



## **G7+ - Global Health Security Initiative (GHSI)**

### **Workshop**

**”Best Practices in Vaccine Production for  
Smallpox and other Potential Pathogens“**

**5-6 September 2002**

**Paul-Ehrlich-Institut, Langen (Germany)**

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**Welcome Address, German Ministry of Health and Social Security**

Distinguished guests,

The events of 9/11 were an act of terrorism on an unprecedented scale. We have to accept that terrorist-related activities will continue and that no country is completely safe when it comes to being a potential target.

We also have to accept that we can become victims of a terrorist attack anywhere and at any time. Although the primary intention of most such attacks is to disrupt political and economic systems rather than to cause harm to individuals, terrorists' aims can be achieved in most cases only by causing severe harm to human beings.

Terrorist attacks have a contained geographical impact when conventional weapons are used. They have an international impact when weapons of mass destruction are deployed, and they are a global menace when specific biological agents are used.

Weaponised human pathogens have been produced in large quantities by many countries around the world in recent decades. This material is obviously readily available and, as the "anthrax letters" demonstrated, will be released intentionally if they get into the wrong hands.

Fortunately, although bacillus anthracis is a dangerous human pathogen, it seems to be inefficient as a wide-ranging tool of bio-terrorism. However, the need for advanced production technology and the availability of efficient therapeutic agents, such as antibiotics, might prompt bio-terrorists to go one step further and release more strongly pathogenic agents such as variola virus. Variola virus would be easy to handle, particularly given the possibility of terrorists protecting themselves by means of vaccination. It is highly contagious and lethal for more than 40% of infected individuals. In addition, there is no efficient therapy for smallpox. We have to anticipate that weaponised variola virus might come into the possession of countries known to host terrorist organizations.

Although there is currently no hard evidence that terrorist organizations are planning to use variola virus, even the hypothetical possibility means that we must make every effort to be prepared for this. Smallpox, the terrible disease caused by variola virus, was declared to be eradicated in 1977. Since then, vaccination programs, vaccine production, preparedness plans and other materials relating to smallpox have lost their earlier importance and, until recently, were no longer available.

More than 20 years have passed since the eradication of smallpox, and we are now confronted with a situation in which we need to revive and up-date our expertise. Such efforts are time-consuming and cost-intensive. Vaccine production needs to be resumed, preparedness plans need to be revised, new vaccination plans are required for the peacetime situation and for the event of an emergency.

We are all worried about this situation and not always sure whether we are managing things properly. Many countries and regions are currently facing the same problems and the question is whether good and bad experience has to be gained by each country for itself or whether pooling knowledge would be the more appropriate and successful strategy for coping with the task in hand.

The G7+ Global Health Security Action Group Workshop on "Best Practices in Vaccine Production for Smallpox and Other Potential Pathogens" which is being hosted by the Paul Ehrlich Institute and co-organized by the German Ministry for Health and Social Security, the European Commission and the World Health Organization is designed to provide a platform for sharing scientific expertise and for coming up with strategies and points to consider for countering the bio-terrorist threat more effectively. Many meetings dealing with related issues have been organized recently at the national level and at the level of the European Union, the Pan American Health Organization and the World Health Organization. These meetings have provided an enormous amount of valuable scientific and other information.

One of the intentions of our Workshop is to distil the results of these previous meetings into information that is generally available to all authorized parties involved in the production and handling of new smallpox vaccines and other vaccines against human pathogens capable of being used by bio-terrorists. This information concerns the optimal use of prophylactic and therapeutic pharmaceuticals and the best possible preparation for managing a potential emergency that will hopefully never become a reality.

Although many efforts to combat bio-terrorism have already been made, it is evident that we still have to step up our activities. We need to reinvestigate the efficacy of old smallpox vaccines and we need clear and reliable data on the effectiveness of second generation vaccines as we are about to develop strategies for the vaccination of million of people all over the world. Therefore, it is of crucial importance to find out as exactly as possible what is the minimum titer of vaccinia virus vaccine when given by a bifurcated needle to induce an adequate immune response to vaccination in addition to a maximum reduction of adverse effects. In summary, we need research on vaccines either to improve the safetiness of existent vaccines or to develop new and better vaccines so that they may be used routinely

without personal risk to the vaccinee no matter whether someone has been vaccinated before or not. We also have to keep in mind that certain numbers of our world population is known to suffer from immunodeficiency owing to a variety of reasons. Therefore, we also need more effective therapeutic agents against viral diseases and last not least we also need better diagnostic tools.

It is also evident that issues relating to the eradication of other viral pathogens, such as measles or polio viruses, need to be reconsidered. Waning immunity to these pathogens globally always harbors the risk of severe harm if these pathogens are intentionally reintroduced into the human population.

Many questions centering on these topics are still unanswered. These represent a new challenge to all of us, scientists and politicians, to do our utmost to find appropriate solutions, preferably by pursuing a co-ordinated and harmonized approach.

I am convinced that this workshop with all the assembled expertise will help to improve means and ways for the scientific as well as political community to cope with bio-terrorist threats.

Therefore, have a good time here at the workshop

Dr. Stefan Winter

Director General for Preventive Health Care  
and Disease Control

### **I Summary of Conclusions by the Paul-Ehrlich-Institut**

A Workshop entitled "Best Practices in Vaccine Production for Smallpox and other Potential Pathogens" initiated by the G7 + Global Health Security Action Group took place at the Paul-Ehrlich-Institut on September 5th and 6th, 2002. Key statements are summarised below:

- Various pathogens can be used as bioterrorist weapons. Many factors are important in judging the risk represented by these agents: the effects on public health, the perception of a hazard for the public, the necessary preparatory measures, the possibility to produce a pathogen in large quantities, and the risk of the pathogen to spread. For risk assessment purposes, the pathogens have been subdivided by the CDC into three categories: A, B and C. Preparing for a bioterrorist attack with Category A pathogens, e.g. smallpox, anthrax, plague, tularaemia, botulinum toxin and filoviruses / arenaviruses has top priority.

- The concrete potential threat arises from the “vulnerability“ of the potential victim, the capacity to use a pathogen and the intention to do so. Recent experience has shown that all three factors are realistic. We can therefore assume that there is a real but barely quantifiable threat.
- The pharmaceutical industry is willing and also skilled enough to contribute to the preparation for such an attack through vaccine development and manufacture. However, the necessary economic conditions must be generated to enable industry to make the necessary decisions and to plan and initiate concrete steps for their implementation. Companies believe that it is high time to act considering the time it takes to implement the measures. The “Note for guidance on the development of vaccines against smallpox“ recently developed by the CPMP creates a regulatory framework which is considered as well suited and helpful by the pharmaceutical industry. Questions on licensing procedures and liability during the use of the vaccine still require clarification.
- In order to counteract bioterrorist threats in future, we will also need the development of new vaccine concepts, last but not least to improve tolerability, and, in doing so, the general usability of vaccines such as smallpox and anthrax vaccines. Such research work should be initiated and supported by appropriate research support programmes. The EU programme for public health planned for the recent future and the 6th framework programme could be used for this purpose to some extent.
- The industrialised countries themselves are in danger of being attacked by smallpox agents in other countries. A strategy for counteracting this global threat and supporting the affected countries in their efforts to curb a smallpox outbreak should be understood as part of our own measures to prepare for an attack.
- The last case of smallpox occurred in Birmingham, UK in 1978. It was caused by a laboratory accident, at first, a false diagnose was made by three physicians. This highlights the difficulty in diagnosing smallpox early and safely. It also represents an example of the importance of a comprehensive and efficient training of physicians in the preparatory measures.
- Both Lister and New York City Board of Health (NYCBH) vaccines were used in the eradication programme of the WHO. There is no conclusive evidence as to a difference in tolerability and efficacy. The vaccines differed in the route of administration (scarification, jet injector, bifurcated needle) Each vaccinee received a dose of 10 or 2.5 µl of vaccine with a titre of at least 10<sup>8</sup> plaque forming units (pfu)/ml.



