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# **WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION**

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Fifty-third Report



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Geneva, 17–21 February 2003

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## **Introduction**

The WHO Expert Committee on Biological Standardization met in Geneva from 17 to 21 February 2003. Dr Asamoah-Baah, Executive Director of the Health Technology and Pharmaceuticals cluster at WHO, opened the meeting on behalf of the Director-General.

Dr Asamoah-Baah recalled the history of high scientific standards set at previous meetings of the Committee and paid tribute to Dr Elwyn Griffiths, the former Chief of the biologicals programme who had recently retired. He reminded the Committee that the written guidance and reference preparations established in accordance with their recommendations define international technical specifications for the quality and safety of biological medicines and in vitro diagnostic procedures. This work contributes to global public health in a fundamental way. WHO is also recognized as a standard-setting body in public health by the World Trade Organization (WTO) which means that standards established by WHO may be recognized by WTO as the basis for discussion and resolution of trade disputes. In considering the heavy agenda, Dr Asamoah-Baah noted that it reflected once again the increasing complexity and growth of the biologicals field. He highlighted the requests for updating recommendations for small-pox vaccine received from a number of countries, and the items concerning poliomyelitis, a disease that WHO had set as a priority for eradication by 2005. With regard to reference materials, he also drew attention to the need for a debate on the role of physical and chemical approaches to characterizations and potency assignment to biologicals. During the meeting, a number of proposals for reference materials for diagnostic testing, especially of blood, and needs in relation to transmissible spongiform encephalopathies were to be considered.

## **General**

### **Developments in biological standardization**

Progress in the spheres of activity of the WHO units responsible for biological standardization (Quality Assurance and Safety of Biologicals and Quality Assurance and Safety of Plasma Derivatives), in meeting the targets set for 2002–2003 was summarized. The Committee noted that a number of these priority topics featured on the agenda of the meeting. In the case of vaccines, there is increasing emphasis on WHO's involvement in standardization activities at an

early stage in the development of new vaccines. The aim is to develop harmonized technical specifications for quality, safety and efficacy early in the product development cycle of a vaccine. In the field of whole blood and blood products, a high priority is to address safety issues through developments in methods for the detection of viral contamination and validation of in vitro diagnostic procedures. The detection of viral contamination involves either nucleic acid amplification technology or immunoassay based tests. These procedures require standardization and their sensitivity has to be established. They have an impact not only on issues related to blood safety, but also on clinical monitoring of disease through measurement of viral load. Priorities for 2004–2005 relating to blood and blood products were being discussed within WHO and would take into consideration requests from WHO Regional Advisers and the Global Collaboration on Blood Safety.

WHO is aware of the need to strengthen the technical capacity of national regulatory authorities in their work in the biologicals field. A key issue is the availability of adequate supplies of reference materials for routine use, and the preparation and characterization of national and/or regional, secondary reference materials is an important element of this. WHO is, therefore, investigating ways of facilitating the development of these materials and of providing technical guidance on their appropriate calibration and use. Requests for support had been received from several national authorities. Workshops on the regulation of biotechnology products and also on blood safety issues had been held at the International Conference of Drug Regulatory Authorities in June 2002.

The Committee's attention was drawn to a plasma proteomics project. The aims of this project are:

- to undertake a comprehensive analysis of plasma proteins in large cohorts of normal (healthy) people;
- to determine the extent of variation in these proteins across populations globally and within the populations of individual countries; and
- to identify biological sources of variation within individuals with time.

The project raises a number of challenging issues, not least in technical aspects of sampling, handling, storage and stability of specimens and in the sensitivity of the analytical methods that might be employed. Standardization would also extend to the enumeration and categorizations of the proteins and the extent of post-translational modifications.

The Committee noted that the WHO Working Group on cytokines, growth factors and endocrinological substances had not met during 2002. At the next meeting of the Working Group, the standardization of unwanted antibody responses to recombinant proteins would be an important topic for discussion.

The Committee was pleased to learn of outreach activities being conducted to draw the attention of the wider scientific community to the major role of WHO in the biological standardization area. One significant emerging sphere is that of gene therapy where WHO has a pivotal role in the development of regulatory guidance on the authorization of gene therapy products and the control of clinical trials that involve these products.

Once again, there had been delays in publication of the reports of the two previous Committee meetings. Nevertheless, reports of the major decisions and the changes in the list of WHO standards had been published. The Committee expressed great concern that detailed information on recommendations, guidelines and other matters of importance remained unavailable to control authorities and manufacturers. Decentralization of publication procedures had recently taken place and the expectation was that the delays would now be much reduced. The Committee requested that this matter be placed on the Agenda for its next meeting.

The Secretariat presented an outline of the initiatives proposed for the period 2003–2004 in the vaccines field. The planned activities of WHO included nonclinical evaluation of new vaccines and delivery systems; clinical evaluation of vaccines for use in developing countries; and establishment of a comprehensive system for ensuring the safety of immunization programmes. Within these overall priorities, standardization and control of biological materials would be of considerable importance, notably with respect to dengue, pneumococcal, rotavirus and human immunodeficiency virus (HIV) vaccines; reference reagents for polio neurovirulence testing; laboratory studies on *Simian virus 40* (SV40) and *Simian Cytomegalovirus* (sCMV) and finalization of the guidelines for nonclinical evaluation of vaccines.

In the blood field, safety issues have the highest priority and have consequences for the preparation, availability and characterization of a wide range of reference materials for blood safety testing as noted elsewhere in the report. The Committee requested that additional implications for ensuring that blood products were safe from contamination with emerging viral disease organisms should be kept under review. Another high priority is improving the quality and availability of anti-venom preparations and ensuring the availability and

appropriate use of reference materials for blood products and in vitro diagnostic procedures.

***National Institute for Biological Standards and Control, Potters Bar***

The Committee was informed of the work programme proposed by the National Institute for Biological Standards and Control (NIBSC) for the period 2003–2008 including work in progress, reference materials due for replacement and new projects. Although NIBSC's overall programme covered national as well as international concerns, every attempt was being made to ensure integration of the programme with the priorities of WHO, and this issue would be kept under rolling review. The Committee requested that mechanisms for setting priorities, taking into account those of WHO as well as those perceived by the international laboratories, should be reviewed at its next meeting.

A new facility for the preparation of reference materials, funded by the Department of Health of the United Kingdom (UK), had been constructed and would be commissioned during 2003. This would provide better facilities and offer the possibility of provision of secondary standards as well as allowing research and development relating to standardization.

The Committee was also informed of a new area of activity assigned a high priority in the UK in the field of genetic testing. This would involve activities related to screening and diagnosis, and reference materials had been identified as having a critical role. The initial activities relate to haematological disease, particularly thrombosis.

The Committee was interested to learn that the NIBSC had been given responsibility for the establishment and hosting of the UK national stem cell bank, which would become operational during 2003. This role would raise novel issues relating to quality control. A high-level steering committee representing many interests had been established to oversee operation of the bank, and to ensure that the cells going in and out of it are derived and used ethically. In principle, cells could be made available outside the UK subject to stringent controls.

***The Sanquin Blood Foundation, Amsterdam, the Netherlands***

The Central Laboratory of the Blood Transfusion Service at the Sanquin Blood Foundation holds and distributes a number of WHO International Standards and Reference Reagents. The Laboratory also makes available well characterized reference materials for use in international proficiency programmes to ensure that laboratories

perform tests correctly and accurately in the blood field. The Committee was informed of work being carried out together with NIBSC associated with the development of heat-inactivated HIV-RNA reference reagents that had shown that both lyophilization and heat result in significantly reduced recovery of material. The Committee was also informed about several other studies on viral materials and antisera connected to the implementation of European legislation for in vitro diagnostics, such as monospecific antisera against various proteins of hepatitis C. The Secretariat had identified the replacement of blood grouping reagents as a priority activity, together with finalizations of ongoing projects on nucleic acid amplification testing (NAT) and immunoassay candidate standards.

### ***Center for Biologics Evaluation and Research***

The Committee was informed of extensive changes that had taken place recently at the Centre for Biologics Evaluation and Research, Bethesda, MD, USA. These changes affected personnel and also involved the re-allocation of responsibilities for monoclonal antibodies and therapeutic proteins to the Center for Drug Evaluation and Research. The vaccine and blood products areas had been relatively unaffected by these changes. Issues of vaccine safety continued to have high priority.

Another area of high priority related to bio-terrorism and counter-measures to this threat. In particular, much work is in progress on new vaccines and the development of modern vaccines for infectious agents for which former vaccines may no longer be available or are now inadequate. These activities had resulted in the initiation of a number of new research programmes.

### **Scientific basis for preparation and characterization of International Standards**

The Committee recalled that, at its fifty-first meeting, attention had been drawn to the draft standard prEN/ISO 17511 addressing the standardization of in vitro diagnostic procedures. The draft was based on a reference system using a hierarchy of metrological determinations that were intended to provide traceability of measurement and an indication of the level of uncertainty attached to measurements. This approach differed from that historically used for biological reference materials. The Committee confirmed its previous view that procedures and considerations for the establishment of such biological materials differed from those applied to in vitro diagnostics, although it would be desirable to apply the same principles if this were possible. It was pointed out that biological activity is not an SI unit and that the

approach adopted by WHO is valid. Although the International Unit is assigned arbitrarily, the continuity of the International Unit with replacement reference materials is ensured by inclusion of the previous materials in the collaborative studies.

Where multi-molecular species are being studied, it is desirable to use several methods that provide valuable scientific information about specificity; the use of a single reference method to generate a value in SI units with an associated uncertainty is not appropriate. In addition, the Committee noted that scientific developments may mean that reliance on a single method is inappropriate because newer methods may well be more specific, accurate and precise and some methods, such as those that depend on extensive testing in animals, are no longer acceptable. In some cases, improved specificity has resulted in the establishment of WHO reference materials with a defined, restricted use, e.g. for bioassay or immunoassay. Furthermore, scientific advances have resulted in the discontinuation of a number of biological reference materials because the substances can be fully characterized by physical and chemical methods alone. The Committee confirmed that full evaluation of an analyte by biological, physical, chemical and medical properties should guide their decision on the assignment of activity.

A further consideration is that the WHO reference materials are primary standards for the calibration of therapeutic products and the ISO Guide 35 on reference materials explicitly indicates that reference materials for pharmaceutical use should not have an uncertainty stated with their assignment of content.

The Committee recommended that issues relating to the scientific basis for characterization and value assignment to biological reference materials be addressed in the revision of the guidelines for establishment of such materials (see p. 17).

### **WHO biologicals web site**

The Committee was informed of developments relating to the establishment of a specific website for biologicals at [www.who.int/biologicals/](http://www.who.int/biologicals/). It contains background information; guidelines and recommendations; the catalogue of the international biological reference preparations with information about the custodian laboratories; meeting reports classified by subject (blood products, gene therapy products, transmissible spongiform encephalopathies (TSEs), vaccines); the mission and all the reports of the Expert Committee for Biological Standardization; announcements and useful links to other sites, including other WHO web sites, the international laboratories

and professional societies. The site has a search engine that allows searches of the reference materials by name of substance or year of establishment. Complete meeting reports can be viewed and downloaded. The site can also be searched for Guidelines and Recommendations by topic. The Committee warmly welcomed these developments and their impact in meeting the need for wider availability of work on biological standardization in an easily accessible form. The Committee requested the Secretariat to explore ways of making the decisions of the Committee, including establishment of reference materials, available soon after the meeting, separately from the eventual inclusion of the formal report of the meeting on the site. The Committee also recommended that resources be made available by WHO to maintain the site and ensure that it is kept up to date.

### **International Nonproprietary Names for biotechnology derived medicinal products**

The Committee was reminded of the role and status of International Nonproprietary Names (INNs). The Committee was also informed of discussions that had taken place during the meeting of the Monitoring Group for gene transfer medicinal products relating to the need for and development of a nomenclature system for these products. The conclusion was that any nomenclature system adopted should be as simple as possible. There are three categories of gene therapy products: cells, viral vectors and plasmid vectors. It should be borne in mind that different manufacturers' products that are apparently the same material may not be interchangeable because the vectors used are not identical. The Committee noted that there would be further discussion of this issue at the next meeting of the Monitoring Group.

The Committee was also informed of recent developments on the strategy and process for assignment of INNs to biological medicinal products derived by biotechnology. A WHO Consultation on this topic had been held in January 2002 and the conclusions from that meeting had been endorsed by the WHO Committee on International Nonproprietary Names at their meeting in April 2002. Special sessions at that meeting and at a subsequent meeting had been devoted to consideration of the names of biological products. The Committee noted the progress made in this area and encouraged the Secretariat to maintain and strengthen the links between the two WHO Committees, and to ensure that personnel with appropriate biological expertise were available to advise on nomenclature.

## **Feedback on WHO recommendations and guidelines and capacity building in quality assurance**

The Committee was reminded that WHO has assigned priority to the assurance of quality, safety and efficacy of vaccines used in global immunization programmes. Part of this assurance is the strengthening of national regulatory authorities. Much had been achieved during the past few years through consultations, publication of guidance documents, and defining the functions to be performed by these authorities depending on the sources of the vaccines employed. Assessments of national authorities had been carried out in nearly 50 countries by more than 100 experts over a 4-year period. By 2002, 91% of all countries were using vaccines of assured quality. In addition, a global training network of laboratories had been established and more than 500 members of national regulatory authorities had been trained. From this programme, a number of quality issues and concerns had been identified.

The Committee was also reminded of the existing pre-qualification procedure to provide UN agencies purchasing vaccines with independent advice on the quality and suitability of vaccines intended for use in given countries. The procedure also permits monitoring of compliance of vaccines and provides a mechanism for handling defects and other issues. It has benefits in raising quality standards globally and provides added value to manufacturers and the regulatory authorities. It also provides feedback from the field on questions regarding Recommendations and Guidelines that may help the Committee in deciding whether these documents should be reviewed and revised. The Committee welcomed this feedback and asked the Secretariat to keep it informed when the need for revision of Recommendations and Guidelines arose.

## **International recommendations, guidelines and other matters related to the manufacture and quality control of biologicals**

### **Recommendations for the production and quality control of smallpox vaccine**

The Committee reviewed the draft Recommendations for production and control of smallpox vaccine (BS/03.1954), prepared as an update of the Requirements for smallpox vaccine published in 1966 (WHO Technical Report Series No. 323, 1966). The new document had been drafted following an Informal WHO Consultation held at WHO Headquarters, Geneva, in May 2002 and at a further review at an

Informal WHO Consultation in January 2003 also held at WHO Headquarters. The need for stringent requirements for a vaccine intended only for emergency use and the changes necessary to update the Requirements had been discussed. The main changes in the draft included clarification of the need for regulatory approval; deletion of the monkey neurovirulence test; strengthening of the sections addressing source animals; giving more flexibility in testing viral harvests for adventitious contamination and allowing for expiry dates to be displayed on secondary packaging. After making a number of changes to the text, the Committee adopted the revised text as Recommendations for the production and control of smallpox vaccine and agreed that it should be annexed to its report (Annex 1).

The Committee was informed about the policies in certain countries regarding the administration of vaccine to specific groups within the population.

The Committee was also given an update on the collaborative study in progress to identify a preparation that might be appropriate for use as a replacement international reference material for calibration of smallpox vaccine assays.

### **Guidelines for the safe production and quality control of poliomyelitis vaccine (inactivated)**

The Committee was informed of the current situation worldwide regarding the incidence of polio and the move towards eradication of the disease. A Global Action Plan for laboratory containment of wild polioviruses used in vaccine production had been developed in 1999 and a revised version was published in 2003. The Committee was reminded of the special biohazard risk factors associated with the manufacture of inactivated polio vaccine when wild type polioviruses are used as starting materials for vaccine production. Although the risk to workers is low, the consequences of inadvertent release from manufacturing facilities would increase as the levels of wild poliovirus in the community decrease. To minimize this risk, the Global Action Plan has proposed increased biosafety containment of wild polioviruses during the manufacture and quality control of inactivated poliovirus vaccine. To facilitate this process it had been decided that guidance relating to the manufacture of inactivated polio vaccine under improved biosafety conditions should be drafted. Initial drafts had been the subject of considerable discussion with industry and regulatory authorities. It became clear that it would not be commercially feasible nor scientifically justified to meet the

most demanding requirements at biosafety level 4 for manufacture. Containment at a biosafety level 3 is practicable and will meet the goal of decreasing risk of accidental release from manufacturing sites. The Committee reviewed the draft Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses (BS/03.1951) proposed as an addendum to the Recommendations for the production and quality control of poliomyelitis vaccine (inactivated). After making further modifications, the Committee adopted the text as an addendum to the Recommendations for the production and control of poliomyelitis vaccine (inactivated) and agreed that it should be annexed to its report (Annex 2).

### **Recommendations for the production and control of group C meningococcal conjugate vaccines (addendum)**

The Committee was informed of developments in the use of group C meningococcal conjugate vaccines for routine immunizations of children and infants. These had resulted in the proposal at the fifty-second meeting (WHO Technical Report Series, 924) to draft an addendum to the already adopted Recommendations for the production and control of group C meningococcal conjugate vaccines. This addendum would provide guidance to manufacturers and national regulatory authorities on serological assays to evaluate the immunogenicity of these vaccines as part of the process to demonstrate efficacy before large-scale immunization took place. Two such assays are available: a serum bactericidal antibody assay and an enzyme-linked immunosorbent assay (ELISA). The Committee reviewed the draft document (BS/03.1952) and, after making some modifications, adopted the text as an Addendum 2003 to the recommendations for the production and control of group C meningococcal conjugate vaccines (revised) 2001 and agreed that it should be annexed to its report (Annex 3).

### **Guidelines on regulatory expectations related to the elimination, reduction or replacement of thiomersal in vaccines**

The Committee reviewed the draft WHO Guidelines on regulatory expectations related to the elimination, reduction or replacement of thiomersal in vaccines (BS/03.1956). The Guidelines are intended to provide information and guidance to national regulatory authorities and vaccine manufacturers regarding changes in the thiomersal content of already licensed vaccines that contain thiomersal. The proposals were based on a Consultation held in Geneva on 15–16

April 2002.<sup>1</sup> After making several changes to the text, the Committee adopted the revised text as Guidelines on regulatory expectations related to the elimination, reduction or replacement of thiomersal in vaccines, and agreed that it should be annexed to its report (Annex 4).

### **Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives**

The Committee was informed of the need to update the Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (WHO Technical Report Series, No. 840, 1994). During the past few years, two particular areas where additional guidance was considered to be necessary had been identified (BS/03.1964). The first concerned procedures for virus inactivation and removal intended to assure the viral safety of human blood plasma products. The second was the need to prepare a revised document covering collection, processing and quality control of human plasma. The goals of revision are to ensure the safety of blood and blood products; to assure plasma of a quality suitable for fractionation, and to set out the principles on which formulation of local requirements by regulatory authorities could be based. It was proposed to identify those sections in the current Requirements requiring modification and the new sections that would be required to improve blood safety.

The Committee agreed that the Requirements were outdated in some respects and should be revised. It discussed possible strategies for revision and concluded that complete revision of the existing Requirements would be preferable. Although it might be possible to divide the document into several separate documents, the Committee counselled against division and, on balance, favoured retention of all guidance in a single document. However, different areas of expertise would be required to undertake revision of the different parts of the document and this work could be undertaken in distinct stages. It was considered important that WHO continues to provide recommendations in this area. Guidance on good manufacturing practice in the blood products field was also needed and should be integrated into the Requirements. The Committee welcomed a proposal to begin revision and requested the Secretariat to consult with appropriate

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<sup>1</sup> A summary of the meeting was subsequently published as "Knezevic I, Griffiths E, Reigel F, Dobbelaer R (2004). Thiomersal in vaccines: a regulatory perspective. Vaccine, volume 22, pages 1836–41.

bodies, such as the Global Collaboration on Blood Safety, and to report back at the next meeting of the Committee.

### **Recommendations for the production and quality control of poliomyelitis vaccines (oral)**

The Committee was informed of the conclusions of a joint WHO/European Directorate for the Quality of Medicines/Agence Française de Sécurité Sanitaire de Produits de Santé meeting on quality control of monovalent bulks of oral poliovirus vaccine held in Lyon, France, in November 2002. The meeting reviewed the experience gained over 20 years of applying the monkey neurovirulence test for polio to more than 1000 batches of vaccine. Although some deviations from the recommended procedure had occurred, the results had remained valid. Because expertise in conducting the test procedure is diminishing, the participants had recommended that WHO should ensure that competence and good practice is maintained for the monkey and transgenic mouse neurovirulence tests in at least two laboratories, and that suitable funding for this be obtained. The histopathological slides that exist are a unique resource and it was proposed that they be held in an archive for training purposes and that those performing the evaluation of the slides should meet informally on a regular basis. It would be desirable for the transgenic mouse neurovirulence test to be approved by regulatory authorities using consistent criteria. The molecular analysis by PCR restriction enzyme cleavage (MAPREC) assay is coming into extensive use. Moves to revise the specification in the European Pharmacopoeia should help this process. It was recommended that batches exceeding the limits specified by WHO should be examined by the *in vivo* procedure and could be considered as potential reference reagents (positive control materials). Finally it was recommended that all remaining stocks of WHO reference materials be transferred to the WHO Laboratory at the National Institute for Biological Standards and Control, so that they can be made available rapidly from a single centre. The Committee endorsed the recommendations from the meeting, in particular those relating to maintenance of expertise in animal testing and assessment of histopathological data. The Committee requested the Secretariat to raise the issues involved at the highest appropriate level; to take the necessary steps to facilitate their implementation; and to publish the report of the meeting as soon as possible.

### **Stability evaluation of vaccines: regulatory expectations**

The Committee was reminded that the existing stability guidelines do not address the special considerations relating to vaccines and other

biologicals. Following discussion at, and recommendations from, its forty-ninth and fifty-first meetings, a WHO Drafting Group had been formed to prepare Guidelines for regulatory expectations on the stability evaluation of vaccines. The Committee was informed that the Group had met at Langen, Germany, in February 2002 and had reviewed existing Guidelines and identified the major issues that would need to be addressed in the new WHO document. The concept of the document and its structure were outlined and the general approach and the plan of action were endorsed by the Committee.

### **Guidelines on nonclinical evaluation of vaccines: regulatory expectations**

The Committee was informed that a Guideline on regulatory expectations for nonclinical evaluation of vaccines had been drafted to provide guidance to regulatory authorities and manufacturers. This Guideline is intended as a companion document to the Guidelines on clinical evaluation of vaccines adopted by the Committee at its fifty-second meeting in 2001 (WHO Technical Report Series, 924). The document is intended to apply to vaccines for preventive use. It had been reviewed at a WHO Informal Consultation held in Geneva in December 2002. The Committee was advised of a number of changes proposed to the draft and of issues that would need to be resolved before the document could be completed. The Committee recommended that the scope of the document be focused on preventive and therapeutic vaccines for infectious diseases at this stage, although the scope could be extended in the future. It also made a number of suggestions to improve the draft that should be referred back to the Group responsible.

