Annex 2

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

Replacement of Annex 2 of WHO Technical Report Series, No. 980

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

Abbreviations

5′ UTR	5′ untranslated region
bOPV	bivalent oral poliomyelitis vaccine
CBER	FDA Centre for Biologics Evaluation and Research
$CCID_{50}$	cell culture infectious dose 50%
cVDPV	circulating vaccine-derived poliovirus
EPI	Expanded Programme on Immunization
EUL	WHO emergency use listing
GAPIII	WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use
GAPIV	WHO Global Action Plan for Poliovirus Containment
GPEI	Global Polio Eradication Initiative
HTS	high-throughput sequencing
IPV	inactivated poliomyelitis vaccine
MAPREC	mutant analysis by polymerase chain reaction and restriction enzyme cleavage
MCB	master cell bank
MHRA	Medicines and Healthcare products Regulatory Agency
MNVT	monkey neurovirulence test
mOPV	monovalent oral poliomyelitis vaccine
NAT	nucleic acid amplification technique
NCL	national control laboratory
nOPV	novel oral poliomyelitis vaccine
NRA	national regulatory authority
NVT	neurovirulence test
OPV	oral poliomyelitis vaccine
PCR	polymerase chain reaction
rcDNA	residual cellular DNA
rct40	reproductive capacity at elevated temperature

RSO	RNA-plaque-purified Sabin Original
SAGE	WHO Strategic Advisory Group of Experts on Immunization
SNP	single nucleotide polymorphism
SO	Sabin Original
SOM	Sabin Original Merck
SOP	standard operating procedure(s)
SV40	simian virus 40
TgmNVT	transgenic mouse neurovirulence test
TgPVR21 mice	transgenic mice expressing the human poliovirus receptor
tOPV	trivalent oral poliomyelitis vaccine
VAPP	vaccine-associated paralytic poliomyelitis
WCB	working cell bank
WPV	wild poliovirus

Introduction

WHO Requirements for oral poliomyelitis vaccine (OPV) were first formulated in 1962 (*1*) and then revised in 1966 (*2*) and 1972 (*3*) when an appendix describing the production of OPV in human diploid cells was added. The Requirements were further updated in 1982 (*4*) to reflect an accumulation of data, particularly on the performance and evaluation of the monkey neurovirulence test (MNVT) and tests on the karyology of human diploid cells. The Requirements were then updated in full in 1989 (*5*) to take account of the WHO Requirements for continuous cell lines used for biologicals production, which had been adopted in 1985 (*6*) – with a WHO Study Group concluding that, in principle, such cell lines were acceptable for use as substrates for the production of biologicals (*7*). An addendum was subsequently adopted (*8*) that: (a) introduced changes in tests for freedom from detectable DNA sequences of simian virus 40 (SV40); (b) introduced the mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) assay as an optional additional in vitro test for poliovirus type 3; (c) increased levels of laboratory containment for wild polioviruses (WPVs); and (d) provided guidance on additional antibody screening tests (for foamy viruses) for animals from closed primate colonies used as a source of primary monkey kidney cells.

The Requirements were subsequently revised in full in 1999 (*9*) when they became the WHO Recommendations for the production and control of poliomyelitis vaccine (oral). The changes introduced included the use of transgenic mice expressing the human poliovirus receptor (TgPVR21 mice) (*10*) as an alternative to the MNVT for type 3 virus, and the introduction of the MAPREC assay as the in vitro test of preference for the evaluation of filtered bulk suspensions of poliovirus type 3 (*11*). The previously mandated reproductive capacity at elevated temperature (rct40) test then became an optional additional test if MAPREC was performed. In 2000, following the completion of studies of poliovirus types 1 and 2 in TgPVR21 mice, an addendum to the Recommendations was adopted (*12*) that included the transgenic mouse neurovirulence test (TgmNVT) as an alternative to the MNVT for all three poliovirus serotypes.

In 2012, the fully revised WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) were adopted. The revised Recommendations provided updated information on the origins of different virus strains used for OPV production, as well as consideration of new monovalent OPV (mOPV) and bivalent OPV (bOPV) vaccine formulations (*13*). Updated sections were also provided on international standards and other reference materials, general manufacturing recommendations and control tests, and on WHO standard operating procedures (SOP) for the TgmNVT and MAPREC in light of technical developments. Other changes included the provision of new sections on the nonclinical and clinical evaluation of OPV,

updating of terminology and the introduction of the "virus sub-master seed lot" concept applicable only to the virus master seed supplied by WHO. Updated guidance was also given on the use of neurovirulence testing (MNVT and TgmNVT) and on the MAPREC assay, which was extended to include all three types of virus seeds and vaccine bulks. A rationale was also provided to guide the choice of monkey or mouse neurovirulence testing.

Since then, significant progress has been made towards global polio eradication, and important advances made in scientific knowledge, novel laboratory techniques (including the use of high-throughput sequencing (HTS), also known as next generation sequencing, massively parallel, or deep sequencing), and the development of new non-pathogenic strains of polioviruses for use in quality control tests. The global eradication of WPVs of serotypes 2 and 3 was declared by WHO in 2015 and 2019, respectively. In early 2016, following the declaration of WPV serotype 2 eradication, the global use of trivalent OPV (tOPV) for routine immunization was replaced by the exclusive use of bOPV containing only serotypes 1 and 3. Therefore, the routine use of tOPV was discontinued, with bOPV now being used for routine and supplemental immunization. mOPV against type 2 (mOPV2) is used to control outbreaks of type 2 circulating vaccine-derived poliovirus (cVDPV2). In addition, tOPV was approved by the WHO Strategic Advisory Group of Experts on Immunization (SAGE) for use in the control of cVDPV2 outbreaks occurring alongside WPV1 circulation. Rationally designed and more genetically stable strains of Sabin 2 poliovirus were developed to minimize reversion of the vaccine strain to virulence and have been used to manufacture novel OPV2 (nOPV2). At the end of 2020, nOPV2 was introduced for cVDPV2 outbreak control under the WHO emergency use listing (EUL) procedure (*14*).

Since the 2012 revision of the WHO Recommendations, new WHO guidance documents have also been issued. The WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) was adopted in 2014 (*15*), with a subsequently revised 4th edition (GAPIV) produced in 2022 (*16*). The resulting tightened biosafety and biosecurity requirements for handling live polioviruses led to the adoption of the revised WHO Guidelines for the safe production and quality control of poliomyelitis vaccines in 2018 (*17*), and its subsequent amendment in 2020 (*18*). Also in 2020, the WHO Expert Committee on Biological Standardization recommended that the 2012 OPV Recommendations should be revised. In response, WHO convened a drafting group composed of national regulators to prepare the revised document. A virtual informal consultation was held by WHO on 15–17 November 2021 attended by experts and representatives from academia, national regulatory authorities (NRAs)/national control laboratories (NCLs), industry, and other international organizations and institutions involved in the research, manufacture,

authorization and testing/release of OPV to discuss and reach consensus on the issues to be addressed in the revision process (*19*).

The major issues addressed in the revised Recommendations include:

- the use of HTS in quality control of OPV as an alternative to the MAPREC assay as a preferred in vitro test;
- analysis of whole genome mutational profiles generated by HTS as a possible future replacement of the MNVT and TgmNVT for routine lot release once manufacturing consistency has been established – practical experience in these areas is currently limited and further guidance will be provided in due course;
- removal of the rct40 test due to its insufficient sensitivity and requirement for WPVs as control strains which complicates GAPIV compliance;
- consideration of the design, manufacture and quality control of nOPV strains;
- use of new non-pathogenic strains for the measurement of neutralizing antibodies to polioviruses;
- updates on international reference materials relevant to OPV manufacture and control, and inclusion of a new appendix on such materials;
- updating of terminology;
- introduction of the "virus sub-master seed" concept for nOPV strains in addition to Sabin OPV; and
- the clinical evaluation of new and safer OPV strains that may be developed.

Additional changes have also been made to refer to, and align the current document with, other WHO recommendations published since its previous revision.

Purpose and scope

These WHO Recommendations provide guidance to NRAs and manufacturers on the manufacturing processes, quality control, and nonclinical and clinical evaluation needed to assure the quality, safety and efficacy of live attenuated poliomyelitis vaccines (oral).

The scope of these Recommendations encompasses live attenuated poliomyelitis vaccines (oral) derived from the original Sabin strains – some by simple passage and others by more complex routes, including plaque purification.

The document is intended to apply to all OPV products prepared from Sabin poliovirus strains and their derivatives.

The document also includes consideration of the issues raised in the manufacture and control of nOPV made from rationally designed strains created by targeted genetic manipulation of Sabin viruses, and by the introduction of HTS as a quality control method for both nOPV and Sabin OPV.

In the current document, "OPV" refers to oral poliomyelitis vaccines made from any attenuated poliovirus – both the original Sabin strain and novel, genetically modified strains. In some cases, the terms "Sabin OPV" and "nOPV" are used to distinguish between classical OPV and novel OPV.

These WHO Recommendations should be read in conjunction with other relevant WHO guidelines and guidance documents, such as those on nonclinical (*20*) and clinical (*21*) evaluation of vaccines, good manufacturing practices for biological products (*22*), characterization of cell banks (*23*), lot release (*24*) and the safe production and quality control of poliomyelitis vaccines (*17*, *18*).

Terminology

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

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

Cell culture infectious dose 50% ($CCID_{50}$): the quantity of a virus suspension that will infect 50% of cell cultures.

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

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

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

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

High-throughput sequencing (HTS): a next generation sequencing (NGS) technology based on sequencing of individual nucleic acid molecules that allows each nucleotide to be sequenced multiple times (massively parallel or deep sequencing), thereby enabling the detection and quantitation of sequence heterogeneities including single nucleotide polymorphisms (SNPs).

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

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

Novel OPV (nOPV): any OPV manufactured using rationally designed genetically modified derivatives of the live attenuated Sabin vaccine strain. nOPV strains have enhanced genetic stability and lower risk of reversion to neurovirulence compared to the original Sabin strain.

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

RSO (re-derived Sabin Original): RNA-plaque-purified Sabin Original (*25*). All subsequent passages are designated by an additional number – for example, RSO+1 (master seed) is one passage on from RSO. It is distributed to vaccine manufacturers that create their own **virus master seed lot**, **virus sub-master seed lot** and **virus working seed lot** for the manufacture of monovalent bulks of OPV3.