- From the epidemiological point of view, the natural human to human spread of smallpox was comparatively slow in populations that were at least partly immunised before and during the eradication campaign. The explanation is as follows. The disease is transmitted primarily by infection through droplets. Infected individuals show a significant increase in temperature at the beginning of their period of infectivity. This period is very short (less than one day), and at least in the beginning of this phase patients are not restricted in their mobility. Furthermore they don't show any pustules on hands or face. But feeling seriously ill patients are soon bed-ridden and contact to other persons is limited. However, epidemiological behaviour of smallpox might be different in case of a deliberate release of the virus in an unvaccinated population.
- When cases of smallpox occur, the identification of the contact persons of individuals suffering from smallpox after the beginning of their phase of fever, the vaccination of the contact persons and the monitoring of their body temperature are suitable means of curbing a smallpox outbreak. Recent mathematical models (Kaplan et al. 2002) are based on a number of unrealistic/false premises. The conclusion that mass vaccinations are the preferred treatment of choice compared with ring vaccinations cannot be maintained. An example of sufficiently careful and accurate modelling of a ring vaccination has not yet been published.
- Compulsory vaccinations of contact persons have proved to be a less effective means of eradicating smallpox, since the avoidance of such forced measures led to further spreading of possibly infected individuals rather than curbing the disease.
- A note for guidance was developed in the EU for the development of 2nd generation vaccines. In agreement with US regulations, the "take rate" together with the neutralising antibody titre are the decisive criteria for judging the efficacy of these vaccines. The WHO recommendations have also been revised recently.
- 3rd generation vaccines on the basis of highly attenuated strains, e.g. MVA can be manufactured under GMP conditions and are expected to be clearly more tolerable. However, the lack of evidence for their protective effect against a smallpox infection represents the crucial hurdle for their use. For these smallpox vaccines, the two above criteria do not suffice for judging their efficacy. Although clinical safety and immunogenicity data can be collected, clinical data on the efficacy in humans cannot be provided. The question regarding the efficacy of these vaccines can at present only be answered in connection with results from preclinical animal models, which will probably

mean a fair amount of uncertainty regarding the protective efficacy against a challenge with variola virus. The same applies to other potential vaccines, e.g. replication deficient vaccinia virus.

- The ability of the control authorities to test the “potency” of the vaccines experimentally probably varies to a great extent. However, it should be restored as soon as possible and verified by the appropriate collaborative studies.
- Most member states (MS) of the EU currently possess stockpiles of vaccines prepared on animal skin. Some MS are currently stocking up their cell culture vaccine supplies to an amount sufficient for immunising the entire population if required. The intention to establish joint vaccine stockpiles at an EU level has been met with reservations on the part of the MS.
- In addition to the existing stockpiles of vaccines prepared on animal skin, the USA are creating supplies for the entire population. Contracts exist for the provision of NYCBH based cell culture vaccines. Clinical trials are also planned for MVA vaccines. If the outcome of these trials is favourable, first purchases of such vaccines for the USA might be possible by 2004.
- Japan has stockpiles of vaccines prepared on animal skins and will produce cell culture vaccines in 2002 and 2003 based on the lister derived strain LC16m8 which has revealed a take rate similar to that of lister vaccines and did not show any neurovirulent properties in various animal models.
- The intention of the WHO to compile an overview of current international vaccine supplies has proved to be difficult in view of the insufficient response, especially from the MS of the EU. The WHO is examining incoming notifications of suspected cases of smallpox, which, however, could never be confirmed.
- The American vaccine Dryvax, which is produced on animal skin, showed a satisfactory take rate in primary vaccinees when administered with a bifurcated needle, both undiluted and at a dilution of 1:5 or 1:10. For the vaccine, a starting titre of  $10^{8.1}$ /ml was calculated. Per vaccinee, depending on the dilution, doses of  $10^{5.0}$ ,  $10^{4.3}$  and  $10^{4.0}$  were administered. Absolute values must be interpreted with care, since no details on titres are available for a reference material, and titrations were carried out in a cell culture rather than the chorion allantois membrane (CAM) of embryonated hen's eggs. For this reason, the real titre is easily underestimated. It is also possible that the take rate was influenced

by the fact that the vaccination site was covered with gauze and a semi-permeable film. Another noteworthy observation is that a higher administered dose led to a stronger local reaction but a reduced incidence of "satellite lesions".

- Cidofovir is approved for the treatment of Cytomegalo virus-based retinitis in AIDS patients. Cidofovir is effective in vitro against a number of DNA viruses including poxviruses. In the mouse model, Cidofovir proved to be effective against vaccinia and cowpox virus burden if administered at the time of infection. Data on humans are currently available only in isolated cases in which Cidofovir was used successfully for the treatment of infections with Molluscum contagiosum and Orf (two other types of smallpox virus). When administering Cidofovir, major possible adverse events can be observed. Effectiveness of Cidofovir in the treatment of adverse effects of vaccinations (eczema vaccinatum, vaccinia generalisata) is unproven.

## **II Summaries of Presentations**

### **Session I: General Overview**

Chairpersons: S. Winter, German Ministry of Health, D.A. Henderson, U.S. DHHS, Office of Public Health Preparedness, Washington DC, USA

### **Assessment of Microbial Agents as Tools for Bioterrorists**

Lisa D. Rotz, CDC, Atlanta, USA

Assessment parameters for the identification of higher risk agents for public health were developed by the CDC. The evaluation process is based on general considerations such as the potential to threaten a large population, the extent of the damage done to the health of the people, and the morbidity and mortality caused by the agents. Further points include the availability and the complexity of handling of a specific organism (natural reservoir, biosafety level), the stability of the agent in the environment and the route of infection. Three categories of risk agents were assigned. Category A agents have a high public health impact and public perception, a moderate to high dissemination potential and require comprehensive public health preparedness. Category B agents have a less comprehensive public health impact, low to moderate dissemination potential and require less comprehensive public health preparedness. Category C agents have an unknown or unclear impact and are related to emerging infectious diseases, where only limited information is available.

Using this evaluation process, six agents and/or groups of agents with the highest priority for preparedness were identified: Variola virus, Bacillus anthracis, Yersinia pestis, Francisella tularensis, Clostridium botulinum toxins, viral haemorrhagic fever (Filoviruses and Arenaviruses). These priority agents are characterised by their potential to infect via aerosols and the high morbidity and mortality rates following infection. The susceptibility of the civilian population has also been identified, and furthermore a person-to-person transmission. These priority agents are difficult to diagnose and/or treat and some of them were previously developed for biowarfare.

