Annex 2

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated)

Replacement of Annex 1 of WHO Technical Report Series, No. 904, and Addendum to Annex 1 of WHO Technical Report Series, No. 910

Recommendations published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes recommendations for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Recommendations be made only on condition that such 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 or examples intended to provide additional guidance to manufacturers and NRAs.

Introduction

WHO Requirements for oral poliomyelitis vaccine (OPV) were first formulated in 1962 (*1*), and revised in 1965 (*2*), and then again in 1971 (*3*), when an appendix describing the production of OPV in human diploid cells was added. The Requirements were further updated in 1982 (*4*) following an accumulation of data, particularly on the performance and evaluation of the monkey neurovirulence test (MNVT) and tests on the karyology of human diploid cells. The Requirements for poliomyelitis vaccine (oral) were updated in full in 1989 (*5*) to take account of the general requirements for the characterization of continuous cell lines for the preparation of biologicals, which were adopted in 1985 (*6*), and after a WHO study group concluded that, in principle, such cell lines are acceptable as substrates for the production of biologicals (*7*). An addendum was subsequently adopted (*8*) that introduced changes in the tests used to confirm freedom from detectable DNA sequences of simian virus 40 (SV40); introduced the mutant analysis by polymerase chain reaction (PCR) and restriction enzyme cleavage (MAPREC) assay as an optional additional in vitro test for poliovirus type 3; increased levels of laboratory containment for wild polioviruses (WPVs) (*9*); and provided guidance on additional antibody screening tests (for foamy viruses) for animals from closed primate colonies used as a source for primary monkey kidney cells.

The Requirements (now Recommendations) were last revised in full in 1999 (*10*) when the use of transgenic mice expressing the human poliovirus receptor (TgPVR21 mice) (*11*) as an alternative to the MNVT for type-3 virus was included in the revision, and the MAPREC test was introduced as the in vitro test of preference for the evaluation of filtered bulk suspensions for poliovirus type 3 (*12*). The previously mandated reproductive capacity at elevated temperature (rct40) test then became an optional, additional test. The studies with poliovirus types 1 and 2 in TgPVR21 mice were completed by June 2000, and an addendum to the WHO Recommendations for the production and control of poliomyelitis vaccine (oral) was adopted in 2000 (*13*) that included the neurovirulence test in TgPVR21 mice as an alternative to the MNVT for all three poliovirus serotypes.

Since then, advances in scientific knowledge have been made, novel laboratory techniques have become available and new vaccine formulations (such as monovalent and bivalent OPV) are being used. In 2008, the WHO Expert Committee on Biological Standardization advised that the Recommendations for OPV should be revised. In addition, various tests are now applicable to all three types of polioviruses, and their significance needs to be better explained and rationalized. Sections on the nonclinical and clinical evaluation of new candidate OPVs are also required. To facilitate this process, WHO convened a working group to initiate the revision of the Recommendations for the production and control of OPV, as outlined in WHO Technical Report Series No. 904 and

No. 910. Experts from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry involved in the research, manufacture, authorization and testing or release of OPV from countries around the world met from 20–22 July 2010 to identify and discuss the issues to be considered in revising Technical Report Series No. 904 and No. 910 (*14*).

The major issues addressed during this revision process included:

- updating information on the origin of different strains for OPV production, and the addition of a new Appendix 1;
- updating the section on international standards and reference preparations;
- updating the section on general manufacturing recommendations and control tests;
- updating information on neurovirulence tests in monkeys (MNVTs) and in transgenic mice (TgmNVTs), and on the MAPREC test, which is extended to all three types of seeds and bulks;
- a new Appendix 2, giving rationales for the choice of monkey or mouse neurovirulence tests;
- consideration of new vaccine formulations (monovalent OPV and bivalent OPV);
- an update on terminology, and the introduction of the "virus submaster seed lot", which is applicable only to the master seed supplied by WHO;
- inclusion of new sections on the nonclinical and clinical evaluation of OPV;
- updating the appendices;
- updating the standard operating procedures (SOPs) for TgmNVTs and MAPREC assays, and for new MNVTs in light of technical developments.

Additional changes have been made to bring the document into line with other WHO Recommendations published since the last revision.

General considerations

Poliomyelitis is an acute communicable disease of humans caused by three distinct poliovirus serotypes (types 1, 2 and 3) distinguishable by a neutralization test (*15*). Poliovirus is a species C human enterovirus of the *Picornaviridae* family, and is composed of a single-stranded, positive-sense RNA genome and a protein capsid.

Where sanitation is poor, these viruses are believed to be spread mainly by faecal-to-oral transmission, whereas the oral-to-oral mode of transmission probably dominates in settings with higher standards of sanitation. However, in most settings, mixed patterns of transmission are likely to occur. In the pre-vaccine era, roughly one in 200 susceptible individuals infected by polioviruses developed paralytic poliomyelitis (*15*).

Progress in polio control (and, since 1988, polio eradication) has occurred mainly due to the widespread use of vaccines. An inactivated poliomyelitis vaccine (IPV Salk vaccine) was licensed in 1955; live-attenuated OPV (Sabin vaccine) was licensed as a monovalent OPV (mOPV) in 1961, and as a trivalent OPV (tOPV) in 1963. The Sabin strains of poliovirus used in the production of OPV were shown to be both immunogenic and highly attenuated when administered orally to susceptible children and adults. Most countries that initially introduced vaccination with IPV later changed to OPV because OPV provided many advantages, including easier administration, suitability for mass vaccination campaigns, superior induction of intestinal mucosal immunity, and lower production costs. In 1974, OPV was recommended as part of the Expanded Programme on Immunization, and OPV was again the vaccine of choice in 1988 when the World Health Assembly resolved to eradicate polio globally by the year 2000. By 2010, three of the six WHO Regions had been certified as free of WPVs, and WPV2 has not been detected worldwide since 1999 (*15*).

In addition to tOPV, which is used in many countries for routine or supplementary vaccination, monovalent OPV against type 1 (mOPV1) and against type 3 (mOPV3), and bivalent OPV against type 1 and type 3 (bOPV) (*15*), as used by the Global Polio Eradication Initiative (GPEI) have been licensed for use in endemic countries or for outbreak control in situations where one or two types may re-emerge. In addition, mOPV against type 2 has been licensed but is expected to be used primarily for emergency response stockpiles. In 2012, the Strategic Advisory Group of Experts on Immunization was asked by WHO to consider the possibility of replacing tOPV with bOPV for routine immunization globally.

Following the introduction and widespread use of mOPV1 and mOPV3 in supplementary immunization activities in 2005, the GPEI reported substantial reductions in these poliovirus types. The last reported case of polio in India involved poliovirus type 1 and occurred in January 2011. Since polio is now considered to have been eradicated in India, the country has been removed from the list of endemic countries. However, the co-circulation of WPV1 and WPV3 in the three remaining polio-endemic countries requires that huge quantities of bOPV be used to supplement the tOPV given during routine immunization and mass immunization campaigns. A clinical trial to evaluate the immunogenicity of different OPV formulations (mOPV1, mOPV3 and bOPV) compared with tOPV in an Indian population was conducted by WHO. The seroconversion

rates to poliovirus type 1 and type 3 following immunization with bOPV were significantly higher than those induced by tOPV, and they were not lower than those induced by immunization with either mOPV1 or mOPV3 (*16*).

Although OPV is a safe vaccine, adverse events may occur on rare occasions (*15*) with vaccine-associated paralytic poliomyelitis (VAPP) being the most serious of these rare adverse events. Cases of VAPP are clinically indistinguishable from poliomyelitis caused by WPV, but can be distinguished by laboratory analysis. The incidence of VAPP has been estimated at 4 cases/1 000 000 birth cohort per year in countries using OPV (*17*). Sabin viruses can also spread in populations where the coverage of OPV is low. In such situations, Sabin viruses can acquire the neurovirulence and transmissibility characteristics of WPV, and can cause polio cases and outbreaks as circulating vaccine-derived poliovirus (cVDPV) (*18*).

Live vaccines prepared from the Sabin strains of poliomyelitis viruses types 1, 2 and 3 were introduced for large-scale immunization in 1957. In 1972, Albert Sabin proposed that WHO should be the custodian of his poliovirus seed strains. The Director-General of WHO agreed to assume responsibility for ensuring the proper use of the strains, and established a scientific committee, the Consultative Group on Poliomyelitis Vaccines, to advise WHO on all matters pertaining to their use. Detailed information on the work of the consultative group, and the preparation of the strains by Behringwerke of Marburg, Germany, has been published by Cockburn (*19*). NRAs should decide on which strains to use and on the appropriate procedures for preparing virus seed lots for OPV in their own countries.

The original poliovirus seeds produced by Sabin – Sabin original (SO) (*20*) – were sent to Merck, which generated seeds from them designated Sabin original Merck (SOM). Aliquots of SOM were supplied to other manufacturers to enable them to develop their own seeds. Some seed lots were contaminated with SV40, which had been present in the primary Rhesus kidney cells, the preferred cell-culture system at that time for virus propagation. OPV manufacturers used various strategies to reduce the contamination, including passage in the presence of a specific antibody, treatment with toluidine blue or thermal inactivation of SV40 in the presence of 1M magnesium chloride ($MgCl_2$), which stabilizes poliovirus. In 1974, Behringwerke generously agreed to produce SO+1 seeds for WHO free of charge. The Behringwerke type 1 and type 2 seeds have been widely used since the 1970s.

In the 1950s, it was established that, particularly for the type-3 strain, increases in the passage number correlated with an increase in reactivity in the MNVT. This finding led to the establishment of rigorous limits on the passage level for vaccine production for all types of OPV.

The type-3 vaccine was found to be less stable on passage than either type 1 or type 2; this was manifested in a higher number of type-3 vaccine lots

failing the MNVT. In order to develop a more stable strain, a new seed was prepared by Pfizer; susceptible cells were transfected with viral RNA extracted from poliovirus at the SO+2 level. One plaque, designated 457-III, was identified as having particularly favourable properties (*21*). Theoretically, vaccine derived from this stock was at passage SO+7 level. However, the purpose of tracking the passage history of seed viruses is to reduce the accumulation of mutations that takes place during the course of their serial propagation. Since plaque purification represents the cloning of a single infectious particle, it eliminates the heterogeneity of the viral population, and the passage level is effectively reset to zero. Thus the cloned stock 457-III was renamed RNA-derived Sabin original (RSO).

Two additional passages were used to prepare virus master seeds (RSO1) and working seeds (RSO2), and vaccines produced from this virus are at RSO3 level. Retrospectively, the RSO sequence has been shown to be the same as the consensus of SO (*22*), but more homogeneous and containing smaller quantities of mutant viruses.

The RSO seed was not used for the production of type-3 vaccine until the 1980s when it became clear that the stocks of material passaged from the SOM and other SO+1 seeds were inadequate. Since then, it has been widely used by European and American manufacturers because it is of lower virulence in laboratory tests than the SO+1 type-3 seed. The RSO seeds were bought from Pfizer by Sanofi Pasteur which donated them to WHO.

The virus seeds available from WHO (WHO master seeds) are types 1, 2 and 3 at SO+1 level produced by Behringwerke from SO seeds, and the type-3 RSO seed donated by Sanofi Pasteur. The seeds are kept at the National Institute for Biological Standards and Control (NIBSC) in England, and include a proportion of the stocks of the SO+1 seeds formerly held at Istituto Superiore di Sanità in Italy (*19*, *21*).

In addition to vaccines based upon the RSO type-3 seed, a number of manufacturers in China, Japan and the Russian Federation have produced vaccines using their own purified seed stocks of the Sabin 3 strain derived by plaque purification (cloning). Sequencing of these seed viruses demonstrated that, although they had only a low content of neurovirulent mutants, there were differences among these strains and the consensus sequence of SO virus (*22*). However, there are no reports of any differences in clinical safety between OPV produced from Pfizer stocks and the alternative seeds of Sabin 3 virus. An overview of virus seeds used in OPV production is given in Appendix 1.

The MNVT, as described in the 1989 Requirements (*5*), has been used as a quality-control test, and is based on the level and the distribution of virus-specific lesions within the central nervous system produced by vaccine virus when compared with an appropriate reference preparation (*23*). Because nonhuman primates are used, efforts to complement and eventually replace the test are of

considerable importance. WHO has encouraged and supported research on various aspects of poliovirus biology, including the development of alternative animal models, as part of its initiative to promote the development of new norms and standards for vaccines. Two groups of scientists developed transgenic mice by introducing into the mouse genome the human gene encoding the cellular receptor for poliovirus (*24*, *25*). This receptor, known as CD155, makes TgPVR mice susceptible to poliovirus infection with clinical signs of flaccid paralysis and with histological lesions in the central nervous system similar to those observed in monkeys.

In 1992, WHO initiated a project to evaluate the suitability of such transgenic mice for testing the neurovirulence of OPV, with the aim of replacing monkeys with mice. The advantages of a neurovirulence test in transgenic mice are:

- a reduction in the number of primates used for quality control of OPV;
- the use of animals with highly defined genetic and microbiological quality standards;
- a reduction in hazards to laboratory personnel through a reduced need to handle primates;
- in some countries, a reduction in the cost of quality-control tests for OPV.

Studies were carried out initially on mOPV3 vaccines using the TgPVR21 mouse line, provided free of charge by the Central Institute for Experimental Animals in Japan. Researchers at the Japan Poliomyelitis Research Institute and at the United States Food and Drug Administration Center for Biologics Evaluation and Research (CBER) developed an intraspinal inoculation method suitable for testing vaccine lots. This method was evaluated in an international collaborative study designed to establish a standardized TgmNVT test for OPV (*26*). Several laboratories participated in the study, and the results were assessed by WHO at meetings held in 1995, 1997, 1998 and 1999. As a result, the revised WHO Recommendations for the production and control of poliomyelitis vaccine (oral) (*10*) introduced the murine model as an alternative to the MNVT for type-3 poliovirus, and further studies demonstrated that this test was also suitable as an alternative to the MNVT for poliovirus type 1 and type 2 (*13*). Laboratories must comply with specifications for containment of the transgenic animals (*27*). As with the MNVT, the TgmNVT can also provide evidence of the consistency of production.

The molecular mechanisms and genetic determinants of attenuation and of reversion to virulence of all three types of Sabin polioviruses used to manufacture OPV have been well studied. Evidence strongly suggests that mutations in the 5′ noncoding region of the poliovirus genome, especially for the Sabin type-3

strain, are critical in determining the attenuated phenotype (*28*). A molecular biological test, known as the MAPREC assay, was developed by researchers at CBER to quantify reversion at the molecular level (*29*). Studies showed that all analysed batches of type-3 OPV contained measurable amounts of revertants, with C instead of U at nucleotide 472. Batches that failed the MNVT contained significantly higher quantities of 472-C than batches that passed the test. Studies with coded samples at CBER identified 100% of lots that failed the MNVT (*30*).

In 1991, WHO initiated a series of international collaborative studies to evaluate the MAPREC assay for all three types of poliovirus, and to validate appropriate reference materials. Several laboratories participated in the collaborative studies, and the results were assessed by WHO at meetings held in 1995 and 1997 in Geneva, Switzerland. It was concluded that the MAPREC assay was a sensitive, robust and standardized molecular biological assay suitable for use by manufacturers and NRAs for monitoring the consistency of the production of type-3 OPV. The revised WHO Recommendations for the production and control of poliomyelitis vaccine (oral) (*10*) introduced MAPREC as the preferred in vitro test for type 3 poliovirus in place of the rct40 test. Reference materials for the MAPREC assay for comparable positions in type 1 and type 2 have now been established. While the results do not correlate with neurovirulence in the range studied, they provide a measure of production consistency. The quantity of other mutants (such as 2493-U in Sabin 3 virus) can also be used to identify types of seed virus, and to monitor the consistency of manufacturing. After appropriate validation, quantitative profiles of other mutations in stocks of OPV could be used for this purpose.

The manufacturer of the final lot must be responsible for ensuring conformity with all of the recommendations applicable to the final vaccine (see Part A, sections A.5–A.11), even where manufacturing involves only the filling of final containers with vaccine obtained in bulk from another manufacturer. The manufacturer of the final lot must also be responsible for any production and control tests performed, with the approval of the NRA, by an external contract laboratory, if applicable.

OPV has been in worldwide use since the 1960s, and although vaccines produced from human diploid cells or continuous cell lines have been used to a lesser extent than those produced in cultures of primary monkey kidney cells, experience has indicated that all three cell substrates produce safe and effective vaccines.

In 1986, a WHO study group (*7*) stated that the risks for residual cellular DNA (rcDNA) in vaccines produced in continuous cell lines should be considered negligible for preparations given orally. This conclusion was based on the finding that polyomavirus DNA was not infectious when administered orally (*31*). For such products, the principal requirement is the elimination of potentially contaminating viruses. Additional data on the uptake of DNA via the oral route

have been published (*32*). These studies demonstrated that the efficiency of the uptake of DNA introduced orally was significantly lower than that of DNA introduced intramuscularly. Nevertheless, the specifics of the manufacturing process and the formulation of a given product should be considered by NRAs (*33*) and, where possible, data should be accumulated on the levels of rcDNA in OPV produced in Vero cells.

There is increasing interest in developing alternative strains of poliovirus for use in OPV production using molecular-manipulation techniques. The poliovirus-specific quality evaluation of such strains – e.g. for neurovirulence testing or for the MAPREC assay – as described in these Recommendations and associated SOPs, may not be appropriate. The testing of such vaccines – which is likely to include extensive preclinical and clinical studies to demonstrate attenuation, genetic stability, and the safety and transmissibility of the proposed strains – will need to be considered on a case-by-case basis, and may differ fundamentally from the approaches described in the current document.

Scope of the Recommendations

The scope of the present Recommendations encompasses poliomyelitis vaccines (oral, live, attenuated) derived from the original Sabin strains, some by simple passage and others by more complex routes, including plaque purification. This document is intended to apply to all Sabin poliovirus strains regardless of their history. It does not necessarily apply to other strains that may be developed.

This document should be read in conjunction with other relevant WHO Guidelines, such as those on the nonclinical (*34*) and clinical evaluation (*35*) of vaccines.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be poliomyelitis vaccine (oral, live, attenuated) with additions to indicate the virus serotype or serotypes of the vaccine. The proper name should be the equivalent of the international 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

Poliomyelitis vaccine (oral, live, attenuated) is a preparation of live-attenuated poliovirus type 1, 2 or 3 grown in in vitro cultures of suitable cells containing any

one type or any combination of the three types of the Sabin strains, prepared in a form suitable for oral administration and satisfying all the recommendations formulated in this document.