Sabin strain: any preparation of an attenuated poliovirus of type 1, 2 or 3 derived by a limited number of passages from stocks developed by Dr Albert Sabin (*26*) and which retain attenuated properties as measured by biological and molecular markers.

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

Sabin Original (SO): as described by Sabin and Boulger (*26*). All subsequent passages are designated by an additional number – for example, SO+1 is one passage on from SO.

Virus master seed lot: a quantity of virus suspension that has been processed at the same time in a single production run to assure a uniform composition, and which has been characterized to the extent necessary to

support development of the **virus working seed lot** or a **virus sub-master seed lot** (if applicable).

Virus sub-master seed lot: a quantity of virus suspension produced by a single passage from the **virus master seed** and made at a multiplicity of infection that ensures the development of cytopathic effect within an appropriate timeframe, and which has been processed at the same time in a single production run to assure a uniform composition. Sub-master seed lots should be made by the manufacturer when the supply of a well-characterized master seed of Sabin OPV supplied by WHO is insufficient to meet production needs. They may also be produced from qualified nOPV master seeds if necessary. The virus sub-master seed lot should be characterized as extensively as the virus master seed lot 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).

Virus working seed lot: a quantity of virus suspension of uniform composition, fully characterized, derived by only one passage from a master or sub-master virus seed lot and approved by the NRA for the manufacturing of vaccine, and made at a multiplicity of infection that ensures the development at cytopathic effect within an appropriate timeframe (for example, 3 days).

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 will be used for vaccine production. All containers are treated identically and once removed from storage are not returned to stock.

General considerations

Poliomyelitis is an acute communicable disease of humans caused by three distinct poliovirus serotypes (types 1, 2 and 3) that can be distinguished through neutralization with type-specific antibodies (*27*). Poliovirus is a species C human enterovirus of the Picornaviridae family and consists of a single-stranded, positive-sense RNA genome and a protein capsid.

Where sanitation is poor, polioviruses are believed to spread mainly by faecal-to-oral transmission, with the oral-to-oral mode of transmission probably dominating in areas with a higher standard of sanitation. Mixed patterns of transmission are likely to occur in most settings. In the pre-vaccine era, around 1 in every 200 susceptible individuals infected by polioviruses developed paralytic poliomyelitis, while the rest were asymptomatic or had mild symptoms (*27*).

Progress in polio control (and, since 1988, in polio eradication) has been mainly due to the widespread use of vaccines. An inactivated poliomyelitis vaccine (IPV Salk vaccine) was licensed in 1955. The use of live, attenuated OPV (Sabin vaccine) for mass immunizations started in the Soviet Union and a few

other countries in 1959. In the United States and some European countries, an mOPV was licensed in 1961 followed by a tOPV in 1963. The strains of poliovirus used in the production of Sabin OPV were shown to be both immunogenic and highly attenuated when administered orally to susceptible children and adults. Most countries that initially introduced vaccination with IPV later changed to OPV because of its ease of administration, suitability for mass vaccination campaigns, induction of superior intestinal mucosal immunity and lower production costs. In 1974, OPV was recommended as part of the Expanded Programme on Immunization (EPI), and was again the vaccine of choice in 1988 when the World Health Assembly resolved to eradicate polio globally by the year 2000. The last cases of poliomyelitis caused by WPV type 2 (WPV2) and 3 (WPV3) were reported in October 1999 in India and in November 2012 in Nigeria, respectively. Subsequently, the global eradication of WPV2 and WPV3 was certified on 20 September 2015 and 24 October 2019, respectively (27). By the end of 2021, WPV1 remained endemic in only two countries – Afghanistan and Pakistan.

Although OPV is safe, adverse events may occur on rare occasions (*27*). Vaccine-associated paralytic poliomyelitis (VAPP) is the most important of these rare adverse events and is clinically indistinguishable from poliomyelitis caused by WPV. The identification of VAPP requires laboratory analysis of the virus isolated from the case. VAPP incidence has been estimated at 2–4 cases per million annual birth cohort in countries using OPV (*27*). Sabin viruses can spread in populations where OPV coverage is low. In such situations, Sabin viruses can acquire the neurovirulence and transmissibility characteristics of WPV, thus becoming cVDPV that can cause outbreaks of the disease (*28*), presenting a significant challenge to the global eradication campaign. cVDPV2 is the predominant type, and its continued circulation is fuelled by inadequate population immunity. To prevent gaps in population immunity, the switch from tOPV to bOPV (containing only type 1 and type 3 vaccine viruses) was supposed to be accompanied by the introduction of supplemental immunization with trivalent IPV. However, the shortage of IPV in some countries led to a decline in population immunity to type 2 poliovirus and to an increase in cVDPV2 cases from 2 in 2016 to over 1000 in 2020. Outbreaks of cVDPV2 have been controlled through the targeted use of mOPV2, but where the vaccination campaigns have been poor due to difficulties in delivery, they have triggered the emergence of new cVDPV2 outbreaks. To a lesser extent, outbreaks caused by type 1 and 3 cVDPVs have also occurred, and have continued to occur in recent years.

cVDPVs will continue to emerge as long as classical Sabin OPV is used and gaps in population immunity exist. To overcome this problem, in 2011 an international consortium of scientists sponsored by the Bill & Melinda Gates Foundation set out to develop novel vaccine strains with a lower risk of losing their attenuated phenotype and evolving into neurovirulent cVDPVs. One such

virus has now been used to produce an nOPV2 which has been granted EUL by WHO for use in cVDPV2 outbreaks (*29–32*). Additional strains may be developed in the future, including similar genetically stabilized type 1 and type 3 strains. The design of such novel strains is based on an understanding of the molecular biology of polioviruses and vaccines gained over the years. The attenuation of the Sabin strains is associated in part with a highly base-paired hairpin structure (domain V) in the 5′ untranslated region (5′ UTR) of the virus, which is involved in the initiation of protein synthesis. The three Sabin strains have less thermally stable domain V structures compared to the respective wild strains as a result of the introduction of a single base change in this section of the RNA – which is different for each serotype, but which changes the strength of a base pair. As it is a single base change, all three serotypes can readily revert following a single mutational event to the wild-type sequence at this position, as has been observed in vaccine recipients. Viruses have therefore been designed in which it is harder for the hairpin structure of domain V to become stronger by mutation. This was achieved by replacing stronger GC pairs and weaker GU pairs with intermediately strong AU pairs so that the overall thermostability of the hairpin, and therefore virus neurovirulence, remain unchanged. However, this makes the attenuated phenotype more stable because in this redesigned structure two simultaneous mutations at any given position would be required to revert to the wild-type base pair strength. The nOPV strains should therefore be at least as attenuated as the Sabin strains and genetically more stable. This was demonstrated to be the case in vitro, in animal models and in human trials. Modifications were also introduced into the viral polymerase to increase virus genetic stability by reducing mutation and recombination rates. In addition, an essential cis-acting replicative element was moved from the centre of the genome to the 5′ UTR to minimize the risk of the genetically modified domain V region being removed by recombination.

The key to nOPV safety lies in the low level of reversion at key known sites. Consistency has therefore been monitored by molecular means rather than animal tests – though animal tests are retained as a final check. Because nOPV strains have different properties to the classical Sabin strains with respect to optimal growth conditions, the production and quality control of vaccines made from them may differ from those made using classical Sabin strains. Such differences could include growth and titration properties, optimal temperature of growth, dose required, thermal stability and other parameters. The nucleotide sequence of the nOPV2 strain is available in GenBank[9] (accession number MZ245455) and a graphical representation of its structure is provided below in Fig. 3 of Appendix 1. Similar nOPV1 and nOPV3 strains are currently in early clinical development and may be used in future trivalent formulations. Such

[9] https://www.ncbi.nlm.nih.gov/nucleotide/

novel strains are of great importance to the polio eradication programme and are therefore considered in these revised WHO Recommendations.

Trivalent formulations of conventional (Sabin) OPV were created in the early 1960s to ensure that the immune response against all three poliovirus serotypes was adequate. However, subsequent studies demonstrated that the Sabin 2 virus had higher fitness and interfered with the immunogenicity of serotypes 1 and 3, leading to lower seroconversion (*33*). In 2008, a clinical trial to evaluate the immunogenicity of alternative OPV formulations (mOPV1, mOPV3 and bOPV) compared to tOPV was conducted in India by WHO. Seroconversion rates to poliovirus type 1 and type 3 following immunization with bOPV were significantly higher than those induced by tOPV, and were not lower than those induced by immunization with either mOPV1 or mOPV3 alone (*34*). The introduction and widespread use of mOPV1 and mOPV3 in supplementary immunization activities in 2005 led to substantial reductions in cases caused by the respective serotypes. This resulted in the cessation of WPV1 circulation in India and to WPV3 eradication worldwide in 2019. However, the continued circulation of WPV1 in the two remaining polio-endemic countries still requires huge quantities of bOPV to be given in routine and mass campaigns conducted in around 140 countries throughout the world.

In addition to bOPV, which is used in most countries for routine or supplementary vaccination, mOPVs of all three serotypes are used by the Global Polio Eradication Initiative (GPEI)[10] and have been licensed for use in endemic countries and for outbreak control in situations where one or two types can re-emerge. In 2020, SAGE recommended that tOPV be made available to countries for cVDPV2 outbreak response in subnational areas in which there was co-circulation (or a high risk of co-circulation) of cVDPV2 with cVDPV1, cVDPV3 or WPV1 instead of dual mOPV2 and bOPV campaigns (*35*). As a result, there is still a need for all current formulations of OPV.

Live vaccines prepared from Sabin poliovirus strains of types 1, 2 and 3 were introduced for large-scale immunization in 1959. In 1972, Sabin proposed that WHO should become the custodian of his poliovirus seed strains. The Director-General of WHO agreed to assume responsibility for ensuring the proper use of the strains, and established the Consultative Group on Poliomyelitis Vaccines to advise WHO on all matters pertaining to their use. Detailed information on the work of the Consultative Group and on the preparation of the seed stocks made by Behringwerke has been published (*36*). NRAs should decide on the use of virus strains and on the detailed procedures applicable to the preparation of virus seed lots for the production of OPV in their own countries.

