Animal biosafety

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Chapter Outline

Institutional Biosafety Committees (IBCs) are comprised of a chair, community members, biosafety personnel, and individuals with other areas of technical, scientific, and legal expertise. These other experts may include bacteriologists, virologists, veterinarians, or animal model experts, as well as plant researchers. The chair must have a vast understanding of scientific techniques and knowledge. The other members of the committee may also have a wide and varying range of scientific knowledge. Animal models are an essential resource for research related to disease and infection. Animal models can be used in many different ways, including mimicking human infection, replacing the model animal's genes with others – including genes of human origin – to create transgenic animals, or "knocking out" certain genes to determine how loss of function of a given gene affects a particular disease state or the animal's overall health and development.

Many standard animal models sufficiently resemble humans that they can meaningfully predict the effect of infection and disease in humans. Mice are a prime example; mice are surprisingly similar to humans in terms of genetics, physiology, and anatomy – a fact that the average person may not appreciate. Perhaps the greatest factor contributing to the utility of mice as a model of human disease is the fact that 80% of the genes in the mouse genome have a direct counterpart in the human genome [1]. These similarities make it possible to identify and model genetic risk factors in mice that are relevant to human disease. Other animal models can also be useful for more specific research applications. Rats, for instance, are often used for neurological studies as their brains function in a manner similar to humans. The rat and human genomes are also highly similar, and up to 90% of rat genes have direct human counterparts; however, genomic manipulation has proven more difficult in the rat relative to the mouse, and mouse models therefore remain more popular [2].

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Mouse genomes have been more extensively studied to this point, though strides are being made in genetic manipulation of rat genomes, as some symptoms of disease are closer to those of humans in rats than in mice. Non-human primates (NHPs) are the animal models most closely related to humans; the sequences of the chimpanzee and human genomes are 96% identical [3]. However, there are numerous challenges associated with the research use of NHPs, including cost, housing, containment, as well as ethical and public perception issues related to the high intelligence and cognitive function of these species.

In addition to serving as surrogates for humans in experimental research, animal models are useful for studying diseases in natural hosts. These can include diseases that are endemic within a given animal population as well as zoonotic diseases that can be transmitted to human hosts. These include infectious diseases such as foot and mouth disease, Q-fever, herpes B, and brucellosis.

Animals and biosafety

While extremely valuable from a research perspective, the use of animal models also raises specific concerns, many of which relate to biosafety and biosecurity. As one simple but important example, allergies can be of concern for researchers, animal husbandry staff, or anybody else whose job brings them into proximity of the animals. People may be allergic to animal dander, saliva, or urine, or even to the animal bedding. In these instances, personal protective equipment (PPE) such as gowns, sleeves, respirators, or masks may be necessary to prevent allergic reactions to animals in vivariums, satellite animal housing units, or other spaces used for animal procedures. Because allergies represent the most basic level upon which biosafety and biosecurity interventions are built, animal allergy postings, animal biosafety level postings or other relevant information may be provided to inform those entering a certain area that animals are present and provide certain warnings to those that may be affected.

Beyond the risk from biological agents that are shed or excreted after being administered to animals, exposure to metabolites, specifically from drug or chemical treatment, can also be a potential trigger for allergies. While an individual may not have a reaction to a certain agent, drug, or chemical, when excreted in an animal model, these metabolites may cause an adverse reaction. In many cases, these metabolites can be derived from administered toxins of biological origin. Taking this risk into consideration, certain PPEs may be prescribed and standard operating procedures (SOPs) may be modified to address the proper handling and disposal of bedding, as well as how to conduct cage changes. As part of this review process, the IBC may be tasked with determining how the aforementioned issues are to be handled, in conjunction with other chemical or biosafety professionals. In many cases, it may be necessary to develop a cage card system that warns of specific hazards associated with certain agents that were administered to the animal and the risks that may be involved in working with that animal.

It is also important to be aware of the physical challenges of working with animals in a research environment. Animals behave unpredictably, can cause injury with their sharp claws or teeth, and may harbor endemic or administered zoonotic diseases. In the case of large animals, the animals may be physically imposing and able to maim, crush, charge, scratch, bite or throw objects in a research environment. Accordingly, an animal's behavioral disposition or attitude and the degree of personnel experience with that particular species can be an important factor in risk assessments and containment practices as well. It is therefore important to establish effective barriers between workers and animals, and to determine the restraints needed for the animals.