A.1.3 International reference materials

A trivalent virus mixture is available as the Second WHO International Reference Reagent for live-attenuated poliovirus (Sabin) types 1, 2 and 3 for determination of virus titre.

Three monotypic virus suspensions of types 1, 2 and 3 have been established as WHO Reference Reagents for use in reference laboratories to measure the sensitivity of cell cultures for poliovirus infection.

International standards for MAPREC analysis of poliovirus types 1, 2 and 3 (Sabin) and international reference reagents for control of MAPREC assays of poliovirus type 1, 2 and 3 (Sabin) are available.

International standards for antipoliovirus types 1, 2 and 3 antibodies (human) are available for standardization of neutralizing antibody tests for poliovirus.

> The reference materials listed above are available from the NIBSC, Potters Bar, England.

Reference preparations at the SO+2 passage level, designated WHO/I for type-1 virus, WHO/II for type-2 virus and WHO/III for type-3 virus are available upon request from WHO.[1] These reference preparations are for use in in vivo neurovirulence tests of homotypic vaccines. The relevant reference materials should be included in each test of vaccine (see section A.4.4.7.2).

A.1.4 Terminology

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

Adventitious agents: contaminating microorganisms of the cell substrate or source materials used in their cultures; these may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced.

Cell culture infectious dose 50% ($CCID_{50}$): the amount of a virus sufficient to cause a cytopathic effect in 50% of inoculated replicate cell cultures, as determined in an end-point dilution assay in monolayer cell cultures.

[1] Contact the Coordinator, Quality, Safety and Standards, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/biologicals/vaccines/en/).

Cell seed: a quantity of vials containing well-characterized cells derived from a single tissue or cell of human or animal origin, stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which may be used for the production of a master cell bank.

Comparator vaccine: an approved vaccine with established efficacy, or with traceability to a vaccine with established efficacy, that is tested in parallel with an experimental vaccine and serves as an active control in nonclinical or clinical testing.

Final bulk: the finished vaccine from which the final containers are filled. The final bulk may be prepared from one or more monovalent bulks, and may contain more than one virus type.

Final lot: a collection of sealed final containers of finished vaccine that is homogeneous with respect to the risk of contamination during the filling process. Therefore, all of the final containers must have been filled from a single vessel of final bulk in one working session.

Master cell bank (MCB): a quantity of fully characterized cells of human or animal origin derived from the cell seed and frozen in aliquots of uniform composition at –70 °C or below. The MCB is itself an aliquot of a single pool of cells that has been dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks. The testing performed on a replacement MCB – derived from the same cell clone or from an existing master or working cell bank – is the same as that for the initial MCB unless a justified exception is made.

Monovalent bulk: a pool of a number of single harvests of the same virus type.

Production cell culture: a cell culture derived from one or more ampoules of the working cell bank or from primary tissue, and used for the production of vaccines.

RNA-derived Sabin original type-3 virus (RSO) (*21*)**:** All subsequent passages are designated by an additional number – e.g. RSO1 (master seed) is one passage on from RSO. The working seed passage level is therefore RSO2, and the vaccine is RSO3.

Single harvest: a quantity of virus suspension of one virus type harvested from cell cultures derived from the same working cell bank, and prepared from a single production run.

Sabin original virus (SO): as described by Sabin and Boulger in 1973 (*20*). All subsequent passages are designated by an additional number – e.g. SO+1 is one passage on from Sabin original.

Virus master seed lot: a quantity of virus suspension that has been processed at the same time to ensure a uniform composition, and that has been characterized to the extent necessary to support development of the virus working seed lot. The characterized virus master seed lot is used for the preparation of virus working seed lots or a virus submaster seed (if applicable).

Virus submaster seed lot (applicable only to master seed supplied by WHO): a quantity of virus suspension produced by a single passage from the virus master seed supplied by WHO, and made at a multiplicity of infection that ensures the development of a cytopathic effect within an appropriate time frame; the virus submaster seed lot must have been processed at the same time to ensure a uniform composition. The virus submaster seed lot should be characterized to the extent necessary to support the development of the virus working seed lot. The characterized virus submaster seed lot is used for the preparation of virus working seed lots (see section A.3.2.2 and Part B).

Virus working seed lot: a quantity of virus of uniform composition, fully characterized, derived from only one passage made at the multiplicity of infection, ensuring that a cytopathic effect develops within an appropriate time frame (e.g. three days), from a virus master seed lot or submaster seed lot by a method approved by the NRA.

Working cell bank (WCB): a quantity of cells of uniform composition derived from one or more ampoules of the MCB at a finite passage level, stored frozen in aliquots at –70 °C or below, one or more of which may be used for vaccine production. All containers must be treated identically, and once removed from storage must not be returned to stock.

A.2 General manufacturing recommendations

The general manufacturing recommendations contained in WHO good manufacturing practices for pharmaceutical products: main principles (*36*) and Good manufacturing practices for biological products (*37*) should apply to establishments manufacturing OPV, with the addition of the following recommendations:

- The production of OPV should be conducted by staff who are healthy and who are examined medically at regular intervals. Steps should be taken to ensure that all persons in the production areas are immune to poliomyelitis. Personnel working in monkey quarters should also be examined for tuberculosis as outlined in Part A, section 2 of Recommendations to assure the quality, safety and efficacy of BCG vaccines (*38*).
- The establishment should be in compliance with current global recommendations for poliovirus containment.

A.3 Control of source materials

General production precautions, as formulated in Good manufacturing practices for biological products (*37*), should apply to the manufacture of OPV, with the additional recommendation that during production only one type of cell should

be introduced or handled in the production area at any one time. Vaccines may be produced in cell lines such as MRC-5 and Vero cells (see section A.3.1) or in primary monkey kidney cells (see Part E).

A.3.1 Cell lines

A.3.1.1 Master cell bank and working cell bank

The use of a cell line for the manufacture of OPVs should be based on the cell-bank system. The cell seed and cell banks should conform with the Recommendations for the evaluation of animal-cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*33*). The cell bank should be approved by the NRA. The maximum number of passages (or population doublings) allowed between the cell seed, the MCB, the WCB and the production passage level should be established by the manufacturer, and approved by the NRA. Additional tests may include but are not limited to propagation of the MCB or WCB cells to or beyond the maximum in vitro age for production, and examination for the presence of retroviruses and tumorigenicity in an animal test system (*33*).

It is important to show that the cell banks (cell seed, MCB and WCB) are free from adventitious agents relevant to the species used in their derivation. Cell banks should be assessed for the absence of adventitious agents that may have been present during production.

> The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB (*39*), and is available to manufacturers on application to the Coordinator, Quality, Safety and Standards, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

A.3.1.2 Identity tests

Identity tests on the MCB and WCB are performed in accordance with WHO Recommendations for the evaluation of animal-cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*33*), and should be approved by the NRA.

The WCB should be identified by means of, inter alia, biochemical tests (e.g. isoenzyme analysis), immunological tests, tests for cytogenetic markers, and DNA fingerprinting or sequencing. The tests should be approved by the NRA.

A.3.1.3 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate that it is free from infectious viruses as well as from bacteria, fungi and mycoplasmas using appropriate tests as specified in Part A, sections A.5.2 (*40*) in the General requirements for the sterility of biological substances no. 6 (1973) and A.5.3 (*41*) in the General requirements for the sterility of biological substances no. 6

(amended 1995). Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of the WHO Recommendations for the evaluation of animal-cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*33*).

Validated molecular tests for bovine viruses may be used instead of cell culture tests of bovine serum if approved by the NRA. As an additional means of monitoring quality, serum may be examined to ensure it is free from bacteriophages and endotoxins. Gamma radiation may be used to inactivate potentially contaminating viruses, while recognizing that some viruses are relatively resistant to gamma radiation.

The source or sources of animal components used in the culture medium should be approved by the NRA. These components should comply with the *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*42*).

Human serum should not be used. If human serum albumin is used at any stage of manufacturing, the NRA should be consulted regarding requirements because these may differ from country to country. As a minimum, the serum should meet the Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (*43*). In addition, human albumin and materials of animal origin should comply with current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*42*).

Penicillin and other beta-lactam antibiotics should not be used at any stage of manufacturing because of their nature as highly sensitizing substances.

> Other antibiotics may be used at any stage of manufacturing provided that the quantity present in the final lot is acceptable to the NRA.

> Nontoxic pH indicators may be added, such as phenol red at a concentration of 0.002%.

> Only substances that have been approved by the NRA may be added.

Bovine or porcine trypsin used for preparing cell cultures should be tested and found free from cultivable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate. The methods used to ensure this should be approved by the NRA.

> In some countries, irradiation is used to inactivate potentially contaminating viruses. If irradiation is used, it is important to ensure that a reproducible dose is delivered to all batches and to the component units of each batch. The irradiation dose must be low enough for the biological properties of the reagents to be retained but high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process (*33*).

> Recombinant trypsin is available and its use should be considered; however, it should not be assumed to be free from the risk of contamination, and should be subject to the usual considerations for any reagent of biological origin (*33*).

The source or sources of trypsin of bovine origin, if used, should be approved by the NRA, and should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*42*).

A.3.2 Virus seeds

A.3.2.1 Virus strains

Strains of poliovirus used in the production of OPV should be identified by historical records, which should include information on their origin. Producers of OPV can obtain virus master seeds from WHO. Manufacturers receiving this virus may prepare a submaster seed by a single passage, and then prepare their working seed. However, only virus strains that are approved by the NRA should be used (see General considerations in the Introduction).

A.3.2.2 Virus-seed lot system

Vaccine production should be based on the seed lot system. Virus-seed lots should not be purified. The virus master seed lot and virus working seed lot used for the production of vaccine batches should be prepared by a single passage from the virus strain and the virus master seed lot, respectively, using a method and a passage level from the original seed virus approved by the NRA. A virus submaster seed lot may be prepared by a single passage from WHO master seed, and the characterized virus submaster seed lot (see Part B) may be used for the preparation of virus working seed lots by a single passage.

Virus master seed lots, submaster seed lots and working seed lots should be stored in dedicated, monitored freezers at a temperature that ensures stability on storage – that is, ≤ –60 °C. Guidance on the additional characterization of master and submaster seeds is provided in Part B.

A.3.2.3 Tests on virus master seed, submaster seed and working seed lots

The virus master seed is provided by WHO as well characterized seed material. The virus submaster seed lot and working seed lot used for the production of vaccine batches should be shown to be free from detectable extraneous viruses and from detectable SV40 DNA as determined by a validated nucleic acid amplification test; the submaster seed lot and the working seed lot should conform to the recommendations set out in Part A, sections A.4.3 (single

harvests) and A.4.4.1–A.4.4.4 (monovalent bulks). The control cell cultures should conform to section A.4.1 (control of cell cultures).

> DNA from SV40 is widely used as a molecular biological reagent, and contamination of PCR assays is potentially a major problem. One approach is to identify separate genomic regions of SV40 for amplification, and to use one region for screening purposes and the other for the confirmation of repeatedly positive samples. It is useful if the genomic region used for confirmation varies between isolates from different sources because it is then possible to show that it has a unique sequence, and that positive results are not due to contamination with laboratory strains of SV40. The sensitivity of the PCR assays for the genomic regions used should be established.

A.3.2.4 Tests to monitor molecular characteristics of the virus

A.3.2.4.1 *Tests in vitro*

Seed viruses should be tested with MAPREC assays or temperature-sensitivity assays (such as the rct40 test) (see section A.4.4.7.1). If the NRA agrees, then at least three consecutive monovalent bulks prepared from the seed virus should meet the criteria for acceptability given in section A.4.4.7.1.

> Historically, four consecutive monovalent bulks prepared from the seed virus have been tested to monitor the molecular characteristics of the virus and production consistency.

A.3.2.4.2 *Neurovirulence tests*

New virus working seeds should be evaluated for neurovirulence. Summaries of the MNVT and TgmNVT, including pass/fail criteria, are given in Appendix 2 along with considerations on the choice of assay. The test should be approved by the NRA for the specific product, and transgenic mice, nonhuman primates, or both, may be used.

The test for neurovirulence in nonhuman primates should be carried out as summarized in Appendix 2, and following the SOPs available from WHO[2] for neurovirulence tests for types 1, 2 or 3 live-attenuated OPV in monkeys.

The use of the TgmNVT should be approved by the NRA, and it should be carried out as summarized in Appendix 2, and described in detail in the SOPs available from WHO[2] for the neurovirulence tests for type 1, 2 or 3 live-attenuated OPV in transgenic mice susceptible to poliovirus.

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[2] Contact the Coordinator, Technologies, Standards and Norms, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/biologicals/vaccines/en/).

Under normal circumstances, a new virus working seed will be prepared using the same production protocol and from the same virus master seed as the currently approved virus working seed. If the TgmNVT has been approved by the NRA for the release of vaccine batches, and if the virus working seed is generated by the same production process, the new seed can be qualified using the TgmNVT and supporting in vitro data.

If there are any major changes in the production process for a new virus master seed, full characterization using tests in nonhuman primates and transgenic mice will be required (see Part B).

If the NRA agrees, then the neurovirulence of the virus working seeds and at least three consecutive monovalent bulks prepared from it should meet the criteria for acceptability given in section A.4.4.7.2 and the appropriate SOP before the working seed can be considered suitable for use in the production of OPV.

Historically, four consecutive monovalent bulks prepared from the seed virus have been tested in monkeys to monitor production consistency.

A.3.2.5 Genotype characterization

Advances have been made in the development and application of molecular methods such as deep sequencing. For any new virus working seed, it may be useful for information purposes to analyse the new virus working seed and at least three consecutive monovalent bulks for nucleotide sequence changes from the seed virus (deep genome sequence). If such tests are performed for regulatory purposes, they should be scientifically validated and approved by the NRA.

A.4 Control of vaccine production

Part E contains additional or alternative recommendations for OPV prepared in cultures of primary monkey kidney cells, and information on testing the cell substrate used for the production of the vaccine.

A.4.1 Control of production cell cultures

When human diploid or continuous cell lines are used to prepare cultures for the production of vaccine, a fraction equivalent to at least 5% of the total or 500 ml of cell suspension, or 100 000 000 cells, at the concentration and cell passage level employed for seeding vaccine production cultures, should be used to prepare control cultures. (See Appendix 3 for an example of a flowsheet for tests in cell cultures.)

If fermenter technology is used, the NRA should determine the size and treatment of the cell sample to be examined.

A.4.1.1 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 so they can be used as control cultures for detecting adventitious agents.

These control cell cultures should be incubated for at least two weeks under conditions as similar as possible to the inoculated cultures, and they should be tested for the presence of adventitious agents as described below. For the test to be valid, 20% or fewer of the control cultures should have been discarded for nonspecific, accidental reasons.

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

A.4.1.2 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 these cells have been stored, the duration of storage should not have exceeded seven days, and the storage temperature should have been in the range of 2–8 °C. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

> Some NRAs require that as an additional test for haemadsorbing viruses, other types of red cells – including cells from humans (blood group IV O), monkeys and chickens (or other avian species) – should be used in addition to guinea-pig cells.

A reading should be taken after 30 minutes' incubation at 2–8 °C, and after incubation for an additional 30 minutes at 20–25 °C.

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

A.4.1.3 Tests for other adventitious agents in cell fluids

At the end of the observation period, a sample of the pooled fluid from each group of control cultures should be tested for adventitious agents. For this purpose, 10 ml from each pool should be tested in the same cells, but not the same batch of cells, as those used for the production of vaccine.

A second indicator cell line should be used to test an additional 10 ml sample from each pool. When a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When

a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line (*33*).

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 fall below 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of cell culture should remain uninoculated to serve as a control.

The inoculated cultures should be incubated at 35–37 °C, and should be observed for at least 14 days.

> Some NRAs require that at the end of this observation period a subculture is made in the same culture system and observed for at least an additional 14 days. Furthermore, some NRAs require that these cells be tested for the presence of haemadsorbing viruses.

For the tests to be valid, 20% or fewer of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the test period.

If any cytopathic changes caused by 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.

Some selected viruses may be screened for by using specific validated assays that have been approved by the NRA, such as molecular techniques (e.g. nucleic acid amplification) (*33*).

If these tests are not performed immediately, the samples should be kept at –60 °C or below.

A.4.1.4 Identity test

At the production level, the cells should be identified by means of tests approved by the NRA. Suitable methods include but are not limited to biochemical tests (e.g. isoenzyme analyses), immunological tests, cytogenetic tests (e.g. for chromosomal markers) and tests for genetic markers (e.g. DNA fingerprinting or sequencing).

A.4.2 Cell cultures for vaccine production

A.4.2.1 Observation of cultures 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 visually for degeneration caused by infective agents. If such examination of a cell culture shows evidence of any adventitious agent, the culture should not be used for vaccine production (see section A.4.1.3).

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.

A.4.3 Control of single harvests

A.4.3.1 Single harvest

After inoculation of the production cells with the virus working seed lot, inoculated cell cultures and control cultures should be held at a fixed temperature that has been shown to be suitable and that falls within the range 33–35 °C for the relevant incubation periods. The temperature should not vary by more than 0.5 °C from the set temperature. The optimal range for pH, multiplicity of infection, cell density, virus recovery and time of incubation should be established for each manufacturer, and should be approved by the NRA.

The virus suspension should be harvested not later than four days after virus inoculation.

> The inoculated cell cultures should be processed so that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until the results of all tests have been obtained as described in Part A sections A.4.1.2–4.1.4, A.4.3.3.1–4.3.3.3, and A.4.3.3.4 and A.4.3.3.5.

A.4.3.2 Sampling

Samples required for testing single harvests should be taken immediately on harvesting. If the tests for adventitious agents described in Part A section A.4.3.3.3 are not performed immediately, the samples taken for these tests should be kept at –60 °C or lower and subjected to no more than one freeze–thaw cycle.

A.4.3.3 Tests on single harvests

A.4.3.3.1 *Identity*

Each single harvest should be identified as the appropriate poliovirus serotype by immunological assay on cell culture using specific antibodies or by a molecular method that has been validated and approved by the NRA.