### **How Real is the Biological Threat?**

Richard F. Pilch, Monterey Institute for International Studies, Monterey, USA

A potential bioterrorist threat is defined by three factors, the vulnerability of a population, the capability of an adversary to attain, develop and deploy a pathogenic agent, and the intention to do so. Recent attempts as in the case of the anthrax attack in the US have shown that all three factors are realistic. Agents of the most concern, i.e., those agents considered to have

a low probability but high impact, are available on international markets. They can be acquired by strain collections or through free international scientific exchange. Furthermore, it cannot be excluded that pathogenic agents can be received from poorly paid scientists at biowarfare facilities of the former Soviet Union, as well as from other sources such as hospital laboratories or the environment. The risk which arises from international exchange students or scientists can only be judged on a personal basis. The know-how on the production of biological agents is widely available on the internet, and vaccines against pathogenic agents can conceivably be converted into biological weapons because the equipment, methodologies, and pathogens employed in vaccine production are essentially dual-use. So far, there is no evidence for transfer of BW agents from state sponsors to terrorist groups. Past experiences such as those involving the group Aum Shinrikyo, however, suggest that independent religious cults or large terrorist groups, namely transnational networks, may have the intention to use biological weapons, and whether these groups can overcome the technical hurdles of acquisition, production, and delivery may ultimately determine whether such use is realized. According to many security analysts, however, the largest domestic threat in the US still arises from single operators. In the final analysis, although we can assume that there is a real but barely quantifiable threat, from a policy-making standpoint it is nevertheless prudent to prepare for a worst-case scenario in this respect.

### **Biological Industry Perspective on Addressing Biosecurity Issues and Related Preventive Strategies**

Michel Greco, International Federation of Pharmaceutical Manufacturers (IFPMA)

Thanks to its scientific expertise, its technological skills and its experience with clinical development, the pharmaceutical industry is well prepared to develop vaccines and anti-toxins against potential bioterrorist pathogens. Industry acknowledges the need to do so and is willing to contribute to the efforts made. However, several severe constraints have to be realised by all partners involved. After operational decisions have been taken by industry, a significant time span is needed to plan, implement, and finalise the developmental, regulatory and manufacturing process, i.e. before vaccines become available. Hence, it is of crucial importance, that a clear and reliable basis for these strategic decisions is made by pharmaceutical companies and provided by governments as soon as possible. The amount of vaccines needed, immunisation strategies, logistics, legal, contractual as well as financial issues must be urgently defined. The 'Note for guidance on development of Vaccinia based vaccines against smallpox', recently established by the European Agency for the Evaluation

of Medicines (EMA), is regarded as very helpful with respect to regulatory issues. However, the question of licensing procedures and liability remain to be addressed. In conclusion, the appropriate strategy for the pharmaceutical industry to cope with the biological threat not only depends on the biological agents. It rather relies on political/governmental decisions and the corresponding legal, regulatory, and financial framework, which have to be defined with highest priority.

### **EC Co-operation Programme on the “Preparedness and Responses to Biological and Chemical Agent Attacks – Health Security“**

Albrecht Werner, European Commission, Luxembourg

A ‘Task Force for Bioterrorism’ was established by the European Commission in May 2002, to support the measures of European Member States (MS) in their efforts to prepare and respond to biological and chemical attacks. Major purposes of this Task Force include the capability to detect and identify a biological/chemical attack, the co-ordination of the responses by MS, possible improvements of the expert and management capacities as well as mechanisms of information exchange. The laboratory capacities as well as the surveillance and response capacities are currently under evaluation. A rapid alert system is already available between MS. Collaborations with third countries and other relevant international organisations are being established. The work of the group is expected to be finalised on a short time frame, i.e. the Task Force will have completed its work in November 2003.

### **WHO Concept for the Assistance of Underprivileged Countries in the Management of Biological Threats**

Ottorino Cosivi, WHO, Geneva, Switzerland

Biological threats are not confined to industrialised countries. They may also affect less developed countries. However, any deliberate release of pathogens, and especially of smallpox, must be viewed as a global health threat and will have an impact on industrialised nations. Consequently, strategies of industrialised nations to protect themselves should include measures to cope with attacks, initially taking place outside of their own territory, but bearing the potential for world-wide spread. WHO assists underprivileged countries in preparing the management of biological attacks, e.g. in developing and implementing national action plans and strengthening laboratory capacities and expertise. This is achieved by organising meetings, performing training programmes and providing written guidance

documents. In addition, WHO maintains a global surveillance system for detecting and identifying disease outbreaks.

### **Research and Development: Focus on Novel Preventive Strategies against Bacterial Biothreat**

Stefan H.E. Kaufmann, Max Planck Institute for Infection Biology, Berlin, Germany

Several bacterial pathogens cause a high risk of misuse. The CDC e.g. lists under Category A as high-risk group: *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis* and *Francisella tularensis*, the etiologic agents of anthrax, botulism, plague, and tularemia. Although these bacterial agents still cause disease naturally, they are extremely rare in the industrialised world. Therefore, the impetus to develop vaccines to protect the general public against these bacteria has been minimal. In contrast, the military has developed vaccines against these diseases which, however, have never been tested in phase III clinical trials. Hence, they are of questionable protective efficacy. Moreover, these vaccines potentially cause side effects that would not be acceptable if used as vaccines for the general public. Hence, novel strategies should be considered on the basis of recent advances in research. From a vaccinology point of view, the above-mentioned bacteria can be divided into three groups: extracellular bacteria (*B. anthracis*, *Y. pestis*), toxin producers (*C. botulinum*), and intracellular bacteria (*F. tularensis*). Extracellular bacteria are typically attacked by antibodies. Neutralising antibodies represent the basis for vaccines against botulinum. In contrast, protection against intracellular bacteria depends on T lymphocytes rather than antibodies. Novel vaccination strategies directed at counteracting bioterrorism should comprise both preventive therapeutic approaches. Classical vaccination which specifically stimulates acquired immunity should be complemented by immune modifiers and generic vaccines that stimulate specific immune responses and rapidly activate the innate immune system. Moreover, antidotes provide novel approaches towards toxin neutralisation. Finally, immune intervention strategies curtailing exaggerated host responses need to be considered. Although recent advances in genomics, basic immunology and molecular biology provide helpful guidelines for the construction of novel vaccine candidates, their availability for the civil population depends on a comprehensive research agenda ranging from basic science to applied field studies.

**Session II: Smallpox**

Chairpersons: J. Löwer, Paul-Ehrlich-Institut, Germany; L.D. Rotz, CDC, Atlanta, USA

**Smallpox – Pathology and Clinical Features**

Alasdair Geddes, University of Birmingham, UK

The variola virus which causes smallpox, is a member of the Poxviridae family. It enters the body via the respiratory tract and causes transient viraemia followed by a latent period of four to 14 days during which it multiplies in the body. After that, another period of viraemia occurs followed by the prodromal illness when the virus invades the mucosa of the mouth and the skin. Neutralising antibodies to the virus appear during the first week of illness. Haemagglutination-inhibition antibodies are detectable in the blood by day six of the rash and complement-fixation antibodies by day eight. The clinically important form of 'variola major' is a life-threatening disease with a mortality of 30%. Death is probably due to toxæmia associated with circulating immune complexes and soluble viral antigens.

In terms of modern scientific analysis, the pathology of the disease is not well understood. However, the clinical features of the disease are well known and described in the literature. Nevertheless, the problem of obtaining a fast and valid diagnosis of a smallpox case must not be underestimated. Differential diagnosis against influenza, chickenpox, Eczema herpeticum, Eczema vaccinatum and drug eruptions /erythema multiforme is necessary. The last smallpox case in Birmingham, UK, caused by a lab infection in 1978, was initially misdiagnosed by 3 physicians, leading to a significant delay in treatment and containment measures. This fact underlines the importance of an appropriate education and training of physicians to diagnose disease caused by bioterroristic agents as part of the preparedness plans.