### **Gene transfer medicinal products**

The Committee was informed of a Consultation of the WHO Clinical Gene Transfer Monitoring Group held in Geneva in May 2002. The report of the meeting summarized the relevant vectors and gene transfer methods currently in development, addressed regulatory aspects and guidance documents and considered issues of nomenclature. The Committee was advised of recent developments and progress in clinical trials, most of which are in phase I or II and directed to orphan disease conditions. The results have been variable. The positive results obtained have been offset by recent severe adverse reactions such as the death of one patient and the development of lymphomas in two others. The Committee noted the information and requested the Secretariat to keep it informed of further developments.

## **Task force on cell substrates**

The Committee was informed of the outcome of a meeting of an ad hoc WHO Task Force on Cell Substrates held in Geneva in June 2002. The Committee noted that the major subjects discussed at the meeting were contamination with reverse transcriptase, SV40 and simian CMV. Since the previous WHO consultation no evidence of infection in recipients of vaccines showing low levels of reverse transcriptase had emerged nor was there any evidence of pseudotype formation. From studies of SV40, it appeared that viral DNA was detectable in certain tumours. However, there is no evidence of an increased incidence of these tumours in children who had received contaminated polio vaccines nearly 40 years ago. Finally, studies of polio vaccine showing simian CMV DNA contamination have failed to detect infective virus. Nevertheless, the introduction of specific tests for simian CMV should be considered in a revision of the WHO Recommendations for the production and quality control of poliomyelitis vaccine, oral. The Committee endorsed the conclusions and recommendations of the Task Force.

## **World Health Organization cell banks**

The Committee was informed of the outcome of a meeting of the WHO Monitoring Group on Cell Banks held at the National Institute for Biological Standards and Control in October 2002. The Group had reviewed the requirements of cell banking, issues relating to the WHO Vero cell bank 10–87, and cell banks for diagnostic purposes. The Monitoring Group had drawn a number of conclusions on these topics. The Committee was advised that records relating to the Vero cell bank may be incomplete and that the status of the cells should therefore be revised to a well-characterized cell seed from which master cell banks may be derived. The Committee noted that it would be necessary to revise the Requirements for the use of animal cells as in vitro substrates for the production of biologicals (WHO Technical Report Series No. 878, 1998) with respect to the status of the WHO Vero cell bank. The Committee endorsed the conclusions and recommendations of the Monitoring Group. In particular, the Committee agreed that the proposal to prepare and validate an MDCK cell bank for influenza surveillance was a high priority. Such a cell bank should be characterized to the standard required for production because viruses isolated in such cells may be used as virus seed for vaccine production.

## **Human and animal transmissible spongiform encephalopathies and the safety of biologicals**

The Committee was reminded of the Report of a WHO Consultation on medicinal and other products in relation to human and animal transmissible spongiform encephalopathies (TSEs) (WHO/EMC/ZOO/97.3; WHO/BLG/97.2). The Committee was informed that a further WHO Consultation on TSEs in relation to biological and pharmaceutical products had been held in Geneva in February 2003. This Consultation had considered the risks of transmission from animal to humans and between humans and there had been a high level of agreement among the invited experts. Bovine spongiform encephalopathy (BSE) presents a global risk and is transmitted through the food chain. Cases of variant Creutzfeld-Jacob disease (vCJD) had occurred in a number of countries; the cases were both of indigenous origin and probably through external infection. Since 1997, there had been changes in the epidemiology of BSE and of vCJD. In view of these and other developments, it was intended that the information in the 1997 report should be revised to provide up-to-date guidance, for example on the categorization of tissues in humans, cattle, sheep and goats as to whether they present high risk, low risk or no detectable risk of infectivity.

The Committee welcomed this review of the current information on TSEs. The final report of the 2003 Consultation had not yet been completed and approved by the participants. However the Committee recommended that the report be finalised<sup>1</sup> as soon as possible and be presented at its next meeting so that its wider implications for biological standardization could be considered.

## **Requirements for diphtheria, tetanus, pertussis and combination vaccines**

The Committee was informed of the outcome of a WHO Consultation on the potency assay and measurement of consistency of diphtheria and tetanus vaccines, held at Bilthoven, the Netherlands, in December 2002. These vaccines had been used successfully over a long period of time in many countries. However, there is increasing trade of vaccines between countries and combination vaccines are growing in importance. The absence of an international consensus regarding assay procedures and minimum potency requirements is adversely affecting the international exchange of vaccines. Changes

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<sup>1</sup> The report was finalised after the ECBS meeting and is available as "WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products (WHO/BCT/QSD/2003. 01)."

had taken place over a number of years to improve standardization of requirements, but international differences remain in assay methods as well as in vaccination schedules. These have an impact on WHO vaccination programmes. The meeting had been convened to review and discuss progress towards simplified lot release assay and resolution of technical issues and it had been concluded that such a development is achievable. In some parts of the world, regulatory authorities are moving towards the adoption of the simplified lot release assay, using test and reference vaccines, for routine use after the vaccine had been authorized on the basis of data generated using a full, functional assay system. It was agreed that the control vaccine should not be a clinical lot, but a heterologous, generic preparation. The lack of comparability between assays was recognized as a barrier to trade in vaccines internationally. Clear guidance will have to be developed to describe the circumstances under which the simplified assay will be used how it will be used, and acceptance criteria for the assay. The developments that had been discussed will entail revision of the present requirements for diphtheria, tetanus, pertussis and combined vaccines (WHO Technical Report Series 800, 1990).

The Committee welcomed the positive steps that had been taken. It recommended that further global consultation should take place before proposals for revision of the current Requirements are presented to it. The Committee noted that the availability of suitable reference materials is under review.

### **Proposals for discontinuation of Requirements and Guidelines**

At its forty-ninth (WHO Technical Report Series, No. 897, 2000) and fifty-second meetings (WHO Technical Report Series, 924), the Committee had decided not to retain responsibility for recommendations and reference materials for veterinary use and to discontinue them. The Committee had requested that the Office International des Epizooties (OIE) be informed of this decision and had asked for confirmation that the Office had no objection. The Committee was informed that OIE had not raised objections. The Committee, therefore, discontinued the following Requirements and Guidelines:

- Requirements for anthrax spore vaccine (live, for veterinary use) 1966 (WHO Technical Report Series, No. 361, 1967);
- Requirements for rinderpest cell culture vaccine (live) and rinderpest vaccine (live) 1969 (WHO Technical Report Series, No. 444, 1970);
- Requirements for *Brucella abortus* strain 19 vaccine (live, for veterinary use) 1969 (WHO Technical Report Series, No. 444,

- 1970) and Addendum 1975 (WHO Technical Report Series, 594, 1976);
- Requirements for *Brucella melitensis* strain rev 1 vaccine (live, for veterinary use) 1976 (WHO Technical Report Series, No. 610, 1977);
  - Requirements for rabies vaccine for veterinary use 1980 (WHO Technical Report Series, 658, 1981) and Amendment 1992 (WHO Technical Report Series, 840, 1994);
  - Requirements for Rift Valley fever vaccine (live attenuated) for veterinary use 1983 (WHO Technical Report Series, 700, 1984).

## **International reference standards**

### **Recommendations for the preparation, characterization and establishment of international and other biological reference standards**

The Committee was informed that a proposed revision of the current Guidelines for preparation, characterization and establishment of international and other standards and reference reagents for biological substances (WHO Technical Report Series, 800, 1990) had been drafted. Many changes had been made including the addition of a new section on quality assurance, more details on processing and introduction of the concept of regional reference materials. Editorial changes had also been made. The Committee recommended the addition of an expanded section addressing the scientific basis for calibration of, and potency assignment to, the first and replacement reference materials; how to design such collaborative studies and their statistical analysis. Additional guidance on the role and use of WHO reference materials was considered important. Guidance on when the standards should be used, how frequently and under what circumstances is required. It should also include the reasons for and ways in which the use of WHO primary reference materials as working standards can be avoided through the establishment of national, regional and other reference materials. It may be necessary to prepare a separate Appendix or to draft a separate document to provide this additional guidance. The Committee recommended that the draft should be revised as noted, on the basis of input from interested experts.

### **Antibodies**

The Committee noted inconsistencies in the designation and activity assignment to antibody reference materials and requested the Secretariat to investigate this issue and make proposals for amendments at a future meeting.

## **Anti-parvovirus B19 plasma**

The Committee was informed that measurement of levels of parvovirus in plasma is an important diagnostic procedure and that stocks of the current International Standard for parvovirus B19 are almost exhausted. The Committee noted a proposal to establish a replacement reference material for anti-parvovirus B19 plasma, (BS/03.1960), based on a collaborative study performed by nine laboratories in eight countries. The study showed that the different test methods employed in the collaborative study have different specificities and only the results obtained with kits containing the VP2 antigen were used for calibration of the candidate material. The participants in the study had agreed with this approach and that a replacement reference material is desirable. The candidate preparation showed adequate stability in studies that will be continued. After discussion and on the basis of the results obtained, the Committee established the preparation, in ampoules coded 91/602, as the Second International Standard for anti-parvovirus B19, plasma, human and assigned an activity of 77 International Units per ampoule to it. The Committee recommended that attention be drawn in the documentation accompanying the standard to the fact that only the results for VP2 antigen were used in the potency assignment.

## **Anti-human platelet antigen 5b**

The Committee recalled that a decision to establish a reference material for anti-human platelet antigen (Anti-HPA) 5b had been taken at its fifty-second meeting, subject to endorsement by the Scientific Sub-Committee of the International Society on Thrombosis and Haemostasis (ISTH), which had now been received. The Committee, therefore, confirmed its establishment of the preparation, coded 99/566, as the First WHO Reference Reagent for anti-human platelet antigen 5b. It should be used as a minimum sensitivity reagent at a dilution of 1 in 2; this is the minimum dilution at which a test should be positive, and laboratories should use the Reference Reagent to validate new assays or alterations to existing assays.

## **Antigens and related substances**

### **Flavivirus reference materials**

The Committee received a report on progress towards the preparation of reference materials for flaviviruses. In the past the results of cell-based assays for yellow fever vaccine had not correlated with data derived from mouse potency assays. Reference materials are being

evaluated to improve this correlation. In a second study, which concerned dengue virus, sera from infected individuals were being analysed for calibration of VERO cell neutralizing antibody assays. A similar study on antibodies to Japanese encephalitis virus is also in progress.

## **Blood products and related substances**

### **Anti-D immunoglobulin**

The Committee was informed that the existing International Standard had been found positive for hepatitis C virus and should be replaced. The Committee was also informed that stocks of the US Standard were almost exhausted and that the European authorities were interested in establishing a secondary, regional reference preparation. Consequently, an extensive study had been performed on several preparations using a variety of methods. The Committee noted the proposal to establish a replacement reference material for anti-D immunoglobulin (BS/03.1962 and Addendum 1), based on a collaborative study performed by 25 laboratories in 15 countries. Because the candidate preparation showed no evidence of degradation after storage at 37 °C for 1 year, it appeared to have good stability. The stability studies will be continued. On the basis of the results obtained, the Committee established the preparation, in ampoules coded 01/572, as the Second International Standard for anti-D immunoglobulin and assigned an activity of 285 International Units per ampoule to it. Portions of the same batch of material will be supplied to and used by the Center for Biologics Evaluation and Research and the European Directorate for the Quality of Medicines.

The Committee disestablished the remaining stock of the first International Standard but recommended that the National Institute for Biological Standards and Control continues to hold the stock.

### **Reference panel for human immunodeficiency virus-1 RNA genotype**

The Committee was informed that human immunodeficiency virus (HIV)-1 exhibits substantial genetic diversity. However, NAT-based assays often have narrow specificity and may therefore give inaccurate or false-negative results. Consequently the availability of a reference panel would aid the evaluation and application of such assays. The Committee noted a proposal to establish a reference panel for HIV-1 RNA genotype (BS/03.1961), based on a collaborative study performed by 28 laboratories in 16 countries. The panel consists of a

set of 10 viral strains and genotypes, where possible having a full-length genomic sequence. The cell-free culture supernatants from propagation of viral isolates were diluted until they contained comparable numbers of copies/ml based on four different NAT assay systems. However, quantitation of HIV-1 RNA through the collaborative study was not possible. The preparations are stored at  $-70^{\circ}\text{C}$  and their stability will be monitored. Similar materials had already been stored under these conditions for more than two years without change. On the basis of the results obtained, the Committee established the reference panel of 10 individual genotypes, in vials coded 01/466, as the first International Reference Panel for HIV-1 RNA genotypes, but did not assign unitages to its components. For traceability, the individual genotypes within the Panel should be uniquely identified. The Committee also recommended that the Panel should be made available to laboratories to allow them to gain experience in its use and that studies should continue with the ultimate aim of assigning a suitable quantitative property to the constituent materials.

## **Hepatitis A RNA**

The Committee noted a proposal to establish an International Standard for hepatitis A RNA (BS/03.1959), based on a collaborative study performed by 16 laboratories in 10 countries in which five candidate materials had been examined. The assays employed were in-house assays and the variation observed between assays was similar to that observed with related RNA determinations. Of the five preparations in the study, the preparation assigned the code AA, was considered by the participants at the fifteenth meeting of the Working Group on the Standardisation of Gene Amplification Techniques for the Virological Safety Testing of Blood and Blood Products (SoGAT) as the most suitable for use as the primary reference material.

The preparation has shown adequate stability in testing so far and the stability studies will be continued. On the basis of the results obtained, the Committee established the preparation, in vials coded 98/552, as the first International Standard for hepatitis A RNA and assigned an activity to it of 50000 International Units per vial. The Committee requested that results of the further stability studies be reported to it in due course. The Committee noted that the preparation coded BB in the collaborative study might be a suitable candidate replacement when stocks of the First International Standard are exhausted.

## **Hepatitis C RNA**

The Committee was informed that stocks of the first International Standard for hepatitis C virus RNA were almost exhausted. The Committee noted a proposal to establish a replacement International Standard for hepatitis C RNA (BS/03.1958), based on a collaborative study performed by three laboratories in three countries. The study, which had been designed to provide reassurance of the validity of the data obtained previously, had a limited number of participants due to the unavailability of sufficient samples. The candidate replacement standard had been included in the original collaborative study and no significant difference between the two preparations had been detected. The preparation showed adequate stability as demonstrated by lack of change in its behaviour relative to that of the First International Standard. However, the Committee noted that the stability data did not exclude the possibility that both the First International Standard and its candidate replacement had degraded to a similar extent. On the basis of the results obtained, the Committee established the preparation, in vials coded 96/798, as the second International Standard for hepatitis C RNA and assigned to it an activity of 50 000 International Units per vial. The Committee requested that recommendations regarding the design of stability studies should be considered at a future meeting.

## **Thrombin**

The Committee was informed that stocks of the current International Standard were almost exhausted. The Committee was also made aware that the use of the reference material had changed in recent years to reflect increasing use of new therapeutic products. The Committee noted a proposal to establish a replacement reference material for thrombin (BS/03.1955 and Addendum 1), based on a collaborative study performed by 25 laboratories in 15 countries, in which both clotting and chromogenic assays had been employed. The results obtained using the chromogenic methods had been analysed separately and had not been used in the determination of the potency of the candidate material. The preparation showed adequate stability with a predicted loss of activity of 0.10% per year at  $-20^{\circ}\text{C}$ . On the basis of the results obtained, the Committee established the preparation, in ampoules coded 01/580, as the second International Standard for thrombin and assigned a potency of 110 International Units per ampoule to it.

Differences had been observed in the past in measurements of the same sample when calibrated against the WHO and the US standards. The Committee was informed that the current US Standard would be

replaced with the same material as the second International Standard to allow use of a common reference material and common unitage.

### **Fibrinogen, plasma**

The Committee was informed that high levels of fibrinogen had been shown to correlate with high risk of thromboembolic disease. The current International Reference materials have low concentrations of fibrinogen and concern has been expressed that they may be unsuitable for determination of high levels of the analyte. The Committee noted the progress report on the collaborative study of three preparations having high levels of fibrinogen being undertaken by 17 laboratories in 10 countries using a variety of assay methods. The value of the study was limited in that the laboratories had used a range of reference materials and therefore not all results could be compared. It was proposed that the raw data from the study be sent to the National Institute for Biological Standards and Control for analysis.

The Committee expressed concern that the peer review requirements normally applied by WHO for candidate materials and collaborative studies appear not always to be properly understood by outside organizations. The Secretariat agreed to review experience in the standards setting process with external bodies and report back at a future meeting.

## **Cytokines, growth factor and endocrinological factors**

### **Erythropoietin**

The Committee was informed that stocks of the first International Standard for Erythropoietin, recombinant, are almost exhausted. A number of preparations had been included in the collaborative study on the basis of which the first Standard had been established, and three of these had been recognized at the time as candidate replacement materials. The Committee noted a proposal to establish a replacement reference material for recombinant erythropoietin (BS/03.1963), based on the results of the original collaborative study performed by 26 laboratories in 11 countries (BS/90.1650). The Committee also noted that the relative potencies of the candidate preparation and the current standard as determined by bioassay and immunoassay are very similar. A recent stability study of the preparation stored for more than 13 years at 37°C compared with material stored at -20°C for the same period of time showed excellent stability. On the basis of the information supplied, the Committee established the preparation,

in ampoules coded 88/574, as the second International Standard for erythropoietin, recombinant and assigned to it an activity of 120 International Units per ampoule, based on results obtained by in vivo bioassay. The Committee noted that the potency assigned may not be appropriate for use in types of assay other than in vivo bioassays. The Committee recommended that the National Institute for Biological Standards and Control undertake studies to assess whether a separate standard for immunoassay is required.

### **Interferon beta**

The Committee was informed of progress in the international collaborative study of new and existing reference preparations for interferon beta. The study involved 16 laboratories in eight countries and the results were being analysed. The Committee noted that the completed analysis of this study was likely to be presented at its next meeting.

## **Diagnostic reagents**

### **Lipoprotein (a)**

The Committee was informed that the presence of high levels of lipoprotein (a) is a genetically determined marker of predisposition to coronary heart disease. Because accurate measurement of lipoprotein (a) is important for diagnosis, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) had established a Working Party for standardization of lipoprotein (a). The Working Party had performed a series of studies to calibrate the lipoprotein (a) content of pooled human serum in terms of purified preparations of lipoprotein (a). The Committee noted a proposal to establish a reference material for lipoprotein (a) (BS/03.1953), based on a collaborative study performed by 18 laboratories in 10 countries. The stability of the preparation had been assessed in various real-time and accelerated studies and appeared to be adequate. After discussion, the Committee requested that additional information be supplied before a final decision on adoption of this reference material is taken, and welcomed the further collaboration of the IFCC.

### **International Reference Materials for diagnosis and study of transmissible spongiform encephalopathies**

The Committee noted a proposal from the WHO Working Group on International Reference Materials for Diagnosis and Study of TSEs to establish WHO Reference Reagents for in vitro assays of

specimens for CJD prions (BS/03.1965). The Secretariat recalled that various meetings had been held to facilitate the development and comparison of procedures for the diagnosis and study of TSEs. The Committee noted the results, obtained with brain tissue using immunoblot methods, from a study by six laboratories in five countries. The materials consisted of 10% w/v homogenates of human brain tissue in 0.25 M unbuffered sucrose, stored frozen at  $-86^{\circ}\text{C}$ . The tissues were obtained from one healthy human, two individuals who had developed sporadic CJD and one who had developed vCJD. Having considered the data, the Committee established the preparation, coded RU 97/03, as the first Reference Reagent for human brain, CJD control; the preparation, coded RU 99/009, as the first Reference Reagent for human brain, sporadic CJD preparation 1; the preparation, coded RU 99/008, as the first Reference Reagent for human brain, sporadic CJD preparation 2; and the preparation, coded RU 98/148, as the first Reference Reagent for human brain, variant CJD. The Committee did not assign activities to them. The Committee noted that the preparations would usually be supplied as a panel of reference reagents including the human brain control, but that the individual preparations would also be available separately. Their availability would allow different assay systems to be compared and guidance on their use is available. The Committee noted that their use is being evaluated further in *in vitro* and *in vivo* assays.

## **WHO Consultation on International Standards for *in vitro* clinical diagnostic procedures based on nucleic acid amplification techniques**

The Committee was informed of a WHO Consultation on International Standards for *in vitro* clinical diagnostic procedures based on nucleic acid amplification techniques held in Geneva in April 2002. At this meeting, the participants agreed to study the use of synthetic target nucleic acids as candidate reference materials. The objective of the study was to compare quantitative values obtained from different NAT assays for a panel of samples positive for hepatitis C (HCV) RNA using the synthetic transcript as the calibrant. The Committee noted the results of the feasibility study on HCV performed by five industrial laboratories to evaluate a synthetic HCV RNA as a candidate standard. The study had shown that the approach adopted had promise, but that further studies are necessary to characterize the material; examine the matrix in which the materials would be diluted and to include all relevant NAT assays. The Committee agreed that

this work should continue and requested the Secretariat to consider the implications for eventual replacement of the current International Standard for hepatitis C RNA.

## **Miscellaneous**

### **Mutant analysis by polymerase chain reaction and restriction enzyme cleavage assays for poliovirus**

The Committee was informed that a single base change in poliovirus type 2 was associated with an increase in neurovirulence. A mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) assay had been developed that could monitor this change and be used to check consistency of vaccine production. Reference preparations had been developed to calibrate the assay. The Committee noted a proposal to establish these reference materials for the MAPREC assay of poliovirus type 2. On the basis of a collaborative study performed by nine laboratories in seven countries, the preparations appeared to be suitable for this purpose (BS/03.1957). The stability of the preparations had been demonstrated in studies conducted over a period of four years. The Committee therefore established the preparation, in ampoules coded 97/758, as the first International Standard for MAPREC assay of poliovirus type 2 and assigned an activity of 0.67% 481-G per ampoule to it. The Committee also established the preparation, in ampoules coded 98/596, as the first Reference Reagent (high mutant virus) for MAPREC assay of poliovirus type 2 and assigned an activity of 1.21% 481-G per ampoule to it; the preparation, in ampoules coded 97/756, as the first Reference Reagent (low mutant virus) for MAPREC assay of poliovirus type 2 and assigned an activity of 0.65% 481-G per ampoule to it; and the preparation, in ampoules coded 98/524, as the first Reference Reagent (100% 481-G) for MAPREC assay of poliovirus type 2 and assigned an activity of 93.5% 481-G per ampoule to it. These three reagents form a reference panel for calibration of the MAPREC assay of poliovirus type 2. In the Recommendations for the production and control of poliomyelitis vaccine (oral) (WHO Technical Report Series, No. 904, 2002), the MAPREC assay is recommended for testing the consistency of batches of polio vaccine. The Committee noted some discrepancies in the report BS/03.1957 and requested that these be corrected in an Addendum to be provided to the Secretariat.