> Neutralization tests can distinguish the serotype of polioviruses. Molecular methods, such as sequencing or deep sequencing, can distinguish Sabin virus from wild-type virus.
>
> Care should be taken to ensure that the serum samples used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

A.4.3.3.2 *Titration for virus content*

The virus titre per millilitre of single harvest should be determined for cell cultures by comparing them with an existing reference preparation (see Appendix 4).

A.4.3.3.3 *Tests of neutralized single harvests for adventitious agents*

Some selected viruses may be screened by using specific assays, such as molecular techniques (e.g. nucleic acid amplification) (*33*). For the recommendations set out in this section of Part A, the volume of each single harvest taken for neutralization and testing should be at least 10 ml, and should ensure that a total of at least 50 ml or the equivalent of 500 doses of the final vaccine, whichever is greater, has been withheld from the corresponding single harvest.

The antiserum used for neutralization should be of nonhuman origin, and should have been prepared in animals other than monkeys 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 and at least one other sensitive cell system.

The neutralized suspensions should be inoculated into bottles of these cell cultures so that the dilution of the suspension in the nutrient medium does not fall below 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of neutralized suspension. At least one bottle of each kind of cell culture should remain uninoculated to serve as a control; it should be maintained using nutrient medium containing the same concentration of the specific antiserum used for neutralization.

> Animal serum may be used to propagate the cells but the maintenance medium used after the test material has been inoculated should not contain any added serum other than the poliovirus neutralizing antiserum or fetal calf serum of controlled origin.

The inoculated cultures should be incubated at 35–37 °C, and should be observed for at least 14 days.

> If adequately justified and validated, lower temperatures may be used.

For the tests to be valid, 20% or fewer of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the test period.

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

> New molecular methods with broad capabilities are being developed to detect adventitious agents. These methods include degenerate nucleic acid amplification testing for whole virus families that analyses the amplicons by hybridization, sequencing or mass spectrometry; nucleic acid amplification testing with random primers that is followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and high throughput sequencing. These methods may be used in the future to supplement existing methods, or as alternatives to both in vivo and in vitro tests after appropriate validation and approval by NRAs (*33*).

A.4.3.3.4 *Sterility tests for bacteria, fungi and mycoplasmas*

A volume of at least 10 ml of each single harvest should be tested for bacterial, fungal and mycoplasmal contamination using the appropriate tests specified in Part A, sections 5.2 and 5.3 of the General requirements for the sterility of biological substances (*41*) or by a method approved by the NRA.

> Nucleic acid amplification techniques, used alone or in combination with cell culture and an appropriate detection method, may be used as alternatives to one or both of the compendial mycoplasma detection methods if they have been validated and the NRA agrees (*33*).

A.4.3.3.5 *Test for mycobacteria*

The virus harvest should be shown to be free from mycobacteria using an appropriate method approved by the NRA.

> Molecular assays may be used as alternatives to microbiological culture tests for detecting mycobacteria after they have been validated and approved by the NRA (*33*).
>
> With NRA approval, some manufacturers test for mycobacteria only at the monovalent bulk stage.

A.4.3.3.6 *Tests for molecular consistency of production*

> Some manufacturers perform a test for the molecular consistency of production on single harvests using the MAPREC assay (see section A.4.4.7.1.1). If performed, the acceptance and rejection criteria for this test should be updated periodically and approved by the NRA.

A.4.4 Control of monovalent bulk

A.4.4.1 Preparation of monovalent bulk

The monovalent bulk may be prepared by pooling a number of single harvests of the same virus serotype into a single vessel. The filter used for this bulk should be able to retain cell debris.

The NRA may require further purification of harvests derived from continuous cell lines. However, if the harvests are derived from human diploid cells or monkey kidney cells, further purification is not required.

A.4.4.2 Sampling

Samples of the monovalent bulk prepared as described in section A.4.4.1 should be taken immediately, and if not tested immediately should be kept at –60 °C or below until the tests described in the following sections are performed.

A.4.4.3 Identity test

Each monovalent bulk should be identified as the appropriate poliovirus serotype by immunological assay on cell culture using specific antibodies, or by a molecular method that has been validated and approved by the NRA.

> Neutralization tests can distinguish the serotype of polioviruses. Molecular methods, such as sequencing or deep sequencing, can distinguish Sabin virus from wild-type virus.
>
> Care should be taken to ensure that the serum samples used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

A.4.4.4 Titration for virus content

The virus titre per millilitre of filtered monovalent bulk should be determined for cell cultures by comparing them with an existing reference preparation (see Appendix 4).

The virus titre as determined by this test should be the basis for the quantity of virus used in the neurovirulence tests in monkeys or in TgPVR mice (see Part A, section A.4.4.7.2), and for formulation of the final bulk (see Part A, section A.4.5).

The detailed procedures for carrying out this test and for interpreting the results should be approved by the NRA.

A.4.4.5 Sterility tests for bacteria and fungi

The final vaccine bulk should be tested for bacterial and fungal sterility as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (*40*).

A.4.4.6 Test for mycobacteria

The virus harvest should be shown to be free from mycobacteria by an appropriate method approved by the NRA.

> Molecular assays may be used as alternatives to microbiological culture tests for detecting mycobacteria after they have been validated and approved by the NRA (*33*).

A.4.4.7 Tests to monitor molecular characteristics of the virus (consistency)

The poliovirus in the filtered monovalent bulk, prepared as described in section A.4.4.1, should be compared with the seed lot or a reference virus preparation (see Part A, section A.1.3) to ensure that the vaccine virus has not undergone changes during its multiplication in the production cell culture.

A.4.4.7.1 ***Tests in vitro***

The virus in the monovalent bulk should be tested by at least one in vitro test. The test used should be approved by the NRA. The MAPREC assay provides a sensitive and quantitative measure for consistency purposes. However, other assays are acceptable after they have been validated. Historically, the assay used tests the property of reproducing virus at temperatures of 36 °C and 40 °C in comparison with the seed lot or a reference virus preparation of poliovirus of the same type.

A.4.4.7.1.1 **The MAPREC assay**

The MAPREC assay is suitable for all three serotypes. Implementation of the assay should be fully validated by each manufacturer, and performed according to the WHO SOP for the MAPREC assay for oral poliovirus (Sabin) vaccine, which was developed from collaborative studies and is available from WHO,[3] or according to a validated alternative procedure.

Once the test has been validated and normal values for the standards have been determined, the MAPREC assay should be used to establish the consistency of production. Depending on a laboratory's experience with the MAPREC test, an approach using "warning limits" of ±2 standard deviations and "rejection limits" of ±3 standard deviations may be appropriate. Acceptance and rejection criteria should be specific to each manufacturer and each working seed, and should be continually updated as each new bulk is prepared. An investigation of consistency should take place if a batch gives results that are inconsistent with previous production batches.

Results should be expressed as ratios relative to the type-specific International Standard for MAPREC analysis of poliovirus (Sabin). The acceptable variation of mutant content from batch to batch should be agreed with the NRA in light of experience with production and testing.

For type-3 OPV (with revertant 472-C), a batch should be rejected if the level of mutations is above 1.0% when normalized against the International Standard. The limits for type 1 and type 2 should be approved by the NRA.

> Levels of mutations obtained by manufacturers who have implemented tests for type 1 and type 2 virus have been less than 2.0% for type-1 Sabin (for the sum of both mutations, 480-A and 525-C) and less than 1.5% for type-2 Sabin (481-G) (*14*).

[3] Contact the Coordinator, Technologies, Standards and Norms, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/biologicals/vaccines/en/).

If a filtered monovalent bulk fails a MAPREC assay, it cannot be used in the manufacturing of the finished product, and an evaluation of the manufacturing process, including the suitability of the virus working seed, should be undertaken and discussed with the NRA. Filtered monovalent bulks that pass the MAPREC assay should be tested subsequently for in vivo neurovirulence.

The assay for type-3 OPV is highly predictive of in vivo neurovirulence in animal models. No such correlation exists for type 1 and type 2 at the level of revertants present in vaccine bulks. For these types, the assay results provide a measure of consistency (*14*).

Nonradioactive methods for performing MAPREC analysis are available and may be introduced after being validated and approved by the NRA.

Alternative molecular biological methods that demonstrate an equivalent or better level of discrimination may be used after being validated and approved by the NRA.

A.4.4.7.1.2 Temperature sensitivity

The monovalent bulk may be tested for the property of reproducing at 36 °C and 40 °C in comparison with the seed lot or a reference virus preparation for the marker tests, and with appropriate rct/40– and rct40+ strains of poliovirus of the same type. The wild-type viruses (defined as field isolates or reference strains from polioviruses known or believed to have circulated persistently in the community), which are used as rct40+ controls in this test, should be maintained within the laboratory at progressively higher levels of containment in accordance with the GPEI global action plan and the timetable for the safe handling of WPVs. The incubation temperatures used in this test should be controlled to within ±0.1 °C.

The monovalent bulk passes the test if, for both the virus in the monovalent bulk and that in the appropriate reference material, the titre determined at 36 °C is at least 5.0 $\log_{10}$ greater than that determined at 40 °C. If all of the titres obtained for the reference viruses are not in line with the expected values, the test should be repeated.

> An additional specification that the virus titre must not exceed 10 $CCID_{50}$/ml at the higher temperature may also be applied.
>
> It is desirable that the temperatures used in the test should also include one in the region of 39.0–39.5 °C, at which the titre of the reference material should be reduced by a factor in the range of 3.0–5.0 $\log_{10}$ of its value at 36 °C. In one laboratory, a temperature of 39.2 °C was found to be suitable.
>
> It is important to show that the behaviour of the monovalent bulk is comparable to that of the Sabin reference strain over a range of temperatures so that a more-accurate comparison can be made.

A.4.4.7.2 *Neurovirulence tests*

An appropriate in vivo test should be used to evaluate virus seeds and monovalent bulks. Summaries of the MNVT and TgmNVT, including pass and fail criteria, are given in Appendix 2, along with considerations on the choice of assay.

The test should be approved by the NRA for the specific product, and may use transgenic mice or nonhuman primates, or both. The test for neurovirulence in nonhuman primates should be carried out as summarized in Appendix 2 and described in the SOP on neurovirulence tests for types 1, 2 or 3 live-attenuated OPV in monkeys, available from WHO.[4]

Where the TgmNVT has been approved by the NRA, it should be carried out as summarized in Appendix 2 and described in detail in the SOP on neurovirulence tests for type 1, 2 or 3 live-attenuated OPV in transgenic mice susceptible to poliovirus, available from WHO.[4] Its use for batch-release purposes should follow the appropriate validation and implementation processes, according to national and international regulations. This SOP has been validated for vaccines made from Behringwerke SO-derived seeds (type 1 and type 2) and RSO-derived seeds (type 3).

To qualify as competent to perform the TgmNVT test, there is a requirement for laboratories to complete a standard implementation process as detailed in the SOP. Once qualified as competent, each laboratory should continue to monitor its performance routinely.

A collaborative study organized by WHO demonstrated that the MNVT and TgmNVT are equivalent for testing vaccines prepared from RSO seeds, but lots prepared from derivative strains containing additional mutations may be found acceptable by the MNVT but fail the TgmNVT (*26*). Therefore, the TgmNVT can be used as a replacement for the MNVT for vaccines made from RSO Sabin 3 strain, but the TgmNVT may require further validation for other derivative strains. This validation may include developing an appropriate homologous reference.

A.4.5 Final bulk

Different final bulks can be formulated.

Final tOPV bulk, mOPV1 bulk, mOPV3 bulk and bOPV bulk (bOPV1+3) can be manufactured using a defined virus concentration of each component.

[4] Contact the Coordinator, Technologies, Standards and Norms, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/biologicals/vaccines/en/).

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

> The dilution and mixing procedures involved in preparing the final vaccine bulk should be approved by the NRA.

A.4.5.1 Stabilizers

Any stabilizers that may be added to the final bulk should have been shown to the satisfaction of the NRA to improve the stability of the vaccine in the concentrations used, and not to impair the safety of the vaccine.

All of the tests described in Part A, sections A.4.3.3 and A.4.4 should be performed on samples taken before any stabilizers are added.

A.4.5.2 Sterility tests for bacteria and fungi

The final vaccine bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (*40*).

A.5 Filling and containers

The requirements concerning filling and containers given in Good manufacturing practices for biological products (*37*) apply to vaccine filled in the final form.

> Care should be taken 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 final filtration stage may be included just before the filling operations.

The manufacturer should provide the NRA with adequate data to prove that the product is stable under appropriate conditions of storage and shipping.

A.6 Control tests on final lot

Samples should be taken from each filling lot for the tests described in the following sections. The following tests should be performed on each final lot of vaccine (i.e. in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers taken from each final lot by means of validated methods approved by the NRA. The permissible limits for the different parameters listed under this section, unless otherwise specified, should be approved by the NRA.

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded.

A.6.1.1 Appearance

The appearance of the vaccine should be described with respect to its form and colour.

A.6.2 Extractable volume

Unless otherwise justified and authorized, the extractable volume (in ml) and the number of drops (using an approved dropper) should be determined in a minimum of five individual final containers.

A.6.3 pH

The pH of the final lot should be tested in a pool of final containers, and an appropriate limit set to guarantee virus stability.

A.6.4 Identity

Each final lot should be identified by immunological assay on cell culture using specific antibodies, or by a molecular method that has been validated and approved by the NRA.

> Neutralization tests can distinguish the serotype of polioviruses. Molecular methods, such as sequencing or deep sequencing, can distinguish Sabin virus from wild-type virus.
>
> Care should be taken to ensure that the serum samples used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be used for this purpose.

A.6.5 Sterility tests for bacteria and fungi

Liquid vaccine should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the General requirements for the sterility of biological substances (*40*), or by methods approved by the NRA.

A.6.6 Potency

At least three final containers should be selected at random from each final lot, and should be individually tested with a single assay. The poliovirus content of each serotype, and the total virus content, should be determined by assay as described in Appendix 4 of these Recommendations, using assays that include a reference preparation. When the vaccine contains more than one poliovirus type, each type should be titrated separately, using appropriate type-specific antiserum to neutralize each of the other types present. The NRA should specify the minimum virus titre per human dose.

An internal upper limit may be established by each manufacturer to monitor the consistency of production (e.g. based on the mean titre of the $CCID_{50}$ +3 standard deviations). The upper limit should be approved by the NRA.

It is recommended that as determined by assay described in Appendix 4, the estimated mean virus titres for a single human dose of tOPV should be: for type 1: not less than $10^{6.0}$ $CCID_{50}$; for type 2: not less than $10^{5.0}$ $CCID_{50}$; and for type 3: not less than $10^{5.5}$ $CCID_{50}$. The 95% confidence intervals for the assays should not differ by a factor of more than 0.3 $\log_{10}$ from the estimated number of infectious units in the vaccine.

In 1986, the WHO Region of the Americas began using a trivalent formulation with $10^{5.8}$ $CCID_{50}$ of poliovirus type 3 (*44*) following a study in Brazil that demonstrated improved immunogenicity when the amount of type-3 virus in the trivalent vaccine was increased (*45*). The subsequent success in controlling poliomyelitis in the Americas using this formulation led the Global Advisory Group for the Expanded Programme on Immunization to recommend a formulation of tOPV for use worldwide with $10^{6.0}$ $CCID_{50}$ per dose for type 1, $10^{5.0}$ $CCID_{50}$ per dose for type 2, and $10^{5.8}$ $CCID_{50}$ per dose for type 3 (*16*, *46*).

A.6.7 Thermal stability

Thermal stability should be considered as a vaccine characteristic that provides an indicator of the consistency of production. The thermal stability test is not designed to provide a predictive value of real-time stability but to evaluate whether the product complies with a defined specification. Additional guidance on the evaluation of vaccine stability is provided in WHO Guidelines on stability evaluation of vaccines (*47*).

Three final containers of the vaccine should be incubated at 37 °C for 48 hours. The total virus content in both exposed and unexposed containers should be determined concurrently with that of a suitable, validated reference preparation. For trivalent vaccines, the vaccine passes the test when the loss on exposure is not greater than a factor of 0.5 $\log_{10}$ $CCID_{50}$ per human dose.

Several OPV manufacturers have demonstrated that the thermal stability specification applied to tOPV formulations (loss on exposure is not greater than a factor of 0.5 $\log_{10}$ $CCID_{50}$ per human dose) is not applicable to some mOPVs and bOPVs. Some manufacturers have shown that mOPV formulations that failed to meet the specification of 0.5 $\log_{10}$ have an acceptable stability profile throughout the product's shelf-life. Therefore, a specification of 0.6 $\log_{10}$ has been accepted by NRAs and by the WHO prequalification programme on the basis of

documented evidence that mOPV1 is stable over two years when stored at –20 °C or below, and is stable for six months when stored at 2–8 °C.

A.6.8 Residual antibiotics (if applicable)

If any antibiotics are added during vaccine production, the content of the residual antibiotics should be determined and should be within limits approved by the NRA. This test may be omitted from routine lot release once the consistency of production has been established to the satisfaction of the NRA.

A.6.9 Stabilizer (if applicable)

If a stabilizer is added during vaccine production, the content of the stabilizer should be determined, and should be within limits approved by the NRA.

A.7 Records

The recommendations given in section 8 of Good manufacturing practices for biological products (*37*) apply.

A.8 Retained samples

The requirements given in section 9.5 of Good manufacturing practices for biological products (*37*) apply.

A.9 Labelling

The requirements given in section 7 of Good manufacturing practices for biological products (*37*) apply, but the following information should be added.

The label on the container or package should include:

- the designation(s) of the strain(s) of poliovirus contained in the vaccine;
- the minimum amount of each type of virus contained in one recommended human dose;
- the cell substrate used to prepare the vaccine, and the nature and amount of any stabilizer present in the vaccine;
- a statement that the vaccine is not to be injected;
- the number of doses in each vial;
- the volume of the dose.

It is desirable for the label to carry the names of both 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.10 Distribution and transport

The requirements given in section 8 of Good manufacturing practices for biological products (*37*) apply. Further guidance is provided in WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (*48*).

A.11 Stability, storage and expiry date

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Guidance on the evaluation of vaccine stability is provided in WHO Guidelines on stability evaluation of vaccines (*47*). Stability testing should be performed at different stages of production, namely on single harvests, monovalent bulk, final bulk and final lot. Parameters that indicate stability should be defined or selected according to the stage of production. A shelf-life should be assigned to all in-process materials during vaccine production, particularly intermediates such as single harvests, monovalent bulk and final bulk.

