workers becoming infected is calculated to be 0.0038, which is roughly a 1-in-300 chance.

Table K–19. Central estimate calculations for centrifuge release of *F. tularensis*, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 2.3 \times 10^{-3}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 7.2 \times 10^{-5}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 400 years** **$\lambda_{\geq 2} \approx$ 1 in 10,000 years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 7.9 \times 10^{-5}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 7.8 \times 10^{-8}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 10,000 years** **$\lambda_{\geq 2} \approx$ 1 in 10 million years**
	Full or Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 2.4 \times 10^{-3}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 7.7 \times 10^{-5}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 400 years** **$\lambda_{\geq 2} \approx$ 1 in 10,000 years**

The calculations in Table K–19 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–18) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–19 provides estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event would be placed in the B frequency category (between 1 in 100 years and 1 in 10,000 years). The estimated frequency of events leading to two-or-more initial infections is near the boundary between the B and C frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–20, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–20: Uncertainty results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for *F. tularensis*.[a]

Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Literature-based	1 or more	1513 (15%)	8487 (85%)	0	0
	2 or more	56 (< 1%)	7036 (70%)	2908 (29%)	0
	3 or more	0	451 (5%)	7854 (79%)	1695 (17%)
	4 or more	0	2 (<0.1%)	929 (9%)	9069 (91%)
Expert-based	1 or more	348 (3%)	7557 (76%)	2095 (21%)	0
	2 or more	5 (<0.1%)	1380 (14%)	5123 (51%)	3492 (35%)
	3 or more	0	23 (< 1%)	1806 (18%)	8171 (82%)
	4 or more	0	0	32 (< 1%)	9968 (>99%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations which resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results may differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–20 show that most input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within the B frequency category. A percentage of estimates also fall in the A frequency category, with the estimates that use the literature-based dose-response model producing more results with average return period less than 100 years. The distribution of estimates using the expert-based dose-response curves produces more results in the lower frequency categories. The frequency of centrifuge releases resulting in multiple infections of *F. tularensis* is most often estimated to be in the B frequency category (with literature-based dose-response) or the C category (with expert-based dose-response), with some estimates falling into each extreme category as well.

Sensitivity Results

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–21. The output variable selected for sensitivity evaluation was the

frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–21: Sensitivity results – PRCC. The effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection).

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Literature-based	0.75	0.75	0.71	0.96
Expert-based	0.48	0.52	0.95	0.85

Because each PRCC in Table K–21 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–20. For the estimates using the literature-based dose-response curve, the PRCC for the release frequency input is the highest, which means that reducing the uncertainty in estimating the frequency with which releases occur from centrifuges in BSL-3 laboratories would have the greatest effect on reducing the uncertainty in the results. For the estimates using the expert-based dose-response curve, the PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with *F. tularensis* would have the greatest impact in reducing the uncertainty in those results.

K.3.2.3 *Yersinia pestis*

This section discusses the potential for infection with *Y. pestis* among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of *Y. pestis* was 0–0.09 CFU for laboratory workers with full respiratory protection and 0–9 CFU for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of *Y. pestis* in either of these dose ranges. The lowest inhalational dose to which nonhuman primates have succumbed in published experimental studies was about 580 CFU; however, through intratracheal inoculation, nonhuman primates have succumbed to doses as low as 120–270 CFU. These doses are more than a factor of 10 higher than the maximum dose estimated for workers with partial respiratory protection. However, because data from exposures to lower doses are not plentiful, the possibility remains that doses less than 9 CFU would result in a non-zero probability of infection, which is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the assumed values and distributions are shown in Tables K–22, K–23, and K–24.

Table K–22. Central estimate calculations for centrifuge release of *Y. pestis*, part I

Output	Respiratory protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(0.0225) = 1.8 \times 10^{-6}$ $p(0.045) = 3.7 \times 10^{-6}$ $p(0.0675) = 5.5 \times 10^{-6}$ $p(0.09) = 7.3 \times 10^{-6}$ $q_1 = [\, p(0) + p(0.0225) + p(0.045) + p(0.0675) + p(0.09) \,]/5$ **$q_1 = 3.7 \times 10^{-6} \approx 0.0004\%$**
	Partial	$p(0) = 0$ $p(2.25) = 1.8 \times 10^{-4}$ $p(4.5) = 3.7 \times 10^{-4}$ $p(6.75) = 5.5 \times 10^{-4}$ $p(9) = 7.3 \times 10^{-4}$ $q_2 = [\, p(0) + p(2.25) + p(4.5) + p(6.75) + p(9) \,]/5$ **$q_2 = 3.7 \times 10^{-4} \approx 0.04\%$**

Table K–23. Central estimate calculations for centrifuge release of *Y. pestis*, part II.

Output	Respiratory protection	Calculation
Probability of x initial infections per release (μ_x).	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ $\boldsymbol{\mu_1 = 7.3 \times 10^{-6} \approx 0.0007\%}$ $\boldsymbol{\mu_2 = 1.3 \times 10^{-11} \approx 0.000000001\%}$
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ $\boldsymbol{\mu_1 = 1.8 \times 10^{-6} \approx 0.0002\%}$ $\boldsymbol{\mu_2 = 8.4 \times 10^{-13} \approx 0.00000000008\%}$
	Full or partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ $\boldsymbol{\mu_1 = 9.1 \times 10^{-6} \approx 0.0009\%}$ $\boldsymbol{\mu_2 = 2.1 \times 10^{-11} \approx 0.000000002\%}$

The results in Table K–22 above provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. For workers with full respiratory protection, the function estimates up to 7.3×10^{-6} probability of infection at the maximum dose $d = 0.09$. The average probability of infection across the five doses shown calculates to 3.7×10^{-6}, or an approximately 1-in-300,000 chance of infection per worker. For workers with partial respiratory protection, the function estimates up to 7.3×10^{-4} probability of infection at the maximum dose $d = 9$. The average probability of infection across the five doses shown calculates to 3.7×10^{-4}, or an approximately 1-in-3000 chance of infection per worker.

The calculations in Table K–23 above continue where the calculations from the previous table left off, now including the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, that one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability p_1 of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection

(assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–22). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the probability that one becomes infected with respiratory protection and the other does not, multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection (assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–22).

The final set of calculations in Table K–23 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–23 show that, under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. While this result may seem counterintuitive (because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection), this is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, and given the rarity of occurrence of these higher-level exposures results in the lower derived probabilities experiencing one or two infections.

Overall, the results show that, under the central estimate example input values, the probability of one out of two workers being infected after exposure resulting from a centrifuge release of *Y. pestis* is estimated at about 9.1×10^{-6}, which is about a 1-in-100,000 chance. The calculated probability of both workers becoming infected is vanishingly small.

Table K–24. Central estimate calculations for centrifuge release of *Y. pestis*, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 1.5 \times 10^{-7}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 2.7 \times 10^{-13}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 7 million years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 3.7 \times 10^{-8}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.7 \times 10^{-14}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in > 10 million years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Full or partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 1.8 \times 10^{-7}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 4.2 \times 10^{-13}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 5 million years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**

The calculations in Table K–24 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–23) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–24 provides estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event would be placed in frequency category D (less than one in one million years).

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–25, display how many

different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–25: Uncertainty results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for *Y. pestis*.[a]

Dose-Response Estimate	Number of Initial Infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Literature-based	1 or more	0	0	1,156 (12%)	8,844 (88%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	24 (< 1%)	3,281 (33%)	6,695 (67%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations that resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results might differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–25 show that most input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within frequency category D. A percentage of estimates also fall in the C frequency category, with the estimates that use the expert-based dose-response model producing more results with average return period less than one million years, including a small percentage in the B frequency category. The frequency of centrifuge releases resulting in multiple infections of *Y. pestis* is was estimated to be in the D frequency category for 100% of the inputs.

Sensitivity Results

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–26. The output variable selected for sensitivity evaluation was the frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–26: Sensitivity results – PRCC. The effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection)

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Literature-based	0.65	0.67	0.86	0.93
Expert-based	0.46	0.55	0.97	0.84

Because each PRCC in Table K–26 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–25. For the estimates using the literature-based dose-response curve, the PRCC for the release frequency input is the highest, which means that reducing the uncertainty in estimating the frequency with which releases occur from centrifuges in BSL-3 laboratories would have the greatest effect on reducing the uncertainty in the results. For the estimates using the expert-based dose-response curve, the PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with *Y. pestis* would have the greatest impact in reducing the uncertainty in those results.

K.3.2.4 1918 H1N1 influenza virus

This section discusses the potential for infection with 1918 H1N1V among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of 1918 H1N1V was 0–0.9 PFU for laboratory workers with full respiratory protection and 0–90 PFU for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of 1918 H1N1V in either of these dose ranges. There are numerous data sets from human volunteers exposed to other strains of influenza virus, but in the vast majority of those studies, volunteers were exposed to high doses. One study reported outcomes from human volunteers exposed to low-dose aerosols of an H2N2 strain, concluding that unprotected individuals were infected by doses as low as 1 $CCID_{50}$. These data suggest that humans could potentially be infected by doses in the estimated exposure ranges for the centrifuge release, considering that quantities of virus are generally similar or higher in units of $CCID_{50}$ compared to PFU.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the assumed values and distributions are shown in Tables K–27, K–28, and K–29.

Table K–27. Central estimate calculations for centrifuge release of 1918 H1N1V, part I.

Output	Respiratory protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(0.225) = 3.0 \times 10^{-4}$ $p(0.45) = 8.2 \times 10^{-4}$ $p(0.675) = 1.4 \times 10^{-3}$ $p(0.9) = 2.1 \times 10^{-3}$ $q_1 = [\, p(0) + p(0.225) + p(0.45) + p(0.675) + p(0.9)\,]/5$ $\boldsymbol{q_1 = 9.3 \times 10^{-4} \approx 0.09\%}$
	Partial	$p(0) = 0$ $p(22.5) = 0.062$ $p(45) = 0.11$ $p(67.5) = 0.14$ $p(90) = 0.17$ $q_2 = [\, p(0) + p(2.25) + p(4.5) + p(6.75) + p(9)\,]/5$ $\boldsymbol{q_2 = 0.095 \approx 10\%}$

The results in Table K–27 above provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. For workers with full respiratory protection, the function estimates up to 0.0021 probability of infection at the maximum dose $d = 0.9$. The average probability of infection across the five doses shown calculates to 9.3×10^{-4}, or an approximately 1-in-1000 chance of infection per worker. For workers with partial respiratory protection, the function estimates up to 0.17 probability of infection at the maximum dose $d = 90$. The average probability of infection across the five doses shown calculates to 0.095, or an approximately 1-in-10 chance of infection per worker.

Table K–28. Central estimate calculations for centrifuge release of 1918 H1N1V, part II.

Output	Respiratory protection	Calculation
Probability of x initial infections per release (μ_x)	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ $\boldsymbol{\mu_1 = 1.9 \times 10^{-3} \approx 0.2\%}$ $\boldsymbol{\mu_2 = 8.7 \times 10^{-7} \approx 0.00009\%}$
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ $\boldsymbol{\mu_1 = 4.8 \times 10^{-4} \approx 0.05\%}$ $\boldsymbol{\mu_2 = 5.7 \times 10^{-8} \approx 0.000006\%}$
	Full or partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ $\boldsymbol{\mu_1 = 2.3 \times 10^{-3} \approx 0.2\%}$ $\boldsymbol{\mu_2 = 1.4 \times 10^{-6} \approx 0.0001\%}$

The calculations in Table K–28 above continue where the calculations from the previous table left off, now including the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, that one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability p_1 of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection (assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–27). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the probability that one becomes infected with respiratory protection and the other does not, multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection

(assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–27).

The final set of calculations in Table K–28 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–28 show that, under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. This result seems counterintuitive because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection. However, this is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, and the rarity of occurrence of these higher-level exposures results in the lower derived probabilities of seeing one or two infections.
Overall, the results show that, under the central estimate example input values, the probability of one out of two workers being infected after exposure resulting from a centrifuge release of 1918 H1N1V is estimated at about 2.3×10^{-3}, which is about a 1-in-400 chance. The calculated probability of both workers becoming infected is about 1.4×10^{-6}, which is about a 1-in-700,000 chance.

Table K–29. Central estimate calculations for centrifuge release of 1918 H1N1V, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 3.7 \times 10^{-5}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.7 \times 10^{-8}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 30,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 9.5 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.1 \times 10^{-9}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 100,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Full or Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 4.7 \times 10^{-5}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 2.7 \times 10^{-8}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 20,000 years** **$\lambda_{\geq 2} \approx$ one in > 10 million years**

The calculations in Table K–29 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–28) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–29 gives the estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event would be placed in the C frequency category (between 1 in 10,000 years and 1 in 1 million years), and the frequency estimate for an event infecting two or more workers would be placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–30, display how many

different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–30: Results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for 1918 H1N1V.[a]

Dose-Response Estimate	Number of Initial Infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Expert-based	1 or more	28 (< 1%)	3916 (39%)	4467 (45%)	1589 (16%)
	2 or more	0	21 (< 1%)	2524 (25%)	7455 (75%)
	3 or more	0	0	7 (0.1%)	9993 (99.9%)
	4 or more	0	0	0	10,000 (100%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations that resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results might differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–30 show that most input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within the B or C frequency categories. A small percentage of estimates also fall in the A frequency category, and about 16% fall in the D frequency category. The frequency of centrifuge releases resulting in multiple infections of 1918 H1N1V was mostly estimated to be in the D frequency category, though about a quarter of the inputs resulted in return periods of less than one million years.

Sensitivity Results

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–31. The output variable selected for sensitivity evaluation was the frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–31: Sensitivity results – PRCC. The effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection).

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Expert-based	0.40	0.43	0.97	0.76

Because each PRCC in Table K–31 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–30. The PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with 1918 H1N1V would have the greatest impact in reducing the very wide uncertainty range shown in the Table K–30 results.

K.3.2.5 SARS-associated coronavirus

This section discusses the potential for infection with SARS-CoV among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of SARS-CoV was 0–0.09 PFU for laboratory workers with full respiratory protection and 0–9 PFU for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of SARS-CoV in either of these dose ranges. However, there are published experimental studies on viruses related to SARS-CoV in which individuals have been infected by low inhalational doses, including humans becoming infected by doses as low as 4 $CCID_{50}$, so it is possible that doses less than 9 PFU would result in a non-zero probability of infection, which is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the assumed values and distributions are shown in Tables K–32, K–33, and K–34.

Table K–32. Central estimate calculations for centrifuge release of SARS-CoV, part I.

Output	Respiratory protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(0.0225) = 5.5 \times 10^{-5}$ $p(0.045) = 1.1 \times 10^{-4}$ $p(0.0675) = 1.7 \times 10^{-4}$ $p(0.09) = 2.2 \times 10^{-4}$ $q_1 = [\, p(0) + p(0.0225) + p(0.045) + p(0.0675) + p(0.09)\,]/5$ $\boldsymbol{q_1 = 1.1 \times 10^{-4} \approx 0.01\%}$
	Partial	$p(0) = 0$ $p(2.25) = 0.0055$ $p(4.5) = 0.011$ $p(6.75) = 0.016$ $p(9) = 0.022$ $q_2 = [\, p(0) + p(2.25) + p(4.5) + p(6.75) + p(9)\,]/5$ $\boldsymbol{q_2 = 0.011 \approx 1\%}$

The results in Table K–32 above provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. For workers with full respiratory protection, the function estimates up to 2.2×10^{-4} probability of infection at the maximum dose $d = 0.09$. The average probability of infection across the five doses shown calculates to 1.1×10^{-4}, or an approximately 1-in-9000 chance of infection per worker. For workers with partial respiratory protection, the function estimates up to 0.022 probability of infection at the maximum dose $d = 9$. The average probability of infection across the five doses shown calculates to 0.011, or an approximately 1-in-90 chance of infection per worker.

Table K–33. Central estimate calculations for centrifuge release of SARS-CoV, part II.

Output	Respiratory protection	Calculation
Probability of x initial infections per release (μ_x).	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ $\boldsymbol{\mu_1 = 2.2 \times 10^{-4} \approx 0.02\%}$ $\boldsymbol{\mu_2 = 1.2 \times 10^{-8} \approx 0.000001\%}$
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ $\boldsymbol{\mu_1 = 5.5 \times 10^{-5} \approx 0.005\%}$ $\boldsymbol{\mu_2 = 7.5 \times 10^{-10} \approx 0.00000008\%}$
	Full or partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ $\boldsymbol{\mu_1 = 2.8 \times 10^{-4} \approx 0.03\%}$ $\boldsymbol{\mu_2 = 1.9 \times 10^{-8} \approx 0.000002\%}$

The calculations in Table K–33 above continue where the calculations from the previous table left off, now including the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability p_1 of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection (assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–32). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the probability that one becomes infected with respiratory protection and the other does not, multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection

(assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–32).

The final set of calculations in Table K–33 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–33 show that, under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. This result seems counterintuitive because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection. However, this is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, and the rarity of occurrence of these higher-level exposures results in the lower derived probabilities of seeing one or two infections.

Overall, the results show that under the central estimate example input values, the probability of one out of two workers being infected after exposure resulting from a centrifuge release of SARS-CoV is estimated at about 2.8×10^{-4}, which is approximately a 1-in-4000 chance. The calculated probability of both workers becoming infected is 1.9×10^{-8}, which is less than a 1-in-10 million chance.

Table K–34. Central estimate calculations for centrifuge release of SARS-CoV, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 4.4 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 2.4 \times 10^{-10}/\text{year}$ **$\lambda_{\geq 1} \approx$ one in 200,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 1.1 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.5 \times 10^{-11}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 900,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Full or partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 5.5 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 3.8 \times 10^{-10}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 200,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**

The calculations in Table K–34 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–33) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–34 provides the estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event would be placed in the C frequency category (between 1 in 10,000 years and 1 in 1 million years). The estimate for the frequency of a two-or-more infection event would be placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–35, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–35: Uncertainty results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for SARS-CoV.[a]

Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Literature-based	1 or more	0	282 (3%)	9694 (97%)	24 (< 1%)
	2 or more	0	0	1 (<0.1%)	9,999 (>99.9%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	28 (< 1%)	2792 (28%)	7,180 (72%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations that resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results might differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–35 show that for the estimates that use the literature-based dose-response estimates, most input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within the C frequency category, while for the estimates that use the expert-based dose-response estimates, most estimates fall in the D frequency category. In both cases, a small percentage of estimates also fall in the B frequency category. The frequency of centrifuge releases resulting in multiple infections of SARS-CoV is was estimated to be D frequency except for a single input combination.

Sensitivity Results

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–36. The output variable selected for sensitivity evaluation was the frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–36: Sensitivity results – PRCCs and the effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection).

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Literature-based	0.69	0.71	0.82	0.94
Expert-based	0.40	0.50	0.93	0.78

Because each PRCC in Table K–36 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–35. For the estimates using the literature-based dose-response curve, the PRCC for the release frequency input is the highest, which means that reducing the uncertainty in estimating the frequency with which releases occur from centrifuges in BSL-3 laboratories would have the greatest effect on reducing the uncertainty in the results. For the estimates using the expert-based dose-response curve, the PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with SARS-CoV would have the greatest impact in reducing the uncertainty in those results.

K.3.2.6 Rift Valley fever virus

This section discusses the potential for infection with RVFV among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of RVFV was 0–9 $MICLD_{50}$ or 0–0.9 for laboratory workers with full respiratory protection and 0–900 $MICLD_{50}$ or 0–90 PFU for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of RVFV in either of these dose ranges. However, there are numerous published experimental studies on animal exposures to aerosols in which individuals have been infected by low inhalational doses well within the estimated ranges for the centrifuge release, so it is possible that doses in these ranges would result in a non-zero and perhaps substantial probability of infection for humans, which is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the assumed values and distributions are shown in Tables K–37, K–38, and K–39.

Table K–37. Central estimate calculations for centrifuge release of RVFV, part I

Output	Respiratory Protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(2.25) = 0.15$ $p(4.5) = 0.28$ $p(6.75) = 0.38$ $p(9) = 0.48$ $q_1 = [\, p(0) + p(2.25) + p(4.5) + p(6.75) + p(9) \,]/5$ $\boldsymbol{q_1 = 0.26 \approx 30\%}$
	Partial	$p(0) = 0$ $p(225) = 0.9999999$ $p(450) \approx 1$ $p(675) \approx 1$ $p(900) \approx 1$ $q_2 = [\, p(0) + p(225) + p(450) + p(675) + p(900) \,]/5$ $\boldsymbol{q_2 = 0.80 \approx 80\%}$

The results in Table K–37 above provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. For workers with full respiratory protection, the function estimates up to 0.48 probability of infection at the maximum dose $d = 9$. The average probability of infection across the five doses shown calculates to 0.26, or an approximately 1-in-4 chance of infection per worker. For workers with partial respiratory protection, the function estimates close to certainty of infection at dose points $d = 225$ and greater. The average probability of infection across the five doses shown calculates to 0.80, or about a 4/5 chance of infection per worker.

Table K–38. Central estimate calculations for centrifuge release of RVFV, part II.

Output	Respiratory protection	Calculation
Probability of x initial infections per release (μ_x)	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ **$\mu_1 = 0.38 \approx 40\%$** **$\mu_2 = 0.066 \approx 7\%$**
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ **$\mu_1 = 4.0 \times 10^{-3} \approx 0.4\%$** **$\mu_2 = 4.0 \times 10^{-6} \approx 0.0004\%$**
	Full or partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ **$\mu_1 = 0.38 \approx 40\%$** **$\mu_2 = 0.067 \approx 7\%$**

The calculations in Table K–38 above continue where the calculations from the previous table left off, now including the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability p_1 of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection (assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–37). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the probability that one becomes infected with respiratory protection and the other does not, multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection

(assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–37).

The final set of calculations in Table K–38 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–38 show that, under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. This result may seem counterintuitive because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection. However, this is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, as well as the rarity of occurrence of these higher-level exposures results in the lower derived probabilities of seeing one or two infections. Overall, the results show that under the central estimate example input values, the probability of one out of two workers being infected after exposure resulting from a centrifuge release of RVFV is estimated at about 40%. The calculated probability of both workers becoming infected is estimated to be about 7%.

Table K–39. Central estimate calculations for centrifuge release of RVFV, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 8.9 \times 10^{-3}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.3 \times 10^{-3}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 100 years** **$\lambda_{\geq 2} \approx$ 1 in 800 years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 8.0 \times 10^{-5}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 8.0 \times 10^{-8}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 10,000 years** **$\lambda_{\geq 2} \approx$ 1 in 10 million years**
	Full or partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 9.0 \times 10^{-3}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 1.3 \times 10^{-3}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 100 years** **$\lambda_{\geq 2} \approx$ 1 in 800 years**

The calculations in Table K–39 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–38) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–39 gives the estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event is near the boundary of the A and B frequency categories. The estimate for the frequency of a two-or-more infection event would be placed in the B frequency category (between once in 100 years and once in 10,000 years).

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–40, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–40: Uncertainty results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for RVFV.[a]

Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Literature-based	1 or more	5400 (54%)	4600 (46%)	0	0
	2 or more	1679 (17%)	8313 (83%)	8 (0.1%)	0
	3 or more	310 (3%)	7608 (76%)	2080 (21%)	2 (<0.1%)
	4 or more	31 (< 1%)	2517 (25%)	4068 (41%)	3384 (34%)
Expert-based	1 or more	10 (0.1%)	2222 (22%)	7361 (74%)	407 (4%)
	2 or more	0	8 (0.1%)	1091 (11%)	8901 (89%)
	3 or more	0	0	2 (<0.1%)	9998 (>99.9%)
	4 or more	0	0	0	10,000 (100%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations that resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results might differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–40 show that, for the estimates that use the literature-based dose-response estimates, all input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within the A or B frequency categories, while for the estimates that use the expert-based dose-response estimates, most estimates fall in the B or C category with only a small percentage in the A category. The frequency of centrifuge releases resulting in multiple infections of RVFV is was estimated to be mostly B frequency category for the estimates using the literature-based dose-response and mostly in the D frequency category for the estimates using expert-based dose-response, although the uncertainty range is wide in both cases.

Sensitivity Results

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–41. The output variable selected for sensitivity evaluation was the

frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–41: Sensitivity Results – PRCC. The effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection)

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Literature-based	0.69	0.68	0.76	0.96
Expert-based	0.37	0.42	0.90	0.75

Because each PRCC in Table K–41 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–40. For the estimates using the literature-based dose-response curve, the PRCC for the release frequency input is the highest, which means that reducing the uncertainty in estimating the frequency with which releases occur from centrifuges in BSL-3 laboratories would have the greatest effect on reducing the uncertainty in the results. For the estimates using the expert-based dose-response curve, the PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with RVFV would have the greatest impact in reducing the uncertainty in those results.

K.3.2.7 Andes virus

This section discusses the potential for infection with ANDV among laboratory workers as a result of a centrifuge release. In Chapter 4, the estimated exposure range resulting from a centrifuge release of ANDV was 0–0.009 $CCID_{50}$ for laboratory workers with full respiratory protection and 0–0.9 $CCID_{50}$ for workers with partial respiratory protection. As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of ANDV in either of these dose ranges. The lowest dose of ANDV that infected Syrian hamsters intranasally was about 20 PFU, which is presumed to be at least 10 times higher than the maximum estimated dose under partial respiratory protection. However, data on low dose exposures are scarce, so it is possible that doses less than 1 $CCID_{50}$ could result in a non-zero probability of infection, which is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

First, the calculations described in Section K.2.2.3 were conducted for base-case assumptions for each of the input values described in Section K.2.2.2. Step-by-step calculations and results under the above assumed values and distributions are shown in Tables K–42, K–43, and K–44.

Table K–42. Central estimate calculations for centrifuge release of ANDV, part I.

Output	Respiratory protection	Calculation
Expected probability of infection per worker (q)	Full	$p(0) = 0$ $p(0.00225) = 2.5 \times 10^{-5}$ $p(0.0045) = 5.0 \times 10^{-5}$ $p(0.00675) = 7.4 \times 10^{-5}$ $p(0.009) = 9.9 \times 10^{-5}$ $q_1 = [\, p(0) + p(0.00225) + p(0.0045) + p(0.00675) + p(0.009)]/5$ **$q_1 = 5.0 \times 10^{-5} \approx 0.005\%$**
	Partial	$p(0) = 0$ $p(0.225) = 0.0025$ $p(0.45) = 0.0049$ $p(0.675) = 0.0074$ $p(0.9) = 0.0099$ $q_2 = [\, p(0) + p(0.225) + p(0.45) + p(0.675) + p(0.9)]/5$ **$q_2 = 0.0049 \approx 0.5\%$**

The results in Table K–42 above provide information about what the example dose-response function $p(d)$ estimates for the probability of infection across the two dose ranges. For workers with full respiratory protection, the function estimates up to 9.9×10^{-5} probability of infection at the maximum dose $d = 0.009$. The average probability of infection across the five doses shown calculates to 5.0×10^{-5}, or an approximately 1-in-20,000 chance of infection per worker. For workers with partial respiratory protection, the function estimates up to 0.0099 probability of infection at the maximum dose $d = 0.9$. The average probability of infection across the five doses shown calculates to 0.0049, or an approximately 1-in-200 chance of infection per worker.

Table K–43. Central estimate calculations for centrifuge release of ANDV, part II.

Output	Respiratory protection	Calculation
Probability of x initial infections per release (μ_x)	Full	$p_1 = 0.9975 \times q_1$ $\mu_1 = 2 \times p_1 \times (1 - p_1)$ $\mu_2 = (p_1)^2$ $\boldsymbol{\mu_1 = 9.9 \times 10^{-5} \approx 0.01\%}$ $\boldsymbol{\mu_2 = 2.4 \times 10^{-9} \approx 0.0000002\%}$
	Partial	$p_2 = 0.0025 \times q_2$ $\mu_1 = 2 \times p_2 \times (1 - p_2)$ $\mu_2 = (p_2)^2$ $\boldsymbol{\mu_1 = 2.5 \times 10^{-5} \approx 0.002\%}$ $\boldsymbol{\mu_2 = 1.5 \times 10^{-10} \approx 0.00000002\%}$
	Full or partial	$p_T = p_1 + p_2$ $\mu_1 = 2 \times p_T \times (1 - p_T)$ $\mu_2 = (p_T)^2$ $\boldsymbol{\mu_1 = 1.2 \times 10^{-4} \approx 0.01\%}$ $\boldsymbol{\mu_2 = 3.8 \times 10^{-9} \approx 0.0000004\%}$

The calculations in Table K–43 above continue where the calculations from the previous table left off, now including the central estimate assumption that there will be exactly two workers in the biocontainment area of a centrifuge release who might become infected after exposure. Calculations were conducted for the probability that one or both of the workers are infected with full respiratory protection, one or both of the workers are infected without full respiratory protection, and that one or both of the workers are infected with either level of protection.

The probability p_1 of a particular worker both having full respiratory protection and becoming infected with that level of protection is the product of the probability of having full respiratory protection (assumed to be 0.9975) and the average probability of infection across the estimated dose range for that level of protection (q_1, calculated in Table K–42). Next, the probability that exactly one of two workers becomes infected with full respiratory protection (μ_1) is the product of p_1 and $(1 - p_1)$, referring to the probability that one becomes infected with respiratory protection and the other does not, multiplied by two because there are two ways to choose one out of two people. The probability of both of two workers having full respiratory protection and becoming infected is the square of p_1.

A similar set of calculations are shown for workers with partial respiratory protection, this time using p_2, which is the product of the probability of a particular worker having partial respiratory protection

(assumed to be 0.0025) and the average probability of infection across the estimated dose range for partial protection (q_2, calculated in Table K–42).

The final set of calculations in Table K–43 is for the probability that one or both of the workers become infected, regardless of respiratory protection level. Here the value p_T, which is the sum of p_1 and p_2, is the estimated probability of a particular worker becoming infected, and this value is used in the calculation of μ_1 and μ_2 as described above.

The results in the Table K–43 show that under the central estimate input values, the probability of one or two infections occurring among workers with full respiratory protection is higher than the corresponding probabilities among workers with partial respiratory protection. This result may seem counterintuitive because a worker with partial respiratory protection has a chance to inhale a much higher dose than a worker with full protection. However, this is outweighed by the fact that it is much less likely that a worker would have only partial protection in the first place, and the rarity of occurrence of these higher-level exposures results in the lower derived probabilities of experiencing one or two infections. Overall, the results show that, under the central estimate example input values, the probability of one out of two workers being infected after exposure resulting from a centrifuge release of ANDV is estimated at about 1.2×10^{-4}, which is roughly a 1-in-8000 chance. The calculated probability of both workers becoming infected is 3.8×10^{-9}, which is vanishingly small.

Table K–44. Central estimate calculations for centrifuge release of ANDV, part III.

Output	Respiratory protection	Calculation
Frequency of an *x*-or-more infection event ($\lambda_{\geq x}$)	Full	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 2.0 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 4.9 \times 10^{-11}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 500,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 4.9 \times 10^{-7}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 3.1 \times 10^{-12}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 2 million years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**
	Full or partial	$\lambda_{\geq 1} = (\mu_1 + \mu_2) \times 0.02/\text{year} = 2.5 \times 10^{-6}/\text{year}$ $\lambda_{\geq 2} = \mu_2 \times 0.02/\text{year} = 7.6 \times 10^{-11}/\text{year}$ **$\lambda_{\geq 1} \approx$ 1 in 400,000 years** **$\lambda_{\geq 2} \approx$ 1 in > 10 million years**

The calculations in Table K–44 above continue where the calculations in the previous table left off. To calculate the frequency of a centrifuge release resulting in one-or-more initial infections, the values of μ_1 and μ_2 corresponding to the given level of respiratory protection (calculated in Table K–43) are summed and multiplied by the central estimate example frequency of the occurrence of centrifuge releases (0.02/year, or once per 50 years on average).

The bottom row of Table K–44 gives the estimates for the overall frequency of centrifuge releases leading to infections in laboratory workers. The estimate for the frequency of a one-or-more infection event would be placed in the C frequency category (between once in 10,000 years and once in 1 million years). The estimate for the frequency of a two-or-more infection event would be placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.2.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.2.2. The results, presented in Table K–45, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–45: Uncertainty results for the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) for ANDV.[a]

Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Literature-based	1 or more	0	92 (1%)	9,211 (92%)	697 (7%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	25 (< 1%)	2,332 (23%)	7,643 (76%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
	4 or more	0	0	0	10,000 (100%)

[a] Entries are the number (and approximate percentage) of 10,000 input combinations that resulted in an estimated frequency in the given category. Because inputs are drawn randomly, exact results might differ under different sets of 10,000 input combinations, but generally agree with the given results within a percentage point.

The results in Table K–45 show that for the estimates that use the literature-based dose-response estimates, most input combinations result in an estimated frequency of at least one infection occurring from a centrifuge release within the C frequency category, while for the estimates that use the expert-based dose-response estimates, most estimates fall in the D frequency category. In both cases, a small percentage of estimates also fall in the B frequency category. The frequency of centrifuge releases resulting in multiple infections of ANDV is was estimated to be in the D frequency category for all input combinations.

Sensitivity Results

The sensitivity analysis procedure described in Section K.2.2.5 was applied. The results of the sensitivity analysis are shown in Table K–46. The output variable selected for sensitivity evaluation was the frequency of a centrifuge release leading to one or more initial infections (with full or partial respiratory protection).

Table K–46: Sensitivity results – PRCC. The effect of changing each input on the frequency of at least one initial infection (with full or partial respiratory protection).

	Distribution of number exposed	Distribution of amount of exposure	Dose-response curve	Frequency of release
Literature-based	0.68	0.70	0.83	0.93
Expert-based	0.37	0.46	0.97	0.77

Because each PRCC in Table K–46 is statistically significantly greater than zero, it can be concluded that each source of uncertainty played a significant role in contributing to the uncertainty apparent in Table K–45. For the estimates using the literature-based dose-response curve, the PRCC for the release frequency input is the highest, which means that reducing the uncertainty in estimating the frequency with which releases occur from centrifuges in BSL-3 laboratories would have the greatest effect on reducing the uncertainty in the results. For the estimates using the expert-based dose-response curve, the PRCC for the dose-response curve is the highest, which means that achieving greater certainty in appropriate dose-dependent probabilities of infection for humans with ANDV would have the greatest impact in reducing the uncertainty in those results.

K.3.2.8 Summary of infection frequency estimates

This section summarizes the results from Sections K.3.1.1 to K.3.1.7. Table K–47 compiles the central estimate results while Table K–48 compiles the uncertainty results for all seven BSL-3 pathogens.

Table K–47. Central estimate overall calculations for BSL-3 centrifuge release (with full or partial respiratory protection).

Pathogen	Number of initial infections (x)	Probability of x or more initial infections ($\mu_{\geq x}$)	Frequency of release leading to x or more initial infections ($\lambda_{\geq x}$)
B. anthracis	1 or more	6.5×10^{-5}	1.3×10^{-6}/year ≈ 1 in 800,000 years
F. tularensis	1 or more	0.12	2.4×10^{-3}/year ≈ 1 in 400 years
	2 or more	3.8×10^{-3}	7.7×10^{-5}/year ≈ 1 in 10,000 years
Y. pestis	1 or more	9.1×10^{-6}	1.8×10^{-7}/year ≈ 1 in 5 million years
1918 H1N1V	1 or more	2.3×10^{-3}	4.7×10^{-5}/year ≈ 1 in 20,000 years

Pathogen	Number of initial infections (x)	Probability of x or more initial infections ($\mu_{\geq x}$)	Frequency of release leading to x or more initial infections ($\lambda_{\geq x}$)
SARS-CoV	1 or more	2.8×10^{-4}	5.5×10^{-6}/year ≈ 1 in 200,000 years
RVFV	1 or more	0.38	9.0×10^{-3}/year ≈ 1 in 100 years
	2 or more	0.067	1.3×10^{-3}/year ≈ 1 in 800 years
ANDV	1 or more	1.2×10^{-4}	2.5×10^{-6}/year ≈ 1 in 400,000 years

Table K–48: Summary of uncertainty results: number of 10,000 input combinations that resulted in the frequency of centrifuge releases leading to the given number of initial infections (among workers with full or partial protection) falling into each frequency category.

Pathogen	Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
B. anthracis	Literature-based	1 or more	0	20 (< 1%)	7,606 (76%)	2,374 (24%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	217 (2%)	7,484 (75%)	2,299 (23%)
		2 or more	0	0	0	10,000 (100%)
F. tularensis	Literature-based	1 or more	1,513 (15%)	8,487 (85%)	0	0
		2 or more	56 (1%)	7,036 (70%)	2,908 (29%)	0
		3 or more	0	451 (5%)	7,854 (79%)	1,695 (17%)
		4 or more	0	2 (<0.1%)	929 (9%)	9,069 (91%)
	Expert-based	1 or more	348 (3%)	7,557 (76%)	2,095 (21%)	0
		2 or more	5 (<0.1%)	1,380 (14%)	5,123 (51%)	3,492 (35%)
		3 or more	0	23 (< 1%)	1,806 (18%)	8,171 (82%)
		4 or more	0	0	32 (< 1%)	9,968 (>99%)
Y. pestis	Literature-based	1 or more	0	0	1,156 (12%)	8,844 (88%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	24 (< 1%)	3,281 (33%)	6,695 (67%)
		2 or more	0	0	0	10,000 (100%)
1918 H1N1V	Expert-based	1 or more	28 (< 1%)	3,916 (39%)	4,467 (45%)	1,589 (16%)
		2 or more	0	21 (< 1%)	2,524 (25%)	7,455 (75%)
		3 or more	0	0	7 (0.1%)	9,993 (99.9%)
SARS-CoV	Literature-based	1 or more	0	282 (3%)	9,694 (97%)	24 (< 1%)
		2 or more	0	0	1 (<0.1%)	9,999 (>99.9%)

Pathogen	Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
	Expert-based	1 or more	0	28 (< 1%)	2,792 (28%)	7,180 (72%)
		2 or more	0	0	0	10,000 (100%)
RVFV	Literature-based	1 or more	5,400 (54%)	4,600 (46%)	0	0
		2 or more	1,679 (17%)	8,313 (83%)	8 (0.1%)	0
		3 or more	310 (3%)	7,608 (76%)	2,080 (21%)	2 (<0.1%)
		4 or more	31 (< 1%)	2,517 (25%)	4,068 (41%)	3,384 (34%)
	Expert-based	1 or more	10 (0.1%)	2,222 (22%)	7,361 (74%)	407 (4%)
		2 or more	0	8 (0.1%)	1,091 (11%)	8,901 (89%)
		3 or more	0	0	2 (<0.1%)	9998 (>99.9%)
		4 or more	0	0	0	10,000 (100%)
ANDV	Literature-based	1 or more	0	92 (1%)	9,211 (92%)	697 (7%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	25 (< 1%)	2,332 (23%)	7,643 (76%)
		2 or more	0	0	0	10,000 (100%)

K.3.2.9 Summary of potential health consequences

The results described above estimate a non-zero risk of a laboratory worker developing an infection after exposure to any of the BSL-3 pathogens as a result of loss of biocontainment after an event involving a centrifuge. The route of exposure considered is via inhalation. As it is assumed that the exposure from the centrifuge event is undetected and/or unreported, the health consequences are based on the assumption that post-exposure prophylaxis (if available), quarantine, and supportive measures are not instituted unless and until the laboratory worker exhibits symptoms and seeks medical attention. Table K–49 summarizes the potential health consequences in the laboratory worker and subsequent risk to the public in the event of an inhalational infection occurring in the laboratory worker.

The issues of pre-existing immunity and potentially available vaccines for each pathogen are further discussed in Appendix J. Full descriptions of the clinical diseases caused by the pathogens are provided in Chapter 4 and Appendix C. The mortality estimates provided below are also summarized in Chapter 4, Appendix C, and Mahmoud (2008), and are presumed to apply to heterogeneous populations containing individuals from medically vulnerable groups. Therefore, applying these mortality estimates to laboratory workers may be conservative, as it is presumed that laboratory workers are healthy adults as discussed further in Appendix I. Potential risks to the public from laboratory workers infected with transmissible pathogens is further discussed in Chapter 10 and Appendix L.

Table K–49. Summary of health consequences in laboratory worker from centrifuge event.[a]

Pathogen	Pre-existing immunity to pathogen in laboratory worker	Resulting illness via inhalation route	Case fatality estimates from infection	Potential for secondary transmission to members of the public if initial infection is undetected and/or unreported
B. anthracis	Possible, as vaccine is available	Inhalational anthrax	45%	No
F. tularensis	No	Pneumonic form of tularemia	< 2%	No
Y. pestis	No	Pneumonic plague	15%	Yes
1918 H1N1V	Possible, due to cross-protection from past influenza vaccines or infections	Influenza	2.5%	Yes
SARS-CoV	No	SARS	10%	Yes
RVFV	Possible, as vaccine is available	Rift Valley fever	0.5–2%	No
ANDV	No	Hantavirus pulmonary syndrome	50%	Yes

[a] Adapted from Mahmoud et al. (2008).

Finally, the case fatality rate estimates in Table K–49 were integrated into the initial infections calculations to compute estimates for the frequency of centrifuge releases leading to mortalities among laboratory workers. For *F. tularensis* and RVFV, the conservative value of 2% case fatality was applied. The results under the central estimate example inputs are shown in Table K–50.

Table K–50. Central estimate mortality results for BSL-3 centrifuge infectious aerosol release among laboratory workers (with full or partial respiratory protection).

Pathogen	Number of deaths among laboratory workers (x)	Probability of x or more deaths among laboratory workers	Frequency of release leading to x or more deaths among laboratory workers
B. anthracis	1 or more	2.9×10^{-5}	5.9×10^{-7}/year ≈ 1 in 2 million years
F. tularensis	1 or more	2.5×10^{-3}	5.0×10^{-5}/year ≈ 1 in 20,000 years
	2 or more	1.5×10^{-6}	3.1×10^{-8}/year = 1 in > 10 million years

Pathogen	Number of deaths among laboratory workers (*x*)	Probability of *x* or more deaths among laboratory workers	Frequency of release leading to *x* or more deaths among laboratory workers
Y. pestis	1 or more	1.4×10^{-6}	2.7×10^{-8}/year = 1 in > 10 million years
1918 H1N1V	1 or more	5.8×10^{-5}	1.2×10^{-6}/year ≈ 1 in 900,000 years
SARS-CoV	1 or more	2.8×10^{-5}	5.5×10^{-7}/year ≈ 1 in 2 million years
RVFV	1 or more	0.010	2.1×10^{-4}/year ≈ 1 in 5,000 years
	2 or more	2.7×10^{-5}	5.3×10^{-7}/year ≈ 1 in 2 million years
ANDV	1 or more	6.2×10^{-5}	1.2×10^{-6}/year = 1 in 800,000 years

Under the central estimate inputs, centrifuge releases leading to one or more deaths among laboratory workers would be placed in the B frequency category for RVFV; the C frequency category for *F. tularensis*, 1918 H1N1V, and ANDV; and the D frequency category for *B. anthracis*, *Y. pestis*, and SARS-CoV. Centrifuge releases leading to two or more deaths among laboratory workers would be placed in the D frequency category for all pathogens.

Uncertainty results pertaining to these mortality estimates are presented in Table K–51.

Table K–51: Summary of mortality uncertainty results: percentage of 10,000 input combinations that resulted in the frequency of centrifuge releases leading to the given number of deaths (among workers with full or partial protection) falling into each frequency category.

Pathogen	Dose-response estimate	Number of deaths among laboratory workers	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
B. anthracis	Literature-based	1 or more	0	0	3,969 (40%)	6,031 (60%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	77 (1%)	6,278 (63%)	3,645 (36%)
		2 or more	0	0	0	10,000 (100%)
F. tularensis	Literature-based	1 or more	0	3,353 (34%)	6,647 (66%)	0
		2 or more	0	0	407 (4%)	9,593 (96%)
		3 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	820 (8%)	8,121 (81%)	1,059 (11%)
		2 or more	0	0	40 (< 1%)	9,960 (> 99%)
		3 or more	0	0	0	10,000 (100%)
Y. pestis	Literature-based	1 or more	0	0	93 (1%)	9,907 (99%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	0	754 (8%)	9,246 (92%)
		2 or more	0	0	0	10,000 (100%)
1918 H1N1V	Expert-based	1 or more	0	102 (1%)	4,780 (48%)	5,118 (51%)
		2 or more	0	0	0	10,000 (100%)
SARS-CoV	Literature-based	1 or more	0	2 (< 0.1%)	3,866 (39%)	6,132 (61%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	0	537 (5%)	9,463 (95%)
		2 or more	0	0	0	10,000 (100%)
RVFV	Literature-based	1 or more	69 (1%)	9,249 (92%)	682 (7%)	0
		2 or more	0	54 (1%)	6,409 (64%)	3,537 (35%)
		3 or more	0	0	14 (0.1%)	9,986 (99.9%)
		4 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	34 (< 1%)	2,914 (29%)	7,052 (71%)
		2 or more	0	0	0	10,000 (100%)
ANDV	Literature-based	1 or more	0	21 (< 1%)	7,196 (72%)	2,783 (28%)
		2 or more	0	0	0	10,000 (100%)
	Expert-based	1 or more	0	10 (0.1%)	1,713 (17%)	8,277 (83%)
		2 or more	0	0	0	10,000 (100%)

B. anthracis mortality frequency is placed mostly in the D frequency category under the literature-based dose-response models and mostly in C under the expert-based models.

F. tularensis mortality frequency is placed mostly in the C category. Under the literature-based dose-response models, a significant percentage (34%) placed in B frequency category, compared to only 8% under the expert-based models.

Y. pestis mortality frequency is placed mostly in the D frequency category, with small percentages (1% or 8%) in the C frequency category.

1918 H1N1V mortality frequency was split close to evenly between C and in the D frequency category, with a small percentage (1%) in B frequency category.

SARS-CoV mortality frequency was placed mostly in the D frequency category, with a significant percentage (39%) placed in the C frequency category under the literature-based dose-response models, compared to only 5% in C frequency category under the expert-based models.

RVFV mortality frequency (one or more deaths) was placed mostly in B frequency category under the literature-based dose-response models and mostly in the D frequency category under the expert-based models, with uncertainty ranges across three frequency categories in each case.

ANDV mortality frequency was placed mostly in the C frequency category under the literature-based dose-response models and mostly in the D frequency category under the expert-based models, with small percentages (< 0.3%) in B frequency category in both cases.

K.3.3 Earthquake Beyond Design Basis Release

The calculations described in Sections K.2.3.3 to K.2.3.4 were performed using the exposure estimates from the (BDB Release scenario described in Chapter 4 and Appendix F. As the calculations resulted in very low risk estimates for every pathogen under every population scenario, the results are displayed here only in summary form. A more detailed discussion of aspects of each pathogen relevant to potential initial infections in the public is provided in Section K.3.4.

Table K–52. Central estimate calculations for urban site BDB earthquake release.

Pathogen	Number of initial infections overall (x)	Probability of x or more initial infections overall ($\mu_{\geq x}$)	Frequency of release leading to x or more initial infections ($\lambda_{\geq x}$)
B. anthracis	1 or more	1.5×10^{-9}	1.5×10^{-14}/year = 1 in > 10 million years
F. tularensis	1 or more	6.1×10^{-7}	6.1×10^{-12}/year = 1 in > 10 million years
Y. pestis	1 or more	4.0×10^{-11}	4.0×10^{-16}/year = 1 in > 10 million years
1918 H1N1V	1 or more	6.6×10^{-20}	6.6×10^{-25}/year = 1 in > 10 million years
SARS-CoV	1 or more	1.8×10^{-8}	1.8×10^{-13}/year = 1 in > 10 million years
RVFV	1 or more	5.2×10^{-5}	5.2×10^{-10}/year = 1 in > 10 million years
ANDV	1 or more	8.0×10^{-9}	8.0×10^{-14}/year = 1 in > 10 million years
EBOV	1 or more	1.5×10^{-9}	1.5×10^{-14}/year = 1 in > 10 million years
MARV	1 or more	9.0×10^{-10}	9.0×10^{-15}/year = 1 in > 10 million years
LASV	1 or more	8.6×10^{-10}	8.6×10^{-15}/year = 1 in > 10 million years
JUNV	1 or more	1.5×10^{-10}	1.5×10^{-15}/year = 1 in > 10 million years
TBEV-FE	1 or more	5.6×10^{-52}	5.6×10^{-57}/year = 1 in > 10 million years
NIPV	1 or more	5.5×10^{-11}	5.5×10^{-16}/year = 1 in > 10 million years

For the central estimate example, all pathogens are estimated to produce a low probability that one or more infections will occur among members of the public. The highest estimated probability is for RVFV (5.2×10^{-5}), which is about a 1-in-20,000 chance. Combined with the central estimate estimated frequency of the occurrence of an earthquake release (once in 100,000 years), the frequency of an earthquake BDB release resulting in one or more infections of any pathogen is estimated to be well into the D frequency category frequency category.

Table K–53: Summary of uncertainty results: number of 10,000 input combinations that resulted in the frequency of earthquake BDB release (urban site) leading to the given number of initial infections falling into each frequency category.

Pathogen	Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Both	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Both	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
EBOV	Both	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Both	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Both	1 or more	0	0	0	10,000 (100%)
NIPF	Both	1 or more	0	0	0	10,000 (100%)

These uncertainty results apply for estimates using both the literature-based and the expert-based range of dose-response estimates. Every combination of dose-response estimates and earthquake release frequency estimates results in an estimated frequency of at least one initial infection in frequency category D. This means that even the most conservative dose-response models combined with the most conservative earthquake frequency (once per 10,000 years) results in an average return period estimate greater than one million years.

Results for urban residents, suburban site, and rural site

The results in Tables K–52 and K–53 were calculated using the overall estimated urban population, which includes estimates of area residents as well as daytime students, workers, hospital patients, and passersby within a 1km radius. If the population inputs are restricted to residents only, the resulting probabilities in Table K–52 are even lower, and the same conclusions are drawn regarding the frequency category for one or more infections occurring (frequency category D).