The Committee was advised that a collaborative study is in progress on validation of MAPREC assays for poliovirus type 1 and to assess candidate reference materials. In the case of type 1 poliovirus,

increased neurovirulence is associated with changes at two sites in the nucleic acid. The study has shown the greater difficulty in developing a suitable method for type 1 than for type 2. The Committee noted the urgent need for this work to be completed.

### **Papillomavirus reference materials**

The Committee was informed that over 99% of cases of cervical cancer are associated with sexually transmitted human papillomavirus DNA. Cervical cancer is thus the main disease associated with the virus although the virus is also associated with other diseases. Cervical cancer is a major type of cancer in many female populations. The Committee was also informed that candidate vaccines are under development and initial testing had shown them to be safe, immunogenic and well tolerated and to offer complete protection against infections with human papillomavirus. Reference reagents are required to evaluate antibody responses to these vaccines and to monitor the incidence of disease. Collaborative studies on the value of such reagents in diagnostic procedures for human papillomavirus DNA detection and in serological type-specific assays have been initiated. It is intended to initiate similar studies relating to assays for cellular mediated immunity. The results of these studies are being analysed and proposals could be made to the Committee at its next meeting.

## Annex 1

# **Recommendations<sup>a</sup> for the production and quality control of smallpox vaccine, revised 2003**

Recommendations published by WHO are intended to be scientific and advisory. Each of the following sections constitutes guidance for national regulatory authorities and for the manufacturers of biological products. If a national regulatory authority so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by a national regulatory authority. It is recommended that modifications to these Recommendations be made only on condition that the modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the Recommendations set out below. The parts of each section printed in small type are comments for additional guidance intended for manufacturers and national regulatory authorities, which may benefit from those details.

In these Recommendations, Part A describes the general provisions for the production and quality control of smallpox vaccine and is applicable to production of vaccine in all permissible substrates, including the use of a cell bank. Part B describes national control requirements. The terms “national regulatory authority” and “national control laboratory”, as used in these Recommendations, always refer to the country in which the vaccine is manufactured.

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<sup>a</sup> Previously called Requirements for Smallpox vaccine.

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## Introduction

The Recommendations (formerly Requirements) for Production and Control of Smallpox Vaccines were last revised in 1965 (1). Since that time an intensified global eradication programme implemented from 1967 to 1980, and led by WHO, has resulted in the global eradication of smallpox (2). This was achieved by the globally coordinated use in national immunization programmes of effective vaccines that met the quality specifications in the 1965 Requirements. The last naturally occurring case of smallpox was reported in 1977. In addition to the availability of effective vaccines, an efficient infrastructure was established worldwide embracing the production, supply and administration of smallpox vaccine. Good surveillance, diagnosis of disease, training and public health information were additional important elements in successfully combating smallpox.

After human-to-human transmission of smallpox had been interrupted worldwide the Global Commission that certified eradication was of the opinion that the likelihood of reintroduction of smallpox from laboratories or natural or animal reservoirs was negligible. Nevertheless it recommended that it would be prudent for WHO and national health authorities to be prepared for unforeseen circum-

stances. One measure was to ensure that adequate reserves of potent vaccine remained available (3). Accordingly a global stockpile of vaccine was established, as were national stockpiles. In addition seed lots of vaccinia virus suitable for the preparation of smallpox vaccine were maintained in a designated WHO collaborating centre. However in March, 1986, the Committee on Orthopoxvirus Infections concluded that the maintenance of a global reserve by WHO was no longer indicated (4), and interest in maintaining stocks rapidly declined. A survey conducted by WHO in 2001 found that only small amounts of stockpiled smallpox vaccines still existed. These stocks are distributed quite unevenly around the world and are accessible to only a very selected part of the global population. Additional production would be needed to meet any major demand on vaccine supply such as might follow an intentional release of smallpox vaccine.

Global resumption of smallpox vaccine production would benefit from modern concepts of production and control, and modern regulatory expectations should be adhered to in the licensing process. These general principles should apply to new vaccine manufactured in embryonated eggs, or in primary or continuous cell lines, or animal skins. In addition, contemporary international reference materials to determine the potency of new vaccines and their immunogenicity in vaccinated individuals would be beneficial. The WHO Secretariat has followed these principles in producing this reformulated document and has included changes to bring the document into line with other WHO Recommendations published since the last revision.

## **General considerations**

Since the cessation of routine smallpox vaccination after the successful eradication of the pathogen, population susceptibility has increased with each unvaccinated birth cohort. After the terrorist events of 11 September 2001 in the USA, and the subsequent anthrax-related incidents in the United States, heightened concern has been expressed by Member States about potential intentional release of microorganisms. Even though the risk of the deliberate use of smallpox against civilian populations is considered to be low, there is consensus regarding the need to:

- develop a coordinated response effort;
- include sufficient numbers of doses in a strategic reserve of vaccines;
- enhance the public health infrastructure; and
- improve disaster mitigation capacity.

Since the last revision of the WHO requirements for smallpox vaccines, the principles governing regulation of medicinal products, in particular biological medicinal products including vaccines, have evolved significantly in response to increased knowledge in this field and to advanced technologies. New vaccine production should take into account these developments, and all new vaccines, including those intended for strategic reserves, should be subject to the current national licensing processes for biological medicines. It is recognized however that the use of vaccines stockpiled from the eradication programmes or of new vaccines still undergoing evaluation may be justified, if supported by an appropriate risk–benefit analysis, in cases of national emergency.

Licensing of a new vaccine usually requires the demonstration of its efficacy against the natural infection in a clinical trial. This is not possible in the case of new smallpox vaccines because the natural infection has been eradicated. One approach that has been taken is to develop a new vaccine that is phenotypically similar to a vaccine known to be successful in the eradication initiative, but other approaches are also possible. Immunological correlates of protection are not defined for vaccinia virus. However pock formation in humans after smallpox vaccination is a marker of vaccine effectiveness. The formation of papules, vesicles and pustules with an appropriate appearance within a predefined time frame may be used as a marker to support the acceptability of a new smallpox vaccine. Other parameters such as levels of neutralizing antibodies (NA) or haemagglutination inhibiting (HI) anti-vaccinia virus antibodies can presently be considered only as supportive information. Further research is needed in this area. Challenge studies in a relevant animal model (e.g. mouse/vaccinia virus and monkey/monkeypox virus) may provide additional evidence on the protective efficacy of new smallpox vaccines.

Many general and specific aspects of the production and control of (live) viral vaccines, such as the origin, quality and certification of starting materials, cell substrate issues, specific pathogen free status of eggs, viral safety, method and process validation, testing procedures and principles of good manufacturing practice are not adequately covered by the 1965 WHO Guidelines. All of these issues have been addressed in this revision.

The 1965 requirements for smallpox vaccines focused on production on animal skin, in embryonated eggs and in chick embryo fibroblast cells (CEFs). Production in other cell lines was not covered. There is considerable interest in the production of new smallpox vaccines in

either Vero cells, human diploid cells or primary rabbit kidney cells, and other cells such as quail cells have been used in the past. A new section is therefore included that covers the production and control of cell substrate produced vaccine. Cell culture vaccines were not used in areas endemic for smallpox during the eradication campaigns and their efficacy against smallpox has not been demonstrated. Therefore an important parameter to establish is that the cell substrate does not have a negative effect on the safety and/or efficacy of the vaccine virus. Such effects are not predictable and considerable efforts are thus needed to show that a new cell culture-derived vaccine has similar preclinical and clinical properties to a comparator vaccine with a known safety and efficacy profile.

Adventitious agent testing for viruses in the vaccine virus seeds and product intermediates is complicated because complete neutralization of vaccinia virus is difficult to achieve. Testing for viral adventitious agents in eggs, animals and tissue culture might give ambiguous results. Although these tests remain the gold standard, supplementary testing to detect specific viral adventitious agents using validated polymerase chain reaction (PCR)-methodology or immunochemical methods is envisaged. The scope of the test programme depends on many parameters such as the nature and origin of the virus seed. For smallpox vaccines produced on animal skin, special attention should be given to the health status of the animals. Testing for viral adventitious agents in animal skin vaccine should depend on the animal species, the origin and on epizootiological considerations in the source country of the animals. Reference has been made to the guidelines on transmissible spongiform encephalopathy (TSE) and these should be adhered to. The bioburden of new vaccines produced in animal skin can be reduced by state-of-the art animal husbandry in dedicated facilities. A revised (stricter) specification for bioburden in the final product is introduced in these guidelines. However, since the production process on animal skin may be very difficult to validate, consistent sterility of the finished product may be difficult to achieve. The use of non-sterile final product may be justified because smallpox vaccine is administered in a very low volume by scarification rather than by intramuscular or intravenous inoculation, and specified pathogens are excluded from the vaccine. In addition the history of use of the vaccine produced on animal skin defined its safety profile and unambiguously demonstrated its efficacy.

An animal model to predict the neurovirulence of vaccinia virus has been introduced. It had previously been shown that vaccinia viruses could be classified according to their high, medium and low neuropathogenic potential in humans (5). Although the histological pattern

that is seen in the mouse brain following intracerebral inoculation of different vaccinia strains is different to the histological changes found in the human brain following encephalitis after vaccination, the mouse model reflects the neuropathogenic potential of vaccinia strains in humans. The mouse model is introduced for phenotypic characterizations of both the seed material and of each lot of bulk suspension produced until sufficient validation data are available to reduce the frequency of testing.

Future research is anticipated to focus on highly attenuated vaccinia virus strains, or on other approaches such as inactivated vaccines, subunit vaccines or DNA vaccines, to facilitate the safe and effective immunization of vulnerable sectors of certain populations (such as the immunocompromised, the elderly, pregnant women and children with eczema). If such strains or approaches do not induce pock formation, then alternative markers of efficacy will be needed. Vaccinia strains that do not induce pocks are not covered in these guidelines.

The terms “national regulatory authority” and “national control laboratory”, as used in these recommendations, always refer to the country in which the vaccine is manufactured.

## **Part A. Manufacturing recommendations**

### **A.1 Definitions**

#### **A.1.1 *International name and proper name***

The international name shall be “*Vaccinum variolae*”. The proper name shall be the equivalent name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the recommendations formulated below.

#### **A.1.2 *Descriptive definition***

*Vaccinum variolae* is a preparation of live vaccinia virus grown in the membranes of the chick embryo or in in vitro cultures of suitable tissues or in the skin of living animals. The preparation should satisfy all the recommendations formulated below.

#### **A.1.3 *International standard and reference preparations***

To allow for standardization of potency assays an International Reference Preparation of smallpox vaccine was established in 1962 (6, 7). This standard was derived from the Lister strain of virus and was

produced on the flanks of sheep before lyophilization and storage at  $-20^{\circ}\text{C}$ . Initially held at the Statens Seruminstitut in Copenhagen, Denmark, the Reference Preparation was passed to the National Institute for Biological Standards and Control (NIBSC) in England in 1997. This Reference Preparation is available from NIBSC for calibration and establishment of in-house potency reference materials.

An international collaborative study of two new candidate International Reference Preparations is in progress. One of these candidate preparations will later be selected to replace the dwindling stocks of the current International Reference Preparation. Updated information may be found at [www.who.int/biologicals](http://www.who.int/biologicals).

An International Standard for Anti-Smallpox Serum was established in 1966 (8). Initially held at the Statens Seruminstitut in Copenhagen, Denmark, the Reference Preparation was passed to the National Institute for Biological Standards and Control (NIBSC) in England in 1997. Subsequently this material was found to be contaminated with hepatitis B virus and was destroyed.

A WHO informal consultation in 2002 recommended that a replacement preparation be obtained and studied for suitability in an international collaborative study. Such a standard would be used for the assay of variola and vaccinia antibodies and to calibrate vaccinia immunoglobulin preparations. National standards that are calibrated in International Units are known to exist and may serve as interim calibrants until a new international standard is prepared and characterized.

#### A.1.4 **Terminology**

*Cell seed:* A quantity of cells of human or animal origin stored frozen at  $-100^{\circ}\text{C}$  or below in aliquots of uniform composition, one or more of which may be used for the production of a manufacturer's working cell bank.

*Manufacturer's working cell bank (MWCB):* A quantity of cells of uniform composition derived from one or more ampoules of the cell seed, which may be used for the production cell culture.

In normal practice, a cell bank is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the MWCB. One or more of the ampoules from such a pool may be used for the production cell culture.

*Production cell culture:* A cell culture derived from one or more ampoules of the MWCB, or primary tissue, used for the production of a single harvest.

*Adventitious agents:* Contaminating microorganisms including bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses.

*Original seed:* A batch of vaccine, or a seed preparation, with proven effectiveness and safety in the eradication initiative, from which the master seed is derived.

*Virus master seed lot:* A quantity of virus, physically homogeneous, derived from an original seed processed at one time and passaged for a number of times that does not exceed the maximum approved by the national regulatory authority.

*Virus working seed lot:* A quantity of virus, physically homogeneous, derived from the master seed by a limited number of passages by a method approved by the national regulatory authority. The virus working seed is used for production of vaccine without intervening passage.

*Single harvest:* A virus suspension harvested from a group of embryonated eggs or a group of cell cultures prepared from a single production run. For vaccines produced in animal skin, a single harvest is a quantity of material harvested from one animal.

*Bulk suspension:* A pool of a number of single harvests.

*Final bulk:* The finished biological preparation after completion of preparations for filling, homogeneous with respect to mixing of all components, and present in the container from which the final containers are filled. The final bulk may be prepared from one or more bulk suspensions.

*Filling lot (final lot):* A collection of sealed, final containers of freeze-dried vaccine that are homogeneous with respect to the risk of contamination during the filling process or the preparation of the finished vaccine. A filling lot must therefore have been filled or prepared in one working session.

*Pock-forming unit:* The smallest quantity of a virus suspension that will produce a single pock on the chick chorio-allantoic membrane.

*Plaque-forming unit:* The smallest quantity of a virus suspension that will produce a single plaque in monolayer cell cultures.

## A.2 **General manufacturing recommendations**

The general manufacturing recommendations contained in Good manufacturing practices for biological products (9) should apply to establishments manufacturing smallpox vaccine, with the addition of the following:

Production areas should be decontaminated before they are used for the manufacture of smallpox vaccine.

The production of smallpox vaccine should be conducted by a separate staff which should consist of healthy persons, who should receive regular medical examinations. Steps should be taken to ensure that all such persons in the production areas and all relevant quality control staff are protected against vaccinia virus infection by immunization or other means. Steps should also be taken to minimize the risks of transmission of vaccinia virus from the production facility to the outside environment.

For new vaccine production in animal skins method strict adherence to good manufacturing practices will not be possible. It will also be very difficult to validate the manufacturing process. Therefore every effort should be made to minimize contaminating microbial agents in the vaccine by meticulous controls of facilities, personnel, animals used for production and by specific tests on the product.

## **A.3 Production control**

### **A.3.1 Control of source materials**

#### **A.3.1.1 Virus strains**

Strains of vaccinia used in the production of smallpox vaccine should be identified by historical records, which should include information on their origin. Only vaccinia strains that are approved by the national regulatory authority should be used. They should be shown to yield immunogenic vaccines that produce typical vaccinal lesions in the skin of humans. For new vaccines, neutralizing antibodies or haemagglutination inhibition antibodies, or an inhibition to response to revaccination, may be used to assess immunogenicity.

Vaccine strains known to protect humans against variola include, but are not restricted to, the Lister-Elstree, and the New York City Board of Health (NYCBOH) strains. The Tiantan strain, and other derivatives of the Lister-Elstree strain, were also used in some countries.

The WHO seed virus, based on the Lister-Elstree strain, is held by WHO Collaborating Centres. Although WHO has taken every possible precaution to ensure that these seeds meet the recommendations for smallpox vaccine it should be emphasized that, in each country, the national regulatory authority must accept responsibility for the quality of vaccines produced from the seeds and used in that country. Requests for the seed virus should be made through WHO.

#### **A.3.1.1.1 Virus seed lot system**

Vaccine production should be based on the virus seed lot system. The number of passages required to produce vaccine single harvests from

the original seed should be limited and approved by the national regulatory authority.

The virus master seed lot may be produced by cloning from an original seed.

The passages between master and working virus seeds should be in the same general type of substrate as used for vaccine production. For example if the vaccine is produced in embryonated eggs, the working seed lot should be produced from the master seed by passage in embryonated eggs and not by passage in cell cultures or in animal skin.

If different substrates have been utilized for master and working virus seeds, adequate validation of this change must be conducted to rule out changes in the quality of the vaccine.

Vaccine should be produced from the virus working seed with no intervening passage.

Phenotypic differences between clonal derivatives of vaccinia have been shown. It is theoretically possible that multiple passages may select variants present in the original seed. Restricting the number of passages from original seed to vaccine single harvests should minimize this risk.

A large working seed lot should be set aside as the basic material to be used by the manufacturer for the preparation of batches of vaccine.

All virus seed lots in liquid form should be stored at a temperature of  $-60^{\circ}\text{C}$  or below. Seed lots that are freeze-dried need not be stored at  $-60^{\circ}\text{C}$  or below. The available data show that potency is retained when seed lots are stored at temperatures of  $-20^{\circ}\text{C}$  or below.

#### **A.3.1.1.2 Tests on virus seed lots**

The virus master and working seed lots should be identified as vaccinia by suitable tests. A sensitive test should be conducted to exclude the presence of other orthopoxviruses.

Molecular tests such as restriction fragment length polymorphism or partial sequencing, especially of terminal DNA sequences which show the greatest variation between vaccinia strains, may be useful as identity tests.

The potency of the virus master and working seed lots should be determined as described in section A.3.3.4.

The virus master and working seed lot used for the production of vaccine batches in embryonated eggs or cell cultures should be free from detectable adventitious agents. Seed lots produced in embryonated eggs should comply with the recommendations in sections A.3.1.2 and A.3.2.1 and seed lots produced in cell cultures should comply with the recommendations in section A.3.1.3 and A.3.2.2 or A.3.2.3 as appropriate.

Whereas testing for adventitious bacteria, mycoplasma and fungi should use standardized methods, testing of vaccine virus seeds for viral adventitious agent's might be more complex because complete neutralization of vaccinia virus may be difficult to achieve. Should this be the case, the seed lot may be diluted to the dilution used as inoculum for production of vaccine prior to testing for viral adventitious agents. Supplementary specific testing for viral adventitious agents using validated PCR-methodology or the use of immunochemical methods could also be appropriate.

Seed lots to be used for production in embryonated eggs or cell culture should also be tested for carry-over of potential adventitious agents from the original seed. Given that the complete passage history of the original seed is unlikely to be known, and that more than one species may have been used in the passage history, this additional testing should at least cover important adventitious agents of concern.

The passage history of the original seed is likely to have included sheep, calves and humans and may have included rabbits, goats or water buffaloes.

The burden of contaminating microbial agents in virus master and working seeds prepared in animal skins should be limited by meticulous controls of facilities, personnel, animals used for production and by specific tests on the seeds. However it may be difficult to ensure that seed lots produced in animal skins are totally free from adventitious agents. Such lots should also comply with the recommendations in sections A.3.1.4 and A.3.2.4. The absence of specific human pathogens should be confirmed by additional testing procedures (bacterial and fungal cultures, virus culture and PCR testing for viral agents).

All master and working seed lots should comply with the current guidelines to minimize the risks of transmission of animal transmissible spongiform encephalopathies (10).

The neurovirulence of master and working seed viruses should meet the criteria for acceptability given in section A.3.3.5.1 (test for neurovirulence in mice). The original seed should be used, where possible, as comparator in these tests. Where original seeds are not available for this purpose equivalent materials may be used.

As an alternative to mice, a neurovirulence test may be conducted in rabbits.

#### A.3.1.2 Eggs

If the vaccine is to be produced in embryonated eggs or primary chick embryo fibroblasts, the eggs to be used should be from a closed, specific-pathogen-free, healthy flock. This flock should be monitored at regular intervals for agents pathogenic to birds. These include

*Mycobacterium avium*, fowlpox virus, avian leucosis virus (ALV) and other avian retroviruses; Newcastle disease virus and other avian parainfluenza viruses; avian encephalomyelitis virus; infectious laryngotracheitis virus; avian reticulo-endotheliosis virus; Marek's disease virus; infectious bursal disease virus; *Haemophilus paragallinarum*; *Salmonella gallinarum*; *Salmonella pullorum*; *Mycoplasma gallisepticum*, and *Mycoplasma synoviae*.

In some countries, all birds are bled when a colony is established, and thereafter 5% of the birds are bled each month. The resulting serum samples are screened for antibodies to the relevant pathogens. Any bird that dies should be investigated to determine the cause of death.

The flock must not have been vaccinated with live Newcastle disease virus vaccine.

It is recommended that eggs should be obtained from young birds.

#### A.3.1.3 Cell cultures

Smallpox vaccines may be produced in human diploid cells, in continuous cell lines, in primary rabbit kidney cells or in primary chick embryo fibroblast cells. For vaccines produced in human diploid cells and continuous cell lines sections A.3.1.3.1 and A.3.1.3.2 should apply; for production in primary rabbit kidney cells section A.3.1.3.3 should apply; and for production in primary chick embryo fibroblasts section A.3.1.2 should apply to the source materials. Section A.3.1.3.4 applies to all types of cell culture.

##### A.3.1.3.1 Cell seed and manufacturer's working cell bank

The use of a cell line such as MRC-5 or Vero cells for the manufacture of smallpox vaccines should be based on the cell seed system. The cell seed should be approved by the national control authority. The maximum number of passages (or population doublings) by which the MWCB is derived from the cell seed should be established by the national regulatory authority.

WHO has established a cell bank of Vero cells characterized in accordance with the requirements in the report of the WHO Expert Committee on Biological Standardization (11), which is available as a well characterized starting material to manufacturers for preparation of their own master and working cell seeds on application to the Coordinator, Quality Assurance and Safety of Biologicals, World Health Organization, Geneva, Switzerland.

##### A.3.1.3.2 Identity test

Cell seed should be characterized according to the requirements for animal cells lines used as substrates for biologicals production (14), as appropriate to continuous cell lines or human diploid cells.

The MWCB should be identified by means, inter alia, of biochemical (e.g. isoenzyme analysis), immunological and cytogenetic marker tests, approved by the national regulatory authority.

#### **A.3.1.3.3 Primary rabbit kidney cells for production of smallpox vaccines**

Rabbits, 2–4 weeks old, may be used as the source of kidneys for cell culture. Only rabbit stock approved by the national regulatory authority should be used as the source of tissue and they should be derived from a closed, healthy colony. A closed colony is a group of animals sharing a common environment and having their own caretakers who have no contact with other animal colonies. The animals are tested according to a defined programme to ensure freedom from specified pathogens or their antibodies.