The stability of the vaccine in its final container and at the recommended storage temperatures should be demonstrated to the satisfaction of the NRA on at least three consecutive lots of final product. Accelerated thermal stability tests may be undertaken to give additional information on the overall characteristics of a vaccine.

The formulation of vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with NRAs. Following licensure, continual monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (*47*). Data should be provided to the NRA in accordance with local requirements.

Where vaccine is to be stockpiled, manufacturers should conduct real-time stability studies on monovalent bulks at –40 °C or below, or on finished monovalent, bivalent and trivalent compositions at –20 °C.

Any extension of the shelf-life should be approved by the NRA.

The final stability testing programme should be approved by the NRA, and should include an agreed set of parameters, procedures for the continuing collection and sharing of data on stability, and criteria for the rejection of vaccines.

A.11.2 Storage conditions

Before being released by the manufacturer, all vaccines in final containers should be kept continuously frozen at a temperature below –20 °C.

The manufacturer should indicate the conditions for storage and shipping that will ensure the vaccine conforms to the requirements of potency until the expiry date stated on the label. These conditions must be approved by the NRA.

Although the recommended storage temperature is –20 °C, vaccine may be stored at 2–8 °C for six months. During shipment or in the field, the vaccine may be thawed and refrozen.

> Manufacturers should demonstrate that multiple freeze–thaw cycles do not adversely affect the quality of the product. The number of freeze–thaw cycles permitted should be approved by the NRA.

The total storage period at 2–8 °C should not exceed six months. Stability data should be generated for each formulation of OPV to support storing the formulation at 2–8 °C following thawing, and these data should be approved by the NRA.

A.11.3 Expiry date

The expiry date should be based on the shelf-life, and should be supported by stability studies and approved by the NRA. The expiry date should relate to the date of filling or to the date of the first valid titration for virus content after filling (i.e. the date of the potency test), which should be performed as an assay of virus concentration as described in Appendix 4.

The label should specify only one storage temperature and expiry date.

Part B. Nonclinical evaluation of poliomyelitis vaccines (oral, live, attenuated)

The nonclinical evaluation of candidate poliomyelitis vaccines (oral, live, attenuated) should be based on the WHO guidelines on nonclinical evaluation of vaccines (*34*). In addition to the tests described in sections A.3.2.3 and A.3.2.4, the following specific issues should be considered in the context of a change in virus seed or manufacturing process for OPV.

B.1 Characterization of a new virus submaster seed from the WHO master seed

In the event that a new virus submaster seed is prepared by a single passage from the WHO master seed, it should be subjected to extensive characterization; this should include evaluation of the virus working seeds and at least three monovalent bulks derived from it, as described in section A.4.4.7. Characterization studies must include the evaluation of identity by complete nucleotide sequencing to prove that the new submaster seed consensus sequence is identical to conventional Sabin master seeds, and that the mutational composition is consistent (e.g. in a MAPREC assay). Massively parallel sequencing may also be undertaken to determine the distribution of mutants. These approaches have not yet been

formally validated, other than the MAPREC tests used for base positions in the 5′ noncoding region, which are described in section A.4.4.7.1.1. A new submaster seed should be tested for neurovirulence using the MNVT or the TgmNVT. Summaries of the MNVT and TgmNVT are given in Appendix 2, along with considerations on the choice of assay.

B.2 Characterization of virus working seeds from an established master seed where passage level between master seed and working seed is increased

The acceptable passage level of live polio vaccines relative to the original seeds is rigidly specified because there is evidence that for some seeds, increases in virulence have occurred with increases in passage. However, due to the limited stocks of master seeds, in the future it may be necessary for some manufacturers to prepare working seed lots by expanding current seed lots with an additional passage. Studies will be required that carefully compare new working seed lots with the previously approved working seed lot, and the new lots will need to meet the criteria outlined in sections A.3.2.3 and A.3.2.4. At least three monovalent bulks produced from the new virus working seed lot should also be tested and shown to meet the requirements of section A.4.4.7.

B.3 Characterization following changes in the manufacturing process

If the OPV manufacturing process is new or major changes are implemented in production – such as changing from primary monkey cells to cell lines – extensive assessment should be conducted to ensure that the mutational composition is not significantly altered by the new process. This evaluation may include the use of nucleotide sequencing and studies of mutant accumulation during passage in production cultures by MAPREC assay and other molecular methods, such as massively parallel sequencing. The new virus working seed lots will need to meet the criteria outlined in sections A.3.2.3 and A.3.2.4. In addition, at least three monovalent bulks produced from the new lots will need to be tested and shown to meet the requirements outlined in section A.4.4.7. In addition, clinical studies may be required, depending on the results of the genetic characterization and animal neurovirulence tests (see Part C).

Part C. Clinical evaluation of poliomyelitis vaccines (oral, live, attenuated)

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice for trials on pharmaceutical products (*49*) and

Guidelines on clinical evaluation of vaccines: regulatory expectations (*35*). All clinical trials should be approved by the relevant NRA.

Some of the issues that are specific to the clinical evaluation of OPVs are discussed in the following sections. These sections should be read in conjunction with the general guidance mentioned above. It is also recommended that manufacturers consult with the relevant NRAs regarding their overall clinical development programme.

Part C considers the provision of clinical data required for:

- new formulations based on licensed OPVs that are derived from Sabin poliovirus strains, including monovalent, bivalent and trivalent vaccines;
- situations where there have been major changes to the manufacturing process of an established vaccine (e.g. changing from primary monkey kidney cells to a cell line).

Clinical evaluation is not required for a vaccine manufactured using a new virus working seed lot, provided that the passage level is not more than one from the master seed lot, the working seed has been characterized, and the consistency of the manufacturing process has been demonstrated (see sections A.3.2.3 and A.3.2.4). Generating a new submaster seed requires extensive characterization but not clinical trials (see Part B).

Vaccine formulations containing one or two poliovirus serotypes have been licensed based on the findings from clinical trials in endemic countries. The results of clinical trials in Egypt and northern India have demonstrated that the efficacy of mOPV1 is superior to that of tOPV in terms of inducing immunity against poliovirus type 1 (*16*, *50*). Health authorities have recommended widespread use of this vaccine to eliminate transmission of poliovirus type 1 in India. In addition, studies on bOPV containing type 1 and type 3 have demonstrated that bOPV is noninferior to mOPV1 and mOPV3 individually, and superior to tOPV. As a result of these findings, the Advisory Committee on Poliomyelitis Eradication recommended that bOPV should be used as a complement to tOPV in routine immunization programmes and to complement tOPV and mOPVs during supplementary immunization activities.

C.1 General considerations

The Global Polio Eradication Initiative was prompted by World Health Assembly resolution WHA41.28 in 1988, and has led to a dramatic decrease in poliomyelitis cases globally (*15*). As a result, efficacy studies for poliovirus vaccines are not feasible, and clinical evaluations and seroprevalence studies should compare the safety and immunogenicity of candidate vaccines with a licensed vaccine (comparator vaccine). The assessment of seroconversion

should be based on the elicitation of neutralizing antibodies, which are the basis of protection (*15*). The approval of a candidate OPV should be based on a clear demonstration of noninferiority compared with licensed OPVs. The relative risk of VAPP for a new candidate vaccine when compared with approved vaccines cannot be estimated from pre-approval studies but should be addressed as part of post-marketing surveillance.

C.2 Safety and immunogenicity studies

C.2.1 Assessment of the immune response

A serum neutralizing antibody titre of 1/4–1/8 is considered to be a marker of protection against poliovirus (*51*). The demonstration of an immune response to OPV vaccination should be based on the pre-vaccination and post-vaccination measurement of neutralizing antibody titres. Geometric mean titres (GMTs), seroconversion rates and reverse cumulative distributions should be provided. Seroconversion for polio antigen is defined as:

- for subjects who are seronegative at the pre-vaccination time point, antibody titres above the cut-off titre (1/4–1/8);
- for subjects who are seropositive at the pre-vaccination time point, antibody titres that are four-fold above the expected titre for maternal antibodies (based on the pre-vaccination titre declining with a half-life of 28 days) (*52*);
- in populations with high antibody titres, a change from below the highest dilution tested (< 8192) to above the highest dilution tested (> 8192) will also indicate seroconversion.

WHO has made an effort to standardize polio virology methods, leading to the publication in 1990 of the Manual for the virological investigation of polio (*53*). It is recommended that a standardized technique for measuring neutralizing antibodies, involving standard cell lines and other standard reagents, should be used, such as International Standards for antipoliovirus sera for types 1, 2 and 3, and that the results should be expressed in IUs of neutralizing antibody (*54*, *55*).

C.2.2 Immunogenicity studies

Candidate OPVs manufactured using different vaccine compositions (e.g. monovalent or bivalent) should be compared with a licensed formulation. Candidate vaccines should be compared with at least one well established and licensed OPV. The comparator vaccine or vaccines selected should have been in use for a number of years so that some data on effectiveness are available in addition to a reliable description of the safety profile.

C.2.3 Population

The evaluation of new OPV formulations based on Sabin strains, including monovalent, bivalent and trivalent vaccines, may be conducted in infants and neonates since safety profiles in these populations have already been established.

The study exclusion criteria should reflect contraindications to the administration of OPVs.

C.2.4 End-points and analyses

The clinical study protocol should state the primary objectives of the study. The neutralizing antibody response to the candidate vaccine should be demonstrated to be noninferior when compared with an appropriate, licensed OPV using primarily GMTs or seroconversion rates, or both. The primary end-point should be selected according to the study population and the anticipated immune response. For example, very high seroprevalence rates are expected in highly immunized populations, and this has implications for the selection of the noninferiority margin and therefore the calculation of the sample size. Further details on demonstrating noninferiority are given in WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*35*).

Other immunological parameters should be compared by using planned secondary analyses (e.g. percentages reaching predefined titres).

C.2.5 Dose–ranging studies

As of 2012, all licensed OPV formulations (monovalent, bivalent and trivalent) contained the recommended dose for each poliovirus type – namely, for type 1: not less than $10^{6.0}$ $CCID_{50}$; for type 2: not less than $10^{5.0}$ $CCID_{50}$; and for type 3: not less than $10^{5.5}$ $CCID_{50}$. However, the development of novel formulations with improved stability (owing to the addition of stabilizers or excipients) or immunogenicity (used in combination with an adjuvant) may require dose–ranging studies to determine the minimum dose of virus required in the $CCID_{50}$ to provide adequate immune responses (*35*). These data could also be used to support the derivation of the minimum viral titre that should be present in the vaccine at the end of its shelf-life.

C.2.6 Vaccine virus shedding and transmission

Although the primary objective of immunization with OPV is to provide individual protection against paralytic disease, information on virus shedding is desirable for a better understanding of the underlying biological mechanisms of protection. Therefore guidance is provided for optional studies on virus shedding.

As changes in vaccine composition may impact virus replication in the intestinal tract, and may influence the ability to induce an immune response

manufacturers may undertake studies to determine the profile of the vaccine virus (if applicable, by serotype) excreted in the stools of vaccinees, and the duration of shedding. Evaluation of the virus excretion of new vaccine formulations containing one, two or three serotypes (i.e. monovalent, bivalent or trivalent) should be compared, if done, with the licensed trivalent formulation (*16*).

For evaluation of modified strains (intentionally containing additional mutations compared to Sabin strains) then virus excretion studies may be required rather than being optional.

C.2.7 Challenge studies with attenuated Sabin poliovirus

Although the primary objective of immunization with OPV is to provide individual protection against paralytic disease, information on mucosal immunity is desirable for a better understanding of the underlying biological mechanisms of protection. Therefore guidance is provided for optional studies on mucosal immunity.

Induction of mucosal immunity by candidate and comparator vaccines may be determined by assessing virus excretion after administering a challenge dose of mOPV. Excretion of poliovirus in stool specimens is determined immediately before the challenge (day 0) and on days 7, 14, 21 and 28 thereafter (*50*).

For evaluation of modified strains (intentionally containing additional mutations compared to Sabin strains) then studies of mucosal immunity may be required rather than being optional.

C.2.8 Concomitant administration with other vaccines

An evaluation of the effects of co-administration of an OPV with other vaccines should be considered, taking into account which vaccines are most likely to be given concomitantly in different age groups and populations.

When OPVs are used in the Expanded Programme on Immunization simultaneously with other vaccines, it is particularly important that the effects of co-administration should be evaluated (e.g. studies may evaluate co-administration with rotavirus vaccines, which are also administered by the oral route).

Immune responses to all other antigens co-administered with a new OPV should be measured at least in subsets. While a study will usually be powered only to demonstrate noninferiority with respect to neutralizing antibodies against the different poliovirus types used in the vaccine, the protocols should at least include planned secondary analyses of antigen-specific responses. If these analyses indicate that immune responses are lower on co-administration with a new OPV compared with a licensed vaccine, NRAs will need to consider the potential clinical consequences on a case by case basis.

C.2.9 Prelicensure safety data

The general approach to assessing the safety of a new OPV during clinical studies should follow WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*35*). Planned safety studies should be supported by a clear, scientific rationale. Given the long history of the use of vaccines based on Sabin strains, an NRA may decide that additional prelicensure safety studies are not required. In cases in which a new vaccine formulation, which has not been used previously, is investigated, larger-scale studies will be needed.

An appropriate pharmacovigilance plan should be developed and approved by the NRA prior to licensure.

C.3 Post-marketing studies and surveillance

Enhanced safety surveillance, particularly for detecting VAPP, should be undertaken during the initial post-approval years in collaboration with NRAs. Manufacturers and health authorities should collaborate with the Global Polio Laboratory Network to monitor new vaccines once they are introduced into immunization programmes. These laboratories have extensive experience in poliovirus surveillance, and can provide excellent surveillance and post-marketing support.

The total duration of enhanced surveillance should be regularly reviewed by NRAs. If particular issues arise during prelicensure studies or during post-licensure safety surveillance, it may be necessary to conduct specific safety studies after licensure.

Part D. Recommendations for NRAs

D.1 General

The general recommendations for NRAs and NCLs given in the Guidelines for national authorities on quality assurance for biological products (*56*) and the Guidelines for independent lot release of vaccines by regulatory authorities (*57*) should apply.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of live-attenuated OPV, should be discussed with and approved by the NRA.

For control purposes, the International Standards currently in force should be obtained for the purpose of calibrating the national, regional and working standards (*58*). The NRA may obtain the product-specific working reference from the manufacturer and use this for lot release until the international standard preparation or national standard preparation has been established.

Only a monovalent bulk approved by the NRA with regard to the neurovirulence test can be used by the manufacturer for the formulation of a final bulk.

If the NCL does not perform the MNVT itself, it should carry out a second reading of the histological sections provided by the manufacturer for each monovalent bulk. In addition, the NCL should perform a second reading of at least four neurovirulence tests on the reference preparations using the MNVT in order to obtain the necessary baseline data for comparison with the neurovirulence of test vaccines.

The NCL should encourage the use of the standard form for reporting data on virus activity in the sections taken for histopathological examination.

If the NCL performs the TgmNVT itself, it should complete the standard implementation process.

If the NCL does not perform the TgmNVT, it should carry out a clinical scoring of mice in parallel with the manufacturer for each monovalent bulk at least at days 3 and 4, plus day 14. Moreover, once a year the injection of mice should be observed by the NCL. Only appropriately trained staff from a competent NCL can carry out a clinical scoring of mice in parallel with the manufacturer.

> In one region of the world 1 in 10 bulks is also independently tested by an NCL certified as competent to carry out the test. Countries or other regions that implement the TgmNVT may wish to follow this approach.

Consistency of production has been recognized as an essential component in the quality assurance of live-attenuated OPV. In particular, NRAs should carefully monitor production records and quality control test results for clinical lots, as well as a series of consecutive lots of the vaccine.

D.2 Release and certification by the NRA

A vaccine should be released only if it fulfils all national requirements or satisfies Part A of these Recommendations, or both (*57*).

A protocol based on the model given in Appendix 5, signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request for release of the vaccine for use.

A statement signed by the appropriate official of the NRA should be provided to the manufacturing establishment, and should certify that the lot of vaccine in question meets all national requirements, as well as Part A of these Recommendations. The certificate should provide sufficient information about the vaccine lot. A model certificate is given in Appendix 6. The official national release certificate should be provided to importers of the vaccines. The purpose of the certificate is to facilitate the exchange of vaccines between countries.

Part E. Recommendations for poliomyelitis vaccines (oral, live, attenuated) prepared in primary monkey kidney cells

The following additional or alternative recommendations are for OPV prepared in cultures of primary monkey kidney cells, and concern the testing of the cell substrate used for the production of the vaccine. They should therefore be added to – or used as an alternative to – the appropriate sections in Part A.4 as follows:

- sections E.1.1.1, E.1.3.1, E.1.4.1 and E.1.4.2 are additions to the corresponding Part A.4 sections as indicated below;
- sections E.1.2.1, E.1.2.2 and E.1.2.3 are replacements for the corresponding Part A.4 sections as indicated below.

All the other recommendations given in Parts A and B of this document are also applicable to this type of vaccine.

E.1 Control of vaccine production

E.1.1 Control of source materials

E.1.1.1 Monkeys used for preparation of kidney-cell cultures and for testing of virus

Addition to section A.4.1

If vaccine is prepared in monkey kidney-cell cultures, animals should be from a species approved by the NRA, and the animals should be in good health and not previously have been used for experimental purposes.

Manufacturers should use animals from closed or intensively monitored colonies.

The monkeys should be kept in well constructed and adequately ventilated animal rooms in cages separated in such a way as to prevent cross-infection among cages. Cage-mates should not be interchanged. The monkeys should be kept in the country where the vaccine will be manufactured in quarantine groups[5] for a period of not less than six weeks before use. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5% (excluding deaths from accidents or where the cause was specifically determined not to be an infectious disease), monkeys from that entire shipment should continue in quarantine for a further period of not less than six weeks. The monkeys used should be free from infection. At the end of

[5] A quarantine group is a colony of selected healthy monkeys kept in one room, with feeding and cleaning facilities separate from those of other groups; each group should have no contact with other monkeys during the quarantine period.

the extended quarantine period, and following thorough investigations, if any additional monkeys die from the same infectious disease, the entire group must be discarded from production.