The average, per-person exposure estimates at the suburban and rural sites are slightly higher than at the urban site (see also Chapter 4 and Appendix F), but the estimated suburban and rural populations are lower, which results in lower probabilities of at least one infection compared to the urban results in Table K–52. Therefore, the same conclusions are drawn regarding the frequency category for one or more infections occurring at the suburban and rural sites (frequency category D).

A more detailed discussion of the effects of site differences on the earthquake initial infections calculations is provided in Section K.3.4, in which site and population differences lead to placement of some results in different frequency categories under the larger exposure estimates for the MRF release quantities.

K.3.4 Earthquake Maximum Reasonably Foreseeable Release

This section is organized by pathogen. For each pathogen, the results from Chapter 4 and Appendix F detailing the MRF exposure estimates for the urban site are synthesized with the dose-response information from Appendix J. Next, the results from the calculations, uncertainty analysis, and sensitivity analysis described in Sections 2.3.3 to 2.3.5 are displayed. At the end of this section, the results for urban residents and the suburban and rural sites are summarized.

K.3.4.1 *Bacillus anthracis*

This section discusses the potential for initial infection and mortality with *B. anthracis* among members of the public after a MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.053 CFU for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-4} CFU at 1 km on the centerline. If it is assumed 1 CFU represents a single, potentially infectious bacterial cell, and under the assumption of Poisson-distributed

variability around the average dose, the MEI exposure estimate of 0.053 CFU can be interpreted to mean that, for people very close to a release and in the path of the plume, the chance of inhaling one or more cells of *B. anthracis* is, at most, about 5%. Further, this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of *B. anthracis* on the order of 1 CFU. The lowest dose to which nonhuman primates have succumbed in published experimental studies was about 200 CFU. However, there is also no direct evidence that infection from inhaling very low doses is impossible, especially in a heterogeneous population containing individuals who have respiratory health problems or are immunocompromised. The dose-response models fit to the available data and to the expert estimates reflect the possibility that low doses result in a non-zero probability of infection.

It is important to note that *B. anthracis* is the only pathogen examined in this RA for which there is a documented historical example of a large-scale aerosol release from a biological research facility that caused infections in members of the public downwind of the release point (the Sverdlovsk incident, discussed in Appendix J). Certain aspects of the spatial and temporal distribution of the observed cases of disease that resulted from the release were used to justify the assumed form of the literature-based dose-response model described in Appendix J and applied here. However, the quantity of *B. anthracis* that was released from the facility is not known and could have been much different than the stock inventory estimates assumed for NEIDL. Therefore, there is a limited amount of inference that can be drawn from the number of infections that occurred in Sverdlovsk to the number likely to occur in the scenarios described here.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–54. Central estimate calculations for urban site earthquake MRF release of *B. anthracis*.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	5.3×10^{-5}	5.3×10^{-10}/year = 1 in > 10 million years
2 or more	1.4×10^{-9}	1.4×10^{-14}/year = 1 in > 10 million years
3 or more	2.5×10^{-14}	2.5×10^{-19}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	2.4×10^{-5}	2.4×10^{-10}/year = 1 in > 10 million years
2 or more	2.9×10^{-10}	2.9×10^{-15}/year = 1 in > 10 million years
3 or more	2.5×10^{-15}	2.5×10^{-20}/year = 1 in > 10 million years

The results in Table K–54 show that the base-case estimate for the probability of one or more *B. anthracis* infections occurring, should a maximum earthquake release occur, is 5.3×10^{-5}, or roughly a 1-in-20,000 chance. This result, combined with the low estimated frequency of occurrence of a MRF earthquake release, leads to the frequency of occurrence of initial infections or deaths from *B. anthracis* being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–55, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–55: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections or deaths for *B. anthracis*.

Dose-response estimate	Number of initial infections or deaths	A < 1 per yr > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–55 show that all input combinations result in an estimated frequency of at least one infection occurring from an earthquake within the D frequency category. This means that even the most conservative dose-response estimates from the extremes of the either the literature-based or the expert-based models, combined with the most conservative estimate of earthquake frequency (once per 10,000 years), does not lead to an estimate of a return period less than 1 million years for the occurrence of an infection or mortality.

K.3.4.2 *Francisella tularensis*

This section discusses the potential for infection with *F. tularensis* among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.0088 CFU for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 2×10^{-5} CFU at 1 km on the centerline. If it is assumed 1 CFU represents a single, potentially infectious bacterial cell, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 0.0088 CFU can be interpreted to mean that, for people very close to a release and in the path of the plume, the chance of inhaling one or more cells of *F. tularensis* is,

at most, close to 1%, and this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there exist published data from studies on human volunteers who inhaled doses of *F. tularensis* measured as low as 10 CFU and became infected. There is no direct evidence of humans being infected from inhaling doses on the order of 1 CFU, although the high rate of infection observed in the range of about 10 to 50 CFU suggests that infection at even lower doses might be possible, a notion that is reflected in the dose-response models derived in Appendix J.

Frequency of Initial Infections – Central estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–56. Central estimate calculations for urban site earthquake MRF release of *F. tularensis*.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	0.021	2.1×10^{-7}/year ≈ 1 in 5 million years
2 or more	2.3×10^{-4}	2.3×10^{-9}/year = 1 in > 10 million years
3 or more	1.6×10^{-6}	1.6×10^{-11}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	4.3×10^{-4}	4.3×10^{-9}/year = 1 in > 10 million years
2 or more	9.2×10^{-8}	9.2×10^{-13}/year = 1 in > 10 million years
3 or more	1.3×10^{-11}	1.3×10^{-16}/year = 1 in > 10 million years

The results in Table K–56 show that the base-case estimate for the probability of one or more *F. tularensis* infections occurring, should an MRF earthquake release occur, is 0.021, or roughly a 1-in-10 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF

earthquake release, leads to the frequency of occurrence of one or more initial infections with *F. tularensis* being approximately once in five million years, which is in the D frequency category. A one-or-more death event would also be placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–57, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–57: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for *F. tularensis*.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	1,760 (18%)	8,240 (82%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	196 (2%)	9,804 (98%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–57 show that some input combinations result in an estimated frequency of at least one infection occurring from an earthquake within the C frequency category and others in the D frequency category. About 18% of the results using the literature-based dose-response estimates are placed in the C category, compared to only about 2% for results using the expert-based dose-response estimates. All results for the frequency of multiple infections and for mortalities are placed in the D frequency category.

K.3.4.3 *Yersinia pestis*

This section discusses the potential for infection with *Y. pestis* among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.00044 CFU for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-6} CFU at 1 km on the centerline. If it is assumed 1 CFU represents a single, potentially infectious bacterial cell, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 0.00044 CFU can be interpreted to mean that, for people very close to a release and in the path of the plume, the chance of inhaling one or more cells of *Y. pestis* is, at most, about 0.04% (about a one in 2000 chance), and this probability becomes significantly lower for individuals located farther away.

In the rare case of an individual potentially inhaling one cell of *Y. pestis*, the evidence described in the dose-response appendix (Appendix J) suggests low infectivity, as the lowest dose to which nonhuman primates succumbed to exposure in published data sets was in the range of 120–270 organisms, and several monkeys withstood exposure to both lower and higher doses. However, there is no direct evidence that infections resulting from very low inhaled doses are impossible, a notion that is reflected in the dose-response models derived in Appendix J, which generally estimate low but non-zero probabilities of infection at a dose of one organism.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–58. Central estimate calculations for urban site earthquake MRF release of *Y. pestis*.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	1.4×10^{-6}	1.4×10^{-11}/year = 1 in > 10 million years
2 or more	9.5×10^{-13}	9.5×10^{-18}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	2.1×10^{-7}	2.1×10^{-12}/year = 1 in > 10 million years
2 or more	2.1×10^{-14}	2.1×10^{-19}/year = 1 in > 10 million years

The results in Table K–58 show that the base-case estimate for the probability of one or more *Y. pestis* infections occurring, should an MRF earthquake release occur, is 4.8×10^{-5}, or roughly a 1-in-200,000 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from *Y. pestis* being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–59, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–59: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for *Y. pestis.*

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–59 show that all input combinations for *Y. pestis* result in an estimated frequency of at least one infection or mortality occurring from an earthquake MRF release within the D frequency category.

K.3.4.4 1918 H1N1 influenza virus

This section discusses the potential for infection with 1918 H1N1V among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.066 PFU for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-4} PFU at 1 km on the centerline. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 0.066 PFU can be interpreted to mean that, for people very close to a release and in the path of the plume, the chance of inhaling one or more potentially infectious units of 1918 H1N1V is, at most, about 7%, and this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of 1918 H1N1V on the order of 1 PFU. There are numerous data sets from human volunteers exposed to other strains of influenza virus, but in the vast majority of these studies, volunteers were exposed to high doses. One study reported outcomes from human volunteers exposed to low-dose aerosols of an H2N2 strain, concluding that unprotected individuals were infected by doses as low as 1 $CCID_{50}$. These data suggest that humans could potentially be infected by very low doses of influenza virus, considering that quantities of virus are generally similar or higher in units of $CCID_{50}$ compared to PFU. No literature-based dose-response estimate was derived for 1918 H1N1V. The expert-based dose-response range includes models that estimate greater than 1% probability of infection at an average inhaled dose of 1 PFU.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–60. Central estimate calculations for urban site earthquake MRF release of 1918 H1N1V.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	4.5×10^{-4}	4.5×10^{-9}/year = 1 in > 10 million years
2 or more	1.0×10^{-7}	1.0×10^{-12}/year = 1 in > 10 million years
3 or more	1.6×10^{-11}	1.6×10^{-16}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	1.1×10^{-5}	1.1×10^{-10}/year = 1 in > 10 million years
2 or more	6.5×10^{-11}	6.5×10^{-16}/year = 1 in > 10 million years
3 or more	8.8×10^{-17}	8.8×10^{-22}/year = 1 in > 10 million years

The results in Table K–60 show that the base-case estimate for the probability of one or more 1918 H1N1V infections occurring, should an MRF earthquake release occur, is 4.5×10^{-4}, or roughly a 1-in-

2000 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from 1918 H1N1V being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–61, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–61: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for 1918 H1N1V.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Expert-based	1 or more	0	0	755 (8%)	9,245 (92%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–61 show that most input combinations result in an estimated frequency of at least one infection occurring from an earthquake MRF release within the D frequency category. About 8% of the estimates fall in the C frequency category. All results for the frequency of multiple infections and mortalities fall in the D frequency category.

K.3.4.5 SARS-associated coronavirus

This section discusses the potential for infection with SARS-CoV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.0066 PFU for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-5} PFU at 1 km on the centerline. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average

dose, the MEI exposure estimate of 0.0066 PFU can be interpreted to mean that, for people very close to a release and in the path of the plume, the chance of inhaling one or more potentially infectious units of SARS-CoV is less than 1%, and this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of SARS-CoV on the order of 1 PFU. However, there are published experimental studies on viruses related to SARS-CoV in which individuals have been infected by low inhalational doses, including humans becoming infected by doses as low as 4 $CCID_{50}$, so it is possible that very low doses would result in a non-zero probability of infection, which is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–62. Central estimate calculations for urban site earthquake MRF release of SARS-CoV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	6.3×10^{-4}	6.3×10^{-9}/year = 1 in > 10 million years
2 or more	2.0×10^{-7}	2.0×10^{-12}/year = 1 in > 10 million years
3 or more	4.1×10^{-11}	4.1×10^{-16}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	6.3×10^{-5}	6.3×10^{-10}/year = 1 in > 10 million years
2 or more	2.0×10^{-9}	2.0×10^{-14}/year = 1 in > 10 million years
3 or more	4.1×10^{-14}	4.1×10^{-19}/year = 1 in > 10 million years

The results in Table K–63 show that the base-case estimate for the probability of one or more SARS-CoV infections occurring, should an MRF earthquake release occur, is 6.3×10^{-4}, or roughly a 1-in-2,000 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from SARS-CoV being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–64, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–64: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for SARS-CoV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–64 show that all input combinations result in an estimated frequency of at least one infection or mortality from SARS-CoV occurring from an earthquake MRF release within the D frequency category.

K.3.4.6 Rift Valley fever virus

This section discusses the potential for infection with RVFV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.66 $MICLD_{50}$ (0.066 PFU) for the urban site. Further, the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-3} $MICLD_{50}$ (1×10^{-4} PFU) at 1 km on the centerline.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling low doses of RVFV. However, there are numerous published experimental studies on animal exposures to aerosols in which individuals have been infected by low inhalational doses near or less than the above estimated MEI exposure. Therefore, it is possible that doses in these ranges would result in a non-zero and potentially substantial probability of infection for humans, which is especially reflected in the literature-based dose-response models fit to the available data.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–65. Central estimate calculations for urban site earthquake MRF release of RVFV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	0.84	8.4×10^{-6}/year ≈ 1 in 100,000 years
2 or more	0.54	5.4×10^{-6}/year ≈ 1 in 200,000 years
3 or more	0.27	4.6×10^{-6}/year ≈ 1 in 400,000 years
4 or more	0.11	1.1×10^{-6}/year ≈ 1 in 900,000 years
5 or more	0.038	3.8×10^{-7}/year ≈ 1 in 3 million years
Deaths among initially infected		
1 or more	0.036	3.6×10^{-7}/year ≈ 1 in 3 million years
2 or more	6.4×10^{-4}	6.4×10^{-9}/year = 1 in > 10 million years
3 or more	7.8×10^{-6}	7.8×10^{-11}/year = 1 in > 10 million years

The results in Table K–65 show that, should an MRF earthquake release occur, the base-case estimates state that there is about a 84% chance that 1 or more initial infections would occur within 1 km of the Boston site. This results in an estimated return period for a 1 or more infection event of just above 100,000 years, which was the assumed central estimate return period of the occurrence of the earthquake release itself. A 4-or-more infection event has an estimated return period of 900,000 years, which is also within the C frequency category, though a 5-or-more infection event would be placed in the D frequency category. The above results also reveal that the median number of infections expected under the central estimate inputs would be about two. Note that the annular ring-specific calculations for RVFV revealed an approximately 2% probability that at least one infection would occur in the annular ring farthest from the release source (between 900 and 1000 m); thus, including population at distances farther than 1 km would slightly increase the probabilities in Table K–65.

The estimated probability for one or more deaths from RVFV occurring after an MRF earthquake release is 0.036, or about a 1-in-30 chance. A one-or-more death event has an estimated return period of about 3 million years, which falls in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–66, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring. As described in the RVFV section of Appendix J, the exposures in units of $MICLD_{50}$ are applied to the literature-based dose-response estimates, and the exposures in units of PFU are applied to the expert-based dose-response estimates.

Table K–66: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for RVFV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	9,638 (96%)	362 (4%)
	2 or more	0	0	8,743 (87%)	1,257 (13%)
	3 or more	0	0	7,430 (74%)	2,570 (26%)
	4 or more	0	0	5,668 (57%)	4,332 (43%)
	5 or more	0	0	3,700 (37%)	6,300 (63%)
	10 or more	0	0	182 (2%)	9,818 (98%)
Expert-based	1 or more	0	0	355 (4%)	9,645 (96%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	3,039 (30%)	6,961 (70%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

For estimates using the literature-based range of dose-response estimates, the results in Table K–66 show that about 87% of input combinations result in an estimated frequency of at least one infection occurring

from an earthquake MRF release within the C frequency category. The majority of results also fall in the C category for 2-, 3-, and 4-or-more infection events. The extreme ≈ 2% of inputs result in 10-or-more infection events being placed in the C frequency category, with the rest falling in D frequency category. Overall, the median number of expected initial infections across all parameter combinations using the literature-based dose-response estimates was approximately two, and 95% of the estimates fell within the range 1–6 expected initial infections. For this set of results, about 30% of the estimates for frequency of one or more deaths fell in the C frequency category.

The estimates that use the expert-based dose-response models provide a different outcome, with only about 4% of the estimates resulting in a frequency of a 1-or-more infection release in the C category, and 100% in the D frequency category for a 1-or-more death release. The lower risk expert estimates may be more relevant if it is true that humans are significantly less susceptible to low-dose infection with RVFV than are the animals that formed the basis for the literature-based dose-response estimates.

K.3.4.7 Andes virus

This section discusses the potential for infection with ANDV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 6.6×10^{-4} $CCID_{50}$ for the urban site. The estimates decrease steadily with distance away from the release, down to approximately 1×10^{-6} $CCID_{50}$ at 1 km on the centerline.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling low doses of ANDV. The lowest dose of ANDV that infected Syrian hamsters intra-nasally was about 20 PFU, which is presumed to be at least 30,000 times higher than the estimated MEI exposure scenario described above (i.e., the exposure estimates would likely be lower in PFU). However, data on low dose exposures are scarce, so it is possible that doses less than 1 $CCID_{50}$ could result in a low but non-zero probability of infection. This is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–67. Central estimate calculations for urban site earthquake MRF release of ANDV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	2.8×10^{-4}	2.8×10^{-9}/year = 1 in > 10 million years
2 or more	3.9×10^{-8}	3.9×10^{-13}/year = 1 in > 10 million years
3 or more	3.7×10^{-12}	3.7×10^{-16}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	1.4×10^{-4}	1.4×10^{-9}/year = 1 in > 10 million years
2 or more	9.8×10^{-9}	9.8×10^{-14}/year = 1 in > 10 million years
3 or more	4.6×10^{-13}	4.6×10^{-18}/year = 1 in > 110 million years

The results in Table K–67 show that the base-case estimate for the probability of one or more ANDV infections occurring, should an MRF earthquake release occur, is 2.8×10^{-4}, or roughly a 1-in-4,000 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from ANDV and falls in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–68, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–68: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for ANDV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–68 show that all input combinations result in an estimated frequency of at least one infection or death from ANDV occurring from an earthquake MRF release within the D frequency category.

K.3.4.8 Ebola virus

This section discusses the potential for infection with EBOV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure at the urban site for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.033 $CCID_{50}$, which is equivalent to approximately 0.0027 PFU according to the units conversion factor cited in Appendix J. These estimates decrease steadily with distance away from the release, down to approximately 8×10^{-5} $CCID_{50}$ or 7×10^{-6} PFU at 1 km on the centerline. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 0.0027 PFU can be interpreted to mean that for people very close to a release and in the path of the plume, the chance of inhaling one or more potentially infectious units of EBOV is, at most, about 0.3%. This probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling low doses of EBOV on the order of 1 PFU. Among nonhuman primates in laboratory studies, a 100% infection rate has been observed for monkeys inhaling as low as 20 PFU. Infections were also observed for monkeys inhaling as low as 3.5 $MICLD_{50}$, which as discussed in Appendix J, was shown to be equivalent to approximately 1 PFU. Given these data, it is possible that individuals inhaling doses of EBOV amounting to about 1 PFU could become infected, and this is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–69. Central estimate calculations for urban site earthquake MRF release of EBOV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	0.017	1.7×10^{-7}/year ≈ 1 in 6 million years
2 or more	1.5×10^{-4}	1.5×10^{-9}/year = 1 in > 10 million years
3 or more	8.3×10^{-7}	8.3×10^{-12}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	0.015	1.5×10^{-7}/year ≈ 1 in 7 million years
2 or more	1.2×10^{-4}	1.2×10^{-9}/year = 1 in > 10 million years
3 or more	6.1×10^{-7}	6.1×10^{-12}/year = 1 in > 10 million years

The results in Table K–69 show that the base-case estimate for the probability of one or more EBOV infections occurring, should an MRF earthquake release occur, is 0.017, or roughly a 1-in-60 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections with EBOV being estimated at approximately once in 6 million years. This is in the D frequency category. The results for the

probability and frequency of deaths among those initially infected are similar because of the high case fatality rate of EBOV.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–70, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–70: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for EBOV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	1,333 (13%)	8,667 (87%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	1,098 (11%)	8,902 (89%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

For estimates using the literature-based range of dose-response estimates, the results in Table K–70 show that the majority of input combinations result in an estimated frequency of at least one infection or death occurring from an earthquake MRF release within the D frequency category. However, about 13% of results for infections and 11% for deaths fall in the C frequency category.

The estimates that use the expert-based dose-response models produced no results for the frequency of a 1-or-more infection release in the C frequency category. The lower risk expert estimates may be more relevant if it is true that humans are significantly less susceptible to low-dose infection with EBOV than are the nonhuman primates that formed the basis for the literature-based dose-response estimates.

K.3.4.9 Marburg virus

This section discusses the potential for infection with MARV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure at the urban site for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.0066 $CCID_{50}$. This is equivalent to approximately 5.5×10^{-4} PFU according to the units conversion factor cited in Appendix J, and the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-5} $CCID_{50}$ or 1×10^{-6} PFU at 1 km on the centerline. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 5.5×10^{-4} PFU can be interpreted to mean that for people very close to a release and in the path of the plume, the chance of inhaling one or more potentially infectious units of MARV is, at most, about 0.05% (1-in-2,000 chance). Again, this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling low doses of MARV on the order of 1 PFU. Among nonhuman primates in laboratory studies, inhaled doses as low as 2 PFU and possibly lower have caused infection. Given these data, it is possible that individuals inhaling doses of MARV amounting to about 1 PFU could become infected, and this is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–71. Central estimate calculations for urban site earthquake MRF release of MARV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	0.0034	3.4×10^{-8}/year = 1 in > 10 million years
2 or more	5.9×10^{-6}	5.9×10^{-11}/year = 1 in > 10 million years
3 or more	6.7×10^{-9}	6.7×10^{-14}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	0.0034	3.4×10^{-8}/year = 1 in > 10 million years
2 or more	5.9×10^{-6}	5.9×10^{-11}/year = 1 in > 10 million years
3 or more	6.7×10^{-9}	6.7×10^{-14}/year = 1 in > 10 million years

The results in Table K–71 show that the base-case estimate for the probability of one or more MARV infections occurring, should an MRF earthquake release occur, is 0.0034, or roughly a 1-in-300 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from MARV being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–72, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–72: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for MARV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	4 (< 0.1%)	9,996 (> 99.9%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	4 (< 0.1%)	9,996 (> 99.9%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

For estimates using the literature-based range of dose-response estimates, the results in Table K–72 show that only 4 of 10,000 input combinations result in an estimated frequency of at least one infection or death occurring from an MRF release earthquake within the C frequency category. The estimates that use the expert-based dose-response models result in none of the estimates resulting in a frequency of a 1-or-more infection release in the C category.

K.3.4.10 Lassa virus

This section discusses the potential for infection with LASV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure at the urban site for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.0066 PFU. These estimates decrease steadily with distance away from the release, down to approximately 1×10^{-5} PFU at 1 km on the centerline. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 0.0066 PFU can be interpreted to mean that for people very close to a release and in the path of the plume, the chance of inhaling one or more potentially infectious units of LASV is less than 1%. Further, this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling low doses of LASV on the order of 1 PFU. In laboratory experiments, guinea pigs have become infected after inhaling doses as low as 5 PFU. Given these limited data, the possibility that individuals inhaling doses of LASV amounting to about 1 PFU could become infected cannot be ruled out. This is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–73. Central estimate calculations for urban site earthquake MRF release of LASV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	0.010	1.0×10^{-7}/year ≈ 1 in 10 million years
2 or more	5.0×10^{-5}	5.0×10^{-10}/year = 1 in > 10 million years
3 or more	1.7×10^{-7}	1.7×10^{-12}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	2.0×10^{-4}	2.0×10^{-9}/year = 1 in > 10 million years
2 or more	2.0×10^{-8}	5.0×10^{-13}/year = 1 in > 10 million years
3 or more	1.3×10^{-12}	1.7×10^{-17}/year = 1 in > 10 million years

The results in Table K–73 show that the base-case estimate for the probability of one or more LASV infections occurring, should an MRF earthquake release occur, is 0.010—or roughly a 1-in-100 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections with LASV being estimated at approximately once in 10 million years. This falls in the D frequency category. The frequency of one or more deaths from LASV would also be placed in the category D.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–74, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–74: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for LASV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	382 (4%)	9,618 (96%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–74 show that all input combinations result in an estimated frequency of at least one infection or death from LASV occurring from an earthquake MRF release within the D frequency category, except for about 4% of the estimates for infection that use the literature-based dose-response models.

K.3.4.11 Junin virus

This section discusses the potential for infection with JUNV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30m from the release point at the centerline of the simulated plume, was approximately 0.0066 PFU for the urban site. The estimates decrease steadily with distance away from the release, down to approximately 1×10^{-5} PFU at 1 km on the centerline. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average dose, the MEI exposure estimate of 0.0066 PFU can be interpreted to mean that for people very close to a release and in

the path of the plume, the chance of inhaling one or more potentially infectious units of JUNV is less than 1%. This probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of JUNV on the order of 1 PFU. Laboratory nonhuman primates have been infected by doses as low as 32 PFU, and the possibility that even lower doses might also be infective cannot be ruled out in the absence of further data. No literature-based dose-response estimate was derived for JUNV. The expert-based dose-response range includes models that estimate small but non-zero probabilities of infection at an average inhaled dose of 1PFU.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–75. Central estimate calculations for urban site earthquake MRF release of JUNV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	0.0018	1.8×10^{-8}/year = 1 in > 10 million years
2 or more	1.6×10^{-6}	1.6×10^{-11}/year = 1 in > 10 million years
3 or more	9.2×10^{-10}	9.2×10^{-13}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	1.8×10^{-5}	1.8×10^{-10}/year = 1 in > 10 million years
2 or more	1.6×10^{-10}	1.6×10^{-15}/year = 1 in > 10 million years
3 or more	7.5×10^{-16}	9.2×10^{-21}/year = 1 in > 10 million years

The results in Table K–75 show that the base-case estimate for the probability of one or more JUNV infections occurring, should an MRF earthquake release occur, is 0.0018, or roughly a 1-in-600 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake

release, leads to the frequency of occurrence of one or more initial infections or deaths from JUNV being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–76, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–76: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for JUNV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Expert-based	1 or more	0	0	58 (1%)	9,942 (99%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–76 show that all input combinations result in an estimated frequency of at least one infection or death occurring from an earthquake MRF release within the D frequency category, except for about 1% of the estimates for infection that fall in the C frequency category. All results for the frequency of the occurrence of multiple infections and for mortalities fall in the D frequency category.

K.3.4.12 Tick-borne encephalitis virus, Far Eastern subtype

This section discusses the potential for infection with TBEV-FE among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.066 MID_{50} for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 1×10^{-4} MID_{50} at 1 km on the centerline. As stated and explained in Appendix J, it is conservatively assumed that exposure estimates in units of MID_{50} can be applied to dose-response estimates that are in units of PFU for TBEV-FE. If it is assumed 1 PFU represents a single, potentially infectious unit, and under the assumption of Poisson-distributed variability around the average dose, an

MEI exposure estimate of 0.066 PFU can be interpreted to mean that, for people very close to a release and in the path of the plume, the chance of inhaling one or more potentially infectious units of TBEV-FE is, at most, about 7%, and this probability becomes significantly lower for individuals located farther away.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling doses of TBEV-FE on the order of 1 PFU. No low-dose inhalational animal data were found in the literature, so no literature-based dose-response estimate was derived for TBEV-FE. The expert-based dose-response range includes models that estimate small but non-zero probabilities of infection at an average inhaled dose of 1PFU.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–77. Central estimate calculations for urban site earthquake MRF release of TBEV-FE.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	2.5×10^{-5}	2.5×10^{-10}/year = 1 in > 10 million years
2 or more	3.0×10^{-10}	3.0×10^{-15}/year = 1 in > 10 million years
3 or more	2.5×10^{-15}	2.5×10^{-20}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	9.8×10^{-6}	9.8×10^{-11}/year = 1 in > 10 million years
2 or more	4.8×10^{-11}	4.8×10^{-16}/year = 1 in > 10 million years
3 or more	2.6×10^{-16}	2.6×10^{-21}/year = 1 in > 10 million years

The results in Table K–77 show that the base-case estimate for the probability of one or more TBEV-FE infections occurring, should an MRF earthquake release occur, is 2.5×10^{-5}, or roughly a 1-in-40,000

chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from TBEV-FE being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–78, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–78: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for TBEV-FE.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Expert-based	1 or more	0	0	412 (4%)	9,588 (96%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–78 show that all input combinations result in an estimated frequency of at least one infection occurring from an earthquake within the D frequency category, except for about 4% of the estimates that fall in the C frequency category. All results for the frequency of the occurrence of multiple infections and for mortalities fell in the D frequency category.

K.3.4.13 Nipah virus

This section discusses the potential for infection with NIPV among members of the public after an MRF earthquake release. In Chapter 4, the estimated average exposure for an MEI, an individual located 30 m from the release point at the centerline of the simulated plume, was approximately 0.013 $CCID_{50}$ or PFU for the urban site, and the estimates decrease steadily with distance away from the release, down to approximately 3×10^{-5} $CCID_{50}$ or PFU at 1 km on the centerline.

As described in the dose-response appendix (Appendix J), there is no direct evidence that humans have become infected after inhaling low doses of NIPV. The lowest dose of NIPV that infected ferrets by the oral-nasal route of exposure was about 500 $CCID_{50}$. Because low-dose exposure data are scarce, it is possible that much lower doses could result in a low but non-zero probability of infection. This is reflected in the dose-response models fit to the available data and to the expert estimates.

Frequency of Initial Infections – Central Estimate

The calculations described in Section K.2.3.3 were applied to the central estimate inputs described in Section K.2.3.2.

Table K–79. Central estimate calculations for urban site earthquake MRF release of NIPV.

Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections		
1 or more	6.5×10^{-4}	6.5×10^{-9}/year = 1 in > 10 million years
2 or more	2.1×10^{-7}	2.1×10^{-12}/year = 1 in > 10 million years
3 or more	4.5×10^{-11}	4.5×10^{-16}/year = 1 in > 10 million years
Deaths among initially infected		
1 or more	4.5×10^{-4}	4.5×10^{-9}/year = 1 in > 10 million years
2 or more	1.0×10^{-7}	1.1×10^{-12}/year = 1 in > 10 million years
3 or more	1.5×10^{-11}	1.5×10^{-16}/year = 1 in > 10 million years

The results in Table K–79 show that the base-case estimate for the probability of one or more NIPV infections occurring, should an MRF earthquake release occur, is 6.5×10^{-4}, or roughly a 1-in-2,000 chance. This result, combined with the central estimate estimated frequency of occurrence of an MRF earthquake release, leads to the frequency of occurrence of one or more initial infections or deaths from NIPV being placed in the D frequency category.

Frequency of Initial Infections – Uncertainty

The uncertainty procedure described in Section K.2.3.4 was applied. Uncertainty ranges for each input parameter are described in Section K.2.3.2. The results, presented in Table K–80, display how many different input combinations (of 10,000) lead to a frequency estimate in each category. These results provide a sense of the uncertainty associated with estimating the frequency of a given event occurring.

Table K–80: Percentage of results for the frequency of maximum earthquake releases leading to the given number of initial infections for NIPV.

Dose-response estimate	Number of initial infections or deaths	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections					
Literature-based	1 or more	0	0	31 (< 1%)	9,969 (> 99%)
	2 or more	0	0	12 (< 1%)	9,988 (>9 9%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)
Deaths among initially infected					
Literature-based	1 or more	0	0	27 (< 1%)	9,973 (>99%)
	2 or more	0	0	6 (<0.1%)	9,994 (> 99.9%)
	3 or more	0	0	0	10,000 (100%)
Expert-based	1 or more	0	0	0	10,000 (100%)
	2 or more	0	0	0	10,000 (100%)
	3 or more	0	0	0	10,000 (100%)

The results in Table K–80 show that all input combinations result in an estimated frequency of at least one infection occurring from an earthquake MRF release within the D frequency category, except for a small percentage (< 1%) of the results from the estimates that use the literature-based dose-response estimates, which fall in the C frequency category. The results for the frequency of mortalities are similar.

K.3.4.14 Results summary – urban site

This section summarizes the results from Sections K.3.4.1–K.3.4.13. The base-case example results are shown in Tables K–81 and K–82, while the uncertainty results are shown in Tables K–83 and K–84.

Table K–81. Central estimate initial infection and mortality results for urban MRF earthquake release, BSL-3 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
B. anthracis	1 or more	5.3×10^{-5}	5.3×10^{-10}/year = 1 in > 10 million years
F. tularensis	1 or more	2.1×10^{-2}	2.1×10^{-7}/year ≈ 1 in 5 million years
Y. pestis	1 or more	1.4×10^{-6}	1.4×10^{-11}/year = 1 in > 10 million years
1918 H1N1V	1 or more	4.5×10^{-4}	4.5×10^{-9}/year = 1 in > 10 million years
SARS-CoV	1 or more	6.3×10^{-4}	6.3×10^{-9}/year = 1 in > 10 million years
RVFV	1 or more	0.84	8.4×10^{-6}/year ≈ 1 in 100,000 years
	2 or more	0.54	5.4×10^{-6}/year ≈ 1 in 200,000 years
	3 or more	0.27	4.6×10^{-6}/year ≈ 1 in 400,000 years
	4 or more	0.11	1.1×10^{-6}/year ≈ 1 in 900,000 years
	5 or more	0.038	3.8×10^{-7}/year ≈ one in 3 million years
ANDV	1 or more	2.8×10^{-4}	2.8×10^{-9}/year = one in > 10 million years
Deaths among initially infected			
B. anthracis	1 or more	2.4×10^{-5}	2.4×10^{-10}/year = 1 in > 10 million years
F. tularensis	1 or more	4.3×10^{-4}	4.3×10^{-9}/year = 1 in > 10 million years
Y. pestis	1 or more	2.1×10^{-7}	2.1×10^{-12}/year = 1 in > 10 million years
1918 H1N1V	1 or more	1.1×10^{-5}	1.1×10^{-10}/year = 1 in > 10 million years
SARS-CoV	1 or more	6.3×10^{-5}	6.3×10^{-10}/year = 1 in > 10 million years
RVFV	1 or more	3.6×10^{-2}	3.6×10^{-7}/year ≈ 1 in 3 million years
ANDV	1 or more	1.4×10^{-4}	1.4×10^{-9}/year = 1 in > 10 million years

Table K–82. Central estimate initial infection and mortality results for urban MRF earthquake release, BSL-4 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu \geq x$) or deaths ($\delta \geq x$)	Frequency of release leading to x or more initial infections or deaths ($\lambda \geq x$)
Initial infections			
EBOV	1 or more	1.7×10^{-2}	1.7×10^{-7}/year ≈ 1 in 6 million years
MARV	1 or more	3.4×10^{-3}	3.4×10^{-8}/year = 1 in > 10 million years
LASV	1 or more	1.0×10^{-2}	1.0×10^{-7}/year ≈ 1 in 10 million years
JUNV	1 or more	1.8×10^{-3}	1.8×10^{-8}/year = 1 in > 10 million years
TBEV-FE	1 or more	2.5×10^{-5}	2.5×10^{-10}/year = 1 in > 10 million years
NIPV	1 or more	6.5×10^{-4}	6.5×10^{-9}/year = 1 in > 10 million years
Deaths among initially infected			
EBOV	1 or more	1.5×10^{-2}	1.5×10^{-7}/year ≈ 1 in 7 million years
MARV	1 or more	3.4×10^{-3}	3.4×10^{-8}/year = 1 in > 10 million years
LASV	1 or more	2.0×10^{-4}	2.0×10^{-9}/year = 1 in > 10 million years
JUNV	1 or more	1.8×10^{-5}	1.8×10^{-10}/year = 1 in > 10 million years
TBEV-FE	1 or more	9.8×10^{-6}	9.8×10^{-11}/year = 1 in > 10 million years
NIPV	1 or more	4.5×10^{-4}	4.5×10^{-9}/year = 1 in > 10 million years

Table K–83: Summary of uncertainty results for BSL-3 pathogens (MRF release, urban site).

Pathogen	Dose-Response Estimate	Number of Initial Infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Literature	1 or more	0	0	1,760 (18%)	8,240 (82%)
		2 or more	0	0	0	10,000 (100%)
	Expert	1 or more	0	0	196 (2%)	9,804 (98%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Expert	1 or more	0	0	755 (8%)	9,245 (92%)
		2 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Literature	1 or more	0	0	9,638 (96%)	362 (4%)
		2 or more	0	0	8,743 (87%)	1,257 (13%)
		3 or more	0	0	7,430 (74%)	2,570 (26%)
		4 or more	0	0	5,668 (57%)	4,332 (43%)
		5 or more	0	0	3,700 (37%)	6,300 (63%)
		10 or more	0	0	182 (2%)	9,818 (98%)
	Expert	1 or more	0	0	355 (4%)	9,645 (96%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
Deaths among initially infected						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Expert	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Literature	1 or more	0	0	3,039 (30%)	6,961 (70%)
		2 or more	0	0	0	10,000 (100%)
	Expert	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)

Table K–84: Summary of uncertainty results for BSL-4 pathogens (MRF release, urban site).

Pathogen	Dose-Response Estimate	Number of Initial Infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections						
EBOV	Literature	1 or more	0	0	1,333 (13%)	8,667 (87%)
	Expert	1 or more	0	0	0	10,000 (100%)
MARV	Literature	1 or more	0	0	4 (<0.1%)	9,996 (> 99.9%)
	Expert	1 or more	0	0	0	10,000 (100%)
LASV	Literature	1 or more	0	0	382 (4%)	9,618 (96%)
	Expert	1 or more	0	0	0	10,000 (100%)
JUNV	Expert	1 or more	0	0	58 (1%)	9,942 (99%)
TBEV-FE	Expert	1 or more	0	0	412 (4%)	9,588 (96%)
NIPV	Literature	1 or more	0	0	31 (< 1%)	9,969 (> 99%)
	Expert	1 or more	0	0	0	10,000 (100%)
Deaths among initially infected						
EBOV	Literature	1 or more	0	0	1,098 (11%)	8,902 (89%)
	Expert	1 or more	0	0	0	10,000 (100%)
MARV	Literature	1 or more	0	0	4 (<0.1%)	9,996 (> 99.9%)
	Expert	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Expert	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Expert	1 or more	0	0	0	10,000 (100%)
NIPV	Both	1 or more	0	0	0	10,000 (100%)

K.3.4.15 Results summary – urban residents

The results summarized in the previous section were calculated using the overall estimated urban population including estimates of area residents as well as daytime students, workers, hospital patients, and passersby in a 1km radius. Another set of results was calculated for population inputs restricted to estimates of the resident population only. The base-case example results are shown in Tables K–85 and K–86, while the uncertainty results are shown in Tables K–87 and K–88.

Table K–85. Central estimate initial infection and mortality results among urban residents for MRF earthquake release, BSL-3 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
B. anthracis	1 or more	8.7×10^{-6}	2.4×10^{-10}/year = 1 in > 10 million years
F. tularensis	1 or more	3.5×10^{-3}	9.7×10^{-8}/year ≈ 1 in 10 million years
Y. pestis	1 or more	2.3×10^{-7}	6.3×10^{-12}/year = 1 in > 10 million years
1918 H1N1V	1 or more	2.1×10^{-6}	3.1×10^{-9}/year = 1 in > 10 million years
SARS-CoV	1 or more	1.0×10^{-4}	9.5×10^{-9}/year = 1 in > 10 million years
RVFV	1 or more	0.26	2.6×10^{-6}/year ≈ 1 in 400,000 years
	2 or more	0.037	3.7×10^{-7}/year ≈ 1 in 3 million years
ANDV	1 or more	4.6×10^{-5}	4.6×10^{-10}/year = 1 in > 10 million years
Deaths among initially infected			
B. anthracis	1 or more	3.9×10^{-6}	3.9×10^{-11}/year = 1 in > 10 million years
F. tularensis	1 or more	7.0×10^{-5}	7.0×10^{-10}/year = 1 in > 10 million years
Y. pestis	1 or more	3.4×10^{-8}	3.4×10^{-13}/year = 1 in > 10 million years
1918 H1N1V	1 or more	5.2×10^{-8}	5.2×10^{-13}/year = 1 in > 10 million years
SARS-CoV	1 or more	1.0×10^{-5}	1.0×10^{-10}/year = 1 in > 10 million years
RVFV	1 or more	6.0×10^{-3}	6.0×10^{-8}/year = 1 in > 10 million years
ANDV	1 or more	2.3×10^{-5}	2.3×10^{-10}/year = 1 in > 10 million years

Under the base-case inputs, an earthquake MRF release resulting in one or more infections among urban residents would be placed in the D frequency category for every BSL-3 pathogen except for RVFV. It is estimated that one or more RVFV infections among residents would occur with about 26% probability

given a MRF release. A MRF release resulting in multiple RFVF infections among residents would be placed in the D frequency category.

Table K–86. Central estimate initial infection and mortality results among urban residents for MRF earthquake release, BSL-4 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
EBOV	1 or more	2.8×10^{-3}	2.8×10^{-8}/year = 1 in > 10 million years
MARV	1 or more	5.6×10^{-4}	5.6×10^{-9}/year = 1 in > 10 million years
LASV	1 or more	1.6×10^{-3}	1.6×10^{-8}/year = 1 in > 10 million years
JUNV	1 or more	2.9×10^{-4}	2.9×10^{-9}/year = 1 in > 10 million years
TBEV-FE	1 or more	4.8×10^{-9}	4.8×10^{-14}/year = 1 in > 10 million years
NIPV	1 or more	1.1×10^{-4}	1.1×10^{-9}/year = 1 in > 10 million years
Deaths among initially infected			
EBOV	1 or more	2.5×10^{-3}	2.5×10^{-8}/year = 1 in > 10 million years
MARV	1 or more	5.6×10^{-4}	5.6×10^{-9}/year = 1 in > 10 million years
LASV	1 or more	3.3×10^{-5}	3.3×10^{-10}/year = 1 in > 10 million years
JUNV	1 or more	2.9×10^{-6}	2.9×10^{-11}/year = 1 in > 10 million years
TBEV-FE	1 or more	1.9×10^{-9}	1.9×10^{-14}/year = 1 in > 10 million years
NIPV	1 or more	7.4×10^{-5}	7.4×10^{-10}/year = 1 in > 10 million years

Under the base-case inputs, an earthquake MRF release resulting in one or more infections among urban residents would be placed in the D frequency category for every BSL-4 pathogen. The highest estimated probabilities are for EBOV and LASV, for which it is estimated that one or more infections among residents would occur with about 0.2% or 0.3% probability given a MRF release. Combined with the central estimate estimated frequency of the earthquake release (once per 100,000 years), these

probabilities result in an estimated return period for releases leading to infection among residents greater than ten million years.

Table K–87: Summary of uncertainty results for BSL-3 pathogens (MRF release, urban residents).

Pathogen	Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Literature	1 or more	0	0	0	10,000 (100%)
	Expert	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Expert	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Literature	1 or more	0	0	7,275 (73%)	2,725 (27%)
		2 or more	0	0	3,268 (33%)	6,732 (67%)
		3 or more	0	0	430 (4%)	9,570 (96%)
	Expert	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
Deaths among initially infected						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Expert	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Literature	1 or more	0	0	94 (1%)	9,906 (99%)
	Expert	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)

The uncertainty analysis reveals that, except for RVFV under the literature-based dose-response estimates, the majority of input combinations place the frequency of one or more infections with BSL-3 pathogens among urban residents in the D frequency category. The extreme 2.43% of input combinations for *F. tularensis* using the literature-based models fell in the C frequency category, while 10.61% of 1918 H1N1V inputs fell in the C frequency category. For RVFV, the median number of infections among urban residents expected across all input combinations using the literature based model was about 3, with 95% of estimates falling between about 1 and 9 infections. However, the expert-based dose-response models lead to D frequency category for even one RVFV infection among urban residents.

Table K–88: Summary of uncertainty results for BSL-4 pathogens (MRF release, urban residents).

Pathogen	Dose-response estimate	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections						
EBOV	Literature	1 or more	0	0	2 (< 0.1%)	9,998 (> 99.9%)
	Expert	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Expert	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Expert	1 or more	0	0	0	10,000 (100%)
NIPV	Literature	1 or more	0	0	14 (0.1%)	9,986 (99.9%)
	Expert	1 or more	0	0	0	100%
Deaths among initially infected						
EBOV	Both	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Expert	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Expert	1 or more	0	0	0	10,000 (100%)
NIPV	Literature	1 or more	0	0	10 (0.1%)	9,990 (99.9%)
	Expert	1 or more	0	0	0	10,000 (100%)

The uncertainty analysis reveals that the majority of input combinations place the frequency of one or more infections or deaths with BSL-4 pathogens among urban residents in the D frequency category. Very few input combinations (0.1% or less) for EBOV and NIPV using the literature-based model fell in the C frequency category.

K.3.4.16 Results summary – suburban site

Another set of results was calculated for exposure and population inputs from estimates based on characteristic of the suburban site (see Chapter 4 and Appendix F). The base-case example results are shown in Tables K–89 and K–90, while the uncertainty results are shown in Table K–91.

Table K–89. Central estimate initial infection and mortality results at suburban site for MRF earthquake release, BSL-3 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
B. anthracis	1 or more	1.1×10^{-6}	1.1×10^{-11}/year = 1 in > 10 million years
F. tularensis	1 or more	4.6×10^{-4}	4.6×10^{-9}/year = 1 in > 10 million years
Y. pestis	1 or more	3.0×10^{-8}	3.0×10^{-13}/year = 1 in > 10 million years
1918 H1N1V	1 or more	1.6×10^{-7}	1.6×10^{-12}/year = 1 in > 10 million years
SARS-CoV	1 or more	1.3×10^{-5}	1.3×10^{-10}/year = 1 in > 10 million years
RVFV	1 or more	3.8×10^{-2}	3.8×10^{-7}/year ≈ 1 in 3 million years
ANDV	1 or more	6.0×10^{-6}	6.0×10^{-11}/year = 1 in > 10 million years
Deaths among initially infected			
B. anthracis	1 or more	5.2×10^{-7}	5.2×10^{-12}/year = 1 in > 10 million years
F. tularensis	1 or more	9.2×10^{-6}	9.2×10^{-11}/year = 1 in > 10 million years
Y. pestis	1 or more	4.5×10^{-9}	4.5×10^{-14}/year = 1 in > 10 million years
1918 H1N1V	1 or more	4.0×10^{-7}	4.0×10^{-12}/year = 1 in > 10 million years
SARS-CoV	1 or more	1.3×10^{-6}	1.3×10^{-11}/year = 1 in > 10 million years
RVFV	1 or more	7.8×10^{-4}	7.8×10^{-9}/year = 1 in > 10 million years
ANDV	1 or more	3.0×10^{-6}	3.0×10^{-11}/year = 1 in > 10 million years

Table K–90. Central estimate initial infection and mortality results at suburban site for MRF earthquake release, BSL-4 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
EBOV	1 or more	3.7×10^{-4}	3.7×10^{-9}/year = 1 in > 10 million years
MARV	1 or more	7.4×10^{-5}	7.4×10^{-10}/year = 1 in > 10 million years
LASV	1 or more	2.2×10^{-4}	2.2×10^{-9}/year = 1 in > 10 million years
JUNV	1 or more	3.8×10^{-5}	3.8×10^{-10}/year = 1 in > 10 million years
TBEV-FE	1 or more	2.6×10^{-6}	2.6×10^{-11}/year = 1 in > 10 million years
NIPV	1 or more	1.4×10^{-5}	1.4×10^{-10}/year = 1 in > 10 million years
Deaths among initially infected			
EBOV	1 or more	3.3×10^{-4}	3.3×10^{-9}/year = 1 in > 10 million years
MARV	1 or more	7.4×10^{-5}	7.4×10^{-10}/year = 1 in > 10 million years
LASV	1 or more	4.3×10^{-6}	4.3×10^{-11}/year = 1 in > 10 million years
JUNV	1 or more	3.8×10^{-7}	3.8×10^{-12}/year = 1 in > 10 million years
TBEV-FE	1 or more	1.0×10^{-6}	1.0×10^{-11}/year = 1 in > 10 million years
NIPV	1 or more	9.7×10^{-6}	9.7×10^{-11}/year = 1 in > 10 million years

For the central estimate example, all pathogens are estimated to produce a low probability that one or more infections occur among members of the public at the suburban site. The highest estimated probability is for RVFV (3.8×10^{-2}), which is about a 1-in-30 chance. Combined with the central estimate estimated frequency of the occurrence of an earthquake release (once in 100,000 years), the frequency of an earthquake MRF release resulting in one or more infections of any pathogen at the suburban site is estimated to be in the D frequency category.

Table K–91: Summary of uncertainty results for initial infections and deaths (MRF release, suburban site).

Pathogen	Dose-response estimates	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Both	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Literature	1 or more	0	0	3191 (32%)	6809 (68%)
		2 or more	0	0	0	10,000 (100%)
	Expert	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
EBOV	Both	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Both	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Both	1 or more	0	0	0	10,000 (100%)
NIPF	Both	1 or more	0	0	0	10,000 (100%)
Deaths among initially infected						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Both	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Both	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
EBOV	Both	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Both	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Both	1 or more	0	0	0	10,000 (100%)
NIPF	Both	1 or more	0	0	0	10,000 (100%)

The uncertainty results in the rows labeled *Both* apply for estimates using both the literature-based and the expert-based range of dose-response estimates. Every combination of dose-response estimates and earthquake release frequency estimates results in an estimated frequency of at least one initial infection at the suburban site in frequency category D, except for about 32% of the estimates using the literature-based dose-response models for RVFV.

K.3.4.17 Results summary – rural site

Another set of results was calculated for exposure and population inputs from estimates based on characteristic of the suburban site (see Chapter 4 and Appendix F). The base-case example results are shown in Tables K–92 and K–93, while the uncertainty results are shown in Table K–94.

Table K–92. Central estimate initial infection and mortality at rural site for MRF earthquake release, BSL-3 pathogens.