[10] https://polioeradication.org/

The Sabin Original (SO) poliovirus seeds (*26*) were sent on to Merck which generated seeds designated as Sabin Original Merck (SOM). Aliquots of SOM were supplied to a number of other manufacturers to enable them to develop their own seeds. Some seed lots were contaminated with SV40 which had been present in the primary monkey kidney cells that were the preferred cell culture system at that time for virus propagation. OPV manufacturers used various strategies to reduce the contamination, including passage in the presence of specific antibody, treatment with toluidine blue and thermal inactivation of SV40 in the presence of 1M $MgCl_2$ that stabilizes poliovirus. In 1974 Behringwerke AG of Marburg/Lahn, Germany generously agreed to produce SO+1 seeds for WHO free of charge. The Behringwerke type 1 and type 2 seeds have been widely used from the 1970s up to the present time. In the 1950s, it had been established that, particularly for the type 3 strain, an increase in passage number correlated with increased reactivity in the MNVT. This finding led to the establishment of rigorous limits on the passage level used for vaccine production for all types.

In order to develop a more stable type 3 strain, a new seed was prepared by Pfizer from a single plaque after transfecting susceptible cells with viral RNA extracted from poliovirus at the SO+2 level. This also reduced any residual risk of SV40 contamination. One plaque (designated 457-III) was identified with particularly favourable properties (*25*). Theoretically, vaccine derived from this stock was at passage SO+7. However, the purpose of tracking the passage history of seed viruses is to reduce the accumulation of mutations that occur during their serial propagation. Since plaque purification represents the cloning of a single infectious particle, it eliminates the heterogeneity of the viral population and the passage level is effectively reset to zero. Thus, the cloned stock 457-III was renamed RNA-plaque-purified Sabin Original (RSO). Two additional passages were used to prepare virus master seeds (RSO+1) and working virus seeds (RSO+2), with vaccines produced from this virus at RSO+3 level. Retrospectively, the consensus sequence of RSO has been shown to be the same as the consensus sequence of SO (*37*) but it is more homogeneous and contains lower quantities of viruses with sequence polymorphisms. Consensus sequences of all three Sabin strains are available in GenBank under accession numbers AY184219, AY184220 and AY184221.

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

The virus seeds available from WHO ("WHO master seeds") are therefore types 1 and 2 at SO+1 level produced by Behringwerke from SO seeds and the type 3 RSO "Pfizer" seed donated by Sanofi Pasteur. The seeds are kept at the Medicines and Healthcare products Regulatory Agency (MHRA) in the United Kingdom, and the U.S. Food and Drug Administration Center for Biologics Evaluation and Research (CBER) in the United States, and include a proportion of the stocks of the SO+1 seeds formerly held at Istituto Superiore di Sanità in Italy which kindly transferred them (*25*, *36*). These virus seed stocks are available to vaccine manufacturers upon request to WHO.

In addition to the RSO type 3 seed, a number of manufacturers in China, Japan and the Russian Federation have used their own purified seed stocks of Sabin 3 strain that were derived by a combination of passage and plaque purification (cloning). Sequencing of these seed viruses demonstrated that, while they contained low quantities of neurovirulent mutants, there were differences at other genomic sites between these strains and the consensus sequence of SO virus in the form of single nucleotide polymorphisms (SNPs) (*36*). However, there are no reports of any differences in clinical safety or immunogenicity between OPV produced from Pfizer stocks and from the alternative seeds of Sabin 3 virus. An overview of virus seeds used in OPV production is given below in Appendix 1.

The MNVT described in the 1989 WHO Requirements (*5*) has been used as a quality control test and is based on the level and distribution of virus-specific lesions within the central nervous system produced by vaccine virus upon intraspinal inoculation into the anterior horns of rhesus or cynomolgus monkeys compared against an appropriate reference preparation (*38*). Because nonhuman primates are used, efforts to complement and eventually replace the test are of considerable importance. WHO has encouraged and supported research on various aspects of poliovirus biology, including the development of alternative animal models, as part of the WHO initiative to promote the development of new norms and standards for vaccines. Two groups of scientists have developed transgenic (TgPVR) mice by introducing the human gene encoding the cellular receptor for poliovirus into the mouse genome (*39*, *40*). This receptor, known as CD155, renders TgPVR mice susceptible to poliovirus infection, with clinical signs of flaccid paralysis along with histological lesions in the central nervous system similar to those observed in monkeys.

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

- a reduction in the number of primates used in the quality control of OPV;
- the use of animals of highly defined genetic and microbiological quality standards;

- a reduction in the hazards to laboratory personnel associated with handling primates; and
- a reduction in the time and cost of quality control tests for OPV.

Studies were carried out initially on type 3 mOPV using the TgPVR21 mouse line generously provided free of charge for the study by the Central Institute for Experimental Animals in Japan. Researchers at CBER developed an intraspinal inoculation method suitable for testing vaccine lots. The method was then evaluated in an international collaborative study (*41*) and the results assessed by WHO during a series of meetings held between 1995 and 1999. As a result of these studies, the revised WHO Recommendations for the production and control of poliomyelitis vaccine (oral) (*9*) introduced the murine model as an alternative to the MNVT for type 3 poliovirus. Further studies subsequently demonstrated that this test was also suitable as an alternative to the MNVT for poliovirus types 1 and 2 (*12*). In all cases, laboratories must comply with specifications for the containment of transgenic animals (*42*).

The molecular mechanisms and genetic determinants of attenuation and reversion to virulence of all three types of Sabin polioviruses used for the manufacture of OPV have been the subject of several studies. As discussed above in the context of nOPV, evidence strongly suggests that mutations in domain V of the internal ribosome entry site in the 5′ UTR of the poliovirus genome are critical determinants of attenuation and reversion (*43*). To quantify reversion at the molecular level, the MAPREC assay was developed by researchers at CBER (*44*). Studies showed that all batches of type 3 OPV contained measurable amounts of revertants, with C instead of U at nucleotide 472. Batches that failed the MNVT contained significantly higher quantities of 472-C than batches that passed the test. The CBER MAPREC studies identified 100% of vaccine lots that failed the MNVT (*45*).

In 1991, WHO initiated a series of international collaborative studies to evaluate the MAPREC assay for use with all three types of polioviruses and to validate appropriate international reference materials. Study results were assessed by WHO at two meetings held in 1995 and 1997 and it was concluded that the MAPREC assay was a sensitive, robust and standardized molecular biological assay suitable for use by manufacturers and NRAs for monitoring the consistency of OPV3 production. As a result, the subsequently revised WHO Recommendations for the production and control of poliomyelitis vaccine (oral) (*9*) introduced the MAPREC assay as the in vitro test of preference for OPV3 in place of the rct40 test. In addition, international reference materials for the MAPREC assay were established for all three serotypes. For type 3, the WHO international standard defines the threshold of 472-C content above which vaccine lots will have a high probability of failing the MNVT. Reference materials

for type 1 and type 2 are used to provide a measure of production consistency, but do not define the pass/fail threshold because the amount of domain V mutants that would cause the vaccine preparations to fail the MNVT is much higher than the amount found in production lots.

High-throughput sequencing (HTS), also known as deep sequencing or next generation sequencing, is a powerful technique with potentially numerous applications in the regulation of biological products. Classical (Sanger) sequencing determines the consensus or average sequence of a population of nucleic acid molecules, whereas HTS determines the sequence of individual molecules in a population. HTS generates multiple reads of each base position and produces large amounts of sequence data very rapidly. Although the technology is still evolving rapidly, determining the sequence of complete viral genomes is relatively straightforward, and usually involves amplifying sequences by polymerase chain reaction (PCR) using primers which may be either specific for a given sequence or random to pick up any nucleic acid sequence present. HTS could therefore be used in principle to detect adventitious agents whose presence was not even suspected. As HTS determines the sequence of individual molecules, it will also detect minority populations and polymorphisms so that revertants can be accurately quantitated. HTS therefore has applications in the quality control of live vaccines and could reduce the need for in vivo testing by demonstrating consistency of production on a previously impossible scale.

The bioinformatic analysis required for HTS is significant and the validation of the method for a specific purpose remains a major issue. However, it would be possible to determine if the frequency of a particular mutation – that is, a single nucleotide polymorphism (SNP) – varied between production runs. It remains to be determined to what extent this occurs and what limits could be allowed for the runs to be acceptable. In the context of OPV, HTS could be a replacement for MAPREC when used to monitor the frequency of one or two particular mutations, and studies are underway to validate this application. Early evidence indicates that HTS can be used to accurately measure the 472-C content of type 3 OPV lots and could provide an alternative to the MAPREC assay (*46*, *47*). Whole genome HTS has the potential to become a unique tool for determining product consistency and has already been extensively applied during nOPV development, where it is a potentially more sensitive procedure for monitoring product consistency than animal neurovirulence testing.

Further developmental work needs to be completed before HTS can be introduced for general regulatory purposes. At its meeting in 2019, the WHO Expert Committee on Biological Standardization recommended that a study be performed to explore the utility of HTS technology for the quality control of OPV made from Sabin strains. Study results indicated that HTS could accurately quantify 472-C mutants in monovalent bulks of OPV3 and in the final

product (*48*). A second phase of the same study showed that HTS could also accurately quantify mutations of 480-A/525-C and 481-G for OPV1 and OPV2, respectively (*49*). The results generated by HTS and MAPREC methods were very well correlated (*48–50*) indicating that HTS could in principle be used as an alternative to MAPREC, providing an appropriate test format and analytical processes for establishing assay validity and pass/fail criteria were agreed with the NRA.

HTS makes it possible to conduct whole-genome sequencing on a routine basis. The degree of sequence heterogeneity expressed in terms of the number of SNPs at nucleotide positions in the genome not necessarily linked to any tangible biological properties provides a unique molecular "fingerprint" for a particular virus preparation. HTS is thus ideally suited to generating quantitative whole-genome SNP profiles of individual vaccine lots that can be used to identify types of polio seed virus and monitor consistency of manufacture. After appropriate validation and the establishment of manufacturing consistency, quantitative whole-genome SNP profiles of OPV lots could be used for routine lot release instead of the MNVT or TgmNVT. In all cases, appropriate acceptance criteria would need to be approved by the NRA.