The groups should be kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used. After the last monkey in a group has been taken, the room that housed the group should be thoroughly cleaned and decontaminated before being used for a new group.

> In countries in which the kidneys from near-term monkeys are used, the mother should be quarantined for the term of pregnancy.

All actions taken by working personnel should be based on the assumption that a great potential hazard exists at all times in the quarantine area. Personnel should be provided with protective clothing, including gloves, footwear, and masks or visors. Street clothes should not be permitted to be worn in the rooms where the animals are kept. Smoking, eating and drinking should be forbidden while personnel are in the rooms where the animals are kept.

A supervisor should be responsible for reporting unusual illnesses among employees, and for ensuring that all injuries are properly treated. No worker who has cuts or abrasions on exposed areas of the body should enter the animal area. Any unexplained febrile illness, even one that occurs while off duty, should be considered as potentially related to the employee's occupation.

Monkeys from which kidneys are to be removed should be anaesthetized and thoroughly examined, particularly for evidence of tuberculosis and herpes B virus infection.

Before the preparation of a seed lot or vaccine, if a monkey shows any pathological lesion relevant to the use of its kidneys, the animal should not be used, nor should any of the remaining monkeys in the same quarantine group be used unless it is evident that their use will not impair the safety of the product.

All the operations described in this section should be conducted outside the areas where the vaccine is made.

The monkeys should be free from antibodies to SV40 and simian immunodeficiency virus.

> It is desirable that kidney-cell cultures are derived from monkeys shown to be free from antibodies to foamy viruses. In some countries, monkeys are tested for antibodies to herpes B virus.

E.1.2 Production precautions

The general production precautions called for by Good manufacturing practices for biological products (*37*) apply to the manufacture of vaccines, with the addition of the following tests.

E.1.2.1 Monkey kidney-cell cultures for vaccine production

Replacement of section A.4.2.1 – in conjunction with section E.1.2.2 (below).

Cultures of monkey kidney cells should be prepared from kidneys that have no pathological signs. Virus for the preparation of vaccine should be grown by aseptic methods in such cultures. If animal serum is used in the propagation of the cells, the maintenance medium used after virus inoculation should contain no added serum.

> To reduce animal use, the virus may be grown in serially passaged monkey kidney-cell cultures from primary monkey kidney cells.

Each group of cell cultures derived from a single monkey, or from no more than 10 near-term monkeys, should be prepared and tested as a group.

E.1.2.2 Tests of cell cultures used for vaccine production (see Appendix 7)

Replacement of section A.4.2.1 – in conjunction with section E.1.2.1 (above).

On the day of inoculation with virus working seed lot, each cell culture should be examined for degeneration caused by an infective agent. If during this examination evidence is found in a cell culture of any adventitious agent, the entire group of cultures should not be used for vaccine production.

On the day of inoculation with the virus working seed lot, a sample of at least 30 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey, or from no more than 10 near-term monkeys, should be divided into two equal portions. One portion of the pooled fluid should be tested in monkey kidney-cell cultures prepared from the same species but not the same animal as that used for vaccine production. The other portion of the pooled fluid should be tested in kidney-cell cultures from another species of monkey, provided that the tests are done in cell cultures from at least one species known to be sensitive to SV40. 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 fall below 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of cell culture should remain uninoculated to serve as a control.

> When the monkey species used for vaccine production is known to be sensitive to SV40, a test in a second species may be omitted if the NRA approves.
>
> Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody or other inhibitors, but the maintenance medium used after inoculation of the test material should contain no added serum except as described below.

The cultures should be incubated at 35–37 °C, and should be observed for at least four weeks. During this observation period, and after not less than two weeks' 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 two weeks.

> Serum may be added to the original culture at the time of subculturing, provided that the serum does not contain SV40 antibody or other inhibitors. Immunochemical techniques may be useful for detecting SV40 and other viruses in the cells.

A further sample of at least 10 ml of the pooled fluid should be tested in rabbit kidney-cell cultures for the presence of herpes B virus and other viruses. Serum used in the nutrient medium of these cultures should be free from inhibitors.[6] The sample 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 fall below 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures should remain uninoculated to serve as a control.

The cultures should be incubated at 35–37 °C, and should be observed for at least two weeks.

> It is suggested that in addition to these tests, a further sample of 10 ml of pooled fluid removed from the cell cultures on the day of inoculation with the seed lot virus should be tested for the presence of adventitious agents by inoculation into cell cultures sensitive to measles virus.

For the tests to be valid, 20% or fewer of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the respective test periods.

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

If the presence of herpes B virus is demonstrated, the manufacturing of the vaccine should be discontinued and the NRA should be informed. Manufacturing should not be resumed until a thorough investigation has been completed and precautions have been taken against any reappearance of the infection; manufacturing should be resumed only with the approval of the NRA.

[6] Human herpesvirus (herpes simplex) has been used as an indicator of freedom from B-virus inhibitors because of the danger of handling herpes B virus.

If these tests are not carried out immediately, the samples of pooled cell-culture fluid should be kept at –60 °C or below, with the exception of the sample for the test for herpes B virus, which may be held at 4 °C, provided that the test is done not more than seven days after the sample has been taken.

E.1.2.3 Test of control cell cultures

Replacement of section A.4.1.

Cultures prepared on the day of inoculation with the virus working seed lot from 25% of the cell suspension (but not more than 2.5 litres of suspension) obtained from the kidneys of each single monkey, or from not more than 10 near-term monkeys, should remain uninoculated to 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, 20% or fewer of the control cultures should have been discarded for nonspecific, accidental reasons. At the end of the observation period, the control cultures should be examined for degeneration caused by an infectious agent. If this examination or any of the tests required in this section shows evidence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures from the same group should not be used for vaccine production.

E.1.2.3.1 *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 tested for haemadsorbing viruses. At the end of the observation period, the remaining control cultures should be similarly tested. The tests should be carried out as described in Part A, section A.4.1.2.

E.1.2.3.2 *Tests for other adventitious agents*

At the time of harvest, or no more than seven days after the day of inoculation of the production cultures with the virus 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 two kinds of monkey kidney-cell cultures as described in section E.1.2.2.

At the end of the observation period for the original control cultures, similar samples of the pooled fluid should be taken, and the tests referred to in this section in the two kinds of monkey kidney-cell cultures and in the rabbit-cell culture should be repeated as described in section E.1.2.2.

If the presence of herpes B virus is demonstrated, the production cell cultures should not be used, and the measures concerning vaccine production described in section E.1.2.2, should be taken.

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 being tested for adventitious agents.

E.1.3 Control of single harvests

Addition to section A.4.3.

E.1.3.1 Tests for neutralized single harvests in monkey kidney-cell cultures

A sample of at least 10 ml of each single harvest should be neutralized by type-specific poliomyelitis antiserum prepared from animals other than monkeys. In preparing antiserum for this purpose, the immunizing antigens used should be prepared in nonsimian cells.

Care should be taken to ensure that the antiserum used is monospecific. This may be demonstrated by titration of the antiserum against homotypic and heterotypic viruses of known virus titres, using the same dilution of the antiserum as that used for neutralization.

Half of the neutralized suspension (corresponding to at least 5 ml of a single harvest) should be tested in monkey kidney-cell cultures prepared from the same species but not the same animal as that used for vaccine production. The other half of the neutralized suspension should be tested in monkey kidney-cell cultures from another species, provided that the tests are done in cell cultures from at least one species known to be sensitive to SV40.

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 fall below 1 part in 4. The area of the cell sheet should be at least 3 cm^2 per ml of neutralized suspension. At least one bottle of each kind of cell culture should remain uninoculated to serve as a control; it should be maintained using nutrient medium containing the same concentration of the specific antiserum used for neutralization.

Animal serum may be used to propagate the cells provided that it does not contain inhibitors, but the maintenance medium used after the test material has been inoculated should not contain any added serum other than the poliovirus neutralizing antiserum, except as described below.

The cultures should be incubated at 35–37 °C, and should be observed for at least four weeks. During this observation period, and after no less than two weeks' incubation, at least one subculture of fluid should be made from each of these cultures in the same tissue-culture system. The subcultures should also be observed for at least two weeks.

Serum may be added to the original cultures at the time of subculturing, provided that the serum does not contain inhibitors. Immunohistochemical techniques may be useful for detecting SV40 and other viruses in the cells.

It is suggested that in addition to these tests, a further sample of the neutralized single harvest should be tested by inoculating 10 ml into human cell cultures sensitive to measles virus.

For the tests to be valid, 20% or fewer of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the respective test periods.

If any cytopathic changes occur in any of the cultures, the causes of these changes should be investigated. If the cytopathic changes are due to unneutralized poliovirus, the test should be repeated. If there is evidence of the presence of SV40 or other adventitious agents attributable to the single harvest, that single harvest should not be used for vaccine production.

E.1.4 Control of monovalent bulk

Addition to section A.4.

E.1.4.1 Monovalent bulk (before filtration)

E.1.4.1.1 *Tests in rabbits*

A sample of the monovalent bulk should be tested for the presence of herpes B virus and other viruses by injection into at least 10 healthy rabbits, each weighing between 1.5 kg and 2.5 kg. The sample should consist of at least 100 ml. Each rabbit should receive not less than 10 ml and not more than 20 ml, of which 1 ml is given intradermally at multiple sites and the remainder is given subcutaneously. The rabbits should be observed for between three and five weeks for signs of illness or death.

It is suggested that the sample should consist of at least 1% of monovalent bulk, provided that this is not less than 100 ml and is not more than 500 ml.

All rabbits that die after the first 24 hours of the test should be examined by necropsy, with the brain and organs removed for detailed examination to establish the cause of death. Animals showing signs of illness should be humanely killed and subjected to a similar necropsy.

The monovalent bulk passes the test if 20% or fewer of the inoculated rabbits show signs of intercurrent infection during the observation period, and if none of the rabbits shows evidence of infection with herpes B virus or other adventitious agents, or lesions of any kind attributable to the bulk suspension.

If the presence of herpes B virus is demonstrated, the measures concerning vaccine production described in section E.1.2.2, should be taken.

A test for the presence of Marburg virus may be carried out in guinea-pigs.

E.1.4.2 Monovalent bulk (after filtration) – tests for retroviruses

Test samples from the filtered monovalent bulk should be examined for the presence of retroviruses by an assay for reverse transcriptase that has been approved by the NRA (*36*).

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A third draft was then prepared by Dr M. Ferguson, England, and Dr T.Q. Zhou, Department of Immunization, Vaccines and Biologicals, World

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A fourth draft was prepared by Dr M. Ferguson, Norfolk, England, and Dr T.Q. Zhou, Department of Immunization, Vaccines and Biologicals, World Health Organization, Switzerland, following comments received during a WHO-sponsored public consultation between November 2011 and February 2012. Comments were received from Mr A. Azhari, BioFarma, Indonesia; Dr L.A. Bigger, who coordinated and compiled comments from the Vaccines Committee of the International Federation of Pharmaceutical Manufacturers and Associations, Switzerland; Dr L. Fiore, Istituto Superiore di Sanità, Italy; Dr J. Fournier-Caruana, Department of Immunization, Vaccines and Biologicals, World Health Organization, Switzerland; Dr E. Leal, Fiocruz, on behalf of the National Institute of Quality Control in Health, Brazil; Dr A. Lopez, Laboratorios de Biológicos y Reactivos de México, Mexico; Dr S. Morgeaux, Agence Française de Sécurité Sanitaire des Produits de Santé, France; Dr S.R. Pakzad, Food and Drug Control Laboratory, Islamic Republic of Iran; Ms V. Pithon, Agence Française de Sécurité Sanitaire des Produits de Santé, France; Dr A.L. Salvati, Istituto Superiore di Sanità, Italy; Dr Y. Tano, Japan Poliomyelitis Research Institute, Japan; and Dr G. Waeterloos, Scientific Institute of Public Health, Belgium.

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Italy; Mr J.B. González, Laboratorios de Biológicos y Reactivos de México, Mexico; Dr M.A. González, Federal Commission for the Protection from Sanitary Risks, Mexico; Professor V. Grachev, Russian Academy of Medical Sciences, Russian Federation; Mrs T. Jivapaisarnpong, Ministry of Public Health, Thailand; Dr I. Knezevic, Department of Immunization, Vaccines, and Biologicals, World Health Organization, Switzerland; Dr D. Kusmiaty, Directorate General of Drug and Food Control, Ministry of Health, Indonesia; Dr K. Katayama, National Institute of Infectious Diseases, Japan; Dr C.G. Li, National Institutes for Food and Drug Control, China; Dr J. Martin, National Institute for Biological Standards and Control, England; Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr R. Modi, Cadila Pharmaceuticals, India; Ms E. Niogret, Sanofi Pasteur, France; Dr H. Okayasu, Research, Policy and Product Development, World Health Organization, Switzerland; Dr L.V. Phung, National Institute for Control of Vaccine and Biologicals, Viet Nam; Ms V. Pithon, Agence Française de Sécurité Sanitaire des Produits de Santé, France; Dr A. Sinyugina, Federal State Unitary Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides, Russian Federation; Dr R. Sutter, Research, Policy and Product Development, World Health Organization, Switzerland; Mr D. Ugiyadi, BioFarma, Indonesia; Dr G. Waeterloos, Scientific Institute of Public Health, Belgium; Ms H. Wang, Tiantan Biological Products Company, China; Dr S. Yamazaki, Japan Poliomyelitis Research Institute, Japan; Mr L. Yi, Kunming Institute of Medical Biology, China; and Dr T.Q. Zhou, Immunization, Vaccines and Biologicals, World Health Organization, Switzerland.

The final draft document was prepared by Dr M. Ferguson, England and Dr T.Q. Zhou, Department of Immunization, Vaccines and Biologicals, World Health Organization, Switzerland following comments received on the fifth draft from Mr B.S. Chauhan, Bharat Biotech International, India; Dr K. Chumakov, United States Food and Drug Administration Center for Biologics Evaluation and Research, USA; Dr E. Coppens, Sanofi Pasteur, France; Dr C. Dubeaux, GlaxoSmithKline Biologicals, Belgium; Dr J. Fournier-Caruana, Department of Immunization, Vaccines and Biologicals, World Health Organization, Switzerland; Dr M.A. González, Ministry of Health, Mexico; Dr C.G. Li, National Institutes for Food and Drug Control, China; Dr P. Minor, Dr A. Heath, Ms G. Dunn and Ms G. Cooper, National Institute for Biological Standards and Control, England; Dr V. Pithon, Agence Nationale de Sécurité du Médicament et des Produits de Santé, France; Dr D. Ugiyadi, BioFarma, Indonesia; Dr E. Vitkova, European Directorate for the Quality of Medicines & HealthCare, France; and Dr S.Yamazaki, Japan Poliomyelitis Research Institute, Japan.

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References

1. Requirements for Poliomyelitis Vaccine (oral) (Requirements for Biological Substances, No. 7). In: *Requirements for Biological Substances. Report of a Study Group*. Geneva, World Health Organization, 1962 (WHO Technical Report Series, No. 237), Annex.
2. Requirements for poliomyelitis vaccine (oral) (Requirements for Biological Substances, No. 7): revised 1965. In: *Requirements for Biological Substances. Manufacturing establishments and control laboratories – poliomyelitis vaccine (inactivated) – poliomyelitis vaccine (oral) – smallpox vaccine. Revised 1965. Report of a WHO Expert Group*. Geneva, World Health Organization, 1966 (WHO Technical Report Series, No. 323), Annex 3.
3. Requirements for poliomyelitis vaccine (oral). In: *WHO Expert Committee on Biological Standardization. Twenty-fourth report*. Geneva, World Health Organization, 1972 (WHO Technical Report Series, No. 486), Annex 1.
4. Requirements for poliomyelitis vaccine (oral) (Revised 1982). In: *WHO Expert Committee on Biological Standardization. Thirty-third report*. Geneva, World Health Organization, 1983 (WHO Technical Report Series, No. 687), Annex 4.
5. Requirements for poliomyelitis vaccine (oral) (revised 1989). In: *WHO Expert Committee on Biological Standardization. Fortieth report*. Geneva, World Health Organization, 1990 (WHO Technical Report Series, No. 800), Annex 1.
6. Requirements for continuous cell lines used for biologicals production. In: *WHO Expert Committee on Biological Standardization. Thirty-sixth report*. Geneva, World Health Organization, 1987 (WHO Technical Report Series, No. 745), Annex 3.
7. *Acceptability of cell substrates for production of biologicals. Report of a WHO Study Group*. Geneva, World Health Organization, 1987 (WHO Technical Report Series, No. 747) (apps.who.int/iris/bitstream/10665/38501/1/WHO_TRS_747.pdf, accessed 20 June 2013).
8. 1990 Requirements for poliomyelitis vaccine (oral) (Addendum 1998). In: *WHO Expert Committee on Biological Standardization. Forty-ninth report*. Geneva, World Health Organization, 2000 (WHO Technical Report Series, No. 897), Annex 2.