Pathogen	Number of initial infections / deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
B. anthracis	1 or more	5.8×10^{-7}	5.8×10^{-12}/year = 1 in > 10 million years
F. tularensis	1 or more	2.3×10^{-4}	2.3×10^{-9}/year = 1 in > 10 million years
Y. pestis	1 or more	1.5×10^{-8}	1.5×10^{-13}/year = 1 in > 10 million years
1918 H1N1V	1 or more	9.1×10^{-6}	9.1×10^{-11}/year = 1 in > 10 million years
SARS-CoV	1 or more	6.8×10^{-6}	6.8×10^{-11}/year = 1 in > 10 million years
RVFV	1 or more	1.9×10^{-2}	1.9×10^{-7}/year ≈ 1 in 5 million years
ANDV	1 or more	3.1×10^{-6}	3.1×10^{-11}/year = 1 in > 10 million years
Deaths among initially infected			
B. anthracis	1 or more	2.6×10^{-7}	2.6×10^{-12}/year = 1 in > 10 million years
F. tularensis	1 or more	4.7×10^{-6}	4.7×10^{-11}/year = 1 in > 10 million years
Y. pestis	1 or more	2.3×10^{-9}	2.3×10^{-14}/year = 1 in > 10 million years
1918 H1N1V	1 or more	2.3×10^{-7}	2.3×10^{-12}/year = 1 in > 10 million years
SARS-CoV	1 or more	6.8×10^{-7}	6.8×10^{-12}/year = 1 in > 10 million years
RVFV	1 or more	3.9×10^{-4}	3.9×10^{-9}/year = 1 in > 10 million years
ANDV	1 or more	1.5×10^{-6}	1.5×10^{-11}/year = 1 in > 10 million years

Table K–93. Central estimate initial infection and mortality results at the rural site for MRF earthquake release, BSL-4 pathogens.

Pathogen	Number of initial infections/deaths (x)	Probability of x or more initial infections ($\mu_{\geq x}$) or deaths ($\delta_{\geq x}$) overall	Frequency of release leading to x or more initial infections or deaths ($\lambda_{\geq x}$)
Initial infections			
EBOV	1 or more	1.9×10^{-4}	1.9×10^{-9}/year = 1 in > 10 million years
MARV	1 or more	3.7×10^{-5}	3.7×10^{-10}/year = 1 in > 10 million years
LASV	1 or more	1.1×10^{-4}	1.1×10^{-9}/year = 1 in > 10 million years
JUNV	1 or more	1.9×10^{-5}	1.9×10^{-10}/year = 1 in > 10 million years
TBEV-FE	1 or more	1.3×10^{-6}	1.3×10^{-11}/year = 1 in > 10 million years
NIPV	1 or more	7.0×10^{-6}	7.0×10^{-11}/year = 1 in > 10 million years
Deaths among initially infected			
EBOV	1 or more	1.7×10^{-4}	1.7×10^{-9}/year = 1 in > 10 million years
MARV	1 or more	3.7×10^{-5}	3.7×10^{-10}/year = 1 in > 10 million years
LASV	1 or more	2.2×10^{-6}	2.2×10^{-11}/year = 1 in > 10 million years
JUNV	1 or more	1.9×10^{-7}	1.9×10^{-12}/year = 1 in > 110 million years
TBEV-FE	1 or more	5.4×10^{-7}	5.4×10^{-12}/year = 1 in > 10 million years
NIPV	1 or more	4.9×10^{-6}	4.9×10^{-11}/year = 1 in > 10 million years

For the central estimate example, all pathogens are estimated to produce a low probability that one or more infections occur at the rural site. The highest estimated probability is for RVFV (1.9×10^{-2}), which is about a 1-in-50 chance. Combined with the central estimate estimated frequency of the occurrence of an earthquake release (once in 100,000 years), the frequency of an earthquake MRF release resulting in one or more infections or deaths from any pathogen at the rural site is estimated to fall in the D frequency category.

Table K–94: Summary of uncertainty results for initial infections and deaths (MRF release, rural site).

Pathogen	Dose-response estimates	Number of initial infections	A < 1 per year > 1 per 100 yrs	B < 1 per 100 yrs > 1 per 10K yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
Initial infections						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Both	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Literature	1 or more	0	0	1,744 (17%)	8,256 (83%)
		2 or more	0	0	0	10,000 (100%)
	Expert	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
EBOV	Both	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Both	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Both	1 or more	0	0	0	10,000 (100%)
NIPF	Both	1 or more	0	0	0	10,000 (100%)
Deaths among initially infected						
B. anthracis	Both	1 or more	0	0	0	10,000 (100%)
F. tularensis	Both	1 or more	0	0	0	10,000 (100%)
Y. pestis	Both	1 or more	0	0	0	10,000 (100%)
1918 H1N1V	Both	1 or more	0	0	0	10,000 (100%)
SARS-CoV	Both	1 or more	0	0	0	10,000 (100%)
RVFV	Both	1 or more	0	0	0	10,000 (100%)
ANDV	Both	1 or more	0	0	0	10,000 (100%)
EBOV	Both	1 or more	0	0	0	10,000 (100%)
MARV	Both	1 or more	0	0	0	10,000 (100%)
LASV	Both	1 or more	0	0	0	10,000 (100%)
JUNV	Both	1 or more	0	0	0	10,000 (100%)
TBEV-FE	Both	1 or more	0	0	0	10,000 (100%)
NIPF	Both	1 or more	0	0	0	10,000 (100%)

The uncertainty results in the rows labeled *Both* apply for estimates using both the literature-based and the expert-based range of dose-response estimates. Every combination of dose-response estimates and earthquake release frequency estimates results in an estimated frequency of at least one initial infection at the rural site in frequency category D, except for about 17% of the estimates using the literature-based dose-response models for RVFV.

K.3.5 Medically Vulnerable Sub Populations

In this section, results from calculations described in Section K.2.4 are presented. The results are shown only for the three pathogens that produced the highest estimated probabilities of infection and death under the earthquake MRF release scenario as described in Section K.3.4. These three pathogens also represent the highest estimated infection and death probabilities among bacteria (*F. tularensis*), BSL-3 viruses (RVFV), and BSL-4 viruses (EBOV). Results from these three pathogens are sufficient to demonstrate the estimated influence of the MVSP profile at the three sites in comparison to U.S. average rates of MVSP.

K.3.5.1 Earthquake MRF release overall results adjusted for MVSP

In this section, the earthquake MRF release results presented in Section K.3.4, for which dose-response and case fatality estimates assumed to be applicable to a general U.S. population were used, are compared to adjusted results that apply site-specific MVSP data and estimates for increased susceptibility of MVSP (described in Section K.2.4).

Table K–95. Central estimate results for earthquake MRF release.

Pathogen	Adjusted results using local site MVSP estimates		Baseline results from Section K.3.4	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: initial infections				
F. tularensis	2.1×10^{-2}	≈ 4.7 million years	2.1×10^{-2}	≈ 4.7 million years
RVFV	0.84	≈ 120,000 years	0.84	≈ 120,000 years
EBOV	1.7×10^{-2}	≈ 5.9 million years	1.7×10^{-2}	≈ 5.9 million years
URBAN SITE: deaths among initially infected				
F. tularensis	4.3×10^{-4}	> 10 million years	4.3×10^{-4}	> 10 million years
RVFV	3.6×10^{-2}	≈ 2.8 million years	3.6×10^{-2}	≈ 2.8 million years
EBOV	1.5×10^{-2}	≈ 6.5 million years	1.5×10^{-2}	≈ 6.5 million years
SUBURBAN SITE: initial infections				
F. tularensis	4.5×10^{-4}	> 10 million years	4.6×10^{-4}	> 10 million years
RVFV	3.7×10^{-2}	≈ 2.7 million years	3.8×10^{-2}	≈ 2.6 million years
EBOV	3.6×10^{-4}	> 10 million years	3.7×10^{-4}	> 10 million years
SUBURBAN SITE: deaths among initially infected				
F. tularensis	8.9×10^{-6}	> 10 million years	9.3×10^{-6}	> 10 million years

Pathogen	Adjusted results using local site MVSP estimates		Baseline results from Section K.3.4	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
RVFV	7.5×10^{-4}	> 10 million years	7.8×10^{-4}	> 10 million years
EBOV	3.3×10^{-4}	> 10 million years	3.3×10^{-4}	> 10 million years
RURAL SITE: initial infections				
F. tularensis	2.4×10^{-4}	> 10 million years	2.3×10^{-4}	> 10 million years
RVFV	2.0×10^{-2}	≈ 5.0 million years	1.9×10^{-2}	≈ 5.1 million years
EBOV	1.9×10^{-4}	> 10 million years	1.9×10^{-4}	> 10 million years
RURAL SITE: deaths among initially infected				
F. tularensis	5.0×10^{-6}	> 10 million years	4.7×10^{-6}	> 10 million years
RVFV	4.2×10^{-4}	> 10 million years	3.9×10^{-4}	> 10 million years
EBOV	1.8×10^{-4}	> 10 million years	1.7×10^{-4}	> 10 million years

For the urban site, adjusting the overall results according to local proportions of MVSP does not change the estimates (to two significant Tables) for probability and frequency of at least one infection or death. This result can be explained by the observation that, while the estimated proportions of people with diabetes and HIV/AIDS are higher at the urban site than the U.S. averages, the estimated proportions of children under five and adults over 65 are lower.

For the suburban site, the estimated probabilities of at least one infection or death are slightly lower after adjusting for the local proportions of MVSP. The largest contributor to this small difference is the estimated suburban site proportion of adults over 65 years of age, which is just over half the U.S. average proportion. However, these new results do not change the assigned frequency category for the occurrence of a MRF release resulting in one or more infections or deaths at the suburban site (D frequency category).

For the rural site, the estimated probabilities of at least one infection or death are slightly higher after adjusting for the local proportions of MVSP. The largest contributor to this small difference is the estimated rural site proportion of adults over 65, which is appreciably higher than U.S. average proportion. These results do not change the assigned frequency category for the occurrence of a MRF release resulting in one or more infections or deaths at the rural site (D frequency category).

It is noted that the local site MVSP adjustments for the probability of deaths has a smaller effect on the estimates for EBOV than for *F. tularensis* and RVFV. This can be explained by the fact that the estimated EBOV case fatality rate is already quite high, even for healthy adults, so there is little room for increase when the estimates of MVSP-specific case fatality rates are calculated.

Because the inputs for susceptibility of MVSP relative to healthy adults shown in Table K–8 are not based on data, it is possible that they significantly underestimate the true differences in susceptibility. However, the results shown in Table K–95 exhibit very low sensitivity to those inputs. For example, even if the relative susceptibility values in Table K–8 are multiplied by a factor of 100, the results do not change appreciably, and all estimated return periods correspond to the same frequency categories.

Table K–96. Uncertainty results for earthquake MRF release (frequency categories for release leading to one or more initial infections or deaths).

Pathogen	Adjusted results using local site MVSP estimates		Baseline results from Section K.3.4	
	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
URBAN SITE: initial infections				
F. tularensis	1,768 (18%)	8,232 (82%)	1,760 (18%)	8,240 (82%)
RVFV	9,640 (96%)	360 (4%)	9,638 (96%)	362 (4%)
EBOV	1,341 (13%)	8,659 (87%)	1,333 (13%)	8,667 (87%)
URBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	3,057 (31%)	6,943 (69%)	3,039 (30%)	6,961 (70%)
EBOV	1,108 (11%)	8,892 (89%)	1,098 (11%)	8,902 (89%)
SUBURBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	3,138 (31%)	6,862 (69%)	3,191 (32%)	6,809 (68%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	1,814 (18%)	8,186 (82%)	1744 (17%)	8,256 (83%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

K.3.5.2 Earthquake MRF release results for each MVSP

This section contains results for estimated probabilities and frequencies of initial infections and deaths among members of each individual MVSP at each site. Each set of results is compared to what the results would be if the population at each site was the same estimated size but with MVSP in line with overall U.S. proportions.

Children under 5

Table K–97. Central estimate results for earthquake MRF release among children under 5.

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: initial infections				
F. tularensis	1.5×10^{-3}	> 10 million years	1.8×10^{-3}	> 10 million years
RVFV	0.12	≈ 830,000 years	0.14	≈ 720,000 years
EBOV	1.2×10^{-3}	> 10 million years	1.4×10^{-4}	> 10 million years
URBAN SITE: deaths among initially infected				
F. tularensis	3.3×10^{-5}	> 10 million years	3.9×10^{-5}	> 10 million years
RVFV	2.8×10^{-3}	> 10 million years	3.3×10^{-3}	> 10 million years
EBOV	1.1×10^{-3}	> 10 million years	1.3×10^{-4}	> 10 million years
SUBURBAN SITE: initial infections				
F. tularensis	3.0×10^{-5}	> 10 million years	3.8×10^{-5}	> 10 million years
RVFV	2.6×10^{-3}	> 10 million years	3.2×10^{-3}	> 10 million years
EBOV	2.4×10^{-5}	> 10 million years	3.1×10^{-5}	> 10 million years
SUBURBAN SITE: deaths among initially infected				
F. tularensis	6.7×10^{-7}	> 10 million years	8.4×10^{-7}	> 10 million years
RVFV	5.6×10^{-5}	> 10 million years	7.0×10^{-5}	> 10 million years
EBOV	2.3×10^{-5}	> 10 million years	2.8×10^{-5}	> 10 million years
RURAL SITE: initial infections				
F. tularensis	1.6×10^{-5}	> 10 million years	1.9×10^{-5}	> 10 million years
RVFV	1.4×10^{-3}	> 10 million years	1.6×10^{-3}	> 10 million years
EBOV	1.3×10^{-5}	> 10 million years	1.6×10^{-5}	> 10 million years
RURAL SITE: deaths among initially infected				
F. tularensis	3.6×10^{-7}	> 10 million years	4.2×10^{-7}	> 10 million years
RVFV	3.0×10^{-5}	> 10 million years	3.6×10^{-5}	> 10 million years
EBOV	1.2×10^{-5}	> 10 million years	1.4×10^{-5}	> 10 million years

The results in the left columns of this table are the central estimate estimated probability and return period for initial infection and mortality among children under 5 years of age at each site after an earthquake MRF release. The calculations of these values incorporated the estimated proportion of children under five present at each site and increased vulnerability of children under five to disease and death. At each site, the estimated return periods would be placed in the D frequency category, except for at least one initial infection with RVFV at the urban site that would be placed in the C frequency category.

The results in the right columns are displayed for comparison purposes—they are the equivalent estimates for a site with the same population size but with a typical U.S. rate of children under five. There are some small differences across each row, but all of the return periods still fall in the same frequency category across each row. The direction of the small differences in each row reflect the fact that all three sites were estimated to have a smaller-than-average proportion of children under five.

Table K–98. Uncertainty results for earthquake MRF release among children under 5 (frequency categories for release leading to one or more initial infections or deaths).

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs >1 per 1M yrs	D < 1 per 1M yrs	C < 1 per 10K yrs >1 per 1M yrs	D < 1 per 1M yrs
URBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	5,643 (56%)	4,357 (44%)	5,974 (60%)	4,026 (40%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
URBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	12 (0.1%)	9,988 (99.9%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	6 (0.1%)	9,994 (99.9%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

The uncertainty analysis reveals that about 56% of input combinations result in the estimated frequency of one or more infections with RVFV among children under five at the urban site in the C frequency category. All other results are D frequency category. If the U.S. proportion of children under five were

applicable to the urban site population, the percentage of results in the C frequency category would increase slightly.

Adults over 65

Table K–99. Central estimate results for earthquake MRF release among adults over 65.

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: initial infections				
F. tularensis	3.6×10^{-3}	> 10 million years	3.6×10^{-3}	> 10 million years
RVFV	0.27	≈ 380,000 years	0.27	≈ 380,000 years
EBOV	2.9×10^{-3}	> 10 million years	2.9×10^{-3}	> 10 million years
URBAN SITE: deaths among initially infected				
F. tularensis	1.0×10^{-4}	> 10 million years	1.0×10^{-4}	> 10 million years
RVFV	8.4×10^{-3}	> 10 million years	8.4×10^{-3}	> 10 million years
EBOV	2.8×10^{-3}	> 10 million years	2.8×10^{-3}	> 10 million years
SUBURBAN SITE: initial infections				
F. tularensis	5.1×10^{-5}	> 10 million years	7.9×10^{-5}	> 10 million years
RVFV	4.3×10^{-3}	> 10 million years	6.6×10^{-3}	> 10 million years
EBOV	4.1×10^{-5}	> 10 million years	6.3×10^{-5}	> 10 million years
SUBURBAN SITE: deaths among initially infected				
F. tularensis	1.4×10^{-6}	> 10 million years	2.2×10^{-6}	> 10 million years
RVFV	1.2×10^{-4}	> 10 million years	1.8×10^{-4}	> 10 million years
EBOV	4.0×10^{-5}	> 10 million years	6.1×10^{-5}	> 10 million years
RURAL SITE: initial infections				
F. tularensis	6.9×10^{-5}	> 10 million years	4.0×10^{-5}	> 10 million years
RVFV	5.7×10^{-3}	> 10 million years	3.3×10^{-3}	> 10 million years
EBOV	5.5×10^{-5}	> 10 million years	3.2×10^{-5}	> 10 million years
RURAL SITE: deaths among initially infected				
F. tularensis	1.9×10^{-6}	> 10 million years	1.1×10^{-6}	> 10 million years
RVFV	1.6×10^{-4}	> 10 million years	9.1×10^{-5}	> 10 million years
EBOV	5.3×10^{-5}	> 10 million years	3.1×10^{-5}	> 10 million years

The results in the left columns of this table are the central estimate estimated probability and return period for initial infection and mortality among adults over 65 years of age at each site after an earthquake MRF release. The calculations of these values incorporated the estimated proportion of adults over 65 present at each site and increased vulnerability of adults over 65 to disease and death. At each site, the estimated return periods would be placed in the D frequency category, except for infections at the urban site, for which the estimated return period is in the C frequency category.

The results in the right columns are displayed for comparison purposes – they are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of adults over 65. Although there are some small differences across each row, all of the return periods fall in the same frequency category across each row. The direction of the small differences in each row reflects the fact that the urban and suburban sites were estimated to have a smaller-than-average proportion of adults over 65 and the rural site a higher-than-average proportion.

Table K–100. Uncertainty results for earthquake MRF release among adults over 65 (frequency categories for release leading to one or more initial infections or deaths).

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs > 1 per 1M yrs	**D** < 1 per 1M yrs	**C** < 1 per 10K yrs >1 per 1M yrs	**D** < 1 per 1M yrs
URBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	7322 (73%)	2,678 (27%)	7326 (73%)	2,674 (27%)
EBOV	2 (< 0.1%)	9,998 (> 99.9%)	2 (< 0.1%)	9,998 (> 99.9%)
URBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	293 (3%)	9,707 (97%)	298 (3%)	9,702 (97%)
EBOV	2 (< 0.1%)	9,998 (> 99.9%)	2 (<0.1%)	9,998 (> 99.9%)
SUBURBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	27 (0.3%)	9,973 (99.7%)	129 (1%)	9,871 (99%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs	C < 1 per 10K yrs >1 per 1M yrs	D < 1 per 1M yrs
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	80 (1%)	9,920 (99%)	11 (0.1%)	9,989 (99.9%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

The uncertainty analysis reveals that about 73% of input combinations result in the estimated frequency of one or more infections with RVFV among adults over 65 at the urban site in the C frequency category. All other results are in the D frequency category, except for small (less than 5%) portions of results RVFV deaths at the urban site, EBOV infections at the urban site, and RVFV infections at the suburban and rural sites. If the U.S. proportion of adults over 65 were applicable to each population, the percentage of results in the C frequency category are not appreciably different.

People with diabetes

Table K–101. Central estimate results for earthquake MRF release among people with diabetes.

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: initial infections				
F. tularensis	2.1×10^{-3}	> 10 million years	1.4×10^{-3}	> 10 million years
RVFV	0.16	≈ 640,000 years	0.11	≈ 910,000 years
EBOV	1.6×10^{-3}	> 10 million years	1.1×10^{-3}	> 10 million years

URBAN SITE: deaths among initially infected				
F. tularensis	4.7×10^{-5}	> 10 million years	3.3×10^{-5}	> 10 million years
RVFV	3.9×10^{-3}	> 10 million years	2.7×10^{-3}	> 10 million years
EBOV	1.5×10^{-3}	> 10 million years	1.0×10^{-3}	> 10 million years
SUBURBAN SITE: initial infections				
F. tularensis	4.2×10^{-5}	> 10 million years	3.1×10^{-5}	> 10 million years
RVFV	3.4×10^{-3}	> 10 million years	2.5×10^{-3}	> 10 million years
EBOV	3.3×10^{-5}	> 10 million years	2.4×10^{-5}	> 10 million years
SUBURBAN SITE: deaths among initially infected				
F. tularensis	9.6×10^{-7}	> 10 million years	7.0×10^{-7}	> 10 million years
RVFV	7.8×10^{-5}	> 10 million years	5.7×10^{-5}	> 10 million years
EBOV	3.0×10^{-5}	> 10 million years	2.2×10^{-5}	> 10 million years
RURAL SITE: initial infections				
F. tularensis	1.8×10^{-5}	> 10 million years	1.6×10^{-5}	> 10 million years
RVFV	1.5×10^{-3}	> 10 million years	1.3×10^{-3}	> 10 million years
EBOV	1.4×10^{-5}	> 10 million years	1.2×10^{-5}	> 10 million years
RURAL SITE: deaths among initially infected				
F. tularensis	4.1×10^{-7}	> 10 million years	3.6×10^{-7}	> 10 million years
RVFV	3.3×10^{-5}	> 10 million years	2.9×10^{-5}	> 10 million years
EBOV	1.3×10^{-5}	> 10 million years	1.1×10^{-5}	> 10 million years

The results in the left columns of this table are the central estimate estimated probability and return period for initial infection and mortality among people with diabetes at each site after an earthquake MRF release. The calculations of these values incorporated the estimated proportion of people with diabetes present at each site and increased vulnerability of people with diabetes to disease and death. At each site, the estimated return periods would be placed in the D frequency category except for initial infections with RVFV at the urban site, which places it in the C frequency category.

The results in the right columns are displayed for comparison purposes. They are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of people with diabetes. While there are some small differences across each row, all of the return periods fall in the same frequency category across each. The directions of the small differences in each row reflect the fact that all three sites were estimated to have a higher-than-average proportion of people with diabetes.

Table K–102. Uncertainty results for earthquake MRF release among people with diabetes (frequency categories for release leading to one or more initial infections or deaths).

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
URBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	6211 (62%)	3,789 (38%)	5431 (54%)	4569 (46%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
URBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	22 (0.2%)	9,978 (99.8%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	14 (0.1%)	9,986 (99.9%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

The uncertainty analysis reveals that about 62% of input combinations result in the estimated frequency of one or more infections with RVFV among people with diabetes at the urban site in the C frequency category, while all other results are in the D frequency category, except for a small (< 1%) portion of results for RVFV deaths at the urban site and RVFV infections at the suburban site. If the U.S. proportion

of people with diabetes were applicable to the urban site population, the percentage of results in the C frequency category would drop to 54%.

People with HIV/AIDS

Table K–103. Central estimate results for earthquake MRF release among people with HIV/AIDS.

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: initial infections				
F. tularensis	1.4×10^{-4}	> 10 million years	1.1×10^{-4}	> 10 million years
RVFV	1.1×10^{-2}	≈ 8.7 million years	9.1×10^{-3}	> 10 million years
EBOV	1.1×10^{-4}	> 10 million years	8.7×10^{-5}	> 10 million years
URBAN SITE: deaths among initially infected				
F. tularensis	3.2×10^{-6}	> 10 million years	2.6×10^{-6}	> 10 million years
RVFV	2.7×10^{-4}	> 10 million years	2.2×10^{-4}	> 10 million years
EBOV	1.0×10^{-4}	> 10 million years	8.2×10^{-5}	> 10 million years
SUBURBAN SITE: initial infections				
F. tularensis	1.2×10^{-6}	> 10 million years	2.3×10^{-6}	> 10 million years
RVFV	9.8×10^{-5}	> 10 million years	2.0×10^{-4}	> 10 million years
EBOV	9.4×10^{-7}	> 10 million years	1.9×10^{-6}	> 10 million years
SUBURBAN SITE: deaths among initially infected				
F. tularensis	2.8×10^{-8}	> 10 million years	5.5×10^{-8}	> 10 million years
RVFV	2.3×10^{-6}	> 10 million years	4.6×10^{-6}	> 10 million years
EBOV	8.8×10^{-7}	> 10 million years	1.8×10^{-6}	> 10 million years
RURAL SITE: initial infections				
F. tularensis	3.2×10^{-7}	> 10 million years	1.2×10^{-6}	> 10 million years
RVFV	2.7×10^{-5}	> 10 million years	9.9×10^{-5}	> 10 million years
EBOV	2.6×10^{-7}	> 10 million years	9.5×10^{-7}	> 10 million years
RURAL SITE: deaths among initially infected				
F. tularensis	7.7×10^{-9}	> 10 million years	2.8×10^{-8}	> 10 million years
RVFV	6.5×10^{-7}	> 10 million years	2.3×10^{-6}	> 10 million years
EBOV	2.4×10^{-7}	> 10 million years	8.9×10^{-7}	> 10 million years

The results in the left columns of this table are the central estimate estimated probability and return period for initial infection and mortality among people with HIV/AIDS at each site after an earthquake MRF release. The calculations of these values incorporate the estimated proportion of people with HIV/AIDS present at each site and increased vulnerability of people with HIV/AIDS to disease and death. At each site, the estimated return periods would be placed in the D frequency category.

The results in the right columns are displayed for comparison purposes. They are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of people with HIV/AIDS. Although there are some small differences across each row, all of the return periods still fall in the D frequency category. The direction of the small differences in each row reflect the fact that the urban site was estimated to have a higher-than-average proportion of people with HIV/AIDS and the suburban and rural sites a lower-than-average proportion.

Table K–104. Uncertainty results for earthquake MRF release among people with HIV/AIDS (frequency categories for release leading to one or more initial infections or deaths).

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs > 1 per 1M yrs	**D** < 1 per 1M yrs	**C** < 1 per 10K yrs > 1 per 1M yrs	**D** < 1 per 1M yrs
URBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	699 (7%)	9,301 (93%)	383 (4%)	9,617 (96%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
URBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: initial infections,				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
RURAL SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

The uncertainty analysis reveals that about 7% of input combinations result in the estimated frequency of one or more infections with RVFV among people with HIV/AIDS at the urban site in the C frequency category, while all other results are in the D frequency category. If the U.S. proportion of people with HIV/AIDS were applicable to the urban site population, the portion of results in the C frequency category would drop to 4%.

Pregnant women

Table K–105. Central estimate results for earthquake MRF release among pregnant women.

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
URBAN SITE: initial infections				
F. tularensis	2.5×10^{-4}	> 10 million years	2.5×10^{-4}	> 10 million years
RVFV	2.4×10^{-2}	≈ 4.1 million years	2.4×10^{-2}	≈ 4.1 million years
EBOV	2.3×10^{-4}	> 10 million years	2.3×10^{-4}	> 10 million years
URBAN SITE: deaths among initially infected				
F. tularensis	5.5×10^{-6}	> 10 million years	5.5×10^{-6}	> 10 million years
RVFV	5.4×10^{-4}	> 10 million years	5.4×10^{-4}	> 10 million years
EBOV	2.2×10^{-4}	> 10 million years	2.2×10^{-4}	> 10 million years

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths	Probability of 1 or more initial infections or deaths	Return period of release leading to 1 or more initial infections or deaths
SUBURBAN SITE: initial infections				
F. tularensis	5.4×10^{-6}	> 10 million years	5.4×10^{-6}	> 10 million years
RVFV	5.2×10^{-4}	> 10 million years	5.2×10^{-4}	> 10 million years
EBOV	5.0×10^{-6}	> 10 million years	5.0×10^{-6}	> 10 million years
SUBURBAN SITE: deaths among initially infected				
F. tularensis	1.2×10^{-7}	> 10 million years	1.2×10^{-7}	> 10 million years
RVFV	1.1×10^{-5}	> 10 million years	1.1×10^{-5}	> 10 million years
EBOV	4.6×10^{-6}	> 10 million years	4.6×10^{-6}	> 10 million years
RURAL SITE: initial infections				
F. tularensis	2.7×10^{-6}	> 10 million years	2.7×10^{-6}	> 10 million years
RVFV	2.6×10^{-4}	> 10 million years	2.6×10^{-4}	> 10 million years
EBOV	2.5×10^{-6}	> 10 million years	2.5×10^{-6}	> 10 million years
RURAL SITE: deaths among initially infected				
F. tularensis	6.0×10^{-8}	> 10 million years	6.0×10^{-8}	> 10 million years
RVFV	5.8×10^{-6}	> 10 million years	5.8×10^{-6}	> 10 million years
EBOV	2.3×10^{-6}	> 10 million years	2.3×10^{-6}	> 10 million years

The results in the left columns of this table are the central estimate estimated probability and return period for initial infection and mortality among pregnant women at each site after an earthquake MRF release. The calculation of these values incorporated the estimated proportion of pregnant women present at each site and their increased vulnerability to disease and death. At each site, the estimated return periods would be placed in the D frequency category.

The results in the right columns are displayed for comparison purposes. They are the equivalent estimates for a site with the same population size but with a typical U.S. proportion of pregnant women. There are no differences across any of the rows, because the proportion of pregnant women at each site and in the U.S. overall was estimated to be the same (1% of the population).

Table K–106. Uncertainty results for earthquake MRF release among pregnant women (frequency categories for release leading to one or more initial infections or deaths).

Pathogen	Results using local site MVSP estimates		Results using U.S. average MVSP estimates	
	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs	C < 1 per 10K yrs > 1 per 1M yrs	D < 1 per 1M yrs
URBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	2,186 (22%)	7,814 (78%)	2,186 (22%)	7,814 (78%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
URBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
SUBURBAN SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: initial infections				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)
RURAL SITE: deaths among initially infected				
F. tularensis	0	10,000 (100%)	0	10,000 (100%)
RVFV	0	10,000 (100%)	0	10,000 (100%)
EBOV	0	10,000 (100%)	0	10,000 (100%)

The uncertainty analysis reveals that about 22% of input combinations result in the estimated frequency of one or more infections with RVFV among pregnant women at the urban site in the C frequency category, while all other results are in the D frequency category. The U.S. proportion of pregnant women was estimated to be the same as that of the urban site, so the results in the right-hand columns are the same.

Because the inputs for susceptibility of MVSP relative to healthy adults shown in Table K–6 are not based on data, it is possible that they significantly underestimate the true differences in susceptibility. The numerical results in Tables K–97 to K–106 are sensitive to these inputs. The largest change in the results for a particular MVSP would occur if the relative susceptibility of that MVSP is increased and the relative susceptibility of all other MVSP remains the same, in which case the particular MVSP would be disproportionately affected relative to not only healthy adults, but also to the other MVSP. If the relative sensitivities for all MVSP are increased at the same time, then the estimated risk to each MVSP would still increase, but to a much lesser extent. In all cases, however, the results regarding the risk estimates to each local population as compared to what those results would be in a population with typical U.S. proportions of MVSP would not change substantially. Therefore, the relative differences across each row of Tables K–97 to K–106 are not sensitive to potential inaccuracies of the estimated susceptibility differences of the MVSP.

K.4 References

Blower, S. M. and H. Dowlatabadi (1994). "Sensitivity and uncertainty analysis of complex models of disease transmission: and HIV model, as an example." International Statistical Review **62**(2)**,** 229-243.

Iman, R. and W. Conover (1980). "Small sample sensitivity analysis techniques for computer models, with an application to risk assessment." Commun. Statist.-Theor. Meth **A9**(17): 1749-1874.

Likos, A. M., D. J. Kelvin, et al. (2007). "Influenza viremia and the potential for blood-borne transmission." Transfusion **47**(6): 1080-1088.

Mahmoud, A., D. Burke, et al. (2008). NIH Blue Ribbon Panel to Advise on the Risk Assessment of the National Emerging Infectious Diseases Laboratory at Boston University Medical Center, Finding and Recommendations, Part I: Risk Assessment; Briefing of the Advisory Committee to the Director, NIH, June 6, 2008**:** 1-40.

McKay, M. D., W. Conover, et al. (1979). "A comparison of three methods for selecting values of input variables in the analysis of output from a computer code." Technometrics **21**: 239-245.

Stramer, S. L., F. B. Hollinger, et al. (2009). "Emerging infectious disease agents and their potential threat to transfusion safety." Transfusion **49 Suppl 2**: 1S-29S.

Appendix L:
Health Effects – Secondary Transmission

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L. Health Effects – Secondary Transmission

L.1 Introduction

Secondary transmission analyses for this risk assessment (RA) consist of qualitative and quantitative estimates of potential infection and health effects in those exposed to a pathogen because of contact with an individual already infected as a result of one of the events from the event sequence analyses. In this RA, the term "secondary transmission" refers to the transfer of pathogenic organisms from an initially infected person to another person, causing the establishment of infection in the second person, as well as any and all subsequent generations of transmission. Secondary transmission is distinguished from initial infection, which describes an infection caused by exposure to pathogens released directly from a NEIDL-associated event.

Under scenarios in which a laboratory worker is infected (e.g., via needlestick or centrifuge aerosol release), the worker may become contagious and transmit infection to other individuals, who may in turn transmit to others, and a chain of transmission could continue through any number of generations. Under this scenario, the infection of the original laboratory worker is considered an initial infection, while all subsequent infections result from secondary transmission. The potential for secondary transmission increases when the exposure is undetected by the laboratory worker or if protocol in reporting or responding to a known mishap occurring in the laboratory is not followed. In these cases, the exposed worker may develop infection and become contagious while outside the laboratory among members of the public.

A member of the public who becomes infected as a result of an initial infection (e.g., via an earthquake release) or a secondary transmission may also transmit infection to others. Infected members of the public may or may not know or suspect that they have been exposed, and if symptoms develop they may not recognize the source of their disease. In assessing the possibility of extended outbreaks in the public, lessons can be learned from outbreaks that have occurred in the past regardless of whether or not they originated from loss of containment at a biological laboratory.

L.2 Methodology

The assessment of secondary transmission potential for each pathogen is based on observations from past outbreaks that have occurred among humans. Most historical outbreaks did not occur as a result of a release from a laboratory. Most of the documented cases of infections occurring from a large-scale laboratory release or among laboratory workers who subsequently interacted with contacts occurred with

pathogens that are very rarely or not at all transmissible from person to person. The cases of laboratory-related initial infections with transmissible pathogens are too few to draw meaningful conclusions about the likelihood of transmissions under such a scenario in general. Therefore, for purposes of this RA, it is assumed that information from previous outbreaks are applicable to the initial infection scenarios analyzed, regardless of whether those historical outbreaks occurred as a result of a laboratory release.

It is possible that specific circumstances surrounding a laboratory release would make information from "natural" outbreaks less relevant. For example, laboratory workers developing disease symptoms would presumably be more likely than an average person to link their symptoms to pathogens they had been working with and take necessary precautions to avoid potential spread to others. However, there is no guarantee that such precautions would be followed. In addition, some of the pathogens studied at the lab cause disease in humans that are initially difficult to distinguish from the symptoms of common diseases, so the worker may not initially realize the source of the infection and not take the necessary precautions to avoid transmission to others. Furthermore, for some pathogens it is possible that transmission from the laboratory workers could occur simultaneously with or even before symptoms develop, so that laboratory workers could transmit to others before they themselves are aware or confirm that they are infected.

When evaluating the potential for secondary transmission, it is important to assess the likelihood that a particular infected person will transmit infection to a given number of others. Data from previous infections and outbreaks can shed light on this question, especially when adequate contact tracing is performed, in which public health officials track each infected case and how many others were infected by each case. However, such data are sparse for many pathogens, and the data that do exist may be unreliable because there is often difficulty in determining who infected whom after the fact. As an alternative, researchers often attempt to estimate the reproductive number, the *average* number of transmissions from each primary case, by indirect means using more easily measureable data from outbreaks and translating those data into a reproductive number through the use of mathematical formulas.

Even when outbreak data are relatively rich and reproductive numbers are well established, there is still uncertainty in any attempt to predict the number of transmissions that will occur from *a particular* individual, because there is normally high variation from case to case. For example, studies of SARS-associated coronavirus (SARS-CoV) outbreaks consistently place the basic reproductive number in the range 2–4. This means that there is relatively high certainty that, early in an outbreak involving several individuals, the *average* number of transmissions per individual would be in that range. However, in

examining the number of transmissions that actually occurred from particular individuals during SARS-CoV outbreaks, one finds that most individuals did not transmit infection to 2–4 others. In fact, most infected individuals did not transmit to anyone, while some individuals transmitted to far greater than four others. Understanding this high variation between cases is important in assessing the risk posed by a single infected individual entering a public community. The variation is partially caused by case-to-case differences in characteristics of the infection or of the human host and partially caused by chance events that determine whether or not transmission occurs. Two factors that contribute to determining how many transmissions occur in a particular case are a) the infected person's social contact rate and b) the likelihood that transmission occurs from each contact.

a) **Social contact rate**

The number of social contacts of an infectious person during the infectious period determines how many transmissions can potentially occur. A social contact refers to any person-to-person interaction that might result in a transmission of pathogens. A social contact may or may not involve direct physical contact between individuals. Normally a social contact with potential for transmission involves two people being in the vicinity of one another at the same time (in the same room, vehicle, or outdoor area). However, transmission can also occur between people in different rooms (for example, through a ventilation duct) or through touching the same contaminated object, or fomite, at different times.

Assessing social contact rates (number of social contacts per day) of infectious people is important for comparing the likelihood of transmission from different individuals or from people living and/or working in different areas (e.g., urban vs. suburban vs. rural). These rates are difficult to measure with certainty because of the complicated nature of social interactions and the high variation in contact rates from person to person. Furthermore, the studies that do attempt to measure contact rates tend to assess typical rates of contact in people's daily lives. However, a person with disease symptoms may behave atypically resulting in an abnormal contact rate.

Further assessments of attempts to quantify rates of social contact are discussed in later sections of this appendix.

b) **Transmissions occurring from different types of contacts**

It is important to note that not all contacts result in the same probability of transmission. Here, characteristics of the pathogen are important. Some pathogens can be transmitted through almost

any type of contact (although shorter duration contacts may result in lower probability of transmission). Others are only transmissible through physical or extended contact typical of what would occur between family members or close friends or colleagues. Still others are only transmissible through the most intimate of contacts involving the exchange of bodily fluids. Several other pathogens require a vector, such as a biting insect, for transmission to occur from person-to-person. Pathogen-specific discussion of these issues is important in comparing the likelihood of transmission across different individuals and different locations.

The issues described above are discussed on a pathogen-by-pathogen basis in Section L.3. For each pathogen, the discussion involves the following items.

- Description of the quantity, quality, and relevance of data from transmissions occurring in past outbreaks involving the pathogen.
- Assessment of the likelihood for given numbers of transmissions occurring under the scenarios specific to this RA.
- Assessment of the uncertainty associated with the likelihood of transmission due to lack of knowledge of the pathogen's characteristics.
- Assessment of the variability in transmissions exhibited by observations of past outbreaks.
- Discussion of the implications of uncertainty and variability on the assessment of transmission risk.

The above points are discussed qualitatively for each pathogen. For some of the more highly transmissible pathogens, quantitative analysis is performed through the use of mathematical modeling and statistical procedures. Quantitative analysis allows one to express assumptions in a precise manner, to accurately assess the implications of those assumptions, and to sort out the relative importance of input values, uncertainties, and variabilities, which can interact in complicated ways that are difficult to understand through non-quantitative techniques.

L.2.1 Branching Process Modeling

Pathogens for which quantitative analysis of secondary transmission is performed are analyzed using branching process models. Branching process models are stochastic mathematical models for populations of individuals in which each individual in one generation produces some number of additional individuals in the next generation. In epidemiology, branching process models have been used, dating back to Reed

and Frost in the 1920's (Abbey 1952), to simulate the transmission of infection from person to person in a population.

When the number of infected individuals in a population is small (for example, one infected lab worker entering the community), elements of chance can play an important role in determining the ultimate size of a potential outbreak. Even for highly contagious diseases, there is the possibility that one infected individual does not transmit to anyone because the individual has an atypically low rate of contact with others, or simply because of random luck. On the other hand, one individual could transmit to many others and start a large outbreak that extends to many generations of transmission. The branching process modeling framework is useful for capturing the range of possibilities that could occur, starting from one or a few initially infected individuals.

A recent study (Lloyd-Smith 2005) attempted to quantify the variation in the number of possible transmissions from each infected individual under the branching process modeling framework, using contact-tracing data from real human outbreaks of infectious diseases. We start the description of our branching process model with the basic parameters and variables used in this study.

- R_0 is the basic reproductive number, a constant parameter for the population-wide average number of secondary transmissions per infected individual in a fully susceptible population.
- ν is the individual reproductive number, a random variable describing the expected number of secondary transmissions from any particular infected individual. Values of ν are randomly drawn for each infected individual from a continuous probability distribution that has mean R_0.
- Z is the number of transmissions, a random integer variable used in simulations to represent the actual number of transmission from each infected individual. Values of Z are randomly drawn for each infected individual from a discrete probability distribution that has mean ν.

The randomization of ν encompasses heterogeneity in a population, i.e., the ways in which individuals differ from one another with regard to transmission (different number of contacts, different rate of shedding pathogens, different behavioral tendencies, etc.). Then, randomization of Z represents the element of chance, or how the actual number of transmissions may differ from what is expected for each individual.

Note that the parameter R_0, according to the definition, is only technically valid when there is exactly one infected individual in a population where everyone else is susceptible. When multiple individuals are

infected, there's a chance that one or more contacts of any one of the infected individuals will already be infected, so that the expected number of transmissions would be less than if every contact were guaranteed to be susceptible. However, this effect is likely to be small in the early stages of a small outbreak within a large population, so for simplicity we assume that ν has a mean of R_0 for each individual in early generations of small simulated outbreaks. For extended outbreaks and when simulating the effect of public health intervention, this assumption is relaxed as described in later sections.

In a branching process simulation, random values for ν and Z are generated for each initially infected individual to determine the number of transmissions by each individual to the next generation. A sample simulation of a small outbreak is presented to illustrate the basic process, as follows. The simulation was seeded by one initially infected individual, R_0 was set to 1.8, each ν was drawn randomly from the exponential distribution (with mean R_0) and each Z was drawn randomly from the Poisson distribution (with mean ν). Note that both the exponential and Poisson distributions are one-parameter distributions, so that each distribution is fully defined when the mean is specified. Table L–1 displays the results for one simulation under these specifications.

Table L–1: Example simulation of secondary transmission

Infected ID	Infected by	ν value	Z value
A	(initial)	3.1	2
B	A	0.57	0
C	A	5.1	4
D	C	0.022	0
E	C	0.76	1
F	C	0.068	1
G	C	0.22	0
H	E	0.32	0
I	F	0.15	0

The simulated outbreak consisted of nine total infections over the course of four generations (the first generation consisting of individual A), before the outbreak extinguished when generation four (individuals H and I) did not transmit to anyone else. The fact that the outbreak extinguished relatively quickly was random luck; other simulations run under the same assumptions resulted in large outbreaks in which dozens of individuals had been infected after just a few generations.

L.2.2 Timing of Disease Progression

The sample simulation in the previous section generated results based on generations of transmission, but the timing of transmission events was not modeled. Timing of transmissions is important in estimating the speed of possible outbreaks, particularly in relation to the timing of intervention measures put in place to curb transmission, once public health officials become aware that an outbreak is occurring. The following values are used to simulate disease progression for infected individuals.

- t_I is the incubation period, or the time between exposure to pathogens and the development of symptoms.
- t_G is the generation time (also called serial interval), or the time between the onset of symptoms of a primary case and the onset of symptoms of a secondary case caused by transmission from the primary case.

The incubation period and the generation time are treated as random variables, with a distribution derived from data for each pathogen. See the sections on modeling each pathogen for details. The incubation period is only simulated for the initially infected individual(s), for whom the time of exposure is fixed at the time of the initiating incident. For secondarily infected individuals, the incubation period is included within the generation time, because the generation time incorporates both the timing of the transmission and the period before the secondary case develops symptoms.

There are several advantages to using the generation time in simulations, rather than explicitly modeling the infectious period and the timing of transmission events. When data on the generation time is available from real outbreaks, it is relatively reliable in that the development of symptoms are observable events, whereas the timing of a transmission event is generally not observable and may be highly uncertain if the primary and secondary individuals had contact over several days. Also, the generation time includes information on the average and variation of the timing of transmission events during the primary individual's infectious period, including the possibility that an individual may transmit before becoming symptomatic (which could explain observations of very low generation times). If the incubation period, infectious period and timing of transmission events are all modeled explicitly, then one has to make assumptions about the likelihood of transmission during various portions of the incubation and infectious periods, which are difficult to validate with reliable data.

For each infectious individual in the simulation, each of the transmissions from that individual is assigned an associated generation time, which is used to calculate the time at which each secondary case becomes

symptomatic. It assumed that, if there are multiple transmissions from one individual, the generation time associated with each transmission is independent of the others. Each generation time is drawn independently from the distribution associated with that pathogen.

L.2.3 Timing and Effect of Intervention Control Measures

For simulated outbreaks starting with initially infected individuals, it is assumed that transmission continues to occur under the values of R_0 and distribution of ν as described above until control measures are implemented. Until this intervention, the simulation is a model for a period of unchecked transmission before public health officials or hospitals are aware that an outbreak is occurring. The delay in implementing control measures (T_c) is modeled by first assuming a value for the delay in implementation of control measures, or the time between the onset of symptoms of the first case and the time at which control measures are put in place.

The following are some factors that may play a role in determining the length of the delay in implementation of control measures.

- The first symptomatic individual may delay going to see a doctor about the symptoms.
- There may be a delay in diagnosing the patient once he or she is examined.
- There may be a delay in mobilizing and implementing an intervention strategy once the patient is diagnosed.

All these delays contribute to the overall delay time. For each pathogen undergoing quantitative modeling, a range of values for the delay time was implemented in simulations in order to assess the sensitivity of the results to varying the length of the delay.

The effect of intervention measures is modeled by changing the parameters that contribute to the distribution of the individual reproductive number ν. As described above, the mean of ν was fixed at R_0, which describes the average number of transmissions per infected individual early in an outbreak, before control measures are implemented. Once control measures are implemented, a new parameter is utilized called R_c, which is the post-control reproductive number, a constant parameter for the population-wide average number of secondary transmissions per infected individual, once control measures are implemented.

If a simulated individual becomes symptomatic at a time after control measures are implemented, the value of ν for that individual is drawn from the new distribution with mean R_c. The assumption of a sudden transition from R_0 to R_c for the average number of transmissions is potentially unrealistic. For example, individuals becoming symptomatic just before the transition time are assumed to transmit to the pre-control average R_0, even though the transmissions themselves are likely to occur after the transition-to-control time. On the other hand, individuals becoming symptomatic just after the transition time are assumed to transmit to the post-control average R_c, even though the control measures are unlikely to be fully implemented very soon after they have begun. It is assumed that occurrence of these two examples balance each out in the simulations and that a more complicated model of the transition to control measures would not provide insight beyond what is obtained by varying the assumed time of delay.

A branching process model containing most of the features described above was employed by Wallinga and Teunis (2004) as a simulation for outbreaks of SARS. To test whether the branching process simulation codes used for this risk assessment were in good working order, values and distributions for parameters matching what was used in Wallinga and Teunis (2004) (as close as possible given the published details) were used as input, and the output was in good agreement with the values published in the study.

L.2.4 Large Outbreaks

If the control measures are implemented relatively quickly and the value of R_c is small, then it is likely that the outbreak will extinguish before a large number of individuals become infected. However, if R_c is approximately one or greater, and/or if a string of individuals in early generations of the outbreak are atypical and transmit to a large number of individuals, then an outbreak has the potential to become large even if it was started by a single initial case.

The use of R_0 or R_c as the average number of transmission from each individual as the outbreak progresses assumes that infected individuals in later generations have the same average chance of contacting susceptible individuals as did infected individuals in earlier generations. However, if an outbreak is large enough that a significant portion of the local population becomes infected, infected individuals in later generations should have a higher chance that one or more of their contacts have already been infected, thereby eliminating the possibility of would-be transmissions that may have occurred had those contacts happened earlier in the outbreak.

A simple way to model the effect of decreasing susceptibility of the population is to introduce a new parameter N, the size of the local population, and to adjust R_c based on the fraction of the population that are still susceptible when the number of transmission from an infected individual is being calculated. The equation

$$R^* = R_c \frac{S}{N},$$

where R^* is the effective reproductive number and S is the number of susceptible individuals in the population, was proposed by Gumel et al. (2004) as a good estimate of the average reproductive number as the number of susceptible individuals relative to the total population decreases. The full model in Gumel at al. (2004) is a considerably more complicated differential equation model that includes a large number of parameters that contribute to the value of R_c, including rates of placing exposed and infected individuals into quarantine and isolation and the relative effectiveness of quarantine and isolation in decreasing transmission. To test whether employing a similarly complicated model would be useful for purposes of this risk assessment, the model in Gumel et al. (2004) and the branching process model employing the above equation were each run for the same values of R_c and with the same average values for the incubation period and generation time. Values of $R_c > 1$ and a large number of infected individuals entering the post-control period were used to ensure that the simulated outbreaks would reach high levels compared to the total population. The average result from the branching process simulations for total number of infections agreed with the result from the more complicated model within approximately 5%. Given that these test-case results were not much different, and in the interest of simplicity, employing the above equation to model the effect of decreasing susceptible individuals, in conjunction with varying the value of R_c for uncertainty and sensitivity analyses, was determined to be appropriate for this risk assessment.

In the branching process model, S is calculated just before determining the value of Z for an infected individual by subtracting the current total number of infections from N. This assumes that everyone in the population who has not already been infected is susceptible. For large values of N (e.g., for $N \approx 1$ million daytime population in Boston), the number of infections would have to increase substantially in order to have a noticeable effect on changing the value of R_c. Therefore, this adjustment would only have a significant effect for outbreaks with very large numbers of cases in the local area.