No generally agreed testing programme is available. Agents that may be considered include the following viruses: myxoma virus, rabbit poxvirus, parainfluenza viruses, Sendai virus, reovirus type 3, rotavirus, and rabbit parvovirus; mycoplasma species; the following bacteria: *Bordetella bronchiseptica*, *Clostridium perfringens*, *Clostridium pilliforme*, *Chlamydia psittaci*, *Citrobacter rodentium*, *Clostridium spiroforme*, *Francisella tularensis*, *Listeria*, *Mycobacterium tuberculosis*, *Pasteurellaceae*, *Pseudomonas aeruginosa*, *Salmonella species*, *Staphylococcus aureus*, *Yersinia enterocolitica*; *Toxoplasma gondii*; ticks and endoparasites.

When new animals are introduced into the colony, they should be maintained in quarantine in vermin-proof quarters for a minimum of 2 months and shown to be free from specified pathogens. Animals to be used to provide kidneys should not previously have been used for experimental purposes, especially those involving infectious agents. The colony should be monitored for zoonotic viruses and markers of contamination at regular intervals.

At the time the colony is established, all animals should be tested to determine freedom from antibodies to possible viral contaminants for which there is evidence of capacity for infecting humans or evidence of capacity to replicate in vitro in cells of human origin. A test for retroviruses using a sensitive polymerase chain reaction (PCR)-based reverse transcriptase (Rtase) assay should also be included. The results of such assays may need to be interpreted with caution because Rtase activity is not unique to retroviruses and may derive from other sources, such as retrovirus-like elements that do not encode a complete genome (12). Nucleic acid amplification tests for retrovirus may also be used.

After the colony is established, it should be monitored by testing a representative group of animals consisting of at least 5% of the animals that are bled at intervals acceptable to the national regulatory authority.

As an example of how often to monitor the rabbit colony, it is suggested that they are bled at monthly intervals in line with the sampling frequency of birds used in the production of chick embryo fibroblast cells (section A.3.1.2).

In addition, the colony should be screened for pathogenic bacteria, including mycobacteria, fungi and mycoplasma, as agreed with the national regulatory authority. The screening programme should test all of the animals over a defined period of time, as agreed with the national regulatory authority.

Any animal that dies should be investigated to determine the cause of death. If the presence of an infectious agent is demonstrated in the colony, the national control authority should be informed and the manufacture of smallpox vaccine may be discontinued. In this case, manufacture should not be resumed until a thorough investigation has been completed and precautions have been taken against the infectious agent being present in the product, and only then with the approval of the national control authority.

At the time of kidney harvest, the animals should be examined for the presence of any abnormalities and if kidney abnormalities or other evidence of pathology is found, those animals are not be used for smallpox vaccine production.

Each group of control cultures derived from a single group of animals used to produce a single virus harvest should remain identifiable as such until all testing, especially for adventitious agents, is completed.

#### **A.3.1.3.4 Cell culture medium**

Serum used for the propagation of cells should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, according to the requirements given in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (13), and from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of the Recommendations for Production and Control of Poliomyelitis Vaccine (Oral) (14).

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera.

As an additional monitor of quality, sera may be examined for freedom from phage and endotoxin.

Irradiation may be used to inactivate potential contaminant viruses.

The acceptability of the sources(s) of any components of bovine, sheep or goat origin used in culture media should be approved by the national regulatory authority. These components should comply with current guidelines in relation to animal transmissible spongiform encephalopathies (10).

Human serum should not be used. If human albumin is used it should meet the revised Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (Requirements for Biological Substances No. 27) (15), as well as current guidelines in relation to human transmissible encephalopathies (10).

The use of human albumin as a component of a cell culture medium requires careful consideration due to potential difficulties arising from the shorter expiry period of albumin in relation to the intended long-term storage of smallpox vaccines.

**Penicillin and other  $\beta$ -lactams should not be used at any stage of the manufacture.**

Other antibiotics may be used at any stage in the manufacture provided that the quantity present in the final product is acceptable to the national control authority. Nontoxic pH indicators may be added, e.g. phenol red in a concentration of 0.002%. Only substances that have been approved by the national regulatory authority may be added.

If trypsin is used for preparing cell cultures should be tested and found free of cultivable bacteria, fungi, mycoplasmas and infectious viruses, especially bovine or porcine parvoviruses, as appropriate. The methods used to ensure this should be approved by the national regulatory authority.

The source(s) of trypsin of bovine origin, if used, should be approved by the national regulatory authority. Bovine trypsin, if used, should comply with current guidelines in relation to animal transmissible spongiform encephalopathies (10).

#### *A.3.1.4 Animals used for production of animal skin vaccine*

If vaccine is prepared in animal skins, animals of a species approved by the national control authority, in good health, and not previously employed for experimental purposes should be used.

Manufacturers are encouraged to use animals from closed or intensively monitored colonies where these are available.

The animals should be kept in well-constructed and adequately ventilated animal rooms in cages spaced as far apart as possible. Adequate precautions should be taken to prevent cross-infection between stalls or cages. For large animals, not more than one animal should be housed per stall. For small animals, not more than two animals should be housed per cage and cage-mates should not be interchanged. The animals should be kept in the country of manufacture of the vaccine in quarantine groups for a period of not less than six weeks before use.

A quarantine group is a colony of selected, healthy animals kept in one room, with separate feeding and cleaning facilities, and having no contact with other animals during the quarantine period.

If at any time during the quarantine period the overall death rate of a group of animals reaches 5%, animals from that entire group should not be used for vaccine production. The groups should be kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the animals are used. After the last animal of a group has been taken, the room that housed the group should be thoroughly cleaned and decontaminated before being used for a fresh group.

Animals to be inoculated should be anaesthetized and thoroughly examined. If an animal shows any pathological lesion relevant to its use in the preparation of a seed lot or vaccine, it should not be used, nor should any of the remaining animals from that quarantine group be used unless it is evident that their use will not impair the safety of the product.

If ruminants are to be used for vaccine production, special attention is required to ensure that the animals comply with the current guidelines on animal transmissible spongiform encephalopathies given in *the Report of a WHO Consultation on Medical and Other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies (10)*. This means the animals used should be less than two years of age and sourced from herds that have had no cases of BSE, have been actively monitored, and have never been fed mammalian-derived protein (other than milk).

Where possible ruminants used for vaccine production should also be from a BSE-free country, have a fully documented breeding history, and have come from a herd in which any new genetic material introduced is from herds with the same BSE-free status.

### **A.3.2 Control of vaccine production**

#### **A.3.2.1 Production in specific pathogen free embryonated eggs**

##### **A.3.2.1.1 Tests on uninoculated eggs**

A sample of 2% of, but in any case not less than 20 and not more than 50, uninoculated embryonated eggs from the batch used for vaccine production should be incubated under the same conditions as the inoculated eggs. At the time of virus harvest, the uninoculated eggs should be processed in the same manner as the inoculated eggs, and the extract from the control embryos should be shown to be free from haemagglutinating agents and from adenoviruses, avian retroviruses such as avian leukosis virus, and other extraneous agents by tests approved by the national control authority.

A test for retroviruses using a sensitive PCR-based reverse transcriptase (Rtase) assay may be used. The results of such assays need to be interpreted with caution because Rtase activity is not unique to infectious retroviruses and may derive from other sources, such as mammalian polymerase or incomplete retrovirus-like elements that do not encode a complete genome (12). Nucleic acid amplification tests for retrovirus may also be used.

#### **A.3.2.1.2 Single harvests**

After inoculation and incubation at a controlled temperature, only living, typical chick embryos should be harvested. The age of embryos at the time of harvest should be reckoned from the initial introduction of the eggs into the incubator and should be no more than 12 days. After homogenization and centrifugation, the embryonic extract should be kept at  $-60^{\circ}\text{C}$  or below until further processing.

#### **A.3.2.1.3 Bacterial and fungal sterility and mycoplasma tests**

A volume of at least 10ml of each single harvest should be tested for bacterial and fungal sterility, and mycoplasma, according to the revised Requirements for Biological Substances No. 6 (16)

#### *A.3.2.2 Production in primary chick embryo fibroblasts, human diploid cells or continuous cell cultures*

##### **A.3.2.2.1 Control of cell cultures**

At least 5% or 1000 ml of the cell suspension at the concentration and cell passage level employed for seeding vaccine production cultures should be used to prepare control cultures.

If bioreactor technology is used, the national regulatory authority should determine the size and treatment of the cell sample to be examined.

##### **A.3.2.2.1.1 Tests of control cell cultures**

The treatment of the cells set aside as control material should be similar to that of the production cell cultures, but they should remain uninoculated for use as control cultures for the detection of extraneous viruses.

These control cell cultures should be incubated under similar conditions to the inoculated cultures for at least two weeks, and should be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific, accidental reasons.

At the end of the observation period, the control cell cultures should be examined for degeneration caused by an extraneous agent. If this examination, or any of the tests specified in this section, shows

evidence of the presence in a control culture of any adventitious agent, the vaccinia grown in the corresponding inoculated cultures should not be used for vaccine production.

#### A.3.2.2.1.2 Tests for haemadsorbing viruses

At the end of the observation period, 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter have been stored, the duration of storage should not have exceeded seven days and the storage temperature should have been in the range 2–8 °C .

In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

As an additional test for haemadsorbing viruses the national regulatory authority may require that other types of red blood cells, including cells from humans (blood group O), monkeys and chickens (or other avian species), should be used in addition to guinea-pig cells.

A reading should be taken after incubation for 30 minutes at 2–8 °C and again after a further incubation for 30 minutes at 20–25 °C.

If a test with monkey red cells is performed, readings should also be taken after a final incubation for 30 minutes at 34–37 °C.

#### A.3.2.2.1.3 Tests for other adventitious agents

At the end of the observation period, a sample of the pooled fluid from each group of control cultures, and a sample of pooled cell homogenate from each group of control cultures, should be tested for adventitious agents. For this purpose, 10 ml of each pool should be tested in the same cells, but not the same batch of cells, as those used for the production of virus, and additional 10-ml samples of each pool should be tested in human cells sensitive to measles and at least one other sensitive cell system.

The test of cell homogenates is added as an additional test for adventitious agents because of the potential difficulties in neutralizing vaccinia virus in single harvests.

The pooled fluid should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. At least one bottle of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated cultures should be incubated at a temperature of 35–37 °C and should be observed for a period of at least 14 days.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvests produced from the batch of cells from which the control cells were taken should be discarded.

If these tests are not performed immediately, the samples should be kept at a temperature of  $-60^{\circ}\text{C}$  or below.

#### A.3.2.2.1.4 Additional tests if chick cell cultures are used for production

If chick cell cultures are used, a sample of fluids pooled from the control cultures should be tested for adenoviruses and for avian retroviruses such as avian leukosis virus, by a method approved by the national regulatory authority.

A test for retroviruses using a sensitive PCR-based Rtase assay may be used. The results of such assays need to be interpreted with caution because Rtase activity is not unique to retroviruses and may derive from other sources, such as retrovirus-like elements that do not encode a complete genome (12). Nucleic acid amplification tests for retrovirus may also be used.

#### A.3.2.2.1.5 Identity test

At the production level, and for vaccines produced in human diploid cells or continuous cells only, the cells should be identified by means of tests approved by the national regulatory authority.

Suitable tests are isoenzyme analysis, immunological tests and cytogenetic marker tests.

### A.3.2.2.2 Cell cultures for vaccine production

#### A.3.2.2.2.1 Tests for adventitious agents

On the day of inoculation with the virus working seed lot, each cell culture or a sample from each culture vessel should be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the culture should not be used for vaccine production.

If animal serum is used for cell cultures before the inoculation of virus, the medium should be removed and replaced with serum-free maintenance medium, after the cells have been washed with serum-free medium, if appropriate. If suspension cultures are used, testing should be conducted to ensure that serum levels are reduced to acceptable levels. This testing may be performed at a later stage of production.

#### A.3.2.2.2.2 Tests for bacteria, fungi and mycoplasmas

A volume of at least 20 ml of the pooled supernatant fluids from the production cell culture should be tested for bacterial and fungal sterility and for mycoplasmas. The tests for bacterial, fungal and

mycoplasmal sterility should be performed as described in the revised General Requirements for the Sterility of Biological Substances (Requirements for Biological Substances No. 6) (13).

#### **A.3.2.2.3 Control of single harvests**

##### **A.3.2.2.3.1 Single harvest**

After inoculation of the production cells with the virus working seed lot, neither inoculated nor control cell cultures should at any time be at a temperature outside the range approved by the national regulatory authority for the relevant incubation periods. The optimal range for pH, multiplicity of infection, cell density and time of incubation should be established for each manufacturer, and be approved by the national regulatory authority.

The virus suspension should be harvested not later than that number of days after virus inoculation agreed by the national regulatory authority.

It is advisable that the inoculated cell cultures should be processed in such a manner that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until the results of all the tests described in Part A sections A.3.2.2.1, A.3.2.2.2, A.3.2.2.3.3, A.3.2.2.3.4 and A.3.2.2.3.5 have been obtained.

##### **A.3.2.2.3.2 Sampling**

Samples required for the testing of single harvests should be taken immediately on harvesting. If the tests for adventitious agents as described in Part A, section A.3.2.2.3.3 are not performed immediately, the samples taken for these tests should be kept at a temperature of  $-60^{\circ}\text{C}$  or lower, and subjected to no more than one freeze-thaw cycle.

##### **A.3.2.2.3.3 Tests of neutralized single harvests for adventitious agents**

For the purposes of the recommendations specified in this section of Part A, the volume of each single harvest taken for neutralization and testing should be at least 10ml and should be such that a total of at least 50ml or the equivalent of 500 doses of final vaccine, whichever is the greater, has been withheld from the corresponding bulk suspension.

The antisera used for neutralization should be of nonhuman origin and should have been prepared using virus cultured in cells from a species different from that used in the production of the vaccine. Samples of each virus harvest should be tested in human cells sensitive to measles and at least one other sensitive cell system.

Complete neutralization of vaccinia virus may be difficult to achieve at high virus concentrations. If this is the case, specific tests can supplement non-

specific testing with standard tissue culture tests or eggs. Specific tests could include PCR, immunochemical tests or antibody production tests in animals. The extent of testing for specific adventitious agents may vary and depends on the agents that could be present based on the nature and origin of the substrate used for vaccine production and the origin of the virus seed. The national regulatory authority should approve the test programme for viral adventitious agents. Use of scarce biological reagents, such as high-titred vaccinia neutralizing sera, may be decreased by not testing for viral adventitious agents at the level of the single harvest but testing instead at later stages of the manufacturing process, for example at the level of the final bulk. This option should first be approved by the national regulatory authority.

The neutralized suspensions should be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm<sup>2</sup> per ml of neutralized suspension. At least one bottle of each kind of cell culture should remain uninoculated; it should serve as a control; it should be maintained by nutrient medium containing the same concentration of the specific antiserum used for neutralization.

Animal serum may be used in the propagation of the cells, but the maintenance medium used after inoculation of the test material should contain no added serum other than the smallpox neutralizing antiserum.

The inoculated cultures should be incubated at a temperature of 35–37 °C and should be observed for a period of at least 14 days.

If adequately justified lower temperatures may be used.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvest should be discarded.

#### A.3.2.2.3.4 Additional tests if chick cell cultures are used for production

A volume of each neutralized virus pool equivalent to 100 human doses of vaccine or 10 ml, whichever volume is greatest, should be tested in a group of fertilized chicken's eggs by the allantoic route of inoculation, and a similar sample should be tested in a separate group of eggs by the yolk-sac route of inoculation. In both cases 0.5 ml of inoculum should be used per egg.

The virus pool passes the test if, at the end of a 3–7-day observation period, there is no evidence of the presence of any adventitious agents. If an adventitious agent is detected in the uninoculated controls, the test should be repeated.

#### A.3.2.2.3.5 Sterility tests

A volume of at least 10ml of each single harvest should be tested for bacterial, fungal and mycoplasmal sterility according to the requirements given in the revised Requirements for Biological Substances No. 6 (16).

#### A.3.2.3 ***Production in primary rabbit kidney cells***

##### A.3.2.3.1 *Tests of cell cultures used for vaccine production*

On the day of inoculation with virus working seed lot, each cell culture should be examined for degeneration caused by an infective agent. If this examination reveals evidence of the presence in a cell culture of any adventitious agent, none of the entire group of cultures concerned should be used for vaccine production.

On the day of inoculation with the virus working seed lot, a sample of at least 30ml of the pooled fluid is removed from the cell cultures of the kidneys of each group of animals used to prepare the primary cell suspension. The pooled fluid should be tested in primary kidney-cell cultures prepared from the same species, but not the same group of animals, as that used for vaccine production. The pooled fluid should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm<sup>2</sup> per ml of pooled fluid. At least one bottle of each primary cell culture should remain uninoculated and should serve as a control.

The cultures should be incubated at a temperature of 34–36°C and should be observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks of incubation, from each of these cultures at least one subculture of fluid should be made in the same tissue culture system. The subculture should also be observed for at least 2 weeks.

Serum may be added to the original culture at the time of subculturing, provided that the serum does not contain antibody or other inhibitors to adventitious agents of the cell culture donor species.

For the tests to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the respective test periods.

If, in these tests, evidence is found of the presence of an adventitious agent, the single harvest from the whole group of cell cultures concerned should not be used for vaccine production.

If these tests are not done immediately, the samples of pooled cell-culture fluid should be kept at a temperature of –60°C or below.

#### A.3.2.3.2 *Tests of control cell cultures*

Cultures prepared on the day of inoculation with the virus working seed lot from 25% of the cell suspension obtained from the kidneys of each group of animals used to prepare the primary cell suspension should remain uninoculated, and should serve as controls. These control cell cultures should be incubated under the same conditions as the inoculated cultures for at least two weeks, and should be examined during this period for evidence of cytopathic changes. For the tests to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific, accidental reasons.

At the end of the observation period, the control cell cultures should be examined for degeneration caused by an infectious agent. If this examination or any of the tests specified in this section shows evidence of the presence in a control culture of any adventitious agent, the vaccinia grown in the corresponding inoculated cultures from the same group should not be used for vaccine production.

##### A.3.2.3.2.1 *Tests for haemadsorbing viruses*

At the time of harvest or not more than four days after the day of inoculation of the production cultures with the virus working seed lot, a sample of 4% of the control cell cultures should be taken and should be tested for haemadsorbing viruses. At the end of the observation period, the remaining control cell cultures should be similarly tested. The tests should be made as described in Part A, section A.3.2.2.1.2.

##### A.3.2.3.2.2 *Tests for other adventitious agents*

At the time of harvest, or not more than seven days after the day of inoculation of the production cultures with the working seed lot, a sample of at least 20 ml of the pooled fluid from each group of control cultures should be taken and tested in primary kidney-cell cultures, as described in Part A, section A.3.2.3.1.

At the end of the observation period for the original control cell cultures, similar samples of the pooled fluid should be taken and the tests in primary kidney-cell cultures should be repeated, as described in Part A, section A.3.2.3.1.

In some countries, fluids are collected from the control cell cultures at the time of virus harvest and at the end of the observation period. Such fluids may then be pooled before testing for adventitious agents.

##### A.3.2.3.3 *Tests for neutralized single harvests in primary kidney-cell cultures*

The specifications given in part A section A.3.2.2.3.3 should apply with the addition that each neutralized single harvest should be

additionally tested in primary kidney-cell cultures prepared from the same species, but not the same group of animals, as that used for vaccine production.

#### A.3.2.3.4 *Sterility tests*

A volume of at least 10 ml of each single harvest should be tested for bacterial, fungal and mycoplasmal sterility according to the requirements given in the revised Requirements for Biological Substances No. 6 (13).

#### A.3.2.4 **Production in animal skins**

##### A.3.2.4.1 *Vaccines produced in the skin of living animals*

The animals should be free of ectoparasites, and each animal should be kept under veterinary supervision for at least two weeks prior to the inoculation of the seed virus. Before inoculation the animals should be cleaned, and thereafter kept in scrupulously clean stalls until the vaccinal material is harvested.

The use of bedding, unless sterilized and changed frequently, should be avoided. The stalls, including feed boxes, should be designed so as to make cleaning easy, and dust-producing food should be avoided.

During a period of five days before inoculation, and during incubation, the animals should remain under veterinary supervision; they should remain free from any sign of disease, and rectal temperatures should be recorded daily. If any abnormal rise in temperature occurs, or if any clinical sign of disease is observed, the production of vaccine from the group of animals concerned should be suspended until the cause of these irregularities has been resolved. The prophylactic and diagnostic procedures adopted to exclude the presence of infectious disease should be submitted for approval to the national regulatory authority.

According to the species of animal used and the diseases to which that animal is liable in the country where the vaccine is being produced, the prophylactic and diagnostic procedures to be used will vary. They must exclude the possibility of transmitting diseases within the country where the vaccine is prepared, but consideration should also be given to the danger of spreading diseases to other countries or continents to which the vaccine may be shipped.

Special attention should always be given to foot-and-mouth disease, transmissible spongiform encephalopathies, brucellosis, Q fever, tuberculosis and dermatomycosis, but in some areas it will also be necessary to consider diseases such as contagious pustular dermatitis (orf), pulpy kidney disease, sheep pox, anthrax, rinderpest, haemorrhagic septicaemia, Rift Valley fever and many others.

The inoculation of seed virus should be made on parts of the animal that are not liable to be soiled by urine and faeces. The surface used

for inoculation shall be so shaved and cleaned as to procure the nearest possible approach to surgical asepsis. If any antiseptic substance deleterious to the virus is used in the cleaning process it shall be removed by thorough rinsing with sterile water prior to inoculation. During inoculation, the exposed surface of the animal not used for inoculation shall be covered with sterile covering.

Many workers prefer to inoculate the ventral surface of female animals. If male animals are used this area is more liable to soiling by urine and faeces than the flank, which may be equally susceptible to vaccinia virus and easier to keep clean, especially since the animal tends to rest on the uninoculated side.

It is recommended that the animal be anaesthetized during the process of shaving, cleaning and inoculation.

After inoculation the area may be covered with suitable antibiotics.

Before the collection of the vaccinal material, any antibiotic should be removed and the inoculated area should be subjected to a repetition of the cleaning process. The uninoculated surfaces should be covered with sterile covering.

Before harvesting, the animal should be killed painlessly. The animals should be exsanguinated before harvesting to avoid heavy admixture of the vaccinal material with blood.

The vaccinal material from each animal should be collected separately taking aseptic precautions.

All animals used in the production of vaccine should be examined by autopsy. Special attention should be paid to examining the central nervous system for evidence of transmissible spongiform encephalopathy. If evidence of any generalized or systemic disease other than vaccinia is found, or evidence of encephalopathy, the vaccinal material from the entire group of animals exposed should be discarded.

### **A.3.3 Control of bulk suspension**

#### **A.3.3.1 *Preparation of bulk suspension***

The bulk suspension should be treated to remove cell debris.

The national regulatory authority may require the further purification of harvests derived from continuous cell lines to remove cellular DNA, and/or the use of DNAase treatment to reduce the size of DNA fragments. If the harvests are derived from human diploid or primary cell cultures, further purification is not required.