For large animals, one should consider a buddy system in place with experienced large-animal handlers, because of inherently erratic animal behavior. For these reasons, large animal research usually deals with less automation and fewer engineering controls, with heavier reliance on PPE and species-specific knowledge and experience. It is obviously impossible to fit a cow, sheep, or bison into a biosafety cabinet, and in many of these cases – especially in an Animal Biosafety Level-3 Agriculture (ABSL-3Ag) laboratory – the laboratory or research space will serve as the primary containment. With this in mind, special consideration is needed to safely house large research animals, as well as perform research activities and necropsies.

Beyond the fundamental risks related to animal husbandry and manipulation, there are risks associated with the administration of specific agents of biological origin. It is the task of IBCs and its cadre of professions to develop preventative measures and response plans to ensure safety and security by agent and by animal.

Risk assessment, risk groups, and biosafety levels

There are a number of different actions that need to take place before a biological agent is used in an animal model. The initial risk assessment is typically conducted by a biosafety professional, but the entire IBC may provide consultation even at this stage. This risk assessment needs to take into account the type of agent being used, the risk group it belongs to, and different routes of exposure as well as factors such as agent-specific potential for splash or aerosolization. After this initial step, other components of the risk assessment – including review of location, engineering controls, PPE prescriptions and medical surveillance protocols – should be conducted.

Biological agents can be classified into risk groups that start at Risk Group 1 (RG1) and work up to RG4, in increasing order of threat level. RG1 agents are not associated with disease in healthy adult humans. These include a variety of commonly used animal-specific viruses such as adeno-associated viruses. RG2 agents cause diseases in humans that are rarely serious, and for which preventive or therapeutic interventions are often available. Examples include: hepatitis B virus, and many RG2 bacteria easily treated with antibiotics if exposed. RG3 agents are associated with serious or lethal human diseases such as *Mycobacterium tuberculosis* (one of the agents causing tuberculosis), severe acute respiratory syndrome, *Yersinia pestis* (plague), or *Brucella abortus*, for which preventive or therapeutic interventions may be available; this typically means high risk for individual researchers, but lower risk

to the general community. Finally, RG4 agents cause serious or lethal human diseases for which preventive or therapeutic interventions are generally not available, creating high levels of risk for both individuals and the outside community [4]. RG4 agents include Ebola virus, Marburg virus and herpes B virus to name a few.

Once a determination of the risk group has been made, the next step is to establish the biosafety level at which experiments will be conducted. The ABSL correlates with the risk group of the agent in most cases, with the lowest level of protection required at ABSL-1 [5]. It is important to note the process for determining the biosafety level at which a particular agent is to be handled. Biological agents are not classified according to biosafety levels; rather, the biosafety level takes into account the containment requirements for a specific agent and experiment based on the risk group category designated at the risk assessment stage. For example, the potential creation of an aerosol for experimental purposes of a RG2 organism may increase the biosafety level from BSL-2 to BSL-2 enhanced or possibly BSL-3. In the biosafety world (and even in IBCs) individuals may get too comfortable classifying agents based on biosafety levels without truly understanding how a risk assessment is conducted. It is therefore important to ensure that the IBC is trained and knowledgeable in these concepts. Subject matter experts on the IBC may already be well-versed in the practice of risk assessment, but it is worthwhile to also communicate to community members the process by which risk group is determined, and how this eventually informs what animal biosafety level is used. Depending on how a given agent is being manipulated, risk group classifications may either be raised or lowered and this in turn can affect their stratification in terms of biosafety levels.