L.2.5 Effect of Commuting

Additional model details are included to account for the possibility that an outbreak does not remain local. There are a number of reasons why a person infected locally might travel outside the local area and potentially transmit to others at new location. One large group of travelers that is relatively easy to quantify is commuters, or people who work outside their area of residence. Demographic data from the U.S. Census Bureau was obtained for areas in which the three sites are located to determine the percentage of residents in each area who work outside the area, as well as the percentage of the daytime population that reside elsewhere. These percentage are used to randomly assign simulated individuals a classification for having potential to transmit to someone outside the local area.

Non-local transmissions are considered branches of the transmission chain that have escaped from the local area. Transmissions from non-local individuals continue to be tracked in the model, so that the final output from each simulation includes the portion of the overall number of infections that were local and non-local. These results provide two potential insights. First, they provide an adjustment to the estimated risk to individuals local to each site. Second, they provide a measure of the risk that NEIDL might pose to individuals outside the local area at each site. For outbreaks originating at the suburban and rural sites, given their location relatively close to Boston and the large amount of travel that occurs between Boston and surrounding areas, the number of non-local transmissions is a measure of the risk to individuals in Boston. While not all of the non-local transmissions would occur in Boston, it is assumed that if the number of non-local cases is large, there is a strong likelihood that outbreak will have spread into Boston. Thus, these results allow for a rough comparison between the risk that NEIDL might pose at its current locations and at the other locations to individuals in Boston.

The following data are taken from the U.S. Census Bureau's estimated daytime population tables (U.S. Census 2011) derived from the 2000 census. Tables using data collected later than the year 2000 had not been provided by the U.S. Census Bureau as of July 2011. For the urban site, data from the city of Boston was used to derive commuter estimates. Data restricted to specific areas of Boston close to the NEIDL location are not available in the U.S. census tables. The suburban site is located in Tyngsborough, MA, and data from that town are not available in the U.S. census tables. As a replacement, data from Wilmington, MA, which is a suburban town in the same county as Tyngsborough, were used. The rural site abuts the towns of Peterborough, NH, and Hancock, NH. Data from the Peterborough census-designated place (CDP), which is a portion of the overall town of Peterborough, were used.

Table L–2: Commuting data and calculations

Data from 2000 U.S. Census (U.S. Census 2011)					
Place Name	Total resident population (A)	Total workers working in the place (B)	Total workers living in the place (C)	Estimated daytime population (D)	Workers who lived and worked in the same place (E)
Boston city, MA	589141	520555	278463	831233	184954
Wilmington CDP, MA	21363	20584	11357	30590	2287
Peterborough CDP, NH	2944	2779	1375	4348	599

Additional Calculations			
Place Name	Percent of workers in the place who are in-commuters = (B – E) / B	Percent of daytime population who are in-commuters = (B – E) / D	Percent of residents who are out-commuters = (C – E) / A
Boston city, MA	64.5%	40.4%	15.9%
Wilmington CDP, MA	88.9%	59.8%	42.5%
Peterborough CDP, NH	78.4%	50.1%	26.4%

The percentages in the lower part of the table above are used to randomly assign simulated individuals a classification for having potential to transmit to someone outside the local area. Each individual is assigned one of the following four categories. In these desriptions, the local area is defined as the city of Boston for the urban site, the town of Tyngsborough for the suburban site, and the towns of Peterborough and Hancock, NH for the rural site.

1. Fully local. These individuals live in the local area and either do not work or work in the local area. If infected they could potentially transmit to individuals in category 1, 2, or 3.

2. Out-commuter. These individuals reside in the local area and commute to work outside the local area. If infected, they could potentially transmit to individuals in category 1, 2, or 4.

3. In-commuter. These individuals reside outside the local area but commute to the local area for work. If infected, they could potentially transmit to individuals in category 1, 3, or 4.

4. Non-local. These individuals reside outside the local area and either do not work or work outside

the local area. If infected, it is assumed that they would only transmit to other individuals in category 4.

Under scenarios in which one or more laboratory workers are initially infected, those initial individuals are randomly assigned to category 1 or 3 according to a probability equal to the "percent of workers in the place who are in-commuters" from Table L–2. This assumes that NEIDL workers would be commuters at the same average rate as indicated for workers in the areas shown in Table L–2.

When transmissions occur in a simulation, the category of each secondary case must be chosen based on the category of the primary case. When deriving these probabilities, in some cases it is important to consider whether the transmission occurs during working hours (called "daytime") or during home hours (called "nighttime", although home hours also include non-working days). A person infected with SARS-CoV could potentially develop symptoms and become infectious at any time during a typical day. For the simulations, it is assumed that each transmission has a 1/3 probability of occurring during "daytime" and a 2/3 probability of occurring during "nighttime." One week consists of 168 hours, and a 40-hour work week represents about 24% of those hours. This number was increased to 1/3 to account for commuting time and for the possibility that more contacts may occur during work hours than would occur at home.

The following procedures are used to randomly determine the category of each secondary case of a transmission.

- If the primary case is category 1:
  For a daytime transmission, the secondary case will be category 3 according to "percent of daytime population who are in-commuters" from Table L–2, and category 1 otherwise. For a nighttime transmission, the secondary case will be category 2 according to "percent of resident population who are out-commuters" from Table L–2, and category 1 otherwise.

- If the primary case is category 2:
  For a daytime transmission, the secondary case will be category 4. For a nighttime transmission, the secondary case will be category 2 according to "percent of resident population who are out-commuters" from Table L–2, and category 1 otherwise.

- If the primary case is category 3:
  For a daytime transmission, the secondary case will be category 3 according to "percent of

daytime population who are in-commuters" from Table L–2, and category 1 otherwise. For a nighttime transmission, the secondary case will be category 4.

- If the primary case is category 4:
  The secondary case will also be category 4.

For transmissions occurring after the implementation of control measures, the adjustment to the parameter R_c based on the local population, described in Section L.2.4, is applied for primary cases in categories 1, 2, and 3, but not category 4, as non-local individuals are assumed not to have contacts in the local population.

The results of these simulations are reported in terms of the risk to local individuals as well as non-local individuals. For this purpose, local individuals are defined as anyone in category 1, 2, or 3 as defined above. The results for risk to local residents (categories 1 and 2) are also reported.

L.2.6 Initial Conditions and Epidemiological Parameters

The branching process model requires an initial condition in the form of the number of initially infected individuals entering a community of susceptible individuals with potential for secondary transmission to occur. The exact number of initially infected individuals is important, because outbreaks starting with more infected individuals should have a higher probability of leading to an extended chain of transmission. Some events leading to initial infections have the potential to infect only one individual at a time (e.g., needlestick), while others have the potential to infect many individuals at once (e.g., earthquake maximum reasonably foreseeable release). In the initial infections analyses (Chapter 9 and Appendix K), each pathogen was paired with each event to determine the likelihood that one or multiple individuals would be infected by that pathogen if the event were to occur. The results of these analyses are used to determine possible initial conditions for each pathogen undergoing secondary transmission modeling.

The literature on epidemiological features of outbreaks of each pathogen was carefully examined to determine base-case values and uncertainty ranges for each input parameter that is used in the branching process model. The chosen values are shown in tables within the section dedicated to each pathogen.

L.2.7 Analysis of Base Case Model

When the base case parameter values for a pathogen are assumed, the branching process model is fully specified. However, because the model is stochastic, i.e., assumes variability in the events being modeled, the outcome for any particular simulated outbreak is not knowable in advance even when the input parameters are specified. Instead, results are given in terms of average outcomes or probabilities that various outcomes will occur. It is sometimes possible to calculate the probability that a specified event will occur directly from the input parameters. However, for most outputs of interest (such as the average size of an outbreak), such calculations are unwieldy due to the large number of random input variables that interact and contribute to the specified output. In these cases, Monte Carlo simulations are employed to simulate a large number of outbreaks all starting from the same initial conditions and the same input parameters. In each simulation, values for random variables (number of transmissions, generation time, etc.) are drawn from the assumed distributions specified by the base case parameter values. Finally, the outcomes of all the simulations are examined as a whole to estimate expected outcomes and probabilities of events of interest.

The following types of outcomes are evaluated:

- **Probability that the number of transmissions or fatalities exceeds various thresholds** – Probabilities are presented for both transmissions and fatalities for various thresholds, generally one or more, ten or more, 100 or more, and higher factors of ten if applicable, in tabular form, based on sorted outcomes of the Monte Carlo simulations.

- **Frequency of initial infections leading to given numbers of transmissions or deaths** – This is the product of the probabilities from the previous outcomes and the frequency of initial infections occurring from each of the event sequence analyses. These results are presented in both tabular and graphical form.

L.2.8 Uncertainty and Sensitivity Analysis

The base case results stem from fixing the input parameters to their base case values, all of which are uncertain. An uncertainty analysis attempts to systematically assess how varying the input values across their probable ranges affects the estimation of outcomes of interest. A sensitivity analysis attempts to separate the relative contribution from each uncertain input value to the uncertainty in each output in order to gain insight into which parameters affect the estimated outcomes in what ways.

Assumed distribution of input values

The uncertainty analysis techniques employed here require that each input variable be assigned a probability distribution. Each parameter was assigned a base case value along with an uncertainty range. The values and distributions assigned to each parameter are described in the Section L.3 for each pathogen, along with associated justification for each assumption in light of the relevant literature.

Sampling of input values

Given the distribution assumed for each value, random combinations of input parameters were drawn from each distribution using Latin Hypercube Sampling. This sampling scheme was also employed for initial infections modeling, and an explanation of the technique can be found in Appendix K. For each combination of input parameters, a large number of Monte Carlo simulations were run to generate statistics for the evaluation of uncertainty in the output values described in the previous section.

Sensitivity analysis

Sensitivity refers to the effect that variation in particular input values has on variation in results. A qualitative discussion of sensitivity is provided where notable results are observed in the uncertainty analysis. For example, if certain parameters cross a threshold above which results are appreciably different, that insight is provided. A quantitative sensitivity analysis is provided in the SARS-CoV section, using Partial Rank Correlation Coefficients, which were also used in the initial infections analysis (where the full description of the technique can be found). In this section, all relevant input parameters were tested against their effect on outputs for the frequency of public fatalities. The insights gained here for SARS-CoV are applicable to the other pathogens, which are modeled under similar frameworks.

L.2.9 Site-specific analyses

Characteristics of the population near each of the sites considered in this RA might affect the inputs and results for the secondary transmission analyses. Three site-specific characteristics are already incorporated into the analyses described above.

- **Population size** – As described in Section L.2.4, the size of the local population, which differs at the three sites, is incorporated by adjusting the average number of transmissions as the size of an outbreak becomes larger. It is again noted that this adjustment would only have a noticeable effect for very large outbreaks.

- **Initial conditions for earthquake release** – As described in Section L.2.6, the initial infections analyses provide the initial conditions for the number of infected individuals with the potential to transmit to others and start an outbreak among the general public. For the earthquake release scenario, the initial infections results are different for the three different sites because of differences in the size of the population potentially exposed to an aerosol release. Therefore, the results for the frequency of releases leading to outbreaks of different sizes will vary by site.

- **Effect of Commuting** – As described in Section L.2.5, commuting data from the area near each site are applied in simulations to determine the risk to local and non-local individuals.

There are other site-specific characteristics that could affect the assumed input values relevant for secondary transmission. For example, the population density, or number of residents and workers per unit of land area, near the three sites is different, and may affect the rate at which transmissions occur even before an outbreak becomes large, because the local population density is a factor in determining how many person-person contacts an infected individual is likely to have while moving about the local area. Attempting to specify the nature and magnitude of correlation between population density and rate of transmission necessarily requires the use of assumptions that are difficult to verify with data. The difficulty lies in the fact that human interactions are complicated and depend on many factors other than overall population density, and many of those factors are highly variable from person to person. For example, a person's family size, work or school environment, travel patterns, underlying health characteristics, typical social behavior, and how that social behavior changes when experiencing illness are all factors that play important roles in determining the likelihood of transmission.

In addition, the transmissibility characteristics of particular pathogens could affect the role that population density plays in determining the rate of transmission. For example, it is reasonable to assume that a person moving from place-to-place in a high-density urban area would likely come into brief, close proximity with many more others on average than would a person moving around a low-density rural area. The difference highlighted in this example would play a larger role for pathogens that are highly transmissible via an aerosol route than it would for pathogens that require more intimate contact for transmission. For pathogens of the latter type, an increase in the number of brief, casual contacts would have little to no effect on the rate of transmission. Even for highly transmissible pathogens, the relative importance of brief, casual contacts compared to more intimate contacts during historical outbreaks has often been unclear.

The formulation of quantitative models to address the issues described above is an open area of research, and at present there are no well established or validated methods for estimating the effects of these characteristics on rates of transmission for specific sites or populations. Nevertheless, because of the importance of some of these issues for this RA, a few relatively simple calculations are applied to investigate the possibility that certain site difference have a notable effect on the most relevant input parameters and, in turn, the resulting output of the quantitative models.

L.2.9.1 Contact rate estimates for local populations

Individuals differ with respect to the number of contacts with whom they interact on a daily basis. If an individual is infected with a transmissible pathogen, that person's contact rate is presumed to have an effect on the probability that the person would transmit infection to others. The contact rate depends on the number of people in the person's home, school, work location, places of service, or other locations where the person typically spends time. When considering average contact rates over populations at the three different sites, it is worth investigating whether there exist systematic differences between the local populations with respect to typical contact rates for individuals living in each place.

As contact rates are difficult to quantify through direct observation, researchers have developed techniques to simulate synthetic populations, based on available data from real populations, to computationally create interaction networks that can be used to estimate contact rates. One such procedure, described in Eubank et al. (2006) and Bisset et al. (2009), was used to create a synthetic population for areas in and around Boston, Massachusetts. Information from these synthetic populations and associated contact networks were provided for use in this RA by Dr. Stephen Eubank, as follows.

For specific zip codes within the study area, a synthetic resident population was created that is demographically representative of the actual population. A simulation of the synthetic population on a single day (a weekday in the Spring) spending time in various activity locations, such as homes, offices, and schools, was used to estimate the number of contacts of at least 10-minute duration for each individual. The results are as follows:

Urban site

For a synthetic population of 17,437 residing in zip code 02118 (the zip code at the NEIDL site in Boston), the average number of contacts per person was 44.0 (standard deviation 29.8 and range 0–246).

Suburban site

For a synthetic population of 12,192 residing in zip code 01879 (Tyngsborough, Massachusetts), the average number of contacts per person was 37.6 (standard deviation 24.2 and range 0–252).

Rural site

The towns of Hancock and Peterborough, NH were not included in the study area. As a replacement, the town of Ashby, MA was chosen. This town is geographically similar to the rural site, being about 20 miles southeast of Peterborough along the New Hampshire border and having a similar population density (about 129 people per square mile) to that of the combined area of Hancock and Peterborough (about 115 people per square mile). For a synthetic population of 2741 residing in Ashby, the average number of contacts per person was 20.83 (standard deviation 17.47, range 0–199).

The average numbers of contacts estimated for each location are used to calculate adjusted *R* values. For this purpose, it is illuminating to decompose *R* into a product of two components,

$$R = C \times p$$

Here, *C* = *average number of contacts*, and *p* = *average probability of transmission per contact*. Assuming that the value of *p* would be the same across the three sites, the value of *R* would be directly proportional to the value of *C*. Under this assumption and using the relative average contact rates derived from synthetic populations, the following relationships between *R* values from infected individuals residing at the three sites are obtained.

$$R_{\text{suburban}} = \frac{37.6}{44.0} \times R_{\text{urban}} \approx 0.855 \times R_{\text{urban}}$$

$$R_{\text{rural}} = \frac{20.83}{44.0} \times R_{\text{urban}} \approx 0.473 \times R_{\text{urban}}$$

These conversions are applied to separate sets of simulations to investigate the implications of these assumptions on site-specific results for this RA.

L.2.9.2 Medically vulnerable sub-populations

Estimates of increased susceptibility to disease and mortality of medically vulnerable sub-populations (MVSP) are detailed in Appendix I. Differential susceptibility has potential implications for affecting the

average number of transmissions from an infected individual interacting with a susceptible population that includes members of MVSP. Recall that the population-wide reproductive number R (R_0 before intervention or R_c after) is used to model the average number of transmissions per infected individual. In the context of considering the effect of vulnerable portions of the susceptible population, it is illuminating to decompose R into a product of two components,

$$R = C \times p$$

Here, C = *average number of contacts*, and p = *average probability of transmission per contact*. Under this framework, one can consider the portion of contacts that is likely to represent contacts with individuals in each MVSP (based on the portion of the available contacts that are members of the sub-population) and then how the probability of transmission would change based on the differential susceptibility of that MVSP.

The general estimates of R that are used for this risk assessment were based on transmission data from real outbreaks among populations that contained some portion of individuals belonging to MVSP. In this sense, the effect of differential susceptibility is already incorporated into the R values being applied. However, because this risk assessment is concerned with specific populations for which data on sub-populations are available, it was determined to be worthwhile to include a framework for adjusting values of R based on how the local population characteristics may differ from a typical population.

The following assumptions are made.

- For a given scenario, the base case R value is relevant for a population containing portions of each sub-population that are in line with their overall frequency in the total U.S. population.

- The probability that any given contact of an infectious individual is a member of a given MVSP is equal to the portion of the local population that is a member of that MVSP.

- The average probability of transmission when an infectious individual contacts a member of a MVSP is higher than the probability resulting from contact with an individual who is not a member of an MVSP. The increase in this probability is calculated under similar methodology to that used in the context of initial infections.

- No differences in transmission *from* infected individuals belonging to different sub-populations are applied.

Input Values

This section specifies the values that are used in the calculations. For this part of the analysis, only single point estimates are applied for each input. While uncertainties do exist for these inputs, the given values are sufficient for the purpose of this exercise, which is to explore the potential role that MVSP proportions might play at each site in affecting secondary transmission estimates.

First, the index k is defined, which represents an index for the five MVSP described in Appendix I, as follows.

- $k = 1$ for children under five
- $k = 2$ for adults over 65
- $k = 3$ for people with diabetes
- $k = 4$ for people with HIV/AIDS
- $k = 5$ for pregnant women

With this index in hand, the following inputs are defined and specified.

Proportion of U.S. and local populations belonging to MVSP k (x_k, y_k). Appendix I provides estimates for proportions of each MVSP according to U.S. data and data and estimates from areas near the three sites. The assumed U.S. proportions are termed x_k, for $k = 1$ to 5, and the values are taken directly from Table 1 in Appendix I. The assumed urban resident proportions are taken from the column in Table 1 of Appendix I that estimates the proportion of each MVSP residing in the city of Boston. For the portion of the urban population working but not residing in Boston, as well as the contacts of these individuals during non-working hours, the Massachusetts statewide MVSP estimates were assumed. The suburban site estimates were taken from the Tyngsborough, MA data for residents and Massachusetts statewide data for non-resident local workers and their contacts outside of working hours. The rural site estimates were calculated by combining the data from Peterborough, NH and Hancock, NH for residents and New Hampshire statewide data for non-resident local workers and their contacts outside of working hours. Table L–3 specifies the values applied in this analysis.

Table L–3: Inputs for MVSP population values

MVSP (k)	Proportion of MVSP in U.S. population (x_k)	Proportion of MVSP in local population (y_k)				
		Urban resident	Suburban resident	Rural resident	Urban / Suburban non-resident	Rural non-resident
1: Children under five	0.069	0.059	0.055	0.058	0.063	0.061
2: Adults over 65	0.126	0.102	0.082	0.217	0.135	0.12
3: People with diabetes	0.057	0.092	0.078	0.066	0.074	0.067
4: People with HIV/AIDS	0.0045	0.0094	0.0020	0.0011	0.0028	0.00086
5: Pregnant Women	0.01	0.01	0.01	0.01	0.01	0.01

See Appendix I for sources of these estimates.

The values in the columns of Table L–3 are applied based on the classification of each infected individual with regard to commuting as described in Section L.2.5. When a transmission occurs in the local area during non-working hours, the MVSP population numbers applied to the secondary case are the local resident numbers. When a transmission occurs in the local area during working hours, the MVSP numbers applied to the secondary case are calculated as a weighted average between the local resident and the local non-resident numbers, weighted according to the numbers in Table L–2. When a transmission occurs non-locally but from an individual who either lives or works locally, the MVSP numbers applied to the secondary case are the local non-resident numbers. When a transmission occurs from a non-local individual (someone who neither lives nor works locally), the MVSP numbers applied to the secondary case are the overall U.S. numbers.

Increased susceptibility of MVSP *k* to pathogens (q_k). Appendix I includes a discussion of evidence for susceptibility to the 13 pathogens of members of the five MVSP. In rare cases, there exist enough data on the experience of a particular MVSP with a particular pathogen to derive a quantitative estimate for the mortality rate of that group (for example, the mortality rate among elderly from infection with SARS-CoV). In most cases, however, the quantitative estimates from experts on the Delphi panel (listed in Appendix I), which were estimates of percentage increase in susceptibility to disease and mortality for viruses and bacteria of each MVSP compared to healty adults (denoted q_k), are used. For this analysis, the maximum estimate from each set of expert estimates was applied, to reduce the possibility of producing non-conservative results. These values are listed in Table L–4.

Table L–4: Inputs for MVSP relative susceptibility values

MVSP (k)	Increased susceptibility compared to healthy adult (q_k)			
	to disease from bacteria	to mortality from bacteria	to disease from viruses	to mortality from viruses
1: Children under five	0.33	0.2	0.33	0.2
2: Adults over 65	0.5	0.5	0.5	0.5
3: People with diabetes	0.3	0.25	0.25	0.25
4: People with HIV/AIDS	0.4	0.3	0.4	0.3
5: Pregnant Women	0.3	0.2	0.5	0.2

See Appendix I for sources of these estimates. The q_k numbers are the maximum values from the expert estimates

Calculations

To derive quantitative estimates, a procedure is applied that is similar to what was described in Appendix K for initial infections. In that case, the probability p represented the probability of infection at a particular inhaled dose, whereas in this case, p represents the probability that a transmission occurs as a result of a contact between an infectious and a susceptible individual. In Appendix K, the probability of infection of a member of MVSP k, p_k, was related to the probability of infection of a "healthy adult" (defined as an individual not belonging to any of the five MVSP), p_h, using the estimated increased susceptibility q_k and formulas based on the dose-response models being assumed. For example, for the exponential dose-response model, the following formula was applied:

$$p_k = 1 - (1 - p_h)^{1+q_k}$$

For purposes of the secondary transmission analysis in this Appendix, a linearized version of this equation is applied:

$$p_k = (1 + q_k)p_h$$

This linearized equation is approximately equivalent to the more complicated version when the values of p are small, which is a reasonable assumption in the context of transmission probability per contact. For example, the R_0 value of SARS-CoV is approximately 3 (see Section L.3.5 below), which, considering that individuals presumably average hundreds of contacts over an infectious period lasting 2-3 weeks, translates to a value of p on the order of 1% or less. The linear approximation is applied so that the

following calculations may be performed without requiring an absolute estimate of the average number of contacts, which is difficult to obtain.

As in Appendix K, x_k refers to the portion of the U.S. population belonging to MVSP k and y_k refers to the portion of a particular local population in MVSP k. When it is assumed that a baseline R value is applicable to a typical U.S. population, then the assumptions described above can be applied to derive an adjusted value, R_{local} for a local population.

$$R = Cp = C\left[\sum_{k=1}^{5}(1+q_k)p_h x_k + p_h\left(1-\sum_{k=1}^{5}x_k\right)\right] = Cp_h\left(1+\sum_{k=1}^{5}q_k x_k\right)$$

$$R_{\text{local}} = Cp_h\left(1+\sum_{k=1}^{5}q_k y_k\right)$$

$$R_{\text{local}} = R\left(\frac{1+\sum_{k=1}^{5}q_k y_k}{1+\sum_{k=1}^{5}q_k x_k}\right)$$

The final equation above is used to calculate an adjusted overall R value for each of the three sites.

Probability of *x* or more infections or deaths among each individual MVSP

Simulations were run in which infections and deaths among each MVSP k were recorded, using the y_k values for that site. Each time a transmission occurs in a simulation, the appropriate set of y_k values for the secondary case is selected and used in the following formula.

$$\text{Probability that transmission is to MVSP } k = \frac{(1+q_k)y_k}{1+\sum_{k=1}^{5}q_k y_k}$$

A uniformly-distributed random number is drawn and compared to these probabilities to determine to which MVSP, if any, each secondary case belongs.

Once this MVSP identity is determined, the appropriate fatality rate is applied to randomly determine if the simulated case results in death. The case fatality rate (CFR) for each MVSP and for individuals not belonging to any MVSP is determined using the same procedure and calculations described in Appendix K.

This process is repeated using the values x_k instead of y_k, for the purpose of comparing what the estimated risk to the MVSP population at each site would be if all the local population proportions were in line with U.S. averages.

L.2.9.3 Effectiveness of control measures

It is possible that differences between the sites would lead to different expectations for the timing and effectiveness of control measures, as quantified by the parameters T_c and R_c in the quantitative transmission analyses. However, no convincing evidence was found to justify concrete assumptions about site differences in this regard, as explained in the following points.

- Any evaluation of the current facilities, resources, personnel, and outbreak preparedness at the suburban and rural sites may not be relevant if the NEIDL was actually located there. Presumably, the presence of NEIDL in the area would bring new medical resources to the area and potentially enhance the preparedness of the local area hospitals.

- Even if it could be concluded that there are differences in overall preparedness of hospitals at different sites, there are important factors contributing to the timing and effectiveness of control measures that are beyond the control of health officials. For example, i) decisions made by infected individuals in the pre-control phase of an outbreak, such as whether or not to seek medical attention, could contribute to delays; ii) the human factor, i.e., errors made by individual health care workers could contribute to delays and decrease control effectiveness; iii) variation in the biology infectious cases, for example, unusually contagious patients, could hinder control efforts. These examples could occur at any hospital, which supports the notion that the full range of possible delays and control effectiveness could be relevant at any site.

- There is no guarantee that medical facilities closest to the NEIDL location would be the ones primarily affected by an outbreak. Given the high rates of commuting observed at the three sites, an outbreak could easily spread outside the local area in the early stages, making it difficult to predict which area or hospital would begin seeing the first cases. For example, in the case of an outbreak started by an infected laboratory worker, the initial cases could be family members or individuals near the worker's home, which may or may not be close to the NEIDL.

L.3 Results

L.3.1 *Bacillus anthracis*

Bacillus anthracis (*B. anthracis*) causes inhalational, gastrointestinal or cutaneous forms of anthrax. There is no evidence of secondary transmission of inhalational or gastrointestinal anthrax. There are rare reports of person-to-person transmission of cutaneous anthrax. There are reports of humans acting as vectors in physically carrying spores on hands or inanimate items such as clothing to close contacts resulting in infection in the close contact (World Health Organization. 2008). The most recent example of this is the cutaneous anthrax that developed in a 7-month old infant who most likely came into contact with *B. anthracis* spores while being held by co-workers of his mother at her workplace in New York City that was contaminated with spores during the 2001 intentional release (Freedman, Afonja et al. 2002). As the person-to-person transmission of cutaneous anthrax is rare, secondary transmission modeling of spread of infection in the community after loss of bio-containment is not performed for *B. anthracis.*

L.3.2 *Francisella tularensis*

Francisella tularensis (*F. tularensis*) is the causative pathogen of tularemia, which is a disease of animals that also affects humans. *F. tularensis* can infect humans through the skin, mucous membranes, gastrointestinal tract, and lungs (Dennis, Inglesby et al. 2001; Ellis, Oyston et al. 2002). There are no reports of direct person-to-person transmission of *F. tularensis*, even from the pneumonic form of tularemia**.** There is one published report that suggests that bacteria are aerosolized from patients and in animal models of pneumonic tularemia and this could potentially cause secondary human infections (Jones, Nicas et al. 2005); these conclusions have not been validated by other authors or experts. As there is no known direct person-to-person transmission of *F. tularensis,* secondary transmission modeling of spread of infection in the community after loss of bio-containment is not performed for this pathogen

L.3.3 *Yersinia pestis*

Yersinia pestis (*Y. pestis*) causes plague and is transmissible from person to person, particularly in pneumonic form, as described in Chapter 3. Some detailed studies exist in the literature on epidemiological parameters and quantitative transmission modeling of pneumonic plague outbreaks that have occurred. Therefore, *Y. pestis* was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. The remainder of this section describes the specification of the quantitative transmission modeling procedure for *Y. pestis* and summarizes the modeling outputs.

L.3.3.1 Specification of branching process model

This section describes the parameters, distributions, and assumptions used for specifying the branching process model to outbreak simulations for *Y. pestis*.

Individual reproductive number ν

The individual reproductive number ν is a random variable describing the expected number of transmissions from an infected individual. Gani and Leach (2004) tested two different assumptions on the distribution of ν, one equivalent to assuming an exponential distribution and one equivalent to assuming a constant value with no variation, and evaluated them on their ability to describe transmission data from several documented outbreaks of pneumonic plague. They found that the exponentially distributed ν assumption was superior. Lloyd-Smith et al. (2005), using the same data, also tested those two distributions as well as the gamma distribution, and also chose the exponential distribution as being more accurate than the constant-ν assumption and close to as accurate but more parsimonious than the gamma distribution, needing only one parameter to adequately describe the data instead of two. Therefore, the exponential distribution was chosen to be applied to ν in this RA. Specifically, ν is assumed to have the probability density function f defined as

$$f(x) = \frac{1}{R}\, \mathrm{e}^{-\mathrm{x}/R}.$$

As described in the methodology section, when generating numbers of transmissions from each case in the simulations, the Poisson distribution with mean ν is applied, which, when ν is exponentially distributed with mean R, is equivalent to applying the geometric distribution with mean R (Lloyd-Smith 2005). This distribution is used both before the implementation of control measures, using R_0, and also after the implementation of control measures, using R_c. The values of these two parameters to be applied are discussed as follows.

Mean reproductive number R_0

Gani and Leach (2004) combined detailed contact-tracing data from several different pneumonic plague outbreaks before control measures were implemented to arrive at a estimate of $R_0 = 1.3$. Lloyd-Smith et al. (2005) analyzed the same data and arrived at the same estimate ($R_0 = 1.32$). No other estimates were found in the literature, so $R_0 = 1.32$ was applied as the base case assumption and the above analyses used as the basis for creating an uncertainty distribution. The raw data were not published, but an uncertainty distribution for R_0 can be constructed without the data by using the parametric bootstrap technique

described by Lloyd-Smith et al. (2005). The bootstrap sampling distribution was created by simulating 10,000 data sets of size $N = 74$ (the size of the data set from which the above R_0 estimate was generated) of randomly drawn numbers from the geometric distribution with mean $R_0 = 1.32$, and then calculating the average value of each simulated dataset (the R_0 estimate that would have been found under those data). The 10,000 values of R_0 were then used to generate random values of the paramter under the latin hypercube sampling scheme in the same manner that confidence intervals were calculated using the bias-corrected percentile method in Lloyd-Smith et al. (2005) and references therein. This procedure resulted in a 99% of randomly chosen R_0 values falling in the range (0.8 – 1.9), which is close to the range tested by Gani and Leach (2004) in their sensitivity analyses.

Mean post-control reproductive number R_c

Gani and Leach (2004) observed that in all but one of the six documented 20th century pneumonic plague outbreaks, transmissions were essentially completely curbed almost immediately after control measures were implemented. This observation is consistent with the notion that standard control measures can be highly effective at preventing transmission once awareness of an outbreak occurs. In their simulations, Gani and Leach essentially assume that $R_c = 0$ once control measures are put in place. The one exception among the historical outbreaks analyzed occurred in Mukden, China (Tieh 1948), during which 27 cases occurred after implementation of fairly substantial control measures. Lloyd-Smith et al. (2005) analyzed data from the post-control portion of the outbreak and arrived at an estimate $R_c = 0.41$. Since this outbreak was exceptional rather than typical in this regard, the value $R_c = 0.41$ is regarded as a worst feasible case, and the range $R_c = 0$–0.4 (uniform distribution) is applied for the uncertainty analysis, with the midpoint $R_c = 0.2$ applied as the base case.

Generation time (T_g)

Gani and Leach (2004) fit lognormal distributions to data on the incubation period and infectious periods from over 200 pneumonic plague cases. The average infectious period was short (2.5 days), and they argue that most transmissions would occur within one day of the end of the incubation period. In this sense, the incubation period, plus one day, would be a reasonable estimate for the generation time. Therefore, the Gani and Leach estimate of the incubation period (lognormal distribution with mean 4.3 days and standard deviation 1.8 days), shifted up by one day, is applied as the generation time distribution. This, then, gives an average generation time of 5.3 days. Uncertainty in the lognormal distribution parameters is not included in the analysis. The distribution provided a good fit to a large dataset consisting of observed events, so it is assumed that the estimated mean and standard deviation are relatively accurate values for pneumonic plague. Furthermore, the generation time affects the estimates

for the overall size of outbreaks through its relationship to the delay in implementation of control measures. Therefore, it is assumed that testing the uncertainty range of this delay also covers the potential implications of the average generation time being less or greater than 5.3 days.

Delay in implementation of control measures (T_c)

For six different pneumonic plague outbreaks, Gani and Leach (2004) documented the delay in implementation of control measures as measured in days from the onset of symptoms of the first case. The six different delays were 11, 12, 19, 20, 24, and 32 days, which average to approximately 20 days. Massin et al. (2007) ran simulations of potential pneumonic plague outbreaks in France and assumed a reference scenario in which the control delay was 10 days, and they tested a range of 1–41 days. Given that the outbreaks analyzed by Gani and Leach occurred in the early 20th century before modern medical advances and/or in underdeveloped countries, the average delay of 20 days is considered a worst feasible case for an outbreak occurring in modern-day U.S. A range of 0–20 days (continuous uniform distribution) is applied in the uncertainty analysis, and the midpoint of 10 days is applied for the base case, consistent with what was assumed by Massin et al. (2007) for modern day France.

Case fatality rate

Estimating the CFR for pneumonic plague essentially comes down to estimating the probability that patients would receive prompt care. At one extreme, patients who receive antibiotics before or very soon after development of symptoms would have a very low mortality rate approaching zero. At the other extreme, untreated patients would have a very high mortality rate approaching 100%. The time window for effectiveness of antibiotic treatment is estimated at approximately 24 hours after the onset of symptoms. Given this short window, it is assumed to be less likely that treatment would be delivered in time to patients who develop symptoms before control measures are put in place, because patients and caregivers may not immediately realize the cause of illness during this time. For these individuals, a CFR of 75% is applied. For individuals developing symptoms after control measures are in place, a CFR of 15% (the overall observed CFR for all forms of plague) is applied.

Summary

Table L–5: Summary of assumed parameter values for *Y. pestis* transmission model

Parameter	Base Case Value	Uncertainty Distribution
R_0	1.32	Parametric Bootstrap (99% range: 0.8–1.9)
R_c	0.2	Uniform (min = 0, max = 0.4)
T_g	4.3 days	N/A
T_c	10 days	Uniform (min = 0, max = 20)
CFR	75% before intervention, 15% after	N/A

L.3.3.2 Transmission Results – Base Case

Given the introduction of one initially infected individual into the population, 100,000 simulations were run under the base-case input parameters listed in Table L–5. A summary of the results of the output from these simulations for secondary infections and deaths are presented in the following table.

Table L–6: *Y. pestis* base case results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Number of simulations in which consequence occurred (of 100,000)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	56776 (57%)	34227 (34%)
	10 or more	16925 (17%)	5586 (5.6%)
	100 or more	0 (<0.001%)	0 (<0.001%)
Number of Public Fatalities	1 or more	52214 (52%)	29743 (30%)
	10 or more	3521 (3.5%)	750 (0.8%)
	100 or more	0 (<0.001%)	0 (<0.001%)

These results suggest that, under the base case assumptions, a laboratory worker infected with pneumonic plague who enters the public would have about a 57% chance of transmitting infection to at least one contact. Under the commuting assumptions described in Section L.2.5, there is a chance that the worker would not live in Boston, which explains why the chance drops to 34% for at least one secondary

infection occurring among Boston residents. The estimated chance of at least 10 public infections is 17% (5.6% among Boston residents), while the chance of at least 100 infections is very small, as no outbreaks that large occurred in 100,000 simulations. The chances for each number of fatalities occurring are somewhat smaller, as not every infection would result in fatality.

L.3.3.3 Transmission Results – Uncertainty

The input parameter uncertainty distributions summarized in Table L–5 were simultaneously applied within the Latin hypercube sampling scheme described in the methodology. One hundred sets of input parameters were generated, and 10,000 simulations were run under each set of parameters to generate alternate estimates for the chance of public infections and fatalities after an undetected or unreported initial infection. The following table summarizes the range of estimates found for each designated consequence. Each range was generated by sorting the 100 different estimates and dropping the lowest two and the highest two, so that 96% of the estimates are within or at the boundaries of the range. The base case results are also displayed in the table for reference.

Table L–7: *Y. pestis* base case and uncertainty results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	**57%** (48% to 63%)	**34%** (26% to 40%)
	10 or more	**17%** (0.8% to 34%)	**5.6%** (0.1% to 15%)
	100 or more	**<0.001%** (≈0 to 2.4%)	**<0.001%** (≈0 to 0.2%)
Number of Public Fatalities	1 or more	**52%** (18% to 59%)	**30%** (8.1% to 36%)
	10 or more	**3.5%** (≈0 to 26%)	**0.8%** (≈0 to 9.2%)
	100 or more	**<0.001%** (≈0 to 0.05%)	**<0.001%** (≈0)

"≈0" at the lower end of an uncertainty range means that the given consequence was not observed in any of the simulations under at least three of the 100 input parameter combinations; when appearing as the entire range, then the consequence was not observed for at least 98 of the 100 parameter combinations.

The uncertainty ranges for the consequence of one or more public infections are relatively narrow (plus or minus less than 10 percentage points from the base case estimate), suggesting that the base case estimates are relatively robust to uncertainties in the likelihood of transmission occurring from the initial case. For the consequence of one or more public fatalities, the base case estimates of 52% (total) and 30% (local residents) could be substantial overestimates given that the ranges drop as low as 18% and 8%, but are

unlikely to be substantial underestimates. On the other hand, the chance of ten or more fatalities could be as high as 26% (total) and 9% (resident), appreciably higher than the base case estimates. Outbreaks leading to 100 or more fatalities are rare under all parameter combinations, less than 0.05% (1-in-2,000 chance) for total fatalities and much smaller for fatalities among Boston residents.

L.3.3.4 Transmission results linked to event sequences

The results in the previous section are estimates of the chance of public infections and fatalities *given that one initially infected individual enters the community*. The initial infection analyses from Chapter 8 and Appendix K provide estimates for the average frequency of such an initiating event occurring, for three different scenarios. The estimates for *Y. pestis* were as follows.

Table L–8: Summary of *Y. pestis* initial infections results

Event	Frequency Range
Undetected / Unreported Needlestick Infection	1 in 100 to 10,000 years
Undetected / Unreported Centrifuge Release and Infection	1 in 56,000 to >10 million years
Earthquake Release and Infection	1 in >10 million years

Of these three events, an undetected or unreported needlestick infection is estimated as the most frequent initial infection event by which an individual could pose a secondary transmission risk to the public. Therefore, the given frequency range for needlestick is applied in the remainder of this section as the representative event for estimating the frequency of public infections and fatalities.

The exact frequency for the needlestick event is uncertain, and no basis for was found for assuming a most likely frequency within the range. Therefore, a distribution of frequencies across the given range is applied as an additional layer of uncertainty within the overall Latin hypercube sampling scheme. Random return periods are drawn from a uniform distribution across the range (100 years to 10,000 years), and the frequency is calculated by taking the inverse. A given needlestick frequency is then multiplied by a probability of a given consequence (public infections or fatalities due to transmission) to calculate the estimated frequency of that consequence. This results in a range of estimated frequencies for each consequence. A sampling of these ranges is shown in the following table.

Table L–9: *Y. pestis* uncertainty results – frequency of public infections and fatalities due to an undetected / unreported needlestick (urban site)

Consequence		Frequency Range	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	1 in 510 to 18,000 years	1 in 850 to 32,000 years
	10 or more	1 in 1,500 to 740,000 years	1 in 4,600 to 3.5 million years
	100 or more	1 in 130,000 to >10 million years	1 in 1.8 million to >10 million years
Number of Public Fatalities	1 or more	1 in 560 to 38,000 years	1 in 970 to 86,000 years
	10 or more	1 in 6,500 to >10 million years	1 in 21,000 to >10 million years
	100 or more	1 in 5.8 million to >10 million years	1 in >10 million years

The following table provides more insight into the distribution of estimated frequencies for each consequence, in terms of the portion of each distribution falling in each of the four frequency categories.

Table L–10: Y. pestis uncertainty results – frequency categories for public infections and fatalities due to undetected / unreported needlestick (urban site)

Consequence		Percentage of 100 estimates falling in each category							
		Total				Among Boston Residents			
		A	B	C	D	A	B	C	D
Number of Public Infections	1 or more	0	54%	46%	0	0	33%	67%	0
	10 or more	0	17%	82%	1%	0	4%	85%	11%
	100 or more	0	0	11%	89%	0	0	1%	99%
Number of Public Fatalities	1 or more	0	41%	59%	0	0	21%	79%	0
	10 or more	0	4%	61%	35%	0	2%	53%	45%
	100 or more	0	0	0	100%	0	0	0	100%

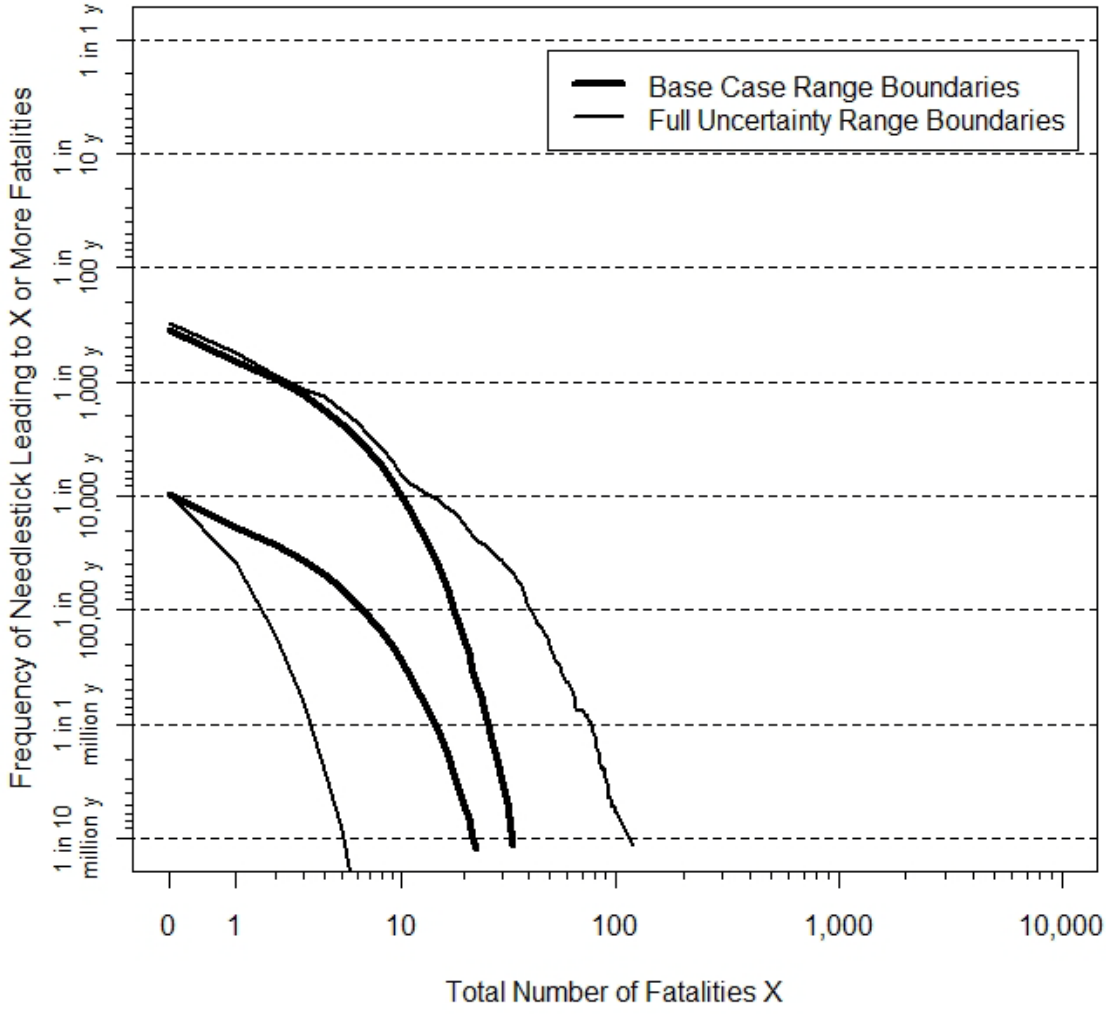
A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years; C = 1 in 10,000 to 1 million years; D = 1 in >1 million years

Finally, estimated ranges of frequencies for different consequences are conveyed graphically in Figure L–1. In the figure, numbers of fatalities are plotted against the estimated frequency with which a needlestick event leading to at least that number of fatalities in the public would be expected to occur. Under a given set of transmission parameters and a given needlestick frequency, a curve can be generated by connecting the points calculated by multiplying the needlestick frequency by the estimated chance that at least the

given number of fatalities would occur in the public. A different curve exists for each of the 100 sets of input values that were used in the uncertainty analysis. Rather than plotting all 100 curves, four curves are displayed, which form base case boundaries (thick curves) and full uncertainty boundaries (thin curves).

The base case boundaries were formed by multiplying the 95% uncertainty range for the needlestick frequency by the base case estimates from the secondary transmission analysis. The full uncertainty range represents the combined effects of uncertainty in the needlestick frequency and uncertainty in the secondary transmission parameters. By choosing a given number of fatalities on the horizontal axis, one can move vertically up the figure to find the boundaries for the estimated frequency range for that consequence.

Figure L–1: Risk curves for total public fatalities from *Y. pestis* (pneumonic plague) due to undetected / unreported needlestick, urban site



L.3.4 1918 H1N1 influenza virus

1918 H1N1 influenza virus (1918 H1N1V) was transmitted person-person worldwide during the 1918 influenza pandemic, as described in Chapter 3. Numerous studies exist in the literature on epidemiological parameters and quantitative transmission modeling of the 1918 pandemic in addition to other influenza pandemics, including the most recent pandemic in 2009, which was also caused by an H1N1 influenza virus (2009 H1N1V). Therefore, 1918 H1N1V was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the

public under the release scenarios analyzed in this RA. The remainder of this section describes the specification of the quantitative transmission modeling procedure for 1918 H1N1V and summarizes the modeling outputs.

L.3.4.1 Specification of branching process model

This section describes the parameters, distributions, and assumptions used for specifying the branching process model to outbreak simulations for 1918 H1N1V.

Individual reproductive number ν

The individual reproductive number ν is a random variable describing the expected number of transmissions from an infected individual. One study was found (Fraser 2009) that attempted to apply multiple distributions for individual variation in infectiousness for influenza, and they applied the models to data from 2009 H1N1V in Mexico. One was a Poisson model, which is equivalent to assuming that there is no variation in ν, and the other was a negative binomial model, which is equivalent to assuming that ν follows a gamma distribution with mean R and shape parameter k. The Poisson model is a special case of the negative binomial model, with the same mean and k approaching infinity. The best fit negative binomial model in light of the outbreak data produced values of k that led the authors to suggest that the negative binomial model might be more plausible than the Poisson model. Finite values of k produce an overdispersed distribution, which, compared to the Poisson model, allows for a higher probability of a much-larger-than-average number of transmissions from an individual, i.e., a superspreading event (Lloyd-Smith 2005). Given that superspreading events have been observed for influenza (e.g., Moser 1979) and that such events would have important risk implications for scenarios analyzed in this RA, the gamma distribution was chosen to be applied to ν in this RA. Specifically, ν is assumed to have the probability density function f defined as

$$f(x) = \frac{(k/R)^k}{\Gamma(k)} x^{k-1} \mathrm{e}^{-kx/R}.$$

The gamma distribution is used both before the implementation of control measures, using R_0 and k_0 as the two parameters, and also after the implementation of control measures, using R_c and k_c as the two parameters. The values of these four parameters to be applied are discussed as follows.

Mean reproductive number R_0 (and associated generation time T_g)

For the simulations created as part of the quantitative analysis for this RA, the mean reproductive number R_0 is used to estimate the average number of secondary transmissions from each infected individual at the beginning of a potential outbreak. According to the strict definition of R_0, its value applies to a

population that is fully susceptible to the pathogen. Most of the pathogens analyzed in this RA have occurred very rarely among humans in the United States, and it is reasonable to assume that none of the potentially exposed individuals in the public would be immune or partially protected from infection due to past exposure or vaccination. However, in the case of influenza, many members of the public have recovered from or been immunized against infection with one or more strains of the virus, and past experience with newly emerged strains that have caused pandemics suggests that some portion of the population had pre-existing protection due to past infection with or immunization against related strains (see Chapter 3 for references). Therefore, when reviewing the literature on past influenza pandemics for values of R_0 that are appropriate for the way it used in this RA, it is important to focus on estimates of what the average number of transmissions *actually* was in the early stage of the outbreak, rather than what the average number of transmission *would have been* in a population that was fully susceptible. In this sense, the estimated value of R_0 for 1918 H1N1V is interpreted as the average number of transmissions per infected individual early in an outbreak among the current human population, rather than among a fully susceptible population in the strict sense.

Estimates of R_0 from historical records about the 1918 pandemic and other influenza pandemics are mostly based on times series data consisting of the number of new cases or deaths appearing over the course of an outbreak. Detailed contact-tracing data (number of secondary cases infected by each primary case) necessary for a direct calculation of R_0 are scarce even for modern influenza pandemics, so alternate techniques are employed to infer the value of R_0. One difficulty in pinning down the value of R_0 for influenza implied by time series data of case counts is that the estimate of the R_0 value is sensitive to the assumption of the average value and distribution of the generation time T_g (see Section L.2.2) (Wallinga 2007). That is, a range of values of R_0 can be consistent with a given data set depending on what is assumed about the generation time. Therefore, the values of R_0 and T_g to be chosen for 1918 H1N1V analyses in this RA are discussed together here.