#### A.3.3.2 **Sampling**

Samples of the bulk suspension prepared as described in section A.3.3.1 should be taken immediately and, if not tested immediately, should be kept at a temperature of  $-60^{\circ}\text{C}$  or below until the tests described in the following sections are performed.

#### A.3.3.3 **Identity test**

The vaccinia virus in the bulk suspension should be identified by serological or molecular tests.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test. Molecular tests such as restriction fragment length polymorphism or partial sequencing, especially of terminal DNA sequences which show the greatest variation between vaccinia strains, may be useful as identity tests.

#### A.3.3.4 **Virus concentration**

The amount of infective vaccinia virus per ml of filtered bulk suspension should be determined in the chick egg chorioallantoic membrane, or in cell cultures, in comparison with a reference preparation assayed in the same system.

The virus concentration as determined by this test should be the basis for the quantity of virus used in the neurovirulence tests in mice (Part A, section A.3.3.5.1) and for preparing the final bulk (Part A, section A.3.4). The detailed procedures for carrying out this test and for interpreting the results should be those approved by the national regulatory authority.

#### A.3.3.5 **Test for consistency of virus characteristics**

The vaccinia virus in the bulk suspension prepared as described in section A.3.3.1 should be tested in comparison with the working seed virus with regard to certain characteristics, as described below, to ensure that the vaccine virus has not undergone changes during its multiplication in the production culture system. From the results of these tests for successive batches of vaccine a critical assessment may be made of the consistency of vaccine quality (see Part B, section B.2).

##### A.3.3.5.1 **Test for neurovirulence**

The neurovirulence of the bulk suspension should be compared to an original seed (or equivalent) by intracerebral inoculation of suckling mice.

Direct inoculation of vaccinia virus into the central nervous system of suckling mice has been shown to discriminate between clonal derivatives of

vaccinia. It is not a model of post-infectious, demyelinating disease since the pathology is quite distinct. The purpose of the test is to show consistency of production and that the each new filtered bulk suspension does not significantly differ in neurovirulence phenotype from the comparator.

Suckling 3–5-day-old CD-1 outbred mice are inoculated intracerebrally with 20 µl of the filtered bulk suspension or the comparator vaccine. The target titre of the inoculum is  $5.0 \log_{10}$  pfu/ml. The titre of virus in the inoculum should be confirmed by titration of the residual inocula, and should be within  $0.5 \log_{10}$  pfu of the target. The mice are observed for up to 21 days and the mortality ratio and survival times are compared between groups. The mortality ratio of the filtered bulk suspension should not exceed that of the control comparator using by Fisher's exact test, and the filtered bulk suspension should not show more rapid time to death than the comparator control, based on a log rank test.

Other test systems in mice may be used to discriminate between acceptable and unacceptable batches, and should be approved by the national regulatory authority.

The national regulatory authority may approve neurovirulence tests in other species such as rabbit.

#### **A.3.3.6 Tests for cellular DNA**

For viruses grown in continuous cell culture, the bulk suspension should be tested for residual cellular DNA. The removal process should be shown by calculation to consistently reduce the level of cellular DNA to less than 10 ng per human dose. This test may be omitted, with the agreement of the national regulatory authority, if the manufacturing process is validated to achieve this specification.

The cytoplasmic replication cycle and specific structures needed for genome replication and encapsidation of vaccinia virus make it very unlikely that host cell DNA is also encapsidated during smallpox vaccine production. Integration of foreign DNA into the vaccinia virus genome is only possible by specific encapsidation or homologous recombination, the latter process requiring a minimum length of homologous DNA sequences. From these observations it seems extremely unlikely that cellular DNA sequences would be integrated. However, the evolution of viral genes with cellular homologues suggests that under selective pressure this may happen, but only rarely.

#### **A.3.4 Final bulk**

The operations necessary for preparing the final bulk should be conducted in such a manner as to avoid contamination of the product.

The dilution and mixing procedures involved in preparing the final bulk should be those approved by the national control authority.

##### **A.3.4.1 Preservatives, stabilizers and additives**

Any stabilizers, preservatives or additives that may be added to the bulk suspension should have been shown to the satisfaction of the

national control authority not to impair the safety or efficacy of the vaccine and to improve the stability of the vaccine in the concentrations used. If phenol is present its concentration should not exceed 0.5% and it should comply with pharmacopoeial specifications.

Human albumin may present difficulties if used as a stabilizer. The expiry period of albumin may be less than that of the vaccine, especially where long-term stockpiling of smallpox vaccines is intended. There may also be difficulties if a batch of human albumin is subject to a recall from the market.

#### A.3.4.2 **Tests for bacteria and fungi**

For vaccines other than those prepared on animal skins, the final bulk should be tested for bacterial and fungal sterility in accordance with the requirements given in Part A, section A.5, of the revised Requirements for Biological Substances No. 6 (Requirements for the Sterility of Biological Substances) (16). If phenol or other preservatives are used, this test should be performed on samples taken before any preservatives are added.

#### A.3.4.3 **Bioburden tests**

For vaccines produced on animal skins only, a volume of final bulk agreed by the national regulatory authority should be tested after the addition of preservatives for bacterial bioburden using the tests given in the revised Requirements for Biological Substances No. 6 (13). From the number of colonies that appear on the plates the number of living bacteria in 1 ml of final bulk should be calculated. If this number exceeds 50, the final bulk should be discarded. In addition the tests described in part A sections A.3.4.3.1, A.3.4.3.2, A.3.4.3.3 and A.3.4.3.4 shall also be performed.

##### A.3.4.3.1 *Test for the presence of Escherichia coli*

At least three 1-ml samples of a 1:100 dilution of the final bulk after addition of preservatives should be cultured on plates of a medium suitable for differentiating *E. coli* from other bacteria. The plates shall be incubated for 48 hours at 35°C–37°C. If *E. coli* is detected, the final bulk should be subjected to further processing or discarded.

The need for further treatment should be an exceptional occurrence since the presence of *E. coli* in this test might indicate a heavy faecal contamination. It should be justified by a report into the manufacture of the batch. In some countries further treatment is not permitted.

**A.3.4.3.2 Test for the presence of haemolytic streptococci, coagulase-positive staphylococci, or any other pathogenic microorganisms known to be harmful if introduced into the human body by the process of vaccination**

At least three 1-ml samples of a 1: 100 dilution of the final bulk after addition of preservatives should be cultured on plates of blood agar. The plates should be incubated for 48 hours at 35°C–37°C and the colonies appearing should be examined. If any of the organisms mentioned are detected, the final bulk should be discarded.

In some countries culture of the final bulk after addition of preservatives in salt meat broth is made for the purpose of detecting staphylococci.

**A.3.4.3.3 Test for the presence of *Bacillus anthracis***

Any colony seen on any of the plates used in the tests described in Part A, sections A.3.4.3., A.3.4.3.7., A.3.4.3.2 and A.3.4.3.3 that morphologically resembles *B. anthracis* should be examined. If the organisms contained in the colony are non-motile, further tests for the cultural character of *B. anthracis* should be made, including pathogenicity tests in suitable animals. If *B. anthracis* is found to be present, the final bulk, and any other associated bulks, should be discarded.

In countries where anthrax presents a serious risk, this test should be based on tests of each single harvest. The application of molecular tests for *B. anthracis* is encouraged.

**A.3.4.3.4 Test for the presence of *Clostridium tetani* and other pathogenic spore-forming anaerobes**

A total volume of not less than 10 ml of the final bulk after addition of preservatives, preferably taken from the depth of the bulk and not from the upper surface, should be distributed in equal amounts into ten tubes, each containing not less than 10 ml of a medium suitable for the growth of anaerobic microorganisms. The tubes should be held at 65°C for 1 hour to reduce the content of non-spore-forming organisms, after which they should be incubated for at least one week between 35°C and 37°C. From every tube showing growth, subcultures should be made on to plates of a suitable medium which should be incubated anaerobically at the same temperature. All anaerobic colonies should be examined and identified and if *Cl. tetani* or other pathogenic spore-forming anaerobes are present the final bulk should be discarded.

Organisms resembling pathogenic *Clostridia* found in the tube culture from which the subculture was made may be tested for pathogenicity by inoculation into animals as follows: groups of not less than two guinea-pigs and five mice are used for each tube culture to be tested: 0.5 ml of the cultures is mixed with 0.1 ml of a freshly prepared 4% solution of calcium chloride and injected intramuscularly into each of the guinea-pigs; 0.2 ml of

the cultures mixed with 0.1 ml of this calcium chloride solution are injected intramuscularly into each of the mice. The animals are observed for one week. If any animal develops symptoms of tetanus, or if any animal dies as a result of infection with spore-forming anaerobes, the final bulk should be discarded.

If other methods are used for this test, they should have been demonstrated, to the satisfaction of the national regulatory authorities, to be at least equally effective to the test above for detecting the presence of *Cl. tetani* and other pathogenic spore-forming anaerobes.

#### A.4. **Filling and containers**

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (9) should apply to vaccine filled in the final form.

Care should be taken to ensure that the material of which the container is made does not adversely affect the virus content of the vaccine under the recommended storage conditions.

#### A.5. **Control tests on final product**

Samples should be taken from each filling lot for the tests described in the following sections.

##### A.5.1 **Identity test**

The vaccinia virus should be identified by an appropriate method.

Appropriate methods include serology, growth characteristics and molecular methods.

##### A.5.2 **Tests for bacteria and fungi**

Vaccines other than those produced in animal skins should be tested for bacterial and fungal sterility according to the requirements given in Part A, section 5, of the revised Requirements for Biological Substances No.6 (Requirements for the Sterility of Biological Substances) (13).

##### A.5.3 **Virus titration**

The vaccinia titre should be determined using assays that include a reference preparation. Dried vaccine should be reconstituted to the form in which it is to be used for human inoculation before the test is made. The minimum virus titre is  $8.0 \log_{10}$  pock forming units/ml, or the validated equivalent in plaque forming units or TCID<sub>50</sub> units, unless a lower titre is justified by clinical study, and this should be maintained to the end of the shelf-life of the batch.

The 95% confidence intervals of the assays should not differ by a factor of more than 0.5 log<sub>10</sub> from the estimated number of infectious units in the vaccine.

For the test of virus concentration in cell cultures, an international collaborative study is in progress that will provide valuable information on the most appropriate method to recommend. Results of this study are expected in 2003.<sup>1</sup>

#### **A.5.4 Accelerated degradation test**

Representative final containers of the vaccine should be incubated at an elevated temperature for a defined period of time. For freeze-dried vaccines this should be 37°C for 4 weeks. For non-lyophilized vaccines, other temperatures and time periods may be determined on a case-by-case basis by the national regulatory authority.

The purpose of this test is to show that each new batch of vaccine is consistent, when exposed to heat stress, with the batches that were tested in real-time stability studies and used to determine the shelf-life of the vaccine.

The total virus content in both exposed and unexposed vials should be determined concurrently with that of a reference preparation. The vaccine passes the test when the loss on exposure is not greater than a factor of 1.0log<sub>10</sub> infectious units per human dose, and the residual titre after heating is greater than that specified in section A.5.3.

#### **A.5.5 Preservative content**

Where appropriate, each filling lot should be assayed for preservative content if this has not been done for the final bulk. The method used and content permitted should be approved by the national regulatory authority.

#### **A.5.6 Endotoxin content**

Each filling lot should be tested for endotoxin if this has not been done on the final bulk. The method used and content permitted should be approved by the national regulatory authority.

#### **A.5.7 Test for pH**

The pH of each filling lot should be determined and be within limits approved by the national regulatory authority.

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<sup>1</sup> Results of the study are available in document WHO/BS/03.1977

**A.5.8 Protein content**

The protein content of each filling lot, if not done on the final bulk, should be determined and be within limits approved by the national regulatory authority.

**A.5.9 Ovalbumin content**

For vaccines produced in embryonated eggs only, the ovalbumin content of each filling lot, if not done on the final bulk, should be determined and be within limits approved by the national regulatory authority.

**A.5.10 Residual moisture**

The residual moisture content of each filling lot of freeze-dried vaccine shall be determined and be within limits approved by the national regulatory authority.

**A.5.11 General safety (innocuity) test**

Each filling lot should be tested for unexpected toxicity (sometimes called abnormal toxicity) using a general safety (innocuity) test approved by the national regulatory authority.

This test may be omitted for routine lot release once consistency of production has been well established to the satisfaction of the national regulatory authority and when good manufacturing practices are in place. Each lot, if tested, should pass a test for abnormal toxicity. However it should be noted that preliminary experiments may be needed to determine the sample volume to use for this product in this test.

**A.6. Records**

The recommendations given in Good Manufacturing Practices for biological products (9) should apply.

**A.7. Samples**

The requirements given in Good Manufacturing Practices for Biological Products (9) should apply.

**A.8. Labelling**

The requirements given in Good Manufacturing Practices for Biological Products (9) should apply, with the addition of the following.

The label on the container or package should include the following information:

- the designation of the strain of vaccinia virus contained in the vaccine;
- the minimum amount of virus contained in one ml;
- the substrate used for the preparation of the vaccine;
- the nature and amount of any stabilizer, preservative or additives present in the vaccine; and
- the nature and amount of any stabilizer, preservative or additives present in the diluent.

No vaccine should be released for distribution without an adequate indication of the expiry date of the vaccine. This may be displayed on the primary or secondary packaging.

It is desirable for the label to carry the names both of the producer and of the source of the bulk material, if the producer of the final vaccine did not prepare it. The nature and amount of the antibiotics present in the vaccine, if any, may be included.

#### A.9. **Distribution and shipping**

The requirements given in Good Manufacturing Practices for Biological Products (9) should apply.

#### A.10. **Storage and expiry date**

The statements concerning storage temperature and expiry date appearing on the primary or secondary packaging should be based on experimental evidence and should be submitted for approval to the national regulatory authority.

##### A.10.1 **Storage conditions**

Before being released by the manufacturing establishment, all vaccines in final containers should be kept continuously in the frozen state at a temperature below  $-20^{\circ}\text{C}$ .

The maximum duration of storage should be fixed with the approval of the national regulatory authority and should be such as to ensure that the minimum titre specified on the label of the container (or package) will still be maintained after release by the manufacturing establishment until the end of the shelf-life, if the conditions under which the vaccine is stored are in accordance with what is stated on the label. The maximum duration of storage at  $2-8^{\circ}\text{C}$  or below  $-20^{\circ}\text{C}$  may be specified.

Since vaccinia virus batches may be stockpiled for special contingencies, very long-term storage may be envisaged. Under these exceptional circumstances it is permissible for batches to be retested at defined intervals for extension of the storage period. The retesting should involve the accelerated degradation test given in Part A, section A.5.4. If the batch complies with the specifications given in Part A, section A.5, the storage period can be extended by the same amount as the original period.

#### **A.10.2 Expiry date**

The expiry date should be fixed with the approval of the national regulatory authority and should relate to the date of the last satisfactory determination, performed in accordance with Part A, section A.5.3, of virus concentration, i.e. the date on which the test system was inoculated.

## **Part B. Recommendations for national regulatory authorities**

### **B.1 General**

The general recommendations for national regulatory authorities given in Guidelines for National Authorities on Quality Assurance for Biological Products (16), which specify that no new biological substance should be licensed until consistency of production has been established, should apply.

The detailed production and control procedures and any significant changes in them should be discussed with and approved by the national regulatory authority. The national regulatory authority should obtain the International Standard for virus titre and, where necessary, establish national working reference preparations by comparison with this preparation.

### **B.2 Release and certification**

A vaccine lot should be released only if it satisfies Part A of the present Recommendations. Before any vaccine lot is released from a manufacturing establishment, the requirements for consistency of production given in Guidelines for National Authorities on Quality Assurance for Biological Products (16) should be met.

A statement signed by the appropriate official of the national regulatory laboratory should be provided if requested by a manufacturing establishment and should certify whether or not the lot of vaccine in question meets all national requirements as well as Part A of the present Recommendations. The certificate should further state the date of the last satisfactory determination of virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document should be attached.

The purpose of the certificate is to facilitate the exchange of smallpox vaccine between countries. National Regulatory Authorities should consider re-certification of vaccine lots at the time of distribution.

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## References

1. Requirements for smallpox vaccine. (Requirements for Biological Substances No.5.) In: *WHO Expert Group on Requirements for Biological Substances.*, Geneva, World Health Organization, 1965, Annex 4 (WHO Technical Report Series, No. 323).
2. Fenner F et al. *Smallpox and its eradication.* Geneva, World Health Organization, 1988.
3. *The global eradication of smallpox. Final Report of the Global Commission for the Certification of Smallpox Eradication.* Geneva, World Health Organization, 1980.
4. Committee on orthopoxvirus infections. Report of fourth meeting. *Weekly Epidemiological Record*, 1986, **61**:289–293.
5. Marrenikova et al. Characteristics of virus strains for production of smallpox vaccines: In: Gusic, B, ed. *Proceedings of the symposium on smallpox.* Zagreb, Yugoslavia, 2–3 September, 1969.
6. Smallpox Vaccine. In: *WHO Expert Committee on Biological Standardization. Fifteenth report.* Geneva, World Health Organization, 1963 (WHO Technical Report Series, No. 259, page 17).
7. Krag P, Bentzon MW. The International Reference Preparation of Smallpox Vaccine *Bulletin of the World Health Organization* 1963, **29**:299–309.
8. Anti-smallpox serum. In: *WHO Expert Committee on Biological Standardization. Eighteenth report.* Geneva, World Health Organization, 1966 (WHO Technical Report Series, No. 329, page 18).
9. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second report.* Geneva, World Health Organization, 1992, Annex 1 (WHO Technical Report Series, No. 822).
10. *Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products.* Geneva, World Health Organization, 2003 WHO/BCT/QSD/2003.01.
11. Requirements for the use of animal cells as in vitro substrates for the production of biologicals. (Requirements for Biological Substances No. 50.) In: *WHO Expert Committee on Biological Standardization. Forty-seventh report.* Geneva, World Health Organization, 1998 (WHO Technical Report Series, No. 878).
12. Robertson JS et al. Assessing the significance of reverse transcriptase activity in chick cell-derived vaccines. *Biologicals* 1997, **25**:403–414.
13. General requirements for the sterility of biological substances (Requirements for Biological substances No.6, revised 1973). In: *WHO Expert Committee on Biological Standardization. Twenty-fifth report.* Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 530); and Amendment 1995 in *WHO Expert Committee on Biological Standardization. Forty-sixth report.* Geneva, World Health Organization, 1998 (WHO Technical Report Series, No. 872).

14. Recommendations for the production and control of poliomyelitis vaccine (oral). In: *WHO Expert Committee on Biological Standardization. Fiftieth report*. Geneva, World Health Organization, 2002 (WHO Technical Report Series, No. 904).
15. Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives. (Requirements for Biological Substances No. 27, revised 1992) In: *WHO Expert Committee on Biological Standardization. Forty-third report*. Geneva, World Health Organization, 1994 (WHO Technical Report Series, No. 840).
16. Guidelines for national authorities on quality assurance for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second report*. Geneva, World Health Organization, 1992, Annex 2 (WHO Technical Report Series, No. 822).

## Annex 2

# **Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses (Addendum, 2003, to the Recommendations for the Production and Quality Control of Poliomyelitis Vaccine (Inactivated))**

The WHO Recommendations for the Production and Quality Control of Poliomyelitis Vaccine (Inactivated) were last revised in 2000. At that time it was envisaged that the production and quality control of inactivated poliomyelitis vaccine (IPV) manufactured from wild poliovirus strains should, in the near future, comply with more stringent biosafety conditions. This was because of the context of an increasingly polio-free world and the need for effective containment of wild poliovirus strains as a pre-condition of global certification of polio eradication.

These guidelines specify steps to be taken to minimize the risk of reintroducing wild poliovirus from a vaccine-manufacturing facility into the community after global certification of polio eradication. Each of the following sections constitutes guidance for national regulatory authorities and for the manufacturers of inactivated poliomyelitis vaccine. If a national regulatory authority so desires, these Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by a national regulatory authority. It is recommended that modifications to these Guidelines be made only on condition that the modifications ensure that the risks of reintroducing wild poliovirus to the community are no greater than as outlined in the Guidelines set out below.

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## 1. Introduction

In May 1999 the World Health Assembly reaffirmed the commitment of the World Health Organization (WHO) to eradicating poliomyelitis and urged all member states to begin the process leading to the containment of stores of wild poliovirus. In December 1999, after consultation with scientists, ministries of health and vaccine manufacturers worldwide, WHO published the *WHO global action plan for laboratory containment of wild polioviruses*. A second edition of the Global Action Plan published in 2003 replaces the first. The purpose of the plan was to identify steps that all countries should take to minimize the risk of reintroducing wild poliovirus from a laboratory or vaccine production facility into the community after polio eradication. The plan calls for materials infected or potentially infected with poliovirus to be handled and stored under biosafety conditions appropriate to their risk; implementation of these containment levels would be a requirement for the certification of eradication. For facilities such as IPV manufacturers, where infectious materials come into contact with permissive cells or animals, high containment (Biosafety level (BSL-)3/IPV) measures are required.

Biosafety level-3/IPV requires the primary and secondary containment of infectious materials containing wild poliovirus, with provi-

sions governing air, water and materials entering and leaving the facility, specific requirements for personal protective clothing, laboratory design, the use of laboratory equipment and medical surveillance of laboratory staff. Additionally it requires vaccination of all staff against polio, appropriate training in biosafety procedures, and validation and documentation of the physical and operational requirements. Implementation of such containment provisions within IPV production and quality control testing facilities must also take into account the large quantities and concentrations of live vaccine that are produced, the industrial scale of facilities, and the existing rules and regulations governing the manufacture and testing of medicinal products, generally known as good manufacturing practices (GMP).

The control of contamination is a major concern in both biosafety and GMP. Whereas GMP prioritizes the safety of the patient being treated with the medicinal product, biosafety focuses on the protection of the personnel and the surrounding environment. These concepts are not mutually exclusive, and a considerable area of overlap exists between the two. It may not always be possible for facilities and procedures to meet the ideal situation as seen from both GMP and biosafety perspectives, and some degree of flexibility may be required to satisfy both objectives. A number of the internationally harmonized GMP guidelines, such as those of WHO or the European Union, have introduced specialized GMP requirements for biological products. The requirements for IPV producers in the post-eradication era should be viewed as a special addendum to be read with these GMP guidelines. It would apply in the exceptional case where a licensed medicinal product must be manufactured and tested within a large-scale containment facility, when, through the combined efforts of many partners, the disease for which the vaccine has been used has been eliminated globally.