The manufacturer of the final lot must be responsible for ensuring conformity with all recommendations applicable to the final vaccine (see sections A.5–A.11 below) even where manufacturing involves only the filling of final containers with vaccine obtained in bulk form from another manufacturing establishment. 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.

OPV has been in worldwide use since the 1960s and experience has shown that human diploid cells, primary monkey kidney cells and continuous cell lines derived from them (Vero cells) can be used to produce safe and effective vaccines.

In 1986, a WHO study group (*7*) concluded that the risks posed by residual cellular DNA (rcDNA) in vaccines produced in continuous cell lines should be considered to be negligible for preparations given orally. This conclusion was based on the finding that polyoma virus DNA was not infectious when administered orally (*51*). For such products, the principal requirement is the elimination of potentially contaminating viruses. Additional data on the uptake of DNA via the oral route have been published (*52*). These studies demonstrated that the efficiency of uptake of DNA introduced orally was significantly lower than that of DNA introduced intramuscularly. Nevertheless, the specifics of the manufacturing process and the formulation of a given product should be considered by NRAs (*23*) and, where possible, data should be accumulated on the levels of rcDNA in OPV produced in Vero cells.

International reference materials

A number of WHO international reference materials are available to help ensure that the manufacture and quality control testing of OPVs meet appropriate regulatory requirements.

WHO international standards for the potency testing of tOPV have been available since 1995. More recent WHO international standards have also been established for bOPV, mOPV1, mOPV2 and mOPV3, with compositions and potencies similar to the vaccines needed for the final phase of the GPEI. Additionally, low-titre monovalent type 1, 2 and 3 poliovirus WHO reference reagents are available for use by reference laboratories to measure the sensitivity of cell cultures to poliovirus infection. A WHO international standard for anti-poliovirus types 1, 2 and 3 antibodies (human) is also available for the standardization of neutralizing antibody tests for poliovirus.

In addition, WHO international standards for MAPREC analysis of poliovirus types 1, 2 and 3 (Sabin) and WHO international reference reagents for the control of MAPREC assays of poliovirus type 1, 2 and 3 (Sabin) are available. Some of these reference materials might be appropriate for use in HTS assays for Sabin OPV upon suitable validation. Alternatively, new reference materials may be needed for this purpose.

Reference preparations at the SO+2 passage level – designated WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3 virus – are available upon request through WHO. These reference preparations are intended for use during in vivo neurovirulence testing of OPV, both in monkeys and transgenic mice. The relevant reference preparation should be included in each vaccine test (see section A.4.4.7.2 below). Virus panels for the validation and implementation of transgenic mouse neurovirulence testing, as specified in the relevant WHO SOP,[11] are also available.

New non-pathogenic, hyper-attenuated S19 strains of all three serotypes of poliovirus are available for conducting neutralization assays. S19 strains are polioviruses that replicate in tissue culture but are unlikely to replicate in humans, even in those exposed to large amounts, and for this reason can be used outside GAPIV containment requirements.

Some of the reference preparations developed for Sabin OPV might also be suitable for nOPV assays following suitable validation but the establishment of nOPV-specific reference preparations may be required.

The above reference materials are available from MHRA.[12] Full details of these materials, including literature references, are provided in Appendix 8.

---

[11] Available at: https://www.who.int/publications/m/item/neurovirulence-test-sop-of-types-1-2-or-3-opv-in-transgenic-mice-susceptible-to-poliovirus-v8.

[12] Medicines and Healthcare products Regulatory Agency, Potters Bar, United Kingdom: https://www.nibsc.org/.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be "poliomyelitis vaccine (oral, live, attenuated)" with additions to indicate the virus serotype or serotypes of the vaccine and whether the vaccine is a novel or Sabin OPV. The proper name should be the equivalent of the international name in the language of the country in which the vaccine is licensed.

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

A.1.2 Descriptive definition

Poliomyelitis vaccine (oral, live, attenuated) is a preparation of live attenuated poliovirus types 1, 2 or 3 grown in in vitro cultures of suitable cells containing any one type or any combination of the three types of the Sabin strains or novel genetically stabilized attenuated strains, presented in a form suitable for oral administration, and satisfying all of the recommendations set out below, as applicable.

A.2 General manufacturing recommendations

The general guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (*53*) and WHO good manufacturing practices for biological products (*22*) should apply in establishments where OPV is manufactured, with the addition of the following recommendations:

- The production of OPV should be conducted by staff who are healthy and who are examined medically at regular intervals. Steps should be taken to ensure that all individuals in the production areas are immune to poliomyelitis. Personnel working in monkey quarters should also be examined for tuberculosis as outlined in Part A, section 2 of the WHO Recommendations to assure the quality, safety, and efficacy of BCG vaccines (*54*).
- The establishment should be in compliance with the current global recommendations for poliovirus containment (*16–18*, *55*).

A.3 Control of source materials

General production precautions, as described in WHO good manufacturing practices for biological products (*22*) should apply to the manufacture of OPV, with the additional recommendation that during production only one type of cell should be introduced or handled in the production area at any given time.

A.3.1 Cell lines

Some licensed OPV products are produced in primary monkey kidney cells (see Part E below). However, new OPV manufacturers are encouraged to use cell lines such as MRC-5 and Vero cells for vaccine production (*23*).

A.3.1.1 Master cell bank (MCB) and working cell bank (WCB)

The use of a cell line for OPV manufacture should be based on the cell bank system. The cell seed and cell banks used should comply with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*23*). The cell bank should be approved by the NRA. The maximum number of passages (or population doublings) allowed between the cell seed, the MCB, the WCB and the production passage level should be established by the manufacturer and approved by the NRA. Additional tests may include, but are not limited to, propagation of the MCB or WCB cells to or beyond the maximum in vitro age for production, and examination for the presence of retrovirus and tumorigenicity in an animal test system (*23*).

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

> The WHO Vero reference cell bank 10-87 is considered suitable for use as a cell seed for generating an MCB (*23*) and is available to manufacturers on application to the Group Lead, Norms and Standards for Biologicals, Technical Specifications and Standards, Department of Health Product Policy and Standards, Access to Medicines and Health Products Division, World Health Organization, Geneva, Switzerland.

A.3.1.2 Identity test

Identity tests on the MCB and WCBs should be performed in accordance with the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*23*).

The cell banks should be identified using tests such as biochemical tests, immunological tests, cytogenetic marker tests and DNA fingerprinting or sequencing (*23*). The tests used should be approved by the NRA.

A.3.1.3 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacterial, fungal and mycoplasmal contamination using appropriate tests

– as specified in Part A, sections 5.2 (*56*) and 5.3 (*57*) of the WHO General requirements for the sterility of biological substances – as well as freedom from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (*23*).

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 bacteriophages 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. These components should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*58*).

Human serum should not be used. If human serum albumin derived from human plasma is used at any stage of product manufacture, the NRA should be consulted regarding the relevant requirements, as these may differ from country to country. At a minimum, it should meet the WHO Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (*59*). In addition, human albumin should also comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*58*).

Penicillin and other beta-lactams should not be used at any stage of manufacture, as they are highly sensitizing substances. If well justified, other antibiotics may be used during early stages of production, and should be cleared during the downstream manufacturing process. Clearance should be demonstrated through a residual removal study (or studies) and acceptable residual levels should be approved by the NRA (*22*).

Nontoxic pH indicators may be added – for example, 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 cultivable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate (*23*). The methods used to ensure this should be approved by the NRA.

> In some countries, irradiation is used to inactivate potential contaminant viruses in trypsin. 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 so that the biological properties of the reagents are retained while being high

enough to reduce viral contamination. Therefore, irradiation cannot be considered a sterilizing process (*23*). The irradiation method should be validated by the manufacturer and approved by the NRA.

Recombinant trypsin is available and OPV manufacturers are encouraged to use it due to the reduced risk of contamination compared to animal-sourced trypsin – however, it should not be assumed to be free of the risk of contamination and should be subject to the usual considerations for any reagent of biological origin (*23*).

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* (*58*).

A.3.2 Virus seeds

A.3.2.1 Virus strains

Strains of poliovirus used in the production of OPV should be identified by historical records. These should include information on strain origin and subsequent manipulation or passage, including any recombinant DNA technology steps used to modify the viral genome, when applicable.

In addition, it is recommended that the presence of sequence heterogeneities across the entire genome of an OPV virus strain is determined by HTS and documented as a reference for future characterization of the virus seed lots.

Producers of Sabin OPV can obtain virus master seeds from WHO. Manufacturers receiving this virus master seed may prepare a sub-master seed by a single passage and then prepare their working seed from the characterized sub-master seed. Currently, nOPV strains are only available from the respective developers.

Only virus strains approved by the NRA should be used (see **General considerations** above).

A.3.2.2 Virus seed lot system

Vaccine production should be based on the seed lot system. Virus seed lots should not be purified. The virus working seed lot used for the production of vaccine batches should be prepared by a single passage from the virus master seed lot or the virus sub-master seed lot (if used) using a method, and at a passage level from the original seed virus, approved by the NRA.

Virus master, sub-master and working seed lots should be stored as recommended in WHO good manufacturing practices for biological products (*22*) – for example, in temperature-monitored freezers at −60 °C or below to

ensure stability on storage. Guidance on the additional characterization of master and sub-master seeds is provided below in Part B.

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

The Sabin virus master seeds provided by WHO are well characterized and can be used to prepare sub-master seeds using the approved process. All other virus seed lots used for the production of OPV batches (including any sub-master seed derived from the WHO master seed) should be subjected to the tests listed in this section and should conform to the recommendations set out below in sections A.4.3 (single harvests) and A.4.4.1–A.4.4.4 (monovalent bulks). The control cell cultures for virus seed production should conform to section A.4.1 below (control of cell cultures).

A.3.2.3.1 *Tests for adventitious viruses and freedom from detectable SV40 sequences*

The virus seed lots should be shown to be free from detectable adventitious viruses and from detectable SV40 DNA when applicable as determined by a validated nucleic acid amplification technique (NAT)-based assay. The need to test for SV40 DNA, and other human, simian, bovine or porcine adventitious agents, should be based on risk assessment of potential contamination of the cell substrates used to propagate the virus, as well as the risk of adventitious agents being inadvertently introduced through the use of raw materials – for example, animal-derived culture medium components. If necessary, viruses such as bovine polyomavirus, porcine parvovirus and porcine circovirus should be screened for using specific assays, such as molecular NAT-based assays (*23*).