Mills et al. (2004) estimated R (what we are calling R_0 as discussed above) from mortality data from 45 U.S. cities during the 1918 pandemic, and they arrived at range of 2–3 for the value. This range was based on an assumed average generation time of approximately 4 days. Their figure assessing the sensitivity of the R estimate to the assumed generation time shows that their R estimate would drop below 2 for a generation time less than about 3 days and rise above 3 for a generation time more than about 5 or 6 days. Chowell et al. (2007) arrived at the same estimated range of 2–3 for R derived from fitting models to a different data set and under similar assumptions about the generation time. Chowell et al. (2006) estimated a larger R value of 3.75 based model fitting to data from the fall wave 1918 influenza in

Switzerland, but they acknowledge that the data support much smaller values of R depending on what is assumed about the relative contact rate of hospitalized vs. non-hospitalized cases. Gani et al. (2005) also assumed approximately 4 days for the average generation time but came up with lower R estimates in the range 1.55–2.0 based on 1918 data from England and Wales. They state that these numbers were lower than those derived by Mills et al. "probably because our estimates were derived from data throughout England and Wales, thereby incorporating spatial heterogeneity," whereas the Mills et al. estimates were based on urban data from individual U.S. cities treated separately. White and Pagano (2008) estimated R_0 values for various Maryland communities during the 1918 outbreak to be between 1.34 and 3.21 and also attempted estimates of the serial interval based on this data and data from outbreaks that occurred on ships, resulting in estimates as low as 2.83 days but as high as 8.28 days, associated with considerable uncertainty.

Recent studies have assessed the available evidence for the characteristics of influenza A transmission events more closely and found that the assumptions used in many of the above studies may result in overestimates of the generation time, thereby skewing the derived estimates for R. Ferguson et al. (2006) performed an extensive statistical analysis on data from influenza infections among members of households and arrived at an estimate of 2.6 days for T_g (95% interval 2.1–3.0 days), values that would shift the estimated R value by Mills et al. (2004) below 2. The Ferguson et al. (2006) study also provided estimates of R_0 based on 1918 data from U.S. and British cities in the range 1.7–2.0. Wallinga et al. (2007) similarly used household data from a different influenza study to arrive at an estimate of 2.85 days for T_g (standard deviation 0.93 days), from which they derive a range of about 1.6–1.8 for R based on the Mills et al. (2004) estimated growth rates of influenza cases in 1918.

The notion that transmission during the 1918 pandemic may have been characterized by short (three days or less) average generation times and values of R less than 2 is consistent with several results pertaining to the most recent 2009 H1N1 pandemic. Fraser et al. (2009) used detailed data from an outbreak in an isolated community in Mexico to derive an independent estimate of the mean generation time at 1.91 days (95% range 1.30–2.71 days), which was associated with an R_0 estimate of 1.58 (95% range 1.34–2.04). This estimate for R_0 was consistent with estimates based on other epidemiological data sets provided in the same report, which fell in the range 1.4–1.6, with alternative estimates and confidence intervals spanning a range of about 1.1–2.0. Yang et al. evaluated data from 2009 H1N1V in the U.S. and also estimated R_0 to be less than 2 (range 1.3–1.7) along with relatively short generation time estimates (range 2.6–3.2 days). Ghani et al. (2010) performed a detailed analysis of all laboratory-confirmed cases in the UK and arrived at an average generation time estimate of 2.5 days, along with estimates for R in the range

1.2–1.5. Cauchemez et al. (2009) did not estimate R_0 but found a similar estimate for the average generation time (2.6 days) based on household transmission data in the U.S. Similar results (R_0 approximately or less than 2 and T_g less than 3) were found based on data from outbreaks in the United States (after adjusting for ascertainment bias) (White 2009), New Zealand (Paine 2010, Nishiura 2009b), Thailand (de Silva 2009), and Canada (Hsieh, 2009).

At least three studies estimated *R* to be less than two in conjunction with longer generation time estimates of more than three days. These results imply lower risk of large outbreaks occurring in a given horizon (e.g., before control measures can be implemented) than the results outlined above. Tuite et al. (2010) estimated a low R_0 (1.31) in conjunction with a longer average generation time of 4–5 days, based on data from Ontario, Canada. Pourbohloul et al. (2009) estimated a low *R* (about 1.5), in conjunction with assumptions implying a relatively long generation time greater than 4 days, based on data from Mexico City. Hahne et al. (2009) estimated *R* to be less than one (0.5) in conjunction with a generation time of 3 days for cases occurring in the Netherlands, although they caution that this value of *R* may be an underestimate.

A few studies estimated *R* values greater than two in conjunction with short generation times of less than three days, values which imply higher risk of large outbreaks occurring in a fixed time. McBryde et al. (2009) estimated the average generation interval at 2.9 days (standard deviation 1.4 days) based on 2009 H1N1V cases in Australia with an identified primary contact, and the associated estimate of *R* was 2.4, although an alternate model accounting for potential case ascertainment bias shifts the *R* estimate to the range 1.5–1.8. Boelle et al. (2009) used Mexican outbreak data to derive upper bound estimates of *R* of about 2.2 and 2.6 in conjunction with assumed average generation times of 2.6–3.1 days. However, these were upper bound estimates that were uncorrected against potential underreporting bias, which would decrease the estimates of *R*. Nishiura et al. (2009a) estimated *R* at 2.3 (range 2.0–2.6) for an outbreak in Japan, under the assumption of 1.9 days for the average generation time. A potential reason for a higher *R* value and faster spread in Japan and possibly Australia compared to other places is that the outbreaks in those countries were primarily fueled by transmission in schools among children, for whom high age-specific transmission was estimated (McBryde 2009).

The Ghani et al. (2010) study was chosen as the basis for the base case assumed values to characterize the generation time for this RA. This study was based on a large and varied data set for 2009 H1N1V, and the results are consistent with other prominent studies as described above. Thus, the average generation time is assumed to be 2.51 days, with a standard deviation of 1.55 days. It is assumed that the gamma

distribution characterizes variability of the generation time within the simulations for this RA, with a shape parameter of 2.62 and scale parameter of 0.957, which lead to a mean and standard deviation consistent with the Ghani et al. results. For the uncertainty analysis, values for the average generation time are tested over the range 1.5 days to 3.5 days. This range covers the possibility that the average generation time is less than two days, as implied by results from Mexico (Fraser 2009). The range also covers the possibility that the average generation time was longer for the 1918 H1N1V than it was for 2009 H1N1V, as potentially implied by White and Pagano (2008).

The uncertainty analysis techniques employed here require that each input variable be assigned a probability distribution. For T_g, it is not obvious how to assign a probability distribution over the assumed range, especially given the fact that the base case value and range of values were assigned by surveying many research studies that used a wide variety of techniques to arrive at their estimates. To construct a distribution, it was first assumed that the base case value is the *most likely* value for the parameter (i.e., the *mode* of the distribution), and that the limits of the given range are the *minimum* and the *maximum* of the distribution. Given that no criteria other than the mode, minimum, and maximum for choosing a distribution is readily apparent, it was determined that a simple distribution satisfying those three statistics would be appropriate – the triangular distribution, for which the probability density function $T_g(x)$ is defined as follows.

$$T_g(x) = \begin{cases} x - 1.5, & 1.5 \leq x \leq 2.5 \\ 3.5 - x, & 2.5 < x \leq 3.5 \end{cases}$$

For a given choice of the average generation time, the variance of the generation time distribution to be applied in simulations was chosen such that the variance-to-mean ratio is the same as that found by Ghani et al. (2010).

Given the above assumed values characterizing the generation time distribution, the assumptions for R_0 can be presented. For the base case example, a value of $R_0 = 1.6$ is assumed. This value is calculated based on the mean growth rate of 1918 influenza cases in the U.S. estimated by Mills et al. (2004), the most extensive study on 1918 H1N1V spread in the literature, combined with the base case estimate of the generation time distribution (Wallinga 2007). To account for other estimates in the literature associated with average generation times comparable to the base case, a range of 1.2–2.0 (triangular distribution) is assumed for the uncertainty analysis. This distribution is shifted according to the value of the average generation time chosen for a particular sample in the uncertainty analysis, such that range is 1.5–2.3 at the upper end average generation time of 3.5 days, and 0.9–1.7 at the lower end average

generation time of 1.5 days, to account for the fact that higher values of R_0 may be consistent with outbreak data under the assumption of a longer average generation time.

Shape / dispersion parameter k_0

The parameter k_0 found in the probability density function of the gamma distribution (see equation under individual reproductive number ν above) is often called the "shape" parameter of the distribution. If the mean (in this case, R_0) is held constant, and the value of k_0 is changed, the shape of the distribution changes, causing the variance of the distribution to increase as k_0 decreases. Specifically, the variance is calculated as R_0^2/k_0. The random variable ν for the individual reproductive number is assumed to be gamma-distributed with mean R_0 and shape parameter k_0. Then the random variable Z for the actual number of transmissions from an infected individual is assumed to be Poisson distributed with mean ν. A Poisson-distributed random variable with a mean that is gamma-distributed with mean R_0 and shape parameter k_0 has a negative binomial distribution with mean R_0 and "dispersion parameter" k_0.

Fraser et al. (2009) fit a negative binomial model to 2009 H1N1V outbreak data from Mexico and their estimate of k indicated "low-to-moderate overdispersion." Any negative binomial distribution with finite dispersion parameter is overdispersed compared to the Poisson distribution (which is equivalent to the negative binomial distribution with the same mean and infinite dispersion parameter k). The authors imply that k values less than 0.5 would indicate high overdispersion, in that an epidemic associated with k values that low would be characterized by superspreading. Therefore, "low-to-moderate overdispersion" is interpreted to mean a value of k_0 greater than 0.5. For the uncertainty analysis, a continuous uniform distribution is applied for the inverse, $1 / k_0$, on the range 0–2, so that values near 2 imply k_0 near 0.5, and values near 0 imply large k_0. The median of this distribution, at which $k_0 = 1$, is applied as the base case estimate. This value is a special case implying that ν is exponentially distributed, which is a common assumption made in conjunction with differential equation models of disease spread (Lloyd-Smith 2005), including several influenza models.

Mean post-control reproductive number R_c

The parameter R_c is the average number of transmissions per infected individual after control measures for the pathogen have been implemented. For influenza viruses exhibiting relatively short generation times, control measures to reduce transmission can be difficult to implement at the hospital level through anitviral treatment, isolation, and quarantine, because transmissions from a primary case to a secondary case can occur close to, or possibly before, the time that a primary case develops symptoms and is aware of being infected. Nevertheless, increased surveillance for new cases and implementation of community-

wide measures to encourage (through public education) or enforce (through, for example, school closures) social distancing may serve to reduce transmission once implemented (Ferguson 2005).

No studies were found that attempted to measure R_c by averaging estimated reproductive numbers after set dates from which control measures were implemented. However, several studies estimated the evolution of R over time and found that the time-dependent value dropped and remained below one within a few weeks after the first observed cases in the given area (Boelle 2009; Nishiura 2009a; McBryde 2009; Paine 2010). By contrast, one study found that R had not dropped below one more than a month and half after the first known case in the UK (Ghani 2010).

Given the above information, it was decided that a base case value of R_c less than one would be appropriate, but that the uncertainty range should include values of R_c greater than one to account for the possibility that control measures would fail to contain an outbreak that had grown substantially in the initial phase. In addition, given that some values of R_0 in the uncertianty range are close to one, it was decided to calculate R_c as a fraction of the associated R_0 value. This procedure avoids the possibility that $R_c > R_0$ for a given parameter set in the uncertainty analysis. Under the base case, it is assumed that R_c is 50% of R_0, resulting in $R_c = 0.8$. For the uncertainty analysis, it is assumed that the percentage decrease from R_0 to R_c ranges from 25% to 75%, with a uniform distribution over this range.

Shape / dispersion parameter k_c

The same base case value and range applied for k_0 are also applied for k_c.

Delay in implementation of control measures (T_c)

Given that influenza infections are relatively common, initial cases of a potential 1918 H1N1V outbreak might escape notice if they were not linked to a potential source in a laboratory. Ferguson et al. (2005) estimated that it would take an initial cluster of about 20 cases before public health authorities would be aware of an unusual outbreak and initiate control policies, and that this threshold corresponds to about a 14 day delay. They tested a range of 0–28 days for this delay as part of a sensitivity analysis. This range is consistent with the range of control delays seen during the 2009 H1N1V pandemic in various locations, as reflected among the references cited under the R_c section above. Therefore, 14 days is assumed as the base case value of T_c, and a continuous uniform distribution over the range 0–28 days is applied for the uncertainty analysis. A delay close to 0 days would reflect the possibility that the first case (e.g., initially infected laboratory worker) would immediately recognize the source of infection and seek assistance in reducing further transmission from contacts.

Summary

Table L–11: Summary of assumed parameter values for 1918 H1N1V transmission model

Parameter	Base Case Value	Uncertainty Distribution
R_0	1.6	Triangular (mode = 1.3-1.9, min = 0.9-1.5, max = 1.7-2.3)[a]
k_0, k_c	1	Uniform on $1/k$ (min: $1/k$ = 0, max: $1/k$ = 2)
R_c	0.8	Uniform (min = 25% of R_0, max = 75% of R_0)
Average T_g	2.5 days	Triangular (mode = 2.5, min = 1.5, max = 3.5)
T_c	14 days	Uniform (min = 0, max = 28)
CFR	2.5%	N/A

[a] mode, min, and max of R_0 are dependent on value chosen for the average T_g; see text for details.

L.3.4.2 Transmission Results – Base Case

Given the introduction of one initially infected individual into the population, 100,000 simulations were run under the base-case input parameters listed in Table L–11. A summary of the results of the output from these simulations for secondary infections and deaths are presented in the following table.

Table L–12: 1918 H1N1V base case results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Number of simulations in which consequence occurred (of 100,000)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	61666 (62%)	38730 (39%)
	10 or more	39609 (40%)	19715 (20%)
	100 or more	28262 (28%)	8409 (8.4%)
	1,000 or more	3956 (4.0%)	24 (0.02%)
	10,000 or more	0 (<0.001%)	0 (<0.001%)
Number of Public Fatalities	1 or more	36332 (36%)	16193 (16%)
	10 or more	5586 (5.6%)	1447 (1.4%)
	100 or more	15 (0.02%)	0 (<0.001%)
	1,000 or more	0 (<0.001%)	0 (<0.001%)

These results suggest that, under the base case assumptions, a laboratory worker infected with 1918 H1N1V who enters the public would have about a 62% chance of transmitting infection to at least one contact. Under the commuting assumptions described in Section L.2.5, there is a chance that the worker would not live in Boston, which explains why the chance drops to 39% for at least one secondary infection occurring among Boston residents. There is an estimated 28% chance that an outbreak would grow to 100 total cases and less than 5% chance of 1,000 total cases. The relative low case fatality for 1918 H1N1V (compared to the other modeled pathogens) leads to an estimate of a less than 6% chance of 10 or more total fatalities occurring (less than 2% chance among Boston residents).

L.3.4.3 Transmission Results – Uncertainty

The input parameter uncertainty distributions summarized in Table L–11 were simultaneously applied within the Latin hypercube sampling scheme described in the methodology. One hundred sets of input parameters were generated, and 10,000 simulations were run under each set of parameters to generate alternate estimates for the chance of public infections and fatalities after an undetected or unreported initial infection. The following table summarizes the range of estimates found for each designated consequence. Each range was generated by sorting the 100 different estimates and dropping the lowest two and the highest two, so that 96% of the estimates are within or at the boundaries of the range. The base case results are also displayed in the table for reference.

Table L–13: 1918 H1N1V base case and uncertainty results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	**62%** (49% to 81%)	**39%** (30% to 51%)
	10 or more	**40%** (13% to 70%)	**20%** (4.1% to 39%)
	100 or more	**28%** (≈0 to 65%)	**8.4%** (≈0 to 29%)
	1,000 or more	**4.0%** (≈0 to 46%)	**0.03%** (≈0 to 11%)
	10,000 or more	**<0.001%** (≈0 to 30%)	**<0.001%** (≈0 to 2.2%)
	100,000 or more	**<0.001%** (≈0 to 30%)	**<0.001%** (≈0)
Number of Public Fatalities	1 or more	**36%** (8.2% to 69%)	**16%** (3.1% to 36%)
	10 or more	**14%** (≈0 to 53%)	**1.4%** (≈0 to 18%)
	100 or more	**0.02%** (≈0 to 31%)	**<0.001%** (≈0 to 6.3%)
	1,000 or more	**<0.001%** (≈0 to 30%)	**<0.001%** (≈0)

"≈0" at the lower end of an uncertainty range means that the given consequence was not observed in any of the simulations under at least three of the 100 input parameter combinations; when appearing as the entire range, then the consequence was not observed for at least 98 of the 100 parameter combinations.

The results for the consequence of one or more public infections suggest that about 50-80% is a reasonable range for the chance that an initially infected individual would transmit infection, and about 30-50% for the chance that a Boston resident becomes infected. Many of the estimates for the chance of larger outbreaks show substantial uncertainty, which reflects both the difficulty in pinpointing transmission parameters for 1918 H1N1V and the fact that the parameters are close to a threshold of outbreak controllability. For example, some sets of parameters lead to estimates suggesting that an outbreak reaching 100 total infections or 10 total fatalities would be extremely unlikely, while other sets of parameters lead to estimates of a 30% chance that a single initial case would cause an essentially unchecked outbreak.

The most extreme, high consequence estimates are only possible if it is assumed that the parameter R_c is greater than one, meaning that an outbreak that initially grows large and spreads over a wide area would remain uncontrolled. It should be noted that R_c staying above one for an extended period is potentially unrealistic, as public health officials in all areas would presumably step up control measures to more stringent levels in the face of a very large outbreak. However, these upper-limit results reflect the

implications of the possibility of an outbreak in which control of transmission remains elusive, a scenario which cannot entirely be ruled out.

The Boston resident results show a less than 3% chance of an outbreak reaching 10,000 infections among residents, even under extreme parameter assumptions. This is because the commuting assumptions and decreasing population susceptibility tend to reduce the effective R_c below the threshold required for long-term sustained resident-to-resident spread. However, sustained resident-to-resident spread leading to substantially more resident infections is possible for values of R_c just above those that were eliminated in constructing the ≈95% interval (See Table L–16).

L.3.4.4 Transmission results linked to event sequences

The results in the previous section are estimates of the chance of public infections and fatalities *given that one initially infected individual enters the community*. The initial infection analyses from Chapter 8 and Appendix K provide estimates for the average frequency of such an initiating event occurring, for three different scenarios. The estimates for 1918 H1N1V were as follows.

Table L–14: Summary of 1918 H1N1V initial infections results

Event	Frequency Range
Undetected / Unreported Needlestick Infection	1 in 100 to 10,000 years
Undetected / Unreported Centrifuge Release and Infection	1 in 490 to >10 million years
Earthquake Release and Infection	1 in 450,000 to >10 million years

Of these three events, an undetected or unreported needlestick infection extends to a higher frequency range that the other two events. The centrifuge infection event is of comparable frequency at the high end of the uncertainty range, but the range also contains a substantial portion of estimates for much lower frequencies (categories C and D). Therefore, the frequency range for needlestick infection is applied in the remainder of this section as the representative event for estimating the frequency of public infections and fatalities.

The exact frequency for the needlestick event is uncertain, and no basis for was found for assuming a most likely frequency within the range. Therefore, a distribution of frequencies across the given range is applied as an additional layer of uncertainty within the overall Latin hypercube sampling scheme.

Random return periods are drawn from a uniform distribution across the range (100 years to 10,000 years), and the frequency is calculated by taking the inverse. A given needlestick frequency is then multiplied by a probability of a given consequence (public infections or fatalities due to transmission) to calculate the estimated frequency of that consequence. This results in a range of estimated frequencies for each consequence. A sampling of these ranges is shown in the following table.

Table L–15: 1918 H1N1V uncertainty results – frequency of public infections and fatalities due to an undetected / unreported needlestick (urban site)

Consequence		Frequency Range	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	1 in 550 to 16,000 years	1 in 900 to 26,000 years
	10 or more	1 in 980 to 43,000 years	1 in 1,800 to 140,000 years
	100 or more	1 in 1,400 to >10 million years	1 in 3,900 to >10 million years
	1,000 or more	1 in 4,300 to >10 million years	1 in 15,000 to >10 million years
	10,000 or more	1 in 8,300 to >10 million years	1 in 350,000 to >10 million years
	100,000 or more	1 in 23,000 to >10 million years	>10 million years
Number of Public Fatalities	1 or more	1 in 1,100 to 70,000 years	1 in 2,300 to 170,000 years
	10 or more	1 in 2,700 to >10 million years	1 in 10,000 to >10 million years
	100 or more	1 in 5,800 to >10 million years	1 in 50,000 to >10 million years
	1,000 or more	1 in 23,000 to >10 million years	1 in >10 million years

The following table provides more insight into the distribution of estimated frequencies for each consequence, in terms of the portion of each distribution falling in each of the four frequency categories.

Table L–16: 1918 H1N1V uncertainty results – frequency categories for public infections and fatalities due to undetected / unreported needlestick (urban site)

Consequence		Percentage of 100 estimates falling in each category							
		Total				Among Boston Residents			
		A	B	C	D	A	B	C	D
Number of Public Infections	1 or more	0	62%	38%	0	0	38%	62%	0
	10 or more	0	39%	61%	0	0	17%	83%	0
	100 or more	0	28%	63%	9%	0	8%	71%	21%
	1,000 or more	0	11%	49%	40%	0	2%	32%	66%
	10,000 or more	0	3%	19%	78%	0	2%	3%	95%
	100,000 or more	0	2%	7%	91%	0	1%	1%	98%
Number of Public Fatalities	1 or more	0	37%	63%	0	0	15%	85%	0
	10 or more	0	18%	59%	23%	0	2%	56%	42%
	100 or more	0	3%	37%	60%	0	2%	7%	91%
	1,000 or more	0	2%	11%	87%	0	2%	0	98%
	10,000 or more	0	2%	4%	94%	0	0	0	100%

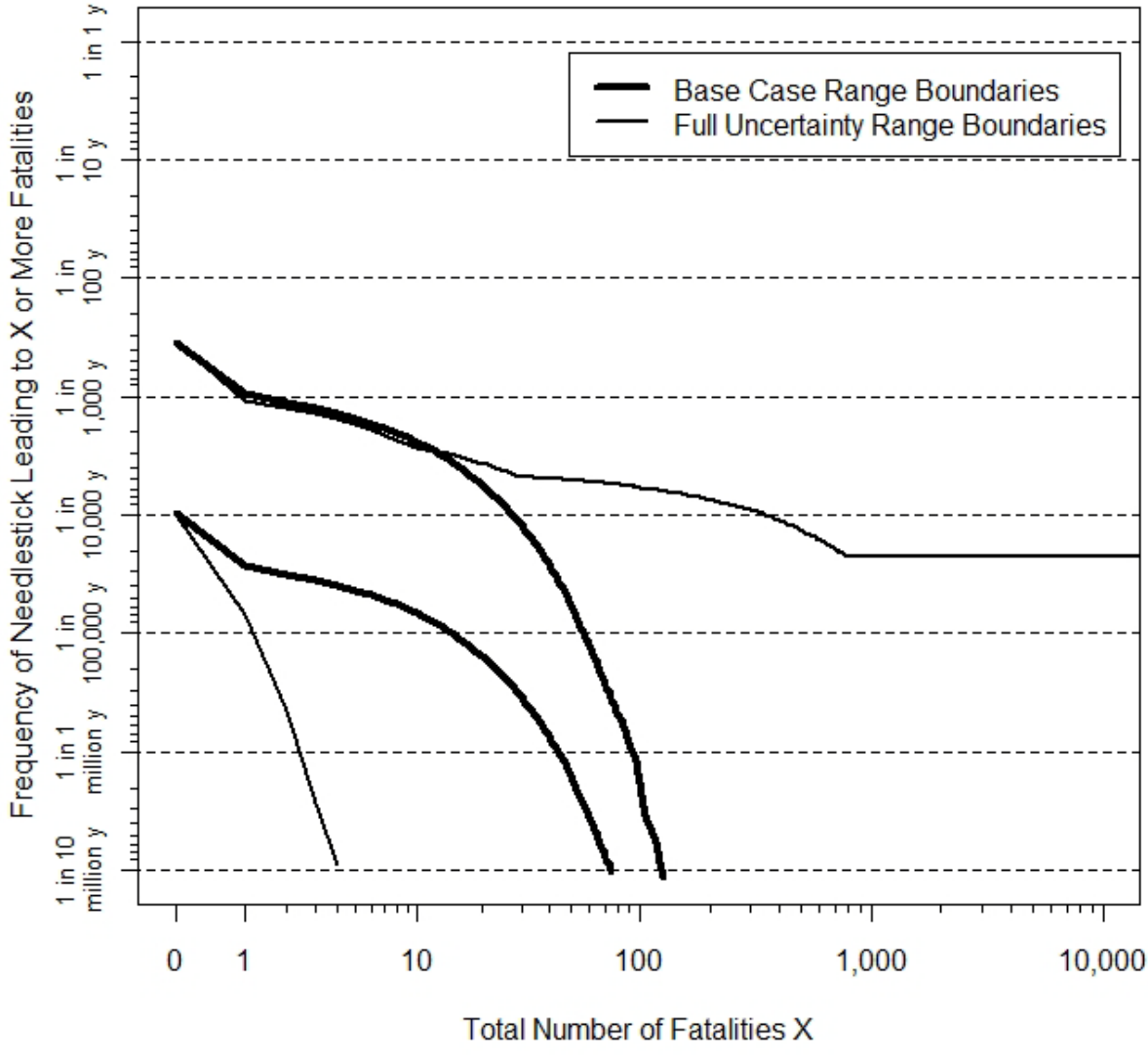
A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years; C = 1 in 10,000 to 1 million years; D = 1 in >1 million years

The results in the above table for the highest consequences reveal that a threshold is crossed for the most extreme 2-3% of parameter estimates, for which high consequence results are estimated to occur within frequency category B. On the other hand, it should also be noted that a substantial portion of estimates for 10 or more fatalities fall in frequency category D (less than one in one million years).

Finally, estimated ranges of frequencies for different consequences are conveyed graphically in Figure L–2. In the figure, numbers of fatalities are plotted against the estimated frequency with which a needlestick event leading to at least that number of fatalities in the public would be expected to occur. Under a given set of transmission parameters and a given needlestick frequency, a curve can be generated by connecting the points calculated by multiplying the needlestick frequency by the estimated chance that at least the given number of fatalities would occur in the public. A different curve exists for each of the 100 sets of input values that were used in the uncertainty analysis. Rather than plotting all 100 curves, four curves are displayed, which form base case boundaries (thick curves) and full uncertainty boundaries (thin curves).

The base case boundaries were formed by multiplying the 95% uncertainty range for the needlestick frequency by the base case estimates from the secondary transmission analysis. The full uncertainty range represents the combined effects of uncertainty in the needlestick frequency and uncertainty in the secondary transmission parameters. By choosing a given number of fatalities on the horizontal axis, one can move vertically up the figure to find the boundaries for the estimated frequency range for that consequence.

Figure L–2: Risk curves for total public fatalities from 1918 H1N1V due to undetected / unreported needlestick, urban site



This figure further emphasizes the extent of uncertainty associated with estimating the likelihood of consequences for 1918 H1N1V. The shape of the upper-limit thin curve is unusual because it reflects the fact that the uncertainty range for the post control reproductive number crosses the threshold $R_c = 1$ at the upper end of the uncertainty range, above which an outbreak will take off if it reaches a substantial size. On the other side, the lower limit curve drops quickly into frequency category D (less than one in one million years) for less than five fatalities. The narrower uncertainty range bounded by the thick curves perhaps represents a more realistic range of frequency estimates, but the more extreme estimates cannot be ruled out.

L.3.5 SARS-associated coronavirus

SARS-CoV is transmissible person-to-person, as observed in many locations during the 2003 outbreak described in Chapter 3. Numerous studies on epidemiological parameters and quantitative transmission modeling of SARS-CoV exist in the literature. Therefore, SARS-CoV was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. The remainder of this section describes the specification of the quantitative transmission modeling procedure for SARS-CoV and summarizes the modeling outputs.

L.3.5.1 Specification of branching process model

This section describes the parameters, distributions, and assumptions used for specifying the branching process model to outbreak simulations for SARS-CoV.

Individual reproductive number ν

The individual reproductive number ν is a random variable describing the expected number of transmissions from an infected individual. Lloyd-Smith *et al.* (2005) examined contact-tracing data from outbreaks of SARS in Beijing and Singapore, and they compared the ability of different distributions of the individual reproductive number, ν, to describe the spread of transmission numbers from each infected individual documented in the data. They demonstrated that using the gamma distribution for ν was superior to the other distributions tried. Specifically, they used a gamma distribution with mean R and shape parameter k, which has a probability density function f defined as

$$f(x) = \frac{(k/R)^k}{\Gamma(k)} x^{k-1} \mathrm{e}^{-kx/R}.$$

It was shown that this distribution is able to capture certain observed features of SARS outbreaks that have important implications for this RA, where other candidate distributions could not. Namely, it is able to estimate a relatively accurate proportion of cases not transmitting, which is important in assessing the likelihood that a single introduced case (e.g., an infected laboratory worker) would cause secondary infections, as well as a relatively accurate frequency of rapidly growing outbreaks, which is important in assessing the likelihood of the occurrence of an explosive outbreak. Given the success of the gamma distribution in describing variation in transmission observed in outbreaks in at least two cities and its ability to capture a range of outcomes with important implications for risk assessment, it was chosen as the distribution to be applied to v in this RA. The gamma distribution is used both before the implementation of control measures, using R_0 and k_0 as the two parameters, and also after the implementation of control measures, using R_c and k_c as the two parameters. The values of these four parameters to be applied are discussed as follows.

Mean reproductive number R_0

The mean reproductive number R_0 was estimated directly by Lloyd-Smith *et al.* (2005) for early generations of transmission during the SARS outbreaks in Beijing and Singapore, using contact tracing data from the first 2-3 generations of transmission. In Beijing, R_0 was estimated at 1.88 (90% confidence interval: 0.41-3.32) over the first two generations of transmission. In Singapore, R_0 was estimated at 2.55 (90% confidence interval: 0.50-4.50) over the first two generations of transmission, and the estimate dropped to 1.63 (90% confidence interval: 0.54-2.65) when the third generation of transmission was included. The authors explain that the drop in the R_0 estimate when including the third generation could potentially be explained by the fact that those transmissions occurred after WHO's global alert on SARS, which could have affected informal changes in behavior among infected individuals and at hospitals, even though centralized control measures were not yet in place in Singapore at that time.

As reflected in the size of the confidence intervals from the Lloyd-Smith et al. results, estimates of R_0 based on early-generation transmission data often suffer from a shortage of documented cases, and contact tracing can be imperfect. Other researchers have developed techniques for estimating R_0 indirectly using more easily observed data from outbreaks, such as case incidence rates, in combination with other epidemiological parameters, such as incubation period and generation time. This information is then translated into R_0 through the use of formulas derived from a mathematical model. For SARS, Bausch et al. (2005) reviewed a number of papers and their estimates for R_0. They determined a consensus estimate of $R_0 = 3$, which is reasonably consistent across quite different statistical and dynamic modeling techniques used in estimation procedures found in the literature. The review carefully

examined studies that estimated R_0 values significantly lower (Choi 2003, Chowell 2003, Wang 2004) or significantly higher (Gumel 2004, Hsieh 2004) than 3 and was able to reconcile those outliers by identifying inaccurate or questionable assumptions that led to the R_0 estimate. Note that $R_0 = 3$ is higher than the Lloyd-Smith et al. (2005) maximum likelihood estimates, but it's within their 90% confidence intervals for the first two generations of transmission in each city.

Based on this review of the literature, a base case value of $R_0 = 3$ was used in the branching process simulations. For uncertainty and sensitivity analysis, values in the range $2 \leq R_0 \leq 4$ were used. The upper limit of $R_0 = 4$ was chosen to reflect the fact that some researchers estimated values in the 3.5–4.0 range in papers whose methods were not called into question by the Bausch *et al.* review. For example, Wallinga and Teunis (2004) estimated $R_0 = 3.6$ for the Hong Kong outbreak, and Lipsitch et al. (2003) estimated $R_0 = 3.5$ for the Singapore outbreak.

The uncertainty analysis techniques employed here require that each input variable be assigned a probability distribution. For R_0, it is not obvious how to assign a probability distribution over the assumed range, especially given the fact that the base case value and range of values were assigned by surveying many research studies that used a wide variety of techniques to arrive at their estimates. To construct a distribution, it was first assumed that the base case value is the *most likely* value for the parameter (i.e., the *mode* of the distribution), and that the limits of the given range are the *minimum* and the *maximum* of the distribution. Given that no criteria other than the mode, minimum, and maximum for choosing a distribution is readily apparent, it was determined that a simple distribution satisfying those three statistics would be appropriate – the triangular distribution, for which the probability density function $R(x)$ is defined as follows.

$$R(x) = \begin{cases} x - 2, & 2 \leq x \leq 3 \\ 4 - x, & 3 < x \leq 4 \end{cases}$$

Using this distribution within the latin hypercube sampling scheme results in 99% of randomly chosen values for R_0 falling between 2.1 and 3.9.

Shape / dispersion parameter k_0

The parameter k_0 found in the probability density function of the gamma distribution (see equation under individual reproductive number ν above) is often called the "shape" parameter of the distribution. If the mean (in this case, R_0) is held constant, and the value of k_0 is changed, the shape of the distribution

changes, causing the variance of the distribution to increase as k_0 decreases. Specifically, the variance is calculated as R_0^2/k_0. The random variable ν for the individual reproductive number is assumed to be gamma-distributed with mean R_0 and shape parameter k_0. Then the random variable Z for the actual number of transmissions from an infected individual is assumed to be Poisson distributed with mean ν. A Poisson-distributed random variable with a mean that is gamma-distributed with mean R_0 and shape parameter k_0 has a negative binomial distribution with mean R_0 and "dispersion parameter" k_0.

Lloyd-Smith et al. (2005) reported an optimal value of $k_0 = 0.16$ for SARS by fitting the negative binomial distribution to transmission data from early generations of the Singapore outbreak. A similar k_0 value was found based on different SARS outbreak data from Beijing. The value of $k_0 = 0.16$ is used as the base case value for the simulations in this RA. This values of k_0 was calculated by Lloyd-Smith *et al.* using maximum likelihood methods that made use of an already calculated maximum likelihood estimate of R_0 that is different that the base case value of $R_0 = 3$ chosen for this RA. However, it was determined that using $R_0 = 3$ and $k_0 = 0.16$ together is not contraindicated by the Singapore and Beijing data in light of other important statistics. For example, when examining the proportion of cases not transmitting (p_0), the authors calculated $p_0 = 0.67$ directly from the Singapore data. For the negative binomial distribution, p_0 has the formula $p_0 = (1 + R_0/k_0)^{-k_0}$. Applying $R_0 = 3$ and $k_0 = 0.16$ to this formular results in $p_0 = 0.62$, close to the value calculated from the data and well within the 90% confidence intervals provided for estimates of p_0 from both Singapore and Beijing data. With this evidence in hand, it is assumed that the k_0 value estimated by Lloyd-Smith et al. is a reasonable estimate for use in this RA in conjunction with the assumed base case R_0 value.

To generate an uncertainty distribution for k_0, one of the procedures used by Lloyd-Smith et al. (2005) was employed (the authors used five statistical different methods to assess uncertainty in k_0, and resulting 90% confidence intervals were similar across all five methods). Specifically, a parametric bootstrap sampling distribution was created by simulating 10,000 data sets of size $N = 40$ (close to the size of the data sets from Singapore and Beijing outbreaks) of randomly drawn numbers from the negative binomial distribution with mean $R_0 = 3$ and dispersion parameter $k_0 = 0.16$, and then calculating the maximum likelihood value of k_0 from each simulated dataset. The 10,000 values of k_0 were then used to generate random values of the paramter under the latin hypercube sampling scheme in the same manner that confidence intervals were calculated using the bias-corrected percentile method in Lloyd-Smith et al. (2005) and references therein. This procedure resulted in a 90% of randomly chosen values falling in the range (0.10 – 0.30), which is very similar to the confidence intervals reported in Lloyd-Smith et al.

(2005). This similarity is evidence that the distribution of k_0 is not very sensitive to the choice of R_0 or to the size of the simulated data sets, which were different in the cited reference.

Mean post-control reproductive number R_c

The parameter R_c is the average number of transmissions per infected individual after control measures for the pathogen have been implemented. For SARS, the value of R_c has been estimated in a number of studies using data from locations in which a SARS outbreak occurred and control measures were implemented to reduce further transmissions. Lloyd-Smith et al. calculated $R_c = 0.68$ for the Singapore outbreak (generations 4 through 7 of transmission) and $R_c = 0.28$ for the Beijing outbreak (generations 3 and 4 of transmission). Wallinga and Teunis (2004) estimated R_c using a likelihood-based estimation procedure on case incidence data after the first global alert on SARS in four locations where an outbreak had already begun at that time (the assumption being that control measures would have started at that time in all locations). They calculated $R_c = 0.7$ for Hong Kong (95% confidence interval: 0.7, 0.8), $R_c = 0.3$ for Vietnam (95% confidence interval: 0.1, 0.7), $R_c = 0.7$ for Singapore (95% confidence interval: 0.6, 0.9), and $R_c = 1.0$ for Canada (95% confidence interval: 0.9, 1.2). Overall, they chose $R_c = 0.7$ for use in simulations.

Following Wallinga and Teunis, a base case value of $R_c = 0.7$ for SARS-CoV was applied for this RA. This value is consistent with the fact that every SARS outbreak in locations around the world was eventually brought under control. To cover lower or higher values estimated in studies cited in the previous paragraph, a range of 0.3–1.1 was applied as part of the uncertainty analysis. Specifically, a triangular distribution with mode 0.7, minimum 0.3, and maximum 1.1 was applied. Values near the maximum serve to test the implications of the possibility that control measures are barely able to stem transmission enough to bring an outbreak under control.

Shape / dispersion parameter k_c

Lloyd-Smith et al. (2005) calculated values of k_c based on transmission data from Singapore and Beijing after control measures were implemented at each location. The interpretation of and procedure for calculating the parameter are the same as described above for k_0. The Beijing dataset was smaller and consisted almost entirely of cases not transmitting at all, so the analysis was unable to provide a confidence interval for k_c under the bootstrap resampling procedures. The Singapore dataset was sizable (114 cases), more amenable to a full uncertainty analysis on k_c, and more consistent with the base case estimate of R_c used for this risk assessment. Therefore, the results from model fitting to the Singapore data set are used as the base case estimate ($k_c = 0.071$), and an uncertainty distribution is generated using

parametric bootstrap resampling as described above in conjunction with the parameter k_0. This procedure generates a 90% of estimates in the range (0.047, 0.13), which is well in line with the results from alternate procedures for generating confidence intervals as described in Lloyd-Smith et al. (2005).

Generation time (T_g)

Lipsitch et al. (2003) analyzed serial interval (generation time) data from 205 probable SARS cases in Singapore and found that the Weibull distribution, with mean 8.4 days and standard deviation 3.8 days, provided a good fit. Wallinga and Teunis (2004) chose the same distribution for use in their stochastic simulations. This distribution is used for simulations of SARS outbreaks for this RA, where the time between the onset of symptoms within each pair of cases involved in each transmission is drawn randomly from the given Weibull distribution. Uncertainty in the Weibull distribution parameters is not included in the analysis. The distribution provided a good fit to a large dataset consisting of observable events (onset of symptoms), so it is assumed that the estimated mean and standard deviation are relatively accurate values for SARS. Furthermore, the generation time affects the estimates for the overall size of outbreaks through its relationship to the delay in implementation of control measures. Therefore, it is assumed that testing the uncertainty range of this delay also covers the potential implications of the average generation time being less or greater than 8.4 days.

Delay in implementation of control measures (T_c)

Singapore: Counting from the date of onset of symptoms of the first case (day zero), the Singapore government was first notified of cases of atypical pneumonia on day 9 but were not yet aware that a SARS outbreak was ocurring. WHO's first global alert on SARS was administered on day 15 and second global alert on day 18, after which transmission rates may have decreased in Singapore due to informal behavioral changes or hospital precautions. Centralized control measures were implemented in Singapore on day 25 and intensified on day 27 and further on day 43. In the analysis of Lloyd-Smith et al. (2005), it was assumed that the first implementation of centralized control measures was the point at which average transmission rates were reduced from R_0 to R_c, representing a value of T_c of about 25 days. This led to their assumption that the first three generations of transmission occurred under pre-control transmission rates and that control measures affected transmission rates starting in generation four.

Beijing: Lloyd-Smith et al. (2005) analyzed a SARS outbreak that occurred at a specific hospital in Beijing. They estimated that two generations of transmission occurred before the hospital implemented control policies to reduce contact rates. Assuming an average generation time of 8.5 days, this assumption would translate to a value of T_c of about 17 days.

Wallinga et al. assumed that the WHO's first global alert on SARS (March 12, 2003) represented the transition from pre- to post-control average transmission rates for outbreaks in Hong Kong, Vietnam, Singapore, and Canada. In those four locations, the first cases were introduced in late February. This, then, is equivalent to an assumption of T_c equalling approximately 2–3 weeks.

Gumel et al. (2004) ran simulations assuming a date of March 30, 2003 for the beginning of quarantine and isolation procedures to reduce the assumed rate of contacts in their model for outbreaks in Toronto, Hong Kong, Singapore, and Beijing. Given that outbreaks started in those cities in late Februrary, the assumption was equivalent to using a value of T_c of approximately 4–5 weeks. They note that "in reality, quite effective control measures were probably put into place at least as early as mid-March, but given we are using a step function as an approximation for their implementation, choosing a slightly later starting date is warranted" (Gumel 2004, Electronic Appendix B).

The studies described above, which used a framework for modeling the effect of control measures as a step function in time similar to what is used in this RA, assumed values for T_c in the range of 2–5 weeks. It is noted that these studies analyzed historical outbreaks of SARS that began during a time when SARS-CoV had not yet been identified as the cause of the cases being seen and the epidemiology of and most effective strategies for curbing SARS transmission were only beginning to be understood. It is assumed that, if a new SARS outbreak were to begin in the present day, health officials would have a much better chance of identifying the source of early cases and implementing effective control strategies in a more timely manner than was possible in 2003 when SARS-CoV was first emerging.

An optimistic scenario would be that the first case to develop symptoms would suspect the source of infection (for example, a laboratory worker who was unknowingly exposed but would likely be wary of SARS-like symptoms during periods of experimental work with SARS-CoV). In this scenario, even if the initial case transmitted infection to others during the early stages of the symptomatic period, health officials would have a good chance of implementing effective control strategies before the second generation of cases became symtomatic themselves, using the lessons learned from the SARS outbreaks that occurred in 2003. This scenario is represented by assuming a minimum value of 3 days for the value of T_c, which is less than half of the assumed average generation time for SARS.

A more conservative scenario would be that early cases of an outbreak would not suspect or report that SARS-CoV was a possible source of infeciton, that health officials would not immediately diagnose early

cases, and/or that hospitals would delay in implementing effective control strategies due to ineffective preparation or imperfect execution of recommended policies. This scenario is represented by assuming a maximum value of 17 days for T_c, which is twice the assumed average generation time for SARS. This allows for the possibility that two and possibly three generations of transmission (or even more for a chain of below-average generation times) could occur before the transition.

The base case value was set at T_c = 10 days, the average of the minimum (3 days) and maximum (17 days) assumed values. It is not assumed that any delay within the range is more likely to occur than another; therefore, a uniform distribution across the range is applied for the uncertainty analysis.

Summary

Table L–17: Summary of assumed parameter values for SARS-CoV transmission model

Parameter	Base Case Value	Uncertainty Distribution
R_0	3.0	Triangular (mode = 3.0, min = 2.0, max = 4.0)
k_0	0.16	Parametric Bootstrap (90% interval: 0.10, 0.30)
R_c	0.7	Triangular (mode = 0.7, min = 0.3, max = 1.1)
k_c	0.071	Parametric Bootstrap (90% interval: 0.047, 0.13)
T_g	8.5 days	N/A
T_c	10 days	Uniform (min = 3, max = 17)
CFR	10%	N/A

L.3.5.2 Transmission Results – Base Case

Given the introduction of one initially infected individual into the population, 500,000 simulations were run under the base-case input parameters listed in Table L–17. A summary of the results of the output from these simulations for secondary infections and deaths are presented in the following table.

Table L–18: SARS-CoV base case results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Number of simulations in which consequence occurred (of 500,000)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	189233 (38%)	139175 (28%)
	10 or more	104160 (21%)	59252 (12%)
	100 or more	44085 (8.8%)	14906 (3.0%)
	1,000 or more	1160 (0.2%)	43 (0.009%)
	10,000 or more	0 (<0.0002%)	0 (<0.0002%)
Number of Public Fatalities	1 or more	119968 (24%)	73948 (15%)
	10 or more	45319 (9.1%)	15956 (3.2%)
	100 or more	1253 (0.3%)	44 (0.009%)
	1,000 or more	0 (<0.0002%)	0 (<0.0002%)

These results suggest that, under the base case assumptions, a laboratory worker infected with SARS-CoV who enters the public would have about a 38% chance of transmitting infection to at least one contact. Under the commuting assumptions described in Section L.2.5, there is a chance that the worker would not live in Boston, which explains why the chance drops to 28% for at least one secondary infection occurring among Boston residents. There is an estimated 8.8% chance that an outbreak would grow to 100 total cases and 0.2% chance of 1,000 total cases. The estimated 10% case fatality for SARS-CoV leads to an estimate of a less than 10% chance of 10 or more total fatalities occurring (less than 5% chance among Boston residents).

L.3.5.3 Transmission Results – Uncertainty

The input parameter uncertainty distributions summarized in Table L–17 were simultaneously applied within the Latin hypercube sampling scheme described in the methodology. One hundred sets of input parameters were generated, and 10,000 simulations were run under each set of parameters to generate alternate estimates for the chance of public infections and fatalities after an undetected or unreported initial infection. The following table summarizes the range of estimates found for each designated consequence. Each range was generated by sorting the 100 different estimates and dropping the lowest two and the highest two, so that 96% of the estimates are within or at the boundaries of the range. The base case results are also displayed in the table for reference.

Table L–19: SARS-CoV base case and uncertainty results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	**38%** (28% to 55%)	**28%** (20% to 39%)
	10 or more	**21%** (15% to 34%)	**12%** (6.7% to 19%)
	100 or more	**8.8%** (1.5% to 20%)	**3.0%** (0.3% to 9.2%)
	1,000 or more	**0.2%** (≈0 to 9.7%)	**0.009%** (≈0 to 2.1%)
	10,000 or more	**<0.0002%** (≈0 to 4.4%)	**<0.0002%** (≈0)
	100,000 or more	**<0.0002%** (≈0 to 2.8%)	**<0.0002%** (≈0)
Number of Public Fatalities	1 or more	**24%** (17% to 36%)	**15%** (10% to 24%)
	10 or more	**9.1%** (1.8% to 20%)	**3.2%** (0.4 to 9.4%)
	100 or more	**0.3%** (≈0 to 9.7%)	**0.009%** (≈0 to 2.0%)
	1,000 or more	**<0.0002%** (≈0 to 4.4%)	**<0.0002%** (≈0)
	10,000 or more	**<0.0002%** (≈0 to 2.8%)	**<0.0002%** (≈0)

"≈0" at the lower end of an uncertainty range means that the given consequence was not observed in any of the simulations under at least three of the 100 input parameter combinations; when appearing as the entire range, then the consequence was not observed for at least 98 of the 100 parameter combinations.

The results for the consequence of one or more public infections suggest that about 28-55% is a reasonable range for the chance that an initially infected individual would transmit infection, and about 20-40% for the chance that a Boston resident becomes infected. The uncertainty ranges for larger outbreaks are relatively narrow compared to those for 1918 H1N1V, but still notable. At one extreme, some sets of parameters lead to estimates suggesting that an outbreak reaching 100 total infections or 10 total fatalities would be less than 2%. At the other extreme, some parameter combinations produce estimates of about a 3% chance that a single initial case would cause an essentially unchecked outbreak.

The most extreme, high consequence estimates are only possible if it is assumed that the parameter R_c is greater than one, meaning that an outbreak that initially grows large and spreads over a wide area would remain uncontrolled. It should be noted that R_c staying above one for an extended period is potentially unrealistic, as public health officials in all areas would presumably step up control measures to more stringent levels in the face of a very large outbreak. However, these upper-limit results reflect the

implications of the possibility of an outbreak in which control of transmission remains elusive, a scenario which cannot entirely be ruled out.

The Boston resident results show about a 2% chance or less of an outbreak reaching 1,000 infections and 100 fatalities among residents, even under extreme parameter assumptions. This is because the commuting assumptions and decreasing population susceptibility tend to reduce the effective R_c below the threshold required for long-term sustained resident-to-resident spread.

L.3.5.4 Transmission results linked to event sequences

The results in the previous section are estimates of the chance of public infections and fatalities *given that one initially infected individual enters the community*. The initial infection analyses from Chapter 8 and Appendix K provide estimates for the average frequency of such an initiating event occurring, for three different scenarios. The estimates for SARS-CoV were as follows.

Table L–20: Summary of SARS-CoV initial infections results

Event	Frequency Range
Undetected / Unreported Needlestick Infection	1 in 100 to 10,000 years
Undetected / Unreported Centrifuge Release and Infection	1 in 9,100 to 600,000 years
Earthquake Release and Infection	1 in >10 million years

Of these three events, an undetected or unreported needlestick infection extends to a higher frequency range that the other two events. The centrifuge infection event is within the needlestick range at the high end of the uncertainty range, but the range also contains a substantial portion of estimates for lower frequencies. Therefore, the frequency range for needlestick infection is applied in the remainder of this section as the representative event for estimating the frequency of public infections and fatalities.