ABSL-1 work involves agents that are well characterized and do not pose any substantive danger to healthy individuals who work with or around these agents. These agents pose a minimal risk to the environment and human or animal health. ABSL-1 facilities are typically segregated from the general laboratory population, with some type of restricted access. This applies to areas within a vivarium or satellite ABSL-1 facilities as well. While most work with agents in ABSL-1 settings may be conducted on the open bench top and generally does not require engineering controls, there are certain circumstances where the latter may be needed. This is one area in which risk assessment is particularly important. The biosafety professional – possibly with the help of the IBC – must do a risk assessment on the agents being used, how they are handled and administered and further take into account what type of animal is being used to prescribe specific engineering controls and appropriate levels of PPE. Engineering controls in this case may relate to requiring administration of the agent within a fume hood or regulating the number of air changes in the room per hour, as stipulated by the *Guide for the Care and Use of Laboratory Animals* (henceforth referred to as *The Guide*), which was developed and is regularly updated by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) [6]. Although many biosafety professionals consider the Centers for Disease Control and Prevention (CDC)'s *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, currently in its fifth edition, to be our "bible," *The Guide* serves a similar function for those involved in the oversight and design of animal research areas and programs, particularly for animal species not covered by USDA regulations [8].

A wide range of other issues comes into play when conducting work at ABSL-1. Personnel working with animals at this very basic level must receive appropriate training, including identification of the hazards that one may come across when working with the animal species and biological agents being used. A medical surveillance program should be in place even for this basic level of work at ABSL-1, and this becomes all the more necessary when dealing with higher animal biosafety levels. It is critical that areas where animal research is being conducted, starting at ABSL-1, have proper signage on the entry doors to these areas, alerting individuals that they are entering an area where biological research is being conducted with animals. General best practices for microbiological facilities should also be communicated to users of these spaces. There must be areas for disposal of sharps and broken glassware. Laboratory workers should also be trained to avoid recapping needles, as numerous injuries occur during recapping procedures, even when these are done "correctly."

The construction of the animal facility becomes more complex as the animal biosafety level increases. Areas where animals are housed within vivariums should have impervious floors, walls, and ceilings. This is important for animal husbandry, as well as decontamination of an area. These areas should be slip-resistant and impermeable not only to water, but also to chemicals that may be used for either general cleaning or decontamination of surfaces. Sinks and floor drains should be filled with water to prevent sewer gases from entering animal areas, and to prevent the introduction of pests or vermin into these areas [5]. Doors should be self-closing and sealed around their outer edges for similar reasons, and may in some cases have a "sweep" around the base of the door to prevent smaller animals – particularly mice – from exiting the room should they escape their cage while being handled or during animal husbandry. Most animal vivariums should not have external windows to the outside. Windows create security problems as well as the complicating maintenance of appropriate temperature, humidity. and other environmental controls.

Ventilation in animal areas should comply with *The Guide* [6]. Ventilation within these areas must take into account heat and humidity loads, and these systems should not recirculate exhaust air either within the room or to other animal-containing areas. Exhaust air is considered "dirty" and may be contaminated by pathogens or allergens present within the animal areas that could affect both workers and the animals themselves. If it is determined that the potential pathogens present in exhaust air could potentially pose an environmental impact, considerations are made for HEPA filtration. In all animal areas, ranging from ABSL-1 through ABSL-4, it is important to limit the use of horizontal surfaces with regard to both features of the room itself or equipment that is being brought into the area. Such surfaces require much more cleaning, as they tend to accumulate dust and dander, and can act as or facilitate the accumulation of fomites. In this scenario, dust could act as a vehicle for infectious organisms to settle upon; if aerosolized, this fomite could then act as a vehicle for transfer to a new host. PPE can also act as a fomite, which is why gowns, booties, gloves, and other PPE should be removed before leaving a room area, vivarium, or building. It is helpful to conduct a risk assessment and develop SOPs to determine when and where PPE is donned and doffed. It is for this specific reason that laboratory workers are strongly discouraged against bringing their lab coats home, as these

may also facilitate transfer of infectious organisms. An animal vivarium should also have a cage-washing system that is capable of reaching a final rinse temperature of 180 F [6]. This applies to either mechanical systems or manual cage washing with chemical disinfecting agents. It is for this reason that most vivariums, especially at larger institutions or facilities, solely use mechanical cage washers for ease of use and efficacy purposes.