**The Genetic Relationship and Virulence Genes of Orthopoxviruses**

Geoffrey L. Smith, Imperial College of medicine, London, UK

Vaccinia virus is the smallpox vaccine, but its origin and natural host are uncertain. Previously, it was suggested that vaccinia virus might have been derived from cowpox or variola by passage and mutation, by recombination between cowpox and variola, or it could be a distinct species from an unrecognised host. The latter seems most probable. Orthopoxviruses are morphologically indistinguishable, immunologically related and infection with any orthopoxvirus confers some protection against other members of the genus. Many vaccinia virus strains were used for smallpox vaccination and these differed in their reactogenicity in man. Attenuated vaccinia virus strains such as LC16m8 (Japan) and MVA



(Germany) were used towards the end of the smallpox eradication campaign but it is uncertain if these viruses protect against smallpox.

Each cell infected by vaccinia virus produces several different types of virus particle, called intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV) and extracellular enveloped virus (EEV). These have different roles in virus dissemination, and immunity to antigens present in the EEV outer envelope, but absent from IMV, is necessary for protection.

Vaccinia virus virulence can be modified by deletion and/or mutation of genes encoding non-essential enzymes, proteins affecting virus dissemination, or proteins aiding evasion of host response to infection. Removal of genes affecting dissemination produces greater attenuation than deletion of immunomodulators. Vaccinia virulence has been studied in several models including primates, mouse, rabbit and CAM. In mice, the virus may be introduced intradermally, intranasally, intracranially or by dermal scarification. The outcome of the gene deletion depends on the model used. Using more models is likely to reveal more phenotypes. A nil phenotype often means the wrong model has been used. The virulence of viruses in the intradermal model broadly reflects their reactogenicity after smallpox vaccination.

Reference. Smith, G.L., Vanderplasschen, A. & Law, M. (2002). The formation and function of extracellular enveloped vaccinia virus. *J. Gen. Virol.* **83**, 2915-2931.

### **Scenarios for the Prevention, Diagnosis and Response to Biological Threats**

Hartmut Hengel, Robert Koch Institut, Berlin, Germany

In Germany, the responsibilities regarding disaster prevention and management are shared out broadly among several institutions due to Germany being a federal state. At the federal level, a variety of ministries are in charge of these tasks which must be implemented at the state level by each of the 16 German states and at the local level by each county or city, respectively. In addition, some governmental institutions have specific tasks. The Robert-Koch-Institut (RKI), for instance, is an epidemiological centre. One of its main functions is the surveillance of outbreaks of infectious diseases. Pathogens listed as CDC category A and B will be controlled as a first step by a syndrome based surveillance. An expert group has been established which has the power to provide facilities to improve public health and, in doing so, to prevent the spreading of infection. Treatment centres, isolation units and laboratories with special safety levels are located all over Germany. An example of managing an exceptional epidemic event in Germany was the anthrax threat in 2001. The RKI offered

information via the Internet and has established both a telephone hotline and an e-mail address for inquiries. It serves as a link between a variety of different administrative bodies in Germany. Cross clearance of the information is an important task.

As we have learned from the anthrax threat, the number of laboratories able to analyse pathogens of safety level 3 is small. Immediate consequences for more laboratory capacity and because of this, better awareness, are required. In conclusion, a great deal of work remains to be done to optimise preparedness. In particular, training of physicians as well as the training for diagnosis of rare or eradicated pathogens should be intensified.

### **Smallpox Control Strategies: Lessons from the WHO Eradication Programme**

Donald A. Henderson, U.S. DHHS; Office of Public Health Preparedness, Washington, DC, USA

The WHO smallpox eradication program began in 1967. Its goal was to eradicate smallpox within 10 years. The lack of an animal reservoir made smallpox eradication feasible. In addition, patients who recover from smallpox infection are immune and cannot serve as carriers. Thus, the programme was planned as a two part strategy: both an immunity of 80% of the population and surveillance-containment were the primary objectives in smallpox eradication.

However, it was traditionally believed that smallpox spreads rapidly and widely. An airborne spread from hospital could not be excluded. Nowadays, as we look back in history, we find that smallpox did not disseminate rapidly – only 58% of secondary household contacts were attacked compared to 76% in the case of measles or 74% in the case of chickenpox. Furthermore, only one case of airborne smallpox was reported. As an example, the transmission rate of primary smallpox contacts in Europe from 1958 to 1973 was 48% in hospital and 25% at home. In October 1977, the last naturally occurring case was reported from Somalia, and in May 1980 the WHO could proclaim eradication. As a result, vaccination was stopped and vaccine production ceased world-wide.

The vaccine strains Lister and NYCBH were used at that time. The vaccines were freeze-dried, and, immediately after reconstitution, applied by either scarification, multiple pressure, subcutaneous injection (jet injector) or multiple puncture (bifurcated needle). It was thought by some to be important to avoid any bleeding after vaccination as they believed that such bleeding might wash out the virus and decrease the 'take' rates. This was found to be totally wrong. Indeed, emphasis was placed on assuring that, after the 15 rapid needle punctures, a trace of blood did appear at the site of vaccination within 15 to 20 seconds. If it did not, vaccination should be repeated with more vigorous strokes of the needle.

An infection with variola major strains in a non-immunised population ends up lethally in up to 30% of all cases. In a typical case, the patient is symptom-free and is not contagious during the first 12 days, followed by high fever and flu-like symptoms on day 13 and 14. From day 15 on, the patient is contagious and develops a rash followed by vesicles and pustules. After scab forming (day 28 to 35 or more), he is no longer contagious.

The WHO eradication program encompassed vaccination and isolation of the patient in a hospital, the identification and vaccination of primary contacts following surveillance of the temperature (twice a day) and finally vaccination of all household contacts (secondary contacts) of the primary contacts.

The WHO's experience has shown that eradication programme containment vaccination is recommended! Compulsory vaccination as well as compulsory isolation would create chaos. Mass vaccination would involve considerable efforts, and the outcome would be questionable compared to ring vaccination. Declaring large areas as quarantine areas or imposing travel restrictions would also bring about major problems. As there is no solution other than isolating infectious persons and contacts, it is necessary that all individuals involved work together.

### **Potential of Cidofovir in the Treatment of Poxvirus Infections**

Erik de Clercq, University of Leuven, Belgium

Cidofovir, a cytosine derivative, inhibits viral DNA synthesis and virus replication and has a broad-spectrum activity against virtually all DNA viruses, including herpes-, adeno-, papilloma- and poxviruses. When covalently linked to a lipid (i.e. hexadecyloxypropyl) tail, cidofovir has a markedly enhanced activity by the oral route of administration. Cidofovir has as such an extremely long intracellular half-life as compared to other antiviral agents. In 1996, Cidofovir was licensed for clinical use for the treatment of cytomegalovirus retinitis in AIDS patients. Among the poxviruses, vaccinia, variola, cowpox, monkeypox, camelpox, molluscum contagiosum and orf have proven sensitive to the inhibitory effect of cidofovir. In vivo, cidofovir has shown high efficacy, even after administration of a single systemic or intranasal dose, in protecting mice from a lethal respiratory infection with either vaccinia or cowpox virus. Cidofovir has also demonstrated high effectiveness in the treatment of vaccinia virus infection in severe combined immune deficiency (SCID) mice. In man, cidofovir has been used successfully in the treatment of recalcitrant molluscum contagiosum and orf in immunocompromised patients both by the topical and by the intravenous route. In conclusion, there are indications that cidofovir should be effective in the therapy and short-term prophylaxis of smallpox and related poxvirus infections in man as well as the treatment

of the complications of vaccinia that may arise in immunocompromised patients inadvertently inoculated with the smallpox vaccine (vaccinia).