This document addresses the period after the interruption of wild poliovirus circulation. At that time, natural immunity acquired as a result of contact with wild poliovirus will start to decline, but immunization coverage is anticipated to remain at levels adequate to protect both individuals and communities. During this period, the implementation of large-scale BSL-3/IPV containment measures will provide sufficient barriers against the accidental release of poliovirus from vaccine manufacturing facilities into the environment. If polio immunization is discontinued in some or all countries after global certification, containment requirements for wild viruses or attenuated viruses used in OPV will need to be reconsidered and may become more stringent, to deal effectively with the consequences of inadvertent transmission of wild poliovirus from the laboratory or vaccine

production facility to an increasingly non-immune community. However, it is acknowledged that the practices and facilities necessary for highest containment laboratories working with Biohazard Level-4 pathogens may restrict the availability of commercially produced polio vaccines. These guidelines have been produced in the full recognition that adequate global capacity for IPV production must be maintained while effective biosafety measures are in place, and this will remain the shared responsibility of the global IPV manufacturers, national oversight authorities for medicines and the environment and the partner organizations active in the global eradication of polio.

## Glossary

The definitions given below apply to the terms as used in these guidelines. They may have different meanings in other contexts.

### *Aerosol*

A dispersion of solid or liquid particles of microscopic size in a gaseous medium.

### *Air balance*

The necessity to keep air supply and exhaust systems in balance by means of measurements of static pressure, fan and motor performance, and air volumes.

### *Airlock*

Areas situated at entrances to or exits from rooms that prevent air in one space from entering another space. Airlocks generally have two doors and a separate exhaust ventilation system. In some cases a multiple-chamber airlock consisting of two or more airlocks joined together is used for additional control.

### *Biocontainment technologies*

The science of measurement or testing of the capability of primary and secondary containment devices.

### *Biosafety committee*

An institutional committee of individuals versed in the subject of containment and handling of infectious materials.

### *Biosafety level (BSL)-3/IPV*

Biosafety level specific for containment of poliovirus in the production and quality control of inactivated poliomyelitis vaccine with specialized air-handling systems, waste effluent treatment, immunization of staff, specialized training, and validation and documentation of physical and operational requirements.

*Biosafety manual*

A comprehensive document describing the physical and operational practices of the laboratory facility with particular reference to infectious materials.

*Biosafety officer*

A staff member of an institution who has expertise in microbiology and infectious materials, and has the responsibility for ensuring that the physical and operational practices of various biosafety levels are carried out in accordance with the standard procedures of the institution and national and international legislation.

*Backflow prevention device*

A device designed to prevent backflow or back-siphoning in a piping system.

*Biohazard risk level*

A risk level or group assigned to a microorganism based on its pathogenicity, mode of transmission and host range, and the local availability of effective preventive and treatment measures.

*Biohazard sign*

A sign posted to provide information on infectious agents in use in the laboratory. Information includes the universal biohazard symbol, the name of the agent, immunization requirements for entry to the laboratory, and emergency response information.

*Biological indicators*

The use of organisms to test the efficacy of sterilization processes.

*Biological safety cabinet*

Primary and partial containment work enclosure used for manipulation of materials that may cause infections or sensitization of workers. They are equipped with high efficiency particulate air (HEPA) filters and may or may not be open-fronted.

*Certification*

Documentation stating that a system qualification, calibration, validation or revalidation has been performed appropriately and the results are acceptable.

*Decontamination*

A process by which an object or material is freed of contaminating agents.

*Double gloving*

The wearing of two pairs of protective gloves, one over the other.

*Electronic data gathering and transmission systems*

Systems for recording and transmitting information such as facsimile (fax) or computer scanning systems.

*Eyewash station*

A dedicated device supplying clean water for emergency cleansing of eyes contaminated with biological or chemical agents.

*Filter housings*

Airtight containment enclosures for the location of high efficiency particulate air (HEPA) or other high efficiency filters.

*Floor dams*

Purpose-built elevations to enclose liquid spills.

*Fumigation*

The process whereby one or more chemicals are applied in the gaseous state to an enclosed space for the purpose of decontaminating the area.

*Good manufacturing practices*

That part of quality assurance that ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

*High efficiency particulate air (HEPA) filter*

A filter capable of removing at least 99.97% of all particles with a mean aerodynamic diameter of 0.3 micrometres.

*Inactivation*

Rendering an organism inert by application of heat, or other means.

*Magnetic badge controller*

A programmable device, which will interact with door locks to allow only authorized personnel entry to restricted areas.

*Master seed lot*

A culture of microorganism distributed from a single bulk container in a single operation, in such a manner as to ensure uniformity and stability and to prevent contamination.

*Mucosal immunity*

The immune defence mechanisms of the host that are associated with mucosal surfaces.

*Neutralizing antibody*

Antibody, which alone or in combination with complement, neutralizes the infectivity of a virus. The level of serum neutralizing antibody

that protects against severe clinical illness such as paralysis has not been determined. Titres of 1:8 are generally assumed to provide protection against contracting poliomyelitis, although it is possible that persons with low but detectable serum antibody may become re-infected with poliovirus.

*Penetrations*

Openings through wall, floors, or ceilings to allow access for mechanical services.

*Positive pressure laminar flow hood*

An enclosure with unidirectional outflowing air, generally used for product protection.

*Primary containment*

A system of containment, usually a biological safety cabinet or closed container, which prevents the escape of a biological agent into the immediate working environment.

*Respirator*

A respiratory protective device with an integral perimeter seal, valves and specialized filtration, used to protect the wearer from toxic fumes or particulates.

*Risk analysis*

A formalized documented process for analysing risks.

*Secondary containment*

A system of containment, usually involving specialized air handling, airlocks, and secure operating procedures, which prevents the escape of a biological agent into the external environment or into other working areas.

*Sharps*

Devices used in the laboratory, which are capable of cutting or puncturing skin (e.g. needles, scissors and glass)

*Sterilization*

A process that destroys and/or removes all classes of microorganisms and their spores.

*Validation*

The documented act of proving that any procedure, process, equipment, activity, or system actually leads to the expected results.

## 2. **Biosafety implementation within a vaccine production facility**

A breach of containment of poliovirus used in a vaccine production or testing facility can theoretically occur through contaminated clothing, liquid effluents or air emissions, or incorrect virus disposal. Transmission from the laboratory or vaccine production facility to the community is most likely to result from either equipment failure or human error. Of greatest concern is the inadvertent transmission of poliovirus to the community through an infected laboratory or vaccine production worker. There is also the possibility that an unexpected emergency could lead to release of infectious materials into the community. The provisions in these guidelines seek to minimize the risk of these occurrences.

- 2.1 The implementation of biosafety level-3/IPV requires that the institution employ a biosafety officer who is knowledgeable in large-scale viral vaccine production and containment, but is independent of production in his or her reporting structure. The biosafety officer is responsible for the independent oversight of the implementation of the biosafety practices, policies and emergency procedures in place within the company or organization. A biosafety officer is needed in addition to a qualified person who, in some countries, has overall responsibility for a medicinal product.
- 2.2 There should also be a biosafety committee comprising representatives of viral vaccine production and quality control facilities that is responsible for reviewing the biosafety status within the company and for coordinating preventive and corrective measures. The institutional biosafety officer must be a member. The chairperson of the committee should be independent of both the production and quality control functions. Due to the importance of containing wild poliovirus following the global cessation of transmission, the management and governing board of the manufacturing company should ensure that adequate priority and resources are made available to the committee to implement the required measures.
- 2.3 A detailed and comprehensive risk analysis should be conducted to define possible contamination sources to personnel or the environment that may arise from the production or testing of live poliovirus within the establishment. For each procedure or system, this analysis should take into account the concentration and stability of the virus at the site, the potential for inhalation, ingestion or injection that could result from accidents, and the poten-

- tial results of a major or minor system failure. The procedural and technical measures to be taken to reduce the risk to workers and the environment should be considered as part of this analysis. The results of this risk analysis should be documented.
- 2.4 A comprehensive biosafety manual must be created and its instructions implemented. The manual must fully describe the biosafety aspects of the production process and quality control activities and define emergency procedures, waste disposal and the requirements for safety practices and procedures as identified in the risk analysis. The manual should be made available to all staff of the production and quality control units, and at least one copy should be kept in the containment area(s). The manual should be reviewed and updated annually.
  - 2.5 Comprehensive guidelines outlining the response to biosafety emergencies and accidents should be prepared and made available to key personnel for information and for coordination with emergency response units. These guidelines should be reviewed and updated annually.
  - 2.6 The implementation of Biosafety level 3/IPV requirements in the production and testing facilities should be verified through an independent assessment. Current national requirements concerning verification mechanisms should be complied with.

### 3. **Personnel**

- 3.1 The manufacturing establishment and its personnel should be under the authority of a qualified person who has been trained in the techniques used in manufacturing biological substances and who possesses the scientific knowledge upon which the manufacture of these products is based.
- 3.2 Personnel required to work in the polio virus containment area(s) should be selected with care to ensure that they may be relied upon to observe the appropriate codes of practice and are not subject to any disease or condition that could compromise the integrity of the product or the safe containment of the poliovirus strains with which they work. The acceptance by staff that the adequate containment of poliovirus is an individual responsibility is a key factor in its implementation and maintenance.
- 3.3 Health examinations of personnel should be required before the start of employment and periodically (at least annually) thereafter. Any changes in health status that could adversely affect the quality of the product or the containment procedures should preclude the person concerned from working in production or

testing of live poliovirus. Immune compromised individuals should not be permitted to work in the containment area. Attention should be paid to ensure that adequate precautions are taken to minimize the risk of transmission of poliovirus from personnel to their immediate family members, and that emergency precautions are ready for implementation in the case that such a danger exists.

- 3.4 All personnel (including those concerned with cleaning, maintenance or quality assurance) employed in areas where live poliovirus is manufactured or tested should receive additional training specific to their work with poliovirus. This should include relevant information and training in hygiene and microbiology as it relates to IPV and poliovirus, as well as basic principles of BSL-3/IPV containment procedures. Personnel coming in direct contact with poliovirus-containing materials in the production and quality control area, including technicians and support personnel, must demonstrate proficiency in standard microbiological practices as outlined in the WHO Biosafety Manual (see Bibliography) as appropriate to the production and testing of IPV. Records of this training should be kept and periodic assessments of the effectiveness of training programmes conducted.
- 3.5 Personnel engaged in IPV production and testing and all visitors to the production and testing facilities, including representatives of regulatory authorities, civil inspectors or emergency personnel should be vaccinated with poliomyelitis vaccines, and adequate blood titres of circulating neutralizing antibody titres against all three serotypes should have been verified. Test results should be no older than 2 years. Results of titre measurements in staff should be kept indefinitely. To ensure adequate mucosal immunity and reduce the likelihood of faecal shedding, consideration should be given to immunizing these personnel with oral poliomyelitis vaccine (OPV) alone or in addition to IPV.
- 3.6 After any major spill or accident where there is reason to believe that the personnel may have been infected with poliovirus, national authorities should be notified and the appropriate emergency containment and monitoring procedures initiated. Diagnostic examinations of personnel should be carried out to determine whether they are shedding poliovirus in their faeces. If poliovirus is being shed in faeces, appropriate measures as detailed in the facility guidelines for responding to biosafety emergencies and incidents should be employed to avoid transmission of poliovirus to other persons or to the public sewage system.

## 4. Premises and equipment

Premises should be designed in such a way as to control both the risk to the product and to the environment. This is accomplished by establishing a primary containment barrier, using appropriate safety equipment to protect the immediate workspace, and enclosing this system within a secondary containment barrier that protects the environment external to it from accidental exposure to infectious materials. These systems must provide adequate safeguards to protect the product against contamination with extraneous agents depending on the level of environmental cleanliness required by the operation.

### General requirements

- 4.1 Live poliovirus and materials with the potential to contain live poliovirus should be handled in contained areas. Contaminated materials, including equipment for repair or maintenance, should be decontaminated by a validated method prior to removal from the containment area.
- 4.2 Whenever possible, polio production facilities and quality control facilities should be in dedicated buildings. If they are located in multipurpose buildings, the polio production facility must have separate entrances and exits for personnel and materials, and dedicated air and biological waste handling systems must be provided. Polio quality control laboratories in multi-purpose buildings should be equipped with air handling and waste disposal systems that preclude the contamination of other areas with material infected with poliovirus.
- 4.3 Use of the poliovirus facility for production of other organisms on a campaign basis may be acceptable provided that the facilities are disinfected following poliovirus production using a validated area fumigation procedure.
- 4.4 Laboratories and production areas should be marked with approved biohazard signs. Information should be posted in prominent locations at the entry to the BSL-3/IPV facility that poliovirus is contained in the area, that immunization against poliomyelitis is required for entry, and that only personnel authorized to work with poliovirus are permitted to enter. The name(s) and contact information of persons to be contacted in the event of an emergency should be displayed and kept up to date at all times.
- 4.5 All exits must be marked. Emergency exit doors from the polio facility must be alarmed and their use treated as a breach of containment.

- 4.6 Protective laboratory or production clothing such as solid-front or wrap-around gowns, scrub suits or coveralls with head and shoe covers should be worn at all times by operators while in the containment facility. Eye covers or full-face masks should be required when there is a potential for generating aerosols. Respirators should be used when conducting procedures with a high probability of aerosol generation. Disposable gloves should be worn when handling infectious materials or contaminated equipment. This protective clothing is removed on leaving the containment facility. Clothing should be decontaminated before being laundered or disposed of.

### **Security access control**

- 4.7 Access to the plant or institution grounds should be monitored and appropriate security measures should be put in place to avoid the entry of unauthorized persons or intruders at any time.
- 4.8 Access to the polio vaccine production facility or polio vaccine quality control testing laboratories should be strictly limited to personnel with authorization to enter the specific area. Entrance into the facility should be monitored at all times. An example of a currently accepted procedure would be a magnetic badge controller for use with self-closing locked entry doors.

### **Primary containment barriers**

- 4.9 Biological safety cabinets should be provided and used within the production and quality control areas where live virus or infected cell cultures are handled or manipulated and where such activities cannot be carried out in closed transfer systems. Positive pressure laminar flow hoods should not be substituted for negative-pressure biological safety cabinets when handling potentially infectious material.
- 4.10 Biological safety cabinets must be constructed and manufactured in accordance with national regulations or standards, such as British Standards Institution (BSI), Deutsche Industrie Norm (DIN) or National Science Foundation (NSF). They must be tested and certified at least annually as meeting those standards. Cabinets with design modifications to meet the requirements of large-scale operations, but providing equivalent containment levels, may be utilized if approved by the responsible national authorities.
- 4.11 When exhaust air from biological safety cabinets is to be discharged through the building exhaust air system, the air handling system must be designed in such a way as to not disturb the

air balance of the cabinet or the room in which the cabinet is situated.

- 4.12 Bio-reactors should be designed wherever possible as closed systems with entry and exit ports that do not require open manipulation of viable poliovirus in the production room, e.g. using steam-through valves or sterile tube welders. Bio-reactors should have the capability of being sterilized while loaded to their maximum capacity. Bioreactor air vents should be provided with high efficiency particulate air (HEPA) filters or an equivalent system to sterilize exhaust gases.

### **Secondary containment barriers**

- 4.13 Containment areas should be separated from access corridors by separate airlocks for personnel and materials. Airlocks should consist of one or more closed chambers and be equipped with interlocking doors or an equivalent system to ensure that both doors cannot be opened simultaneously. Personnel and material airlocks with doors leading to the containment area should be provided with a ducted ventilation system that exhausts air through a HEPA filter. Adequate time should be allowed for the air handling system to flush out contaminants that have entered the airlock from the containment area before opening the door leading to the exterior. When possible, separate airlocks for the entry and exit of personnel should be provided.
- 4.14 Airflow patterns should not present a contamination risk. Care should be taken to ensure that air flows do not distribute poliovirus from a zone of higher contamination risk to a zone of lower contamination risk. A pressure differential of at least 10–15 Pa should be maintained at all times between zones.
- 4.15 An air handling system should maintain a negative pressure (inward directional air flows) in areas where live poliovirus is handled or there is a potential for contamination. The installation of HEPA filters provides a filter efficiency of 99.97% or greater removal of 0.3-micrometre particles. Air from areas where live poliovirus is handled or where there is a potential for contamination should be extracted through HEPA filters at the point of air removal from the chamber or airtight ducts. Although not normally recirculated, air may be recirculated to the same area provided it is HEPA filtered before reuse. A proper system for maintenance and testing of HEPA filters must be in place. Heat exchangers may be utilized to recover warmth from HEPA-filtered exhaust air.

- 4.16 HEPA filters should be installed into the air handling systems in such a manner as to allow gaseous decontamination of the filters before removal or testing by accepted aerosolized challenge methods. Such filters must be tested and certified upon installation and at least annually thereafter.
- 4.17 Indicators of pressure differences should be fitted where these differences contribute to containment. Pressure difference readings for rooms or across HEPA filters should be monitored and recorded regularly.
- 4.18 A warning system consisting of an audible or visual signal that can be readily perceived by personnel in the containment facility should be installed to indicate any failure in the air handling system.
- 4.19 The supply and exhaust air must be interlocked to prevent the positive pressurization of the containment area in the event of a failure of the system.
- 4.20 Exhaust air should provide sufficient air changes in both the quality control and production areas to provide an appropriate level of environmental cleanliness. There should be at least 10 air changes per hour.
- 4.21 Containment premises should be easy to disinfect and should have the following characteristics:
- There should be no windows that can be opened or any direct venting to the outside. Windows must be constructed of break-resistant safety glass with strength characteristics conforming to those required for the purpose for which they are used.
  - Passageways for pipes, tubes and ducts passing through the wall between the containment area and surrounding areas should be completely sealed with materials resistant to contaminants and capable of withstanding disinfectants.
  - Floor drains, where installed, must be capped, fitted with liquid-tight gaskets, or connected to a waste effluent decontamination system to prevent inadvertent release to the sanitary drain.
  - Wherever possible, provisions should be made to contain liquids leaking from bio-reactors or tanks (including waste tanks) by means of floor dams or ramps that enclose a volume equal to the maximum fluid contained in the vessels plus the disinfectant required for inactivation.
  - All liquid and gas services to the containment area must be protected from back flow. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters or 0.2µm hydrophobic membrane filters, or their equivalent.

- 4.22 If circulating water with open taps is used within the containment area, a spill or contamination at the point of use should not result in a breach of containment via the water system. Water loops should be maintained at 80°C or greater, and dead legs should be avoided. Heat exchangers may be used to cool water at the point of use provided that water from the exchanger is not returned to the loop leading outside of the containment area. If there is an accidental release of poliovirus in the areas served by the water loop, or if the circulating temperature of the water system drops below its set point to an extent identified by the risk assessment, an alarm should sound and the system temperature should be raised to a temperature and for a time that have been validated for the ability to kill poliovirus before taps can be opened outside or inside the containment area.
- 4.23 A communication system consistent with the facility containment conditions should be maintained between the support or administrative area and the containment area and shall be kept in working order at all times.
- 4.24 Emergency lighting and power to the containment area and critical containment devices (e.g. biological safety cabinets and air handling systems) should be available and automatically activated in the case of a power failure.

### **Sterilization and waste disposal systems**

- 4.25 Decontamination of solid, liquid and gaseous wastes should take place within the containment area. Should any wastes have to be transported out of the facility prior to decontamination and disposal, they must not be transported through public areas and must be packaged, labelled and transported in accordance with applicable regulations.
- 4.26 The production unit must be provided with one or more interlocking, double door pass-through autoclaves, the performance of each of which is validated at least annually. Liquid effluent from the sterilization chamber of the autoclave should go to the liquid effluent sterilization system of the building. Other means of sterilization of materials, such as fumigation with formaldehyde, gaseous peracetic acid or vapour phase hydrogen peroxide may be used if they have been fully validated for efficacy.
- 4.27 Effluents from equipment, showers and sinks within the containment area must be sterilized by autoclaving or discharge to a liquid effluent decontamination system. Such a system must be fully validated to ensure efficacy and be contained in an area compatible with the requirements of BSL-3/IPV. The effluent

- tanks must be situated in an area with floor dams that will contain the full tank volume. If decontamination is to take place within the floor dam, the volume must be sufficient to add enough disinfectant to fully inactivate its contents.
- 4.28 Viral seeds must be stored within a BSL 3/IPV containment area, or if in a separate location, under BSL 3/IPV conditions in leakproof primary containment containers. Secondary containers for transfer of viral seeds from the storage area to the production area should be leak-proof and unbreakable.
  - 4.29 The areas for the storage of viral seed stock must be dedicated and fully secured against entry by non-authorized personnel. For secondary (back-up) seed storage locations where stocks are not normally used for production, the national regulatory authority may approve storage in leak-proof containment containers within a dedicated freezer that is subject to security and access restrictions appropriate for the storage of poliovirus.
  - 4.30 The viral seed stock must be inventoried. Addition or removal of material must be conducted by authorized personnel following the approval of two authorized signatories on record, or the electronic equivalent of this approval. Records of additions or removal of viral seed must be securely stored.
  - 4.31 The viral seed storage area must be equipped with a back-up emergency power source and recording and alarm systems to monitor freezers.

## **5. Documentation and validation**

- 5.1 Detailed records of operating parameters for the containment facility should be produced and maintained for conducting assessments of the facility performance.
- 5.2 All spills or accidental release of infected materials and the response to such events should be properly investigated and documented. These results of these investigations should be used to review and revise the facility and applicable operating procedures as required.
- 5.3 The production facility must be designed and constructed in such a manner as to allow for full validation and verification of containment processes. It is the responsibility of the institution to ensure that these facilities meet acceptable standards that will ensure containment of poliovirus as well as the protection of the staff and the environment. Tests should be carried out at the completion of construction or renovation. Annual verification that the facility continues to meet the containment conditions should be per-

formed. Records of the annual verification should be maintained throughout the lifetime of the IPV production facility and for at least 5 years after the facility stops production. At the minimum the following containment features should be assessed:

- integrity of containment perimeter, including penetrations through floors, walls and ceilings;
- air-tightness of supply and exhaust ductwork between incoming and first outgoing HEPA filter ducting in the air handling systems. The duct should be considered to be part of the room up to the point of the disinfecting filter or incinerator;
- integrity of all HEPA filters and high efficiency filters and filter housings;
- directional inward air-flow from non-contained areas to containment areas;
- biological safety cabinets and all primary containment devices;
- autoclaves, including cold spot and standard load testing using biological indicators or by means of physical validation;
- waste effluent systems and holding tanks;
- liquid back-flow prevention devices;
- alarm systems for air system failures, room pressure failures, electrical failures and failures of waste treatment systems;
- fire suppression devices and alarms; and
- communication systems.

5.4 Cleaning and disinfecting procedures should be validated and documented. Only procedures that have proven effective in inactivating poliovirus should be employed. Manufacturers are urged to develop and implement monitoring procedures for validating the disinfection of poliovirus contamination of work surfaces.

## 6. **Production**

Production of IPV involves handling large volumes of concentrated preparations of live wild polioviruses. The majority of operations are carried in closed systems. Nevertheless leaks can occur from valves or during procedures such as taking samples for testing purposes. Effective containment therefore requires that all aspects of production, from the specifications for the facility and equipment through to personnel and working procedures, must be in compliance with each of the relevant sections of this guideline.

