

Annex 3

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

Replacement of Annex 2 of WHO Technical Report Series, No. 910

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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 WHO Recommendations are made only on condition that such modifications ensure that the product 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

The Requirements for inactivated poliomyelitis vaccine (IPV) were first formulated in 1959 (1) and revised in 1965 (2). Following several advances in vaccine production technology, the Requirements were further updated in 1981 (3) and amended in 1985 (4). At that time, the introduction of continuous cells for the manufacture of IPV was a novel development. Therefore, when the regulatory control of products manufactured in continuous cells had been standardized, the Requirements were again updated in 2000 (5). An addendum was then developed in 2003 (6), specifying the measures to be taken to minimize the accidental risk of reintroducing wild-type poliovirus from a vaccine manufacturing facility into the community after global certification of polio eradication.

Since the Recommendations for the production and control of poliomyelitis vaccine (inactivated) were last revised in 2000 (5) and 2003 (6), there have been several changes in vaccine production, including the use of seed viruses derived from Sabin strains, which make further revision of the Recommendations necessary. To facilitate this process, a meeting to discuss the international specifications for IPV – attended by experts from academia, national regulatory authorities (NRAs), national control laboratories (NCLs) and industry involved in the research, manufacture, authorization and testing and/or release of IPV around the world – was convened by WHO on 29 March 2012. During the discussions, critical issues were considered in relation to both the quality control and evaluation of IPV, including Sabin-based IPV (sIPV), and the revision of the Recommendations described in Annex 2 of WHO Technical Report Series 910 (5). WHO then convened an international Technical Working Group meeting in Geneva on 14–15 May 2013 (7) – attended by experts from academia, NRAs, NCLs and industry involved in the development, manufacture, authorization and testing and/or release of IPV (including sIPV and other new developments of novel IPV) – to further discuss and reach consensus on critical issues relevant to the revision of Annex 2 of WHO Technical Report Series 910. WHO additionally organized an informal consultation at its headquarters in Geneva on 25–26 March 2014 – attended by academics, researchers, vaccine manufacturers and regulators involved in IPV development, production, evaluation and regulatory licensure – to review the draft Recommendations prepared by a drafting group and to seek consensus on key technical and regulatory issues.

Major issues addressed in these revised WHO Recommendations include:

- updating of “General considerations” and other sections to reflect the future development of IPV in accordance with global programmatic need (for example, the use of Sabin strains and strains derived by recombinant DNA technology);

- inclusion of a new Appendix 1 to update the history of the different virus seed strains used by manufacturers for IPV production;
- updating of the section on international standards and reference preparations;
- updating of the section on general manufacturing recommendations and control tests;
- updating of terminology;
- inclusion of specific tests for sIPV and IPV made from strains derived by recombinant DNA technology;
- updating of the appendices;
- inclusion of new sections on nonclinical and clinical evaluation of IPV.

Additional changes were also made to bring this document into line with other WHO Recommendations published since the last revision.

Scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the quality and nonclinical and clinical aspects of IPV in order to ensure the quality, safety and efficacy of the vaccines. The scope of the present document encompasses IPV derived from: (a) the wild-type strains that have been used in the manufacture of IPV for many years; (b) the attenuated Sabin strains that have been used in the manufacture of oral poliomyelitis vaccine (OPV); and (c) new alternative poliovirus strains currently under development, including those derived by recombinant DNA technology.

This document does not cover vaccines which are based on virus-like particles (VLPs) and replicons. However, some of the aspects discussed may be relevant to these types of seeds and should be taken into consideration during vaccine development using such seeds.

The document should be read in conjunction with other relevant WHO guidelines such as those on the nonclinical (8) and clinical (9) evaluation of vaccines.

Among the most significant changes in production has been the increasing use of IPV in combination with other vaccines, such as those based upon diphtheria toxoid and tetanus toxoid (“DT-based”) combined vaccines, which raises considerations – such as interaction of the poliovirus antigens with other antigens and/or adjuvants – that do not apply when IPV is used as a standalone product. These considerations are dealt with in a separate WHO document (10) but not in these revised WHO Recommendations. However,

to provide further guidance on control of the vaccine, key tests that may be influenced by other antigens and/or adjuvants present in combined vaccines are identified.

General considerations

Poliomyelitis is an acute communicable disease of humans (11) caused by three distinct poliovirus serotypes – types 1, 2 and 3 – which can be distinguished by neutralization tests. Poliovirus is classified as 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, faecal-to-oral transmission predominates, whereas oral-to-oral transmission may be more common where standards of sanitation are high. In most settings, mixed patterns of transmission are likely to occur. In the pre-vaccine era, when poliovirus was the leading cause of permanent disability in children, virtually all children became infected with polioviruses. On average, in the absence of protection by humoral or maternal antibody, 1 in every 200 susceptible individuals develops paralytic poliomyelitis (11).

Progress in polio control (and, since 1988, in polio eradication) has been due mainly to the widespread use of vaccines. An IPV (Salk vaccine, wIPV)¹ was first licensed in 1955; live, attenuated OPV (Sabin vaccine) was licensed in the USA in 1961 as a monovalent (mOPV) vaccine, followed by a trivalent OPV (tOPV) licensed for use in 1963 (11). In May 1988, the World Health Assembly resolved to eradicate poliomyelitis globally by the year 2000 and the Global Polio Eradication Initiative (GPEI) was established. The sustained use of poliomyelitis vaccines worldwide since 1988 has led to a precipitous drop in the global incidence of poliomyelitis by more than 99% (11). Globally, the last case of poliomyelitis caused by naturally circulating wild poliovirus (WPV) type 2 (WPV2) occurred in India in 1999. No case due to WPV type 3 (WPV3) has been detected globally since 10 November 2012. Despite the overall success of the GPEI, Afghanistan, Nigeria and Pakistan remained endemic in 2014 for the transmission of WPV type 1 (WPV1). The Horn of Africa, Cameroon, and parts of the Middle East (Egypt, Israel and the Syrian Arab Republic) also reported WPV1 circulation associated with imported WPV1 in 2013, resulting in clinical cases following a period of elimination (11).

Given the progress made towards polio eradication, countries have increasingly switched from using OPV to wIPV in routine immunization programmes, primarily in order to eliminate the burden of vaccine-associated

¹ In this document the use of the abbreviation “IPV” refers to IPV derived from any strain. “wIPV” indicates IPV derived from wild-type strains only, and “sIPV” represents IPV derived from Sabin strains only.

paralytic poliomyelitis (VAPP), a rare adverse event associated with OPV. The incidence of VAPP has been estimated at two to four cases per million birth cohort per year in countries using OPV (11). The sole use of wIPV successfully eradicated polio in some countries, notably the Netherlands and in Scandinavia. In most of the countries that have introduced wIPV as the only poliomyelitis vaccine over the past decade, there has been no evidence of continued circulation of poliovirus strains, thus indicating that wIPV is able to inhibit the community transmission of poliovirus. However, Israel, which switched to an all-IPV routine immunization schedule in 2004, reported the detection of WPV1 in sewage samples from February 2013 onwards. However, no clinical cases of paralytic poliomyelitis have been reported in Israel, the West Bank or Gaza as of 31 December 2013 (11, 12).

In addition to VAPP, the live poliovirus strains in OPV, currently predominantly Sabin type 2, can occasionally revert to a transmissible form termed circulating vaccine-derived poliovirus (cVDPV) (13, 14), which acts essentially the same as the wild type in causing poliomyelitis. This is an obvious threat to polio eradication that is not posed by the use of IPV.

The WHO GPEI, in conjunction with its partners, developed the comprehensive *Polio Eradication and Endgame Strategic Plan 2013–2018* with the goal of achieving a polio-free world by 2018 (15). This plan involves the detection of poliovirus, the interruption of spread, immunization strengthening, withdrawal of OPV, containment, certification and legacy planning, and provides a timetable of events following identification of the last wild-type poliovirus. Three of the key features of the GPEI strategic plan are: (a) the withdrawal of the type-2 OPV strain from tOPV and the introduction of bivalent (types 1 and 3) OPV (bOPV); (b) the introduction of routine use of IPV for managing long-term poliovirus risks, including type-2 cVDPV; and (c) the cessation of all OPV use following the global certification of total WPV serotype eradication (15).

Although the last WPV2 was detected in 1999, eight countries reported cases of paralytic poliomyelitis in 2013 associated with cVDPVs, most of them derived from Sabin type 2 (11). There are 250–500 VAPP cases per year with 40% of these due to type-2 Sabin poliovirus. The need to synchronize OPV cessation was identified in 2008, and withdrawal of the use of type-2 OPV and the introduction of bOPV began in 2009 (15) in supplementary immunization activities or for outbreak control. In 2012 the WHO Strategic Advisory Group of Experts (SAGE) on Immunization recommended that all countries using OPV should introduce at least one dose of IPV in their routine immunization programmes to mitigate the risks of withdrawal of type-2 OPV (16). One of the prerequisites for type-2 OPV cessation is the availability of an affordable IPV option for all OPV-using countries. This may include full dose, fractional dose and adjuvanted IPV, and the intradermal use of IPV in addition to intramuscular or subcutaneous administration.

When poliomyelitis due to wild-type polioviruses and cVDPVs is eradicated (17), laboratories and manufacturers that store or use wild-type or vaccine-derived polioviruses, or any other related viruses, will become an important potential source of reintroduction of these viruses into the community. To manage and control this risk, WHO is finalizing a Global Action Plan that requires the implementation of: (a) appropriate primary safeguards of biorisk management with poliovirus facility containment specifications; (b) secondary safeguards of population immunity in the country approving manufacturing operations; and (c) tertiary safeguards of facility location in countries with demonstrated good personal, domestic and environmental hygiene standards, including closed sewage systems with effective effluent treatment (18).

To mitigate biosafety and biosecurity concerns associated with virulent wild-type viruses used in the manufacture of wIPV, the use of attenuated strains for IPV production has been proposed (19). Production of IPV from live-attenuated Sabin poliovirus seeds has been shown to be technically feasible (20–24), and the first Sabin IPV has been licensed in Japan in the form of two combination vaccines. Manufacturers in various countries are establishing the production of IPV from live-attenuated Sabin strains or IPV using strains derived by recombinant DNA technology, and are at different stages in the development and licensing process. Additional new manufacturers and IPV manufacturers that currently use wild-type poliovirus strains may wish to consider evaluating the potential offered by a Sabin-based IPV or IPV using strains derived by these alternative means.

Wild-type polioviruses are both transmissible and virulent. They will have to be grown under appropriate and strict containment if they are to be used to produce IPV after the elimination of circulating WPV, according to defined timelines, beginning with type 2 (18). The Sabin vaccine strains are attenuated, and transmission from vaccine recipients is limited. However, they are unstable on passage in cell culture and the human gut, and can revert to give cVDPVs.

Given these uncertainties, assurance is required in relation to the characteristics of the live-attenuated Sabin virus before inactivation in order to justify the implementation of containment measures that may be different from those required for wIPV production (18). Production conditions should be validated by the full range of tests including *in vivo* and *in vitro* testing of the master seed and working seed and successive monovalent bulks (with the number to be approved by the NRA) to ensure that the attenuated phenotype of the Sabin strains in monovalent pools is maintained. Subsequently, a limited range of tests, such as mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency. The number of pools of each type tested each year should be justified and agreed by the NRA. Furthermore, it is important that, at intervals to be agreed with the

NRA, pools should be tested with the full range of tests to ensure that production conditions remain satisfactory.

In addition to the Sabin strains that are used in the manufacture of OPV, alternative attenuation methods utilizing recombinant DNA technology are being investigated (25–29). Strains derived by such a methodology may have properties specifically designed to be suitable for the safe production of vaccine (for example, the inability to replicate in the human gut). Such strains should be considered as they become available and may require specific characterization. Biocontainment requirements for such strains will need to be determined on a case-by-case basis. Only virus strains that are approved by the NRA should be used.

An overview of the history of virus seeds that are currently used in IPV production is shown in Appendix 1.

The *in vivo* potency assay in rats has been standardized and shown to have advantages over those previously described *in vivo* tests for IPV (30). When the *in vivo* assay is required for routine production batches, it should be performed at the level of the final bulk. The assay in rats is described in detail in Appendix 2 of this document. The *in vivo* assay should be used to characterize the vaccine after any changes in the manufacturing process that may influence the quality and immunogenicity of the vaccine, unless otherwise justified and agreed by the NRA.

The *in vivo* potency test described in these WHO Recommendations requires the assay of neutralizing antibodies to each of the three poliovirus types. This test requires the use of live poliovirus and, for historical reasons, many laboratories use wild-type strains of poliovirus. The attenuated Sabin strains of poliovirus have been shown to be suitable for the assay of neutralizing antibodies in the *in vivo* test, in principle, by a collaborative study (30) and should be used, but validation of the use of the Sabin strains by each manufacturer should be provided.

Immunization with OPV will cease at some point in the future when poliomyelitis has been eradicated. After that time, the biocontainment levels for use of the Sabin strains for laboratory work will be reviewed. Laboratories are therefore encouraged to investigate the use of alternatives to live viruses for the assay of poliovirus neutralizing antibodies in order to comply with future biocontainment requirements.