The exact frequency for the needlestick event is uncertain, and no basis for was found for assuming a most likely frequency within the range. Therefore, a distribution of frequencies across the given range is applied as an additional layer of uncertainty within the overall Latin hypercube sampling scheme. Random return periods are drawn from a uniform distribution across the range (100 years to 10,000 years), and the frequency is calculated by taking the inverse. A given needlestick frequency is then multiplied by a probability of a given consequence (public infections or fatalities due to transmission) to

calculate the estimated frequency of that consequence. This results in a range of estimated frequencies for each consequence. A sampling of these ranges is shown in the following table.

Table L–21: SARS-CoV uncertainty results – frequency of public infections and fatalities due to an undetected / unreported needlestick (urban site)

Consequence		Frequency Range	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	1 in 760 to 27,000 years	1 in 1,000 to 37,000 years
	10 or more	1 in 1,100 to 59,000 years	1 in 1,800 to 120,000 years
	100 or more	1 in 2,500 to 440,000 years	1 in 5,600 to 3.2 million years
	1,000 or more	1 in 23,000 to >10 million years	1 in 160,000 to >10 million years
	10,000 or more	1 in 67,000 to >10 million years	1 in >10 million years
	100,000 or more	1 in 260,000 to >10 million years	1 in >10 million years
Number of Public Fatalities	1 or more	1 in 1,000 to 47,000 years	1 in 1,600 to 80,000 years
	10 or more	1 in 2,500 to 350,000 years	1 in 5,300 to 1.9 million years
	100 or more	1 in 23,000 to >10 million years	1 in 170,000 to >10 million years
	1,000 or more	1 in 67,000 to >10 million years	1 in >10 million years
	10,000 or more	1 in 260,000 to >10 million years	1 in >10 million years

The following table provides more insight into the distribution of estimated frequencies for each consequence, in terms of the portion of each distribution falling in each of the four frequency categories.

Table L–22: SARS-CoV uncertainty results – frequency categories for public infections and fatalities due to undetected / unreported needlestick (urban site)

Consequence		Percentage of 100 estimates falling in each category							
		Total				Among Boston Residents			
		A	B	C	D	A	B	C	D
Number of Public Infections	1 or more	0	40%	60%	0	0	30%	70%	0
	10 or more	0	22%	78%	0	0	9%	91%	0
	100 or more	0	7%	93%	0	0	3%	90%	7%
	1,000 or more	0	1%	42%	57%	0	0	14%	86%
	10,000 or more	0	0	7%	93%	0	0	0	100%
	100,000 or more	0	0	4%	96%	0	0	0	100%
Number of Public Fatalities	1 or more	0	28%	72%	0	0	14%	86%	0
	10 or more	0	8%	92%	0	0	3%	91%	6%
	100 or more	0	1%	42%	57%	0	0	15%	85%
	1,000 or more	0	0	7%	93%	0	0	0	100%
	10,000 or more	0	0	4%	96%	0	0	0	100%

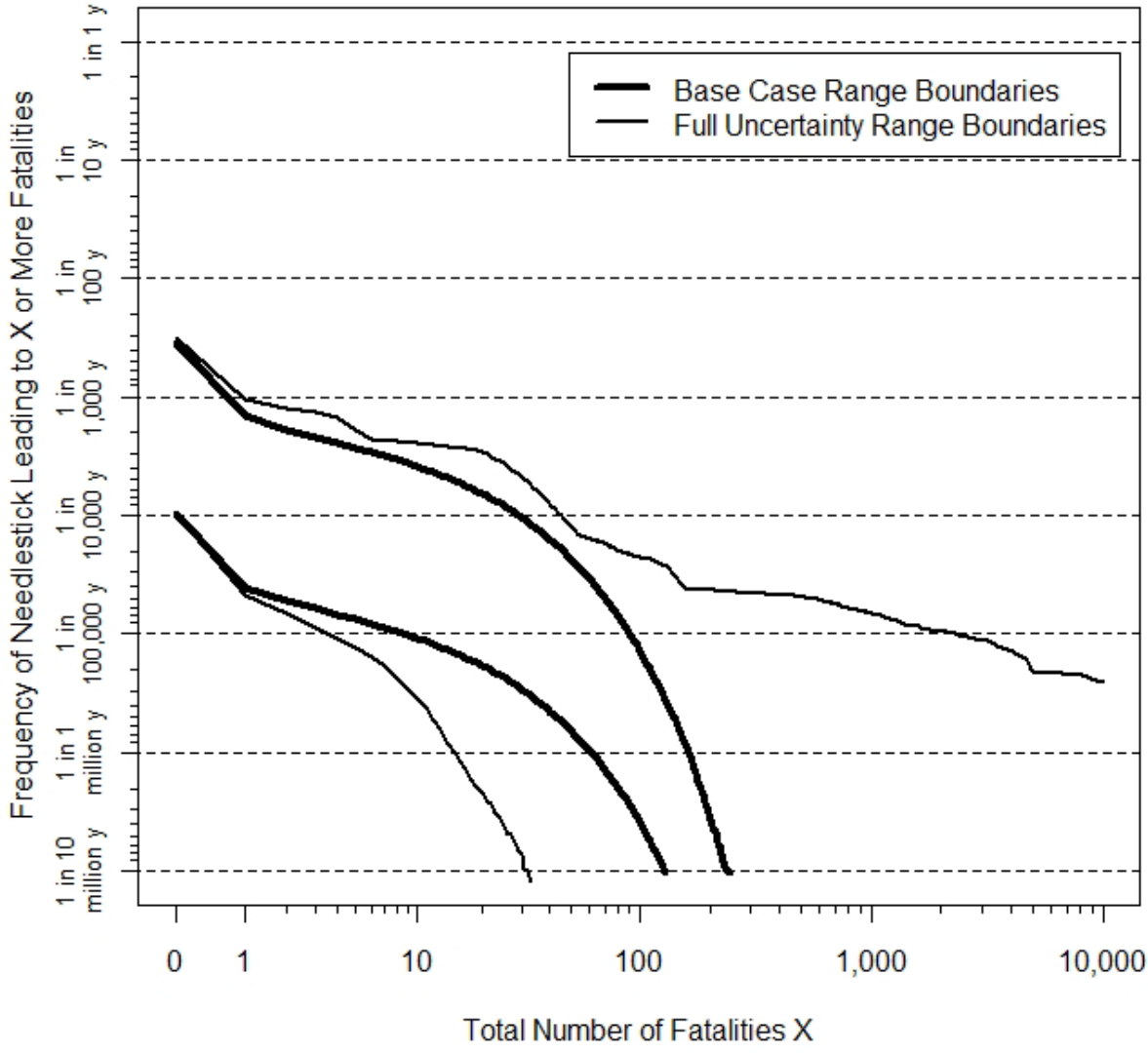
A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years; C = 1 in 10,000 to 1 million years; D = 1 in >1 million years

The results in the above table for the highest consequences reveal that the most extreme 4% of parameter estimates place the frequency of a widespread uncontrolled outbreak within frequency category C. On the other hand, most estimates (57%) for 100 or more fatalities fall in frequency category D (less than one in one million years).

Finally, estimated ranges of frequencies for different consequences are conveyed graphically in Figure L–3. In the figure, numbers of fatalities are plotted against the estimated frequency with which a needlestick event leading to at least that number of fatalities in the public would be expected to occur. Under a given set of transmission parameters and a given needlestick frequency, a curve can be generated by connecting the points calculated by multiplying the needlestick frequency by the estimated chance that at least the given number of fatalities would occur in the public. A different curve exists for each of the 100 sets of input values that were used in the uncertainty analysis. Rather than plotting all 100 curves, four curves are displayed, which form base case boundaries (thick curves) and full uncertainty boundaries (thin curves).

The base case boundaries were formed by multiplying the 95% uncertainty range for the needlestick frequency by the base case estimates from the secondary transmission analysis. The full uncertainty range represents the combined effects of uncertainty in the needlestick frequency and uncertainty in the secondary transmission parameters. By choosing a given number of fatalities on the horizontal axis, one can move vertically up the figure to find the boundaries for the estimated frequency range for that consequence.

Figure L–3: Risk curves for total public fatalities from SARS-CoV due to undetected / unreported needlestick, urban site



L.3.5.5 Sensitivity Analysis

The uncertainty apparent in results from the previous section is driven by uncertainty in the input parameter values. The output for the frequency of needlestick events leading to large numbers of mortalities (500 or more) is most sensitive to the value of the input parameter R_c, the post-control reproductive number. For example, all six outputs for the frequency of a 1000-or-more mortality outbreak that fell in the LOW frequency category occurred using the parameter combinations with the six highest values of R_c (ranging from 0.97 – 1.1), despite the fact that all the other parameters varied extensively within those six combinations. The other parameters play a larger role in affecting output uncertainty for the frequency of events leading to smaller-sized outbreaks. The following table lists PRCC values for the six relevant parameters in relation to various outputs.

Table L–23: Sensitivity Results – Partial Rank Correlation Coefficients. The effect of changing each input on the estimated frequency of needlestick events leading to the given number of mortalities

Number of fatalities	R_0	k_0	R_c	k_c	T_c	Needlestick frequency
1 or more	0.40	0.80	0.19	−0.00*	0.55	0.98
10 or more	0.44	0.28	0.59	0.01*	0.81	0.90
100 or more	0.55	−0.30	0.89	−0.28	0.87	0.59

*Not statistically significantly different than zero ($p > 0.2$). All other entries are statistically significantly different than zero ($p < 0.05$).

Discussion:

- R_0 – the basic reproductive number plays a significant role in determining the estimates for each output, although other parameters play a larger role in each case. As the assumed value of R_0 is increased, the estimated frequency of mortalities tends to increase as well.

- k_0 – the dispersion parameter associated with variation around R_0 plays a large role in the estimated frequency of 1 or more deaths occurring, because the higher value of k_0 results in a higher probability that early cases will transmit and start an outbreak. This effect is diminished for the estimates of higher numbers of deaths. For 100-or-more deaths, the effect of k_0 is reversed, with smaller values of k_0 resulting in higher estimated frequencies. This reversal reflects the fact that smaller values of k_0 result in increased probability of both no transmissions occurring and of a large number of transmission occurring from a particular individual.

- R_c – The post-control reproductive number has a small effect on the estimated frequency of smaller numbers of deaths occurring, and a large effect on the estimated frequency of large outbreaks. As discussed above, R_c plays a dominant role in affecting the estimated frequency of very large outbreaks occurring (the PRCCs are difficult to calculate for these extreme events, as most simulations do not produce any outbreaks that large and thus are not amenable to direct calculation of frequencies).

- k_c – the dispersion parameter associated with variation around R_c does not have a statistically significant effect on the estimated frequencies for smaller numbers of mortalities occurring. It does affect the estimated frequency of a 100-or-more mortality outbreak, with smaller values of k_c resulting in higher frequency estimates. This occurs because smaller values of k_c result in higher estimated probabilities that individuals would transmit to atypically large numbers of contacts despite the fact control measures are in place.

- T_c – the delay in implementation of control measures has a significant effect on all estimates, with longer delays resulting in higher frequency of outbreaks leading to mortalities. This effect becomes large for higher-consequence events.

- Needlestick frequency – the estimated frequency with which needlestick events occur has the largest effect on the estimated frequency of smaller numbers of deaths occurring. The relative effect of this input becomes smaller for the estimates of higher-consequence event frequencies.

L.3.6 Rift Valley fever virus

Rift Valley fever virus is an RNA virus in the larger family of viral hemorrhagic fevers and causes Rift Valley fever. There is no direct person-to-person transmission of RVFV; transmission to humans is via arthropod vectors or by contact with infected animal products. Cattle, sheep, and goats serve as the primary amplifier of the virus. Upon developing adequate viremia, humans may also serve as a virus reservoir for mosquitoes.

There is a limited literature on disease transmission models of RVFV involving animals, arthropod vectors and human hosts. Several models have addressed other complex variables such as climate conditions and livestock in the prediction and transmission of RVFV in endemic areas (Clements, Pfeiffer et al. 2006; Favier, Chalvet-Monfray et al. 2006; Anyamba, Chretien et al. 2009; Metras, Collins et al. 2011; Mpeshe, Haario et al. 2011). The applicability of these published models and epidemiologic,

climate and livestock data to conditions in the US, specifically to NEIDL sites under study in the RA is unknown. Furthermore, data on ruminants and mosquito vectors are not uniformly available in a format suitable for use in secondary transmission modeling. For these reasons, secondary transmission modeling of spread of infection in the community following a loss of bio-containment is not performed for RVFV.

The needlestick, centrifuge release and earthquake release scenarios involving RVFV are considered and the subsequent potential for secondary transmission of RVFV in the community via arthropod vectors and infected animal products is qualitatively analyzed.

An initial infection after a needlestick event without prompt detection and reporting was estimated to occur in frequency category B (between once per 100 years and once per 10,000 years). An initial infection with RVFV following a centrifuge release event was estimated, under a higher risk set of dose response assumptions, to occur in frequency category A or B (between about once per 10 years and once per 300 years). An initial infection occurring in a member of the public after exposure to RVFV released during an earthquake event was estimated to occur in frequency category C (between about once per 10,000 years and once per 1 million years) (Appendix K).

In the event that a laboratory worker or a member of the public develops Rift Valley fever from any of the above events, there is no possibility of directly transmitting the virus to the workers' close social contacts. However, secondary transmission could potentially occur from an initially infected laboratory worker or member of the public developing sufficiently high viremia (virus in the blood) and then being bitten by a mosquito vector belonging to a permissive species. This mosquito could act as a biological vector and transmit the virus to another human while taking its next blood meal. This scenario is possible but considered unlikely because i) the primary amplifiers of RVFV are ruminants; ii) it is rare for humans to serve as a virus reservoir; and iii) very few permissive species of mosquitoes are native to the area.

Similarly, it is possible but unlikely that an initially infected laboratory worker or member of the public would transmit infection via mosquito to ruminants; hence it is considered unlikely that secondary transmission to other humans would occur via infected ruminants. This scenario is distinct from the issue of establishment of RVFV in the environment after a release which is discussed in Chapter 8.

L.3.7 Andes virus

Andes virus is a New World hantavirus that causes a severe cardio-pulmonary syndrome (HPS). A unique feature of ANDV is that direct person-to-person transmissions of ANDV have been reported. There are

reports of HPS in close contacts with genetic evidence of person-to-person transmission, from earlier outbreaks in the 1990s to more recent cases**.** In these circumstances, transmission generally occurred in close family contacts who exchanged bodily fluids, with evidence of ANDV RNA found in saliva of patients. (Enria, Padula et al. 1996; Padula, Edelstein et al. 1998; Martinez, Bellomo et al. 2005; Castillo, Nicklas et al. 2007; Lazaro, Cantoni et al. 2007). A recent study that prospectively studied 476 household contacts of 76 index patients with HPS in Chile found 16 contacts developed confirmed HPS (3.4%) (Ferres, Vial et al. 2007). A third of all the cases occurred in family clusters. Person-to-person transmission was definite in only 3 household contacts and probable in another 9. Sexual contacts were at the highest risk for HPS in this study.

In other reports from Argentina, 16 cases of HPS were suspected to be due to person-to-person transmission, though contact or exposure to rodents could not be completely ruled out for a majority of those patients (Wells, Sosa Estani et al. 1997; Cantoni, Padula et al. 2001). There did not appear to be any hospital- or healthcare-associated person-to-person transmission in one outbreak in Chile (Castillo, Villagra et al. 2004). Other reports have described cases in health care workers (Lopez, Padula et al. 1996; Wells, Sosa Estani et al. 1997; Toro, Vega et al. 1998; Mertz, Hjelle et al. 2006).

In summary, direct person-to-person transmission of ANDV has been documented; though the extent of this transmission is limited to close family and possibly health care contacts and has not resulted in large outbreaks of HPS. Moreover, there are limited studies available to provide epidemiologic data for detailed secondary transmission modeling and no published mathematical models for this pathogen. For these reasons, secondary transmission modeling of spread of infection in the community following a loss of bio-containment is not performed for this pathogen.

L.3.8 Ebola virus

Person-to-person transmission of EBOV has been observed during several outbreaks that have occurred in Africa (Legrand 2006). Several studies on epidemiological parameters and quantitative transmission modeling of EBOV exist in the literature. Therefore, EBOV was selected for detailed quantitative modeling of potential secondary transmission, for purposes of assessing the risk posed to members of the public under the release scenarios analyzed in this RA. The remainder of this section describes the specification of the quantitative transmission modeling procedure for EBOV and summarizes the modeling outputs.

L.3.8.1 Specification of branching process model

This section describes the parameters, distributions, and assumptions used for specifying the branching process model to outbreak simulations for EBOV.

Individual reproductive number ν

The individual reproductive number ν is a random variable describing the expected number of transmissions from an infected individual. Lloyd-Smith *et al.* (2005) attempted to compare the ability of three different distributions of the individual reproductive number, ν, to describe the spread of transmission numbers from each infected individual documented in a small data set from an EBOV outbreak. They were unable to demonstrate that any of the distributions were clearly superior to the others, suggesting that the simplest assumption, i.e., no variation in *v*, might be most appropriate. However, they state that these results must be interpreted with caution, because the data set is small (13 cases), and they suspected that this subset of cases was biased towards cases that had transmitted and therefore was not a fair representation of variation in transmission that occurred during the outbreak.

In addition, Lloyd-Smith *et al.* (2005) identified three documented examples of EBOV transmission that were characterized as superspreading events, or cases where a single infected individual transmitted infection to an unusually high number of others (21–46 transmissions per case). These individuals were not included in the data set described above. If it is assumed that there is no variation in *v*, then numbers of transmissions per case in the simulations would be drawn from a Poisson distribution with mean *R*, and under those assumptions, combined with the base case estimate for R_0 (see below), the probability of one case transmitting to more than 20 others is virtually zero. Given that the potential for occurrence of such extreme events would have important implications for this risk assessment, it was determined that it would be appropriate to assume a distribution for *v* that can allow for the possibility (i.e., small but nonzero probability) of a large number of transmissions from a single case.

Therefore, the gamma distribution for ν is applied with mean *R* and shape parameter k which has a probability density function f defined as

$$f(x) = \frac{(k/R)^k}{\Gamma(k)} x^{k-1} \mathrm{e}^{-kx/R}.$$

Varying the value of the parameter *k* serves to cover all distributions tried by Lloyd-Smith et al. (2005), so that the implications of the commonly used characterizations can be tested as part of the uncertainty

analyis (see discussion below under the parameter k_0). The gamma distribution is used both before the implementation of control measures, using R_0 and k_0 as the two parameters, and also after the implementation of control measures, using R_c and k_c as the two parameters. The values of these four parameters to be applied are discussed as follows.

Mean reproductive number R_0

The mean reproductive number R_0 for EBOV has been estimated by several studies which made use primarily of data from one or both of two relatively large outbreaks, one that occurred in the Democratic Republic of Congo (DRC) in 1995 involving 315 cases (Khan 1999) and one that occurred in Uganda in 2000 involving 425 cases (Oyok 2001). The R_0 estimates derived from these data sets by several authors are summarized in Table L–24.

Table L–24: Estimates of R_0 for EBOV from the literature

Citation	R_0 estimate for DRC 1995 outbreak	R_0 estimate for Uganda 2000 outbreak
Chowell 2004	**1.83** (sd 0.06)	**1.34** (sd 0.03)
Ferrari 2005	**3.65** (3.05–4.33)	**1.79** (1.52–2.30)
Lloyd-Smith 2005	-	**1.50** (0.85–2.08)[a]
Legrand 2006	**2.7** (1.9–2.8)	**2.7** (2.5–4.1)
Lekone 2006	**1.359** (sd 0.128)	-
White 2008	**1.93** (1.74–2.78)	-
Clancy 2008	**1.3–1.7**	-
McKinley 2009	**2.0** (sd 0.21) or **1.5** (sd 0.17)	-

[a] based on a subset of 13 cases for which contact tracing data were available
sd = standard deviation

Conflicting estimates of R_0 based on the same data set can be explained by the fact that the data give only indirect clues to what R_0 might be, and there is a wide variety of mathematical models, statistical techniques, and associated assumptions that can be applied to arrive at an estimate. The highest estimate, by Ferrari et al. (2005) based on the DRC data, is not supported by any of the other estimates based on the same data set, as the confidence interval does not overlap any of the others. The same is true for the estimate by Legrand et al. (2006) for the Uganda outbreak. Their estimate based on the DRC data is also high, although the confidence interval overlaps other estimates. Overall, the estimates seem to favor values for R_0 in the range 1.3–2.0.

It is worth considering the fact that these outbreaks occurred in Africa, in an environment that differs in many ways from the sites at which transmission risk is being assessed for this RA. The value of R_0 is

partially a function of the environment and the community in which an outbreak takes place, because the chance of transmissions occurring is affected by behavior, contact patterns, hygiene, environmental conditions, and cultural practices. Some specific features of the areas where Ebola outbreaks occurred were thought to have played a role in amplifying transmission, such as caregiving systems, certain aspects of indigenous healing practices, and aspects of burial and funeral practices (Dowell 1999, Roels 1999, Hewlett 2003).

However, it is unclear how much these location-specific features might have contributed to the estimates of R_0 above and whether it would be warranted to assume that R_0 would be different during an outbreak in a different location. One follow-up study of the Uganda outbreak (Hewlett 2003) determined that people abandoned the riskiest cultural practices once they realized that an unusual outbreak was taking place, so the potential amplification of transmission would have occurred only very early in the outbreak. A quantitative study (Legrand 2006) attempted separate R_0 into components to determine how much transmission could be attributed to transmission from hospitalized patients, transmission from deceased persons during burial or funerals, and transmission in the general community. Their results suggested that the "traditional burial component" may have been quite significant during the DRC outbreak, but that the Uganda outbreak was dominated by the "community component." However, the uncertainty ranges around their component R_0 estimates were quite large, so these results are statistically inconclusive.

Given the lack of evidence to support a quantitative adjustment of Africa-based R_0 estimates to a different location, the R_0 range 1.3–2.0 as stated above is applied for this RA. A uniform distribution over this range is applied in the uncertainty analysis, and the center of the range, $R_0 = 1.65$, is applied as the base case estimate.

Shape / dispersion parameter k_0

The parameter k_0 found in the probability density function of the gamma distribution (see equation under individual reproductive number ν above) is often called the "shape" parameter of the distribution. If the mean (in this case, R_0) is held constant, and the value of k_0 is changed, the shape of the distribution changes, causing the variance of the distribution to increase as k_0 decreases. Specifically, the variance is calculated as R_0^2/k_0. The random variable ν for the individual reproductive number is assumed to be gamma-distributed with mean R_0 and shape parameter k_0. Then the random variable Z for the actual number of transmissions from an infected individual is assumed to be Poisson distributed with mean ν. A Poisson-distributed random variable with a mean that is gamma-distributed with mean R_0 and shape parameter k_0 has a negative binomial distribution with mean R_0 and "dispersion parameter" k_0.

As described under the discussion of the individual reproductive number ν, there is no reliable estimate of k_0 in the literature due to lack of data. Therefore, a range of values is implemented in order to investigate the implications of various assumptions about the nature of variation in transmission for EBOV. At one extreme, lower values of k_0 would imply a higher likelihood that an atypically large number of transmissions would occur from an individual. Conversely, lower k_0 values also imply a higher likelihood of no transmissions occurring from an individual, such that the average number R_0 is maintained. While there is no estimate of the percentage of cases not transmitting to anyone, there are three documented cases, among approximately 2000 confirmed cases that have occurred (Legrand 2006), of individuals transmitting to 20 or more others (Lloyd-Smith 2005). With these cases in mind, a lower limit value of k_0 = 0.5 is chosen. When combined with the assumed range of R_0 values given above, k_0 = 0.5 leads to approximately a 1-in-400 to 1-in-4000 chance of observing more than 20 transmissions from a single case.

At the other extreme, very high values of k_0 imply very low variation in v from person-to-person (k_0 approaching infinity implies no variation), which could not be ruled out as an appropriate characterization for EBOV transmission (Lloyd-Smith 2005). To capture this possibility along with the low extreme in the uncertainty range, a continuous uniform distribution is applied for the inverse, 1 / k_0, on the range 0–2, so that values near 2 imply k_0 near 0.5, and values near 0 imply large k_0. The median of this distribution, at which k_0 = 1, is applied as the base case estimate. This value is a special case implying that v is exponentially distributed, which is a common assumption made in conjunction with differential equation models of disease spread (Lloyd-Smith 2005), including models that have had success in fitting EBOV outbreak data sets (Chowell 2004).

Mean post-control reproductive number R_c

The parameter R_c is the average number of transmissions per infected individual after control measures for the pathogen have been implemented. All 14 confirmed EBOV outbreaks listed by Legrand et al. (2006) were eventually controlled, with total cases ranging from 12–425, and even the largest outbreak sizes are a very small percentage of the total population of the local area who might have been at risk if the outbreak had continued. This evidence strongly suggests that control measures were successful in reducing transmission such that $R_c < 1$. Control measures consisted of isolation of infected patients, quarantine of potentially exposed contacts (feasible due to relatively long incubation periods), reduction of social gatherings in the area, use of barrier precautious in treating symptomatic cases, and careful handling and burial deceased cases (WHO 2008).

Chowell et al. (2004) quantified values of R_c (R_p in their terminology) based on outbreak data and found $R_c = 0.51$ for the DRC outbreak and $R_c = 0.66$ for the Uganda outbreak, which are two of the largest outbreaks that have been documented. Given these data and the lack of any evidence for R_c being close to or above the $R_c = 1$ threshold, a uniform distribution on the range 0.4 to 0.8 is applied in the uncertainty analysis, with $R_c = 0.6$ as the base case.

Shape / dispersion parameter k_c

The same base case value and range applied for k_0 are also applied for k_c.

Generation time (T_g)

The average generation time for EBOV transmission appears not to have been measured directly, although estimates of the average incubation period of 6.3 days (Breman 1977), estimates of various symptoms lasting between 3.5 and 10.7 days before death or recovery (Piot 1977), and suggestions that transmissions usually occur during the peak or towards the end of the symptomatic period (Dowell 1999), point to an average generation time on the order of 10 days. However, these estimates are subject to substantial variation. For example, incubations periods have been observed to be as short at 1 day and as long as 21 days (Breman 1977).

Some modeling studies estimate average generation times shorter than ten days. White and Pagano (2008) estimated the generation time (serial interval) using statistical inference methods on the DRC 1995 outbreak data set and arrived at an estimate of 5.82 days (interquartile range 5.43–7.60 days), with associated variance 17.40 days^2 (10.02–19.43) under the assumption of a gamma-distributed serial interval. Chowell et al. (2004), based on model fitting to the Uganda outbreak data, estimated a mean incubation period of 3.35 days and a mean infectious period of 3.50 days, which together are roughly consistent with the White and Pagano estimate. For the DRC outbreak, the Chowell et al. estimates of the incubation and infectious periods are higher (5.30 and 5.61 days, respectively), although still potentially consistent with the range of generation times estimated by White and Pagano.

Given that the data from direct observations are spotty and that the estimate from White and Pagano is reasonably consistent with other estimates and observations, their estimate is applied for this RA: a gamma distributed generation time with mean 5.82 days and variance 17.4 days^2. Uncertainty in the gamma distribution parameters is not included in the analysis. The generation time affects the estimates for the overall size of outbreaks through its relationship to the delay in implementation of control

measures. Therefore, it is assumed that testing the uncertainty range of this delay also covers the potential implications of the average generation time being less or greater than 5.82 days.

Delay in implementation of control measures (T_c)

Estimates for the relatively large DRC and Uganda outbreaks suggest that control measures were not implemented until more than one month and perhaps multiple months after the first case (Chowell 2004). However, it is assumed that such a long delay would not occur should a case or cases of EBOV infection appear in the U.S. Given that symptoms are usually quite severe and the mortality rate high, it is presumed that the first case would generate alarm and trigger precautionary measures even if the cause of disease was not immediately identified.

An optimistic scenario would be that health officials would quickly implement effective control strategies before any cases in the second generation of cases became symptomatic themselves, so that the post-control reproductive number would apply to potential transmission to a third generation. A more conservative scenario would be that early cases of an outbreak would be not be identified as an alarming case and/or that hospitals would delay in implementing effective control strategies due to ineffective preparation or imperfect execution of recommended policies. This range of scenarios is represented by assuming a range of 0–6 days for T_c (continuous uniform distribution). The upper limit of 6 days is just over the assumed average generation time, which allows for the possibility that two generations (or more for a chain of below-average generation times) of transmission could occur before the post-control parameters are applied. The midpoint of the range, 3 days, is applied for the base case.

Case Fatality Rate

Given that the CFR has varied widely during different outbreaks of EBOV, from approximately 40% - 90% (Legrand 2006), and given that this is an important parameter in assessing the consequences of potential outbreaks, it was decided to include the mortality rate assumption as part of the uncertainty analysis for EBOV transmission simulations. A uniform distribution on the range 40% - 90% was assumed, with the midpoint of the range (65%) being applied as the base case.

Summary

Table L–25: Summary of assumed parameter values for EBOV transmission model

Parameter	Base Case Value	Uncertainty Distribution
R_0	1.65	Uniform (min = 1.3, max = 2.0)
k_0, k_c	1	Uniform on $1/k$ (min: $1/k$ = 0, max: $1/k$ = 2)
R_c	0.6	Uniform (min = 0.4, max = 0.8)
T_g	5.82 days	N/A
T_c	3 days	Uniform (min = 0, max = 6)
CFR	65%	Uniform (min = 40%, max = 90%)

L.3.8.2 Transmission Results – Base Case

Given the introduction of one initially infected individual into the population, 100,000 simulations were run under the base-case input parameters listed in Table L–25. A summary of the results of the output from these simulations for secondary infections and deaths are presented in the following table.

Table L–26: EBOV base case results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Number of simulations in which consequence occurred (of 100,000)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	61913 (62%)	38073 (38%)
	10 or more	18092 (18%)	5987 (6.0%)
	100 or more	33 (0.03%)	2 (0.002%)
Number of Public Fatalities	1 or more	56095 (56%)	32891 (32%)
	10 or more	11644 (12%)	3202 (3.2%)
	100 or more	0 (<0.001%)	0 (<0.001%)

These results suggest that, under the base case assumptions, a laboratory worker infected with EBOV who enters the public would have about a 62% chance of transmitting infection to at least one contact. Under the commuting assumptions described in Section L.2.5, there is a chance that the worker would not live in

Boston, which explains why the chance drops to 38% for at least one secondary infection occurring among Boston residents. The estimated chance of at least 10 public infections is 18% (6% among Boston residents), while the chance of at least 100 infections is small, as about 0.03% simulations produced outbreaks that large. The chances for each number of fatalities occurring are comparable to the corresponding infection results, as the case fatality rate of EBOV is high.

L.3.8.3 Transmission Results – Uncertainty

The input parameter uncertainty distributions summarized in Table L–25 were simultaneously applied within the Latin hypercube sampling scheme described in the methodology. One hundred sets of input parameters were generated, and 10,000 simulations were run under each set of parameters to generate alternate estimates for the chance of public infections and fatalities after an undetected or unreported initial infection. The following table summarizes the range of estimates found for each designated consequence. Each range was generated by sorting the 100 different estimates and dropping the lowest two and the highest two, so that 96% of the estimates are within or at the boundaries of the range. The base case results are also displayed in the table for reference.

Table L–27: EBOV base case and uncertainty results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection (urban site)

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	**62%** (49% to 83%)	**38%** (31% to 50%)
	10 or more	**18%** (8.3% to 33%)	**6.0%** (2.1% to 13%)
	100 or more	**0.03%** (≈0 to 1.9%)	**0.002%** (≈0 to 0.1%)
Number of Public Fatalities	1 or more	**56%** (42% to 77%)	**33%** (24% to 45%)
	10 or more	**12%** (2.6% to 25%)	**3.2%** (0.5% to 9.3%)
	100 or more	**<0.001%** (≈0 to 1.0%)	**<0.001%** (≈0 to 0.05%)

"≈0" at the lower end of an uncertainty range means that the given consequence was not observed in any of the simulations under at least three of the 100 input parameter combinations; when appearing as the entire range, then the consequence was not observed for at least 98 of the 100 parameter combinations.

L.3.8.4 Transmission results linked to event sequences

The results in the previous section are estimates of the chance of public infections and fatalities *given that one initially infected individual enters the community*. The initial infection analyses from Chapter 8 and

Appendix K provide estimates for the average frequency of such an initiating event occurring, for three different scenarios. The estimates for EBOV were as follows.

Table L–28: Summary of EBOV initial infections results

Event	Frequency Range
Undetected / Unreported Needlestick Infection	1 in 100 to 10,000 years
Undetected / Unreported Centrifuge Release and Infection	N/A
Earthquake Release and Infection	1 in 480,000 to >10 million years

Of these three events, an undetected or unreported needlestick infection is estimated as the most frequent initial infection event by which an individual could pose a secondary transmission risk to the public. The centrifuge event is not applicable because no credible scenario was found for an aerosol exposure going undetected under BSL-4 conditions. Therefore, the given frequency range for needlestick is applied in the remainder of this section as the representative event for estimating the frequency of public infections and fatalities.

The exact frequency for the needlestick event is uncertain, and no basis for was found for assuming a most likely frequency within the range. Therefore, a distribution of frequencies across the given range is applied as an additional layer of uncertainty within the overall Latin hypercube sampling scheme. Random return periods are drawn from a uniform distribution across the range (100 years to 10,000 years), and the frequency is calculated by taking the inverse. A given needlestick frequency is then multiplied by a probability of a given consequence (public infections or fatalities due to transmission) to calculate the estimated frequency of that consequence. This results in a range of estimated frequencies for each consequence. A sampling of these ranges is shown in the following table.

Table L–29: EBOV uncertainty results – frequency of public infections and fatalities due to an undetected / unreported needlestick (urban site)

Consequence		Frequency Range	
		Total	Among Boston City Residents
Number of Public Infections	1 or more	1 in 550 to 18,000 years	1 in 920 to 29,000 years
	10 or more	1 in 1,900 to 76,000 years	1 in 5,000 to 250,000 years
	100 or more	1 in 110,000 to >10 million years	1 in 2.9 million to >10 million years
Number of Public Fatalities	1 or more	1 in 610 to 20,000 years	1 in 1,100 to 36,000 years
	10 or more	1 in 3,100 to 240,000 years	1 in 11,000 to 1.0 million years
	100 or more	1 in 420,000 to >10 million years	1 in 8.9 million to >10 million years

The following table provides more insight into the distribution of estimated frequencies for each consequence, in terms of the portion of each distribution falling in each of the four frequency categories.

Table L–30: EBOV uncertainty results – frequency categories for public infections and fatalities due to undetected / unreported needlestick (urban site)

Consequence		Percentage of 100 estimates falling in each category							
		Total				Among Boston Residents			
		A	B	C	D	A	B	C	D
Number of Public Infections	1 or more	0	65%	35%	0	0	40%	60%	0
	10 or more	0	18%	82%	0	0	6%	94%	0%
	100 or more	0	0	25%	75%	0	0	0%	100%
Number of Public Fatalities	1 or more	0	53%	47%	0	0	36%	64%	0
	10 or more	0	14%	86%	0	0	2%	94%	4%
	100 or more	0	0	11%	89%	0	0	0	100%

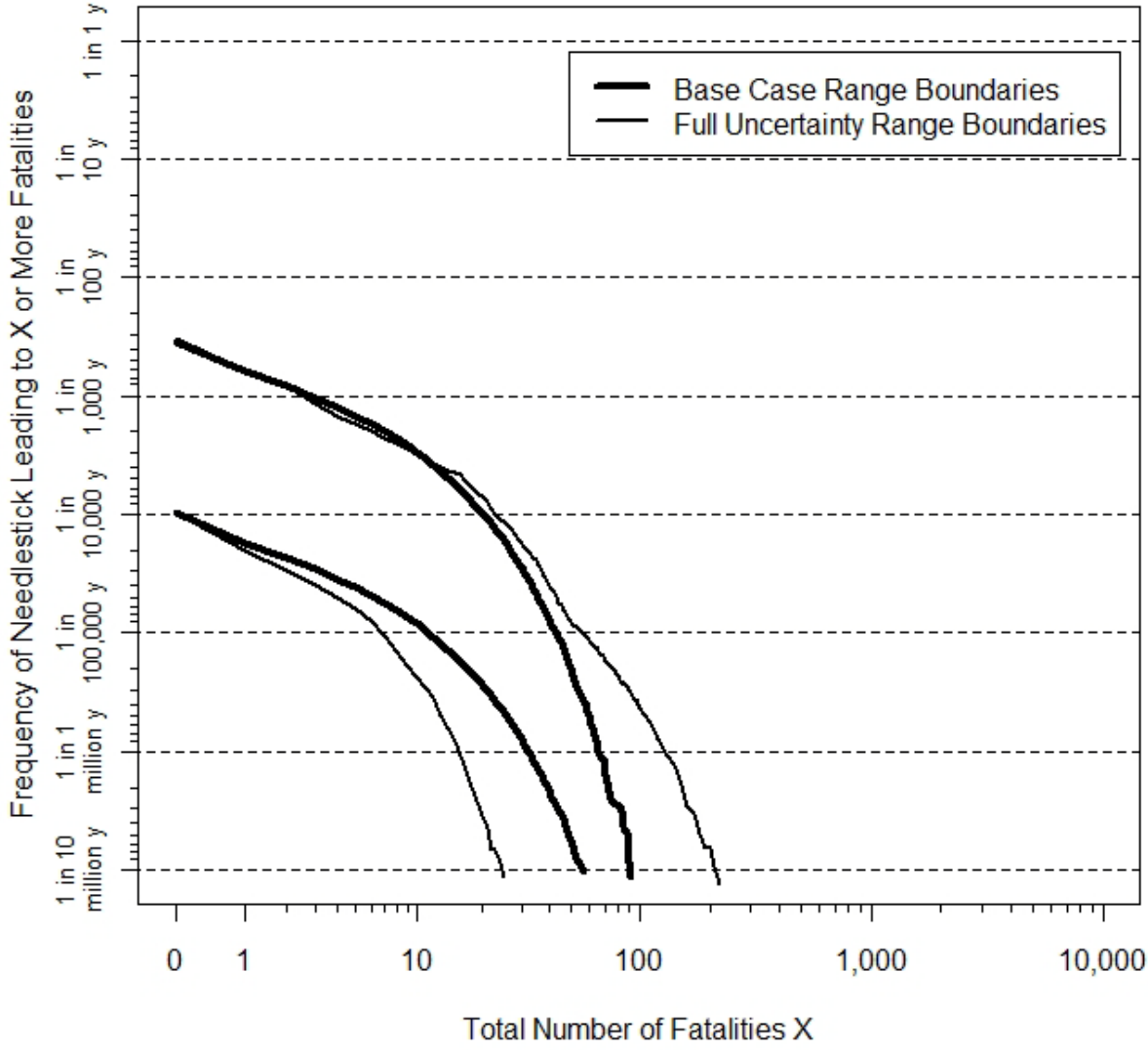
A = 1 in 1 to 100 years; B = 1 in 100 to 10,000 years; C = 1 in 10,000 to 1 million years; D = 1 in >1 million years

Finally, estimated ranges of frequencies for different consequences are conveyed graphically in Figure L–4. In the figure, numbers of fatalities are plotted against the estimated frequency with which a needlestick event leading to at least that number of fatalities in the public would be expected to occur. Under a given set of transmission parameters and a given needlestick frequency, a curve can be generated by connecting the points calculated by multiplying the needlestick frequency by the estimated chance that at least the given number of fatalities would occur in the public. A different curve exists for each of the 100 sets of

input values that were used in the uncertainty analysis. Rather than plotting all 100 curves, four curves are displayed, which form base case boundaries (thick curves) and full uncertainty boundaries (thin curves).

The base case boundaries were formed by multiplying the 95% uncertainty range for the needlestick frequency by the base case estimates from the secondary transmission analysis. The full uncertainty range represents the combined effects of uncertainty in the needlestick frequency and uncertainty in the secondary transmission parameters. By choosing a given number of fatalities on the horizontal axis, one can move vertically up the figure to find the boundaries for the estimated frequency range for that consequence.

Figure L–4: Risk curves for total public fatalities from EBOV due to undetected / unreported needlestick, urban site



L.3.9 Marburg virus

Marburg virus is a member of a group of hemorrhagic fever viruses and is closely related to Ebola virus. Direct person-to-person transmission of Marburg virus from index cases to family and community contacts has been described in the 1967 outbreak and the two large outbreaks in Africa. Secondary transmission is associated with close contact with the ill patient or their bodily fluids, mainly blood (Bausch, Nichol et al. 2006; Feldmann 2006; Towner, Khristova et al. 2006). Other body fluids from

infected humans (feces, vomitus, urine, saliva, and respiratory secretions) with high virus concentrations, especially when these fluids contain blood, have also been implicated in transmission.

Secondary transmission modeling of the spread of infection in the community following loss of bio-containment has been performed for the closely related Ebola virus. The results of this modeling are broadly applicable to Marburg virus; for this reason, secondary transmission modeling of spread of infection in the community following a loss of bio-containment is not performed for this pathogen.

L.3.10 Lassa virus

Lassa viruses are the causative pathogens of Lassa fever, which is a viral hemorrhagic fever**.** Direct person-to-person transmission of Lassa viruses occurs, especially in hospital settings. Person-to-person transmission is associated with direct contact with the blood or other bodily fluids containing virus particles of infected individuals. Airborne transmission has also been postulated to occur. Contact with objects contaminated with virus, such as medical equipment (reused needles), is also associated with transmission in healthcare settings (Centers for Disease Control 2011). The viruses are generally not known to be spread through casual contact, including skin-to-skin contact without exchange of bodily fluids (Ogbu, Ajuluchukwu et al. 2007). Thus, the risk of direct person-to-person transmission is low and involves close contact with infected body fluids. Furthermore, there are limited published mathematical models of such transmission. For these reasons, secondary transmission modeling of spread of infection in the community following loss of bio-containment is not performed for this pathogen.

L.3.11 Junin virus

Junín virus is the causative pathogen of Argentine hemorrhagic fever. As with other hemorrhagic fever viruses in the Family *Arenaviridae* (Lassa fever virus and the New World arenaviruses), there is potential for direct person-to-person transmission of Junín virus, postulated to occur via close contact with infectious blood and body fluids (Borio, Inglesby et al. 2002). It is to be noted that there have been no reports of person-to-person transmission of Junín virus from patients to health care workers, despite the several hundred patients with hemorrhages cared for each year in Argentina (Charrel and de Lamballerie 2003). Thus, the risk of direct person-to-person transmission of Junín virus is considered low and secondary transmission modeling of spread of infection in the community following loss of bio-containment is not performed for this pathogen.

L.3.12 Tick-borne encephalitis virus, Far Eastern subtype

Tick-borne encephalitis virus, Far Eastern sub-type (TBEV-FE) was formerly known as Russian spring-summer encephalitis. This virus is one member of the tick-borne encephalitis virus complex. TBEV-FE is one of the causative pathogens of tick-borne encephalitis (TBE) (Lindquist and Vapalahti 2008). The virus is transmitted to humans through the bite of an infected tick. As there is no direct-to-person transmission of TBEV-FE, secondary transmission modeling of spread of infection in the community following loss of bio-containment is not performed for this pathogen.

L.3.13 Nipah virus

Nipah virus is an emerging pathogen that was first described in 1998 from an outbreak of encephalitis in Malaysia and Singapore (1999). The mode of transmission of Nipah virus has changed between the Malaysian/Singapore outbreaks and those in Bangladesh/India. In the Malaysian/Singapore outbreaks, it is postulated that the virus was transmitted from bats (natural reservoir) to pigs, causing an outbreak in pigs, which subsequently led to an outbreak in humans in close contact with the pigs (abattoir workers and pig farmers). In Bangladesh, the transmission from bats to humans appears to be ongoing and via at least three different routes (Luby, Rahman et al. 2006; Luby, Hossain et al. 2009). The most frequent mode is food-borne through ingestion of Nipah-virus-contaminated date palm sap which is a staple food source in that region. A second mode of transmission appears to be via domestic animals that feed on contaminated fruits or date palm sap that have been licked or partially eaten by fruit bats infected with Nipah virus. There do not appear to be any arthropod vectors in the transmission of Nipah virus.

Evidence from epidemiologic investigations of outbreaks in Bangladesh and India indicates that Nipah virus can be transmitted directly from person-to-person. This has occurred in patients with respiratory illness. Close physical contact with a known Nipah virus patient who later died was found to be the strongest risk factor for direct person-to-person transmission (Gurley, Montgomery et al. 2007). Nipah virus has been found in respiratory secretions of infected patients (Chua, Lam et al. 2001). Though direct transmissions have occurred and are known to be responsible for many of the Bangladeshi outbreaks, the risk of direct transmission appears to be low and requires close contact that may be culture- and region-specific (to Bangladesh) (Luby, Hossain et al. 2009). Given the limitations of conducting field investigations in rural areas of developing countries, this study (Luby, Hossain et al. 2009) estimated that only a few individuals transmitted to others and the generations of transmissions rarely exceeded two. The overall number of secondary cases resulting from an infected person was expected to be less than 0.5, indicating that it was unlikely that any one chain of transmission would result in a large outbreak.

In summary, there is a possibility of person-to-person transmission of Nipah virus via respiratory secretions; however the risk is low and requires close contact that may be culture- and region-specific (Luby, Hossain et al. 2009). Moreover, there are limited studies available to provide epidemiologic data for detailed secondary transmission modeling and no published mathematical models for this pathogen. For these reasons, secondary transmission modeling of spread of infection in the community following loss of bio-containment is not performed for this pathogen.

L.3.14 Site differences

This section describes and compares results from simulations based on details specific to the suburban and rural sites. The following differences in assumptions as compared to those for the urban site are applied.

Local population size – The estimates provided in the results sections above used a local population estimate of one million individuals, which is representative of the daytime population in the city of Boston. The suburban and rural site simulations were run using local populations of 12,000 (approximate population of Tyngsborough, MA) and 8,000 (approximate combined population of Hancock, NH and Peterborough, NH). These changes have an effect only on larger outbreaks in which the infected portion of the local population might become substantial enough to appreciably decrease the effective value of the parameter R_c (see Section L.2.4).

Effect of commuting – The suburban and rural site simulations incorporate the commuting estimates specific to those areas, as described in Section L.2.5. It is noted that chains of transmission occurring among non-locals (who neither work nor live in the local area) are not subject to the local population constraint noted in the previous bullet. I.e., non-locals have contacts among a wider pool of susceptible individuals than just the local area, so that an extreme outbreak could potentially exceed the local population size.

Contact rate differences – The procedure described in Section L.2.9.1 was applied to compare R estimates for transmission at the urban site and the suburban and rural sites. An example using SARS-CoV is described here. The estimates of R_0 and R_c listed in Table L–17 are assumed to be most applicable to the urban site, because the outbreak data used to derive those estimates were mostly observed in large cities (e.g., Beijing, Singapore, Toronto, and others). Therefore, the values used for suburban and rural site simulations are adjusted according to the equations given in Section L.2.9.1. Similar adjustments are made for the other pathogens undergoing quantitative analysis. The adjusted values are only applied to

infected individuals in the simulation if they are classified as local residents. Otherwise, the estimates provided in Section L.2.9.1 are not applicable, and the unadjusted R_0 and R_c estimates are applied.

Table L–31: Summary of assumed, contact-rate-adjusted *R* values for site-specific SARS-CoV transmission model

Parameter	Urban	Suburban	Rural
R_0	3.0 (min = 2.0, max = 4.0)	2.56 (min = 1.71, max = 3.42)	1.42 (min = 0.95, max = 1.89)
R_c	0.7 (min = 0.3, max = 1.1)	0.60 (min = 0.26, max = 0.94)	0.33 (min = 0.14, max = 0.52)

Base case and uncertainty range estimates are provided for all three sites in the following tables, in terms of the estimated chance of public infections and fatalities given that an infected individual is introduced into the public.