**Session III: Smallpox vaccines**

Chairpersons: M. Pfeleiderer, Paul-Ehrlich-Institut, Langen, Germany; R. Dobbelaer, Scientific Institute of Public Health, Brussels, Belgium

**Efficacy and Safety of Smallpox Vaccines Used during the WHO Eradication Programme: The Implications today**

Isao Arita, Agency for Co-operation in International Health; Kumamoto, Japan

A review of the efficacy and safety of smallpox vaccines used during the WHO Smallpox Eradication Program (SEP) demonstrates that there is a need for control of quality, safety, and supply in today's circumstances. At the beginning of the SEP, many vaccine batches not in compliance with potency, microbial contamination or stability were in use for the epidemic control. Furthermore, various strains with an increased frequency of postvaccinal encephalitis were applied. In view of these issues, the quality of the vaccines was improved by several measures taken by the WHO. Independent testing of the vaccines was introduced and seed virus and vaccine reference material were supplied. In addition, the use of the vaccinia virus vaccine strains NYCBOH, Lister and EM-63 was recommended.

Whereas contraindications to vaccination were not apparent in endemic smallpox areas, in non endemic situations, several complications such as eczema, central nervous system disorders and immune disorders were present. Moreover, today the number of patients with immune suppressive therapy is increasing. This implies the need of less reactogenic vaccine strains. At the end of the SEP, several strains with low reactogenicity were developed. One of these strains, LC16m8 was derived from strain Lister-Elstree by multiple passages in primary rabbit kidney cells. A vaccine made of this strain was significantly less reactogenic, especially in neuropathogenicity whilst it produced good skin reactions and elicited neutralising antibodies in humans. However, strains with a less pronounced reactogenicity were not used during the epidemic phase of smallpox. Thus field efficacy was not shown by the attenuated strains. A further implication from the SEP is the perpetuation of the vaccination technique using the bifurcated needle. Millions of people world-wide were successfully vaccinated with this technique. One advantage of the use of the bifurcated needle is four-fold savings of vaccine doses. Furthermore, the final product should be packaged in small quantities of 10-15 doses to reduce wastage, which was unexpectedly high at the time of the SEP.

**Emergency Response to a Smallpox Attack: Comparison of Strategies and Models**

Klaus Dietz, University of Tübingen, Germany

Mathematical models for the evaluation of vaccination strategies controlling a smallpox attack have been compared. The models, as well as the explicit assumptions on the key epidemiologic parameters differ considerably. Kaplan et al. (2002) compared mass vaccination with "traced vaccination" to estimate the number of cases and deaths that would result from an attack in a population of ten million people. However, some of the assumptions used in the model were unrealistic. More realistic parameters have been estimated by Eichner and Dietz on the basis of data collected by Thompson and Foege during a smallpox epidemic in Nigeria in 1967. Using the model of Kaplan, the number of cases was estimated with these more realistic parameters. A model for ring vaccination is currently not available. In conclusion, more realistic models are urgently needed to select strategies for an emergency response.

**Use of Smallpox Vaccine as a Precautionary Measure: The Military Sector**

Ernst J. Finke/Hermann Meyer, German Armed Forces Institute for Microbiology, Munich, Germany

Specific information on the presentation could be made available by the authors upon request.

**Stockpiling of Smallpox Vaccines: The European Plan**

Jan Hendriks, European Commission, Luxembourg

At present, no needs are recognised for common stockpiling at the level of the European Union, nor is there a wish to have formal agreements on sharing national stockpiles. Most Member States follow their own national strategy and have their own national vaccinia vaccine stocks. These national stockpiles are planned to be built and maintained in a sufficient quantity to have at least one vaccine dose per inhabitant. Some states are considering diluting their stocks, so that they can provide a greater number of doses. Moreover, it is planned that second generation vaccines should supplement existing stocks. The superiority of second over first generation vaccines is mainly in the quality of the production methodology, certainly not in efficacy and questionable in safety, because the safety issues are possibly strain and not substrate related. Therefore first generation vaccines will only become obsolete, if new generation vaccines (second and third) have established a better clinical safety profile and equal or better take-rates, which is seen as the best correlate of protection. The next steps at the European Community level are the

promotion of smallpox vaccine dilution studies, the increase in the availability of vaccinia immunoglobulin (VIG), since there is a real shortage in VIG at the moment, and the initiation of a platform for information exchange and vaccine development.

### **Stockpiling of Smallpox Vaccines: The U.S Plans**

Philip Russel, U.S. DHHS, Office of Public Health Preparedness, Washington, DC, USA

The US policy follows the strategy of providing sufficient vaccine for immunising the entire population and for any future needs. So far, 15 Million doses of Dryvax vaccine and 80 Million doses of an Aventis vaccine were stockpiled. Both vaccines, the Dryvax and the Aventis vaccine, were derived from the New York City Board of Health (NYCBOH) strain and were produced on calf skins. The Dryvax vaccine, however, is a freeze-dried formulation, whereas the Aventis vaccine is wet frozen and contains glycerol. The old first generation vaccines should be used in an event of emergency. Stability studies of these first generation vaccines are in progress. More recently, two contracts for the delivery of 250 Million doses of second generation vaccine were signed. These second generation vaccines are produced on cell culture systems using the NYCBOH strain. A first clinical trial with about 100 individuals is under way.

Future plans include the use of third generation vaccines based on the attenuated modified vaccinia virus strain Ankara (MVA). This vaccine is developed especially for immunocompromised patients. Clinical trials are planned for late 2002. If clinical trials and animal studies are appropriate, the immunisation scheme for immunosuppressed patients should be a prime vaccination with MVA followed by a booster immunisation with the second generation vaccine prepared from strain NYCBOH.

However, it must be considered, that this immunisation scheme does not show efficacy under field conditions.

### **Stockpiling of Smallpox Vaccines: The Japanese Plans**

Takeshi Kurata, National Institute of Infectious Diseases, Tokyo, Japan

In Japan, 1.1 Million doses of two old vaccines made from strain Lister-Elstree and Ikeda were stockpiled. These first generation vaccines were produced on calf lymph between 1978 and 1981, followed by storage at -15 to -20°C. The virus titre of the stockpiled vaccine was determined and it was demonstrated, that it was still in the specified range. Furthermore, 2.5 Million vaccine doses were provided in 2002. This new vaccine was produced in cultured primary rabbit kidney (PRK) cells using the attenuated strain LC16m8. In addition, it is

planned to produce another 7.5 Million doses of the highly attenuated smallpox vaccine in 2003. Strain LC16m8 is derived from strain Lister-Elstree by multiple passages in PRK cells at 30°C followed by additional plaque cloning. The highly attenuated strain seems to have the same potential to induce a protective immune response compared to the old type of vaccines as far as evaluated with old criteria of immunity to smallpox vaccine. However, according to animal models and from limited numbers of human trials, LC16m8 shows lower neurovirulence and a decrease in side effects compared to Lister-Elstree and Ikeda. A smallpox vaccine made of this highly attenuated strain was licensed in 1975 in Japan.