The development of transgenic mice that express the human poliovirus receptor (TgPVR mice) (31, 32) has led to the development of an *in vivo* immunization/challenge model (33, 34) that may be useful for assessing the vaccine efficacy of new poliovirus strains. This test is not proposed for lot release. Any work with transgenic mice should comply with WHO guidelines for the maintenance, containment and transport of transgenic animals (35).

The manufacturer of the final lot must be responsible for ensuring conformity with all the recommendations applicable to the final vaccine (Part A,

sections A.5–A.11) even where manufacturing involves only formulating the final bulk from trivalent bulks supplied by another manufacturing establishment and filling the final containers. The manufacturer of the final lot must also be responsible for any production and control tests performed by an external contract laboratory, if applicable, with the approval of the NRA.

If an immunization schedule combining both IPV and OPV is to be claimed which could potentially achieve both high serum antibody levels and intestinal protection (11), clinical studies designed to establish such a combination (sequential) schedule should also examine patterns of virus excretion following poliovirus challenge (with OPV), in addition to serum neutralizing antibodies, in different sequential schedules.

Terminology

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

Adjuvant: a vaccine adjuvant is a substance, or a combination of substances, that is used in conjunction with a vaccine antigen to enhance (for example, increase, accelerate, prolong and/or possibly target) the specific immune response to the vaccine antigen and the clinical effectiveness of the vaccine.

Adventitious agents: contaminating microorganisms of the cell culture, or source materials used in its culture, that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced into the manufacturing process.

Cell-culture infectious dose 50% (CCID₅₀): the quantity of a virus suspension that will infect 50% of cell cultures.

Cell bank: a cell bank is a collection of appropriate containers whose contents are of uniform composition stored under defined conditions. Each container represents an aliquot of a single pool of cells.

The individual containers (for example, ampoules or vials) should be representative of the pool of cells from which they are taken, and should be frozen on the same day by following the same procedure and by using the same equipment and reagents.

Cell seed: a quantity of well characterized cells derived from a single tissue or cell of human or animal origin and 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**.

D-antigen: refers to the antigen found in the sucrose gradient fraction that contains native virus particles which are the target of neutralizing antibodies (36). D-antigen units were originally defined on the basis of an agar precipitin

test performed with D-antigen-specific polyclonal sera. A vaccine preparation that produced a precipitin line at the distance of 25 mm from the centre was arbitrarily assigned a value of 600 D-antigen units using a particular antibody at a particular concentration. This test was used in the initial calibration of reference materials. The D-antigen content of IPV is currently determined by enzyme-linked immunosorbent assay (ELISA).

Final bulk: the finished vaccine present in the container from which the final containers are filled. The final bulk may be prepared from one or more trivalent bulks.

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. All of the final containers must therefore have been filled from a single vessel of **final bulk** in one working session.

Inactivated purified monovalent pool: a filtered and purified monovalent pool that has been inactivated through the use of a validated method.

Master cell bank (MCB): a quantity of well characterized cells of human or animal origin derived from a **cell seed** at a specific population doubling level or passage level, dispensed into multiple containers, cryopreserved and stored frozen under defined conditions, such as the vapour or liquid phase of liquid nitrogen in aliquots of uniform composition. The MCB is prepared from a single homogeneously mixed pool of cells and is used to derive all **working cell banks**. The testing performed on a replacement MCB (derived from the same clone or from an existing master or working cell bank) is the same as for the initial MCB, unless a justified exception is made.

Monovalent pool: a pool of a number of single harvests of the same virus type processed at the same time.

Production cell culture: a collection of cell cultures derived from one or more ampoules of the **working cell bank** used for the production of IPV.

Purified monovalent pool: a concentrated and purified pool of a number of single harvests of the same virus type processed at the same time.

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.

sIPV: inactivated poliomyelitis vaccine derived from Sabin strains only.

Trivalent bulk: a pool of a number of inactivated purified **monovalent pools** processed at the same time and containing all three virus types, blended to achieve a defined **D-antigen** content for each type.

Virus master seed lot: a quantity of virus suspension that has been processed at the same time to ensure a uniform composition, and passaged for a specific number of times that does not exceed the maximum approved by the NRA. It is characterized to the extent necessary to support development of the **virus working seed lot**.

Virus sub-master seed lot (only applicable for Sabin master seed supplied by WHO): a quantity of virus suspension produced by a single passage from the Sabin virus master seed supplied by WHO and made at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate time frame, and that has been processed at the same time to ensure its uniform composition. The virus sub-master seed lot should be characterized to the extent necessary to support the development of the **virus working seed lot**. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.1).

Virus working seed lot: a quantity of virus of uniform composition derived from the **virus master seed lot** made at a multiplicity of infection, ensuring that cytopathic effect develops within an appropriate time frame and used at a passage level approved by the NRA for the manufacturing of vaccine.

wIPV: inactivated poliomyelitis vaccine derived from wild-type polio virus strains only.

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 at -70°C or below in aliquots, one or more of which would be used for vaccine production. All containers are treated identically and once removed from storage are not returned to the stock.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be poliomyelitis vaccine (inactivated). The proper name should be equivalent to 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 (inactivated) should consist of a sterile aqueous suspension of poliovirus types 1, 2 and 3 grown in cell cultures, concentrated, purified and inactivated. The antigen may be formulated with a suitable adjuvant. The preparation should satisfy all the recommendations formulated below.

A.1.3 International reference materials

An International Standard of IPV is available for use in in vitro assays to measure the D-antigen content of IPV containing classical wild-type strains. It is stored frozen in ampoules containing 1 mL of trivalent IPV. This material is for use

in calibrating secondary reference preparations of IPV, which are then used in potency tests to calculate D-antigen content. International standards and reference reagents for the control of *in vivo* potency assays are under investigation. The need for an International Standard for IPV based on the Sabin or other strains is also being investigated.

An International Reference Preparation (IRP) of poliomyelitis vaccine (inactivated) was established by the WHO Expert Committee on Biological Standardization in 1962 (37). This preparation was a trivalent blend prepared in 1959 in primary monkey kidney cells from type-1 (Mahoney), type-2 (MEF) and type-3 (Saukett) strains of poliovirus. After preparation of the IRP, significant advances in the production and control of IPV occurred and vaccines of increased potency and purity were developed. An enhanced potency IPV (PU78-02) from the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) was widely used as a reference preparation for control purposes. When stocks of this reagent were almost exhausted, a new reference material (91/574) was established by the WHO Expert Committee in 1994 as the second WHO International Reference Reagent for *in vivo* and *in vitro* assays of IPV (38). Potencies of 430, 95 and 285 D-antigen units per millilitre were assigned, respectively, to poliovirus types 1, 2 and 3 of this preparation. A separate aliquot of the preparation, established by the European Pharmacopoeia Commission as the Biological Reference Preparation (BRP) batch 1, has an identical assigned titre (39). Material from a concentrated trivalent bulk from a commercially available IPV vaccine was established as the BRP batch 2 in 2003, with assigned potencies of 320, 67 and 282 D-antigen units per millilitre for types 1, 2 and 3, respectively (40). Following inconsistency in the performance of some vials of 91/574, the use of this reference was discontinued in 2010. In 2013, the Third WHO International Standard for inactivated poliomyelitis vaccine (12/104) was established by the WHO Expert Committee using BRP batch 2 as the reference in the study. Potencies of 277, 65 and 248 D-antigen units per millilitre were assigned to poliovirus types 1, 2 and 3, respectively (41).

There are still gaps in the scientific knowledge of biological standardization of IPV, and some inconsistency has been found in results obtained by different laboratories and methods. The validation of international references suitable for vaccines produced from different poliovirus strains will be required.

An International Standard for anti-poliovirus types 1, 2 and 3 antibodies (human) is available for the standardization of neutralizing antibody tests for poliovirus (42).

The International Standards listed above are available from the NIBSC, Potters Bar, England.

A.2 General manufacturing recommendations

The general manufacturing requirements contained in the WHO Good manufacturing practices for pharmaceutical products: main principles (43) and Good manufacturing practices for biological products (44) should apply to establishments manufacturing IPV.

WHO guidance has also been developed on the safe production and quality control of IPV prepared using wild-type poliovirus (6) with the same document also providing guidance on vaccines produced from attenuated poliovirus strains (such as Sabin strains). In addition, facilities that manufacture IPV should comply with the current global recommendations for poliovirus containment appropriate to the particular poliovirus strains used in both vaccine production and quality-control departments (18). WHO guidance on the safe production and quality control of IPV manufactured from wild-type polioviruses (6) may require updating in order to better align such guidance with WHO global recommendations for poliovirus containment (18).

Attenuated strains derived by recombinant DNA technology that are used in IPV production should not be readily transmissible from person to person. The applicable containment conditions will depend on the phenotype and production conditions and should be assessed on a case-by-case basis to ensure an acceptable level of phenotypic stability. In any case, any biocontainment arrangement should comply with the global recommendations for poliovirus containment that are current at the time of production (18).

Staff involved in the production and quality control of IPV should be shown to be immune to all three types of poliovirus.

The manufacturer should also be able to demonstrate the availability of appropriate means to respond adequately to and manage an inadvertent release of unfinished product containing live viruses.

A.3 Control of source materials

A.3.1 Virus strains and seed lot system

A.3.1.1 Virus strains

Strains of poliovirus used in the production of IPV should be identified by historical records, which should include information on the strains' origin and subsequent manipulation (for example, wild, attenuated or manipulated by recombinant DNA technology). The strain identity should be determined by infectivity tests and immunological methods. In addition, Sabin strains and strains derived by recombinant DNA technology should be identified by nucleotide sequence analysis.

Only virus strains that are approved by the NRA and that yield a vaccine complying with the recommendations set out in these WHO Recommendations should be used.

A.3.1.2 Virus seed lot system

Vaccine production should be based on the virus seed lot system. Unless otherwise justified and authorized, the virus in the final vaccine should not have undergone more passages from the virus master seed lot than were used to prepare the vaccine shown to be satisfactory with respect to safety, efficacy and biocontainment requirements.

If Sabin virus master seeds are supplied by WHO, a virus sub-master seed lot should be prepared by a single passage from the WHO master seed at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate time frame. The virus sub-master seed lot should be characterized to the extent that is necessary to support the development of the virus working seed lot. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.2.2 and Part B of WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (45). The virus sub-master seed lot should be subject to the same tests as a virus master seed lot.

Virus master and working seed lots should be stored in dedicated temperature-monitored freezers at a temperature that ensures stability on storage (for example, $\leq -60^{\circ}\text{C}$).

A.3.1.3 Tests on virus master and working seed lots

Each virus master and working seed lot used for the production of vaccine batches should be subjected to the tests listed in this section and certain tests applicable to single harvests listed in sections A.4.3.1–A.4.3.3 below.

Each virus master and working seed lot should have been derived from materials that comply with the recommendations made in sections A.3.2 and A.3.3 and should be approved by the NRA.

A.3.1.3.1 *Tests in rabbit kidney cell cultures (only for virus master seeds derived from strains which have previously been passaged on primary monkey kidney cells)*

Virus master seeds that have previously been passaged on primary monkey kidney cells should be tested in rabbit kidney cell cultures for the presence of herpes B virus and other viruses. A sample of at least 10 mL of virus seeds should be tested. Serum used in the nutrient medium of the cultures should have been shown to be free from B virus inhibitors using herpes simplex virus as an indicator virus. The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm²/mL of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated and control cultures should be incubated at a temperature of 37 °C and should be observed at appropriate intervals for a period of at least 2 weeks.

For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the test period. The sensitivity of each batch of rabbit kidney cells should be demonstrated by challenge with a validated amount of herpes simplex virus. The challenge test should be approved by the NRA.

A.3.1.3.2 *Tests for adventitious viruses and freedom from detectable SV40 sequences*

A.3.1.3.2.1 **Tests for adventitious viruses in cell cultures**

The virus master and working seed lot used for the production of vaccine batches should be free from adventitious viruses in cell culture assays.

A sample of at least 40 mL of each virus master and working seed lot should be tested for the presence of adventitious agents. The sample should be neutralized against the specific type of poliovirus by a high-titred antiserum.

Monoclonal antibodies may be useful in this test.

If polyclonal antisera are used, the Sabin strains may be used as immunizing antigen for the preparation of the antiserum. However, the immunizing antigen used for the preparation of the antiserum should not be the same as the production seed.

The immunizing antigen should be shown to be free from adventitious agents and should be grown in cell cultures free from adventitious microbial agents that might elicit antibodies that could inhibit the growth of any adventitious agents present in the single harvest.

The sample should be tested in primary *Cercopithecus* sp. kidney cell cultures, or cells that have been demonstrated to be of equal susceptibility to SV40 virus, and in human diploid cells. The tissue cultures should be incubated at 37 °C and observed for 2 weeks. At the end of this observation period, at least one subculture of supernatant fluid should be made in the same tissue culture system. The sample should be inoculated in such a way that the dilution of the supernatant fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm²/mL of supernatant fluid. At least one culture vessel of the cell cultures should remain uninoculated and should serve as a control. The cells inoculated with the supernatant fluid and the uninoculated control cultures should be incubated at 37 °C and observed at appropriate intervals for an additional 2 weeks.