Table L–32: *Y. pestis* (pneumonic plague) results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Total			Among Local Residents		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	**57%** (48-63%)	**56%** (47-63%)	**53%** (45-60%)	**34%** (26-40%)	**22%** (16-26%)	**24%** (17-30%)
	≥10	**17%** (0.8-34%)	**16%** (0.8-34%)	**12%** (0.6-27%)	**5.6%** (0.1-15%)	**1.2%** (0.02-5.1%)	**0.2%** (0-1.6%)
	≥100	**0** (0-2.4%)	**0** (0-2.0%)	**0.001%** (0-1.5%)	**0** (0-0.2%)	**0** (<0.01%)	**0** (<0.01%)
	≥1,000	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
Public Fatalities	≥1	**52%** (18-59%)	**52%** (18-59%)	**48%** (16-54%)	**30%** (8.1-36%)	**17%** (3.8-22%)	**19%** (4.1-24%)
	≥10	**3.5%** (0-26%)	**3.4%** (0-24%)	**2.4%** (0-19%)	**0.8%** (0-9.2%)	**0.07%** (0-2.2%)	**0.006%** (0-0.3%)
	≥100	**0** (0-0.05%)	**0** (0-0.05%)	**0** (0-0.03%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
	≥1,000	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)

Table L–33: 1918 H1N1V results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Total			Among Local Residents		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	**62%** (49-81%)	**61%** (50-81%)	**57%** (45-74%)	**39%** (30-51%)	**26%** (19-34%)	**28%** (22-38%)
	≥10	**40%** (13-70%)	**39%** (12-69%)	**32%** (10-58%)	**20%** (4.1-39%)	**8.1%** (0.8-18%)	**2.9%** (0.1-7.0%)
	≥100	**28%** (0-65%)	**27%** (0-63%)	**21%** (0-51%)	**8.4%** (0-29%)	**0.5%** (0-5.0%)	**0** (<0.01%)
	≥1,000	**4.0%** (0-46%)	**3.3%** (0-45%)	**2.4%** (0-34%)	**0.03%** (0-11%)	**0** (0-0.09%)	**0** (<0.01%)
	≥10,000	**0** (0-30%)	**0** (0-29%)	**0** (0-23%)	**0** (0-2.2%)	**0** (<0.01%)	**0** (<0.01%)
	≥100,000	**0** (0-30%)	**0** (0-29%)	**0** (0-23%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
Public Fatalities	≥1	**36%** (8.2-69%)	**35%** (7.8-67%)	**28%** (7.9-56%)	**16%** (3.1-36%)	**5.4%** (1.3-14%)	**2.5%** (1.0-4.6%)
	≥10	**14%** (0-53%)	**13%** (0-50%)	**10%** (0-40%)	**1.4%** (0-18%)	**0.004%** (0-1.6%)	**0** (<0.01%)
	≥100	**0.02%** (0-31%)	**0.02%** (0-29%)	**0.008%** (0-24%)	**0** (0-6.3%)	**0** (<0.01%)	**0** (<0.01%)
	≥1,000	**0** (0-30%)	**0** (0-29%)	**0** (0-23%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
	≥10,000	**0** (0-30%)	**0** (0-29%)	**0** (0-23%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)

Table L–34: SARS-CoV results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Total			Among Local Residents		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	**38%** (28-55%)	**37%** (28-55%)	**36%** (26-51%)	**28%** (20-39%)	**22%** (16-29%)	**23%** (17-31%)
	≥10	**21%** (15-34%)	**21%** (14-33%)	**19%** (12-30%)	**12%** (6.7-19%)	**6.7%** (2.9-13%)	**5.6%** (1.8-10%)
	≥100	**8.8%** (1.5-20%)	**8.4%** (1.3-19%)	**6.5%** (1.0-15%)	**3.0%** (0.3-9.2%)	**0.5%** (0-3.4%)	**0.03%** (0-0.6%)
	≥1,000	**0.2%** (0-9.7%)	**0.2%** (0-8.8%)	**0.1%** (0-6.7%)	**0.009%** (0-2.1%)	**0** (0-0.01%)	**0** (<0.01%)
	≥10,000	**0** (0-4.4%)	**0** (0-4.1%)	**0** (0-3.1%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
	≥100,000	**0** (0-2.8%)	**0** (0-2.9%)	**0** (0-1.9%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
Public Fatalities	≥1	**24%** (17-36%)	**24%** (17-36%)	**22%** (16-33%)	**15%** (10-24%)	**9.4%** (5.8-15%)	**8.8%** (5.9-13%)
	≥10	**9.1%** (1.8-20%)	**8.7%** (1.7-20%)	**6.8%** (1.2-15%)	**3.2%** (0.4-9.4%)	**0.6%** (0.02-3.5%)	**0.08%** (0-0.8%)
	≥100	**0.3%** (0-9.7%)	**0.2%** (0-8.8%)	**0.1%** (0-6.6%)	**0.009%** (0-2.0%)	**0** (0-0.01%)	**0** (<0.01%)
	≥1,000	**0** (0-4.4%)	**0** (0-4.1%)	**0** (0-3.1%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
	≥10,000	**0** (0-2.8%)	**0** (0-2.9%)	**0** (0-1.9%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)

Table L–35: EBOV results – number of public infections and fatalities given the occurrence of one undetected / unreported initial infection

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Total			Among Local Residents		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	**62%** (49-83%)	**62%** (49-82%)	**58%** (46-78%)	**38%** (31-50%)	**24%** (19-31%)	**27%** (22-35%)
	≥10	**18%** (8.3-33%)	**17%** (7.9-31%)	**13%** (5.8-24%)	**6.0%** (2.1-13%)	**1.3%** (0.3-3.1%)	**0.3%** (0.01-1.1%)
	≥100	**0.03%** (0-1.9%)	**0.03%** (0-1.7%)	**0.03%** (0-1.2%)	**0.002%** (0-0.1%)	**0** (<0.01%)	**0** (<0.01%)
	≥1,000	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)
Public Fatalities	≥1	**56%** (42-77%)	**56%** (43-77%)	**52%** (39-72%)	**33%** (24-45%)	**20%** (14-27%)	**21%** (14-30%)
	≥10	**12%** (2.6-25%)	**11%** (2.5-25%)	**8.1%** (1.7-20%)	**3.2%** (0.5-9.3%)	**0.5%** (0.04-2.3%)	**0.05%** (0-0.5%)
	≥100	**0** (0-1.0%)	**0** (0-0.7%)	**0** (0-0.6%)	**0** (0-0.05%)	**0** (<0.01%)	**0** (<0.01%)
	≥1,000	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)	**0** (<0.01%)

In each of the above four tables, the estimated probability for total numbers of infections and fatalities are generally slightly smaller at the suburban and rural sites as compared to the urban site, but the uncertainty ranges largely overlap, so that no statistically significant difference can be concluded. The reason that the overall results from the suburban and rural sites are so similar to the urban site results is that there is a high estimated rate of commuting to and from the towns at those sites, so that a significant portion of transmissions occur among non-residents and are not subject to local population constraints or to the estimates for decreased contact rates that were based on residents only.

The effects of the site difference assumptions are more apparent when comparing the results for local residents. There tends to be a lower estimated chance of each consequence among local residents at the suburban and rural sites compared to the urban site, due to commuting and contact rate differences, although uncertainty ranges overlap in most cases. These differences suggest that a more substantial portion of the risk from an undetected / unreported laboratory worker infection at the suburban and rural sites would be borne by non-residents, particularly areas with a strong connection with the local area via commuting.

L.3.15 Medically Vulnerable Sub Populations

The procedure described in Section L.2.9.2 was applied to SARS-CoV outbreak simulations at the three sites. As described in that section, for most MVSP, the susceptibility to infection and mortality is generally calculated relative to the susceptibility of healthy adults using the values for viruses in Table L–4. However, for one MVSP, adults over 65, there is enough evidence from SARS-CoV outbreaks to infer that the death rate among those individuals could be substantially higher than what would be inferred from Table L–4. For adults over 65, the assumed fatality rate is 50%, which is consistent with observations from the SARS outbreaks in Hong Kong (WHO 2003) and Toronto (Varia 2003).

L.3.15.1 Adjusted overall *R* values and mortality rates

The calculations described in Section L.2.9.2 were applied using the base case *R* values and mortality rate for SARS-CoV, with the following results. Note that these adjustments do not include contact rate adjustments described in Section L.3.14, so that the potential effects of MVSP susceptibility can be separated.

Table L–36: Summary of estimated MVSP-adjusted SARS-CoV parameter values over specific populations

Parameter	U.S.	Boston, MA	Tyngsborough, MA	Peterborough + Hancock, NH	Mass. overall	N.H. overall
R_0	**3.00**	2.99	2.94	3.12	3.02	2.99
R_c	**0.70**	0.70	0.69	0.73	0.70	0.70
Mortality rate	**0.10**	0.09	0.08	0.14	0.10	0.10

The results in the table above show that the different *R* values across each row are well within the uncertainty ranges (2 to 4 for R_0 and 0.3 to 1.1 for R_c) derived in Section L.3.5. Furthermore, the site-specific adjustments based on contact rates that were tested and presented in Section L.3.14 exhibited a much larger effect on site-specific *R* values. The adjusted mortality rates also do not deviate far from the baseline estimate of 10%. Therefore, the MVSP profiles at the three sites do not contribute a substantial effect on site differences for overall risk to the population in light of the overall uncertainty and in comparison to the site differences already explored in Section L.3.14.

Because the inputs for susceptibility of MVSP relative to healthy adults shown in Table L–4 are based on expert opinion and not on data (except for the case fatality rate of adults over 65, as described above), it is

possible that they significantly underestimate the true differences in susceptibility. However, the results shown in Table L–36 exhibit low sensitivity to those inputs. For example, even if the relative susceptibility values in Table L–4 are multiplied by a factor of 10, the results for adjusted *R* values still fall within the overall uncertainty ranges of 2 to 4 for R_0 and 0.3 to 1.1 for R_c.

L.3.15.2 Risk to specific MVSP at the three sites

This section consists of results for the site-specific risk to each of the individual MVSP. For each MVSP, the risk of infections and deaths are presented, as determined by simulations incorporating and tracking MVSP as described in the methodology section. Each set of results is compared to what the results would be if the MVSP profile at each location were in line with overall U.S. proportions.

Table L–37. Base case results for SARS-CoV consequences among children under five, given that an undetected / unreported initial infection has occurred

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Using Local MVSP Estimates			Using U.S. Average MVSP Estimates		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	22%	22%	20%	23%	23%	20%
	≥10	7.6%	7.3%	5.8%	8.0%	7.7%	5.8%
	≥100	0.11%	0.08%	0.06%	0.11%	0.08%	0.06%
Public Fatalities	≥1	7.2%	6.9%	5.7%	7.5%	7.2%	5.8%
	≥10	0.02%	0.01%	0.006%	0.01%	0.008%	0.01%

Among children under five, the estimates are similar at all three sites, with the chance of one or more deaths being between 5 and 8% and ten or more deaths 0.02% or lower. These estimates do not change substantially when U.S. average proportions of MVSP are used as inputs rather than the locally estimated proportions. All differences across each row are small compared to the overall uncertainty range. Therefore, it is not possible to conclude that the differences in population of children under five near each of the three sites relative to the U.S. have a substantial effect on the estimated risk to that group.

Table L–38. Base case results for SARS-CoV consequences among adults over 65, given that an undetected / unreported initial infection has occurred

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Using Local MVSP Estimates			Using U.S. Average MVSP Estimates		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	27%	26%	26%	27%	27%	25%
	≥10	11%	11%	9.9%	12%	11%	9.0%
	≥100	0.95%	0.84%	0.68%	1.1%	0.85%	0.65%
Public Fatalities	≥1	23%	23%	22%	23%	23%	21%
	≥10	7.7%	7.5%	6.5%	8.2%	7.8%	5.9%
	≥100	0.13%	0.08%	0.08%	0.13%	0.10%	0.07%

Among adults over 65, the estimated risk is similar at all three sites, with the estimated chance of ten or more deaths between 6 and 8% and 100 or more deaths about 0.1%. These estimates do not change substantially when U.S. average proportions of MVSP are used as inputs rather than the locally estimated proportions. All differences across each row are small compared to the overall uncertainty range. Therefore, it is not possible to conclude that the differences in population of adults over 65 near each of the three sites relative to the U.S. have a substantial effect on the estimated risk to that group.

Table L–39. Base case results for SARS-CoV consequences among people with diabetes, given that an undetected / unreported initial infection has occurred

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Using Local MVSP Estimates			Using U.S. Average MVSP Estimates		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	23%	22%	20%	22%	21%	19%
	≥10	7.8%	6.7%	5.1%	6.8%	6.3%	4.7%
	≥100	0.11%	0.03%	0.02%	0.04%	0.02%	0.02%
Public Fatalities	≥1	7.5%	6.6%	5.4%	6.5%	6.2%	4.9%
	≥10	0.02%	0.002%	0.003%	0.006%	0.004%	0.002%

Among people with diabetes, the estimated risk is similar at all three sites, with an estimated chance of one or more deaths between 5 and 8% and ten or more deaths 0.2% or less. These estimates decrease slightly when U.S. average proportions of MVSP are used as inputs rather than the locally estimated proportions, because the proportion of people with diabetes is higher than the U.S. average at all three sites, but all differences across each row are small compared to the overall uncertainty range. Therefore,

it is not possible to conclude that the differences in population of people with diabetes near each of the three sites relative to the U.S. have a substantial effect on the estimated risk to that group.

Table L–40. Base case results for SARS-CoV consequences among people with HIV/AIDS, given that an undetected / unreported initial infection has occurred

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Using Local MVSP Estimates			Using U.S. Average MVSP Estimates		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	10%	7.8%	5.8%	9.1%	8.6%	7.1%
	≥10	0.16%	0.03%	0.03%	0.05%	0.05%	0.03%
Public Fatalities	≥1	1.2%	0.74%	0.59%	0.96%	0.84%	0.66%
	≥10	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%

Among people with HIV / AIDS, the estimated risk is similar at all three sites, with the chance of one or more deaths about 1% or less and ten or more deaths less than 0.001%. It is noted that estimated urban site probability of one or more deaths (1.2%) is about twice that of the rural site (0.6%), although both probabilities are low and uncertainty ranges overlap. The estimates do not change substantially when U.S. average proportions of MVSP are used as inputs rather than the locally estimated proportions. All differences across each row are small compared to the overall uncertainty range. Therefore, it is not possible to conclude that the differences in population of people with HIV / AIDS near each of the three sites relative to the U.S. have a substantial effect on the estimated risk to that group.

Table L–41. Base case results for SARS-CoV consequences among pregnant women, given that an undetected / unreported initial infection has occurred

Consequence		Estimated Chance of Consequence (given one undetected / unreported initial infection)					
		Using Local MVSP Estimates			Using U.S. Average MVSP Estimates		
		Urban	Suburban	Rural	Urban	Suburban	Rural
Public Infections	≥1	13%	13%	11%	14%	13%	11%
	≥10	0.81%	0.68%	0.49%	0.82%	0.65%	0.47%
	≥100	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%
Public Fatalities	≥1	1.9%	1.7%	1.4%	1.9%	1.8%	1.4%
	≥10	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%

Among pregnant women, the estimated risk is similar at all three sites, with the chance of one or more deaths between 1 and 2% and ten or more deaths less than 0.001%. The assumed U.S. average proportion

of pregnant women is the same as the estimate for each of the sites (1% of the population), so any differences between the left half and right half of the results in the table are entirely due to random differences in the simulation outcomes.

Because the inputs for susceptibility of MVSP relative to healthy adults shown in Table L–4 are based on expert opinion and not on data (except for the case fatality rate of adults over 65, as described above), it is possible that they significantly underestimate the true differences in susceptibility. The numerical results shown in Tables L–37 to L–41 do exhibit sensitivity to those inputs. The largest change in the results for a particular MVSP would occur if the relative susceptibility of that MVSP is increased and the relative susceptibility of all other MVSP remains the same, in which case the particular MVSP would be disproportionately affected relative to not only healthy adults, but also to the other MVSP. If the relative sensitivities for all MVSP are increased at the same time, then the estimated risk to each MVSP would still increase, but to a much lesser extent. In all cases, however, the results regarding the risk estimates to each local population as compared to what those results would be in a population with typical U.S. proportions of MVSP would not change substantially. Therefore, the relative differences across each row of Tables L–37 to L–41 are not sensitive to potential inaccuracies of the estimated susceptibility differences of the MVSP.

In addition, the expert estimates of increased susceptibility to disease and mortality were based on belonging to each MVSP individually; in reality, it is possible that individuals may have multiple concomitant medical vulnerabilities such as being both elderly and diabetic, or both HIV-positive and pregnant. The susceptibility in these situations is potentially increased due to compounding effects of the individual conditions. Published data about the effects of each combination of vulnerabilities on susceptibility are scarce, and as such the simplified approach for this analysis was to assess each medical vulnerability separately. The sensitivity analysis discussed in the previous paragraph suggests that assuming compounding effects of simultaneous conditions on susceptibility would not have a significant effect on the comparative results.

In the simulations that produced the results in Tables L–37 to L–41, it was assumed that the probability that a contact of an infected individual is a member of a MVSP is equal to the proportion of that MVSP in the population. This assumption may be violated if the social mixing of MVSP in the overall population is not homogenous. For people with diabetes and pregnant women, no data were found that would indicate that individuals in those groups would be more or less likely to be contacted than a typical person in the population. There are numerous studies on the sexual contact patterns of people with HIV/AIDS, but those data have limited applicability to the spread of respiratory pathogens such as SARS-CoV.

Age-specific contact patterns in the context of person-to-person spread of disease have been studied, and it has generally been found that children under five and adults over sixty-five have fewer numbers of contacts as compared to school-age children and younger adults (Wallinga 2006, DelValle 2007). Therefore, the assumption of proportionate mixing likely contributes to overestimating the risk to individuals in those groups (Tables L–37 and L–38). Those studies also found that children under five and adults over 65 tend to preferentially mix with individuals in their own age group (e.g., at day care centers and nursing homes), so it is possible that, if members of those age groups became infected very early in an outbreak, that they would be disproportionately affected in early generations of the outbreak. However, analysis shows that age-specific incidence converges rapidly over the course of an outbreak (Wallinga 2006), so that young children and elderly groups would have fewer contacts with infected cases than other age groups after just a few generations of transmission.

L.4 References

Abbey, H. (1952). "An examination of the Reed-Frost theory of epidemics." Human biology **24**(3): 201-233.

Anyamba, A., J. P. Chretien, et al. (2009). "Prediction of a Rift Valley fever outbreak." Proceedings of the National Academy of Sciences of the United States of America **106**(3): 955-959.

Bausch, C. T., J. O. Lloyd-Smith, et al. (2005). "Dynamically modeling SARS and other newly emerging respiratory illnesses: past, present, and future." Epidemiology **16**(6): 791-801.

Bausch, D. G., S. T. Nichol, et al. (2006). "Marburg hemorrhagic fever associated with multiple genetic lineages of virus." N Engl J Med **355**(9): 909-919.

Bisset, K., X. Feng, et al. (2009). Modeling interaction between individuals, social networks and public policy to support public health epidemiology. Proceedings of the 2009 Winter Simulation Conference.

Boelle, P. Y., P. Bernillon, et al. (2009). "A preliminary estimation of the reproduction ratio for new influenza A(H1N1) from the outbreak in Mexico, March-April 2009." Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin **14**(19).

Borio, L., T. Inglesby, et al. (2002). "Hemorrhagic fever viruses as biological weapons: medical and public health management." JAMA **287**(18): 2391-2405.

Breman, J., P. Piot, et al. (1978). The epidemiology of Ebola haemorrhagic fever in Zaire, 1978. Ebola Virus Haemorrhagic Fever. S. R. Pattyn. Amsterdam, Elsevier/North-Holland Biomedical Press**:** 85-97.

Cantoni, G., P. Padula, et al. (2001). "Seasonal variation in prevalence of antibody to hantaviruses in rodents from southern Argentina." Tropical medicine & international health : TM & IH **6**(10): 811-816.

Castillo, C., C. Nicklas, et al. (2007). "Andes Hantavirus as possible cause of disease in travellers to South America." Travel medicine and infectious disease **5**(1): 30-34.

Castillo, C., E. Villagra, et al. (2004). "Prevalence of antibodies to hantavirus among family and health care worker contacts of persons with hantavirus cardiopulmonary syndrome: lack of evidence for nosocomial transmission of Andes virus to health care workers in Chile." The American journal of tropical medicine and hygiene **70**(3): 302-304.

Cauchemez, S., C. A. Donnelly, et al. (2009). "Household transmission of 2009 pandemic influenza A (H1N1) virus in the United States." The New England journal of medicine **361**(27): 2619-2627.

Centers for Disease Control and Prevention (2011). "Special Pathogens Branch: Arenaviruses." Retrieved September 2, 2011, from http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/arena.htm.

Centers for Disease Control and Prevention (1999). "Update: outbreak of Nipah virus--Malaysia and Singapore, 1999." MMWR Morb Mortal Wkly Rep **48**(16): 335-337.

Centers for Disease Control and Prevention (2001). "Outbreak of Ebola hemorrhagic fever Uganda, August 2000-January 2001." MMWR Morb Mortal Wkly Rep **50**(5): 73-77.

Charrel, R. N. and X. de Lamballerie (2003). "Arenaviruses other than Lassa virus." Antiviral Res **57**(1-2): 89-100.

Choi, B. C. K., Pak, A. W. P. (2003). "A simple approximate mathematical model to predict the number of severe acute respiratory syndrome cases and deaths" Journal of epidemiology and community health **57**: 831-835.

Chowell, G., C. E. Ammon, et al. (2006). "Transmission dynamics of the great influenza pandemic of 1918 in Geneva, Switzerland: Assessing the effects of hypothetical interventions." J Theor Biol **241**(2): 193-204.

Chowell, G., P. W. Fenimore, et al. (2003). "SARS outbreaks in Ontario, Hong Kong and Singapore: the role of diagnosis and isolation as a control mechanism." J Theor Biol **224**(1): 1-8.

Chowell, G., N. W. Hengartner, et al. (2004). "The basic reproductive number of Ebola and the effects of public health measures: the cases of Congo and Uganda." J Theor Biol **229**(1): 119-126.

Chowell, G., H. Nishiura, et al. (2007). "Comparative estimation of the reproduction number for pandemic influenza from daily case notification data." Journal of the Royal Society, Interface / the Royal Society **4**(12): 155-166.

Chua, K. B., S. K. Lam, et al. (2001). "The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia." J Infect **42**(1): 40-43.

Clancy, D. and P. O'Neill (2008). "Bayesian estimation of the basic reproduction number in stochastic epidemic models." Bayesian Analysis **3**(4): 737-758.

Clements, A. C., D. U. Pfeiffer, et al. (2006). "Application of knowledge-driven spatial modelling approaches and uncertainty management to a study of Rift Valley fever in Africa." International journal of health geographics **5**: 57.

Del Valle, S.Y., J.M. Hyman, et al. (2007). "Mixing patterns between age groups in social networks." Social Networks **29**: 539-554.

Dennis, D. T., T. V. Inglesby, et al. (2001). "Tularemia as a biological weapon: medical and public health management." JAMA **285**(21): 2763-2773.

de Silva, U. C., J. Warachit, et al. (2009). "A preliminary analysis of the epidemiology of influenza A(H1N1)v virus infection in Thailand from early outbreak data, June-July 2009." Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin **14**(31).

Dowell, S. F., R. Mukunu, et al. (1999). "Transmission of Ebola hemorrhagic fever: a study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit." J Infect Dis **179 Suppl 1**: S87-91.

Ellis, J., P. C. Oyston, et al. (2002). "Tularemia." Clin Microbiol Rev **15**(4): 631-646.

Enria, D., P. Padula, et al. (1996). "Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission." Medicina **56**(6): 709-711.

Eubank, S., V. Anil Kumar, et al. (2006). Structure of social contact networks and their impact on epidemics. AMS-DIMACS Special Volume on Epidemiology.

Favier, C., K. Chalvet-Monfray, et al. (2006). "Rift Valley fever in West Africa: the role of space in endemicity." Tropical medicine & international health : TM & IH **11**(12): 1878-1888.

Feldmann, H. (2006). "Marburg hemorrhagic fever--the forgotten cousin strikes." N Engl J Med **355**(9): 866-869.

Ferguson, N. M., D. A. Cummings, et al. (2006). "Strategies for mitigating an influenza pandemic." Nature **442**(7101): 448-452.

Ferrari, M. J., O. N. Bjornstad, et al. (2005). "Estimation and inference of R_0 of an infectious pathogen by a removal method." Mathematical biosciences **198**(1): 14-26.

Ferres, M., P. Vial, et al. (2007). "Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in chile." The Journal of Infectious Diseases **195**(11): 1563-1571.

Fraser, C., C. A. Donnelly, et al. (2009). "Pandemic potential of a strain of influenza A (H1N1): early findings." Science **324**(5934): 1557-1561.

Freedman, A., O. Afonja, et al. (2002). "Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old infant." JAMA **287**(7): 869-874.

Gani, R., H. Hughes, et al. (2005). "Potential impact of antiviral drug use during influenza pandemic." Emerging Infectious Diseases **11**(9): 1355-1362.

Gani, R. and S. Leach (2004). "Epidemiologic determinants for modeling pneumonic plague outbreaks." Emerg Infect Dis **10**(4): 608-614.

Ghani, A. C., M. Baguelin, et al. (2009). "The Early Transmission Dynamics of H1N1pdm Influenza in the United Kingdom." PLoS currents **1**: RRN1130.

Gumel, A. B., S. Ruan, et al. (2004). "Modelling strategies for controlling SARS outbreaks." Proc Biol Sci **271**(1554): 2223-2232.

Gurley, E. S., J. M. Montgomery, et al. (2007). "Person-to-person transmission of Nipah virus in a Bangladeshi community." Emerg Infect Dis **13**(7): 1031-1037.

Hahne, S., T. Donker, et al. (2009). "Epidemiology and control of influenza A(H1N1)v in the Netherlands: the first 115 cases." Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin **14**(27).

Hewlett, B. S. and R. P. Amola (2003). "Cultural contexts of Ebola in northern Uganda." Emerging Infectious Diseases **9**(10): 1242-1248.

Hsieh, Y. H., C. W. Chen, et al. (2004). "SARS outbreak, Taiwan, 2003." Emerg Infect Dis **10**(2): 201-206.

Hsieh, Y. H., D. N. Fisman, et al. (2010). "On epidemic modeling in real time: An application to the 2009 Novel A (H1N1) influenza outbreak in Canada." BMC research notes **3**: 283.

Jones, R. M., M. Nicas, et al. (2005). "The Infectious Dose of Francisella tularensis (Tularemia)." Applied Biosafety **10**(4): 227-239.

Khan, A. S., F. K. Tshioko, et al. (1999). "The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit." J Infect Dis **179 Suppl 1**: S76-86.

Lazaro, M. E., G. E. Cantoni, et al. (2007). "Clusters of hantavirus infection, southern Argentina." Emerging Infectious Diseases **13**(1): 104-110.

Legrand, J., R. F. Grais, et al. (2007). "Understanding the dynamics of Ebola epidemics." Epidemiol Infect **135**(4): 610-621.

Lekone, P. E. and B. F. Finkenstadt (2006). "Statistical inference in a stochastic epidemic SEIR model with control intervention: Ebola as a case study." Biometrics **62**(4): 1170-1177.

Lindquist, L. and O. Vapalahti (2008). "Tick-borne encephalitis." Lancet **371**(9627): 1861-1871.

Lloyd-Smith, J. O., S. J. Schreiber, et al. (2005). "Superspreading and the effect of individual variation on disease emergence." Nature **438**(7066): 355-359.

Lopez, N., P. Padula, et al. (1996). "Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina." Virology **220**(1): 223-226.

Luby, S. P., M. J. Hossain, et al. (2009). "Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007." Emerg Infect Dis **15**(8): 1229-1235.

Luby, S. P., M. Rahman, et al. (2006). "Foodborne transmission of Nipah virus, Bangladesh." Emerg Infect Dis **12**(12): 1888-1894.

Martinez, V. P., C. Bellomo, et al. (2005). "Person-to-person transmission of Andes virus." Emerging Infectious Diseases **11**(12): 1848-1853.

Massin, L., J. Legrand, et al. (2007). "Modelling outbreak control for pneumonic plague." Epidemiology and infection **135**(5): 733-739.

McBryde, E., I. Bergeri, et al. (2009). "Early transmission characteristics of influenza A(H1N1)v in Australia: Victorian state, 16 May - 3 June 2009." Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin **14**(42).

McKinley, T., A. Cook, et al. (2009). "Inference in Epidemic Models without Likelihoods." The International Journal of Biostatistics **5**(1): Article 24.

Mertz, G. J., B. Hjelle, et al. (2006). "Diagnosis and treatment of new world hantavirus infections." Current opinion in infectious diseases **19**(5): 437-442.

Metras, R., L. M. Collins, et al. (2011). "Rift Valley Fever Epidemiology, Surveillance, and Control: What Have Models Contributed?" Vector borne and zoonotic diseases.

Mills, C. E., J. M. Robins, et al. (2004). "Transmissibility of 1918 pandemic influenza." Nature **432**(7019): 904-906.

Moser, M. R., T. R. Bender, et al. (1979). "An outbreak of influenza aboard a commercial airliner." American journal of epidemiology **110**(1): 1-6.

Mpeshe, S. C., H. Haario, et al. (2011). "A Mathematical Model of Rift Valley Fever with Human Host." Acta biotheoretica.

Nishiura, H., N. Wilson, et al. (2009). "Estimating the reproduction number of the novel influenza A virus (H1N1) in a Southern Hemisphere setting: preliminary estimate in New Zealand." The New Zealand medical journal **122**(1299): 73-77.

Ogbu, O., E. Ajuluchukwu, et al. (2007). "Lassa fever in West African sub-region: an overview." J Vector Borne Dis **44**(1): 1-11.

Padula, P. J., A. Edelstein, et al. (1998). "Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus." Virology **241**(2): 323-330.

Paine, S., G. N. Mercer, et al. (2010). "Transmissibility of 2009 pandemic influenza A(H1N1) in New Zealand: effective reproduction number and influence of age, ethnicity and importations." Euro

surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin **15**(24).

Piot, P., P. Sureau, et al. (1978). Clinical Aspects of Ebola virus Infection in Yambuku Area, Zaire, 1976. Ebola Virus Haemorrhagic Fever. S. R. Pattyn. Amsterdam, Elsevier/North-Holland Biomedical Press**:** 7-14.

Pourbohloul, B., A. Ahued, et al. (2009). "Initial human transmission dynamics of the pandemic (H1N1) 2009 virus in North America." Influenza and other respiratory viruses **3**(5): 215-222.

Roels, T. H., A. S. Bloom, et al. (1999). "Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: risk factors for patients without a reported exposure." J Infect Dis **179 Suppl 1**: S92-97.

Tieh, T. H., E. Landauer, et al. (1948). "Primary pneumonic plague in Mukden, 1946, and report of 39 cases with three recoveries." The Journal of Infectious Diseases **82**(1): 52-58.

Toro, J., J. D. Vega, et al. (1998). "An outbreak of hantavirus pulmonary syndrome, Chile, 1997." Emerging Infectious Diseases **4**(4): 687-694.

Towner, J. S., M. L. Khristova, et al. (2006). "Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola." J Virol **80**(13): 6497-6516.

Tuite, A. R., A. L. Greer, et al. (2010). "Estimated epidemiologic parameters and morbidity associated with pandemic H1N1 influenza." CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne **182**(2): 131-136.

US Census Bureau. (2011). "Table 3. Selected Places by State. Estimated daytime population. ." U.S. Census Bureau, Population Division, Journey to Work and Migration Statisitcs Branch Retrieved July 29, 2011, 2011, from http://www.census.gov/population/www/socdemo/daytime/daytimepop.html.

Varia, M., S. Wilson, et al. (2003). "Investigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada." CMAJ **169**(4): 285-292.

Wallinga, J. and M. Lipsitch (2007). "How generation intervals shape the relationship between growth rates and reproductive numbers." Proceedings. Biological sciences / The Royal Society **274**(1609): 599-604.

Wallinga, J. and P. Teunis (2004). "Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures." American journal of epidemiology **160**(6): 509-516.

Wallinga, J., Teunis, P., and M. Kretzschmar (2006). "Using data on social contacts to estimate age-specific transmission parameters for respiratory-spread infectious agents." American Journal of Epidemiology **164**(10): 936-944.

Wang, W. and S. Ruan (2004). "Simulating the SARS outbreak in Beijing with limited data." J Theor Biol **227**(3): 369-379.

Wells, R. M., S. Sosa Estani, et al. (1997). "An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Hantavirus Pulmonary Syndrome Study Group for Patagonia." Emerg Infect Dis **3**(2): 171-174.

White, L. F. and M. Pagano (2008). "A likelihood-based method for real-time estimation of the serial interval and reproductive number of an epidemic." Statistics in medicine **27**(16): 2999-3016.

White, L. F. and M. Pagano (2008). "Transmissibility of the influenza virus in the 1918 pandemic." PLoS One **3**(1): e1498.

White, L. F., J. Wallinga, et al. (2009). "Estimation of the reproductive number and the serial interval in early phase of the 2009 influenza A/H1N1 pandemic in the USA." Influenza and other respiratory viruses **3**(6): 267-276.

World Health Organization (2003). Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). Geneva, Switzerland.

World Health Organization (2008). Anthrax in humans and animals. Geneva, Switzerland.

World Health Organization. (2008). "Ebola haemorrhagic fever." Retrieved August 29, 2011, from http://www.who.int/mediacentre/factsheets/fs103/en/index.html.

Appendix M.

Environmental Justice

Contents

Tables and Figures

Demographic Data for the NIH NEIDL BUMC Site, Boston, MA

Demographic Data for the NIH NEIDL Boston University Corporate Education Center Site, Tyngsborough, MA

Demographic Data for the NIH NEIDL Boston University Sargent Center for Outdoor Education (SCOE), Hancock/Peterborough, NH

M. Environmental Justice

M.1 INTRODUCTION

Environmental Justice is defined in Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority and Low Income Populations*, and requires federal agencies to identify and address, as appropriate, disproportionately high and adverse human health or environmental impacts of federal programs, policies, and activities on minority and low income populations. The order also requires agencies to ensure greater public participation in their decision making process. The objective of the environmental justice analysis for this risk assessment (RA) is to identify and address potential disproportionately high and adverse human health and environmental impacts on minority or low income populations from the National Emerging Infectious Diseases Laboratory (NEIDL) within the defined region of analysis for the urban, suburban and rural sites. The analysis characterizes U.S. Census data available for each site and compares U.S. Census data between sites.

M.1.1 Federal Criteria

On February 11, 1994, President William J. Clinton signed Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations*, which directed federal agencies to develop environmental justice strategies to address disproportionately high and adverse human health or environmental effects of their programs on minority and low-income populations, as well as focusing federal attention on the environmental and human health conditions of minority and low-income populations with the goal of achieving environmental protection for all communities. The order is also intended to promote nondiscrimination in federal programs that affect human health and the environment and provide minority and low-income communities access to public information and public participation in matters relating to human health and the environment. In addition to Executive Order 12898, two guidance documents help define how to address environmental justice concerns: The Council on Environmental Quality's (CEQ's) December 1997 document, *Environmental Justice Guidance under the National Environmental Policy Act*, and an April 1998 document produced by an EPA working group titled *Final Guidance for Incorporating Environmental Justice Concerns in EPA's NEPA Compliance Analyses.* The CEQ has oversight of the federal government's compliance with Executive Order 12898 and National Environmental Policy Act (NEPA). EPA has lead responsibility for implementation of the executive order as Chair of the Interagency Working Group on Environmental Justice (CEQ 1997).

M.1.2 Minority Populations

The federal environmental justice criteria identify minority populations as, Black or African American, American Indian and Alaska Native, Asian, Native Hawaiian and other Pacific Islander, persons of two or more races, and persons of Hispanic origin. Minority populations should be identified for environmental justice analyses where, either the minority population of the affected area exceeds 50 percent, or the minority population percentage of the affected area is meaningfully greater than the minority population percentage in the general population or other appropriate unit of geographic analysis (CEQ 1997). The latter guidance was used for this analysis to address the federal environmental justice criteria, identifying census tracts with minority population percentages exceeding the U.S. level. Census tracts are subdivisions of a county and represent a level at which disproportionate effects would be more noticeable. As of, 25 percent of the U.S. population was of a minority race or ethnicity (U.S. Census Bureau 2000a).

M.1.2.1 Low-Income Populations

The federal environmental justice criteria use poverty thresholds established by the Census Bureau to identify low-income populations (CEQ 1997). Poverty status is reported as the number of persons or families with income below a defined threshold level. The Census defines the 2000 poverty level as $8,791 ($10,956 for 2009) of annual income, or less, for an individual and $17,604 ($21,954 for 2009) of annual income, or less, for a family of four (U.S. Census Bureau 2010a). As with the minority populations, the federal environmental justice criteria identify low-income populations for those census tracts with poverty rates exceeding those of the United States. As of the 2000 census, 12 percent (U.S. Census Bureau 2000a) of U.S. residents were classified as living in poverty, and in 2008 there were 13.2 percent of U.S. residents classified as living in poverty (U.S. Census Bureau 2010b).

M.1.2.2 Disproportionately High and Adverse Human Health Effects

Adverse health effects are measured in risks and rates that could result in fatal or nonfatal adverse effects on human health. Adverse health effects could include bodily impairment, infirmity, illness, or death. Agencies are required to identify programs, policies or activities that can cause disproportionately high and adverse human health or environmental effects. The determination, by the agency, of disproportionately high and adverse impacts is made in consideration of whether the impacts, as summarized in the RA, are both significant and disproportionate:

1. The impacts must be significant or above generally accepted norms, such as regulatory limits or state and local statutes and ordinances (NUREG 2003). The significance of

impacts is determined in consideration of both the context and the intensity of the impact. The context includes factors such as extent of the impact whether the impact is local, regional, or national. The context affects the number of people that could be affected. Intensity refers to the severity of the impact. Direct and indirect impacts are considered as well as immediate and long-term impacts (Title 40 of the *Code of Federal Regulations* section 1508.8).

2. The impacts are disproportionate if the risks to a minority individual or low-income individual appreciably exceed the risk to an individual in the general population (CEQ 1997).

M.1.3 State Guidance

The Commonwealth of Massachusetts Executive Office of Energy and Environmental Affairs (EEA) has an Environmental Justice Policy. The state criteria for environmental justice populations are those segments of the population that EEA has determined to be most at risk of being unaware of or unable to participate in environmental decision making or to gain access to state environmental resources. They are defined as neighborhoods that meet *one or more* of the following criteria:

- The median annual household income is at or below 65 percent of the statewide median income for Massachusetts; *or*
- 25 percent of the residents are minority; *or*
- 25 percent of the residents are foreign born, *or*
- 25 percent of the residents are lacking English language proficiency.

Neighborhoods, as defined by EEA's Environmental Justice Policy are U.S. Census Bureau block groups (Massachusetts EEA 2002).

M.2 Methodology

The National Institutes of Health (NIH) performed an environmental justice analysis and an analysis that includes input from the Boston community from January 9, 2004, to present to identify the disproportionate placement of high and adverse environmental or health impacts from the NEIDL at the urban, suburban, or rural sites on minority or low-income populations. The public input gathered since January 9, 2004, assisted in identifying a geographic scale for which demographic information was obtained on the potential impact area(s). Per CEQ, available

demographic data from the U.S. Census Bureau were used to identify the composition of the potentially affected population.

M.2.1 Environmental Justice Data Set

Census data are collected by the U.S. Census Bureau on a decennial (10-year) basis. Census tracts are small, relatively permanent statistical subdivisions of a county. Census tracts are delineated for most metropolitan areas and other densely populated counties by local census statistical areas committees following U.S. Census Bureau guidelines (more than 3,000 census tracts have been established in 221 counties outside metropolitan area's). Census tracts usually include between 2,500 and 8,000 persons and, when first delineated, are designed to be homogeneous with respect to population characteristics, economic status, and living conditions. Census tracts do not cross county boundaries. The spatial size of census tracts varies widely depending on the density of settlement. Census tract boundaries are delineated with the intention of being maintained over a long time so that statistical comparisons can be made from census to census (U.S. Census Bureau 2000b).

M.3 DEMOGRAPHIC DATA FOR THE NIH NEIDL BUMC SITE, BOSTON

A 10-kilometer (km) radius was defined around the proposed NIH Boston University Medical Center (BUMC) NEIDL site. A guideline of 1-km radius study area within the city limits and a 2.4-km radius outside city limits is provided by the U.S. Regulatory Commission as generally sufficient for assessing potential environmental justice impacts associated with activities other than nuclear power plants (NUREG 2003). For NEIDL, environmental justice data were collected for a radius of 10 km of each of the three sites, which is 10 times the city recommendation of the U.S. Nuclear Regulatory Commission. The larger radius is used to ensure that all potentially affected areas are considered. Demographic data were collected from the U.S. Census Bureau's 2000 decennial census for each census tract within the 10-km area; that is the most recent year for which data are available at the census tract geographic level. This 10 km radius was then subdivided into concentric 2-km circles (see Figure 1). For census tracts that covered multiple circles, the tract was included in the circle in which the majority of the tract resided; or, if the tract was largely divided over multiple circles, the data were divided between the defined areas. This 10-km area covers portions of three Massachusetts counties: Suffolk, Middlesex, and Norfolk. For comparative purposes, data for the city of Boston; Suffolk, Middlesex, and Norfolk counties; Massachusetts; and the United States are presented. In addition, this analysis was

performed when the 2007 data were the most recent data available. Therefore, the 2007 data are presented for Boston; Suffolk, Middlesex, and Norfolk counties; Massachusetts; and the United States.

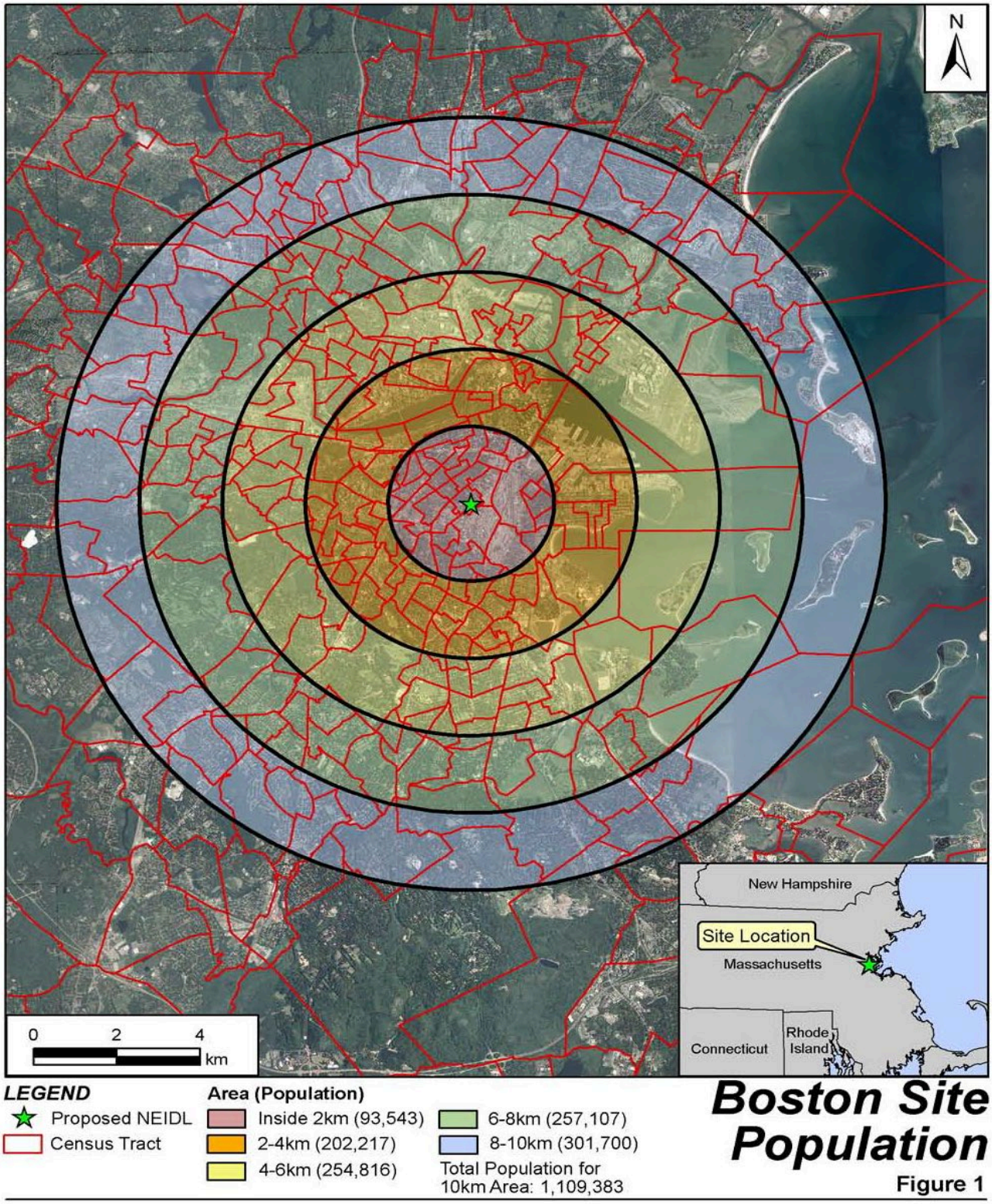


Figure 1. Boston site population

M.3.1 Population

Population data for the census tracts in the 10-km area are listed in Tables 1 through 5. The tracts are sorted first by boundary (inside 2 km, 2–4 km, 4–6 km, 6–8 km, and 8–10 km) and then numerically in ascending order by tract number. Tract 711 corresponds to the BUMC site. The population for the census tracts inside the 2-km boundary ranges from 723 to 9,346; the 2–4 km boundary ranges from 960 to 8,064; the 4–6 km boundary tract populations range from about 200 to 8,880; the 6–8 km population ranges from about 200 to 8,965; and the 8–10 km boundary census tract population ranges from about 200 to more than 8,300 (see Tables 1 through 5).

Table 1. Boston census tracts and population inside the 2-km boundary

Census tract	Population		Census tract	Population
104.01	9,346		708	3,600
104.02	3,769		709	2,853
105	3,061		711	3,120
106	2,406		712	1,344
107	4,908		801	3,381
607	1,384		803	1,682
608	3,726		804	723
610	3,064		805	3,877
611	1,023		806	2,300
612	1,905		814	1,266
702	4,183		817	1,861
703	3,552		818	2,863
704	1,832		904	1,628
705	5,435		906	2,123
706	2,188		907	4,518
707	2,214		913	2,409
Total population inside 2-km boundary				93,543
Total area (square kilometers) inside 2-km boundary				12.5

Source: U.S. Census Bureau 2000, 2008

Table 2. Boston census tracts and population in 2-4 km boundary

Census tract	Population		Census tract	Population
3,521	1,521		810	4,943
3,523	2,229		811	3,754
3,524	1,942		812	2,975
3,531	8,064		813	4,142
3,532	3,143		814	1,266
4,001	4,968		815	1,641
4,008	2,792		817	1,861
4,009	4,135		818	1,432
101.01	5,004		819	3,206
101.02	3,938		820	2,806
102.01	5,464		821	4,251
102.02	2,635		901	4,588
103	5,548		902	1,996
108	6,306		903	3,130
201	4,157		904	1,628
202	3,635		909	3,190
203	5,881		910	2,772

Census tract	Population		Census tract	Population
301	1,963		911	5,086
302	1,534		912	3,458
303	4,074		914	2,440
304	2,222		915	4,795
305	1,160		916	3,448
602	2,054		917	3,347
603	3,077		918	3,547
604	4,946		919	3,684
605	3,326		920	2,674
606	1,401		1,203	4,582
611	1,023		1,204	2,792
701	3,181		1,205	2,480
808	963		1,206	2,368
809	3,564		1,207	2,086
Total population in 2-4km boundary				202,217
Total area (square kilometers) in 2-4 km boundary				37.7

Source: U.S. Census Bureau 2000, 2008

1

2 **Table 3. Boston census tracts and population in 4-6 km boundary**

Census tract	Population		Census tract	Population
5.01	3,683		1,101	6,820
6.02	2,430		1,201.01	2,421
7.01	4,687		1,201.02	3,562
7.02	7,551		1,202	3,423
8.01	3,540		1,204	2,792
8.02	7,984		1,501	211.2
305	1,160		3,512	8,451
401	1,843		3,513	4,336
402	1,652		3,514	8,881
403	3,845		3,515	2,066
404	2,070		3,521	1,521
406	2,131		3,522	2,021
408	3,654		3,525	3,312
501	4,587		3,526	2,652
502	4,897		3,527	2,407
503	2,307		3,528	2,385
504	2,525		3,529	2,553
505	1,897		3,530	3,706
506	2,081		3,533	3,636
507	4,042		3,534	2,430
512	1,334		3,535	2,599
601	3,009		3,537	5,246
920	2,674		3,538	4,636
921	6,859		3,539	5,923
922	3,671		4,002	5,869
923	3,079		4,003	4,448
924	6,470		4,004	5,617
1,001	5,430		4,005	5,312
1,002	2,663		4,006	5,114
1,003	3,661		4,007	3,437
1,004	5,228		4,008	2,792
1,005	6,556		4,010	3,558

Census tract	Population		Census tract	Population
1,006.01	5,666		4,011	1,902
1,006.02	1,941		4,173	1,969
Total population in 4-6km area				254,816
Total area (square kilometers) in 4-6 km boundary				62.8

Source: U.S. Census Bureau 2000, 2008

1 **Table 4. Boston census tracts and population in 6-8 km boundary**

Census tract	Population		Census tract	Population
1	3,968		1,603	2,244
2.01	3,887		1,604	2,771
2.02	3,925		1,605	5,128
3.02	1,503		1,803	3,191
4.01	5,796		3,396	2,521
4.02	3,564		3,398	3,675
5.01	3,683		3,424	5,685
5.02	3,178		3,501	8,964
6.01	3,413		3,502	6,806
6.02	2,430		3,503	2,559
501	2,294		3,504	5,921
509	3,697		3,509	3,139
510	3,914		3,510	6,395
512	1,334		3,511	5,932
1,007	4,384		3,514	8,881
1,008	5,512		3,536	4,742
1,009	4,250		3,540	4,649
1,010.01	6,172		3,541	2,704
1,010.02	5,405		3,542	3,063
1,011.01	3,205		3,544	1,714
1,011.02	4,942		3,545	2,405
1,102	2,115		3,547	2,481
1,103	2,360		3,548	2,049
1,104.01	3,756		3,703	6,070
1,104.02	4,409		4,011	1,902
1,105.02	3,995		4,012	5,261
1,106.01	2,645		4,163	2,383
1,106.02	5,662		4,164	3,014
1,404	3,989		4,172	7,706
1,501	211		4,174	2,626
1,601	7,541		4,175.01	4,929
1,602	3,920		4,175.02	4,537
Total population in 6-8km area				257,107
Total area (square kilometers) in 6-8 km boundary				87.9

Source: U.S. Census Bureau 2000, 2008

Table 5. Boston census tracts and population in 8-10 km boundary

Census tract	Population		Census tract	Population
3.01	2,923		3,418	3,304
3.02	1,503		3,421	7,809
511	5,797		3,422	8,332
1,105.01	3,285		3,423	5,945
1,301	5,041		3,425	6,383
1,302	4,689		3,426	3,883
1,303	4,340		3,505	1,677
1,304.01	3,766		3,506	4,525
1,401.03	3,596		3,507	6,096
1,401.04	4,999		3,508	1,730
1,402.02	2,746		3,543	3,266
1,403	6,214		3,546	4,409
1,404	3,989		3,549	5,235
1,501	211		3,550	2,712
1,605	5,128		3,561	3,104
1,606	8,348		3,562	2,446
1,701	6,789		3,567	7,336
1,702	2,216		3,572	1,602
1,706	4,770		3,573	2,871
1,707	8,315		3,574	2,277
1,708	2,358		3,702	8,049
1,801	2,448		3,704	5,196
1,802	4,120		3,731	4,846
1,804	1,822		3,735	6,395
1,805	2,137		3,736	6,434
3,394	3,512		3,738	2,643
3,395	5,702		3,739	6,322
3,396	2,521		4,161	2,826
3,397	4,039		4,162	6,702
3,398	3,675		4,163	2,383
3,399	5,310		4,164	3,014
3,400	1,285		4,171	4,616
3,412	6,428		4,176.01	5,386
3,413	2,368		4,176.02	4,499
3,414	3,010		4,182	5,811
3,415	2,233			
Total population in 8-10 km area				301,700
Total area (square kilometers) in 8-10 km boundary				113.1

Source: U.S. Census Bureau 2000, 2008

Table 6 summarizes the totals within each 2km (i.e., 2–4 km, 4–6 km, and so on) radius boundary within the 10-km area. As of 2000, the total population in the 10-km boundary was more than 1.1 million, and the area was categorized almost entirely as urban (Table 6). The boundary covers a total area of 314 square km. The population and land area of each 2-km boundary range increases moving outward from the proposed site (Table 6).