We can then build upon the foundational ABSL-1 practices described above to meet the needs of higher animal biosafety levels, where pathogens or infectious substances are in use that are more harmful to the environment, animals, or workers. ABSL-2 is suitable for work involving laboratory animals infected with agents that are associated with human disease and pose moderate hazards both to laboratory personnel and the environment [5]. It also addresses hazards from ingestion as well as percutaneous and mucous membrane exposure. Certain training requirements must be met, and this may involve institution-specific training. At a minimum, training for research personnel should include facility procedures, handling of infected animals and training on the actual administration or manipulation of the pathogenic agents being used in animal studies. Personnel knowledgeable of the potential hazards associated with these agents, as well as the relevant animal manipulation and husbandry procedures, should supervise individuals working at ABSL-2. One of the critical differences between ABSL-1 and ABSL-2 is in the introduction of engineering controls for primary containment at ABSL-2, usually in the form of a biosafety cabinet. These could include Class II A2-type biosafety cabinets that mostly exhaust to the room or Class II B1 or B2 biosafety cabinets that exhaust air to the outside at varying percentages. A biosafety cabinet not only provides a sterile working environment when used correctly, but also offers a primary protective barrier for personnel. When a risk assessment determines that an element of containment is needed, elevation of the agent's risk group and an increase of the biosafety level from ABSL-1 to ABSL-2 are indicated through assessing the risk to humans, animal, environment, or other factors specific to the work being conducted. Other engineering controls may also be administered, but the biosafety cabinet is the most common form of primary containment at ABSL-2. The biosafety cabinet also comes into play when manipulating infectious materials or conducting procedures that have the potential to produce aerosols. Biosafety cabinets may also be used for necropsy, harvesting of tissues, or even animal cage changes if required [5]. If a procedure cannot be conducted in a biosafety cabinet, a combination of PPE and other containment strategies must be used, such as using the actual room as primary containment and possibly considering respiratory protection.

The biosafety professional – in conjunction with the IBC, Institutional Animal Care & Use Committee (IACUC), and institutional veterinarian – can play an important part in researching the shedding rates of biological agents in order to determine when cages or bedding may be considered to contain infectious materials, how bedding or cages should be decontaminated, and when cages or bedding can be deemed to no longer pose an infectious threat. Based on these determinations, it may be appropriate after a certain time period to reduce the animal biosafety level for housing purposes – for example, going from ABSL-2 to ABSL-1. This determination must be

made via risk assessment and must also go through the IBC with notification of the IACUC and comparative medicine department.

There is no uniform prescription for ABSL-2 engineering controls or PPE (as is true of any biosafety level), and the purpose of these interventions is to minimize exposure risk. Personnel that conduct work at ABSL-2 should be enrolled in a medical surveillance program [5] that takes into consideration the agents being used, manipulations taking place, and/or animal species involved. Allergies are still an important consideration, even with widespread use of PPE at this level. Animal workers should be advised to report any sort of immune conditions or general personal health conditions that may make them more susceptible to infection. When possible, one should consider specific practices or restraint devices that reduce the risk of exposure for the worker [5].

As with any animal work, animal protocols must be reviewed and approved by the institution's IACUC, as well as the IBC when biological agents are to be used. As discussed previously, it is vital for the IACUC and IBC to work closely and maintain communication with each other. The biosafety professional often serves as a liaison between the two committees, occasionally by also serving as a member of the IACUC. Site-specific manuals must be developed and implemented for animal facilities. The IACUC and IBC should ensure that workers are being trained and made aware of the potential hazards involved with their specific duties. This training needs to be conducted yearly as well as whenever changes are made to policies, procedures, or agents, and must also be documented.

A small but significant difference in signage is required when moving from ABSL-1 to ABSL-2. When working with multiple agents at ABSL-1, it is only "recommended" to post these agents on the door. However, it is *mandatory* to post the names of the agents being used at ABSL-2 [5]. There may be certain agents posted on the signage that restrict an individual from entering due to personal health status or inadequate experience working with the agents, regardless of whether active work is underway. This signage should also include other basic information that is required at every animal biosafety level, such as contact information, PPE entry requirements, and entry and exit procedures.

PPE at ABSL-2 should be specific for the facility. Lab coats, gowns, scrub suits, or uniforms should be worn while working in the vicinity of infectious agents. They should be removed when contaminated, and never leave the premises to prevent unwitting transfer of known or unknown infectious agents. In many cases, regardless of biosafety level, animal facilities have certain practices in place for the donning and doffing of PPE – not only from facility to facility, but even from room to room or hallway to hallway. These facility-specific SOPs may reduce the risk of environmental contamination that could affect other animal populations within these facilities. Gloves are always to be used within animal facilities, but should never be worn or used outside of these areas. They should also never be reused or washed, as this could compromise the integrity of the glove. It is important to provide training on the proper way to remove gloves in order to prevent contamination. Gloves should be disposed of with other potential or known infectious waste within the facility. Hand-washing

facilities should be available within immediate proximity of the waste disposal areas where gloves are to be removed [5].