If necessary, serum may be added to the primary cultures at this stage, provided that the serum does not contain SV40 antibody or other inhibitors.

The virus master and working seed lot passes the test if there is no evidence of the presence of adventitious agents. For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific accidental reasons by the end of the observation period.

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: (a) degenerate nucleic acid amplification technique (NAT) for whole virus families, with analysis of the amplicons by hybridization, sequencing or mass spectrometry; (b) NAT with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and (c) high-throughput sequencing. These methods might be used in the future to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and with the approval of the NRA (46).

The theoretical risk of the presence of potential human, simian, bovine or porcine adventitious agents in the seed lots, which may be derived from the use of bovine serum or porcine trypsin, should be assessed. If necessary, viruses such as bovine polyomavirus, porcine parvovirus or porcine circovirus (PCV) may be screened by using specific assays, such as molecular NAT-based assays (46).

A.3.1.3.2.2 *Tests for freedom from detectable SV40 sequences*

The virus master seed lot should be shown to be free from detectable SV40 sequences by using specific validated assays which are approved by the NRA, such as molecular NAT-based assays (46).

DNA of SV40 is widely used as molecular biological reagent, and contamination of polymerase chain reaction (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 second genomic region used for confirmation varies between isolates from different sources, as 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 should be established for the genomic regions used.

A.3.1.3.3 *Additional tests on seeds from Sabin strains and other attenuated strains derived by recombinant DNA technology*

If live-attenuated Sabin strains are used for vaccine production, established master seeds should be used and additional tests should be performed. The virus master seed lots used for the production of vaccine batches should be tested to

monitor virus molecular characteristics – for example, by MAPREC – and should meet the specifications established in agreement with the NRA. Specifications for OPV based on Sabin strains are described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (45). Tests to monitor virus molecular characteristics are described in section A.3.2.4 of those Recommendations (see also section A.4.4.2.7 of this document).

Suitable in vitro tests should be performed on the master seed from attenuated strains derived by recombinant DNA technology. The tests may include full genome characterization by nucleotide sequencing or deep sequencing techniques and demonstration of genetic and phenotypic stability on passage under production conditions. Such tests should be validated for this purpose by using appropriate standards and materials, and should be approved by the NRA.

The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology in in vivo neurovirulence tests should be considered and scientifically justified, in agreement with the NRA.

Any new virus working seed derived from an established master seed, including Sabin strains and other attenuated strains derived by recombinant DNA technology, and at least three consecutive monovalent pools should be analysed in tests to monitor virus molecular characteristics such as MAPREC, when relevant (see section A.4.4.2.7.1).

A.3.2 Cell lines

The general production precautions, as formulated in Good manufacturing practices for biological products (44), should apply to the manufacture of IPV, with the additional requirement 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 a human diploid cell line or in a continuous cell line.

A.3.2.1 Master cell bank (MCB) and working cell bank (WCB)

The use of a cell line for the manufacture of IPV should be based on the cell bank system. The cell seed and cell banks should conform to 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 (46). The MCB should be approved by the NRA. The maximum number of passages (or population doublings) by which the WCB is derived from the MCB and the maximum number of passages of the production cultures should be established by the manufacturer and approved by the NRA.

Additional tests may include, but are not limited to: (a) examination for the presence of retrovirus and tumorigenicity in an animal test system (46); and (b) propagation of the MCB or WCB cells to or beyond the maximum in vitro passage for production.

The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB (47) and is available to manufacturers on application to the Coordinator, Technologies Standards and Norms, Department of Essential Medicines and Health Products (EMP), Health Systems and Innovation (HIS) Cluster, World Health Organization, Geneva, Switzerland.

A.3.2.2 Identity test

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 (46) and should be approved by the NRA.

The WCB should be identified by means of tests such as biochemical tests (for example, isoenzyme analysis), immunological tests, cytogenetic marker tests and DNA fingerprinting or sequencing. The tests should be approved by the NRA.

A.3.3 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacterial, fungal and mycoplasmal contamination – as specified in Part A, sections 5.2 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances – and freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of 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 (46).

Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera if approved by the NRA. As an additional monitor of quality, sera may be examined for freedom from bacteriophage and endotoxin. Gamma irradiation may be used to inactivate potential contaminant viruses, while recognizing that some viruses are relatively resistant to gamma irradiation.

The source(s) of animal components used in the culture medium should be approved by the NRA. The components should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (50). The serum protein concentration should be reduced by rinsing the cell cultures with serum-free medium and/or purification of the virus harvests.

In some countries, control tests are carried out to detect the residual animal serum content in the final vaccine (see section A.6.6).

Human serum should not be used. If human serum albumin is used at any stage of product manufacture, the NRA should be consulted regarding the requirements, as these may differ from country to country. As a minimum, it

should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (51). In addition, human albumin and materials of animal origin should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (50).

Manufacturers are encouraged to explore the possibilities of using serum-free media for the production of IPV.

Penicillin and other beta-lactams should not be used at any stage of manufacture because of their nature as highly sensitizing substances in humans.

Other antibiotics may be used at any stage of manufacture, provided that the quantity present in the final product 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 to be free of cultivatable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate (46). The methods used to ensure this should be approved by the NRA.

In some countries, irradiation is used to inactivate potential contaminant 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 also high enough to reduce virological risk. Therefore, irradiation cannot be considered a sterilizing process (46).

Recombinant trypsin is available and should be considered; however, it should not be assumed to be free from risk of contamination and should be subject to the usual considerations for any reagent of biological origin (46).

The source(s) 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* (50).

A.4 Control of vaccine production

A.4.1 Control 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 million cells, at the concentration and cell passage level

employed for seeding vaccine production cultures, should be used to prepare control cultures.

If bioreactor technology is used, the 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 for use as control cultures for the detection of any adventitious agents.

These control cell cultures should be incubated under conditions as similar as possible to the inoculated cultures for at least 2 weeks and should be tested for the presence of adventitious agents as described below. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific accidental reasons.

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

If not tested immediately, samples should be stored at -60°C or below.

A.4.1.2 Tests for haemadsorbing viruses

At the end of the observation period, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded 7 days and the storage temperature should have been in the range of $2-8^{\circ}\text{C}$. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

Some NRAs require, as an additional test for haemadsorbing viruses, that other types of red blood 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 incubation at $2-8^{\circ}\text{C}$ for 30 min, and again after a further incubation for 30 min at $20-25^{\circ}\text{C}$.

If a test with monkey red blood cells is performed, readings should also be taken after a final incubation for 30 min at $34-37^{\circ}\text{C}$.

In some countries the sensitivity of each new batch of red blood cells is demonstrated by titration against a haemagglutinin antigen before use in the test for haemadsorbing viruses.

A.4.1.3 Tests for other adventitious agents in cell supernatant fluid

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

A second indicator cell line should be used to test an additional 10-mL sample of 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 (46).

The pooled fluid should be inoculated into culture vessels of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm²/mL of pooled fluid. At least one culture vessel of each kind of cell culture should remain uninoculated and should serve as a control.

The inoculated cultures should be incubated at a temperature of 35–37 °C and observed at appropriate intervals for a period of 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 should be tested for the presence of haemadsorbing viruses.

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

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

Some selected viruses may be screened using specific validated assays which are approved by the NRA, such as molecular NAT-based assays (46).

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

A.4.1.4 Identity tests

At the production level, the control cells should be identified by means of tests approved by the NRA.

Suitable methods include, but are not limited to, biochemical tests (for example, isoenzyme analyses), immunological tests, cytogenetic tests (for example, for chromosomal markers) and tests for genetic markers (for example, 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 this examination shows evidence of the presence in a cell culture of any adventitious agent, the culture should not be used for vaccine production.

If animal serum is used for cell cultures before the inoculation of virus, the medium should be removed and replaced with serum-free maintenance medium after the cells have been washed with serum-free medium, if appropriate.

A.4.3 Control of single harvests

After inoculation of the production cells with virus, the culture conditions of inoculated and control cell cultures should be standardized and kept within limits agreed with the NRA.

Samples required for the testing of single harvests should be taken immediately on harvesting.

In some countries, samples are taken after storage and filtration with the agreement of the NRA.

A.4.3.1 Sterility test for bacteria, fungi and mycoplasmas

A volume of at least 10 mL of each virus master and working seed lot (see section A.3.1.3) and single harvest should be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests, as specified in Part A, sections 5.2 (48) and 5.3 (49) of the WHO General requirements for the sterility of biological substances, or by a method approved by the NRA. If the test is performed outside the production facilities, adequate containment procedures (18) should be used according to the virus strain used for production.

NAT alone or in combination with cell culture, with an appropriate detection method, may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and the agreement of the NRA (46).

In some countries this test is performed on the purified monovalent harvest instead of on the single harvest.

A.4.3.2 Virus titration

The virus concentration of each virus master and working seed lot (see section A.3.1.3) and single harvest should be determined by titration of infectious virus

using tissue culture methods. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of Hep-2C or Vero cells in microtitre plates is suitable for this purpose (45). The same cells should be used for virus titrations on monovalent pools throughout the production process.

Information on virus titre will help in selecting single harvests that can be expected to meet potency requirements after inactivation.

In some countries the virus titration may be carried out on the purified, pooled monovalent harvest after demonstration of consistency of production at the stage of the single harvest.

A.4.3.3 Identity test

The poliovirus in each virus master and working seed lot (see section A.3.1.3) and single harvest should be tested for serotype and strain identity by neutralization with specific antiserum or molecular methods approved by the NRA.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

The strain identity of each of the three serotypes may be determined by standard or deep nucleotide sequence analysis or by a suitable molecular technique.

In some countries this test is performed on the purified monovalent harvest instead of on the single harvest.

A.4.4 Control of purified monovalent pools

A.4.4.1 Purification of monovalent pools

Each monovalent pool of virus, consisting of several single harvests of the same serotype, should be purified before inactivation. Removal of host cell protein should be assessed during process validation (46).

An acceptable method is to clarify the virus suspension by filtration, to concentrate the virus by ultrafiltration and, thereafter, to collect the virus peak after passing it through a gel-filtration column. Further purification is achieved by passing the virus through an ion-exchange column. Other purification procedures resulting in acceptable release criteria may be used – for example, passing the preparation through an immobilized DNA-ase column.

A.4.4.2 Tests on purified monovalent pools

A.4.4.2.1 *Residual cellular DNA*

For viruses grown in continuous cells, the purified monovalent pools should be tested for residual cellular DNA (46). The purification process should be shown by calculation to reduce consistently the level of cellular DNA to less than 10 ng per human dose.

In some countries, IPV produced in mammalian cells is required to contain less than 100 pg DNA per human dose.

This test may be omitted from routine testing, with the agreement of the NRA, if the manufacturing process is validated to achieve this specification (46).

If assessed, the size distribution of the DNA may be considered as a characterization test, taking into account the amount of DNA detectable using appropriate methods, as approved by the NRA (46).

In some countries this test is performed on the trivalent bulk following validation, and with the agreement of the NRA.

A.4.4.2.2 *Virus titration*

The virus concentration of each purified monovalent pool should be determined by titration of infectious virus using tissue culture methods. This titration should be carried out in not more than 10-fold dilution steps using 10 cultures per dilution, or by any other arrangement yielding equal precision.

The use of Hep-2C or Vero cells in microtitre plates is suitable for this purpose (45).

Information on virus titre will help in selecting purified monovalent pools that can be expected to meet potency requirements following inactivation.

A.4.4.2.3 *Identity test*

The poliovirus in each purified monovalent pool should be tested for serotype and strain identity by neutralization, using specific antiserum or molecular methods approved by the NRA.

Care should be taken to ensure that the sera used are monospecific by titrating them against homotypic and heterotypic viruses of known virus titre. Monoclonal antibodies may be useful in this test.

The strain identity of each of the three serotypes may be determined by nucleotide sequence analysis or a suitable molecular technique.

A.4.4.2.4 *D-antigen content*

The D-antigen content of each purified monovalent pool should be determined by use of a validated immunochemical method and should be calculated by using a reference vaccine calibrated against the WHO International Standard (see section A.1.3).

A.4.4.2.5 *Protein content*

The purified monovalent pool should be shown to contain no more than 0.1 µg of protein per D-antigen unit of poliovirus or should be within the limits approved for that particular product by the NRA.

A.4.4.2.6 *Filtration before inactivation*

Each purified monovalent pool should be filtered before inactivation.

Satisfactory results have been reported with several filter types but a final filtration using a 0.22-µm filter should be used.

Filters containing asbestos should not be used.

Inactivation should be initiated as soon as possible and in any case not later than 72 h after filtration.

It is preferable to start inactivation within 24 h of filtration. Since the purpose of the filtration step is to remove particulate matter and other interfering substances that may diminish the effectiveness of the inactivation process, and since aggregates tend to increase on standing after filtration, efforts should be made to keep within this time limit.