### **Stockpiling of Smallpox Vaccines: The PAHO Plans**

Otavio Oliva, PAHO, Washington DC, USA

In October 2001, a consultation meeting was held in response to concerns of the Member States of the Pan American Health Organisation (PAHO). At that time, no single vaccine dose was available in any of the member states. Participants of the meeting were regional manufacturers, national regulatory authorities, and health officials. A major recommendation of the meeting was to explore the potential for production of quality smallpox vaccines in the Americas, the demand for smallpox vaccine supply and requests for technical advice to start vaccine manufacture. It was concluded that all smallpox vaccines to be eventually produced in the region should be of assured quality and produced following GMP specifications. In addition, only those strains should be used for vaccine production the clinical efficacy of which has been shown (Lister, NYCBOH). However, two problems were identified for production. Only few laboratories in the region have the capacity to engage in smallpox vaccine production without having to incur major investments to reach GMP standards. The majority of countries in the Americas do not have the capacity for immediate smallpox vaccine production without hampering current production of EPI vaccines, i.e. measles vaccine. Meanwhile, Mexico has purchased 5 Million doses of a vaccine produced in cell culture, and Brazil has taken the decision to re-establish its production on embryonated eggs. Furthermore, the US based company Acambis has made the commitment of large scale industrial production within one year to fulfil demands other than from the United States of America. At present, a resolution is prepared for a consensus among the Member States that one case of smallpox in any of them is a threat to the entire region. Therefore, countries that have stocks of smallpox vaccine would make them available for the control of the outbreak in the affected country. Once smallpox vaccines become available on the market, countries in the Americas wishing to stockpile smallpox vaccine should engage in joint procurement through the PAHO Revolving Fund for Vaccines Procurement to ensure an



affordable price and high quality. 90% of the EPI vaccines run through the PAHO revolving fund.

### **WHO Response to the Threat of Smallpox, and Strategic Vaccine Reserves**

C.E. Roth, WHO, Geneva; Switzerland

Arising from the concerns of the Member States, the WHO Smallpox Program focuses on the following areas: research on Variola virus; vaccine policy, supply and standards; surveillance involving both epidemic intelligence and laboratory, response preparedness; and public health information.

In May, 2002 the World Health Assembly, authorised the temporary retention of existing Variola virus stocks for the purpose of further essential international research, to be overseen by the WHO Advisory Committee on Variola Virus Research. This committee reports regularly on the progress of this research.

An inventory of existing smallpox vaccine stocks was conducted by contacting member states and past, present and potential vaccine manufacturers. The results highlighted the inadequate volume of vaccine currently available for potential global needs in the event of an outbreak of smallpox, and the uneven distribution of the existing stocks amongst the WHO Regions. WHO maintains a stockpile of 655,000 doses of vaccine in Geneva for global emergency use. An important issue to be addressed is ensuring that an adequate stockpile be created and maintained, accessible to those countries which do not have the public health resources to commit to creating their own national smallpox vaccine reserves. The WHO Department of Vaccines and Biologicals(V&B) works to ensure maintenance of appropriate standards in manufacture of vaccine.

Surveillance and response is carried out through the Global Alert and Response activities in the Department of Communicable Diseases Surveillance and Response, WHO, and the Global Outbreak Alert and Response Network (GOARN), a network of public health institutions and networks world-wide. Epidemic intelligence is monitored daily, and rumours of smallpox are investigated and verified or refuted. Thus far, 8 smallpox rumours have been investigated; none have turned out to be smallpox. Response preparedness activities include the development of protocols and data management tools for investigation and control of outbreaks. The laboratory component of surveillance and response activities also involves V&B, within WHO, and the network of WHO Collaborating Centres. This component includes an inventory of laboratory capacity for orthopox diagnosis which was performed in 2002, development of consensus laboratory protocols, provision of standards and controls for assays, and specimen transport guidelines.

A variety of information has been made available on the WHO website. This includes basic materials such as Fact Sheets, Frequently Asked Questions, and Press Releases, but also includes training materials for healthcare workers, published monographs, and updates on WHO policy.

**Session IV: Regulatory requirements for historic and new smallpox vaccines**

Chairpersons: J. Scherer, Paul-Ehrlich-Institut, Langen, Germany; I. Arita, Agency for Cooperation in International Health, Kumamoto, Japan

**Review of Regulations in Europe, the US and the WHO**

R. Dobbelaer, Scientific Institute of Public Health, Brussels, Belgium, K. Midthun, CBER, Office of Vaccine Research and Review, Rockville, USA, D. Wood, WHO, Geneva, Switzerland

When smallpox was recognised as a potential bioterrorist weapon, EU authorities started to work on regulatory requirements for smallpox vaccines at three levels:

- The European Pharmacopoeia (Ph. Eur.) Expert Group 15, which is responsible for drafting quality requirements for licensed vaccines for human use, requested authorisation to review the revoked monograph on Smallpox Vaccine.
- The EU CPMP Vaccine Expert Working Group received a mandate from the EU Commission's Bioterrorism Task Force to draft a Note for Guidance covering quality, safety, and efficacy recommendations for cell substrate derived ("second generation") smallpox vaccines.
- The European Directorate for the Quality of Medicines (EDQM) drafted an emergency procedure for Control Authority Batch Release of vaccines for use in pandemic or bioterrorism situations.

The current requirements for smallpox vaccines in the EU as well as the US are based on the so-called first generation vaccines prepared on the skin of calves or other animals or in chicken eggs. They address the characterisation of the seed virus, the production process in calves or embryonated chicken eggs, and the testing process (e.g., potency, bioburden, preservative, etc.).

At present, the only smallpox vaccine licensed in the U.S. is a so-called historical vaccine, Wyeth's Dryvax®. It was derived from the New York City Board of Health (NYCBH) strain of vaccinia, prepared on calf skin, and stored as a lyophilised product. Dryvax® is no longer being manufactured, and remaining supplies are limited.

Second generation smallpox vaccines derive from the already known strains used in the first generation with proven field efficacy, such as the NYCBOH and the Lister/Elstree strains, as well as other strains such as Paris, Copenhagen, Bern, EM-63 and Temple of Heaven, but are produced in cell substrates (e.g. MRC-5, VERO, CEF). Characterisation and qualification of the master and working cell banks (e.g., adventitious agent testing, tumorigenicity) and of the master and working viral seeds (e.g., adventitious agent testing, comparability to licensed vaccine, including animal studies) are essential requirements, together with other common

principles of production and quality control, such as a validated manufacturing process that ensures consistency of manufacture, defined compatible components, product characterisation and specifications, source of materials, and stability. According to US regulations and in agreement with the “Note for Guidance on the Quality, Safety and Efficacy of Second Generation Smallpox Vaccines” the standards for licensure are the same as for any other medicinal product and require the demonstration of safety, purity, potency, efficacy, manufacturing reproducibility, and compliance with current good manufacturing practice. The “take rate” in addition to antibody titres as well as measuring the cellular immune response are the critical parameters for the evaluation of the efficacy of these vaccines.

The efficacy of new vaccines derived from strains without demonstrated efficacy in the field, the so called third generation vaccines, can be based on animal efficacy data, if scientifically appropriate, in addition to comparative human immune response data. As for any biologic, licensure of new smallpox vaccines requires demonstration of safety, efficacy, and quality and consistency of manufacture.