> SV40 DNA is widely used as a molecular biological reagent and the contamination of PCR assays is potentially a major problem. One approach is to identify separate genomic regions of SV40 for amplification and to use one region for screening purposes and the other for the confirmation of repeatedly positive samples. It is useful if the 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 for the genomic regions used should be established.
>
> New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: (a) degenerate 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) HTS. 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 (*23*).

The testing strategy for adventitious virus(es) in seed lots should be based on risk assessment. However, sterility testing for bacteria, fungi and mycoplasmas should always be conducted.

A.3.2.3.2 *In vitro tests to monitor virus molecular consistency*

New virus seed lots used for OPV production should be evaluated for molecular consistency using a suitable test (such as an HTS assay) and should meet the acceptance criteria approved by the NRA. Virus seeds prepared from Sabin strains may be evaluated using the MAPREC assay and should meet the acceptance criteria described below in section A.4.4.7.1. In addition, at least three consecutive monovalent bulks prepared from the new seed virus should meet the acceptance criteria of the applicable in vitro test described in section A.4.4.7.1. Where an HTS assay is used it should be validated using appropriate standards and materials, and acceptance criteria approved by the NRA. At this point, the use of HTS remains developmental and is the subject of international collaborative evaluation that may result in the establishment and availability of appropriate reference materials with defined acceptance criteria.

The acceptance criteria for percentage of mutations at positions that are not examined by MAPREC but found to be variable under the conditions used by the manufacturer should be based on the molecular characteristics of vaccine batches shown to be safe and immunogenic in clinical studies. The acceptance criteria of an HTS assay should be updated periodically based on manufacturing experience. Acceptance criteria should be approved by the NRA.

nOPV seeds and at least three consecutive monovalent bulks prepared from each new working seed should be characterized using an HTS assay, with particular attention given to the regions of the genome that are modified in the parental nOPV strain compared to the Sabin OPV strain. The genetic modifications introduced in domain V of the 5′ UTR of nOPV include changes in specific base pairs of the hairpin structure where GC and GU pairs are replaced by AU base pairs. Strengthening of the hairpin structure leading to neurovirulent reversion would require two simultaneous mutations, and the frequency of such double reversions should be minimal. HTS analysis should therefore be conducted to ensure the absence of undesirable modifications in the 5′ UTR, with particular attention given to changes in base pairing in domain V.

A.3.2.3.3 *Neurovirulence tests for virus seeds prepared from Sabin strain*

New virus seeds prepared from Sabin strains (with the exception of the well-characterized WHO master seed) should be evaluated for neurovirulence using the MNVT or TgmNVT. Summaries of the MNVT and TgmNVT, including pass/fail criteria, are given below in Appendix 2 along with considerations in the choice of assay. The test used should be approved by the NRA for the specific product.

The test for neurovirulence in nonhuman primates should be carried out as summarized in Appendix 2 and following the WHO SOP for the neurovirulence test of types 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in monkeys, available from WHO.[13]

Under normal circumstances, a new virus working seed will be prepared using the same production protocol and from the same virus master seed or sub-master seed as the currently approved virus working seed. If the TgmNVT has been approved by the NRA for the release of vaccine batches, and if the virus working seed is generated by the same production process, the new seed can be qualified by use of the transgenic mouse test and supporting in vitro data alone. The TgmNVT should be carried out as summarized in Appendix 2 and following the WHO SOP for the neurovirulence test of types 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus.[14]

Where there are any major changes in the production process for a new virus working seed or virus sub-master seed, full characterization using an in vivo neurovirulence test and HTS assay will be required (see Part B below).

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

A.3.2.3.4 *Neurovirulence tests for nOPV virus seeds*

The virus seed lot used for nOPV production should be evaluated for neurovirulence. The testing strategy (for example, testing of master virus seed and/or working virus seed) and the method selected (MNVT and/or TgmNVT) should be approved by the NRA. The in vivo neurovirulence test should be carried out as summarized in Appendix 2 below and in the applicable SOP

[13] Available at: https://www.who.int/publications/m/item/neurovirulence-test-of-types-1-2-or-3-opv-in-monkeys.

[14] Available at: https://www.who.int/publications/m/item/neurovirulence-test-sop-of-types-1-2-or-3-opv-in-transgenic-mice-susceptible-to-poliovirus-v8.

available from WHO.[15] The current WHO reference preparation for the MNVT derived from Sabin strain (see Appendix 8 below) is suitable for evaluating the neurovirulence of nOPV virus seeds and vaccine batches.

It is likely that molecular assays will be more sensitive than the animal tests used to justify the limits chosen. All nOPV producers should generate data to support the replacement of in vivo neurovirulence tests with HTS for evaluating neurovirulence in nOPV seeds and vaccine batches by examining the entire genome. The acceptance criteria for percentage of mutations should in the first instance be based on the molecular characteristics of vaccine batches shown to be safe in clinical studies and that have met the in vivo neurovirulence test acceptance criteria. Specifications are likely to change with experience. The data generated will be used to demonstrate consistency and in the longer term the acceptable limits should be set on this basis.

A.4 Control of vaccine production

For OPV prepared in cultures of primary monkey kidney cells, Part E below provides additional or alternative recommendations regarding the testing of the cell substrate used for vaccine production. The guidance provided in Part E should therefore be added to – or used as an alternative to – the relevant guidance provided in this section.

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. An example flowsheet of the cell culture tests performed during OPV production using cell banks is provided below in Appendix 3.

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 the 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 adventitious agents.

The 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

[15] Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis.

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 any reason by the end of test period.

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 2–8 °C. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

> Some NRAs require that, as an additional test for haemadsorbing viruses, other types of red blood cells, including cells from humans, 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 °C for 30 minutes, and again after further incubation for 30 minutes at 20–25 °C.

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

> In some countries the sensitivity of each new lot 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 fluids

At the end of the observation period, a sample of the pooled supernatant fluid from each group of control cultures should be tested for adventitious agents. For this purpose, 10 mL of each pool should be tested in the same cells, but not the same batch of cells, as those used for the production of 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 (*23*).

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 monolayer should be at least 3 cm^2 per 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 should be observed for at least 14 days.

> Some NRAs require that, at the end of this observation period, a subculture is made in the same culture system and observed for at least an additional 14 days. Furthermore, some NRAs require that these cells 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 any reason 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 for using specific validated assays approved by the NRA – such as assays based on molecular techniques (for example, NAT or HTS) (*23*).

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 test

At the production level, the cells should be identified by means of tests approved by the NRA. Suitable methods include, but are not limited to, biochemical tests (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 such examination shows evidence of the presence 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.

A.4.3 Control of single harvests

A.4.3.1 Single harvest

After inoculation of the production cells with the virus working seed lot, inoculated and control cell cultures should be held at a fixed temperature that has been shown to be suitable – for example, within the range 33–35 °C for Sabin strains. The temperature range required to produce a consistent satisfactory product for nOPV manufacture may be different and should be validated.

The temperature should be controlled within a narrow range (for example, ± 0.5 °C from the set temperature). The optimal range for pH, multiplicity of infection, cell density, duration of incubation, and time of virus recovery should be established by each manufacturer and should be approved by the NRA.

The incubation time of the viral culture should be established and validated for each OPV during product development. The virus suspension of Sabin strain should be harvested not later than 96 hours after virus inoculation to limit the number of replication cycles.

Similar appropriate limits should be investigated and set for nOPV harvests.

The inoculated cell cultures should be processed in such a way that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until the results of all the tests described in sections A.4.1.2–A.4.1.4 and sections A.4.3.3.1–A.4.3.3.5 have been obtained. If pooling of single harvests takes place before all the testing results become available, the practice should be justified and a procedure should be put in place to ensure that a monovalent bulk is discarded if any one of the pooled single harvests does not meet all the approved specifications.

A.4.3.2 Sampling

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

A.4.3.3 Tests on single harvest

A.4.3.3.1 *Identity*

Each single harvest should be identified using a suitable method, such as an immunological assay on cell culture using specific antibodies or a molecular method that has been validated and approved by the NRA. If the virus seeds used for production and other poliovirus strains are manipulated or stored at the same production facilities, the identity test should be able to distinguish between these strains as well as distinguish between different poliovirus serotypes.

Neutralization tests can distinguish the serotypes of poliovirus. Molecular methods such as sequencing, HTS or qPCR can distinguish different strains and serotypes of poliovirus.

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.

A.4.3.3.2 *Titration for virus content*

The virus titre per mL of single harvest should be determined in cell cultures in comparison with an existing reference preparation (see Appendix 4), and the result should meet the specification approved by the NRA.

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

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

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

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

Animal serum may be used in the propagation of the cells, but the maintenance medium used after inoculation of the test material should contain no added serum other than the poliovirus neutralizing antiserum or fetal calf serum of controlled origin.

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

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

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

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

> New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: (a) degenerate 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) HTS. These methods might be used 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 (*23*).

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

A volume of at least 10 mL of each single harvest should be tested for bacterial, fungal and mycoplasmal contamination using appropriate tests, as specified in Part A, sections 5.2 and 5.3 of the WHO General requirements for the sterility of biological substances (*56*, *57*), or by methods approved by the NRA.

> Molecular assays (for example NAT-based assays alone or in combination with cell culture) may be used as an alternative to one or both of the compendial mycoplasma detection methods following suitable validation and with the agreement of the NRA (*23*).

A.4.3.3.5 *Test for mycobacteria*

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

> Molecular assays (for example, NAT-based assays) may be used as an alternative to mycobacteria microbiological culture method tests for the detection of mycobacteria following suitable validation and with the agreement of the NRA (*23*).
>
> Some manufacturers test for mycobacteria only at the monovalent bulk stage with the agreement of the NRA.

A.4.3.3.6 *Tests for molecular consistency of production*

OPV producers may monitor the molecular characteristics of single harvests or monovalent bulks using an in vitro test as described in A.3.2.3.2 above. These data may further demonstrate manufacturing consistency.

A.4.4 Control of monovalent bulk

A.4.4.1 Preparation of monovalent bulk

The monovalent bulk may be prepared by pooling a number of single harvests of the same virus serotype into a single vessel. This bulk should be filtered through a filter that is able to retain cell debris.