9. *WHO global action plan to minimize poliovirus facility-associated risk after eradication of wild polioviruses and cessation of routine OPV use*. Draft – 2009. Geneva, World Health Organization, 2009 (http://www.polioeradication.org/Portals/0/Document/Resources/PostEradication/GAP3_2009.pdf, accessed 20 June 2013).
10. 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), Annex 1.
11. *Working group on transgenic mice as animal model for neurovirulence test of OPV, Geneva, 28–30 June 1999*. Geneva, World Health Organization (unpublished document BS/99.1908; available on request from the Department of Quality, Safety and Biologicals, World Health Organization, 1211 Geneva 27, Switzerland).
12. *Use of the MAPREC assay in the quality control of oral poliovirus vaccine. Report of a WHO working group. Geneva, 1–2 July 1999*. Geneva, World Health Organization (unpublished document BS/99.1909; available on request from the Department of Quality, Safety and Biologicals, World Health Organization, 1211 Geneva 27, Switzerland).
13. Recommendations for the production and control of poliomyelitis vaccine (oral) (revised, Addendum 2000). In: *WHO Expert Committee on Biological Standardization. Fifty-first report*. Geneva, World Health Organization, 2002 (WHO Technical Report Series, No. 910), Annex 1.
14. *WHO Working Group Meeting to Discuss the Revision of the WHO Recommendations for OPV: TRS No. 904 and 910*. Geneva, Switzerland 20–22 July 2010. Geneva, World Health Organization, 2010 (http://www.who.int/biologicals/vaccines/OPV_Meeting_report_Final_Clean_13May2011.pdf, accessed 20 June 2013).
15. Polio vaccines and polio immunization in the pre-eradication era: WHO position paper. *Weekly Epidemiological Record*, 2010, 85:213–228.
16. Sutter RW et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. *Lancet*, 2010, 376:1682–1688.
17. Risk assessment: frequency and burden of VAPP, cVDPV and iVDPV. In: *Report of the interim meeting of the Technical Consultative Group (TCG) on the Global Eradication of Poliomyelitis: Geneva, 13–14 November 2002*. Geneva, World Health Organization, 2002.
18. Kew OM et al. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. *Annual Review of Microbiology*, 2005, 59:587–635.
19. Cockburn WC. The work of the WHO Consultative Group on Poliomyelitis Vaccines. *Bulletin of the World Health Organization*, 1988, 66:143–154.
20. Sabin AB, Boulger L. History of Sabin attenuated poliovirus oral live vaccine strains. *Journal of Biological Standardization*, 1973, 1:115–118.
21. Stones PB et al. *Preparation and properties of a derivative of Sabin's type 3 poliovirus strain Leon 12a,b*. 10th Symposium of the European Association against Poliomyelitis and Allied Diseases, 1964, 10:390–397.
22. Rezapkin GV et al. Reevaluation of nucleotide sequences of wild-type and attenuated polioviruses of type 3. *Virus Research*, 1999, 65:111–119.
23. Furesz J, Contreras G. Some aspects of the monkey neurovirulence test used for the assessment of oral poliovirus vaccines. *Developments in Biological Standardization*, 1993, 78:61–70.
24. Ren R et al. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell*, 1990, 63:353–362.
25. Koike S et al. Transgenic mice susceptible to poliovirus. *Proceedings of the National Academy of Sciences of the United States of America*, 1991, 88:951–955.

26. Dragunsky E et al. Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study. *Bulletin of the World Health Organization*, 2003, 81:251–260.
27. Maintenance and distribution of transgenic mice susceptible to human viruses: memorandum from a WHO meeting. *Bulletin of the World Health Organization*, 1993, 71:493–502.
28. Wood DJ, Macadam AJ. Laboratory tests for live attenuated poliovirus vaccines. *Biologicals*, 1997, 25:3–15.
29. Chumakov KM et al. Correlation between amount of virus with altered nucleotide sequence and the monkey test for acceptability of oral poliovirus vaccine. *Proceedings of the National Academy of Sciences of the United States of America*, 1991, 88:199–203.
30. Chumakov K et al. Assessment of the viral RNA sequence heterogeneity for control of OPV neurovirulence. *Developments in Biological Standardization*, 1993, 78:79–89.
31. Israel MA et al. Biological activity of polyoma viral DNA in mice and hamsters. *Journal of Virology*, 1979, 29:990–996.
32. Lebron JA et al. Adaptation of the WHO guideline for residual DNA in parenteral vaccines produced on continuous cell lines to a limit for oral vaccines. *Developments in Biologicals*, 2006, 123:35–44.
33. Recommendations for the evaluation of animal-cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 3.
34. WHO guidelines on nonclinical evaluation of vaccines. In: *WHO Expert Committee on Biological Standardization. Fifty-fourth report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927), Annex 1.
35. 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 1.
36. Good manufacturing practices: main principles for pharmaceutical products. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report*. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 961), Annex 3.
37. Good manufacturing practices for biological products. In: *WHO Expert Committee on Biological Standardization. Forty-second report.* Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 822), Annex 1.
38. Recommendations to assure the quality, safety and efficacy of BCG vaccines. Replacement of Annex 2 of WHO Technical Report Series, No. 745, and Amendment to Annex 12 of WHO Technical Report Series, No. 771. In: *WHO Expert Committee on Biological Standardization. Sixty-second report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 979), Annex 3.
39. Requirements for the use of animal cells as in vitro substrates for the production of biologicals (Addendum 2003). In: *WHO Expert Committee on Biological Standardization. Fifty-fourth report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927), Annex 4.
40. General requirements for the sterility of biological substances (Revised 1973). In: *WHO Expert Committee on Biological Standardization. Twenty-fifth report*. Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 530), Annex 4.
41. General requirements for the sterility of biological substances. (Requirements for Biological Substances No. 6, revised 1973, amendment 1995). In: *WHO Expert Committee on Biological Standardization. Forty-sixth report*. Geneva, World Health Organization, 1998 (WHO Technical Report Series, No. 872), Annex 3.

42. *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products*. Geneva, World Health Organization, 2003 (WHO/BCT/QSD/03.01) (http://www.who.int/biologicals/publications/en/whotse2003.pdf, accessed 22 June 2013).

43. Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (revised 1992). In: *WHO Expert Committee on Biological Standardization. Forty-third report*. Geneva, World Health Organization, 1994 (WHO Technical Report Series, No. 840), Annex 2.

44. de Quadros CA et al. Eradication of poliomyelitis: progress in the Americas. *Pediatric Infectious Disease Journal*, 1991, 10:222–229.

45. Patriarca PA et al. Randomised trial of alternative formulations of oral poliovaccine in Brazil. *Lancet*, 1988, 331:429–443.

46. Expanded Programme on Immunization: Global Advisory Group. *Weekly Epidemiological Record*, 1991, 66:3–7 (Part I); and 9–12 (Part II).

47. Guidelines on stability evaluation of vaccines. In: *WHO Expert Committee on Biological Standardization. Fifty-seventh report*. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 962), Annex 3.

48. Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (jointly with the Expert Committee on Biological Standardization). In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report*. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 961), Annex 9.

49. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: *The use of essential drugs. Sixth report of the WHO Expert Committee*. Geneva, World Health Organization, 1995 (WHO Technical Report Series, No. 850), Annex 3.

50. el-Sayed N et al. Monovalent type 1 oral poliovirus vaccine in newborns. *New England Journal of Medicine*, 2008, 359:1655–1665.

51. Plotkin SA, Vidor E. Poliovirus vaccine-inactivated. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*, 5th ed. London, Elsevier, 2008.

52. Cohen A et al. Seroresponse to trivalent oral poliovirus vaccine as a function of interval. *Pediatric Infectious Disease Journal*, 1995, 14:100–106.

53. *Manual for the virological investigation of polio*. Geneva, World Health Organization 1997 (http://whqlibdoc.who.int/hq/1997/WHO_EPI_GEN_97.01.pdf, accessed 20 June 2013).

54. Wood DJ, Heath AB. The second international standard for anti-poliovirus sera types 1, 2 and 3. *Biologicals*, 1992, 30:203–211.

55. *The Immunological Basis for Immunization Series. Module 6: Poliomyelitis*. Geneva, World Health Organization, 1993 (http://whqlibdoc.who.int/hq/1993/WHO_EPI_GEN_93.16_mod6.pdf, accessed 20 June 2013).

56. 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 (WHO Technical Report Series, No. 822), Annex 2.

57. Guidelines for independent lot release of vaccines by regulatory authorities. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 2.

58. *WHO manual for the establishment of national and other secondary standards for vaccines*. Geneva, World Health Organization, 2011 (WHO/IVB/11.03) (http://whqlibdoc.who.int/hq/2011/WHO_IVB_11.03_eng.pdf, accessed 20 June 2013).

Appendix 1

Overview of virus seeds used in OPV production

The history of the poliovirus strains used in the production of OPV is well documented (*1–3*). This appendix gives an overview of the virus seeds currently used in OPV production.

The flow diagrams shown in Figures 2.1 and 2.2 summarize the history of seed virus and reference materials used to manufacture OPV from Sabin 1 and Sabin 2 (Figure 2.1) and Sabin 3 (Figure 2.2) strains. Concentric circles indicate progressive virus passages made to prepare master seed stocks, working seed stocks and production lots of vaccine. Where relevant, submaster seed stocks are identified in the footnotes. Different seed viruses are identified as SO (Sabin original), SOM (Merck stock of SO), SOB (Behringwerke stock of SO), Pfizer (otherwise known as rederived SO, or RSO), SOJ (Japanese stock of SO) and SOR (Russian stock of SO).

These figures provide only a historical overview of the use of different seeds derived from the Sabin vaccine strain in OPV production (as of June 2012). They do not indicate any WHO "qualification" or "approval" of the strains or vaccines in the context of this document.

Figure 2.1
History of seed virus and reference materials used to produce type 1 and type 2 OPV from Sabin 1 and Sabin 2

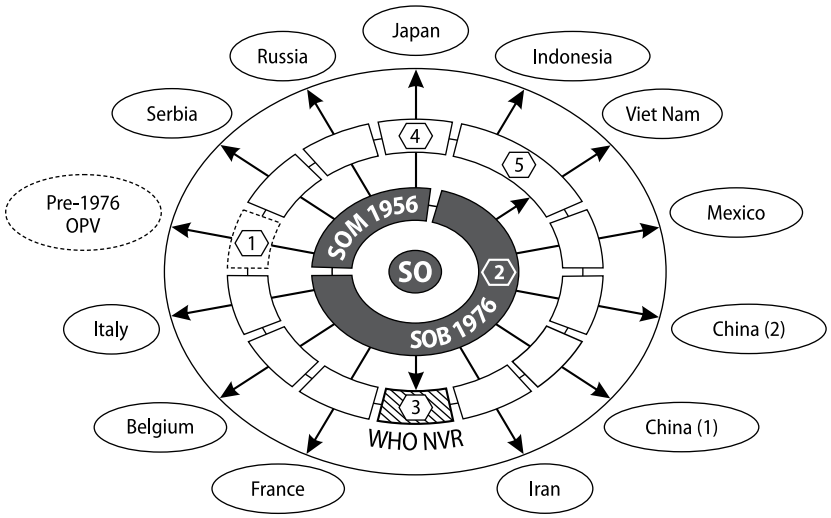
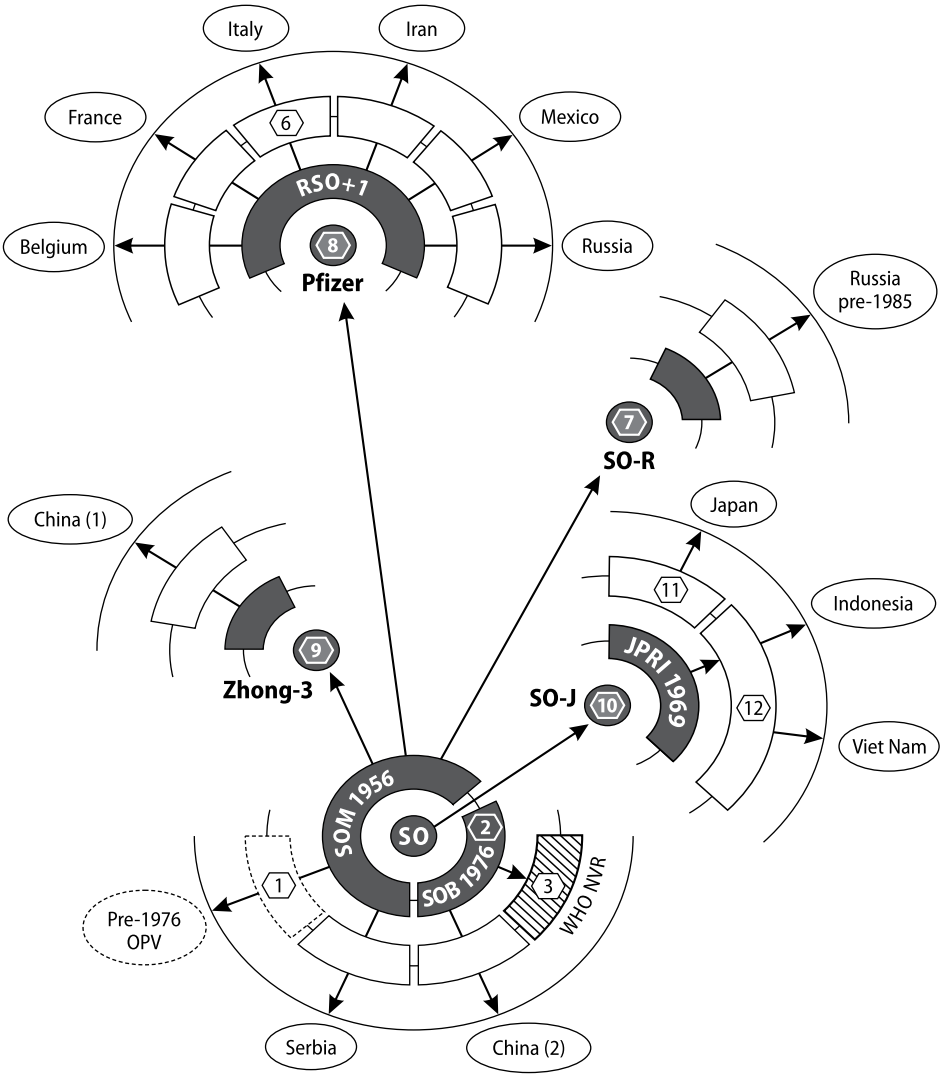


Figure 2.2
History of seed virus and reference materials used to produce type 3 OPV from Sabin 3



Manufacturers corresponding to the countries shown in Figure 2.1 and Figure 2.2

Belgium	GlaxoSmithKline Biologicals
China (1)	Institute of Medical Biology, Kunming
China (2)	China National Biotec Group, Beijing Tiantan Biological Products Company
France	Sanofi Aventis
Indonesia	PT BioFarma
Islamic Republic of Iran	Razi Vaccine and Serum Research Institute
Italy	Novartis Vaccines
Japan	Japan Poliomyelitis Research Institute
Mexico	Biologics and Reagents Laboratories of Mexico
Russian Federation	Federal State Unitary Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides
Serbia	Torlak Institute of Virology, Vaccines and Serum
Viet Nam	Center for Research and Production of Vaccines and Biologicals

Notes 1–12 shown in Figure 2.1 and Figure 2.2

1. Working seeds were produced by different manufacturers before 1976.
2. WHO master seed stock.
3. WHO neurovirulence reference preparation.
4. Type-1 seed stock prepared at Japan Poliomyelitis Research Institute by four passages of SOM, including three terminal dilution passages (passage level SO+5).
5. Type-2 seed stock prepared at Japan Poliomyelitis Research Institute by one passage of SOM (SO+2). Seed stock prepared at Japan Poliomyelitis Research Institute by one passage of SOB (SO+2).
6. Novartis performed an additional passage to prepare submaster seed stock from which a working seed was produced.
7. In the Russian Federation, six plaques were selected, pooled, and grown to produce seed stock.

8. Pfizer (RSO): rederived Sabin original, produced by RNA plaque purification, passage.
9. Zhong-3: plaque purification, passage.
10. Produced by Japan Poliomyelitis Research Institute in 1969 from SO stock by one passage (SO+1).
11. Prepared from SOJ by passages in AGMK cells (SOJ+9), including two plaque purifications and three terminal passages (SO+10).
12. Prepared from SOJ by passages in AGMK cells (SOJ+6), including two plaque purifications (SO+7).

References

1. Cockburn WC. The work of the WHO Consultative Group on Poliomyelitis Vaccines. *Bulletin of the World Health Organization*, 1988, 66:143–154.
2. Sabin AB, Boulger L. History of Sabin attenuated poliovirus oral live vaccine strains. *Journal of Biological Standardization*, 1973, 1:115–118.
3. Stones PB et al. *Preparation and properties of a derivative of Sabin's type 3 poliovirus strain Leon 12a,b*. 10th Symposium of the European Association against Poliomyelitis and Allied Diseases, 1964, 10:390–397.

Appendix 2

In vivo tests for neurovirulence, and considerations in relation to assay choice

Live-attenuated vaccines were developed by Sabin in large part by using nonhuman primates, particularly Old World monkeys, to measure the level of residual neurovirulence. In the 1980s, tests of vaccine bulks and seeds were standardized, as a single dose of test material given by intraspinal inoculation and tested concurrently with a homologous reference. Vaccines derived from the Sabin strains that pass the MNVT have been shown to have an acceptable safety profile. However, in its current form, the MNVT is regarded as a test of consistency, and it is not known whether vaccines that fail the test are virulent in human recipients. Tests designed to replace the MNVT should be able to detect the same changes from batch to batch as the MNVT does, with similar sensitivity. The TgmNVT in mice expressing the human poliovirus receptor (TgPVR21 mice) has been developed as an alternative to the MNVT for all three poliovirus serotypes.

Summaries of the MNVT and TgmNVT are given below, along with the implementation process for the TgmNVT.

1. Summary of the MNVT

1.1 Key features

A detailed SOP for neurovirulence tests for types 1, 2 or 3 live-attenuated OPV in monkeys is available from WHO.[1] To perform the test, between 5.5 $\log_{10}$ $CCID_{50}$ and 6.5 $\log_{10}$ $CCID_{50}$ of monovalent virus is delivered in a single dose by intraspinal inoculation into the lumbar cord. A back titration of the inoculum should be carried out after the inoculation step has been completed. Residual paralysis, if any, occurring during the following 17–22 days should be noted. The animals are killed at the end of the test, or earlier on humanitarian grounds, and prepared for histological examination of the central nervous system. Damage to different regions is scored on a scale from 1 to 4, and a mean lesion score is calculated for each monkey and then for all the monkeys in the test. The clinical signs do not form part of the assessment or of the pass/fail criteria. The homologous WHO/SO+2 reference is tested in parallel. Laboratories that want to introduce the test should agree an implementation process with the NRA.

[1] Contact the Coordinator, Technologies, Standards and Norms, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/biologicals/vaccines/en/).

1.2 Number of animals

The number of monkeys is chosen on statistical grounds, considering the variability of the test, so that a satisfactory vaccine will give the lesion score of a reference preparation only twice in 1% of tests, and therefore be incorrectly scored as a fail. Valid animals must show some sign of histological damage as evidence of correct placement of active virus. The number of "valid" monkeys required per virus preparation is 11 each for type 1 and type 2, and 18 for type 3. Because a reference must be tested at the same time, the total number of monkeys for type 1 and type 2 is at least 22 each; and for type 3 it is 36.