## Personal protection and equipment

- 6.1 Personnel entering the production area must meet all the established requirements for entry and should be restricted to those persons required to meet programme and support needs. They shall be fully knowledgeable in all operating practices, emergency procedures, biohazards and other hazards associated with the work.
- 6.2 Personnel must be provided with the facilities and equipment required to maintain adequate standards of good microbiological practice and personal hygiene. Provision must be made for the changing of clothing and emergency decontamination of personnel in the event of a major spill or other release of infectious materials.
- 6.3 Impervious gloves must be worn at all times in the containment area and discarded as waste for decontamination when leaving the facility. Double gloving is recommended. Outer gloves must be removed and discarded after handling potentially infectious materials. The inner gloves must be discarded on leaving the facility. Where double gloves are not worn, staff must discard gloves after handling potentially infectious materials, disinfect their hands using an adequate procedure, and put on new gloves.
- 6.4 Hands must be washed and disinfected upon leaving the containment area. Hand-washing sinks equipped with automatic (hands-free) controls and a disinfectant shown to be effective against poliovirus should be installed in the personnel air lock. All sinks must be connected with a validated waste decontamination system. The use of water-free (chemical) hand-washing systems with liquid waste decontamination is an acceptable alternative.
- 6.5 A full-body shower should be available within the personnel exit airlock from the containment area. Showers should be taken after spills, in accordance with the facility guidelines for responding to biosafety emergencies and incidents. The biosafety committee may decide, after completion of a risk assessment, that certain personnel are required to shower each time they leave the containment area. Shower drains must be connected with a validated waste decontamination system.
- 6.6 An eyewash station should be available within the personnel exit airlock and at other locations based on a risk assessment by the biosafety committee. Wastewater from eyewash stations within the production facility must be connected to the effluent treatment system.
- 6.7 All laboratory clothing must be sterilized by a validated procedure before reuse or disposal.

- 6.8 Good microbiological practices should be rigorously enforced. These include but are not limited to:
- no eating, drinking, smoking and applying of cosmetics in the containment area;
  - no mouth-pipetting;
  - implementing policies for the safe handling of sharps;
  - decontaminating work surfaces at least once a day and after any spill of viable material; and
  - decontaminating equipment before removing it from the facility for repair or maintenance.
- 6.9 Data sheets and associated materials that have been used in live virus areas must be disinfected upon removal from the containment facility, or an electronic data gathering and transmission system implemented to transfer data from the containment area.

### **Polio strains for biosafety level-3/IPV containment**

- 6.10 All wild type strains of live poliovirus are to be contained within BSL-3/IPV biosafety conditions, including strains commonly utilized for IPV production (e.g. Mahoney, MEF-1, Saukett, Brunhilde and Brunender strains).
- 6.11 Attenuated poliovirus strains (such as Sabin strains) that have been approved by the national regulatory agency in the country of manufacture for use as an oral polio vaccine, when used for manufacturing IPV, do not require containment in BSL-3/IPV facilities provided they are produced under conditions that would make them suitable for oral vaccine use. If conditions other than those approved for production of oral polio vaccine are used (e.g. different multiplicity of infection or fermentation temperatures), it must be verified that the virus so produced poses no greater medical risk than that accepted for oral poliomyelitis vaccine, or BSL-3/IPV containment must be instituted.
- 6.12 Materials containing or potentially contaminated with live poliovirus may be removed from BSL-3/IPV containment conditions when at least one of the following conditions are met:
- the material is decontaminated using a validated process proven to be effective at inactivating poliovirus, such as sterilization in an autoclave, or fumigation; or
  - in the case of in-process or quality control samples, materials have been sealed in leak-proof, unbreakable, wrapped containers appropriate for the containment of pathogenic organisms for transport between production and quality control areas, and the containers have been sealed in

protective wrapping (e.g. a double bag) and the outside of the container disinfected within the poliovirus containment areas; or

- a test for inactivation of poliovirus as described for IPV production and approved by the national regulatory authorities has been completed and the results demonstrate that no residual live virus is present; or
- following a validated inactivation procedure a kinetic measurement of virus inactivation has been completed with at least three time points, with the results of the last two time points indicating that there is no detectable live virus, and a measurement of the formalin concentration in each inactivation container has been conducted and the results indicate that the minimum validated concentration of formalin for inactivation is present. If materials are to be transferred thereafter to an area outside the BSL-3/IPV containment area until such time as all tests of effective inactivation are completed, they must be stored in sealed, leak-proof, unbreakable secondary storage containers.

6.13 Blending, mixing, and formulation of IPV should not be conducted using virus preparations prior to the completion of all tests designed to verify inactivation.

## 7. Quality control

The risks from live poliovirus in testing facilities will be different to those in the production facility. Although the volumes of virus are smaller than in the production facilities, there are many more manual manipulations of samples and infected cell cultures containing viable polioviruses that are not contained in closed systems. In cases of multipurpose quality control laboratories, personnel and materials may move more frequently in and out of the BSL-3/IPV containment area than is the case in a dedicated production facility. The risk assessment should reflect these important differences.

- 7.1 Quality control testing laboratories should maintain BSL-3/IPV conditions for all areas where materials potentially infected with live poliovirus are manipulated.
- 7.2 Prior to using the laboratory to test other products not under BSL-3/IPV containment, or when personnel not qualified to handle poliovirus are to be admitted, the laboratories must be decontaminated by a validated fumigation procedure.
- 7.3 If quality control laboratories are housed within the production facility to enhance containment control, they must be kept sepa-

- rate from the production rooms, with separate air handling systems and dedicated airlocks for personnel and material provided from access corridors.
- 7.4 Quality control laboratories for poliovirus should be equipped with facilities for hand washing and disinfection. Persons must disinfect their hands after handling infectious materials, after removing gloves, and when they leave the laboratory. If sinks are used, the waste water should be collected in a waste disposal tank and disinfected prior to disposal. The use of water-free (chemical) hand-washing systems with liquid waste decontamination is an acceptable alternative.
  - 7.5 Control cell cultures for testing for adventitious agents should be considered to be potentially contaminated with poliovirus and tests conducted under biosafety 3/IPV containment unless either:
    - the cells have been grown in an area where there has been no poliovirus and no physical connection exists between this area and an area containing live poliovirus; or
    - samples have been taken from closed vessels that have been sterilized immediately preceding the introduction of poliovirus-free cells and growth media, and the cells and media must have been introduced into the vessel through steam-through valves or a sterile tubing weld system. If this is the case, the cell control laboratory must have a separate supply and exhaust air handling system which does not circulate air to other areas, and in the event that cytopathic effects of poliovirus are observed on the control cells, the laboratory must be able to be immediately sealed and fumigated.
  - 7.6 Samples within the containment areas should be unwrapped in the quality control testing laboratories within an appropriate biological safety cabinet only after visual inspection indicates that no leaks have occurred during transport. Wrapping should be disposed of as biohazardous waste.
  - 7.7 Tests conducted using manual manipulations of live poliovirus on growth-permissive cell substrates should be considered as high-risk activities, and contained within the biological safety cabinet to the greatest extent possible. Transfers of cell cultures infected with poliovirus between areas of the laboratory should be conducted with special care, and spills or accidents require an immediate and adequate response.
  - 7.8 Special consideration should be given to providing adequate space within the containment area for storage of samples that may contain live poliovirus.

## 8. **Emergency procedures**

Production of IPV using wild polioviruses under BSL-3/IPV containment requires planning for emergencies that could result in the release of live virus within the facility or into the surrounding environment. Failures of containment systems within the facility as well as external events not under the control of the manufacturer could result in the exposure of plant personnel or the public to infectious poliovirus. Response and contingency plans must be established to minimize the impact and consequences of such accidents, and adequate mechanisms must be put in place to ensure the prompt and effective implementation of these plans should an incident occur.

- 8.1 The response to an uncontrolled release of wild poliovirus resulting from a failure in containment systems should be planned and rapidly implemented to limit exposure of persons to the virus and ensure that no further threat of exposure exists. Detailed provisions should also be made for responding to unlikely occurrences such as medical emergencies, fire, earthquake, explosions, exceptional weather conditions and extended power failures, or following access to the facility by unauthorized intruders. Special attention should be paid to events that may require the assistance of emergency personnel who may not be familiar with the facility or with the infectious nature of the agents produced.
- 8.2 In the case of a large-scale release of poliovirus, staff and emergency personnel should be supplied with protective equipment (e.g. respirators, coveralls and gloves) prior to entering BSL-3/IPV production and quality control units. This equipment should be available in sufficient quantities at the entrance to the facilities, kept in good working order, and personnel should be instructed in its use.
- 8.3 Detailed written procedures should be available at the workplace on the actions to be taken after an accident or spill involving the potential contamination of the workplace or personnel with live poliovirus.
- 8.4 All spills and accidents must be promptly reported to the facility manager or the biosafety officer.
- 8.5 Emergency equipment such as disinfectants and other clean-up materials for spills should be available in sufficient quantities for use in response to the release of infected material equivalent to the maximum capacity of the facility.
- 8.6 The area surrounding small spills should be inactivated using adequate concentrations of validated disinfecting agents proven

to be effective in killing poliovirus before decontamination of the area. Such spills would include limited leakage from valves, pipettes or small containers, or accidental dropping of culture flasks and plates in quality control laboratories. Inactivation procedures should be undertaken immediately following detection of the spill. Special attention must be paid to any procedures that may have generated aerosols.

- 8.7 Larger spills, such as breaches in fermentation or liquid waste vessels or explosions, should trigger an immediate assessment of the magnitude of potential contamination. The perimeter for contamination control activities should be large enough to minimize any further spread of poliovirus. The immediate response to a spill should be to evacuate the premises and return with clean-up personnel no sooner than 30 minutes after the incident, to allow time for aerosols to settle.
- 8.8 Special attention should be paid to any potential contamination of floors, walls, ceilings, equipment, airlocks, or plant or outside clothing that may have occurred as a result of the spill. Provisions should be made to verify that all areas have been decontaminated during the clean-up procedure.
- 8.9 Personnel in the containment area at the time of the spill, emergency response personnel, law enforcement, medical or fire-fighting personnel, and persons involved in the risk assessment, clean-up and disinfection of the area all present a risk for a further breach in containment and subsequent poliovirus dissemination to the environment. Emergency personnel should be immunized against poliomyelitis and have adequate training to enable them to understand the need for the containment measures in place. Whenever these precautions are not possible, emergency personnel must be supplied with adequate protective clothing and equipment to ensure that they do not become infected with poliovirus in the course of their duties. Such protective clothing and equipment must be adequately disinfected before removal from the BSL-3/IPV facility.
- 8.10 Appropriate medical evaluation, surveillance and treatment should be provided following spills. Potentially infected personnel should be monitored for oral or faecal shedding of poliovirus.
- 8.11 A full evaluation should be carried out after any emergency involving a breach of containment. The incident and all aspects of the response to that incident should be fully investigated and documented, and revisions made to existing contingency plans and staff training as necessary to minimize its repetition.

8.12 Any major spill, accident, or suspected or confirmed poliovirus infection occurring in the area surrounding an IPV testing or manufacturing facility should be considered an urgent international public health emergency. National public health officials and responsible officials at the World Health Organization should be notified without delay.

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The current version was prepared by the Secretariat and Mrs Mary Ellen Kennedy (as above) taking into account the comments made at the consultation.

## Bibliography

American Society for Microbiology. *Large scale biosafety guidelines*. Washington, American Society for Microbiology, 1999.

- Dowdle WR, Featherstone D, Sanders R. (2001). Biosafety implications of polio eradication. *Applied Biosafety*, 6:13–18.
- European Union. *Commission Directive 91/356/EEC, of 13 June 1991, laying down the principles and guidelines of good manufacturing practice for medicinal products for human use.*
- Health Canada. *Laboratory biosafety guidelines*. 2nd ed. Ottawa, Minister of Supply and Services Canada, 1996.
- World Health Organization. *Public health action in emergencies caused by epidemics*. Geneva, World Health Organization, 1986.
- World Health Organization. *Laboratory biosafety manual*, 2nd ed. Geneva, World Health Organization, 1993.
- World Health Organization. *Biosafety guidelines for personnel engaged in the production of vaccines and biological products for medical use*. Geneva, World Health Organization, 1995.
- World Health Organization. *Quality assurance of pharmaceuticals. Vol. 2. Good manufacturing practices and inspection*. Geneva, World Health Organization, 1999.
- World Health Organization. *WHO global action plan for laboratory containment of wild polioviruses, version 2*. Geneva, World Health Organization, 2003.
- World Health Organization. Recommendations for the production and control of poliomyelitis vaccine (inactivated) In: *WHO Expert Committee on Biological Standardization. Fifty-first report*. Geneva, World Health Organization, 2002 (WHO Technical Report Series, 910).

## Annex 3

# **Recommendations for the production and control of group C meningococcal conjugate vaccines**

(Addendum 2003)

At its fifty-second meeting the Expert Committee on Biological Standardization adopted the Recommendations for the production and control of group C meningococcal conjugate vaccines, which were published in its report (1). The Committee agreed with a proposal to draft an addendum on serological assays to evaluate the immune responses to these vaccines and to review the current recommendations in the light of data emerging from the United Kingdom following the introduction of the vaccine, especially data related to the demonstration of immunological memory.

The addendum is intended to serve as an Appendix to the already adopted Recommendations.

## **Appendix 1**

### **Evaluation of the immunogenicity of group C meningococcal conjugate vaccines**

Each manufacturer should evaluate different lots of single component or combined meningococcal C conjugate vaccines for immunogenicity, including the induction of immunological memory, in the target age group before licensing. National regulatory authorities should ensure that the data made available to them are relevant to individual national immunization programmes, so that appropriate recommendations may be made regarding vaccine coadministration. For combinations of group C meningococcal conjugate vaccine and other antigens, either pre-combined or to be mixed immediately before injection, the national control authority should ensure that there are adequate studies to demonstrate that there is no clinically significant interference with the immunogenicity or induction of immunological memory by the meningococcal C conjugate component.

Two assays are utilized to measure immunogenicity of meningococcal C conjugate vaccines: the serum bactericidal antibody assay that is regarded as the gold standard and the serogroup C-specific IgG

enzyme-linked immunosorbent assay (ELISA). Early studies by Goldschneider *et al.* with polysaccharide vaccines (2) demonstrated that a serum bactericidal titre of 4 measured with human complement is an indicator of clinical protection against serogroup C meningococcal disease. The serum bactericidal antibody assay thus provides a good surrogate measurement of protective immunity associated with natural disease. Following the introduction of meningococcal group C conjugate vaccines in the United Kingdom, a re-evaluation of the correlates of protection for group C was performed (3) utilizing a large database of effectiveness data, the availability of sera for additional testing and serum bactericidal assays utilizing baby rabbit complement (4, 5). Group C meningococci are more susceptible to the bactericidal activity of group C-specific antibodies when using baby rabbit complement than when using human complement, resulting in higher serum bactericidal assay titres for most specimens (6). Nevertheless, there is a general consensus that when baby rabbit serum is used as the source of complement, serum bactericidal assay titres of <8 are predictive of susceptibility to invasive meningococcal disease. From efficacy estimates in the UK and the proportion of responders in various clinical trials of meningococcal C conjugate vaccines, it has been demonstrated that a serum bactericidal assay titre of 8 is the appropriate cut-off correlating with short-term protection (7). This has now been supported by a group C seroprevalence study performed in the UK prior to the introduction of group C conjugate vaccines (8). Additional indicators may be used. These include:

- evidence of a fourfold or greater rise in serum bactericidal antibody titre between preimmunization and 1 month post-primary immunization sera;
- a serum bactericidal titre of  $\geq 4$  utilizing human complement (3).

The ELISA is an antigen-binding assay and is more reproducible than the serum bactericidal assay, which is an assay for functional antibodies (9). The ELISA can measure total or isotype-specific serum antibody responses and is thus a useful adjunct to the serum bactericidal assay. It is however crucial that the ELISA correlates with the serum bactericidal assay. A number of serogroup-C ELISAs have been shown to do this (10–12). Factors reported to increase the correlation include the use of highly purified polysaccharide, solid-phase derivatized polysaccharide antigens, and incorporation of chaotropic agents (thiocyanate) in the serum diluent.

Although the correlates for long-term protection are not currently known, antibody levels decline with time and immunological memory

may have to be relied upon. Immunization with meningococcal C conjugate vaccines primes for the ability to generate memory antibody responses upon subsequent exposure to plain meningococcal polysaccharide (13). Although unproven, the ability of an immunized person to generate a memory antibody response upon exposure to the pathogen may be an important second mechanism of protection, particularly when serum antibody concentrations are below the protective threshold. Recent data (14) demonstrate immunological memory in 4-years-old children who had been immunized with group C conjugate vaccine at 2, 3 and 4 months of age. At 4 years of age the antibody levels had decreased to prevaccination levels.

Laboratory correlates for the induction of immunological memory include

- demonstration of immunological memory by a serum bactericidal titre greater than or equal to that of the primary response 1 month following a 10µg dose of plain polysaccharide administered at least 6 months after the primary series of immunization; or
- evidence of increase in avidity indices of serogroup C-specific IgG antibody 1–6 months after the primary series (3). Long-term monitoring will be necessary to determine whether induction of memory alone is enough to confer long-term protection against meningococcal disease

The serum antibody response to the carrier protein should also be measured in recipients of the meningococcal C conjugate vaccine to ensure that the conjugate vaccine does not interfere with protective immunity that is relevant to that protein. To date, carrier proteins such as diphtheria (CRM<sub>197</sub>) and tetanus toxoids have been used in the conjugation of meningococcal C conjugate vaccines. Since some of carriers are also components of other vaccines administered to infants and children (e.g. diphtheria, tetanus, pertussis), antibody responses to those vaccines should be measured to ensure that there is no immune interference of clinical importance. The assay for these antibodies should be a bioassay or a validated equivalent.

The following reagents are available from the National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England, courtesy of the manufacturers and national regulatory agencies:

- Meningococcal group C polysaccharide, NIBSC code 98/730
- Meningococcal serogroup anticapsular antibody human ref. serum CDC1992, NIBSC code 99/706
- Methylated human serum albumin, NIBSC code 99/592

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## References

1. Recommendations for the production and control of Group C meningococcal conjugate vaccines. In: *WHO Expert Committee on Biological Standardization, Fifty-second report*. Geneva, World Health Organization, Technical Report Series 924, 2004.
2. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus I. The role of humoral antibodies. *Journal of Experimental Medicine*, 1969, **129**:1307–1326.
3. Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: Reevaluation of correlates of protection. *Infection and Immunity*, 2001, **69**:1568–1573.
4. Maslanka SE et al. Standardization and a multilaboratory comparison of *Neisseria meningitidis* serogroup A and C serum bactericidal assays. *Clinical Diagnostic Laboratory Immunology*, 1997, **4**:156–167.
5. Borrow R, Carlone GM. Serogroup B and C serum bactericidal assays. In: Pollard AJ, Maiden MCJ, eds. *Meningococcal vaccines. Methods in molecular medicine*, Totowa, New Jersey: Humana Press, 2001:289–304.
6. Griffiss JM, Goroff DK. IgA blocks IgM and IgG-initiated immune lysis by separate molecular mechanisms. *Journal of Immunology*, 1983, **130**:2882–2885.
7. Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. *Clinical and Diagnostic Laboratory Immunology*, 2003, **10**:780–786.
8. Trotter C, Borrow R, Andrews N, Miller E. Seroprevalence of meningococcal serogroup C bactericidal antibody in England and Wales in the pre-vaccination era. *Vaccine*, 2003, **21**:1094–1098.

9. **Gheesling LL et al.** Multicentre comparison of *Neisseria meningitidis* serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 1994, **32**:1475–1482.
10. **Sikkema DJ et al.** Relationship between serum bactericidal activity and serogroup-specific immunoglobulin G concentration for adults, toddlers, and infants immunized with *Neisseria meningitidis* serogroup C vaccines. *Clinical and Diagnostic Laboratory Immunology*, 2000, **7**:764–768.
11. **Granoff DM et al.** A modified enzyme-linked immunosorbent assay for measurement of antibody responses to meningococcal C polysaccharide that correlate with bactericidal responses. *Clinical and Diagnostic Laboratory Immunology*, 1998, **5**:479–485.
12. **Michon F et al.** Structure activity studies on group C meningococcal polysaccharide–protein conjugate vaccines: Effect of O-acetylation on the nature of the protective epitope. *Developments in Biologicals*, 2000, **103**:151–160.
13. **Richmond P et al.** Meningococcal serogroup C conjugate vaccine is immunogenic in infancy and primes for memory. *Journal of Infectious Diseases*, 1999, **179**:1569–1572.
14. **Borrow R et al.** Antibody persistence and immunological memory at age 4 years after meningococcal group C conjugate vaccine in children in the United Kingdom. *Journal of Infectious Diseases*, 2002, **186**:1353–1357.

## Annex 4

# **Guidelines on regulatory expectations related to the elimination, reduction or replacement of thiomersal in vaccines**

### **Introduction**

Thiomersal (also known as thimerosal, merthiolate) is an organomercurial derivative of ethylmercury that has been used very widely, and for a very long time, as a preservative in vaccines in their final bulk formulations. Its primary purpose has been to prevent microbial growth in the product during storage and use. It has also been used during vaccine production both to inactivate certain organisms and toxins and to maintain a sterile production line. In recent years, safety concerns have been raised over its use in vaccines, especially those given to infants. These concerns have been based primarily on data regarding the toxicity of a related substance, methylmercury, and from data on chronic exposure to mercury from the food chain.

Such safety concerns have led to initiatives in some countries to eliminate, reduce or replace thiomersal in vaccines, both in single dose and multidose presentations. Immune-mediated reactions to products containing mercury (mainly contact allergy as a manifestation of delayed-type hypersensitivity) can occur in some humans (1). Although this reaction has contributed to concerns about vaccine safety, it was not a major force leading to the recommendation by the authorities in some countries for the elimination of thiomersal from vaccines. It is important to note that concerns about the toxicity of thiomersal are theoretical and that there is no compelling scientific evidence of a safety problem related to its use in vaccines, although public perception of risk has been reported in some countries (2–7). WHO policy is clear on this issue, and the Organization continues to recommend the use of vaccines containing thiomersal for global immunization programmes because the benefits of using such products far outweigh any theoretical risk of toxicity (8).

The primary role of thiomersal in vaccines has been considered to be that of a preservative, but data indicate that there are other effects of this additive on vaccine antigens that need to be taken into account when considering its elimination, reduction or replacement. In some

production processes thiomersal is used in the inactivation of vaccine antigen together with heat, for example in the manufacture of whole cell pertussis vaccine. Should a national health authority or a manufacturer decide to eliminate, reduce, remove or replace thiomersal in vaccines, then the strategy chosen may affect not only the subsequent ability of microbial contaminants to grow in vaccine preparations, but also vaccine quality, safety and efficacy. The question therefore arises as to what evidence is needed to ensure that a vaccine in which the thiomersal content has been altered will be as safe and efficacious as the already licensed product.