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

A.4.4.2 Sampling

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

A.4.4.3 Identity test

Each monovalent bulk should be identified using a suitable method, as described in section A.4.3.3.1 above.

A.4.4.4 Titration for virus content

The virus titre per mL of filtered monovalent bulk should be determined in cell cultures in comparison with an existing reference preparation (see Appendix 4), and the result should meet the specification approved by the NRA.

The virus titre as determined by this test should be the basis for the quantity of virus used in the neurovirulence tests in monkeys or transgenic mice (see sections A.4.4.7.2 and A.4.4.7.3 below) and for formulation of the final bulk (see section A.4.5 below).

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

A.4.4.5 Sterility tests for bacteria and fungi

Each monovalent 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 (*56*).

A.4.4.6 Test for mycobacteria

Each monovalent bulk should be shown to be free from mycobacteria by an appropriate method approved by the NRA.

Molecular assays (for example, NAT-based assays) may be used as an alternative to mycobacteria microbiological culture method tests for the detection of mycobacteria following suitable validation and with the agreement of the NRA (*35*).

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

The poliovirus in the filtered monovalent bulk, prepared as described in section A.4.4.1 above, should be tested in comparison with the seed lot or a reference virus preparation (see Appendix 8) to ensure that the vaccine virus has not undergone changes during its multiplication in the production cell culture.

A.4.4.7.1 *In vitro tests to monitor virus molecular consistency*

As with the virus seed lot (see section A.3.2.3.2 above), the virus in the monovalent bulk should also be tested for molecular consistency using at least one in vitro method.

A.4.4.7.1.1 MAPREC

The MAPREC assay is suitable for all three serotypes of Sabin OPV but not nOPV which should be evaluated for molecular consistency using a suitable test such as whole genome HTS. Implementation of the MAPREC assay should be fully validated by each manufacturer and performed according to the WHO SOP[16] developed during WHO collaborative studies or according to a validated alternative procedure.

The MAPREC assay should be used to establish the consistency of production once the test has been validated and normal values for the standards have been established. For all Sabin OPV preparations, and depending on a laboratory's experience with the MAPREC assay, an approach based on "warning limits" of ± 2 standard deviations and "rejection limits" of ± 3 standard deviations from the historical mean may be appropriate. Acceptance and rejection criteria should be specific to each manufacturer and each working seed and should be continually updated as each new bulk is prepared. An investigation of consistency should take place if a batch produces results that are inconsistent with previous production batches.

Results should be expressed as ratios relative to the relevant type-specific WHO international standard for MAPREC analysis of poliovirus (Sabin) (see Appendix 8). The acceptable variation in mutant content from batch to batch should be agreed with the NRA in the light of production and testing experience.

[16] Available at: https://www.who.int/publications/m/item/maprec-sop-for-opv-types-1-2-or-3.

For type 3 (472-C), a batch should be rejected if the level of mutations is above 1.0% when normalized against the international standard. The limits for 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 and 525 C) and less than 1.5% for type 2 Sabin (481-G) (*60*).

If a filtered monovalent bulk fails the MAPREC assay, it cannot be used in the manufacturing of finished product, and an evaluation of the manufacturing process (including the suitability of the virus working seed) should be undertaken and discussed with the NRA. Filtered monovalent bulks that pass the MAPREC assay should be tested subsequently for in vivo neurovirulence (see section A.4.4.7.2 below).

The MAPREC assay for type 3 is highly predictive of in vivo neurovirulence in animal models. No such correlation exists for types 1 and 2 at the level of revertants present in vaccine bulks. For these types, the MAPREC assay results provide a measure of consistency (*60*).

Non-radioactive MAPREC methods are available and may be introduced after validation and with the approval of the NRA.

A.4.4.7.1.2 **HTS**

The MAPREC assay may be replaced by alternative molecular biology methods (such as HTS) that demonstrate an equivalent or better level of sensitivity following validation, and with the approval of the NRA. The current MAPREC reference materials might also be useful for HTS assays for Sabin OPV upon suitable validation. Alternatively, new reference materials might be needed for this purpose.

A.4.4.7.2 *Neurovirulence tests for Sabin OPV*

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

The test should be approved by the NRA for the specific product and may use transgenic mice or nonhuman primates or both. The test for neurovirulence in nonhuman primates should be carried out as summarized below in Appendix 2 and as described in the corresponding WHO SOP.[17]

[17] Available at: https://www.who.int/publications/m/item/neurovirulence-test-of-types-1-2-or-3-opv-in-monkeys.

Where the TgmNVT has been approved by the NRA, it should be carried out as summarized in Appendix 2 and as described in the corresponding WHO SOP.[18] Its use for batch release purposes should follow the appropriate validation and implementation processes according to national and international regulations. The WHO SOP has been validated for vaccines made from Behringwerke SO-derived seeds (types 1 and 2) and RSO seeds (type 3).

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

The WHO collaborative study demonstrated that the MNVT and TgmNVT are equivalent for testing vaccines prepared from RSO seeds but that the TgmNVT may fail otherwise acceptable (by the MNVT) lots prepared from derivative strains containing additional mutations (*41*). Therefore, the TgmNVT can be used as a replacement for the MNVT for vaccines made from RSO Sabin 3 strain but may require further validation for other derivative strains. This may include the development of an appropriate homologous reference preparation.

It is possible that the in vivo neurovirulence test can be omitted once manufacturing consistency has been established based on the results of both in vivo and whole genome HTS. However, additional experience and data are required to establish suitable acceptance criteria for whole genome HTS performed for the control of Sabin OPV.

A.4.4.7.3 *Neurovirulence tests for nOPV*

Where the results of manufacturing, preclinical and clinical studies have demonstrated the genetic stability of the attenuation to the satisfaction of the NRA, the in vivo MNVT may be omitted for routine manufacturing control of nOPV with the agreement of the NRA.

Only monovalent bulks that meet the acceptance criteria using a validated HTS assay are used to formulate the final product.

The acceptance criteria for percentage of mutations at positions found to be variable under the conditions used during manufacture should be based on the molecular characteristics of vaccine batches shown to be safe and immunogenic in clinical studies, or vaccine batches that have met the acceptance criteria of an in vivo NVT. When mutations arise at additional positions, a risk assessment should be performed to assess their potential impact on neurovirulence based on current understanding of the genetic basis for attenuation (*29*, *61*). An in vivo NVT should be performed to assess the suitability of the monovalent bulk when required by the risk assessment. The acceptance criteria of the HTS assay

[18] Available at: https://www.who.int/publications/m/item/neurovirulence-test-sop-of-types-1-2-or-3-opv-in-transgenic-mice-susceptible-to-poliovirus-v8.

should be updated periodically based on manufacturing experience, and should be approved by the NRA.

A.4.5 Final bulk

Final bulk may contain one or more serotypes of poliovirus of the same type of strain (Sabin or nOPV). The operations necessary for preparing the final bulk should be conducted in such a way as to avoid contamination of the product.

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

A.4.5.1 Stabilizers

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

All the tests described in sections A.4.3.3 and A.4.4 above should be performed on samples taken before any stabilizers are added where possible.

A.4.5.2 Sterility tests for bacteria and fungi

The final vaccine bulk should be tested for bacterial and fungal sterility, as specified in Part A, section 5.2 of the WHO General requirements for the sterility of biological substances (*56*).

A.5 Filling and containers

The requirements concerning filling and containers given in WHO good manufacturing practices for pharmaceutical products: main principles (*53*) and WHO good manufacturing practices for biological products (*22*) should apply to OPV filled in the final form. Single- and multi-dose containers may be used.

A final filtration may be included just before the filling operations.

The conditions for storage and shipping, as well as the shelf-life, should be supported by adequate stability data and approved by the NRA.

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 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. In general, the specification used for each test of OPV final lot should be supported by the quality attributes of the clinical lots shown to be safe and sufficiently immunogenic in clinical studies and should be approved by the NRA.

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually or mechanically, and those showing abnormalities should be discarded safely according to applicable regulations. Each abnormality should be recorded.

A.6.1.1 Appearance

The appearance of the vaccine should meet the specifications approved by the NRA with respect to its form and colour.

A.6.2 Extractable volume

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

A.6.3 pH

The pH of the final lot should be tested and the result should be within the range shown to be adequate for preserving virus stability.

A.6.4 Identity

An identity test should be performed on at least one labelled container from each final lot using a suitable method as described in section A.4.3.3.1.

A.6.5 Bacterial and fungal sterility

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 (*56*), or using methods approved by the NRA.

A.6.6 Potency

At least three final containers should be selected at random from each final lot and should be individually tested in a single assay. When the vaccine contains more than one poliovirus type, each type should be titrated separately by using appropriate type-specific antiserum to neutralize each of the other types present. The amount of poliovirus of each serotype present in the vaccine, and its total poliovirus content, should be determined. The assay should include a reference material as described below in Appendix 4. The minimum virus titre per human dose should be shown to induce an adequate immune response in clinical studies, and should be approved by the NRA.

An upper limit may be established by each manufacturer to ensure lot-to-lot consistency (for example, based on mean titre $CCID_{50}$ + 3 standard deviations). The upper limit should be approved by the NRA.

Based on available data, it is recommended that the estimated mean virus titres for a single human dose of tOPV prepared from Sabin strain should be not less than $10^{6.0}$ $CCID_{50}$ for type 1, $10^{5.0}$ $CCID_{50}$ for type 2, and $10^{5.5}$ $CCID_{50}$ for type 3, as determined in an assay described in Appendix 4 below. The 95% confidence intervals of the assays should not differ by a factor of more than 0.3 $\log_{10}$ of the estimated number of infectious units in the vaccine. Different potency limits may be acceptable if supported by clinical data.

In 1986, the WHO Region of the Americas began to use a trivalent formulation containing $10^{5.8}$ $CCID_{50}$ of poliovirus type 3 (*62*) following a study in Brazil which demonstrated improved immunogenicity when the amount of type 3 virus in the trivalent vaccine was increased (*63*). The subsequent success in controlling poliomyelitis in the Americas using this formulation led the Global Advisory Group for the Expanded Programme on Immunization to recommend a formulation of tOPV for use worldwide with $10^{6.0}$, $10^{5.0}$ and $10^{5.8}$ $CCID_{50}$ per dose for types 1, 2 and 3 respectively (*35*, *64*).