A sample of the filtered purified monovalent pool should be retained and its virus titre determined as described in section A.4.4.2.2.

The main purpose of determining the titre of filtered virus pools destined for inactivation is to provide the starting titre to monitor the kinetics of inactivation.

A.4.4.2.7 *Additional tests for purified monovalent pools produced from Sabin vaccine seeds or from other attenuated seeds derived by recombinant DNA technology*

Production conditions should be validated by the full range of tests, including in vivo and in vitro testing of the master and working seed and successive monovalent bulks (the number to be approved by the NRA), to ensure that the attenuated phenotype of the Sabin strains in monovalent pools is maintained. Subsequently, a limited range of tests, such as MAPREC, may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency.

The number of pools of each type tested each year should be justified and should be agreed with the NRA. Furthermore, it is important that, at intervals to be agreed with the NRA, pools should be tested with the full range of tests to ensure that production conditions remain satisfactory.

The use of the rct40 test is discouraged as it requires the use of WPV controls.

In vitro tests to monitor virus molecular characteristics (consistency) and in vivo neurovirulence tests which could be used for this purpose are described in sections A.4.4.2.7.1 and A.4.4.2.7.2, respectively.

Suitable in vitro tests should be performed on purified monovalent pools derived from attenuated strains derived by recombinant DNA technology. Tests may include full genome characterization by nucleotide sequencing or deep sequencing techniques. Such tests should be validated for this purpose by the use of appropriate standards and materials, and should be approved by the NRA.

An in vitro test (described above) for the molecular consistency of production may be performed on single harvests before preparing the purified monovalent pool. If performed, the acceptance/rejection criteria should be updated periodically and should be approved by the NRA.

A.4.4.2.7.1 *Tests to monitor virus molecular characteristics (consistency)*

In vitro tests such as MAPREC, which are used to determine the molecular consistency of production of monovalent pools, should meet the specifications for the test used (45).

Results from MAPREC tests should be expressed as ratios relative to the relevant 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 the light of production and testing experience.

For type 3 (472-C) a purified monovalent pool should be rejected if the level of mutations is more than 1.0% when normalized against the International Standard. The limits for types 1 and 2 should be approved by the NRA.

Levels of mutations obtained by manufacturers who have implemented the test for types 1 and 2 virus have been less than 2.0% for type-1 Sabin (for the sum of both mutations 480-A, 525-C) and 1.5% for type-2 Sabin (481-G) (45, 52).

The test(s) used should be approved by the NRA. The MAPREC assay provides a sensitive and quantitative measure of consistency for monovalent pools derived from Sabin viruses.

A.4.4.2.7.2 **Neurovirulence tests**

Appropriate in vivo tests which may be used to evaluate the phenotype of virus in purified monovalent pools produced from the Sabin vaccine strains are described in section A.4.4.7.2 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (45).

For other attenuated strains derived by recombinant DNA technology, the need for testing purified monovalent pools by in vivo neurovirulence tests should be considered and should be scientifically justified with the agreement of the NRA.

A.4.5 **Control of inactivated purified monovalent pools**

A.4.5.1 **Inactivation procedure**

The virus in the filtered purified monovalent pools should be inactivated by a method approved by the NRA. Prior to inactivation, the concentration of the filtered monovalent pool, based on viral titre or D-antigen content, should be adjusted to the acceptable range established during the process validation. The acceptable range should be established during validation studies.

Most manufacturers during the past 40–50 years have used formaldehyde as the method for inactivation.

The method of inactivation should be shown to give consistent inactivation for the production of acceptable vaccine. The progress of inactivation should be monitored by suitably spaced determinations of virus titres. The inactivation period should exceed the time taken to reduce the titre of live virus to undetectable amounts by a factor of at least 2.

A second filtration should be made during the process of inactivation.

This step is made after the virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

The kinetics of viral inactivation should be established by each manufacturer and should be approved by the NRA. Adequate data on viral inactivation kinetics should be obtained and consistency of the inactivation process should be monitored. For this purpose, the virus titre and D-antigen content of each filtered purified monovalent pool before, during and at the end of inactivation should also be determined, as specified in sections A.4.4.2.2 and A.4.4.2.4, respectively.

A record of consistency (effective inactivation and kinetic of inactivation) should be established by the production of at least five consecutive lots and, if broken, a root-cause analysis should be performed and a further five consecutive filtered purified monovalent pools should be prepared and shown to be satisfactory for establishing this record.

A.4.5.2 Test for effective inactivation

Two samples should be taken of a volume equivalent to at least 1500 human doses of each inactivated purified monovalent pool. One sample should be taken at the end of the inactivation period and the other not later than three quarters of the way through this period. After removal or neutralization of the inactivating agent, the samples should be tested for the absence of infective poliovirus by inoculation into tissue cultures. Kidney cells from some monkey species, such as those of the genera *Macaca*, *Cercopithecus* and *Papio* sp., appear to be more sensitive than others. If other tissue culture systems, including continuous cell lines (for example, L20B), are used, they should have been shown to possess at least the same sensitivity to poliovirus as those specified above by inoculating with partially formalin-inactivated virus (as opposed to infectious, untreated virus) as formalin treatment changes the biological properties of poliovirus (see below). When primary monkey kidney cells are used for this test, the two samples should be inoculated into culture vessels of tissue cultures derived from different batches of cells.

The dilution of the sample in the nutrient fluid should not exceed 1 in 4 and the area of the cell sheet should be at least 3 cm²/mL of sample. One or more culture vessels of each batch of cultures should be set aside to serve as uninoculated control culture vessels with the same medium.

The formaldehyde in samples of vaccine for tissue culture tests is generally neutralized at the time of sampling by the addition of bisulfite. Usually, the samples are subsequently dialysed.

It is possible to conduct tissue culture tests on non-dialysed material. However, this is often found to be toxic to cells, even with a dilution of 1 in 4. If in such tests nonspecific degeneration of cells occurs, or if the sensitivity of the tissue culture system is reduced, the test should be repeated on dialysed material. The virus D-antigen content after dialysis should be determined to ascertain whether any D-antigen was lost during the dialysis process.

The tissue culture vessels should be observed for at least 3 weeks. Not less than two subcultures should be made from each original culture vessel. The first subculture from each culture vessel should be made prior to the first medium change, and the second subculture should be made at the end of the observation period. The subcultures should be observed for at least 2 weeks.

If infectious poliovirus is detected in samples taken at the end of inactivation, or in the samples taken no later than three quarters through the inactivation process, the inactivated purified monovalent pool should not be used for further processing. The isolation of live poliovirus from an inactivated purified monovalent pool must be regarded as a break in the manufacturing

consistency record and a production process review and revalidation should be undertaken.

If primary monkey kidney cells are used in this test, they may contain adventitious agents that could interfere with the test result. It is important to demonstrate that each test retains sensitivity to detect partially inactivated polioviruses. At the end of the observation period, the cell culture used for the detection of residual live virus should be challenged with a validated amount of live Sabin virus of the same type as that of the inactivated purified monovalent pool. The details of the challenge procedure should be approved by the NRA.

If continuous cell lines are used in this test, the ability to detect infectious virus should be checked concurrently for each test by introducing a positive control at the beginning of each test. Positive control flasks should be inoculated with a low quantity of virus close to the detection limit of the method. Alternatively, if no positive control is used, a challenge test should be performed as described above for primary monkey kidney cells.

The problem of detecting residual active poliovirus in an inactivated vaccine is not the same as that of measuring infective virus in untreated suspensions. Poliovirus that has been exposed to the action of formaldehyde without becoming inactivated has been shown to require a much longer period to produce cytopathic changes than untreated virus. For this reason it is desirable that tissue cultures in tests for the presence of residual active virus are observed for as long a time as is technically possible. A satisfactory tissue culture system for this purpose depends, therefore, not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

The serum added to the nutrient fluid should be tested for inhibitors to poliovirus at serum concentrations up to 50%. Only serum free from inhibitors to all three types of poliovirus should be used.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that early changes of fluid may result in unadsorbed virus being removed and the validity of the test would thus be impaired. Therefore, the fluid should be changed no earlier than 5–7 days after inoculation.

A.4.5.3 Sterility test for bacteria and fungi

Each inactivated purified monovalent pool should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.4.5.4 D-antigen content

The D-antigen content of each inactivated purified monovalent pool should be determined by use of a validated immunochemical method and should be

calculated by use of a reference vaccine calibrated against the WHO International Standard (see section A.1.3). The results obtained should be within the required limits established by the NRA.

A.4.6 **Control of trivalent bulk**

Only those inactivated purified monovalent pools that have been shown to be satisfactory should be blended to form a trivalent bulk.

A.4.6.1 **Test for absence of infective poliovirus**

A sample of at least 1500 mL or, if purified and concentrated vaccine is prepared, the equivalent of at least 1500 human doses of each trivalent bulk should be tested in cell cultures for the absence of infective poliovirus by the procedure described in section A.4.5.2. If infective poliovirus is isolated, this trivalent bulk, or product derived from it, should not be used.

In some countries this test may be omitted on the trivalent bulk if, following a review of manufacturing records, the test for inactivation has been performed with satisfactory results on the inactivated purified monovalent pool, subject to approval by the NRA.

When a trivalent bulk is supplied by one manufacturer to another, the validation of inactivation may rely on the inactivation tests performed by the bulk supplier.

A.4.6.2 **Sterility test for bacteria and fungi**

The trivalent bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.4.6.3 **Residual formaldehyde**

The content of free residual formaldehyde in the trivalent bulk should be determined by a method approved by the NRA. The limits should be approved by the NRA.

A.4.6.4 **D-antigen content**

The D-antigen content of each trivalent bulk should be determined by use of a validated immunochemical method and should be calculated by use of a reference vaccine calibrated against the WHO International Standard (see section A.1.3). The results obtained should be within the required limits established by the NRA.

A.4.7 **Control of final bulk**

Preservatives, excipients or other substances that might be added to or combined with the trivalent bulk to form the final bulk should have been shown, to the satisfaction of the NRA, to have no deleterious effect on the immunizing potency and the safety profile of the poliovirus antigens. Preservative efficacy should be demonstrated during product development using a method approved by the NRA.

The operations necessary for preparing the final bulk from trivalent bulk should be conducted in such a manner as to avoid contamination of the product. In preparing the final vaccine bulk, any substances such as diluents, stabilizers or adjuvants that are added to the product should have been shown, to the satisfaction of the NRA, not to impair the safety and efficacy of the vaccine in the concentration used. Until the final bulk is filled into containers, the final vaccine bulk suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity.

A.4.7.1 **Sterility test for bacteria and fungi**

The final bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.4.7.2 **Potency tests**

Each final bulk should be tested in an *in vivo* assay for immunogenicity by tests approved by the NRA. An *in vivo* potency assay in rats has been standardized and shown to be a suitable test for IPV. Product-specific reference preparations may be used in these tests (see Appendix 2).

The D-antigen content of each final bulk should be determined using a validated immunochemical method and calculated using a reference vaccine calibrated against the WHO International Standard (see section A.1.3). The results obtained should be within the required limits established by the NRA. This test may be omitted on the final bulk if conducted on the final lot.

When consistency of production has been established on a suitable number of consecutive final bulks, the *in vivo* assay may be omitted with the agreement of the NRA. This can occur once it has been demonstrated that the acceptance criteria for the D-antigen determination are such that the *in vitro* test yields a comparable result to the *in vivo* assay in terms of acceptance or rejection of a batch. This demonstration must include testing of subpotent batches, produced experimentally if necessary by heat treatment or other means of diminishing the immunogenic activity.

Where there is a change in the manufacturing process of the antigens or in their formulation that might impact on the quality and immunogenicity of the

vaccine, the *in vivo* test should be performed to demonstrate the comparability of the new manufacturing process to the established process. If the process change affects the *in vivo* test, the need for revalidation should be considered and clinical data may be required for the approval by the NRA.

The *in vitro* assay that has been found most suitable for measuring the antigen content is the D-antigen ELISA. Although this assay is widely used, particular attention is required for its standardization. Some NRAs accept the use of polyclonal antisera, whereas others accept the use of monoclonal antibodies in the test. The use of different antibodies may give different results. The D-antigen specificity of the antibodies should be demonstrated. Whichever types of antisera are used, the validation studies should show that the assay can determine consistency of production. For a D-antigen ELISA to be valid, it should comply with specified criteria of linearity and parallelism. The effect of a change in the method of calculation of the D-antigen content on registered specifications should also be taken into account.

Other validated tests such as the multiplex antibody test or plasmon resonance technology (53, 54) may be used subject to the approval of the NRA.

If the use of an adjuvant in the final bulk interferes with the assay, a desorption or treatment step may be necessary before performing the D-antigen ELISA.

If the final bulk is formulated with poliovirus trivalent bulk and with other antigens into a combination vaccine, the suitability of performing the D-antigen ELISA on the final bulk will have to be determined. If the D-antigen ELISA is not suitable for a particular combination, an *in vivo* assay should be used.