Appropriate waste handling is an important aspect of working at ABSL-2, an oft-neglected aspect of containment work. ABSL-2 requires that a form of decontamination be available for any waste generated from the agent being used, including bedding from caging housing an infected animal. Different forms of decontamination may be used, such as autoclaving or chemical decontamination methods. Careful consideration must be given regarding decontamination of equipment used in experimentation as well as husbandry [5]. Equipment that has the potential to become contaminated must be amenable to decontamination in the event that routine maintenance, repairs or removal from a particular area are required. In the event of spills generated during activities involving infectious agents at ABSL-2, staff or personnel must be appropriately trained to contain and decontaminate the affected areas based on the specific policies or SOPs developed for that facility.

Ventilation requirements in ABSL-2 facilities are more stringent than in ABSL-1 sites. Ventilation in ABSL-2 areas should be calibrated to maintain negative pressure relative to areas with lower biosafety levels. This pertains to animal isolation cubicle areas relative to the outer room, as well as housing or procedure rooms relative to hallways. General exhaust air should always be ducted out of the facility and not recirculated anywhere within the facility [6]. There may be a level of filtration at this level before exhausting takes place, most usually HEPA filtration. Class II Type 2 biosafety cabinets that mostly recirculate HEPA-filtered air back into the room are an exception to this requirement. Biosafety cabinets may also be thimble-connected or hard-ducted through the lab or room exhaust. Biosafety cabinets should be certified at least annually or whenever moved from one location to another [7]. Biosafety cabinets should be positioned in a manner that minimizes disruptions in airflow in the room as well as within the biosafety cabinet itself (e.g., near air vents, doors or high-traffic areas). Finally, ventilation within an animal facility should take into account the humidity and temperature requirements for proper animal husbandry, as established by *The Guide* and other resources.

Unsurprisingly, ABSL-3 procedures further build upon those employed at ABSL-2 since agents used in an ABSL-3 environment can cause serious or potentially lethal disease [5]. In particular, careful consideration must be given to the potential of agent transmission via the aerosol route. Accordingly, ABSL-3 labs require more sophisticated airflow systems, procedures, and PPE. Respirators of some type may be used, depending on the risk assessment. These respirators are typically powered air-purifying respirators or N-95 respirators, which require the user to be enrolled in the institution's respiratory protection program. ABSL-3 workers must also be enrolled in a medical surveillance program within their institution. Beyond the access-restriction implemented in ABSL-2, ABSL-3 typically implements multiple safety and security barriers.

All agent manipulation at ABSL-3 must be done within a biosafety cabinet. Airflow and HVAC systems become very important when making the leap to ABSL-3. ABSL-3 laboratories are required to have an inward directional airflow that acts in a cascading manner: from an outer area into an anteroom, and finally into the lab.

The anteroom is a small room between the actual lab or ABSL-3 area and an outside hallway or common area. In ABSL-3 settings, anterooms are always considered clean areas, and are usually where those entering ABSL-3 laboratories don their PPE.

Animal racks and caging in ABSL-3 areas are usually closed systems in which both supply and exhaust air pass through HEPA filtration. Though there may be positive pressure plenums within these caging systems, measures are put into place to prevent reversal of airflow in these systems, which could release potentially infectious materials into the room. This may be accomplished through an interlock system within the blowers or fans within these animal rack systems. These animal-caging systems should be certified at least annually. Caging ventilation and the redundancies involved in the HVAC system should be tested at least annually to ensure proper function of these systems and prevent reversal of airflow in the event of a mechanical failure.

Laboratory-specific SOPs should be developed for ABSL-3 facilities, including information on the agent, hazard communication, lab-specific procedures, waste disposal procedures, inactivation procedures, a detailed medical surveillance program, sharps use or management, and incident response procedures that at an absolute minimum address spill cleanup protocols. ABSL-3 facilities must have impervious floors, ceilings, and walls. The floors should be monolithic in nature. Impermeable surfaces allow these areas to be sealed and decontaminated if needed. These areas should have no penetrations, but are not required to be gas- and bubble-tight, as would be required in an aBSL-3Ag facility. In an aBSL-3Ag facility this is required due to containment of agents of high consequence to agricultural stocks that could affect an economy. Decontamination within these areas can range from surface decontamination to gaseous decontamination, and the sealing of these surfaces is especially vital in this latter scenario.