1.3 Sections examined

Sections are examined from defined regions of the spinal cord and brain, and scored histologically for virus activity on a scale of 1 (cellular infiltration only) to 4 (massive neuronal damage). At least 29 sections are examined per monkey, as specified in the WHO SOP for the MNVT. The readings are used to generate the mean lesion score for the animal, and the mean lesion scores for all animals are then used to generate the mean lesion score for the test as a whole.

1.4 Pass/fail criteria

The pass/fail criteria are based on variations occurring in the test from run to run, established from the scores obtained with the reference preparation, and are specific to each laboratory and operator. The within-test variance is used to calculate the statistical constants C1, C2 and C3. The vaccine is not acceptable if the mean lesion score of the test vaccine is greater than that of the concurrently tested reference by more than C1. If the test vaccine gives a higher score than the reference but the difference in scores lies between C1 and C2, the vaccine may be retested and the results pooled; if the difference for the pooled test results is greater than C3, the vaccine fails.

The values for C1, C2 and C3 are initially established on the basis of the data accumulated after four qualifying tests. These values should then be updated after every test until nine tests have been performed. After that, the C values are based on the 10 most recent tests. The C values must be established for each testing laboratory.

2. Summary of the TgmNVT

2.1 Key features

The detailed SOP for the TgmNVT is available from WHO for neurovirulence tests for type 1, 2 or 3 live-attenuated OPV.[2] The test for the neurovirulence of polio

[2] Contact the Coordinator, Technologies, Standards and Norms, World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland (http://www.who.int/biologicals/vaccines/en/).

vaccines in transgenic mice involves intraspinal inoculation of small volumes of test vaccine into a defined strain of transgenic mice carrying the human receptor for poliovirus. Two virus concentrations are used, and the outcome of the test is based on the clinical response to the dose. A reference preparation is tested at the same time. A clearly defined process has been established for laboratories that want to introduce the test.

2.2 Strain of transgenic mouse

Different transgenic mouse lines differ in their sensitivity to polio infection depending on their particular transgenic construct and genetic background, and only strains from a source approved by WHO should be used. As of 2012, the only approved transgenic mouse strain was TgPVR21, which was developed in Japan and can be sourced from the developers or from an approved subcontractor.

2.3 Titration of virus

Two doses of virus are inoculated in a volume of 5 µl each: for type 1, 1.75 $CCID_{50}$ and 2.75 $CCID_{50}$; for type 2, 5.0 $CCID_{50}$ and 6.0 $CCID_{50}$; and for type 3, 3.5 $CCID_{50}$ and 4.5 $CCID_{50}$. The inocula must be prepared and titrated accurately to ensure that these doses are given; the precision of the determinations should be better than ± 0.3 $\log_{10}$. A back titration of the inoculum should be performed after the inoculation step has been completed.

2.4 Inoculation and observation of animals

Animals procured at age 5–6 weeks are randomly allocated to cages, and allowed to recover for at least seven days. They are then appropriately anaesthetized and inoculated with 5 µl of diluted test virus between the last thoracic vertebra and the first lumbar vertebra. Animals are observed for clinical signs once a day for the next 14 days, and ultimately scored either as normal throughout (slight weakness or no signs) or paralysed (paresis on two consecutive days, or paralysis on a single day). For the test to be valid, the lower doses and higher doses of the reference preparation should cause paralysis in more than 5% and less than 95% of animals, respectively. A test requires 128 mice for one vaccine plus the reference tested concurrently, or 192 mice for two vaccines and the reference. The reference is the same as that used in the monkey test; the use of other references may be acceptable but should be validated.

The vaccine passes if it is not significantly more virulent than the reference as defined in terms of the log of the odds ratio and the statistical constants L_1 and L_2, which are based on the reproducibility of the test and define the pass/fail criteria as well as the grey zone in which a retest is required. The acceptance and rejection limits, L_1 and L_2, have been selected so that a test vaccine that is equivalent to the reference will have a 0.95 probability of passing and a 0.01

probability of failing, respectively. The constants are regularly updated. The statistical evaluation of test validity includes assessments of linearity, and dose and sex effects.

3. Implementation process for the TgmNVT

If a manufacturer wishes to use the TgmNVT, relevant validation data should be available for the specific product to demonstrate the test's applicability. These data may include references to the collaborative studies by which the test was originally developed. A clear, stepwise process for implementing the TgmNVT has been established; it involves training staff in the inoculation technique by first injecting India ink, testing with vaccines, and testing using a blinded evaluation panel containing vaccines that pass, fail or marginally fail the test. Competence in clinical scoring is acquired by working through a standardized training procedure that involves scoring mice in parallel with an experienced scorer; there are also clear criteria for declaring a trainee competent.

Testing should be performed according to the procedures specified in the WHO SOP for the TgmNVT, using appropriate WHO reference materials unless modified procedures have been validated and shown to be suitable. The test chosen should be used to test virus seeds and bulks, as described in sections A.3.2.4.2 and A.4.4.7.2, respectively.

4. Considerations in relation to assay choice

The following specific issues suggest that care should be taken in the selection of the in vivo tests to be performed for neurovirulence, and that the selection should be justified. The report of the WHO working group meeting to discuss the revision of the WHO Recommendations for OPV: TRS Nos. 904 and 910 provides more detailed discussion (*1*).

4.1 Type-1 and type-2 Sabin vaccine viruses

The relative sensitivity of the TgmNVT and MNVT performed according to WHO procedures with respect to the presence of mutations in the 5′ untranslated region in types 1 and 2 polioviruses appears to be comparable, but this sensitivity is significantly lower than that for type 3 (*2*, *3*). It is unknown whether these two models are equally sensitive to other potentially neurovirulent mutations. Most manufacturers use essentially identical seeds of types 1 and 2, in contrast to the situation with type 3.

4.2 Type-3 Sabin vaccine virus

4.2.1 Molecular biology

Studies of the molecular biology of the Sabin polio vaccine virus strains have suggested that few mutations are involved in attenuation, and that for the type-3

strain, there may be only two: one base change in the 5′ noncoding region of the genome at base 472, and one coding change at base 2034 that introduces an amino acid change in the virus protein VP3. A third mutation at position 2493 has been described (*4*). Growth of Sabin 3 virus in cell culture or in vaccine recipients results in rapid accumulation of U instead of C at nucleotide 2493 (changing Thr to Ile at amino acid 6 of capsid protein VP1), and all Sabin 3 OPV batches contain variable amounts of these mutants. This mutation does not affect neurovirulence as determined by the MNVT but there is evidence that it influences the results obtained by the TgmNVT, which is described in the WHO SOP (*5*). Variations in the virulence of vaccine batches as measured in monkeys correlate well with variations in the base in the 5′ noncoding region as measured by the MAPREC assay. Changes in the amino acid in VP3, or changes at other positions that suppress its effect, are not thought to be generated in the course of well-controlled production runs, although this is possible in principle.

4.2.2 Current type-3 seed viruses

Seed viruses used for global vaccine production contain variable proportions of the bases found at position 2493 (C or U):

- The original WHO reference material for neurovirulence testing (passage level SO+2) contained about an equal mixture of both forms (2493 C or U).
- Batches prepared from RSO, the seeds most commonly used in production in Europe, typically contain about 5% or fewer of 2493-U (mutant).
- Seed viruses used in production by some manufacturers (i.e. a plaque purified from SO) result in batches containing 100% of the mutant form (2493-U) (*6*).

All OPVs in use are believed to have an acceptable safety profile.

5. Experience using the MNVT and TgmNVT with type-3 seeds and vaccines

There is evidence that the TgmNVT described in the WHO SOP is sensitive to the presence of 2493-U, whereas the monkey test is not sensitive to this mutation. Thus, batches produced from RSO seed will pass both the MNVT and TgmNVT tests, whereas batches produced from alternative seeds that contain 100% 2493-U will pass the MNVT but may fail the TgmNVT, although they still have an acceptable safety profile for clinical use.

The WHO SOP for the TgmNVT specifies the doses and the WHO reference material to be used, and includes the proportion of mice that need to

be affected at the two doses of virus for the test to be considered valid. The WHO reference material for the TgmNVT is the same as that used in the MNVT, and has approximately 50% 2493-C; it was validated primarily against vaccines made from SO or RSO seeds. However, if this reference material is used to test vaccines derived from seed that contains 2493-U, the seed may fail even if it contains little 472-C and would pass the MNVT. The TgmNVT could be adapted for testing bulks containing 2493-U – e.g. by changing the reference material, the doses or the validity criteria, or a combination of these. Manufacturers may wish to adapt the TgmNVT to make it applicable to their product. Any modified test should be validated and approved by the appropriate NRA.

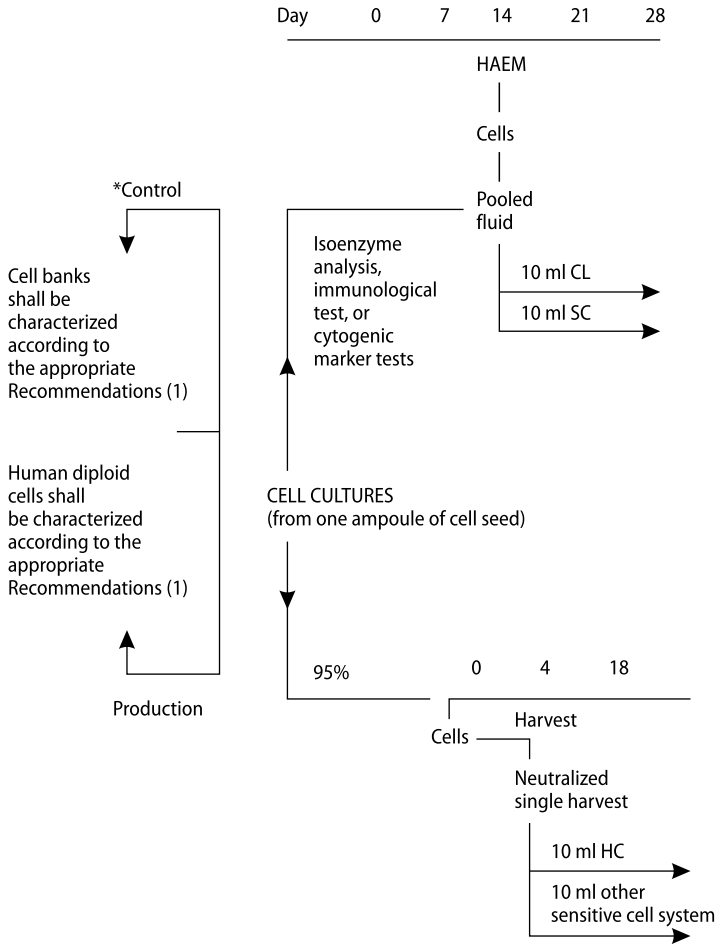
6. References

1. *WHO Working Group Meeting to Discuss the Revision of the WHO Recommendations for OPV: TRS No. 904 and 910*. Geneva, Switzerland 20–22 July 2010. Geneva, World Health Organization, 2010 (http://www.who.int/biologicals/vaccines/OPV_Meeting_report_Final_Clean_13May2011.pdf, accessed 20 June 2013).
2. Gennady V et al. Genetic stability of Sabin 1 strain of poliovirus: implications for quality control of oral poliovirus vaccine. *Virology*, 1998, 245:183–187.
3. Taffs RE et al. Genetic stability and mutant selection in Sabin 2 strain of oral poliovirus vaccine grown under different cell culture conditions. *Virology*, 1995, 209:66–73.
4. Tatem JM et al. A mutation present in the amino terminus of Sabin 3 poliovirus vp1 protein is attenuating. *Journal of Virology*, 1992, 66:3194–3197.
5. Chumakov KM et al. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence. *Journal of Virology*, 1992, 66:966–970.
6. Rezapkin GV et al. Reevaluation of nucleotide sequences of wild-type and attenuated polioviruses of type 3. *Virus Research*, 1999, 65:111–119.

Appendix 3

Preparation of poliomyelitis vaccines (oral, live, attenuated) using cell banks – example of a flowsheet

Figure 2.3
Flowsheet example



[a] Control cells should be 5% of the total or 500 ml of cell suspension or 100 000 000 cells.

HAEM = test for haemadsorbing viruses; CL = cell line used for production but not the same batch of cells as used for production of the virus; SC = when a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line.

Figure 2.3 *continued*

When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line;[1] HC = human cells.

Note: this example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the NRA, this flowsheet should not be considered to be an integral part of the requirements; it has been included solely for guidance. Manufacturers should prepare their own flowsheet to clarify the procedures used.

[1] Recommendations for the evaluation of animal-cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: *WHO Expert Committee on Biological Standardization. Sixty-first report*. Geneva, World Health Organization, 2013 (WHO Technical Report Series, No. 978), Annex 3.

Appendix 4

Cell-culture techniques for determining the virus content of poliomyelitis vaccines (oral, live, attenuated)

This appendix describes a method for determining the virus content of live-attenuated OPV in cell cultures. It is an example that is provided only for guidance.

The preparation to be assayed and the reference preparation are diluted in an appropriate medium. It is convenient to make 10-fold dilution steps of the virus suspensions initially, but for dilutions that are to be inoculated into HEp2 (Cincinnati) cell cultures, the dilutions should be prepared in 1.0 $\log_{10}$ or smaller steps. A preliminary assay may be required to ensure that in the test the dilution range selected encompasses at least three dilutions that will infect between 0% and 100% of the cultures inoculated.

Titrate the vaccine for infectious virus using no fewer than three separate containers of vaccine following the method described below. Titrate one container of an appropriate virus reference preparation in triplicate to validate each assay. The virus titre of the reference preparation is monitored using a control chart, and a titre is established using historical data at each laboratory.

If the vaccine contains more than one type of poliovirus, titration of the individual serotypes is undertaken separately, using mixtures of appropriate type-specific antiserum (or preferably a monoclonal antibody) to neutralize each of the other types present.

To titrate individual serotypes, inoculate a suitable number of wells (ideally 8–10) in a flat-bottomed microtitre plate with equal volumes of the selected dilutions of virus and the appropriate antiserum mixture. Total virus content is determined, without any prior incubation, by directly diluting the vaccine in the assay medium. The assay is then incubated for 1–3 hours at 34–36 °C; this is followed by the addition of an appropriate volume of a suitable cell. The plates are further incubated at 34–36 °C, and examined between day 5 and day 9 for the presence of viral cytopathic effect.

The cytopathic effect can be observed by direct reading or after appropriate staining (vital or fixed staining). The individual virus concentration for each polio serotype and reference preparation is then calculated using an appropriate method.

The assay is considered valid if:

- the estimated virus concentration for the reference preparation is ± 0.5 $\log_{10}$ $CCID_{50}$ of the established value for this preparation;

- the confidence interval ($P = 0.95$) of the estimated virus concentration of the three replicates of the reference preparation is not greater than ± 0.3 $\log_{10}$ $CCID_{50}$.

The assay is repeated and results are averaged if:

- the confidence interval ($P = 0.95$) of the combined virus concentration of the vaccine is greater than ± 0.3 $\log_{10}$ $CCID_{50}$.

Appendix 5

Model protocol for the manufacturing and control of poliomyelitis vaccines (oral, live, attenuated)

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by a manufacturer to the NRA. Information and tests may be added or omitted as necessary, with the authorization of the NRA.

It is possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO Guidelines for a particular product should be given in the protocol submitted.

The section concerning the final product must be accompanied by a sample of the label and a copy of the leaflet that will accompany the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot-release certificate from the NRA or from the NCL in the country where the vaccine was produced or released stating that the product meets national requirements as well as the recommendations in Part A of this annex.

1. Summary information on finished product (final lot)

International name: _____
Trade name/commercial name: _____
Product licence (marketing authorization) number: _____
Country: _____
Name and address of manufacturer: _____
Name and address of licence holder, if different: _____
Virus strain: _____
Origin and short history: _____
_____

2. Summary information on manufacture

Batch number: _____
Final bulk: _____
Type of container: _____
Number of doses per container: _____
Number of filled containers in this final lot: _____

Bulk numbers of monovalent bulk suspensions blended in monovalent/ bivalent/trivalent vaccine:	Type 1	Type 2	Type 3
	_____	_____	_____

Site of manufacture of each monovalent bulk: _____
Date of manufacture of each monovalent bulk: _____
Date of manufacture of final bulk (blending): _____
Date of manufacture (filling) of finished product: _____
Date on which last determination of virus titre was started, or start date of period of validity: _____
Shelf-life approved (months): _____
Expiry date: _____
Storage conditions: _____
Volume of human dose (in drops and/or ml): _____
Virus titre per single human dose:
Type 1: _____
Type 2: _____
Type 3: _____
Nature and concentration of stabilizer: _____
Nature of any antibiotics present in vaccine and amount per human dose: _____
Release date: _____

Starting materials

The information requested below is to be presented for each submission. Full details on master seed and working seed lots should be provided only upon first submission or whenever a change has been introduced.

The following sections are intended for recording the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency in production. If any test has to be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

If any cell lot or virus harvest intended for production is rejected during the control testing, this should also be recorded, either in the following sections or on a separate sheet.