The WHO requirements on smallpox vaccines, based on the revision in 1965, were recently revised. The revision was widely circulated prior to formal adoption by the Expert Committee on Biological Standardisation (ECBS) in February 2003. Both the WHO and the EDQM emphasise the importance of combining know-how and speed in the batch release procedure. International collaborative studies and proficiency studies are useful tools to resuscitate former expertise.

### **EU Scientific Advice for Product Development**

P. Le Courtois, EMEA, London, UK

The provision of scientific advice in the EU can be obtained from regulatory authorities conforming to their national laws. Scientific advice is not mandatory in the EU, if a marketing authorisation is applied for, and is independent from the formal process of starting clinical trials conforming to GCP legal requirements.

Since 1 January 1995, all medicinal products of biotech origin must obtain a marketing approval through the European Agency (EMA) using a centralised procedure. For any other product, this procedure is optional and the approval can be granted by national competent authorities. These rules also apply to vaccines.

The EMA provides sponsors with scientific advice through the Committee for Proprietary Medicinal Products (CPMP) and the Scientific Advice Review Group (SCIARG, now called Scientific Advice Working Group (SAWG)). In December 2001, the CPMP created an expert

group on vaccine (VEG) which meets the need for a multidisciplinary approach, because of the specificity of vaccines. The VEG invite additional specialised experts for participation in its work. These depend on the product and the topic. The VEG has mainly developed, in a very efficacious manner, the CPMP guideline on the development of second generation smallpox vaccines as requested by the European Commission end of 2001.

Advice can be given when aspects of the development plan are not covered in the guideline, or could be subject to interpretation, or when the sponsor would like to diverge from the recommendation. This includes any aspects of the development of the product, manufacturing aspects, pre-clinical or clinical. Scientific advice on products will be given independently from the future procedure for registration.

Where sponsors wish to seek advice from the EMEA on the development of a second generation small pox vaccine or any other product to be used in the context of bioterrorism, it is understood that the structures and processes described above will be involved. Especially the VEG should play an important role.

### **Laboratory Testing of Vaccines against Smallpox**

Johannes Löwer, Paul-Ehrlich-Institut, Langen, Germany

At present, there is no marketing authorisation for smallpox vaccines in Germany or any other Member State of the European Community. The Draft Official Batch Release Guideline and the European Pharmacopoeia Monograph for smallpox vaccines have not yet been formally adopted. Consequently, the extent of laboratory testing depends on the decision of the National Control Laboratories. In order to be prepared for batch release testing of new smallpox vaccines and to assure the quality of the recently purchased old first generation vaccines, the establishment of several smallpox specific release tests was initiated at the Paul-Ehrlich-Institut. These tests include the determination of the virus concentration using cell culture based techniques or titrations on the chorioallantois membrane (CAM) of embryonated hen's eggs. Tests on the thermal stability of the vaccine as well as sterility and identity tests should also be performed. Future demands include the need for updated specific guidelines, and a list of relevant tests to assure the consistent quality of smallpox vaccines from different manufacturers in the EU and in the world. Moreover, reference preparations and standardised methods must be established and qualified in collaborative studies.

**Session V: Old and new vaccines: research and development**

Chairpersons: R. Kurth, Robert Koch-Institut, Berlin, Germany; K. Midthun, CBER, Office of Vaccine Research and Review, Rockville, USA

**Recent Experience Gained from Clinical Trials Conducted with Old Smallpox Vaccines Derived from Animal Skin**

Sharon E. Frey, University of St. Louis, USA

The American vaccine Dryvax, which was produced on animal skin, showed a satisfactory take rate in primary vaccinees when administered with a bifurcated needle, both undiluted and at a dilution of 1:5 or 1:10. For the vaccine, a starting titre of  $10^{8.1}$  p.f.u./ml was calculated. Depending on the dilution, doses of  $10^{5.0}$ ,  $10^{4.3}$  and  $10^{4.0}$  p.f.u. were administered per vaccinee. These absolute values must be interpreted with some care, since no details on titres are available for the reference material, and titrations were carried out in cell culture rather than on the chorioallantoic membrane (CAM). For this reason, the real titre could easily be underestimated. It is also likely that the take rate was influenced by the fact that the vaccination site was covered with gauze and a semipermeable adhesive membrane. Another noteworthy observation is that a higher administered dose led to a stronger local reaction but a reduced incidence of "satellite lesions".

**Relevant Animal Models Available and to be Developed for the Pre-Clinical Evaluation of the Efficacy and Safety of Smallpox Vaccines**

Daniel Garin, Virology Unit, DEF/DCSS/CRS, La Tronche, France

Experiments involving live animals may provide an effective strategy to better understand the complex biological mechanisms involved in the protection induced by a vaccine against a specific pathogen. Animal tests are often required in biological research and vaccine evaluation mainly to estimate safety and efficacy. Although preliminary clinical safety aspects and immunogenicity data of a candidate smallpox vaccine can be collected in clinical trials, assessment of the protective effect of smallpox vaccines cannot be evaluated in man. Therefore, the assessment of the probable protective effect must depend on appropriate animal models. The primary endpoint of the animal studies should be the protection against infection of relevant pathogenic orthopox viruses that results from immunisation with the candidate vaccine and an appropriate comparator vaccine. The EMEA recommended a step-wise approach by demonstrating the cross-protection against two different pathogenic orthopox viruses in two mammalian species. A non-primate model should be used in the early pre-clinical development followed by an assessment of the final product in monkeys.

However, pre-clinical testing of second generation vaccines, even in relevant animal models, can only partly replace clinical studies in man.

### **Highly Attenuated Vaccinia Strains as Safe Third Generation smallpox Vaccines**

Falko G. Falkner, Baxter BioScience, Orth/Donau, Austria

The complications of smallpox vaccination with the standard live vaccines were frequent and sometimes severe. Under normal circumstances, newly developed vaccines which display the spectrum of adverse events of standard smallpox vaccines are not approvable by regulatory authorities. Thus, standard smallpox vaccines are contra-indicated for the immunocompromised, for patients with skin disorders and for pregnant women. The currently produced second generation vaccines (cell-culture derived, administered via scarification) will presumably have a similar spectrum of complications. Therefore, there is an urgent need for safer smallpox vaccines. Since the severe adverse events are associated with replication of the vaccine virus in the vaccinated subject, highly attenuated or genetically disabled ("non-replicating") vaccinia strains that retain their immunising properties are the most promising next generation of smallpox vaccines. Among the candidates are the classical MVA, possibly the NYVAC vector derived from the vaccinia Copenhagen strain by deletion of several nonessential genes, and the dVV vector (defective vaccinia virus), derived from a standard smallpox vaccine strain by deleting one gene essential for viral replication. Development of an MVA-based vaccine belongs to the most promising approaches due to the experience gained with this vector in the past. MVA was previously used in Germany as a pre-vaccine to dampen the effects of standard vaccination. It seems a general consensus that MVA is an intermediate solution of the smallpox vaccine problem. Modern vaccines based on novel genetic principles having no replication risks, such as DNA-based vaccines or genetically disabled vaccinia strains presenting the same antigens and epitopes as the standard vaccine, may be the smallpox vaccine of the future.

### **Clinical Study Design for New Smallpox Vaccines: Ethical Aspects**

Erwin Deutsch, Göttingen, Germany

Clinical trials with vaccines have been known for centuries, beginning with the smallpox inoculation in the 18th century up to "Operation Desert Storm" during the Gulf War in 1991. For new smallpox vaccines, the accepted rules apply for medical experimentation as defined in the Declaration of Helsinki, 1964 (amended in Edinburgh, 2000). The vaccinee must give informed consent. Special care is required if the trial is performed in a vulnerable group.