Work with large animal species that cannot be housed in traditional containment equipment, such as biosafety cabinets, is conducted at what are known as BSL-3-Ag facilities. In this environment, other modifications are made to aid in primary containment. It is important to carefully control the flow in and out of these labs, in terms of both personnel and airflow, especially when working with agents that may be harmful if released into the environment. BSL-3 Ag facilities implement some of the practices used at ABSL-4 (discussed below), although workers do not use positive-pressure hoses or suits, as they become problematic for workers in proximity to large animals. The key concept is this: in BSL-3 Ag areas, the *room itself* acts as the primary containment, with HEPA filtration integrated into the supply air and exhaust ductwork. Gas-tight isolation dampers can be used in place of HEPA filters for the supply air, but as these dampers may not be reliable, HEPA filtration should also be implemented either in conjunction with supply-air isolation dampers or in parallel, within the supply air as a redundancy measure in case the dampers fail. If a reversal of airflow occurs within the supply air plenums due to the failure of exhausts fans, or failure of both supply and exhaust fans, HEPA filtration ensures that this air will not contain any potential contamination from within the ductwork or elsewhere. Directional airflow, air-pressure-resistant doors with gaskets, air locks, effluent decontamination systems, animal renderers, restraint devices, systems that enable pressure decay testing, and the capability to seal any and all access points in the room are just some of

the engineering controls that must be implemented at this level. Pressure decay testing is the administering of pressure to a room or ductwork, and observing the pressure differential to determine the presence of leaks. These areas must provide absolute containment for the protection of both the environment and the public [5]. For all of these reasons, BSL-3Ag facilities are very expensive to design, build, and maintain.

ABSL-4 facilities involve agents that are dangerous and exotic, with no treatment available and high mortality rates for exposed individuals. Differences between ABSL-3 and ABSL-4 include the use of sealed, Class III biosafety cabinets and positive-pressure suits. A complete change of clothes is required upon entry and exit, and personnel are required to shower out upon leaving the lab, which may also be required of ABSL-3+ or ABSL-3Ag labs. Discrete areas within the lab, including the anteroom, should have interlocks.

The risk assessment to determine animal biosafety levels is normally conducted by the biosafety professional in conjunction with the IBC, but it is important to involve the IBC as well. This is useful when there are animal experts or animal model experts on the committee that may have more experience or expertise on a certain subject. The IBC should be responsible for reviewing protocols to make recommendations on containment and animal biosafety levels, and to review work that may pose an unnecessary risk to the personnel involved. IBCs should be composed of individuals with specific expertise, and these experts can provide pertinent information related to experimental protocols that could help determine final recommendations for animal biosafety levels or containment practices.

Transgenic animal models

Transgenic animals that carry a foreign gene – often of human origin – artificially inserted within their genome, have been an integral part of scientific research for decades. Since experimenting on humans is unethical, transgenic animal models can serve as surrogates for the study of human diseases with a genetic component or for infectious diseases for which susceptibility can be modified by certain gene or allele combinations. For instance, insertion of human genes coding for viral receptors may permit infection of the animal model with a pathogen that is otherwise specific to human hosts. Most transgenic animal studies are conducted with mouse models, although smaller numbers of studies are performed with rats and fish. Mice are relatively inexpensive and can be raised in large numbers, their genomes can be easily manipulated, and exhibit many physiological similarities to humans.

It is important for the IBC to note whether transgenic animals are being purchased or are being created at its home institution. The majority of transgenic animals being purchased or transferred fall under the NIH III-F exempt category. This is a category developed by the NIH, which states that any research that falls under the III-F designation is exempt from the NIH guidelines for research involving recombinant or synthetic nucleic acid molecules. Once again there is no singular approach to assigning NIH categories to purchases and transfers; determination is made in conjunction

with the biosafety professional and the IBC and should be documented within the IBC meeting minutes.

