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 characterised.

1.4 Terminology

Cell seed: A quantity of cells of human or animal origin stored frozen at -100 °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.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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.

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 be examined medically at regular intervals. 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.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

For new vaccine production in animal skins method strict adherence to GMP will not be possible. Also it will 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.

3. PRODUCTION CONTROL

3.1 Control of source materials

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 shall be shown to yield immunogenic vaccines which produce typical vaccinal lesions in the skin of man. For new vaccines neutralizing antibodies or haemagglutination inihibition antibodies, or an inhibition to response to revaccination, may be used to assess immunogenicity.

Vaccine strains known to protect man 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.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 without passage in cell cultures or in animal skin.

If different substrates have been utilized between 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.

Clonal derivatives of vaccinia have been shown to exhibit phenotypic differences between each other. 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.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

A large working seed lot should be set aside as the basic material which the manufacturer will use for the preparation of batches of vaccine.

All virus seed lots in liquid form should be stored at a temperature of -60 °C or below. Seed lots that are freeze-dried need not be stored at –60c or below. Data show that potency is retained when stored at temperatures of –20c or below.

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.9

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

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

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

While testing for adventitious bacteria, mycoplasma and fungi should use standardized methods, viral adventitious agent testing of vaccine virus seeds might be more complex due to the fact that complete neutralisation 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 immunochemical methods also could be envisaged.

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

recommendations in part A sections 3.1.4 and 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).

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 part A, section 3.3.5.1. (test for nuerovirulence 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 in rabbits may be used.

3.1.2 Eggs

If the vaccine is to be produced in embryo embryonated eggs or primary chick embryo fibroblasts, the eggs to be used should be from a closed, specific-pathogen-free, healthy flock (19). This flock shall be monitored at regular intervals for agents pathogenic to birds. These include *Mycobacterium avium*, fowl pox 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.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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

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

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 human diploid and continuos cells sections 3.1.3.1 and 3.1.3.2 should apply; for primary rabbit kidney cells section 3.1.3.3 should apply; and for primary chick embryo fibroblasts section 3.1.2 should apply to the source materials. Section 3.1.3.4 applies to all type of cell cultures.

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 (14), 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, WHO, Geneva, Switzerland.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 cells 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.

3.1.3.3 Primary rabbit kidney cells for production of smallpox vaccines

Rabbits 2-4 week 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 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: B.bronchiseptica, C.perfringens, C.piliforme, C.psittaci, C.rodentium, C.spiroforme, Francisella tulerensis, Listeria, M.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

tuberculsosis, Pasteurellaceae, P.aeruginosa, Salmonella species, S.aureus, Y.enterocolitica; Toxoplasma gondii; ticks and endoparasites

When new animals are introduced into the colony, they should be maintained in quarantine in vermin-proofquarters for a minimum of 2 months and shown to be free from these 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 for 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 (15). 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 birds used the production of chick embryo fibroblast cells (part A, section 3.1.2) are bled at monthly intervals.

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

In addition, the colony should be screened for pathogenic bacteria, including mycobacteria, fungi, and mycoplasma, as agreed with the NCA. The screening programme should test 100% of the animals over a defined period of time, as agreed with the NCA.

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 shall be informed and the manufacture of smallpox vaccine may be discontinued. In this case, manufacture shall 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.

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 (16), 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) (17).

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 control authority. These components should comply with current guidelines in relation to 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).

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) (18), 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 with the validity 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,

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

e.g. phenol red in a concentration of 0.002%. Only substances that have been approved by the national control authority may be added.

If trypsin is used for preparing cell cultures it 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).

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

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 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 which are to be inoculated should be anaesthetized and thoroughly examined. If an animal shows any pathological lesion relevant it's use in the preparation of a seed lot or vaccine, it should not be used, nor should any of the remaining animals of the quarantine group concerned 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 current guidelines in relation to 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 under 2 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).

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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

3.2 Control of Vaccine production

3.2.1 Production In SPF Embryonated Eggs

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 shall be incubated under the same conditions as the inoculated eggs. At the time of virus harvest, the uninoculated eggs shall be processed in the same manner as the inoculated eggs, and the extract from the control embryos shall be shown to be free from haemagglutinating agents and for 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 polymerase chain reaction (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

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

mammalian polymerase or incomplete retrovirus-like elements that do not encode a complete genome (15). Nucleic acid amplification tests for retrovirus may also be used.

3.2.1.2 Single harvests

After inoculation and incubation at a controlled temperature, only living, typical chick embryos shall be harvested. The age of embryos at the time of harvest shall be reckoned from the initial introduction

of the eggs into the incubator and shall be no more than 12 days. After homogenization and centrifugation, the embryonic extract shall be kept at -60°C or below until further processing.

3.2.1.3 Bacterial and fungal sterility and mycoplasma tests

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

3.2.2 Production In Primary Chick Embryo Fibroblasts Or Human Diploid Cells Or Continuous Cell Cultures

3.2.2.1 Control of cell cultures

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

At least 5% or 1000 ml (volume to be checked for consistency with other WHO recommendations) 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 control authority should determine the size and treatment of the cell sample to be examined.

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.

3.2.2.1.2 Tests for haemadsorbing viruses

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 exceed 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 control authority also requires that other types of red cells, including cells from humans (blood group O), monkeys and chickens (or other avian species), may 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

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

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.

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 neutralising 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 °C or below.

3.2.2.1.4 Additional tests if chick cell cultures are used for production

Adopted by the 53rd meeting of the WHO Expert Committee on Biological Standardization, 17-21 February 2003. A definitive version of this document, which will differ from this version in editorial but not scientific detail, will be published in the WHO Technical Report Series.