The potency of the final bulk for each virus type should be approved by the NRA.

A.4.7.3 Preservative content

If preservative is added, its content in the final bulk should be determined by a method approved by the NRA. The preservative used and content permitted should be approved by the NRA.

As the use of thiomersal can result in loss of D-antigen content, 2-Phenoxyethanol has been the only preservative used by IPV manufacturers during the past 50 years.

A.4.7.4 Adjuvant (if applicable)

Each final vaccine bulk should be assayed for adjuvant content. This test may be omitted if it is performed on the final lot. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.

A.5 **Filling and containers**

The requirements concerning filling and containers given in Good manufacturing practices for biological products (44) should apply to vaccine filled in the final form. Single- and multiple-dose containers may be used.

A.6 **Control tests on the final lot**

Samples should be taken from each final lot for the tests described in the following sections. The following tests should be performed on each final lot of vaccine (that is, in the final containers). Unless otherwise justified and authorized, the tests should be performed on labelled containers 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.

Differences in the results of tests on novel IPVs – that is, deviations/ excursions from the permissible limits – may be acceptable if justified and 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 and recorded for each relevant abnormality. A limit should be established for the percentage of rejection.

A.6.1.1 **Appearance**

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

A.6.2 **Identity test**

An identity test should be performed on at least one labelled container from each final lot by an appropriate method. The potency test described in section A.6.5 may serve as the identity test.

A.6.3 **Sterility test for bacteria and fungi**

Each final lot should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (48), or by methods approved by the NRA.

A.6.4 **General safety test (innocuity)**

The need to test the final lots of the vaccine for unexpected toxicity (also known as abnormal toxicity) should be agreed with 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, and when reliable good manufacturing practices are in place.

A.6.5 Potency test

The D-antigen content of each final lot should be determined using a validated immunochemical method (see sections A.4.5.4 and A.4.7.2) and calculated using a reference vaccine calibrated against the WHO International Standard (see section A.1.3).

In some countries, this test is omitted provided that the determination of the D-antigen content has been carried out with satisfactory results on the final bulk product and provided that a validation has been performed to demonstrate that there is no loss of potency between the final bulk product and the final lot, subject to approval by the NRA.

If the use of an adjuvant in the final bulk interferes with the assay, a desorption or treatment step may be necessary before performing the D-antigen ELISA. If treatment/desorption is not possible, the interference of the adjuvant should be documented and an *in vivo* assay should be performed (see section A.4.7.2 and Appendix 2).

In general, wIPVs formulated to contain 40, 8 and 32 D-antigen units or more per human dose for types 1, 2 and 3, respectively, are effective (55). Vaccines with lower D-antigen contents may be acceptable if supported by clinical data. Vaccines in which adjuvants are used, or vaccines produced from other seed viruses (for example, Sabin viruses), may also be licensed with a different antigenic composition if supported by clinical data.

If the final bulk is formulated from a trivalent bulk and other antigens into a combination vaccine, the suitability of performing the D-antigen ELISA on the final lot will have to be determined. If the D-antigen ELISA is not suitable for a particular combination, an *in vivo* assay such as that described in Appendix 2 should be used.

The potency of the vaccines for each virus type should be approved by the NRA.

A.6.6 Protein content

Poliomyelitis vaccine (inactivated) should not contain more than 10 µg of protein per human dose. This test may be omitted for routine lot release once consistency of production has been established to the satisfaction of the NRA.

If animal serum is used for the growth of cell cultures, the serum protein concentration (bovine serum albumin) in the final lot should be no more than 50 ng per human dose. The test for bovine serum albumin may be omitted if performed on the trivalent or final bulk, subject to approval by the NRA.

A.6.7 Preservative content

Where appropriate, the preservative content of each final lot should be determined by a method approved by the NRA. The method used and content permitted should be approved by the NRA. This test may be omitted if conducted on the final bulk.

A.6.8 Endotoxin content

The endotoxin content of each final lot should be determined by a method approved by the NRA. Levels should be consistent with levels found to be acceptable in vaccine lots used in pre-licensure clinical trials and approved by the NRA.

A.6.9 Test for residual formaldehyde

The content of free residual formaldehyde in each final lot should be determined by a method approved by the NRA. The limit should be approved by the NRA. This test may be omitted if performed on the trivalent bulk or on the final bulk.

A.6.10 Test for pH

The pH of each final lot should be determined and should be within limits approved by the NRA.

A.6.11 Adjuvant and degree of adsorption (if applicable)

If an adjuvant is used in the formulation, each final lot should be assayed for adjuvant content. Where aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose. This test may be omitted on the final lot if performed on the final bulk.

The degree of adsorption of the antigen to the aluminium compounds (aluminium hydroxide or hydrated aluminium phosphate) in each final lot should be assessed.

A.6.12 Residual antibiotics (if applicable)

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

A.7 Records

The requirements given in Good manufacturing practices for biological products (44) should apply.

A.8 Retained samples

The requirements given in Good manufacturing practices for biological products (44) should apply.

A.9 Labelling

The requirements given in Good manufacturing practices for biological products (44) should apply, and additionally the label on the container or package should include the following information:

- the designation(s) of the strain(s) of poliovirus contained in the vaccine
- the cell substrate used for the preparation of vaccine
- the D-antigen content of each poliovirus type
- the method and inactivating agent used to inactivate the virus
- the nature and amount of any stabilizer and preservative present in the vaccine
- the nature and amount of adjuvant, if applicable.

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

A.10 Distribution and shipping

The requirements given in Good manufacturing practices for biological products (44) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (56).

A.11 Stability, storage and expiry date**A.11.1 Stability testing**

Adequate stability studies form an essential part of vaccine development. Current guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (57). Stability testing should be performed at different stages of production when intermediate product is stored, namely on single harvests, inactivated purified monovalent pool, trivalent bulk, final bulk and final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. During vaccine production a shelf-life should be assigned to all in-process materials – particularly intermediates such as single harvests, inactivated purified monovalent pool, trivalent bulk and final bulk.

The stability of the vaccine in its final containers, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA. As a guide, containers from at least three consecutive final lots, and derived from different monovalent pools and different trivalent bulks, may be tested.

Where manufacturing involves only formulation of the final bulk from trivalent bulks supplied by another manufacturing establishment and the filling of final containers, stability data on the trivalent bulks should be generated if the storage container or storage conditions are changed, and the shelf-life until use should be established by the manufacturer performing the final fill.

The formulation of the vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with the NRA. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine the stability profile (57). Data should be provided to the NRA in accordance with local regulatory requirements.

The final stability testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection and sharing of stability data, and criteria for rejecting vaccine(s).

A.11.2 **Storage conditions**

Poliomyelitis vaccine (inactivated) should be stored at all times at a temperature between 2 °C and 8 °C. For novel vaccines, appropriate storage conditions should be validated and 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 be based on the date of blending of the final bulk, the date of filling or the date of the first valid potency test on the final lot, which should be performed in an assay as described in Appendix 2.

Where an in vivo potency test is used, the date of the potency test is the date on which the test animals were inoculated with the final bulk.

Part B. Nonclinical evaluation of poliomyelitis vaccines (inactivated)

The nonclinical evaluation of candidate IPVs should be based on the WHO guidelines on nonclinical evaluation of vaccines (8). The following issues relate specifically to new IPV candidates and should also be referred to when a significant change is made in the manufacturing process or vaccine formulation of a licensed IPV.

B.1 **Characterization of poliovirus seed lots derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology)**

The virus master and working seed lots derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology) that are used to manufacture a candidate IPV should be extensively characterized, as described in section A.3.1. Ideally, the characterization studies should be performed on seed lots used to prepare the vaccine batches tested in preclinical and clinical studies.

When attenuated poliovirus strains derived by recombinant DNA technology are used to prepare a candidate IPV, the mutations responsible for attenuation should be identified along with the mutations that can revert to a partial or full virulence phenotype. The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology with *in vivo* neurovirulence tests should be considered, and the decision whether to test or not should be scientifically justified and approved by the NRA. In addition, the genetic stability of the strains derived by recombinant DNA technology should be confirmed at the passage level (or beyond) used to prepare the vaccine. Efforts should also be made to develop an *in vitro* test to detect reversion to a partially or fully virulent virus.

B.2 **Antigenic profile**

The available evidence suggests that there may be significant differences in the antigenic composition of various IPV products developed independently (58, 59), particularly when comparing sIPV to wIPV. It is likely that antigenic profiles of IPV are influenced by the virus strains, cell substrates and process parameters used in manufacture. The antigenic structure of a candidate IPV should ideally be established using monoclonal antibodies (59, 60) of known specificity at the early stage of product development and should be used as a characterization tool for investigating vaccine stability and demonstrating manufacturing consistency during product development.

B.3 **D-antigen content of IPV derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology)**

The type-specific antigen content of current licensed wIPV is measured using various ELISA procedures (60) and is reported as D-antigen units relative to a reference preparation traceable to the International Standard. When attenuated strains (for example, Sabin strains and strains derived by recombinant DNA

technology) are used to prepare the candidate IPV, an in-house ELISA should be developed and implemented to determine type-specific D-antigen content. An in-house reference standard should be established at the early stage of product development and should be calibrated against the International Standard. A monitoring programme should be put in place to ensure the stability of the in-house reference standard and the comparability of its subsequent replacement. In addition, the ratio between virus titre (per millilitre) and D-antigen content (per millilitre) of purified monovalent pools prior to inactivation should also be established for each poliovirus type during product development and should be monitored during commercial production. This provides further assurance that the D-antigen content of commercial lots, throughout the product life-cycle, is comparable to lots shown to be safe and immunogenic in clinical studies.

Most licensed wIPV products have been formulated to contain 40, 8 and 32 D-antigen units per human dose. However, the D-antigen unit is not well defined with respect to various poliovirus strains used in manufacture and is known to be influenced by the specificity of the antibodies used as ELISA reagents. Therefore, it is not possible to directly compare the D-antigen content of various IPVs (for example, sIPV versus wIPV) measured using different monoclonal or polyclonal antibody-based ELISA procedures (61). It is recognized that IPV derived from attenuated strains or adjuvanted IPV may require different D-antigen content to induce adequate immune responses in humans.

B.4 Evaluation of immunogenicity in animal models

Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in suitable animal models (for example, rats). Proof-of-concept nonclinical studies should include the comparison of immunogenicity between a candidate IPV and a licensed IPV based on type-specific serum neutralizing antibody titres against both Sabin and wild-type strains. These studies may also assist in the selection of D-antigen content to be tested in the dose-finding studies in humans. However, it is important to note that immunogenicity data in animals do not reliably predict the antigen content that might be appropriate for inclusion as a single human dose in the final vaccine formulation. An assay using transgenic mice may be performed to compare the immune response and protection against virulent challenge induced by a candidate IPV to that induced by a licensed IPV (31, 32). In vivo tests are also important tools to be used as characterization tests to demonstrate comparable manufacturing processes when major changes are introduced.

When an adjuvant is included in the formulation, manufacturers should provide a rationale and immunogenicity data to support the use of an adjuvant in the vaccine (62).

B.5 Nonclinical safety studies

Decisions concerning the need for nonclinical safety studies, types of study and study design should be based on the general guidance provided, and special considerations addressed, in the WHO guidelines on nonclinical evaluation of vaccines (8) – for example, use of a delivery device or alternative administration routes such as intradermal. If a candidate IPV is formulated with a novel adjuvant or excipient (for example, stabilizer) then nonclinical safety studies should be conducted following the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (62).

Part C. Clinical evaluation of poliomyelitis vaccines (inactivated)

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (63) and Guidelines on clinical evaluation of vaccines: regulatory expectations (9).

A number of issues specific to the clinical evaluation of IPV are discussed in the following sections, which are applicable to IPV derived from wild-type strains as well as attenuated strains (for example, Sabin strains and strains derived by recombinant DNA technology).

C.1 General considerations

The establishment of the GPEI following the 1998 World Health Assembly resolution led to a dramatic decrease in poliomyelitis cases globally (11). Consequently, clinical efficacy studies to support the licensure of a candidate IPV are no longer feasible, and clinical evaluation should be based upon comparative assessment of the safety and immunogenicity of a candidate vaccine against an already licensed vaccine (comparator vaccine). The assessment of seroconversion should be based on the elicitation of serum neutralizing antibodies, which have been established as the basis of protection (64). The licensure of a candidate IPV should be based on a clear demonstration of non-inferiority in terms of immunogenicity when compared to a comparator vaccine.

C.2 Immunogenicity studies

C.2.1 Assessment of the immune response

A serum neutralizing antibody titre of ≥ 8 is considered to be a marker of clinical protection against poliomyelitis (65). The demonstration of an immune response to IPV vaccination should be based upon the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for polio antigen is defined as:

- for subjects seronegative at the pre-vaccination time point, post-vaccination antibody titres of ≥ 8 ;
- for subjects seropositive at the pre-vaccination time point, a ≥ 4 -fold rise in antibody titres post-vaccination. In the event that the pre-vaccination titre is due to maternal antibodies, a 4-fold rise above the expected titre of maternal antibodies based on the pre-vaccination titre declining with a half-life of 28 days indicates seroconversion.