In the majority of cases, transgenic animals are created at an institution rather than purchased or transferred. Studies involving these animals do *not* fall under the NIH III-F exempt category, but rather into other, non-exempt categories. If the determination can be made that the research, or the purchase or transfer of transgenic animals may be housed at ABSL-1, it is exempt from the NIH guidelines for research involving recombinant or synthetic nucleic acid molecules. The creation of transgenic animals involves use of recombinant or synthetic nucleic acid molecules, which the NIH respectively defines as "molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell" and "nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but, can base pair with naturally occurring nucleic acid molecules" [4]. The creation of a transgenic animal typically involves recombinant viral vectors that deliver the transgene that in turn becomes integrated into the genome of the recipient animal. This type of *in vivo* work may not always result in the creation of a new transgenic animal line, but can instead result in a foreign protein being expressed from the transgene. As with studies involving pathogens, the IBC is required to review the administration of recombinant DNA technologies studies. The IBC must also classify them and determine biosafety levels and containment practices, as well as possible dual use research of concern (DURC), gain of function studies, and major actions. Major actions are experiments or manipulations that involve the deliberate transfer of antibiotic resistance to an organism. The new DURC policy has established guidelines for research that falls under categories deemed dual use research and dual use research of concern that will take effect in September 2015. DURC committees can also be either separate from the IBC or a sub-committee thereof, with other subject matter experts potentially being involved.

Generally, the *in vivo* use of recombinant or synthetic nucleic acid molecules falls into one of two NIH categories for recombinant or synthetic nucleic acid molecules; III-E or III-D. NIH III-E classification is for studies that require the notification of the IBC simultaneously with study initiation. Briefly, this includes experiments that do not involve more than two-thirds of the genome of any eukaryotic virus, use a RG1 agent, and are conducted at ABSL-1 [4]. A typical example of this type of work would be using recombinant adeno-associated viruses to express certain genes or proteins within an animal. In contrast, studies classified as NIH III-D require IBC approval before the work may be initiated. This category of studies uses higher-risk agents for experiments involving genomic integration, either *in vitro* or *in vivo*. There are many subcategories of the III-D designation, and the specifics depend on the details of a particular experiment. These subcategories range from III-D-1-a for experiments that involve the introduction of synthetic nucleic acids into RG2 organisms usually conducted at BSL-2 containment, to III-D-7-d which covers antiviral susceptibility and containment. The NIH does a very good job of defining what types of experiments fall into these categories, but it is ultimately up to the institutional IBC to make the determination, with consultation from subject matter experts on the committee and an understanding of the NIH guidelines for research with recombinant or synthetic nucleic acid molecules.

Most *in vivo* experiments will fall under these three categories of NIH III-D, -E or -F, although elements of *in vivo* work may fall under the NIH III-B classification. This designation requires NIH Office of Biological Activities (OBA) and IBC approval before initiation, and encompasses experiments involving cloning of toxin molecules with an LD50 less than 100 ng/kg body weight [4]. NIH category III-A designates experiments that require review by the institutional IBC and NIH Recombinant DNA Advisory Committee (RAC), as well as approval from the NIH Director. These experiments constitute "Major Actions" (as detailed above), entailing transfer of a drug resistance trait to microorganisms that are not known to possess this resistance trait naturally [4]. Clearly, it is important that IBC members know the definitions of and differences between these categories. Part of the role of a biosafety professional is to identify the different types of experiments that may fall into these categories, and to reach out to the IBC to make sure they understand why a particular experiment is being reviewed if it potentially falls into one of these higher-level categories. Institutions that receive NIH funding are required to have an IBC with subject matter experts within the institution as well as community members. It is important to have an active, involved IBC that understands how and why they are assigning these NIH category designations to the work under review.