Risks and burdens must be adequate in comparison with the predictable benefits to the subject or other individuals. Controlled clinical trials are desirable. In most cases, a placebo group is unjustified, and usually randomisation is not possible. There should be liability for negligence in performing the trial and the right of the innocent victim for compensation even in the absence of fault.

### **European Manufacturers**

Kim C. Bush, European Vaccine Manufacturers (EVM)

The EVM (European Vaccine Manufacturers) is a specialised group of leading vaccine manufacturers within the European Federation of Pharmaceutical Industries and Associations (EFPIA). EVM's goals are to create a supportive European environment for improved vaccine protection and coverage, to promote vaccine research and development, and to foster a favourable policy climate for innovative vaccine development. EVM's goals regarding bioterrorism are to understand and meet the European requirements, to engage in a continuous dialogue with European authorities and agencies, and to provide state of the art science, products, and support to meet the challenge. The EVM contributes by participating in the European Commission/industry task force on bio-terrorism and provides input to CPMP guidelines on vaccinia based vaccines. The EVM provides vaccine for initial stockpiles and is active in the accelerated investment and development of second and third generation smallpox vaccines. From the perspective of the EVM, the CPMP's vaccinia guidelines are comprehensive, thorough and appropriate. Consequently, they enable multiple suppliers to engage in the production of new smallpox vaccines. The CPMP guidelines cover assessment of quality, safety and immunogenicity, and the requirements of the CPMP guidelines fits EVM ideas. Non-traditional challenges related to smallpox vaccines are to match development and delivery timelines with the depth of requirements, the unique clinical trial requirements, and multiple post-manufacturing implications. Usually, the development of a new vaccine takes 6-10 years. A "fast-track process" to assure rapid development and delivery demands requires adjustment of regulatory framework, manufacturer's capacity and planning, political processes and a legal landscape. Clinical trials with smallpox vaccines present unique challenges, e.g. the efficacy cannot be evaluated in man, or treatment for vaccinia complications is not available. Post marketing issues that need to be addressed include stockpiling, liability and indemnification, crisis logistics and future investment decisions. In conclusion, the EVM fully endorses the CPMP vaccinia guidelines. A fast-track licensing and liability resolution will complement guideline implementation. The implications of the guidelines need to be fully considered in member state preparedness planning.



Industry needs EU support and directions for planning, producing and investing in next generation vaccines, and the EVM is proud to play a role in the European bio-terrorism preparedness.

### **U. S. Manufacturers**

Tom Monath, Acambis Inc., Cambridge, MA, USA

Acambis has developed two second generation smallpox vaccines, ACAM1000 and ACAM2000, respectively. Both vaccines are cell culture derivatives of the Dryvax vaccine (NYCBOH, Wyeth) which was generated on calf skin. Vaccinia virus of ACAM1000 was plaque purified and grown on MRC5-cells, Vaccinia virus of ACAM2000 was grown on Vero-cells under serum free conditions using the ACAM1000 master seed virus. Extensive testing was undertaken using in vitro and in vivo test methods with neutralization of vaccinia, and PCR reactions to demonstrate freedom from adventitious viruses of animal (especially bovine) and human origin.

ACAM1000 vaccine has been shown to be equivalent to parental Dryvax in most biological in vitro and in vivo assays. However, ACAM1000 is significantly less neurovirulent than Dryvax in mouse and monkey models, and it is speculated that it could be less likely to cause postvaccinal encephalitis than Dryvax.

ACAM1000 has been tested in a 60-subject, randomised, double-blind trial in which 30 subjects were given the standard dose of Dryvax and 30 were given an equivalent dose of ACAM1000. The currently accepted indication of protective immunogenicity in the event of smallpox vaccination is the development of a pock-mark on the skin ('take'). This was the primary endpoint of the trial. A 'take' was seen within 10 days after vaccination in 100% of the ACAM1000-treated subjects and in 97% of Dryvax- treated subjects. The size and appearance of the 'takes' were identical across treatment groups. No serious or unexpected adverse events were reported in the ACAM1000 group, whereas one subject in the Dryvax group developed a non-healing pock at the inoculation site.

ACAM2000 is equivalent to ACAM1000 in non-clinical models. The cumulative data suggest that the two vaccines are similar and will have a safety and immunogenicity profile equivalent to or better than calf skin vaccines. However, they differ significantly from Dryvax by improved production methods and freedom from adventitious agents.

Nevertheless, ACAM1000 and ACAM2000 are two different products as pointed out in the discussion. ACAM2000 should be tested in a new clinical trial recruiting different populations. In a phase II clinical trial, at least 1000 subjects are necessary for a relevant statement on the safety profile of the new vaccine.

**Session VI: Lessons for future pathogen eradication**

Chairperson: K. Cichutek, Paul-Ehrlich-Institut, Langen, Germany

**Reflections on the Maintenance of Vaccine Production and Supply for Pathogens Eradicated in the Future**

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Since the polio eradication programme was launched, the number of polio cases decreased from 350 000 in 125 countries in 1988 to 483 cases in 10 countries in 2001. The experience with the eradication of smallpox revealed the importance to contain lab stocks of the virus and to establish regional and global certification as well as post certification immunisation policies. Polio vaccination is still practised in many countries, and a consensus on future OPV use is necessary. A solid scientific basis, country consultation and public information have an important impact on the development of the post-certification polio immunisation policy. The ultimate goal is the cessation of polio immunisation, the medium to long-term objective is the OPV cessation. The benefits of vaccination must outweigh the risks associated with vaccination. The paralytic polio risks can either be vaccine derived (e.g. vaccine-associated polio, vaccine-derived polio outbreak or immunodeficient long-term excretors), or they can be caused by wild-type virus (e.g. IPV vaccine manufacturing sites, inadvertent release of wild-type poliovirus or intentional wild-type poliovirus release). Prevention of adverse events following immunisation with OPV and the financial savings are evident benefits of stopping polio immunisation. Among the options for stopping OPV are targeted campaigns (e.g. ring vaccination as in the case of vaccinia) once poliovirus transmission is limited to a specific focal area, the replacement of OPV with IPV in all countries or the development of a less transmissible polio vaccine. The implications for future vaccine production and supply are a continued OPV production until at least 2010, the creation of an OPV stockpile and the maintenance of reagents and skills necessary for OPV production at both the manufacturer's site and the site of the national control authorities beyond 2010. IPV should be produced under enhanced bio-safety conditions, and the production should be increased even in the absence of a global recommendation for IPV use. In conclusion, the early research on the risk assessment and risk management for "exit strategies", the dialogue with policy makers in low and middle income countries to understand risk perceptions and the proactive management of vaccine stockpile are essential for the post-certification immunisation policy development.

**Acknowledgements:**

This report was drafted by the following staff members of the Paul-Ehrlich-Institut: Christoph Conrad, Eva-Maria Erb, Bettina Grosse, Heidi Meyer, Michael Pfeleiderer and Jürgen Scherer. Editorial help came from Constanze Taylor.

On behalf of Prof. Johannes Löwer, President of the Paul-Ehrlich-Institut, the writers of the report would like to express their gratefulness to all the speakers and participants of the workshop for their commitment and input based on special scientific/medical experience.

Furthermore, the group would like to thank the European Commission, the World Health Organization and the German Ministry for Health and Social Security for co-organizing this workshop.