It is recommended that the assay used to assess serum neutralizing antibodies should be standardized, as described in the WHO *Manual for the virological investigation of polio* (66), particularly with respect to the use of appropriate cell lines, International Standards of anti-poliovirus sera and other important reagents. The level of neutralizing antibody present in a serum sample is expressed as a titre, which is the reciprocal of the highest serum dilution that inhibits the viral cytopathic effect in 50% of cell cultures.

For the evaluation of IPV derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology), serum neutralizing antibody titres against both Sabin and wild-type poliovirus should be determined in order to ensure that the conclusions of clinical studies are applicable to both types of strains. In view of the antigenic differences between the wild-type poliovirus strains, it may be useful to assess the neutralizing antibody titres using both recent wild-type isolates and the conventional strains in a subset of study subjects, if relevant.

The presence of neutralizing antibody against polioviruses is considered a reliable correlate of protection against poliomyelitis. However, immunity induced by one serotype does not provide protection against the other two serotypes.

C.2.2 Comparative immunogenicity studies

A candidate IPV should be directly compared with at least one well established and licensed IPV in prospective controlled studies.

In the event that no IPV products can be used in the country where the clinical studies are conducted due to country-specific regulations, the use of OPV as a comparator may be acceptable to the NRA provided that a high seroconversion rate of the comparator OPV has been well established in the study region. However, the use of OPV in clinical studies should be in compliance with the *Polio Eradication and Endgame Strategic Plan 2013–2018* (15), and it must be noted that the use of tOPV is time limited as bOPV will be introduced and will be followed by the complete cessation of all OPV vaccination.

Non-inferiority studies to evaluate immunogenicity after completion of the primary vaccination series in the target population (for example, naive infants)

are required for regulatory approval of a candidate IPV. Persistence of the serum neutralizing antibodies after the primary series should also be investigated to recommend whether and when a booster dose is required. However, data concerning long-term antibody persistence might not be available prior to regulatory approval. The waning of antibodies over time is inevitable and should not be interpreted as indicating the need for a booster dose per se, as available data suggest that persistent immune memory may be sufficient to protect against poliomyelitis (67, 68).

C.2.3 Study population and region

In general, the first clinical study (Phase I) of a candidate IPV should be performed in healthy adults to assess vaccine safety. Due to the widespread use of IPV and OPV, the immunogenicity of a candidate IPV can be reliably evaluated only in a naive target population, such as infants.

Exposure of study subjects to circulating wild-type or OPV-derived poliovirus may enhance the immune response induced by IPV and, in turn, may affect study conclusions. Therefore, clinical trials to evaluate the immunogenicity of a candidate IPV should ideally be performed in regions where IPV is used exclusively. In the event that a clinical trial is conducted in areas where OPV is used routinely, special measures should be taken to minimize the potential exposure of study participants to live poliovirus (for example, undertaking clinical studies on a new IPV in a region where OPV is used on a campaign basis).

C.2.4 Endpoints and analyses

The primary study analysis should be based on the rate of seroconversion (as described in section C.2.1) against both Sabin and wild-type strains measured at approximately 4 weeks following completion of the primary infant immunization. The primary study objectives should be based on the demonstration of the non-inferiority of the seroconversion rates achieved with the candidate IPV versus the comparator vaccine.

The predefined clinical margins of non-inferiority should be justified, and the calculations of the proposed sample size required should be clearly explained in the study protocol. Further guidance on demonstrating non-inferiority trials is described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (9).

Comparison of geometric mean titres (GMTs) and reverse cumulative distributions of individual titres against both Sabin and wild-type poliovirus at 4 weeks post-primary should also be performed as a secondary analysis. While it may be that the GMT(s) for one or more poliovirus types induced by the candidate IPV derived from attenuated strains is lower than that induced by the comparator, it is not clear if a lower GMT at 4 weeks post-primary affects long-

term antibody persistence. Consequently, any significant differences in observed GMT (for example, not meeting pre-specified criteria) should be carefully considered by the NRA and a decision should be supported by additional studies of antibody persistence (as described in section C.2.2) and by a commitment to post-marketing studies (described in section C.5).

The minimum D-antigen content required for the candidate vaccine at the end of its shelf-life should be based on the D-antigen content of clinical lots shown to induce acceptable immune responses in clinical studies (for example, lots used in the dose-finding study).

C.2.5 Immunization schedule

Different immunization schedules are used for licensed wIPV in various regions and countries. It is common for IPV to be administered according to the same schedule as diphtheria, tetanus and pertussis (DTP) vaccines in order to achieve a high compliance rate. Clinical trial data have shown that the immune response induced by licensed wIPV varies according to the immunization schedule used. In general, longer intervals in the primary immunization series (for example, 2, 4 and 6 months) induce higher neutralizing antibody titres and a higher seroconversion rate (69, 70). An immunization schedule should be defined for the targeted country or region, wherever possible, and dose-finding and non-inferiority studies for a candidate IPV conducted according to this immunization schedule. However, it is not feasible to study a candidate vaccine using every possible schedule in all target regions. Manufacturers should justify the relevance of the clinical data provided to each country in which approval is sought and should discuss the basis for extrapolation of the findings. For example, satisfactory immune responses using a schedule with a short interval between immunizations (for example, 2, 3 and 4 months) would support an expectation that satisfactory immune responses would also be observed using a schedule with a longer interval (for example, 2, 4 and 6 months). However, the local and systemic reactogenicity associated with a candidate IPV may also differ between schedules within a specific population so there is still a need to collect safety data, prior to regulatory approval, for the proposed schedules (for example, 2, 4 and 6 months).

The use of an IPV that is prepared from attenuated poliovirus strains or that contains a fractional antigen dose in a sequential IPV-OPV immunization schedule should be supported by clinical studies to ensure adequate levels of serum neutralizing antibodies against both wild-type and Sabin poliovirus.

C.3 Concomitant administration with other vaccines

IPV is commonly co-administered with other infant and toddler vaccines. Consequently, it is essential to evaluate the immune responses to a candidate IPV as well as to all other antigens co-administered in all the co-administration

situations claimed. Due to the large number of licensed vaccines that may need to be co-administered with IPV in infants and toddlers using a variety of schedules, it is not feasible for manufacturers to study every possible combination. The data on the effects of co-administration that are available at the time of initial licensure may be limited and should be expanded in post-approval studies. If study results indicate that immune responses are lower on co-administration with other vaccine(s), the NRA will need to consider the potential clinical consequences on a case-by-case basis.

C.4 Pre-licensure safety data

The general approach to safety assessment of a candidate IPV during pre-licensure clinical studies should be in accordance with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (9). The safety profile of a candidate IPV derived from attenuated strains is expected to be very similar to that of current licensed wIPV, which is very well tolerated. The NRA may decide that large safety studies are not required. However, if a candidate IPV includes novel adjuvants and/or excipients, or is administered using an alternative route and/or delivery device, a safety database similar in size to that requested for any new vaccine entity might be needed. This should be discussed and approved by the NRA on a case-by-case basis. In addition, it is likely that adverse events at the injection site are more frequent if a candidate IPV contains an adjuvant. This may be acceptable if the incidence of adverse events is comparable to that observed for other licensed adjuvanted vaccines and the benefit clearly outweighs the risks.

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

C.5 Post-marketing studies and surveillance

Post-marketing surveillance should be undertaken during the initial post-approval years in collaboration with the NRA. Manufacturers and health authorities should work in collaboration with the global polio surveillance laboratory network to monitor new vaccines once they are introduced in immunization programmes. Enhanced safety surveillance is particularly important for vaccines which include novel adjuvants and/or excipients. Due to the possibility that the sIPV may induce a lower GMT for one or more poliovirus types, the persistence of antibody and the need for a booster dose should be studied in the post-marketing period.

The total duration of enhanced surveillance should be regularly reviewed by the NRA. If particular issues arise during pre-licensure studies or during

post-licensure safety surveillance, it may be necessary to conduct specific post-licensure safety studies.

Part D. Recommendations for NRAs

D.1 General recommendations

The general recommendations for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (71) and Guidelines for independent lot release of vaccines by regulatory authorities (72) should apply. These recommendations specify that no new biological substance should be released until consistency in batch manufacturing and quality has been demonstrated.

The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of poliomyelitis vaccine (inactivated), should be discussed with and approved by the NRA.

For control purposes, the relevant International Standards currently in force should be obtained for the purpose of calibrating national/regional/working standards (73). The NRA may obtain from the manufacturer the product-specific or working reference to be used for lot release until international/national standard preparation is established.

Consistency of production has been recognized as an essential component in the quality assurance of poliomyelitis vaccine (inactivated). In particular, the NRA should carefully monitor production records and quality-control test results for clinical lots as well as for a series of consecutive lots of the vaccine.

D.2 Official release and certification

A vaccine lot should be released only if it fulfils all national requirements and/or satisfies Part A of these WHO Recommendations (72).

A protocol for the manufacturing and control of poliomyelitis vaccines (inactivated), based on the model protocol provided in Appendix 3 and signed by the responsible official of the manufacturing establishment, should be prepared and submitted to the NRA in support of a request to release vaccine for use.

A lot release certificate signed by the appropriate NRA official should then be provided to the manufacturing establishment, and should certify that the lot of vaccine in question meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot. The purpose of this official national release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines. A model NRA Lot Release Certificate is provided in Appendix 4.

Authors and acknowledgements

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Operations and Research, World Health Organization, Switzerland; Mr D. Ugiyadi, PT Bio Farma, Indonesia; Dr G. Waeterloos, Scientific Institute of Public Health, Belgium; Dr S. Yamazaki, Japan Poliomyelitis Research Institute, Japan; Dr D.J. Wood, Department of Essential Medicines and Health Products, World Health Organization, Switzerland; Mr L. Yi, Institute of Medical Biology, China; Dr T.Q. Zhou, Department of Essential Medicines and Health Products, World Health Organization, Switzerland.

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Republic of Korea; Dr R. Dhere, Serum Institute of India Ltd., India; Dr S. Konda, Panacea Biotec Ltd., India; Ms X. Li, National Vaccine and Serum Institute, China; Mr D. Ugiyadi, PT Bio Farma, Indonesia; Ms H. Wang, Beijing Tiantan Biological Products Corporation Ltd., China; Dr S. de Walque, GlaxoSmithKline Vaccines, Belgium; Dr T. de Rosa, Crucell, Switzerland; Dr E. Vidor, Sanofi Pasteur, France; Dr Y. Kino, The Chemo-Sero-Therapeutic Research Institute, Japan; Dr Q. Li, Kunming Institute of Medical Biology, China; Dr G. Stawski, Statens Serum Institut, Denmark; Dr K. Wakabayashi, Japan Poliomyelitis Research Institute, Japan; Dr D.J. Wood, Department of Essential Medicines and Health Products, World Health Organization, Switzerland.

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The third draft was prepared by Dr M. Lennon, Consultant, Horning, England and Dr T.Q. Zhou, Department of Essential Medicines and Health Products, World Health Organization, Switzerland, and the following drafting group: Dr K. Chumakov, United States Food and Drug Administration Center for Biologics Evaluation and Research, USA; Dr C. Li, National Institutes for Food and Drug Control, China; Dr J. Martin, National Institute for Biological Standards and Control, England; Dr P. Minor, National Institute for Biological Standards and Control, England; Dr T. Wu, Health Canada, Canada; with support from the WHO Secretariat: Dr J. Fournier-Caruana, Dr I. Knezevic and Dr D.J. Wood, Department of Essential Medicines and Health Products, World Health Organization, Switzerland; Dr H. Okayasu, Dr N. Previsani and Dr R. Sutter, Polio Operations and Research, World Health Organization, Switzerland, following a first round of public consultation on the second draft document posted on the WHO Biologicals website December 2013–January 2014, and

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of Infectious Diseases, Japan; Dr Y. Kino (*Industry representative*), The Chemo-Sero-Therapeutic Research Institute, Japan; Ms Y-K. Lee, Ministry of Food and Drug Safety, Republic of Korea; Professor X. Li (*DCVMN representative*), National Vaccine and Serum Institute, China; Dr K. Mahmood, PATH, USA; Dr L. Mallet (*International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) representative*), Sanofi Pasteur, France; Dr W. Meng (*DCVMN representative*), Sinovac, China; Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr M. Mitsuki, Pharmaceuticals and Medical Devices Agency, Japan; Dr A.F. Nkayamba, Tanzania Food and Drugs Authority, United Republic of Tanzania; Dr H. Ogawa (*Industry representative*), Research Foundation for Microbial Diseases of Osaka University, Japan; Dr M. van Oijen, Institute for Translational Vaccinology, Netherlands; Dr S. Pakzad, Food and Drug Control Laboratory, the Islamic Republic of Iran; Dr V. Pithon, Agence Nationale de Sécurité du Médicament et des Produits de Santé, France; Mr D. Ugiyadi (*DCVMN representative*), PT Bio Farma, Indonesia; Dr A.R.T. Utami, National Agency of Drug and Food Control, Indonesia; Dr E. Vidor (*IFPMA representative*), Sanofi Pasteur, France; Dr B. Voordouw, Medicines Evaluation Board, Netherlands; Dr G. Waeterloos, Scientific Institute of Public Health, Belgium; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Mr K. Wakabayashi (*Industry representative*), Japan Poliomyelitis Research Institute, Japan; Dr S. de Walque (*IFPMA representative*), GlaxoSmithKline Vaccines, Belgium; Professor H. Yang and Professor H. Yin, China Food and Drug Administration, China; with support from the WHO Meeting Secretariat: Dr M.R. Balakrishnan, Dr J. Fournier-Caruana, Dr I. Knezevic, Dr D.J. Wood and Dr T.Q. Zhou, Department of Essential Medicines and Health Products, World Health Organization, Switzerland; Dr H. Okayasu and Dr N. Previsani, Polio Operations and Research, World Health Organization, Switzerland.