Regulatory reporting

Institutions that receive NIH funding, have an IBC and conduct research with recombinant or synthetic nucleic acids also have additional important reporting obligations subsequent to an untoward event. Releases of recombinant or synthetic nucleic acid material outside of primary containment, escape from primary containment of an animal that has been administered recombinant or synthetic nucleic acid molecules, or exposure of an individual involving recombinant or synthetic nucleic acid material are all reportable to the NIH. In these cases, the biosafety professional must send a report to the NIH/OBA office upon becoming aware of the incident. They should also meet with the personnel involved in the incident to determine the sequence of events that led to this release, escape, or exposure. Critical details in this process include identifying the particular breach of primary containment, PPE compromise (especially respiratory protection), deviations from SOPs, the extent of decontamination of the area where the incident took place, and any corrective actions that were taken. When dealing with incidents related to transgenic animals or animals infected with recombinant or synthetic nucleic acid molecules, it is important to determine whether PPE was compromised and/or if an animal was able to either bite or scratch the individual. In the latter event, it will be necessary for that individual to receive medical care or treatment at a facility approved by the occupational health program. The personnel involved should let the clinic know the sequence of events, describe any breaches in PPE (including respiratory protection), and be forthright in sharing all relevant information so that the clinic may make a determination of the proper medical treatment, if needed. If the incident involves a breach of primary containment in a

Select Agent laboratory, correspondence with the CDC and a Form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) must also be submitted in conjunction with the NIH reporting. The reporting narrative should be communicated to both agencies in a timely manner. These agencies will follow up with inquiries regarding breaches in PPE, HVAC function during the incident, recapture of the animal, decontamination of the space, and the current and continued health status of the individual who was exposed or potentially exposed. Incidents that are reported to the NIH are public information and should be communicated to the IBC and documented on the IBC agenda and meeting minutes. The IBC plays an important part in determining potential corrective actions. The IBC can be a useful tool for biosafety professionals, as the different areas of expertise and years of experience of committee members may play a vital role in implementing prevention practices. It is important to have a robust reporting process at institutions that conduct work with synthetic nucleic acids, and open lines of communication and well-established relationships between researchers and biosafety professionals are vital to an effective reporting program.

Select agents and toxins

The CDC defines Select Agents and Toxins as biological agents and toxins that could pose a severe threat to public health and safety [5]. A large majority of Select Agent work is conducted *in vivo* in ABSL-3 or ABSL-4 facilities. Though not part of the original mandate, IBCs are often tasked with Select Agent compliance at research institutions. It is important that specific SOPs be developed for this research, and that all CDC rules and regulations are followed. Many of the practices described above for these animal biosafety levels apply to this type of research. Select Agent research requires sophisticated HVAC systems, restricted access, higher levels of PPE and respiratory protection, medical surveillance, training of personnel in biosafety, biosecurity, and incident response, and having written plans in place. It is also important to maintain animal disposition logs that show when each animal has been infected and when it has been inactivated. Entry into these spaces by approved individuals must be tracked in the form of logs and access records, all of which need to be audited internally. It is important that SOPs for Select Agent labs take into consideration animal biosafety concerns with regard to personnel. These may involve specific PPE requirements, such as puncture-proof gloves if working with sharps or if there are concerns about aggressive animals. On the other hand, over-prescribing PPE may lead to a loss in dexterity or agility, which may result in more harm than benefit. Animal handling, especially with smaller animals, can be a very delicate process where reduced dexterity can compromise the safety of both the animal and the personnel. It is important to take all of these factors into consideration when establishing PPE requirements, and to have a thorough discussion with the lab working with the Select Agent. Many Select Agent experiments may allow use of anesthesia to minimize risks of injury or escape, but not all. For example, some studies conducted with Select Agents may focus on the animal's respiratory or neurological function, such that use

of anesthesia may affect the study adversely. In these instances, specific procedures should be developed for animal handling to minimize escape or breach from primary containment in collaboration with the lab, biosafety professionals, and the IBC. The IBC reviews the SOPs for these labs, reviews and approves inactivation protocols, is in charge of Select Agents, and is usually involved with the reporting process if there is a theft, loss, or release of a Select Agent at the institution. The IBC also must be apprised of new labs or Select Agents being implemented at the institution.

Conclusions

IBCs play a vital role in the realm of animal biosafety. The committee is a repository of knowledge, skills, and expertise in a variety of fields that biosafety professionals and institutions can rely on to conduct research with animals that accommodates the animal's well-being, as well as the safety of the personnel involved in the research. Through the risk assessment process, institutions can determine biosafety levels, prescribe PPE, and select appropriate engineering controls to ensure that a successful animal research program can be implemented and executed. It is important that the IBC plays an active role in overseeing all animal work done at an institution, and the implementation of this committee's decisions in work practices will in turn lead to a safe and successful research program.

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