A consultation attended by representatives from national regulatory authorities and the vaccine industry from both industrialized and developing countries was held in Geneva from 15 to 16 April 2002.<sup>1</sup> The objective of the consultation was to review, in a global forum, experiences of eliminating, reducing and/or replacing thiomersal in vaccines and to discuss the potential impact of these changes on the quality, safety and efficacy of the products as well as to consider regulatory requirements and their implications. A report of the meeting is available at [www.who.int/biologicals](http://www.who.int/biologicals). The focus was on already licensed vaccines that include thiomersal as an inactivating agent and/or as a preservative.

Making changes to the thiomersal content of vaccines containing this preservative that are already licensed is a complex issue that requires careful consideration. It should be borne in mind that any change in the formulation may have an important impact on the quality, safety and efficacy of a vaccine. Experience shows that eliminating or reducing thiomersal in an existing product can have some unexpected effects on vaccine quality, safety and efficacy. Effects on vaccine stability might also be expected. The amount of additional data required to demonstrate that a product with an altered thiomersal content is at least of the same quality as the previous licensed one containing thiomersal, including product stability, safety and efficacy, will need to be evaluated on a case-by-case basis. Any decision regarding the elimination or reduction of thiomersal in vaccines should be science-based. There should be a clear rationale for any change in the formulation that takes into account the different implications of reducing or eliminating thiomersal from the production steps and/or from the final stage of production. In some cases the resulting

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<sup>1</sup> A summary of the meeting was subsequently published as: Knezevic I, Griffiths E, Reigel F, Dobbelaer R (2004) Thiomersal in vaccines: a regulatory perspective. *Vaccine*, volume 22, pages 1836–41.

products should be considered as new vaccines and may require further clinical trials.

## Scope

In this guidance document, the general principles of evaluating a vaccine following the elimination, reduction, removal or replacement of thiomersal from an already licensed vaccine are discussed with particular attention being paid to the regulatory expectations for each of the above possibilities. It is not within the scope of these guidelines to discuss the policy of using or not using thiomersal, nor to discuss the effectiveness of reducing levels of thiomersal, or using a new preservative, in preventing microbial contamination. Useful guidance on the reduction, elimination or substitution of thiomersal in vaccines has also been produced by the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products (EMA) (9).

## Terminology

The following terminology may be helpful in clarifying the different options for altering the content of thiomersal in vaccines.

*Thiomersal elimination* indicates that thiomersal is not used at any stage of production. Such a product is considered as a thiomersal-free vaccine.

*Reduction of thiomersal* means that thiomersal is used at some stage of vaccine production, but the amount is smaller than that in the already licensed vaccine. Reduction of thiomersal, even if significant, still leaves residual levels of thiomersal and such a product can not therefore be considered to be a thiomersal-free vaccine.

*Removal of thiomersal* means that thiomersal was used during the production of vaccine and then removed at a later stage by a specific production step. This procedure also results in residual traces of thiomersal.

*Replacement of thiomersal* in vaccines means that thiomersal is not used at all and another preservative is included instead. In this case, there is no thiomersal in the final product, but the replacement preservative is present.

*Reduction and replacement of thiomersal* in vaccines indicates that the amount of thiomersal used is reduced and another preservative is added. There are several possibilities as to stage of vaccine production at which thiomersal could be reduced and replaced. These changes result in traces of thiomersal in the final product.

Residual traces of thiomersal in vaccines may remain following a significant reduction in or removal of thiomersal from the product. Specifications for residual levels of thiomersal with an upper limit should be set. Validated assays should be established to substantiate these specifications. In some cases an amount of less than 1 microgram (per dose) is considered as a trace.

## **Regulatory expectations**

### ***Elimination of thiomersal***

Thiomersal could be used as an inactivating agent, and/or as a preservative to protect the production line from contamination and/or added as a preservative at the final stage of vaccine production. A number of scenarios for elimination of thiomersal can be envisaged, each with different regulatory consequences:

- The elimination of thiomersal from an already licensed vaccine in which thiomersal has been used at all stages (as an inactivating agent and/or as a preservative in the production line, and as a true preservative added during the final formulation steps) might be expected to have the greatest consequences on quality, safety and efficacy. The resulting product will therefore need considerable re-evaluation. Re-evaluation should include: extensive characterization of the active substance and finished product and comparison with the existing product; comparative quality control testing and assessment of in-process controls, for example bioburden and endotoxin; and comparative stability studies on intermediates, final bulk and finished product.
- The elimination of thiomersal used as an inactivating agent and/or as a preservative in the production line will also require considerable re-evaluation, as described above.
- The elimination of thiomersal added simply as a preservative at the final stage of vaccine formulation may be expected to have fewer consequences and therefore a more flexible approach to re-evaluation might be considered.

Comparative pre-clinical data should also be obtained, focusing on immunogenicity and safety testing using *in vitro* and *in vivo* assays appropriate for the type of product being evaluated.

### ***Reduction of thiomersal***

In the case of reduction of thiomersal that has been used as an inactivating agent or to protect the production process, re-validation

of the inactivation process together with some additional tests (see those described above for elimination) in order to re-characterize the product will be required. Reduction of thiomersal added as a preservative at the final stage of production will require justification based on evidence that antimicrobial efficacy is retained. Specifications for residual amounts and/or for reduced content of thiomersal as a preservative need to be set, and validated assays to substantiate these specifications should be established.

### ***Removal of thiomersal***

If thiomersal is used in the manufacturing process (e.g. as an inactivating agent or to protect the production process), its removal will require product characterization similar to that described under elimination. Where thiomersal is used as an inactivating agent, there is no need for any re-validation of the inactivation process because thiomersal removal occurs after inactivation. Thiomersal removal will leave residual traces of thiomersal in the final product. The procedure used to remove thiomersal should be fully described and validated. Specifications for the residual amount of thiomersal in the final product should be set, and validated assays to substantiate these specifications should be established.

### ***Replacement of thiomersal***

Replacing thiomersal in an already licensed vaccine in which thiomersal has been used as an inactivating agent and/or preservative during the production process and/or in the final product, will require considerable product characterization as well as preclinical evaluation. In addition, replacement of thiomersal used as an inactivating agent will require validation of the inactivation process. In the case of the replacement of thiomersal used as a preservative at the final stage, the antimicrobial efficacy of the new preservative should be demonstrated. Specifications for the new preservative in the final product should be set and validated assays to substantiate these specifications should be established. Specific toxicity should be addressed in pre-clinical testing to demonstrate that an alternative inactivating agent and/or preservative which replaces thiomersal has no toxic effects.

### **Clinical trials and postmarketing surveillance**

The need for clinical trials should be considered on a case-by-case basis. More extensive clinical trials are likely to be required in the case of elimination and replacement of thiomersal in vaccines than in the case of its reduction and/or removal.

The design and size of the studies will depend on the vaccine in question, the nature of the changes introduced, and the results of product characterization and preclinical testing. The clinical trials should be based on the principles described in the WHO guidelines on clinical evaluation of vaccines: regulatory expectations (10). In some cases, immunogenicity data may be sufficient for licensure, but every effort should be made to continue safety and efficacy evaluation as a part of postmarketing surveillance.

Postmarketing surveillance is of critical importance especially if the data obtained from clinical trials are limited. A period of active postmarketing surveillance should follow the introduction of a product with altered thiomersal content on to the market.

### ***Antimicrobial efficacy***

Where a reduced quantity of thiomersal, or an alternative reagent, is to be used as a preservative in a multidose presentation, the antimicrobial effectiveness should be evaluated and specifications set. The criteria to be met should be discussed with, and agreed by, the appropriate national regulatory authority on a case-by-case basis.

### ***Labelling***

The information on the label should clearly indicate the presence of thiomersal in the product. The label should follow the guidance given above in the section on Terminology. It is insufficient to indicate that a vaccine is “preservative-free”. “Preservative-free” does not necessarily mean a product is thiomersal-free. Thiomersal might still have been used during production as an inactivating agent, resulting in traces of thiomersal in the final product, which are not intended to have a preservative function.

The label should indicate, as appropriate, the amount of thiomersal in the product or that the product is thiomersal-free.

## **Authors**

The first draft of these guidelines was prepared by Dr Ivana Knezevic, WHO and Dr Elwyn Griffiths, WHO, based on the report of the WHO consultation on the impact of thiomersal on quality, safety and efficacy of vaccines: regulatory perspective, held in Geneva, from 15 to 16 April 2002, attended by the following people:

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Deputy Director, Office of Vaccines, Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD, USA; Dr Thomas Montag-Lessing, Paul Ehrlich Institute, Langen, Germany; Dr Graciella Orefici, Instituto Superiore di Sanita, Rome Italy; Dr María del Pilar Alvarez Castello, Biological Department, Centro para el Control Estatal de la Calidad de los Medicamentos, Havana Cuba; Dr Franz Reigel, Head, Biological Medicines and Laboratories, Swissmedic, Agency for Therapeutic Products, Berne Switzerland; Dr Michael Schwanig, Paul Ehrlich Institute, Langen, Germany; Dr Lucky Slamet, Head, Sub-Directorate of Drug Registration, Directorate-General of Drug and Food Control, Ministry of Health, Jakarta Indonesia; Dr Maryse Surgot, Agence Française de Sécurité Sanitaire de Produits de Santé, Lyon France; Dr Ajay K. Tahlan, Joint Director and Head, Central Drugs Laboratory, Central Research Institute, Kasanli India; Mr Maman Hidayat, Planning and Development Director, P.T. Bio Farma, Bandung Indonesia; Mr Adriansjah Azhari, Head of Pharmaceutical Products Division, P.T. Bio Farma, Bandung Indonesia; Dr Akira Homma, Director, Bio-Manguinhos, Oswaldo Cruz Foundation, Riode Janeiro Brazil; Dr Suresh S. Jadhav, Executive Director, Serum Institute of India Ltd., Poona India; Dr Tony Colegate, Head of Influenza Production, Chiron S. P. A., Siena Italy; Dr Michel Duchêne, Director, Technical Affairs, GlaxoSmithKline Biologicals, Rixemsart Belgium; Mr Ronald Lammers, Project Manager, Rhein Biotech N.V., Maastricht The Netherlands; Dr Luciano Nencioni, Head of Regulatory Affairs, Chiron S. P. A., Siena Italy; Dr Jacques Paturel, Vice President Regulatory Affairs, Aventis Pasteur, Marcy e'Etoile France; Dr Marie-Paule Kieny, Director, IVR, World Health Organization, Geneva, Switzerland; Dr Elwyn Griffiths, Coordinator, QSB, World Health Organization, Geneva, Switzerland; Mr Lahouari Belgharbi, WHO/ATT, World Health Organization, Geneva, Switzerland; Dr John Clements, WHO/EPI, World Health Organization, Geneva, Switzerland; Dr Nora Dellepiane, WHO/ATT, World Health Organization, Geneva, Switzerland; Dr Philippe Duclos, WHO/VAM, World Health Organization, Geneva, Switzerland; Dr Ivana Knezevic, WHO/QSB, World Health Organization, Geneva, Switzerland; Dr Hong-ki Min, WHO/QSB, World Health Organization, Geneva, Switzerland; Dr David Wood, WHO/QSB, World Health Organization, Geneva, Switzerland; Dr Emma Uramis, WHO/ATT, World Health Organization, Geneva, Switzerland.

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## References

1. Cox NH, Forsyth A. Thiomersal allergy and vaccination reactions. *Contact Dermatitis*. 1988, 18:229–233.
2. Halsey NA. Limiting infant exposure to thimerosal in vaccines and other sources of mercury. *Journal of the American Medical Association*, 1999, 282:1763–1766.

3. Clements CJ et al. Thiomersal in vaccines. *Lancet*, 2000, **355**:1279–1280.
4. Pless R, Risher J. Mercury, infant neurodevelopment, and vaccination. *Journal of Pediatrics*, 2000, **136**:571–573.
5. Stajich GV et al. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. *Journal of Pediatrics*, 2000, **136**:679–681.
6. WHO. Thiomersal as a vaccine preservative. *Weekly Epidemiologic Record*, 2000, **2**:12–16.
7. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics*, 2001, **107**:1147–1154.
8. WHO. Vaccines and biologicals: Recommendations from the Strategic Advisory Group of Experts, *Weekly Epidemiologic Record*, 2002, **37**:306.
9. Committee for Proprietary Medicinal Products. *Points to consider on the reduction, elimination or substitution of thiomersal in vaccines*. (CPMP/BWP/2517/00) London, CPMP, 2001.
10. Guidelines on clinical evaluation of vaccines: Regulatory Expectations. In: *WHO Expert Committee on Biological Standardization. Fifty-second report*. Geneva, World Health Organization 2004 (WHO Technical Report Series No. 924).

## Annex 5

### **Biological substances: international standards and reference reagents**

A list of International Biological Standards, International Biological Reference Preparations and International Biological Reference Reagents was issued in WHO Technical Report Series 897, 2000 (Annex 4) and is available on the Internet at <http://www.who.int/technology/biological.html>. Copies may be obtained from appointed sales agents for WHO publications or from: Marketing and Dissemination, World Health Organization, 1211 Geneva 27, Switzerland.

The Expert Committee made the following changes to the previous list.

#### **Additions**

Preparation	Activity	Status
<b><i>Antibodies</i></b>		
Anti-parvovirus B19, plasma, human	77 IU/ampoule	Second International Standard 2003
Anti-human platelet antigen 5b	No assigned unitage	First Reference Reagent 2003

These substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts, EN6 3QG, England.

#### ***Blood products and related substances***

Anti-D immunoglobulin	285 IU/ampoule	Second International Standard 2003
Hepatitis A RNA	50 000 IU/vial	First International Standard 2003
Hepatitis C RNA	50 000 IU/vial	Second International Standard 2003
HIV-1 RNA genotypes (set of 10 genotypes)	No assignment	First International Reference Panel 2003
Thrombin, human	110 IU/ampoule	Second International Standard 2003

## ***Cytokines, growth factors and endocrinological substances***

Erythropoietin, recombinant	120 IU/ampoule	Second International Standard 2003
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This substance is held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts, EN6 3QG, England.

### **Miscellaneous**

Human brain, CJD control	No assignment	First Reference Reagent 2003
Human brain, sporadic CJD preparation 1	No assignment	First Reference Reagent 2003
Human brain, sporadic CJD preparation 2	No assignment	First Reference Reagent 2003
Human brain, variant CJD	No assignment	First Reference Reagent 2003

The above four preparations are usually supplied together as the Human CJD Reference Panel but are also available separately.

Poliovirus type 2 for MAPREC assay	0.67% 481-G	First International Standard 2003
MAPREC assay of poliovirus type 2 (high mutant virus)	1.21% 481-G	First Reference Reagent 2003
MAPREC assay of poliovirus type 2 (low mutant virus)	0.65% 481-G	First Reference Reagent 2003
MAPREC assay of poliovirus type 2 (481-G control)	93.5% 481-G	First Reference Reagent 2003

These substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.

### **Discontinuation**

Anti-D immunoglobulin	First International Reference Preparation 1969
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This substance was held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England.

## Annex 6

# **Recommendations and guidelines for biological substances used in medicine and other documents**

The recommendations (previously called requirements) and guidelines published by the World Health Organization are scientific and advisory in nature but may be adopted by a national regulatory authority as national requirements or used as the basis of such requirements.

These international recommendations are intended to provide guidance to those responsible for the production of biologicals as well as to others who may have to decide upon appropriate methods of assay and control in order to ensure that these products are safe, reliable and potent.

Recommendations concerned with biological substances used in medicine are formulated by international groups of experts and are published in the Technical Report Series of the World Health Organization,<sup>1</sup> as listed here. A historical list of requirements and other sets of recommendations is available on request from the World Health Organization, 1211 Geneva 27, Switzerland.

WHO Technical Report Series: Reports of the Expert Committee on Biological Standardization can be purchased from:

Marketing and Dissemination  
World Health Organization  
1211 Geneva 27, Switzerland  
Telephone: +41 22 7912476  
Fax: +41 22 7914857  
email: [publications@who.int](mailto:publications@who.int)

Individual recommendations and guidelines may be obtained free of charge as offprints by writing to: Quality Assurance and Safety of Biologicals, Department of Immunization, Vaccines and Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

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<sup>1</sup> Abbreviated in the following pages as TRS.

## **Recommendations, Guidelines and other documents**

<b>Recommendations and Guidelines</b>	<b>Reference</b>
Acellular pertussis component of monovalent or combined vaccines	Adopted 1996, TRS 878 (1998)
Animal Cells, use of, as in vitro Substrates for the Production of Biologicals	Revised 1996, TRS 878 (1998)
BCG Vaccine, Dried	Revised 1985, TRS 745 (1987); Amendment 1987, TRS 771 (1988)
Biological products prepared by recombinant DNA technology	Adopted 1990, TRS 814 (1991)
Blood, Blood Components and Plasma Derivatives: Collection, Processing and Quality Control	Revised 1992, TRS 840 (1994)
Blood plasma products, human: viral inactivation and removal procedures	Adopted 2001, TRS 924 (2004)
Cholera vaccine (Inactivated, oral)	Adopted 2001, TRS 924 (2004)
Diphtheria, Tetanus, Pertussis and Combined Vaccines	Revised 1989, TRS 800 (1990)
DNA Vaccines	Adopted 1996, TRS 878 (1998)
<i>Haemophilus influenzae</i> Type b Conjugate Vaccines	Revised 1998, TRS 897 (2000)
Haemorrhagic Fever with Renal Syndrome (HFRS) Vaccine (Inactivated)	Adopted 1993, TRS 848 (1994)
Hepatitis A Vaccine (Inactivated)	Adopted 1994, TRS 858 (1995)
Hepatitis B Vaccine prepared from Plasma	Revised 1987, TRS 771 (1988)
Hepatitis B Vaccines made by Recombinant DNA Techniques	Adopted 1988, TRS 786 (1989); Amendment 1997, TRS 889 (1999)
Human Interferons made by Recombinant DNA Techniques	Adopted 1987, TRS 771 (1988)
Human Interferons prepared from Lymphoblastoid Cells	Adopted 1988, TRS 786 (1989)
Influenza Vaccine (Inactivated)	Revised 1990, TRS 814 (1991)
Influenza Vaccine (Live)	Adopted 1978, TRS 638 (1979)
Japanese Encephalitis Vaccine (Inactivated) for Human Use	Adopted 1987, TRS 771 (1988)
Japanese Encephalitis Vaccine (Live) for Human Use	Adopted 2000, TRS 910 (2002)
Louse-borne Human Typhus Vaccine (Live)	Adopted 1982, TRS 687 (1983)

**Recommendations and guidelines for:**

Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)

Meningococcal Polysaccharide Vaccine

Meningococcal C conjugate vaccines

Monoclonal Antibodies

Poliomyelitis Vaccine (Inactivated)

Poliomyelitis Vaccine (Oral)

Rabies Vaccine (Inactivated) for Human Use, Produced in Continuous Cell Lines

Rabies Vaccine for Human Use

Rift Valley Fever Vaccine

Smallpox Vaccine

Sterility of Biological Substances

Synthetic Peptide Vaccines

Thiomersal for vaccines: regulatory expectations for elimination, reduction or removal

Thromboplastins and Plasma Used to Control Oral Anticoagulant Therapy

Tick-borne Encephalitis Vaccine (Inactivated)

Tuberculins

Typhoid Vaccine

Vaccines, clinical evaluation: regulatory expectations

Varicella Vaccine (Live)

Vi Polysaccharide Typhoid Vaccine

Yellow Fever Vaccine

**Reference**

Adopted 1992, TRS 848 (1994)  
Note TRS 848 (1994)

Adopted 1975, TRS 594 (1976);  
Addendum 1980, TRS 658 (1981)

Adopted 2001, TRS 924 (2004),  
Addendum 2003, TRS 926 (2004)

Adopted 1991, TRS 822 (1992)

Revised 2000, TRS 910 (2002),  
Addendum 2003, TRS 926 (2004)

Revised 1999, TRS 904 (2002),  
Addendum 2000, TRS 910 (2002)

Adopted 1986, TRS 760 (1987);  
Amendment 1992, TRS 840 (1994)

Revised 1980, TRS 658 (1981);  
Amendment 1992, TRS 840 (1994)

Adopted 1981, TRS 673 (1982)

Revised 2003, TRS 926 (2004)

Revised 1973, TRS 530 (1973);  
Amendment 1995, TRS 872 (1998)

Adopted 1997, TRS 889 (1999)

Adopted 2003, TRS 926 (2004)

Revised 1997, TRS 889 (1999)

Adopted 1997, TRS 889 (1999)

Revised 1985, TRS 745 (1987)

Adopted 1966, TRS 361 (1967)

Adopted 2001, TRS 924 (2004)

Revised 1993, TRS 848 (1994)

Adopted 1992, TRS 840 (1994)

Revised 1995, TRS 872 (1998)

## Other documents

A review of tests on virus vaccines

Biological standardization and control: a scientific review commissioned by the UK National Biological Standards Board (1997)

Development of national assay services for hormones and other substances in community health care

Good manufacturing practices for biological products

Guidelines for national authorities on quality assurance for biological products

Guidelines for the preparation, characterization and establishment of international and other standards and reference reagents for biological substances

Guidelines for quality assessment of antitumour antibiotics

Laboratories approved by WHO for the production of yellow fever vaccine, revised 1995

Production and testing of WHO yellow fever virus primary seed lot 213-77 and reference batch 168-73

Recommendations for the assessment of binding-assay systems (including immunoassay and receptor assay systems) for human hormones and their binding proteins. (A guide to the formulation of requirements for reagents and assay kits for the above assays and notes on cytochemical bioassay systems)

Regulation and licensing of biological products in countries with newly developing regulatory authorities

Guidelines on Transmissible Spongiform Encephalopathies in relation to biological and pharmaceutical products

Report of a WHO meeting on Hepatitis B Vaccines Produced by Recombinant DNA Techniques

Report on the Standardization and calibration of cytokine immunoassays

## Reference

TRS 673 (1982)

Unpublished document  
WHO/BLG/97.1

TRS 565 (1975)

TRS 822 (1992)

TRS 822 (1992)

TRS 800 (1990); Addendum 1999,  
TRS 904 (2002)

TRS 658 (1981)

TRS 872 (1998)

TRS 745 (1987)

TRS 565 (1975)

TRS 858 (1987)

WHO/BCT/QSD/2003.01

TRS 760 (1987)

TRS 889 (1997)

**Other documents**

Standardization of interferons (reports of WHO Informal Consultations)

Summary protocol for the batch release of virus vaccines

**Reference**

TRS 687 (1983)

TRS 725 (1985)

TRS 771 (1988)

TRS 822 (1992)