3. Control of source materials (section A.3)

Cell banks (every submission)

Information on cell banking system: _____
Name and identification of substrate: _____
Origin and short history: _____

_____

Authority that approved the cell bank: _____
Master cell bank (MCB) and working cell bank (WCB) lot numbers and date of preparation: _____
Date the MCB and WCB were established: _____
Date of approval by NRA: _____
Total number of ampoules stored: _____
Passage level (or number of population doublings) of cell bank: _____
Maximum number of passages approved: _____
Storage conditions: _____
Method of preparation of cell bank in terms of number of freezes and efforts made to ensure that a homogeneous population is dispersed into the ampoules: _____

Identity tests on MCB and WCB (first submission only) (section A.3.1.2)

Percentage of total cell-bank ampoules tested: _____
Identification of cell substrate: _____
Method: _____
Specification: _____
Date of test: _____
Result: _____
Growth characteristics: _____
Morphological characteristics: _____
Immunological marker: _____
Cytogenetic data: _____
Biochemical data: _____
Results of other identity tests: _____

Tests for adventitious agents

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Tests for bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____

Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C					
30–36 °C					
Negative control					

Test for mycoplasmas

Method used: _____
Volume tested: _____
Media used: _____
Temperature of incubation: _____
Observation period (specification): _____
Positive controls (list of species used and results): _____
_____

	Date of start of test	Date of end of test	Results
Subcultures at day 3			
Subcultures at day 7			
Subcultures at day 14			
Subcultures at day 21			

Indicator cell-culture method (if applicable)

Cell substrate used: _____
Inoculum: _____
Date of test: _____
Passage number: _____
Negative control: _____
Positive controls: _____
Date of staining: _____
Results: _____
Results of tests for tumorigenicity (if applicable): _____

Virus seeds (every submission) (section A.3.2)

Vaccine virus strain(s) and serotype(s): _____
Substrates used for preparing seed lots: _____

Origin and short history of virus seeds: _____
Authority that approved virus strains: _____
Date of approval: _____

Virus strains: information and seed lot preparation (every submission) (section A.3.2.1)

Virus master seed (**VMS**), virus submaster seed and virus working seed (**VWS**)
Source of VMS: _____
VMS and VWS lot numbers: _____
Name and address of manufacturer: _____
VWS passage level from VMS: _____
Dates of inoculation: _____
Dates of harvest: _____
Number of containers: _____
Conditions of storage: _____
Dates of preparation: _____
Maximum passage levels authorized: _____

Tests on VMS, virus submaster seed, and VWS (first submission only)

Tests for adventitious agents

Date(s) of satisfactory test(s) for freedom from adventitious agent: _____
Volume of virus seed samples for neutralization and testing: _____
Batch number of antiserum used for neutralization of virus seed: _____
Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Identity test

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Absence of SV40

Method used: _____
Date of start of test: _____
Date of end of test: _____
Results: _____

In vitro tests: MAPREC or rct/40 marker test

MAPREC test

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A and 525-C in bulk sample to the International Standard or level of mutations: _____

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

Type 2

Ratio of % of 481-G in bulk sample to the International Standard or level of mutations: _____

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

Type 3

Ratio of % of 472-C in bulk sample to the International Standard or level of mutations: _____

Result of test of consistency of production: _____

Result of test of comparison with the International Standard: _____

In vitro rct/40 marker test

Date of test: _____

Reduction of titre in bulk sample: _____

Reduction of titre in negative reference: _____

Reduction of titre in positive reference: _____

Result: _____

Result of test of consistency of production: _____

In vivo tests for neurovirulence

Neurovirulence test in monkeys (MNVT)

Result of blood serum test in monkeys prior to inoculation: _____

Number and species of monkeys inoculated: _____

Quantity ($CCID_{50}$) inoculated into each test monkey: _____

Number of "valid" monkeys inoculated with test sample: _____
Number of positive monkeys observed that were inoculated with test sample or with reference: _____
Reference preparation: _____
Number of "valid" monkeys inoculated with reference: _____
Number of positive monkeys observed: _____
Mean lesion score of test sample: _____
Mean lesion score of reference: (see also attached forms giving details of histological observations and assessment) _____
C_1 constant value: _____

Neurovirulence test in transgenic mice (TgmNVT)

Strain of mice inoculated: _____
For each dose of the seed sample: _____
Number of mice inoculated: _____
Number of mice excluded from evaluation: _____
Number of mice paralysed: _____
Results of validity tests for each dose of the reference virus: _____
Number of mice inoculated: _____
Number of mice excluded from evaluation: _____
Number of mice paralysed: _____
Virus assay results for each dose inoculated (residual inocula): _____
Paralysis rates for test vaccine at each dose: _____
Paralysis rates for reference virus at each dose: _____
Results: _____
Log of the odds ratio: _____
L_1 and L_2 values: _____
Pass/fail decision: _____

Freedom from bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C					
30–36 °C					
Negative control					

Test for mycoplasmas

Method used: _____

Volume tested: _____

Media used: _____

Temperature of incubation: _____

Observation period (specification): _____

Positive controls (list of species used and results): _____

_____

	Date of start of test	Date of end of test	Results
Subcultures at day 3			
Subcultures at day 7			
Subcultures at day 14			
Subcultures at day 21			

Indicator cell-culture method (if applicable)

Cell substrate used: _____

Inoculum: _____

Date of test: _____

Passage number: _____

Negative control: _____

Positive controls: _____

Date of staining: _____

Results: _____

Virus titration

Date of test: _____

Reference batch number: _____

Date of test: _____

Result: _____

Genotype characterization

Method used: _____
Date of test: _____
Result: _____

Test for mycobacteria

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

4. Control of vaccine production (section A.4)

Control cell cultures (section A.4.1)

Lot number of MCB: _____
Lot number of WCB: _____
Date of thawing of ampoule of WCB: _____
Passage number of production cells: _____
Date of preparation of control cell cultures: _____
Results of microscopic observation: _____

Tests of control cell cultures

Ratio of control cell cultures to production cell cultures: _____
Incubation conditions: _____
Period of observation of cultures: _____
Dates observation started and ended: _____
Proportion of cultures discarded for nonspecific reasons: _____
Results of observation: _____
Date supernatant fluid collected: _____

Tests for haemadsorbing viruses

Quantity of cells tested: _____
Method used: _____
Date of start of test: _____
Date of end of test: _____
Results: _____

Tests for adventitious agents in supernatant culture fluids

Method used: _____
Date of start of test: _____

Date of end of test: _____
Result: _____

Identity test

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Control of single harvests (section A.4.3)

Volume harvested: _____
Date of sampling: _____

Identity test

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Virus titration

Date of test: _____
Reference batch number: _____
Date of test: _____
Result: _____

Tests of neutralized single harvests for adventitious agents

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Freedom from bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C					
30–36 °C					
Negative control					

Test for mycoplasmas

Method used: _____
Volume tested: _____
Media used: _____
Temperature of incubation: _____
Observation period (specification): _____
Positive controls (list of species used and results): _____
_____

	Date of start of test	Date of end of test	Results
Subcultures at day 3			
Subcultures at day 7			
Subcultures at day 14			
Subcultures at day 21			

Indicator cell-culture method (if applicable)

Cell substrate used: _____
Inoculum: _____
Date of test: _____
Passage number: _____
Negative control: _____
Positive controls: _____
Date of staining: _____
Results: _____

Test for mycobacteria

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Control of monovalent bulk (section A.4.4)

Date of filtration of bulk: _____
Porosity of filters used: _____
Date of sampling: _____

Identity test

Method used: _____
Date of start of test: _____
Date of end of test: _____
Results: _____
Lot number of reference reagents: _____

Virus titration

Date of test: _____
Reference batch number: _____
Result: _____

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C					
30–36 °C					
Negative control					

Test for mycobacteria

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____

Tests for consistency of virus characteristics

In vitro rct/40 marker test

Date of test: _____

Reference used: _____
Reduction of titre of negative reference: _____
Reduction of titre of positive reference: _____
Result: _____

MAPREC test

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A and 525-C in bulk sample to the International Standard or level of mutations: _____
Result of test of consistency of production: _____
Result of test of comparison with the International Standard: _____

Type 2

Ratio of % of 481-G in bulk sample to the International Standard or level of mutations: _____
Result of test of consistency of production: _____
Result of test of comparison with the International Standard: _____

Type 3

Ratio of % of 472-C in bulk sample to the International Standard or level of mutations: _____
Result of test of consistency of production: _____
Result of test of comparison with the International Standard: _____

Neurovirulence tests in monkeys (MNVT)

Result of blood serum test in monkeys prior to inoculation: _____
Date of inoculation of monovalent bulk: _____
Number and species of monkeys inoculated: _____
Quantity ($CCID_{50}$) inoculated into each test monkey: _____
Number of "valid" monkeys inoculated with test sample: _____
Number of positive monkeys observed that were inoculated with test sample or with reference: _____
Reference preparation: _____
Number of "valid" monkeys inoculated with reference: _____
Number of positive monkeys observed: _____

Mean lesion score of test sample: _____
Mean lesion score of reference: (see also attached forms giving details of histological observations and assessment) _____
C_1 constant value: _____

Neurovirulence test in transgenic mice (TgmNVT)

Strain of mice inoculated: _____
For each dose of the bulk sample: _____
Number of mice inoculated: _____
Number of mice excluded from evaluation: _____
Number of mice paralysed: _____
Results of validity tests for each dose of the reference virus: _____
Number of mice inoculated: _____
Number of mice excluded from evaluation: _____
Number of mice paralysed: _____
Virus assay results for each dose inoculated (residual inocula): _____
Paralysis rates for test vaccine at each dose: _____
Paralysis rates for reference virus at each dose: _____
Results: _____
Log of the odds ratio: _____
L_1 and L_2 values: _____
Pass/fail decision: _____

Final bulk (section A.4.5)

Preparation of bulk (types as appropriate)	Type 1	Type 2	Type 3
Monovalent bulks in blend			
Volume in blend			
Nature and volume of stabilizer			
Nature and volume of diluent			

Total volume of blend: _____

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____
Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C					
30–36 °C					
Negative control					

5. Filling and containers (section A.5)

Total volume for final filling: _____
Date of filling: _____
Number of vials after inspection: _____
Number of vials filled: _____

6. Control tests on the final lot (section A.6)

Inspection of final containers

Appearance: _____
Date of test: _____
Results: _____

Extractable volume

Extractable volume (ml): _____
The number of drops, using the approved dropper,
in a minimum of five individual final containers: _____

pH

Date of test: _____
Result: _____

Identity test

Method used: _____
Date of start of test: _____
Date of end of test: _____
Results: _____
Lot number of reference reagents: _____

Tests for bacteria and fungi

Method used: _____
Number of vials tested: _____

Volume of inoculum per vial: _____
Volume of medium per vial: _____
Observation period (specification): _____

Incubation	Media used	Inoculum	Date of start of test	Date of end of test	Results
20–25 °C					
30–36 °C					
Negative control					

Virus titration

Date of test: _____
Reference batch number: _____
Titre of individual virus types: _____
Batch numbers of antiserum used in test: _____
Date of test: _____

Result	Vaccine	Reference
Type 1		
Type 2		
Type 3		

Thermal stability

Date of test: _____
Batch numbers of antiserum used in test: _____

Results	Vaccine at 37 °C	Vaccine	Difference
Total virus			

Residual antibiotics (if applicable)

Date of start of test: _____
Date of end of test: _____
Results: _____

Level of stabilizer (if applicable)

Date of start of test: _____
Date of end of test: _____
Results: _____

Additional information for production in primary monkey kidney-cell cultures

Production in primary monkey kidney-cell cultures

Control of vaccine production: _____
Control of monkeys: _____
Monkey species used for production: _____
Quarantine batch number: _____
Percentage of monkeys surviving quarantine period: _____
Nature and concentration of antibiotics or selecting agent(s) used in the production cell culture's maintenance medium: _____

Tests for antibodies to simian immunodeficiency virus, SV40, foamy viruses and herpes B virus
Methods used: _____
Date of start of test: _____
Date of end of test: _____
Results: _____

Production details

Production monkey number: _____
Date of trypsinizing: _____
Number of cultures prepared: _____

Cell cultures for vaccine production

Virus-seed lot number: _____
Virus titre/cell ratio: _____
Number of cultures inoculated: _____
Date of inoculation: _____
Date of harvest: _____
Temperature of incubation: _____
Period of incubation: _____
Number of cultures harvested: _____

Tests on pooled supernatant fluids

Date of sampling from production cell cultures: _____
Tests for adventitious agents: _____
Volume tested and cell culture type: _____
Observation period: _____
Date of completion of tests: _____
Results: _____

Date of sampling from cell cultures inoculated with the pooled fluid

Tests for adventitious agents: _____
Volume tested and cell culture type: _____
Date of completion of tests: _____
Results: _____

Tests in rabbit kidney-cell cultures

Volume tested: _____
Date of completion of tests: _____
Results: _____

Control of cell cultures

Ratio of control cell cultures to production cell cultures, or control cell cultures as a proportion of production cell cultures: _____
Period of observation of cultures: _____
Proportion of cultures discarded for nonspecific reasons: _____
Results: _____

Tests for haemadsorbing viruses

Methods: _____
Date of test: _____
Results: _____

Tests for other adventitious agents

Methods: _____
Date of test: _____
Results: _____

Control of single harvests

Volume harvested: _____
Date of sampling: _____
Tests for bacteria, fungi and mycoplasmas: _____
Results: _____

Tests on neutralized single harvests in monkey kidney-cell and human-cell cultures

Batch number of antiserum used: _____
Volume tested: _____
Date primary cell-culture tests started: _____
Period of observation: _____
Date cell-culture fluids sampled: _____

Period of observation: _____
Date of completion of tests: _____
Results: _____

Control of monovalent bulk

Tests in rabbits

Number and weight range of animals: _____
Date of inoculation: _____
Quantity of monovalent bulk injected: _____
Results (survival numbers and other relevant observations): _____
Date of filtration of bulk: _____
Porosity of filters used: _____
Date of sampling: _____

Tests for retroviruses

Methods: _____
Date: _____
Results: _____

7. Certification by the manufacturer

Name of the manufacturer _____

Name of head of production (typed) _____

Certification by the person from the control laboratory of the manufacturing company taking responsibility for the production and control of the vaccine

I certify that lot no. _____ of poliomyelitis vaccine (oral), whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A[1] of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (2014).[2]

Signature _____
Name (typed) _____
Date _____

[1] With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

[2] WHO Technical Report Series, No. 980, Annex 2.

8. Certification by the NRA

If the vaccine is to be exported, attach a certificate from the NRA (as shown in Appendix 6), a label from a final container and an instruction leaflet for users.

Appendix 6

Model certificate for the release of poliomyelitis vaccines (oral, live, attenuated) by NRAs

Lot release certificate

Certificate no. _____

The following lot(s) of poliomyelitis vaccine (oral, live, attenuated) produced by _____[1] in _____,[2] whose numbers appear on the labels of the final containers, complies with the relevant national specifications and provisions for the release of biological products[3] and Part A[4] of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (2014),[5] and complies with WHO good manufacturing practices: main principles for pharmaceutical products;[6] Good manufacturing practices for biological products;[7] and Guidelines for independent lot release of vaccines by regulatory authorities.[8]

The release decision is based on _____[9]

The certificate may include the following information:

- name and address of manufacturer
- site(s) of manufacturing
- trade name and common name of product
- marketing authorization number
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary)

[1] Name of manufacturer.

[2] Country of origin.

[3] If any national requirements have not been met, specify which one(s) and indicate why the release of the lot(s) has nevertheless been authorized by the NRA.

[4] With the exception of provisions on distribution and shipping, which the NRA may not be in a position to assess.

[5] WHO Technical Report Series, No. 980, Annex 2.

[6] WHO Technical Report Series, No. 961, Annex 3.

[7] WHO Technical Report Series, No. 822, Annex 1.

[8] WHO Technical Report Series, No. 978, Annex 2.

[9] Evaluation of the summary protocol, independent laboratory testing, or procedures specified in a defined document etc., as appropriate.

- type of container used
- number of doses per container
- number of containers or lot size
- date of start of period of validity (e.g. manufacturing date) and/or expiry date
- storage conditions
- signature and function of the person authorized to issue the certificate
- date of issue of certificate
- certificate number.

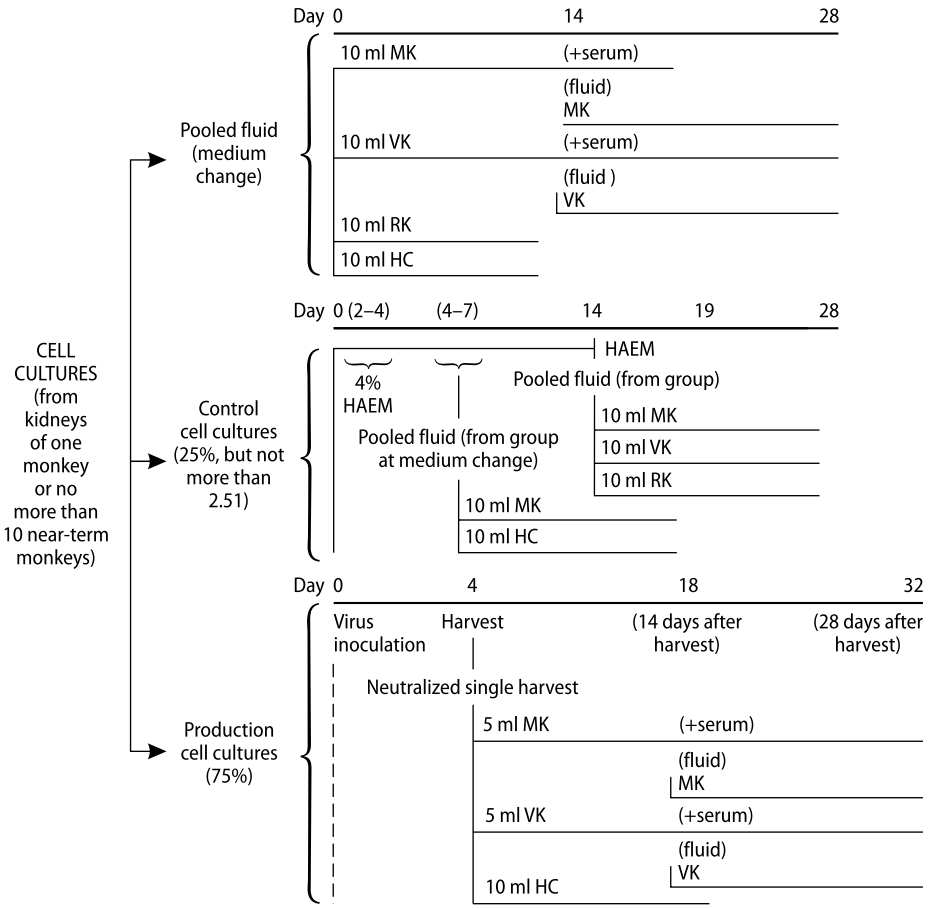
The Director of the NRA (or other appropriate authority)

Name (typed) _____
Signature _____
Date _____

Appendix 7

Preparation of poliomyelitis vaccines (oral, live, attenuated) using primary monkey kidney cells – example of a flowsheet

Figure 2.4
Flowsheet example



HAEM = test for haemadsorbing viruses; MK = monkey kidney cells from species (but not the same animal) used for production; VK = kidney cells from vervet monkey or one sensitive to SV40; RK = rabbit kidney cells; HC = human cells sensitive to measles.

Note: this example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the NRA, this flowsheet should not be considered to be an integral part of the requirements; it has been included solely for guidance. Manufacturers should prepare their own flowsheet to clarify the procedures used.