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of Medical Sciences, China; Dr K. Mahmood, PATH, USA; Dr L. Mallet, Sanofi Pasteur, France; Dr W. Meng, Sinovac, China; Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr. M. van Oijen, Institute for Translational Vaccinology, Netherlands; Dr V. Pithon, Agence Nationale de Sécurité du Médicament et des Produits de Santé, France; Dr E. Vidor, Sanofi Pasteur, France; Dr G. Waeterloos, Scientific Institute of Public Health, Belgium; Dr R. Wagner, Paul-Ehrlich-Institut, Germany; Professor H. Yang, China Food and Drug Administration, China.

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Appendix 1

Overview of virus seeds used in IPV production

This appendix gives an overview of the history of virus seeds that are either currently used in production or may be used in the future. These include the wild strains used in current IPV production and the attenuated Sabin strains which are considered to pose a lower risk and are being developed as alternative seeds. Novel strains intended to be safer for use in production are also in development.

1. IPV made from virulent strains

Both classic IPV, which was developed by Jonas Salk and others and licensed in 1955, and the enhanced-potency IPV introduced in the late 1980s, are prepared from wild (virulent) polioviruses of three serotypes. The strains selected by Salk were Mahoney, MEF-1 and Saukett, representing types 1, 2 and 3, respectively. The Mahoney strain was isolated in 1941 by Drs Thomas Francis and Walter Mack from the pooled faeces of three healthy children in Cleveland, Ohio, USA (1). It was subsequently passaged by Salk, including 14 times in living monkeys and twice in monkey testicular cultures (2). The MEF-1 strain was isolated by the inoculation of monkeys in Egypt in 1940 (3) during a polio outbreak among allied troops of the Mediterranean Expeditionary Force (hence the name MEF). It was adapted by Schlesinger and Olitsky to growth in mice (4), and then transferred by Salk from the spinal cord of a paralysed mouse to tissue culture (2). The original Saukett strain was isolated by Salk in 1950 by direct inoculation of tissue culture with a faecal specimen from a paralysed patient (2). Seed stocks of the viruses were provided by Salk to most manufacturers and were used to establish their virus master seeds. An alternative strain of type-1 poliovirus (Brunhilde) is used by the Statens Serum Institute (SSI) in Denmark. This strain was isolated in 1939 by David Bodian from a pool of stool specimens from seven patients in Maryland, USA (5). The strain was provided to the laboratory of Dr John Enders at Harvard Medical School in Boston, Massachusetts, USA, and from there to Dr Arne Svedmyr's laboratory in Stockholm, Sweden. Dr Svedmyr supplied SSI with the virus. Table 1 summarizes the history of isolation and early passaging of these viruses.

Table 1
History of isolation and early passaging of wild polioviruses (WPVs) used in the production of IPV

Strain name	Source of isolation	Location	Year	Reference
Mahoney	Stool of 3 healthy children	Cleveland, Ohio, USA	1941	Francis & Mack. See Sabin AB, Boulger LR, 1973 (1)
MEF-1	CNS of a paralysed patient	Egypt	1941	van Rooyen CE, Morgan AD, 1943 (3)
Saukett	Stool of a paralysed patient	USA	1950	Salk JE, 1953 (2)
Brunhilde	Stool of 7 patients	Massachusetts, USA	1939	Howe HA, Bodian D, 1941 (5)

Subsequent studies raised questions regarding these strains. The nucleotide sequence of MEF-1 was found to be very close to the sequence of another type-2 strain (Lansing; isolated in 1937 in Michigan, USA) with only 17 nucleotide and 2 amino acid differences (6). Since the strains were isolated 4 years apart in the Middle East and the USA, it is unlikely that the similarity represents a natural relatedness. In 1943, MEF-1 from the spinal cords of monkeys was adapted to growth in mice (4) and was found to be indistinguishable in terms of pathogenicity and immunological properties from Lansing, which was also adapted to growth in mice. A plausible explanation is that the Lansing strain used as a reference strain in Schlesinger and Olitsky's laboratory was inadvertently substituted for MEF-1, and that all subsequent stocks of MEF-1 are derivatives of the Lansing strain. In addition, two common variants of MEF-1, differing by a small number of nucleotides, are in use in different laboratories and production facilities.

Saukett strains obtained from different laboratories and manufacturers differ significantly (7, 8) with the degree of diversity (~10% nucleotide substitutions), demonstrating that they are different strains. Some of the differences were observed in antigenic sites and could affect immunogenicity, suggesting that better characterization of vaccines in the future may need to include determination of the exact nucleotide sequences of virus master seed lots used by manufacturers.

The flow diagrams below (Figs. 1–3) show the history of each of the seed viruses used to prepare, according to IPV manufacturers, the master seed lots used to produce type-1, type-2 and type-3 IPV, respectively. These

diagrams provide only an overview of the use of different seeds based upon a written survey conducted in 2012 by WHO among vaccine manufacturers, and upon information obtained from subsequent consultations. The presentation of these diagrams should not be taken as an indication of any form of WHO qualification or approval of the strains or of the vaccines in the context of these WHO Recommendations.

The names of the manufacturers and agencies shown in the diagrams are given in full in Table 2.

Table 2
Full names of manufacturers and agencies shown in Figs. 1–3

BBio	Bilthoven Biologicals B.V. (formerly NVI), Bilthoven, Netherlands
GSK	GlaxoSmithKline Vaccines, Wavre, Belgium
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment), Bilthoven, Netherlands
Sanofi Pasteur (Canada)	Sanofi Pasteur, Canada
Sanofi Pasteur (France)	Sanofi Pasteur, Marcy L'Etoile, France
SSI	Statens Serum Institute, Copenhagen, Denmark

Fig. 1
History of seed virus used to produce type-1 IPV

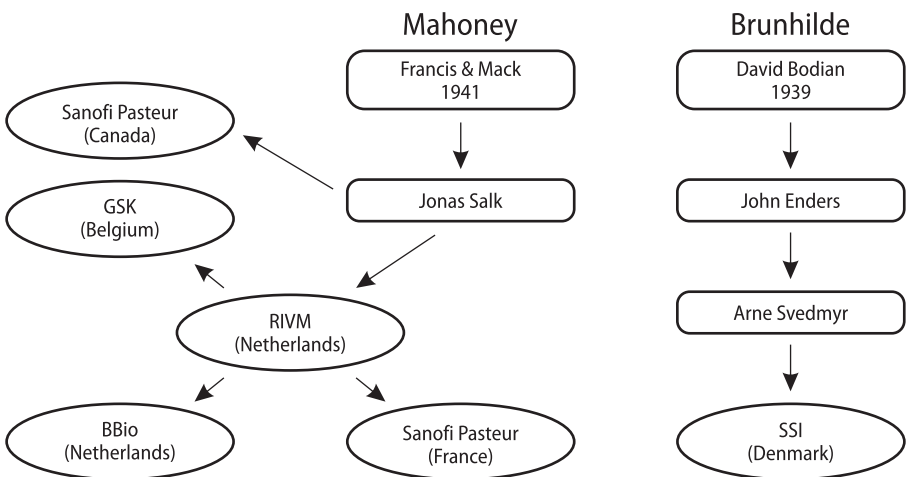


Fig. 2
History of seed virus used to produce type-2 IPV

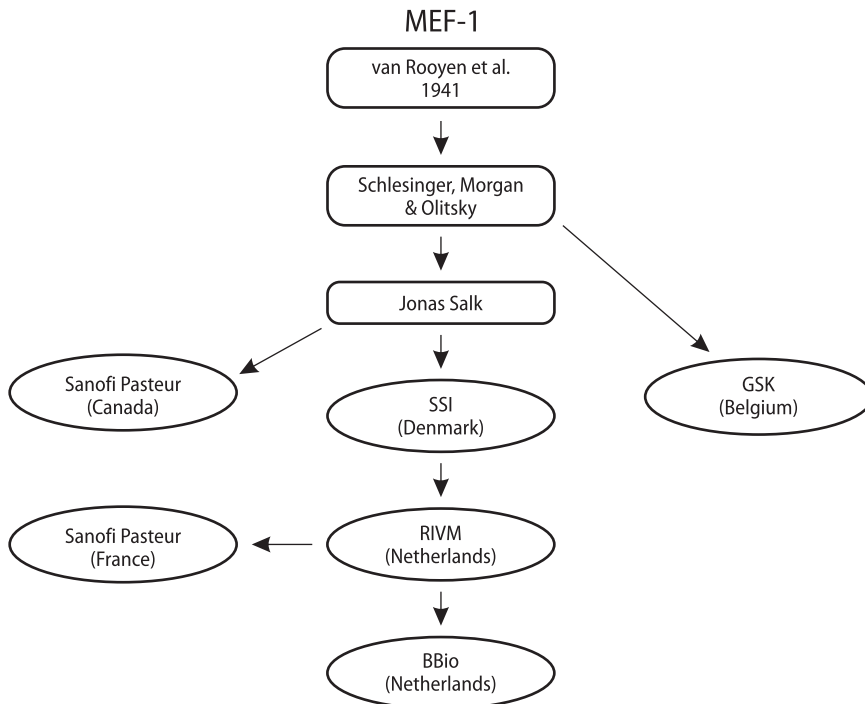
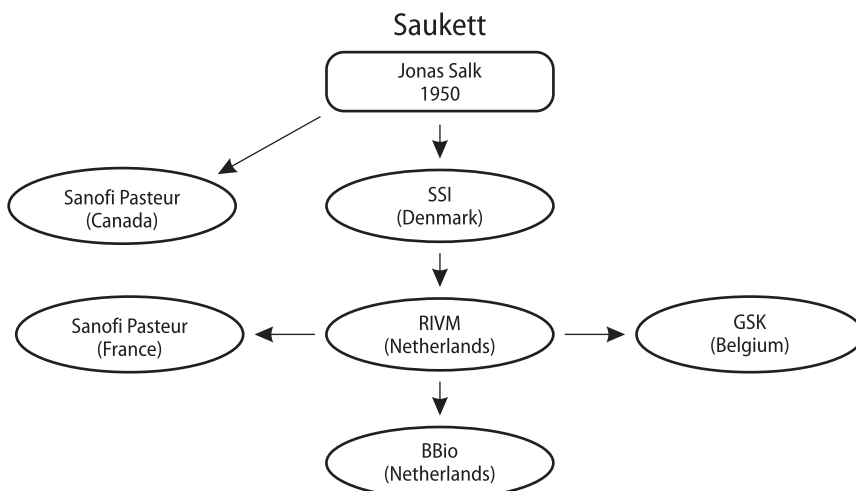


Fig. 3
History of seed virus used to produce type-3 IPV



2. IPV made from attenuated strains (Sabin)

Once the circulation of wild-type polio viruses has been ended, IPV manufacturing establishments will become the most likely potential source of virulent viruses. Such viruses will therefore need to be stringently contained to prevent their reintroduction into the environment. The Sabin vaccine strains used to manufacture OPV are less virulent than the wild strains and have been proposed as less hazardous seeds for IPV production in order to mitigate the risks of potential inadvertent release from production facilities. Sabin strains are known to be genetically unstable in infected humans and, to some extent, in production. To retain the attenuated phenotype the Sabin strains must be propagated under defined and well controlled conditions. In addition, during the manufacture of OPV each harvest is tested to monitor molecular consistency – for example, by MAPREC – and each monovalent bulk is tested for neurovirulence to be sure that the attenuated phenotype is retained. As the IPV product is inactivated, full characterization on every batch is not necessary but some assurance of consistent production is required. Sabin strains are considered less transmissible than the wild type so that, should they escape from the production facility and start to circulate within communities, they would pose a lesser risk. However, they can revert and may give rise to circulating vaccine-derived strains that are both transmissible and capable of causing outbreaks. Therefore, the use of Sabin strains in the manufacture of IPV may reduce biosecurity concerns compared to manufacture from virulent wild strains but does not eliminate such concerns entirely. Some testing or process validation will be required to show that the product is consistent and that the attenuated phenotype is retained in order to justify the level of containment used (see also General considerations and Part A of this document). Two sIPV-containing combination products based on attenuated Sabin strains have been licensed in Japan, and other sIPV vaccines are undergoing clinical evaluation in some countries. The derivation of Sabin strains has been described in the literature (1) and the detailed origin of seed viruses made from them can be found in Appendix 1 of the revised WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (9).