The potency specifications for nOPV should be set based on the potency of vaccine lots shown to induce adequate protective immunity in clinical trials. An upper limit should also be defined based on available human safety data.

A.6.7 Thermal stability

Thermal stability should be considered as a vaccine characteristic that provides an indicator of production consistency. The thermal stability test is not designed to provide a predictive value of real-time stability but rather to evaluate whether the product complies with a defined stability specification. Additional guidance on the evaluation of vaccine stability is provided in the WHO Guidelines on stability evaluation of vaccines (*65*).

Three final containers of each final lot should be incubated at 37 °C for 48 hours. The total virus content in both exposed and unexposed containers should be determined concurrently with that of a suitable validated reference preparation. The loss of potency on exposure should be within the limit approved by the NRA.

For tOPV prepared from Sabin strain, the vaccine passes the test when the loss on exposure is not greater than a factor of 0.5 $\log_{10}$ $CCID_{50}$ per human dose. Several OPV manufacturers have demonstrated that the thermal stability test specification applied to tOPV formulations (loss on exposure is not greater than a factor of 0.5 $\log_{10}$ $CCID_{50}$ per human dose)

is not applicable to some mOPVs and bOPVs. Some manufacturers have shown that mOPV formulations that failed the current tOPV specification of 0.5 $\log_{10}$ have an acceptable stability profile throughout the product shelf-life. Therefore, a specification of 0.6 $\log_{10}$ has been accepted by the NRAs and by the WHO Prequalification Programme in those cases.

Suitable thermal stability test for nOPV should be established and validated.

A.6.8 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.6.9 Stabilizer (if applicable)

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

A.7 Records

The requirements given in section 17 of WHO good manufacturing practices for biological products (*22*) should apply.

A.8 Retained samples

The requirements given in section 16 of WHO good manufacturing practices for biological products (*22*) should apply.

A.9 Labelling

The requirements given in section 14 of WHO good manufacturing practices for biological products (*22*) should apply.

The label on the carton, the container or the leaflet accompanying each container should include the following information:

- the designation(s) of the strain(s) of poliovirus contained in the vaccine;
- the minimum amount of virus of each type contained in one recommended human dose;
- the cell substrate used for the preparation of the vaccine, and the nature and amount of any stabilizer present in the vaccine;

- a statement that the vaccine is not to be injected;
- the number of doses in each vial; and
- the volume of each dose.

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

A.10 Distribution and shipping

The requirements given in WHO good manufacturing practices for biological products (*22*) should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (*66*).

A.11 Stability testing, storage and expiry date

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. These studies should follow the general principles outlined in the WHO Guidelines on stability evaluation of vaccines (*65*) and WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (*67*). The shelf-life of the final product and the hold time of each process intermediate (such as single harvests, monovalent bulk and final bulk) should be established based on the results of real-time, real-condition stability studies, and should be approved by the NRA.

The stability of the vaccine in its final container, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the NRA on at least three consecutive lots of final product. Accelerated thermal stability tests may be undertaken to provide additional information on the overall characteristics of the vaccine and may also aid in assessing comparability should the manufacturer decide to change any aspect of manufacturing.

The formulation of the vaccine should be shown to minimize potency loss throughout its shelf-life. In case of potency loss (for example, when stored at 2–8 °C for 6 months), the manufacturer should implement a higher potency limit at release to ensure that all vaccine lots released will meet the minimum potency specification at the end of shelf-life as described in the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (*67*). 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 (*65*). The ongoing stability testing programme should be approved by the NRA and should include an agreed set of stability-indicating parameters, procedures for the ongoing collection of stability data and criteria for the rejection of vaccine(s). Data should be provided to the NRA in accordance with local regulatory requirements.

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

Any extension of the shelf-life should be based on stability data and approved by the NRA.

A.11.2 Storage conditions

Before being released by the manufacturing establishment, all vaccines in final containers should be kept continuously at a temperature that minimizes potency loss (for example, in the frozen state at a temperature below −20 °C).

To facilitate vaccine distribution, OPV may be stored at a higher temperature for a specified period during shipping and distribution in the field – for example, at 2–8 °C for 6 months. In addition, during manufacturing, shipping or in the field, the vaccine may be thawed and refrozen. Manufacturers should conduct real-time and real-condition stability studies to support the storage conditions at different temperatures as well as the maximum permitted number of freeze–thaw cycles. The stability data should demonstrate that the vaccine conforms to the requirements of potency until the expiry date stated on the label, as approved by the NRA.

A.11.3 Expiry date

The expiry date should be based on the shelf-life as supported by the stability studies and approved by the NRA. The start of the dating period should be specified (for example, based on the date of filling or the date of the first valid potency test on the final lot) and should be approved by the NRA.

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

The nonclinical evaluation of candidate poliomyelitis vaccines (oral, live, attenuated) should be based on the principles outlined in the WHO guidelines on nonclinical evaluation of vaccines (*20*) which provide guidance on the design, conduct, analysis and evaluation of nonclinical studies. In addition, all changes made to a product post-approval should follow the requirements listed in the WHO Guidelines on procedures and data requirements for changes to approved

vaccines (*68*). The following specific issues should be considered in addition to the tests described above in section A.3.2.3 following a change in the virus seed.

B.1 Characterization of a new Sabin virus sub-master seed

In the event that a new Sabin virus sub-master seed is prepared by a single passage from a well-characterized master seed (including the WHO master seed) it should be subjected to extensive characterization. This should include evaluation of at least one virus working seed and three monovalent bulks derived from it, as described in section A.4.4.7 above. Characterization studies must include the evaluation of identity by complete nucleotide sequencing to prove that the new sub-master seed consensus sequence is identical to conventional Sabin master seeds and that the mutational composition (for example, in MAPREC) is consistent. HTS should be undertaken to evaluate the heterogeneity of the virus sequence. These approaches have not yet been formally validated with the exception of the MAPREC assays for base positions in the 5′ UTR of type 3 OPV, as described in section A.4.4.7.1 above. A new virus sub-master seed should be tested for neurovirulence using the MNVT or TgmNVT, subject to the approval of the NRA. Summaries of the MNVT and TgmNVT are provided below in Appendix 2, along with considerations in the choice of assay.

B.2 Characterization of virus seeds for the production of nOPV

Virus strains used for the production of nOPV were constructed using recombinant DNA technology and are genetically stabilized attenuated strains designed based on current knowledge of the molecular mechanisms of attenuation and reversion of poliovirus. The virus master, sub-master (if applicable) and working seed lots used to manufacture a candidate nOPV should be subjected to extensive characterization. This should include evaluation of at least three monovalent bulks derived from the working seed, as described in section A.4.4.7 above. In addition, the genetic stability of the strains used for nOPV production should be confirmed at least at the passage level (or beyond) used to prepare the vaccine, and using a molecular method approved by the NRA, such as whole-genome HTS analysis.

B.3 Evaluation of immunogenicity of nOPV in suitable models

The genomes of nOPV production strains are rationally designed to stabilize attenuation. However, variations can arise in the viral genomes of nOPV production strains on passage in cell cultures. Whether these genome changes (introduced or cumulated) have any impact on the immunogenicity of the candidate nOPV should be studied using suitable methods – for example, evaluation of the antigenicity of the production strain and/or its ability to

grow in in vitro cell culture. If required, based on the outcomes of the in vitro testing, transgenic mice with interferon-receptor knock-out and expression of human poliovirus receptor are available to study vaccine-induced neutralizing antibodies. Proof-of-concept nonclinical studies based on type-specific serum neutralizing antibody titres may also assist in the selection of the doses to be tested in the clinical dose-finding studies.

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

Clinical trials should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (*69*) and WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*21*). All clinical trials should be approved by the relevant NRAs.

A number of issues specific to the clinical evaluation of OPV are discussed in the following sections, which should be read in conjunction with the general guidance mentioned above. It is also recommended that manufacturers should consult with the relevant NRAs regarding the overall clinical development programme.

The following sections consider the provision of clinical data required for:

- nOPV prepared from genetically stabilized attenuated strains;
- new formulations based on licensed OPVs that are derived from Sabin poliovirus strains, including monovalent, bivalent and trivalent vaccines; and
- situations in which major changes have been made to the manufacturing process of an established vaccine (for example, changing from primary monkey kidney cells to a cell line).

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

Vaccine formulations containing one or two poliovirus serotypes have been licensed based on clinical trials in endemic countries. The results of clinical trials in Egypt and northern India indicated that the efficacy of mOPV1 was superior to that of tOPV in terms of inducing immunity against poliovirus type 1 (*35*, *70*). Health authorities therefore recommended the widespread use of mOPV1 to eliminate poliovirus type 1 transmission in India. In addition, studies

of bOPV containing type 1 and type 3 demonstrated that it was non inferior to mOPV1 and mOPV3 individually, and superior to tOPV against poliovirus type 1 and type 3 (*71*).

C.1 General considerations

Prompted by World Health Assembly resolution WHA41.28 in 1988, the GPEI has led to a dramatic decrease in poliomyelitis cases globally (*27*). As a result, efficacy studies for poliomyelitis vaccines are not feasible, and clinical evaluations and seroprevalence studies should therefore compare the safety and immunogenicity of candidate vaccines against a licensed (comparator) vaccine. The assessment of seroconversion should be based on the elicitation of neutralizing antibodies, which are the basis of protection (27). The approval of a candidate OPV should be based on a clear demonstration of non-inferiority compared with a licensed OPV or an OPV used under WHO EUL, as described below in section C.2.2. The relative risk of VAPP for a new candidate vaccine versus approved vaccines cannot be estimated from pre-approval studies but should be addressed as part of post-marketing surveillance. In addition, the genetic stability of any nOPV strain should be verified during clinical studies.

C.2 Immunogenicity and safety studies

C.2.1 Assessment of the immune response

The presence of neutralizing antibodies 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. A serum neutralizing antibody titre of ≥ 8 is considered to be a marker of clinical protection against poliomyelitis (*72*). The demonstration of an immune response to OPV administration should be based on the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for poliovirus 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 four-fold or greater rise in post-vaccination antibody titres. If the pre-vaccination titre is due to maternal antibodies, a four-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, or post-vaccination antibody titres of ≥ 8, whichever is higher.