Table 6. Boston population for 10-km boundary area

Area/boundary	Total population	Total urban population	Total rural population	Land area (square kilometers)
Inside 2 km	93,543	93,543	0	12.5
2–4 km	202,217	202,217	0	37.7
4–6 km	254,816	254,816	0	62.8
6–8 km	257,107	257,107	0	87.9
8–10 km	301,700	301,568	132	113.1
Total for 10-km area	1,109,383	1,109,251	132	314

Source: U.S. Census Bureau 2000, 2008

The most recent population data (2007) for Boston, Suffolk County, and the adjacent counties of Middlesex and Norfolk, Massachusetts, and the United States are in Table 7. Boston and Suffolk had significantly higher population densities compared to the adjacent Middlesex and Norfolk counties and compared to Massachusetts and the United States. Boston and Suffolk County population increased 4 percent and 3 percent, respectively, between 2000 and 2007. That growth rate was higher than Middlesex and Norfolk counties and the state, but lower than U.S. population growth during the same period of 7 percent (U.S. Census Bureau 2007).

Table 7. 2007 Population data for jurisdictions surrounding the proposed BUMC NEIDL site

	Population density 2007 (persons per square km)	2000 Population	2007 Population	Population change, 2000–2007
Boston	4,944	589,141	613,117	4%
Suffolk County	4,691	689,807	713,049	3%
Middlesex County	691	1,465,396	1,473,416	1%
Norfolk County	633	650,308	654,909	1%
Massachusetts	318	6,349,097	6,449,755	2%
United States	33	281,421,906	301,621,159	7%

Source: U.S. Census Bureau 2007, 2008

M.3.2 Environmental Justice

To identify potential environmental justice areas, data were collected on minority and low-income populations for census tracts in the 10-km boundary area of the proposed BUMC NEIDL Site, Boston, Massachusetts. Census tracts are subdivisions of a county and represent a level at which disproportionate impacts would be more noticeable. Tables 8 through 12 list the census tracts and minority and low-income data for the 10-km boundary area. Census tract 711 coincides with the land area of the proposed BUMC NEIDL. Tracts identified by shading indicate those tracts with a percentage of minority residents higher than the U.S. level of 25 percent or a percentage of residents living in poverty higher than the U.S. poverty rate of 12 percent.

Table 8. Boston minority and low-income data by census tract inside the 2-km boundary

Census tract	Minority	Below poverty level		Census tract	Minority	Below poverty level
104.01	28%	38%		708	39%	18%
104.02	31%	39%		709	53%	27%
105	24%	26%		711	50%	27%
106	19%	10%		712	56%	41%
107	9%	7%		801	81%	30%
607	47%	47%		803	91%	30%
608	7%	13%		804	88%	30%
610	39%	48%		805	90%	40%
611	55%	47%		806	76%	39%
612	7%	17%		814	79%	18%
702	77%	35%		817	95%	26%
703	23%	13%		818	87%	27%
704	92%	42%		904	90%	30%
705	45%	22%		906	92%	16%
706	17%	9%		907	36%	17%
707	49%	12%		913	87%	25%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 9. Boston minority and low-income data by census tract in the 2-4 km boundary

Census tract	Minority	Below poverty level		Census tract	Minority	Below poverty level
3,521	28%	20%		810	53%	34%
3,523	23%	15%		811	54%	20%
3,524	63%	26%		812	75%	42%
3,531	50%	24%		813	86%	30%
3,532	32%	15%		814	79%	18%
4,001	20%	10%		815	90%	14%
4,008	20%	12%		817	95%	26%
4,009	26%	10%		818	95%	27%
101.01	23%	23%		819	95%	12%
101.02	26%	57%		820	96%	31%
102.01	27%	27%		821	94%	33%
102.02	27%	48%		901	97%	20%
103	33%	57%		902	94%	34%
108	10%	8%		903	93%	35%
201	4%	4%		904	90%	30%
202	12%	12%		909	63%	32%
203	24%	11%		910	34%	14%
301	3%	8%		911	47%	16%
302	3%	9%		912	67%	20%
303	11%	14%		914	86%	35%
304	4%	11%		915	84%	22%
305	6%	5%		916	80%	22%
602	1%	7%		917	91%	17%
603	2%	7%		918	92%	22%
604	3%	8%		919	94%	29%
605	4%	7%		920	90%	20%
606	11%	7%		1,203	60%	23%
611	55%	47%		1,204	21%	10%
701	44%	34%		1,205	55%	17%

Census tract	Minority	Below poverty level		Census tract	Minority	Below poverty level
808	73%	34%		1206	24%	17%
809	39%	38%		1207	38%	17%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 10. Boston minority and low-income data by census tract in the 4-6 km boundary

Census tract	Minority	Below poverty level		Census tract	Minority	Below poverty level
5.01	17%	24%		1,101	54%	20%
6.02	40%	43%		1,201.01	30%	14%
7.01	31%	27%		1,201.02	10%	5%
7.02	33%	32%		1,202	45%	15%
8.01	28%	27%		1,204	21%	10%
8.02	37%	33%		1,501	49%	70%
305	6%	5%		3,512	24%	10%
401	6%	6%		3,513	21%	19%
402	37%	28%		3,514	37%	14%
403	14%	11%		3,515	34%	12%
404	2%	17%		3,521	28%	20%
406	4%	6%		3,522	27%	17%
408	33%	33%		3,525	51%	21%
501	36%	21%		3,526	32%	12%
502	44%	20%		3,527	40%	23%
503	55%	46%		3,528	33%	10%
504	29%	14%		3,529	15%	5%
505	37%	15%		3,530	35%	14%
506	45%	17%		3,533	25%	10%
507	36%	18%		3,534	58%	13%
512	21%	14%		3,535	43%	15%
601	2%	4%		3,537	24%	16%
920	90%	20%		3,538	22%	15%
921	59%	17%		3,539	34%	26%
922	74%	10%		4,002	27%	17%
923	98%	19%		4,003	17%	11%
924	94%	38%		4,004	17%	10%
1,001	94%	32%		4,005	14%	8%
1,002	97%	19%		4,006	15%	6%
1,003	96%	20%		4,007	17%	8%
1,004	85%	15%		4,008	20%	12%
1,005	84%	28%		4,010	22%	7%
1,006.01	49%	15%		4,011	13%	3%
1,006.02	18%	16%		4,173	18%	5%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 11. Boston minority and low-income data by census tract in the 6-8 km boundary

Census tract	Minority	Below poverty level		Census tract	Minority	Below poverty level
1	26%	13%		1,603	20%	15%
2.01	19%	9%		1,604	64%	30%
2.02	30%	12%		1,605	41%	21%
3.02	17%	6%		1,803	4%	3%
4.01	21%	22%		3,396	16%	6%
4.02	21%	20%		3,398	20%	7%
5.01	17%	24%		3,424	25%	14%
5.02	21%	24%		3,501	34%	20%
6.01	25%	16%		3,502	23%	11%
6.02	40%	43%		3,503	28%	8%
501	36%	21%		3,504	11%	11%
509	30%	20%		3,509	12%	7%
510	13%	19%		3,510	16%	9%
512	21%	14%		3,511	17%	10%
1,007	7%	6%		3,514	37%	14%
1,008	36%	9%		3,536	21%	12%
1,009	73%	10%		3,540	23%	8%
1,010.01	95%	14%		3,541	14%	9%
1,010.02	94%	16%		3,542	7%	4%
1,011.01	99%	20%		3,544	19%	4%
1,011.02	98%	21%		3,545	16%	9%
1,102	58%	11%		3,547	18%	5%
1,103	41%	12%		3,548	17%	5%
1,104.01	49%	19%		3,703	11%	8%
1,104.02	35%	8%		4,011	13%	3%
1,105.02	30%	10%		4,012	18%	6%
1,106.01	8%	2%		4,163	16%	4%
1,106.02	11%	3%		4,164	3%	1%
1,404	83%	12%		4,172	36%	5%
1,501	49%	70%		4,174	3%	4%
1,601	53%	29%		4,175.01	32%	10%
1,602	50%	27%		4,175.02	37%	8%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 12. Boston minority and low-income data by census tract in the 8-10 km Boundary

Census tract	Minority	Below poverty level		Census tract	Minority	Below poverty level
3.01	16%	9%		3,418	33%	9%
3.02	17%	6%		3,421	13%	12%
511	23%	15%		3,422	22%	10%
1,105.01	16%	11%		3,423	19%	9%
1,301	8%	5%		3,425	23%	15%
1,302	5%	3%		3,426	20%	13%
1,303	8%	3%		3,505	9%	11%
1,304.01	34%	14%		3,506	19%	19%
1,401.03	43%	10%		3,507	19%	12%
1,401.04	53%	23%		3,508	21%	5%
1,402.02	32%	9%		3,543	24%	7%
1,403	64%	12%		3,546	41%	12%
1,404	83%	12%		3,549	61%	11%
1,501	49%	70%		3,550	20%	6%
1,605	41%	21%		3,561	9%	4%
1,606	29%	20%		3,562	11%	8%
1,701	11%	15%		3,567	8%	4%
1,702	8%	18%		3,572	9%	4%
1,706	11%	10%		3,573	11%	5%
1,707	39%	27%		3,574	7%	5%
1,708	13%	11%		3,702	6%	3%
1,801	9%	7%		3,704	8%	4%
1,802	4%	5%		3,731	12%	5%
1,804	4%	4%		3,735	8%	2%
1,805	5%	7%		3,736	13%	4%
3,394	12%	10%		3,738	14%	8%
3,395	16%	7%		3,739	14%	4%
3,396	16%	6%		4,161	7%	4%
3,397	16%	13%		4,162	34%	3%
3,398	20%	7%		4,163	16%	36%
3,399	10%	6%		4,164	3%	1%
3,400	13%	7%		4,171	16%	2%
3,412	25%	9%		4,176.01	22%	5%
3,413	43%	12%		4,176.02	18%	4%
3,414	28%	7%		4,182	16%	6%
3,415	40%	12%				

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 13 summarizes the totals within each 2-km radius boundary within the 10-km area. Of the 297 census tracts identified in the 10-km area, 154, or 52 percent, had a higher percentage of minority residents compared to the United States, and 156 tracts, or 53 percent, had a higher percentage of persons living in poverty compared to the United States. As shown in Table 13, the portion of census tracts with a percentage of minority populations or a percentage of persons below poverty that is greater than the U.S. levels decreases going outward from the interior 2-km circle of the proposed BUMC NEIDL site.

Table 13. Boston minority and low-income data by census tract for the 10-km area

Area/boundary	Total census tracts	Number tracts with percentage of minority persons above U.S. level of 25%	Portion of tracts with percentage of minority persons above U.S. level of 25%	Number tracts with percentage of persons in poverty above U.S. level of 12%	Portion of tracts with percentage of persons in poverty above U.S. level of 12%
Inside 2 km	32	25	78%	28	88%
2–4 km	62	42	68%	44	71%
4–6 km	68	42	62%	45	66%
6–8 km	64	28	44%	25	39%
8–10 km	71	17	24%	14	20%
Total for 10-km area	297	154	52%	156	53%

Source: U.S. Census Bureau 2000

The most recent (2007) minority and low-income data for Boston, Suffolk County and the adjacent counties of Middlesex and Norfolk, Massachusetts, and the United States are listed in Table 14. Boston and Suffolk had a notably higher percentage of minority residents and persons living below poverty compared to the adjacent counties of Middlesex and Norfolk and compared to Massachusetts and the United States.

Table 14. 2007 Minority and low-income data for jurisdictions surrounding the BUMC NEIDL site

	Percent minority	Percent living below poverty
Boston	44%	20.4%
Suffolk County	42%	19.7%
Middlesex County	18%	6.8%
Norfolk County	15%	6.6%
Massachusetts	17%	9.9%
United States	26%	13.0%

Source: U.S. Census Bureau 2007

M.3.3 State Criteria

To assess environmental justice per the Massachusetts state criteria, data were collected on minority populations, foreign-born populations, households lacking English language proficiency, and median annual household income for each neighborhood (defined as a census block group) within a 10-km (6-mile) area of the Boston site. Table 15 summarizes the totals within each 2-km (1.2-mile) radius boundary within the 10-k (6-mile) area. Of the 1,112 census block groups identified in the 10-k (6-mile) radius boundary, 622 block groups (56 percent) had a higher percentage of minority residents compared to the state's threshold of 25 percent; 474 census block groups (43 percent) had 25 percent or more residents who are foreign born; 78 block groups (7 percent) had households where 25 percent or more of the residents lacked English language proficiency; and 281 block groups (25 percent) had a median annual household income at or

below 65 percent of the statewide median income of $50,500. As shown in Table 15, the portion of census block groups meeting one or more of the state environmental justice guidance criteria was, for the most part, higher within the boundaries closest to the Boston NEIDL site and decreases moving outward from the site.

Table 15. Massachusetts state criteria for environmental justice for the (10-km) 6-mile boundary area of the urban site

Boundary area radius	Total census block groups	Percent of block groups with a minority population above the state guidance level of 25 percent	Percent of block groups with a foreign born population above the state guidance level of 25 percent	Percent of block groups lacking English language proficiency above the state guidance level of 25 percent	Percent of block groups with a median annual household income at or below 65 % of the Massachusetts statewide median income
Inside 2 km (1.2 mile)	100	79%	35%	17%	55%
2–4 km (1.2–2.4 mile)	203	71%	44%	4%	39%
4–6 km (2.4–3.6 mile)	228	66%	57%	11%	27%
6–8 km (3.6–4.8 mile)	252	53%	48%	8%	17%
8–10 km (4.8–6.0 mile)	329	35%	30%	2%	12%
Total for the10-km area (6-mile)	1,112	56%	43%	7%	25%

Source: U.S. Census Bureau 2000

M.4 DEMOGRAPHIC DATA FOR THE NIH NEIDL BOSTON UNIVERSITY CORPORATE EDUCATION CENTER SITE, TYNGSBOROUGH, MASSACHUSETTS

A 10-km radius was defined around the proposed NIH Boston University Corporate Education Center, NEIDL site in Tyngsborough, Massachusetts. Demographic data were collected from the U.S. Census Bureau's 2000 decennial census for each census tract within the 10-km area; that is the most recent year for which data are available at the census tract geographic level. The 10-km radius was then subdivided into concentric 2-km circles (see Figure 2). For census tracts that covered multiple circles, the tract was included in the circle in which the majority of the tract resided; or, if the tract was largely divided over multiple circles, the data were divided between the defined areas. The 10-km area covers portions of Middlesex County, Massachusetts, and

Hillsborough County, New Hampshire. For comparative purposes, data for Middlesex and Hillsborough counties; Massachusetts and New Hampshire; and the United States are presented. In addition, this analysis was performed when the 2007 data were the most recent data available. Therefore, the 2007 data are presented for Middlesex and Hillsborough counties; Massachusetts and New Hampshire; and the United States.

1

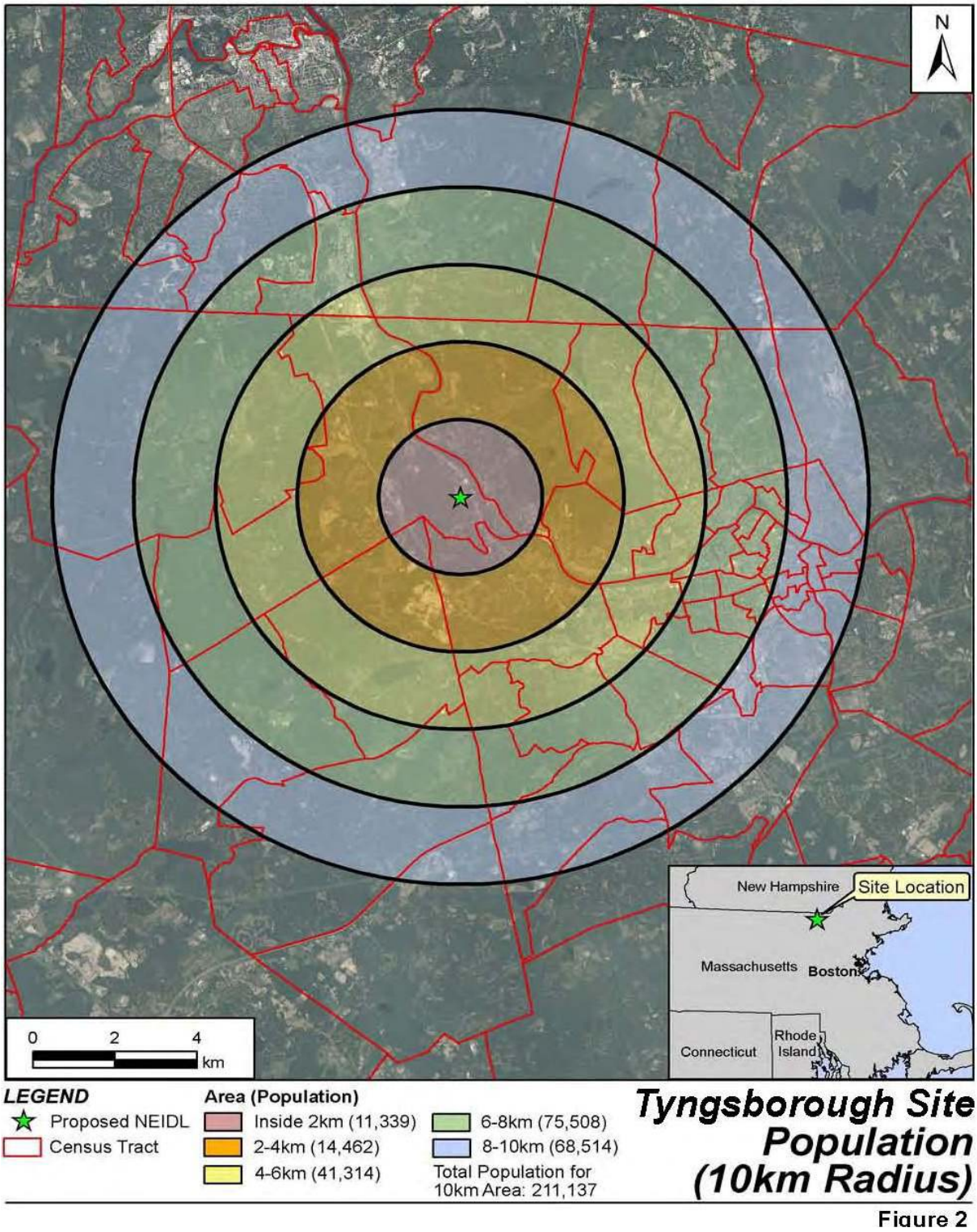


Figure 2. Tyngsborough site population

M.4.1 Population

Population data for the census tracts in the 10-km area are listed in Tables 16 through 20. The tracts are sorted first by boundary (inside 2 km, 2–4 km, 4–6 km, 6–8 km, and 8–10 km) and then numerically in ascending order by tract number. Tract 3131.01 corresponds to the Boston University Corporate Education Center site in Tyngsborough. The population for the census tracts inside the 2-km boundary ranges from about 1,200 to 6,100; the 2–4 km boundary ranges from about 1,200 to 3,900; the 4–6 km boundary tract population ranges from about 700 to 5,800; the 6–8 km population ranges from about 350 to 5,100; and the 8–10 km boundary census tract population ranges from about 350 to more than 6,000 (see Tables 16 through 20).

Table 16. Tyngsborough census tracts and population inside the 2-km boundary

Census tract	Population
3,131.01	1,232
3,131.02	2,031
3,173	1,961
3,181	6,116
Total population inside 2-km boundary	11,339
Total area (square kilometers) inside 2-km boundary	12.5

Source: U.S. Census Bureau 2000, 2008

Table 17. Tyngsborough census tracts and population in the 2-4 km boundary

Census tract	Population
3,106.01	3,613
3,131.01	1,232
3,131.02	2,031
3,141.01	1,831
3,173	3,921
3,181	1,835
Total population in 2-4km boundary	14,462
Total area (square kilometers) in 2-4 km boundary	37.7

Source: U.S. Census Bureau 2000, 2008

Table 18. Tyngsborough census tracts and population in the 4-6 km boundary

Census tract	Population
111	2,252
123	2,476
2,001	1,304
3,106.01	1,779
3,106.02	5,610
3,114	5,857
3,131.01	1,232
3,131.02	2,031
3,141.01	3,717
3,141.02	4,725

Census tract	Population
3,172.01	1,442
3,172.02	1,723
3,172.03	1,441
3,173	1,961
3,181	3,058
3,281	707
Total population in 4-6km area	41,314
Total area (square kilometers) in 4-6 km boundary	62.8

Source: U.S. Census Bureau 2000, 2008

1

2 **Table 19. Tyngsborough census tracts and population in the 6-8 km boundary**

Census tract	Population
111	4,573
112	1,921
123	2,476
2,001	1,304
2,002	357
3,101	1,941
3,103	3,079
3,104	2,399
3,105	3,353
3,107	4,575
3,108	2,457
3,110	2,754
3,111	2,286
3,112	3,374
3,113	3,954
3,115	2,908
3,116	5,099
3,117	4,923
3,118	3,516
3,131.01	1,232
3,141.02	2,327
3,142	3,707
3,172.01	1,442
3,172.02	1,723
3,172.03	2,927
3,181	612
3,182	1,218
3,183	2,368
3,281	707
Total population in 6-8km area	75,508
Total area (square kilometers) in 6-8 km boundary	87.9

Source: U.S. Census Bureau 2000, 2008

3

Table 20. Tyngsborough census tracts and population in the 8-10 km boundary

Census tract	Population
110	2,486
112	3,901
113	2,422
114.01	1,583
123	2,476
2,001	1,304
2,002	1,426
2,003	340
3,101	1,941
3,102	6,070
3,103	3,079
3,104	1,182
3,119	2,666
3,120	2,977
3,121	3,112
3,122	4,741
3,123	1,658
3,124	2,405
3,125.01	1,484
3,125.02	1,320
3,142	1,826
3,143.01	3,903
3,171.01	5,891
3,171.02	2,198
3,171.03	503
3,182	1,218
3,183	2,368
3,184	377
3,261	955
3,281	707
Total population in 8-10 km area	68,514
Total area (square kilometers) in 8-10 km boundary	113.1

Source: U.S. Census Bureau 2000, 2008

Table 21 summarizes the totals within each 2-km radius boundary within the 10-km area. As of 2000, the total population in the 10-km boundary was more than 211,000 and the area was categorized as mostly (96 percent) urban (Table 21). The 10-km area covers a total area of 314 square km. The population and land area of each 2-km boundary range increases moving outward from the proposed site (Table 21).

Table 21. Tyngsborough population for the 10-km area

Area/boundary	Total population	Total urban population	Total rural population	Land area (square kilometers)
Inside 2 km	11,339	11,083	256	12.5
2–4 km	14,462	14,206	256	37.7
4–6 km	41,314	39,414	1,900	62.8
6–8 km	75,508	73,495	2,013	87.9
8–10 km	68,514	65,435	3,080	113.1
Total for 10-km area	211,137	203,632	7,506	314

Source: U.S. Census Bureau 2000, 2008

The most recent population data (2007) for Middlesex County, Hillsborough County, Massachusetts and New Hampshire, and the United States are in Table 22. Middlesex County had a significantly higher population density compared to the adjacent county of Hillsborough and compared to Massachusetts, New Hampshire, and the United States. Middlesex County's population increased by only 1 percent between 2000 and 2007. That growth rate was lower than Hillsborough County, the states, and the U.S. population growth during the same period (U.S. Census Bureau 2007).

Table 22. 2007 Population data for jurisdictions surrounding the proposed Tyngsborough NEIDL site

	Population density 2007 (persons per square km)	2000 Population	2007 Population	Population change, 2000–2007
Middlesex County, MA	691	1,465,396	1,473,416	1%
Hillsborough County, NH	177	380,841	402,302	6%
Massachusetts	318	6,349,097	6,449,755	2%
New Hampshire	57	1,235,786	1,315,828	6%
United States	33	281,421,906	301,621,159	7%

Source: U.S. Census Bureau 2007, 2008

M.4.2 Environmental Justice

To identify potential environmental justice areas, data were collected on minority and low-income populations for census tracts in the 10-km boundary area of the proposed BU Corporate Education Center NEIDL Site Tyngsborough, Massachusetts. Census tracts are subdivisions of a county and represent a level at which disproportionate impacts would be more noticeable. Tables 23 through 27 list the census tracts and minority and low-income data for the 10-km boundary area. Census tract 3131.01 coincides with the land area of the proposed Boston University Corporate Education Center NEIDL site. Tracts identified by shading indicate those tracts with a percentage of minority residents higher than the U.S. level of 25 percent or a percentage of residents living in poverty higher than the U.S. poverty rate of 12 percent.

Table 23. Tyngsborough minority and low-income data by census tract inside the 2-km boundary

Census tract	Minority	Below poverty level
3,131.01	5%	2%
3,131.02	4%	7%
3,173	7%	4%
3,181	9%	0%

Source: U.S. Census Bureau 2000

Table 24. Tyngsborough minority and low-income data by census tract in the 2-4 km boundary

Census tract	Minority	Below poverty level
3,106.01	19%	6%
3,131.01	5%	2%
3,131.02	4%	7%
3,141.01	4%	3%
3,173	7%	4%
3,181	9%	0%

Source: U.S. Census Bureau 2000

Table 25. Tyngsborough minority and low-income data by census tract in the 4-6 km boundary

Census tract	Minority	Below poverty level
111	23%	6%
123	3%	1%
2,001	3%	3%
3,106.01	19%	6%
3,106.02	15%	4%
3,114	41%	10%
3,131.01	5%	2%
3,131.02	4%	7%
3,141.01	4%	3%
3,141.02	7%	4%
3,172.01	3%	0%
3,172.02	6%	2%
3,172.03	9%	3%
3,173	7%	4%
3,181	9%	0%
3,281	3%	2%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 26. Tyngsborough minority and low-income data by census tract in the 6-8 km boundary

Census tract	Minority	Below poverty level
111	23%	6%
112	10%	3%
123	3%	1%
2,001	3%	3%
2,002	3%	2%
3,101	38%	33%
3,103	24%	16%
3,104	33%	25%
3,105	20%	14%
3,107	35%	22%
3,108	37%	36%
3,110	53%	55%

Census tract	Minority	Below poverty level
3,111	68%	33%
3,112	65%	29%
3,113	41%	12%
3,115	31%	7%
3,116	23%	14%
3,117	45%	15%
3,118	60%	17%
3,131.01	5%	2%
3,141.02	7%	4%
3,142	5%	7%
3,172.01	3%	0%
3,172.02	6%	2%
3,172.03	9%	3%
3,181	9%	0%
3,182	4%	5%
3,183	6%	1%
3,281	3%	2%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 27. Tyngsborough minority and low-income data by census tract in the 8-10 km boundary

Census tract	Minority	Below poverty level
110	5%	5%
112	10%	3%
113	5%	1%
114.01	5%	3%
123	3%	1%
2,001	3%	3%
2,002	3%	2%
2,003	3%	4%
3,101	38%	33%
3,102	16%	11%
3,103	24%	16%
3,104	33%	25%
3,119	38%	35%
3,120	39%	32%
3,121	40%	17%
3,122	31%	16%
3,123	11%	7%
3,124	34%	23%
3,125.01	13%	7%
3,125.02	9%	5%
3,142	5%	7%
3,143.01	4%	2%
3,171.01	7%	3%
3,171.02	7%	6%
3,171.03	7%	1%

3,182	4%	5%
3,183	6%	1%
3,184	5%	2%
3,261	3%	4%
3,281	3%	2%

Source: U.S. Census Bureau 2000

Note: Shading for minority indicates a percentage of minority residents higher than the U.S. percentage of 25%. Shading for poverty indicates a percentage of residents living in poverty higher than the U.S. percentage of 12%.

Table 28 summarizes the minority and low-income population totals within each 2-km radius boundary within the 10-km area. Of the 85 census tracts identified in the 10-km area, 19 (22 percent) had a higher percentage of minority residents compared to the United States, and 20 of the census tracts (24 percent) had a higher percentage of persons living in poverty compared to the United States. As shown in Table 28, the portion of census tracts with a percentage of minority populations or a percentage of persons below poverty that is greater than the U.S. levels is higher in the exterior circles (6–8 km and 8–10 km), away from the proposed Tyngsborough NEIDL site. The tracts with the higher percentages of minority and low-income populations are all in the southeast quadrant of the 10-km circle, near the city of Lowell.

Table 28. Tyngsborough minority and low-income data for the 10-km boundary area

Area/boundary	Total census tracts	Number tracts with percentage of minority persons above US level of 25%	Portion of tracts with percentage of minority persons above US level of 25%	Number tracts with percentage of persons in poverty above US level of 12%	Portion of tracts with percentage of persons in poverty above US level of 12%
Inside 2 km	4	0	0%	0	0%
2–4 km	6	0	0%	0	0%
4–6 km	16	1	6%	0	0%
6–8 km	29	11	38%	12	41%
8–10 km	30	7	23%	8	27%
Total for 10-km area	85	19	22%	20	24%

Source: U.S. Census Bureau 2000

The most recent (2007) minority and low-income data for Middlesex and Hillsborough counties; Massachusetts and New Hampshire; and the United States are in Table 29. Middlesex County has a higher percentage of minority residents compared to Hillsborough County and New Hampshire; similar to its home state of Massachusetts; but lower than the United States. The percent living below poverty is the same for both Middlesex and Hillsborough counties (6.8 percent) and less than the state and national levels.

Table 29. 2007 Minority and low-income data for jurisdictions surrounding the proposed Tyngsborough NEIDL site

	Percent minority	Percent living below poverty
Middlesex County	18%	6.8%
Hillsborough County	8%	6.8%
Massachusetts	17%	9.9%
New Hampshire	5%	7.1%
United States	26%	13.0%

Source: U.S. Census Bureau 2007

M.4.3 State Criteria

To assess environmental justice per the Massachusetts criteria, data were collected on minority populations, foreign-born populations, households lacking English language proficiency, and median annual household income for each neighborhood (defined as a census block group) within a 10-km (6-mile) area of the Tyngsborough site. Table 30 summarizes the totals within each 2-km (1.2-mile) radius boundary within the 10-km (6-mile) area. Of the 260 census block groups identified in the 10-km (6-mile) radius boundary around Tyngsborough, 72 block groups (28 percent) had a higher percentage of minority residents compared to the state's threshold of 25 percent; 32 block groups (12 percent) had 25 percent or more residents who are foreign born; 9 block groups (3 percent) had households where 25 percent or more of the residents lacked English language proficiency; and 31 block groups (12 percent) had a median annual household income at or below 65 percent of the statewide median income of $50,500. As shown in Table 30, the portion of census block groups meeting one or more of the state environmental justice guidance criteria is higher in the exterior circles (from 6-8km [3.6–4.8 miles] out to 8-10km [4.8–6.0 miles]). Those census block groups are southeast of the proposed Tyngsborough site, near the city of Lowell.

Table 30. Massachusetts criteria for environmental justice for the 10-km boundary area of the suburban site

Boundary area radius	Total census block groups	Percent of block groups with a minority population above the state guidance level of 25 percent	Percent of block groups with a foreign born population above the state guidance level of 25 percent	Percent of block groups lacking English language proficiency above the state guidance level of 25 percent	Percent of block groups with a median annual household income at or below 65 % of the Massachusetts statewide median income
Inside 2km (1.2 mile)	15	0%	0%	0%	0%
2–4 km (1.2–2.4 mile)	21	5%	0%	0%	0%
4–6 km (2.4–3.6 mile)	48	15%	6%	0%	0%
6–8 km (3.6–4.8 mile)	82	46%	22%	5%	20%
8–10 km (4.8–6.0 mile)	94	28%	12%	5%	16%
Total for the 10k (6-mile) area	260	28%	12%	3%	12%

Source: U.S. Census Bureau 2000

M.5 DEMOGRAPHIC DATA FOR THE NIH NEIDL BOSTON UNIVERSITY SARGENT CENTER FOR OUTDOOR EDUCATION (SCOE), HANCOCK/PETERBOROUGH, NH

A 10-km radius was defined around the proposed NIH BU SCOE, NEIDL site in Hancock/Peterborough, Massachusetts. Demographic data were collected from the U.S. Census Bureau's 2000 decennial census for each census tract within the 10-km area; that is the most recent year for which data are available at the census tract geographic level. This 10-km radius was then subdivided into concentric 2-km circles (see Figure 3). For census tracts that covered multiple circles, the tract was included in the circle in which the majority of the tract resided; or, if the tract was largely divided over multiple circles, the data were divided between the defined areas. This 10-km area covers portions of Hillsborough and Cheshire counties, New Hampshire. For comparative purposes, data for Hillsborough and Cheshire counties; New Hampshire; and the United States are presented. In addition, this analysis was performed when the 2007 data were the most recent data available. Therefore, the 2007 data are presented for Hillsborough and Cheshire counties; New Hampshire; and the United States.

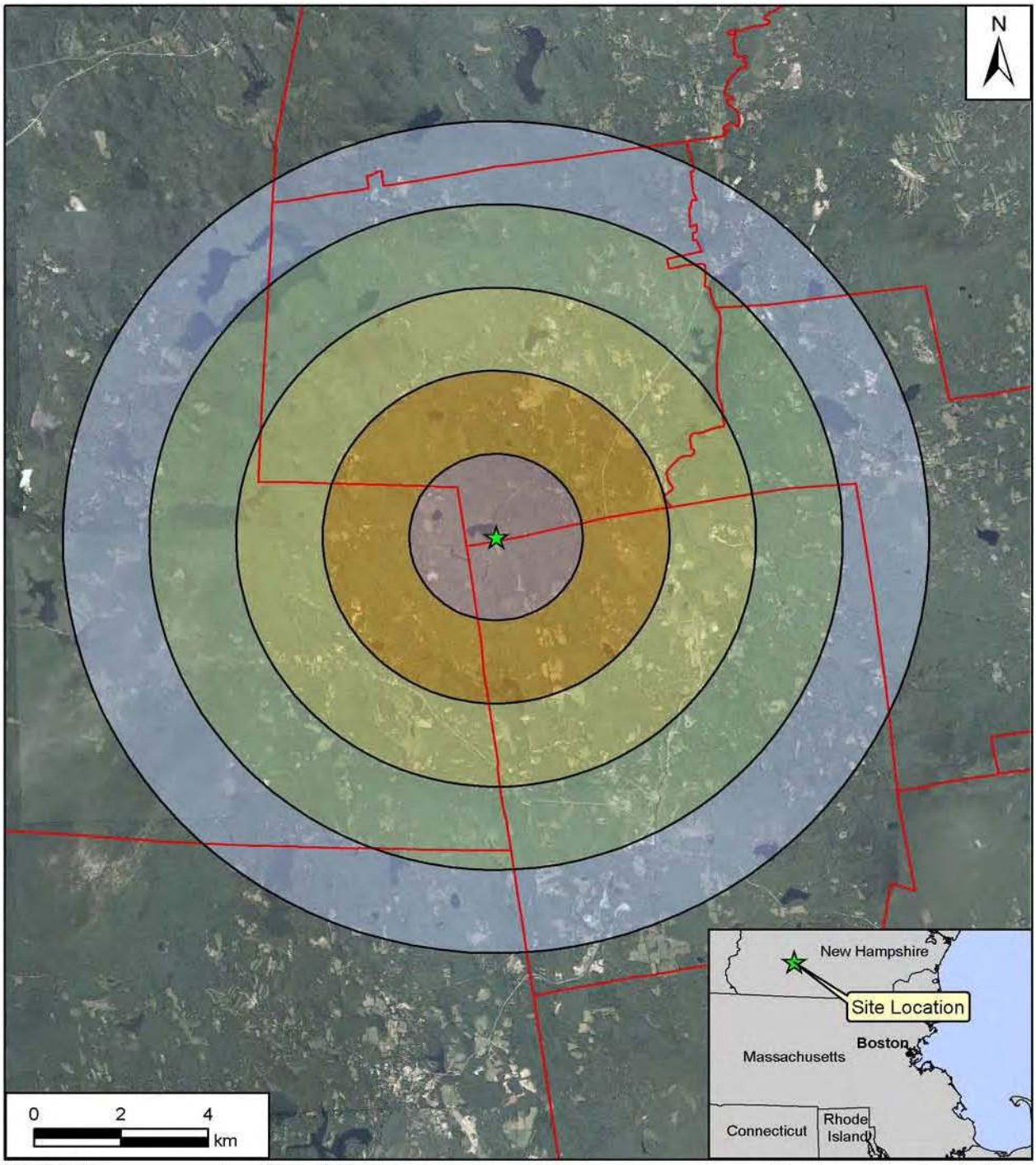


Figure 3. Hancock site population

M.5.1 Population

Population data for the census tracts in the 10-km area are listed in Tables 31 through 35. The tracts are sorted first by boundary (inside 2 km, 2–4 km, 4–6 km, 6–8 km, and 8–10 km) and then numerically in ascending order by tract number. Tract 240 corresponds to the BU SCOE site in Hancock/Peterborough. The population for the census tracts inside the 2-km boundary ranges from about 90 to 300; the 2–4 km boundary ranges from about 300 to almost 600; the 4–6 km boundary tract populations range from about 80 to almost 1,200; the 6–8 km population ranges from about 250 to almost 1,800; and the 8–10 km boundary census tract populations range from about 90 to almost 1,800 (see Tables 31 through 35).

Table 31. Hancock census tracts and population inside the 2-km boundary

Census tract	Population
230	294
240	87
9,704	102
Total population inside 2-km boundary	483
Total area (square kilometers) inside 2-km boundary	12.5

Source: U.S. Census Bureau 2000, 2008

Table 32. Hancock census tracts and population in the 2-4 km boundary

Census tract	Population
230	588
240	348
9,704	306
Total population in 2-4km boundary	1,242
Total area (square kilometers) in 2-4 km boundary	37.7

Source: U.S. Census Bureau 2000, 2008

Table 33. Hancock census tracts and population in the 4-6 km boundary

Census tract	Population
220	83
230	1,177
240	522
9,704	510
Total population in 4-6km area	2,291
Total area (square kilometers) in 4-6 km boundary	62.8

Source: U.S. Census Bureau 2000, 2008

Table 34. Hancock census tracts and population in the 6-8 km boundary

Census tract	Population
220	249
230	1,765
240	696
9,704	612
Total population in 6-8km area	3,321
Total area (square kilometers) in 6-8 km boundary	87.9

Source: U.S. Census Bureau 2000, 2008

Table 35. Hancock census tracts and population in the 8-10 km boundary

Census tract	Population
215	238
220	497
230	1,765
240	87
250	122
9704	1,019
9705	274
Total population in 8-10 km area	4,002
Total area (square kilometers) in 8-10 km boundary	113.1

Source: U.S. Census Bureau 2000, 2008

Table 36 summarizes the totals in each 2-km radius boundary within the 10-km area. As of 2000, the total population in the 10-km boundary was more than 11,300 and the area was categorized as primarily (71 percent) rural (Table 36). The 10-km radius covers a total area of 314 square km and is sparsely populated. The population and land area of each 2-km boundary range increases moving outward from the proposed site (Table 36).

Table 36. Hancock population for the 10-km boundary area

Area/boundary	Total population	Total urban population	Total rural population	Land area (square km)
Inside 2 km	483	167	317	12.5
2–4 km	1,242	334	908	37.7
4–6 km	2,291	667	1,624	62.8
6–8 km	3,321	999	2,321	87.9
8–10 km	4,002	1,157	2,846	113.1
Total for 10-km area	11,338	3,323	8,015	314

Source: U.S. Census Bureau 2000, 2008

The most recent population data (2007) for Hillsborough and Cheshire counties; New Hampshire; and the United States are in Table 37. Hillsborough County had the highest population density compared to the adjacent county of Cheshire and compared to New Hampshire and the United States. Hillsborough County has the highest population of all the counties in New Hampshire and is home to the two largest cities in the state: Manchester and Nashua (the county seat). Manchester and Nashua are on the Route 3 corridor, which connects to Interstate 95 outside Boston. Hancock/Peterborough is 37 miles southwest of Manchester and 30 miles west/northwest of Nashua. Population growth between 2000 and 2007 was very similar among the geographic areas listed in Table 37, ranging from 5 to 7 percent (U.S. Census Bureau 2007).

Table 37. 2007 Population data for jurisdictions surrounding the proposed Hancock NEIDL site

	Population density 2007 (persons per square km)	2000 Population	2007 Population	Population change, 2000–2007
Cheshire County, NH	42	73,825	77,725	5%
Hillsborough County, NH	177	380,841	402,302	6%
New Hampshire	57	1,235,786	1,315,828	6%
United States	33	281,421,906	301,621,159	7%

Source: U.S. Census Bureau 2007, 2008

M.5.2 Environmental Justice

To identify potential environmental justice areas, data were collected on minority and low-income populations for census tracts in the 10-km boundary area of the proposed NEIDL BU SCOE site in Hancock/Peterborough, Massachusetts. Census tracts are subdivisions of a county and represent a level at which disproportionate impacts would be more noticeable. Tables 38 through 42 list the census tracts and minority and low-income data for the 10-km boundary area. Census tract 240 corresponds to the Boston University SCOE site.

Minority populations and poverty rates for each census tract in the 10-km boundary area are listed in Tables 38 through 42. There were no tracts in the 10-km area with a percentage of minority residents higher than the U.S. level of 25 percent or a percentage of residents living in poverty higher than the U.S. poverty rate of 12 percent.

Table 38. Hancock minority and low-income data by census tract inside the 2-km boundary

Census tract	Minority	Below poverty level
230	3%	9%
240	2%	4%
9,704	2%	8%

Source: U.S. Census Bureau 2000

Table 39. Hancock minority and low-income data by census tract in the 2-4 km boundary

Census tract	Minority	Below poverty level
230	3%	9%
240	2%	4%
9,704	2%	8%

Source: U.S. Census Bureau 2000

Table 40. Hancock minority and low-income data by census tract in the 4-6 km boundary

Census tract	Minority	Below poverty level
220	3%	5%
230	3%	9%
240	2%	4%
9704	2%	8%

Source: U.S. Census Bureau 2000

Table 41. Hancock minority and low-income data by Census tract in the 6-8 km boundary

Census tract	Minority	Below poverty level
220	3%	5%
230	3%	9%
240	2%	4%
9704	2%	8%

Source: U.S. Census Bureau 2000

Table 42. Hancock minority and low-income data by census tract in the 8-10 km boundary

Census tract	Minority	Below poverty level
215	2%	5%
220	3%	5%
230	3%	9%
240	2%	4%
250	2%	11%
9,704	2%	8%
9,705	3%	8%

Source: U.S. Census Bureau 2000

Table 43 summarizes the minority and low-income population totals in each 2-km radius boundary within the 10-km area. Of the 21 census tracts identified in the 10-km area, none had a higher percentage of minority residents compared to the United States, and none had a higher percentage of persons living in poverty compared to the United States.

Table 43. Hancock minority and low-income data for the 10-km boundary area

Area/boundary	Total census tracts	Number tracts with percentage of minority persons above US level of 25%	Portion of tracts with percentage of minority persons above US level of 25%	Number tracts with percentage of persons in poverty above US level of 12%	Portion of tracts with percentage of persons in poverty above US level of 12%
Inside 2 km	3	0	0%	0	0%
2–4 km	3	0	0%	0	0%
4–6 km	4	0	0%	0	0%
6–8 km	4	0	0%	0	0%
8–10 km	7	0	0%	0	0%
Total for 10-km area	21	0	0%	0	0%

Source: U.S. Census Bureau 2000

The most recent (2007) minority and low-income data for Cheshire and Hillsborough counties; New Hampshire; and the United States are listed in Table 44. Cheshire and Hillsborough counties and New Hampshire had much lower percentages of minority residents and persons living below poverty compared to the United States.

Table 44. 2007 Minority and low-income data for jurisdictions surrounding the proposed Hancock NEIDL site

	Percent minority	Percent living below poverty
Cheshire County	4%	7.1%
Hillsborough County	8%	6.8%
New Hampshire	5%	7.1%
United States	26%	13.0%

Source: U.S. Census Bureau 2007

M.5.3 State Criteria

Although the rural site of Peterborough is in New Hampshire, environmental justice analysis per the Massachusetts criteria was conducted for the rural site for consistency and comparison with the urban and suburban sites. Data were collected on minority populations, foreign-born populations, households lacking English language proficiency, and median annual household income for each neighborhood (defined as a census block group) within a 10-km (6-mile) area of the Peterborough site. Table 45 summarizes the totals within each 2-km (1.2-mile) radius boundary within the 10-km (6-mile) area. Of the 62 census block groups identified in the 10-km

(6-mile) radius boundary around the rural site, none of the block groups exceeded any of the Massachusetts environmental justice criteria.

Table 45. Massachusetts criteria for environmental justice for the 10-km boundary area of the rural site

Boundary area radius	Total census block groups	Percent of block groups with a minority population above the state guidance level of 25 percent	Percent of block groups with a foreign born population above the state guidance level of 25 percent	Percent of block groups lacking English language proficiency above the state guidance level of 25 percent	Percent of block groups with a median annual household income at or below 65 % of the Massachusetts statewide median income
Inside 2 km (1.2 mile)	10	0%	0%	0%	0%
2–4 km (1.2–2.4 mile)	10	0%	0%	0%	0%
4–6 km (2.4–3.6) mile	11	0%	0%	0%	0%
6–8 km (3.6–4.8 mile)	11	0%	0%	0%	0%
8–10 km (4.8–6.0 mile)	20	0%	0%	0%	0%
Total for the (6-mile) area	62	0%	0%	0%	0%

Source: U.S. Census Bureau 2000

M.6 Results

Environmental justice populations as defined by the federal and Massachusetts criteria were identified at the urban (Boston) and suburban (Tyngsborough) sites. No environmental justice communities meeting the federal or Massachusetts criteria were identified at the rural (Peterborough) site; therefore, no environmental justice impacts would be expected at the rural site. At the Boston site, populations that met or exceeded the criteria are throughout the 10-km (6-mile) study area, with the percentage of neighborhoods meeting one or more of the criteria generally higher toward the center of the study area. At the Tyngsborough site, the environmental justice populations are farther away from the NEIDL location (about 6–10 km [3.6–6 miles]) and are associated with a more urban, higher-density community, which is the city of Lowell (the fourth largest city in Massachusetts), southeast of Tyngsborough.

M.7 References

CEQ (Council on Environmental Quality). 1997. *Environmental Justice Guidance under the National Environmental Policy Act*. Council on Environmental Quality, Executive Office of the President, Washington, DC.

Massachusetts EEA (Executive Office of Environmental Affairs). 2002. *Environmental Justice Policy of the Executive Office of Environmental Affairs.* Commonwealth of Massachusetts, Executive Office of Environmental Affairs, City, MA.

NUREG (Nuclear Regulatory Commission). 2003. *Environmental Review Guidance for Licensing Actions Associated with NMSS Programs* (NUREG-1748). <http://www.nrc.gov/reading-rm/doc-collections/nuregs/staff/sr1748/>. Accessed April 2011.

U.S. Census Bureau. 2000a. Census 2000 Summary Files 1 and 3. <http://factfinder.census.gov/servlet/DatasetMainPageServlet?_program=DEC&_submenuId=&_lang=en&_ts=>. Accessed January 2009.

U.S. Census Bureau. 2000b. Census Tracts and Block Numbering Areas. <http://www.census.gov/geo/www/cen_tract.html>. Accessed July 2011.

U.S. Census Bureau. 2001. *Poverty in the United States: 2000*. <http://www.census.gov/prod/2001pubs/p60-214.pdf>. Accessed April 2011.

U.S. Census Bureau. 2007. *2007 American Community Survey*. <http://factfinder.census.gov/servlet/DatasetMainPageServlet?_program=ACS&_submenuId=&_lang=en&_ts=>. Accessed January 2009.

U.S. Census Bureau. 2008. *State and County QuickFacts*. <http://quickfacts.census.gov/qfd/index.html>. Accessed January 2009.

U.S. Census Bureau, Housing and Household Economic Statistics Division. 2008. Poverty Thresholds 1999. <http://www.census.gov/hhes/www/poverty/threshld/thresh06.html>. Accessed January 2009. U.S. Census Bureau. 2001. *Poverty in the United States: 2000*. <http://www.census.gov/prod/2001pubs/p60-214.pdf>. Accessed April 2011.

U.S. Census Bureau. 2010a. *Historical Poverty Tables—People: Table 1. Weighted Average Poverty Thresholds for Families of Specified Sizes*. <http://www.census.gov/hhes/www/poverty/data/historical/people.html>. Accessed April 2011.

U.S. Census Bureau. 2010b. *USA QuickFacts*. <http://quickfacts.census.gov/qfd/states/00000.html>. Accessed April 2011.