3. Other strains in development

Alternative attenuated strains of poliovirus, such as strains derived from recombinant DNA technology, are under development. They are intended to be both attenuated and genetically stable while possessing low or no infectivity for humans and thus be of negligible transmissibility. Such strains should pose a lower risk of inadvertent release from production facilities or of infecting production workers. They may include strains in which known attenuation

determinants are stabilized by targeted genetic changes, strains with alterations in codon usage in order to introduce multiple mutations to reduce virus replication efficiency, or viruses produced by other strategies. The phenotypes and stability of these strains will require confirmation.

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Appendix 2

In vivo potency assay of IPV

Tests for evaluating the potency of IPV include an *in vivo* assay for immune response. An appropriate WHO International Standard should be used to validate the assay. However, because of the diversity in the reactivity of different vaccines it is unlikely that an International Standard will be suitable for the standardization of *in vivo* assays of vaccines from all manufacturers. If this is shown to be the case, manufacturers should establish a product-specific reference preparation which is traceable to a lot of vaccine shown to be efficacious in clinical trials. The NRA should approve the reference preparation used and should agree with the potency limits applied. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and it should be replaced when necessary.

In recent investigations the *in vivo* potency assay in rats has been standardized (1) and shown to have advantages over previously described *in vivo* tests for IPV (2).

A suitable *in vivo* assay method consists of intramuscular injection into the hind limb(s) of rats of four dilutions of the vaccine to be examined and a reference vaccine, using for each dilution a group of not fewer than 10 rats of a suitable strain and which are free of specific pathogen. The number of animals used should be sufficient to allow for the calculation of potency to be made with 95% confidence limits within the 25–400% range. The number of dilutions and the number of animals used per dilution may differ from that specified here, provided that any alternative scheme gives at least the same sensitivity in the test. For each dilution, the weight of individual animals should not vary by more than 20% from the group mean. An inoculum of 0.5 mL is used per rat. The dose range is chosen so that a dose response to all three poliovirus types is obtained. The animals are bled after 20–22 days. Neutralizing titres against all three poliovirus types are measured separately using 100 CCID₅₀ of the Sabin strains as challenge viruses, Vero or Hep-2C as indicator cells and neutralization conditions of 3 h at 35–37 °C followed by 18 h at 2–8 °C. Results should be read after fixation and staining after 7 days of incubation at 35 °C. For the antibody assay to be valid, the titre of each challenge virus must be shown to be within the range of 30–300 CCID₅₀ and the neutralizing antibody titre of a control serum must be within two 2-fold dilutions of its geometric mean titre. The potency is calculated by comparing the proportions of animals defined as responders to the test vaccine and to the reference vaccine by the probit method. To define an animal as a responder, it is necessary to establish a cut-off neutralizing antibody titre

for each poliovirus type. Owing to inter-laboratory variation, it is not possible to define cut-off values that could be applied by all laboratories. Instead, the cut-off values should be determined by each laboratory on the basis of a minimum series of three tests with the reference vaccine. The mid-point on a $\log_2$ scale of the minimum and maximum geometric mean titres of the series of three or more tests is used as the cut-off value. For each of the three poliovirus types, the potency of the vaccine should not be statistically significantly less than that of the reference preparation. The test is not valid unless:

- the median effective dose (ED_{50}) for both the test and reference vaccines lies between the smallest and the largest doses given to the animals;
- the statistical analysis shows no significant deviation from linearity or parallelism;
- the confidence limits of the estimated relative potency fall between 25% and 400% of the estimated potency.

Laboratories that have established the parallel line method of analysis of antibody titres for the rat test may use it instead of converting titres to proportions of responders as in the probit method of analysis.

Laboratories are encouraged to validate alternative methods for the assay of neutralizing antibody to reduce the use of live polioviruses in laboratories. If IPV is formulated with other antigens into a combination vaccine, then the suitability of performing the rat immunogenicity test will have to be determined. If the immunogenicity test is performed, the potency of the final bulk for each virus type should be approved by the NRA.

The development of transgenic mice that express the human poliovirus receptor (TgPVR mice) (3–5) has led to the development of an immunization/challenge model that may be useful for assessment of vaccine efficacy. This test is not proposed for lot release. Any work with transgenic mice should comply with WHO guidelines (6).

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Appendix 3

Model protocol for the manufacturing and control of poliomyelitis vaccines (inactivated)

The following protocol is intended for guidance. It indicates the information that should be provided as a minimum by the 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 here. The essential point is that all relevant details demonstrating compliance with the licence and with the relevant WHO Recommendations 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 of 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 document.

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: _____

Finished product (final lot)

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

Composition (D-antigen unit) of a single human dose:	Type 1	Type 2	Type 3
Bulk numbers of monovalent pool suspensions:	Type 1	Type 2	Type 3
Site of manufacture of each monovalent pool: _____			
Date of manufacture of each monovalent pool: _____			
Date of manufacture of trivalent bulk (blending): _____			
Date of manufacture of final bulk: _____			
Date on which last determination of potency was started, or date of start of period of validity: _____			
Shelf-life approved (months): _____			
Expiry date: _____			
Storage conditions: _____			
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 on each submission. Full details on master and working seed lots should be provided upon first submission only and 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 of 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 was rejected during the control testing, this should also be recorded either in the following sections or on a separate sheet.

Control of source materials (section A.3)

Virus seed (every submission) (section A.3.1)

Vaccine virus strain(s) and serotype(s): _____

Substrates used for preparing seed lots: _____

Origin and short history: _____

Authority that approved the virus strains: _____

Date of approval: _____

Information on seed lot preparation (every submission) (section A.3.1.3)

Virus master seed (VMS) and virus working seed (VWS) (*to be provided upon first submission only and whenever a change has been introduced*)

Strain used: _____
 Source of VMS: _____
 VMS and VWS lot number: _____
 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 and VWS (first submission only)

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: _____

Virus titration

Date of test: _____

Reference batch number: _____

Date of test: _____

Result: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Test in rabbit kidney cell cultures

Number of cell cultures: _____

Total volume inoculated: _____

Period of observation: _____

Result: _____

*Test 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 antisera used for
neutralization virus seed: _____
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: _____

Tests for neurovirulence (if applicable)

In vitro tests: MAPREC test for attenuated strains (if applicable)

MAPREC

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A
and 525-C of 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 of 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 of 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 vivo tests for neurovirulence (if applicable)

Neurovirulence test in monkeys: _____
 Result of blood serum test in monkeys prior
 to inoculation: _____
 Number and species of monkeys inoculated: _____

 Quantity (CCID₅₀) inoculated in each
 test monkey: _____
 Number of "valid" monkeys inoculated
 with test sample: _____
 Number of positive monkeys observed 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): _____
 C1 constant value: _____

Neurovirulence test in transgenic mice for attenuated strains (if applicable)

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 inoculums)

Paralysis rates for test vaccine at each dose: _____
 Paralysis rates for reference virus at each dose: _____
 Results: _____
 Log odds ratio: _____
 L1 and L2 values: _____
 Pass/fail decision: _____

Cell banks (every submission) (section A.3.2)*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 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 passage 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: _____**Tests on MCB and WCB (first submission only) (section A.3.2)**

Percentage of total cell-bank ampoules tested: _____

Identity test

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: _____

Control of vaccine production (section A.4)

Control cell cultures (section A.4.1)

Virus type (1, 2 or 3) (*a separate protocol should be completed for each type*)

Control of production cell cultures

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 on control cell cultures (provide information on control cells corresponding to each single harvest)

Ratio of control to production cell cultures: _____

Incubation conditions: _____

Period of observation of cultures: _____

Dates observation started/ended: _____

Ratio or proportion of cultures discarded for nonspecific reasons: _____

Results of observation: _____

Tests for haemadsorbing viruses

Quantity of cell tested: _____

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Tests for adventitious agents on 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 cell cultures for vaccine production (section A.4.2)

Observation of cultures for adventitious agents on day of inoculation

Results of microscopic observation: _____

Control of single harvests (section A.4.3)

Batch number(s) and virus type: _____

Date of inoculation: _____

Date(s) of harvest: _____

Volume(s), storage temperature, storage time and
approved storage period: _____*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			

Table continued

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: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Purified monovalent pools before inactivation (section A.4.4)

Batch number(s) and virus type: _____

Date of inoculation: _____

Date(s) of harvest: _____

Volume(s), storage temperature, storage time and
approved storage period: _____

Test for residual cellular DNA

Method used: _____

Date of start of test: _____

Date of end of test: _____

Virus titration

Date of test: _____

Reference batch number: _____

Date of test: _____
Result: _____

Identity test

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

D-antigen content

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

Protein content

Method used: _____
Date of start of test: _____
Date of end of test: _____
Result: _____
Details of filtration and/or clarification and/or
purification (if applied): _____

Date: _____
Additional tests on monovalent pools produced from Sabin vaccine seeds or
from seeds derived by recombinant DNA technology: for example, in vitro
tests (such as MAPREC) for attenuated strains, or in vivo neurovirulence
test in transgenic mice for attenuated strains (if applicable)
(See above in tests on virus seeds) _____

Inactivation of monovalent product (section A.4.5)

Agent(s) and concentration at the beginning and
end of inactivation: _____
Temperature of inactivation: _____
Date of start of inactivation: _____
D-antigen units at start of inactivation: _____
Date of taking first sample: _____
Date of completion of inactivation: _____
D-antigen units at end of inactivation: _____

Test for effective inactivation (after removal/neutralization of inactivating agent)

Sample size tested: _____
 Date of first sample: _____
 Date of second sample: _____
 Details of testing procedure: _____
 Period of observation of cell cultures: _____
 Period of observation of subcultures: _____
 Result: _____
 Result of challenge of used culture with live virus: _____

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					

D-antigen content

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

Trivalent bulk product (monovalent pools incorporated)

Date of preparation: _____
 Preservative (if added, type and concentration): _____

Tests on trivalent bulk (section A.4.6)

Test for absence of infective poliovirus: _____
 Sample size tested: _____
 Details of testing procedure: _____
 Period of observation of cell cultures: _____
 Period of observation of subcultures: _____
 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					

Residual formaldehyde

Method used: _____

Result: _____

D-antigen content

Method used and acceptance limits for test results: _____

Reference used: _____

Date of test: _____

Result: _____

Control of final bulk (section A.4.7)*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					

Potency test(s)*D- antigen test*

Method used and acceptance limits for test results: _____

Reference used: _____

Date of test: _____

Result: _____

Results (and date) of in vivo tests (in rats)

(if performed): _____

Preservative content (if applicable)

Date of test: _____

Method used: _____

Result: _____

Adjuvant (if applicable)

Date of test: _____

Method used: _____

Result: _____

Filling and containers (section A.5)

Final lot number: _____

Total volume for final filling: _____

Date of filling: _____

Number of vials after inspection: _____

Number of vials filled: _____

Control tests on final lot (section A.6)*Inspection of final containers*

Date of test: _____

Results: _____

Appearance

Date of test: _____

Results: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

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					

General safety test (if applicable)

Date of start of test: _____

Date of end of test: _____

Result: _____

Potency test(s)*D-antigen test*

Method used and acceptance limits for test results: _____

Reference used: _____

Date of test: _____

Result: _____

Results (and date) of in vivo tests (in rats)

(if performed): _____

Protein content

Content of protein in mg per human dose: _____

Serum protein tests (if applicable)

Result: _____

Preservative content (if applicable)

Date of test: _____

Method used: _____

Result: _____

Endotoxin content

Date of test: _____

Method used: _____

Result: _____

Test for residual formaldehyde

Date of test: _____

Method used: _____

Result: _____

pH

Date of test: _____

Result: _____

Adjuvant (if applicable)

Date of test: _____

Method used: _____

Result: _____

Residual antibiotics (if applicable)

Date of test: _____

Method used: _____

Result: _____

Certification by the manufacturer

Name of head of quality control (typed) _____

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

I certify that lot no. _____ of trivalent poliomyelitis vaccine (inactivated), whose number appears on the label of the final containers, meets all national requirements and/or satisfies Part A¹ of the 2014 WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated).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. 993, Annex 3.

Certification by the NRA

If the vaccine is to be exported, attach the NRA Lot Release Certificate (as shown in Appendix 4), a label from a final container and an instruction leaflet for users.

Appendix 4

Model NRA Lot Release Certificate for poliomyelitis vaccines (inactivated)

Certificate no. _____

The following lot(s) of poliomyelitis vaccine (inactivated) produced by _____¹ in _____,² whose numbers appear on the labels of the final containers, meet all national requirements³ and Part A⁴ of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (2015)⁵ and complies with WHO good manufacturing practices for pharmaceutical products: main principles;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);
- type of container used;

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. 993, Annex 3.

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 and/or procedures specified in a defined document etc., as appropriate.

- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and 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 _____