The assay used to assess serum neutralizing antibodies in the clinical samples should follow the key parameters described in the WHO *Manual for the*

virological investigation of poliomyelitis (*73*), with the exception of the challenge poliovirus strains. OPV developers are instead encouraged to use genetically modified poliovirus strains that can be manipulated outside of containment facilities (for example, S19 strains) as challenge viruses. 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. A reference serum calibrated against, or traceable to, the appropriate WHO international standard (see Appendix 8) should be used to control assay performance.

Geometric mean titres, seroconversion rates and reverse cumulative distributions should be provided.

C.2.2 Immunogenicity studies

New candidate OPVs manufactured from genetically stabilized attenuated strains or using different vaccine compositions (monovalent, bivalent or trivalent) should be compared with a licensed OPV or an OPV used under WHO EUL. The comparator vaccine(s) selected should have been in use for several years so that data on their effectiveness are available, in addition to a reliable description of their safety profile. When no licensed type-matched OPV is available for use in clinical trials, one or more licensed OPV (or nOPV used under WHO EUL) may be used as the comparator(s) to cover all serotypes included in the candidate vaccine. For example, a candidate tOPV prepared from genetically stabilized attenuated strains may be compared to two suitable comparators – one bivalent and the other monovalent – in a non-inferiority immunogenicity study. In this case, any potential impact on immunogenicity outcomes (for example, a negative immune interference) due to different compositions/serotypes between the comparators and candidate vaccines should be considered in the study design. Further guidance on the selection of comparators is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*21*). In all cases, the study design should be discussed with and approved by the NRA.

C.2.3 Population

The immunogenicity data provided to support the licensure of a candidate OPV as a primary series should include data generated in a naive target population, such as infants. The evaluation of new OPV formulations prepared from Sabin strains may be conducted directly in infants and newborns since safety profiles in these populations have already been established. However, the first clinical study (Phase I) of a candidate nOPV should be performed in healthy adults to assess vaccine safety.

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

C.2.4 End-points and analyses

The clinical study protocol should state the primary objective(s) of the study. The neutralizing antibody response to the candidate vaccine should be demonstrated to be non-inferior versus an appropriate licensed OPV or an OPV used under WHO EUL, as described in C.2.2 above, based primarily on geometric mean titres and/or seroconversion rates. The primary end-point should be selected according to the study population and the anticipated immune response. For example, very high seroprevalence rates are expected in highly immunized populations, with implications for the selection of the non-inferiority margin and therefore the sample size calculation. Further guidance on demonstrating non-inferiority is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*21*).

Other immunological parameters should be compared in planned secondary analyses (for example, percentages reaching predefined titres).

C.2.5 Dose-ranging studies

At the time of publication of this document, all licensed Sabin OPV formulations (monovalent, bivalent and trivalent) contained the recommended dose for each poliovirus type (not less than $10^{6.0}$ $CCID_{50}$ for type 1, $10^{5.0}$ $CCID_{50}$ for type 2 and $10^{5.5}$ $CCID_{50}$ for type 3). However, the development of nOPV or novel formulations with improved stability (through the addition of stabilizers/excipients) or improved immunogenicity (through the use of an adjuvant) may require dose-ranging studies to determine the minimum dose of virus required in $CCID_{50}$ to provide adequate immune responses (*21*). These data could also be used to support the minimum viral titre that should be present in the vaccine at the end of its shelf-life.

C.2.6 Vaccine virus shedding and transmission

Changes in the viral genome of candidate nOPVs, or changes in vaccine composition, may impact virus replication in the intestinal tract and may influence the ability of the vaccine to induce immune responses, with the potential for VAPP or the spread of vaccine viruses to non-target populations. Manufacturers should undertake studies to determine the profile of the vaccine virus (if applicable, by serotype) excreted in the stools of vaccinees, and the duration of shedding. The excretion of candidate nOPV viruses or viruses used in new vaccine formulations should be evaluated alongside a licensed OPV product or an OPV product used under WHO EUL (*35*). For nOPV, virus recovered from stool samples collected from the vaccinees should be evaluated by HTS to verify the genetic stability of the candidate vaccine virus. Genome regions that include key attenuating mutations should be examined, and any genetic variations in the whole genome monitored.

C.2.7 Challenge studies with attenuated Sabin poliovirus

Induction of mucosal immunity by the candidate and comparator vaccines should be determined by the assessment of virus excretion following the administration of a challenge dose of OPV, such as nOPV. Excretion of poliovirus in stool specimens is determined at various intervals immediately before the challenge (day 0) and on days 7, 14, 21 and 28 thereafter (*70*).

C.2.8 Concomitant administration with other vaccines

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

When OPVs are used in an EPI programme simultaneously with other vaccines, it is particularly important that the effects of co-administration are evaluated (for example, in co-administration studies with rotavirus vaccines which are also administered via the oral route).

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

C.2.9 Pre-licensure safety data

The general approach taken to assess the safety of a new OPV during clinical studies should be in accordance with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*21*). Planned safety studies should be supported by a clear scientific rationale. Given the long history of the use of vaccines based on Sabin strains, the NRA may decide that additional pre-licensure safety studies are not required. When a new vaccine formulation that has not previously been used is being investigated, larger-scale studies will be needed.

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

C.3 Post-marketing studies and surveillance

Enhanced safety surveillance, particularly for the detection of VAPP, should be undertaken during the initial post-approval years in collaboration with NRAs. Environmental surveillance should also be conducted. The benefits and risks of using mOPV and bOPV derived from Sabin strains should be carefully

considered, as in areas with sub-optimal polio vaccine coverage this may lead to the emergence of cVDPVs. Manufacturers and health authorities should work in collaboration with the global polio surveillance laboratory network[19] to monitor new vaccines once they are introduced into immunization programmes. These laboratories have extensive experience in poliovirus surveillance and can provide excellent surveillance and post-marketing support.

The total duration of enhanced surveillance should be regularly reviewed by 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 guidance for NRAs and NCLs given in the WHO Guidelines for national authorities on quality assurance for biological products (*74*) and WHO Guidelines for independent lot release of vaccines by regulatory authorities (*24*) should be followed. These guidelines specify that no new biological product should be released until consistency of manufacturing and product quality have been established and demonstrated by the manufacturer.

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

For control purposes, the relevant international reference materials currently in force should be obtained for the purpose of calibrating national, regional and working standards as appropriate (*75*). The NRA may obtain the product-specific or working reference from the manufacturer to be used for lot release until the international/national standard is established.

Only a monovalent bulk approved by the NRA can be used by the manufacturer for the formulation of a final bulk.

Where the MNVT is performed for the control of the monovalent bulk and the NCL does not perform this test itself, the NCL should carry out a second evaluation of the histological sections provided by the manufacturer for each monovalent bulk. In addition, the NCL or a contract organization certified by the NRA for proficiency in conducting NVTs should perform a second evaluation of the results of at least four MNVTs on the reference preparations to obtain the necessary baseline data for comparison with the neurovirulence of the test

[19] https://polioeradication.org/polio-today/polio-now/surveillance-indicators/the-global-polio-laboratory-network-gpln/

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

Where the TgmNVT is performed for the control of the monovalent bulk and the NCL performs this test itself, the standard implementation process should be followed. If the NCL does not perform the test, it should carry out a clinical scoring of mice in parallel with the manufacturer at least at day 3 or day 4. Only appropriately trained staff from a competent NCL can carry out a clinical scoring of mice in parallel with the manufacturer. Whether or not a clinical scoring at day 14 is needed should be justified for each monovalent bulk. Moreover, once a year, the injection of mice should be observed by the NCL.

> In one region of the world, 1 in 10 bulks are also independently tested by an NCL competent in carrying out the test. Other regions that implement the TgmNVT may wish to follow this approach.

Consistency of production has been recognized as an essential component in the quality assurance of live attenuated OPV. In particular, 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 (*24*).

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

A lot release certificate signed by the appropriate NRA/NCL official should then be provided if requested by the manufacturing establishment, and should certify that the lot of vaccine meets all national requirements and/or Part A of these WHO Recommendations. The certificate should provide sufficient information on the vaccine lot, including the basis of the release decision (by summary protocol review and/or independent laboratory testing). The purpose of this official national lot release certificate is to facilitate the exchange of vaccines between countries, and should be provided to importers of the vaccines.

A model NRA/NCL Lot Release Certificate for poliomyelitis vaccines (oral, live, attenuated) is provided below in Appendix 6.

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

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

- sections E.1.1.1, E.1.3.1, E.1.4.1 and E.1.4.2 are additions to the corresponding section A.4 text (as individually indicated below); and
- sections E.1.2.1–E.1.2.3 are replacements for the corresponding section A.4 text (as individually indicated below).

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

E.1 Control of vaccine production

E.1.1 Control of source materials

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

[Addition to section A.4.1]

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

Manufacturers should use animals from closed or intensively monitored colonies.

The monkeys should be kept in well-constructed and adequately ventilated animal rooms in cages separated in such a way as to prevent cross-infection between cages. Cage mates should not be interchanged. The monkeys should be kept in the country of manufacture of the vaccine in quarantine groups[20] for a period of not less than 6 weeks before use. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5% (excluding deaths from accidents or where the cause was specifically determined not to be an infectious disease), all the monkeys from that entire shipment should continue to be quarantined for a further period of

[20] A quarantine group is a colony of selected healthy monkeys kept in one room, with separated feeding and cleaning facilities, and having no contact with other monkeys during the quarantine period.

not less than 6 weeks. The monkeys used should be free of infection. At the end of the extended quarantine period, and following thorough investigations, if any additional monkeys die of the same infectious disease, the entire group is to be discarded from production.

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

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

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

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

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

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

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

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

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

E.1.2 Production precautions

The general production precautions called for in WHO good manufacturing practices for biological products (*22*) should apply to the manufacture of the vaccine, with the addition of the following tests.

E.1.2.1 Monkey kidney cell cultures for vaccine production

[Replacement of section A.4.2.1 above – in conjunction with section E.1.2.2 below]

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

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

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

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

[Replacement of section A.4.2.1 above – in conjunction with section E.1.2.1 above]

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

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

> When the monkey species used for vaccine production is known to be sensitive to SV40, a test in a second species may be omitted with the approval of the NRA.

> Animal serum may be used in the propagation of the cells provided that it does not contain SV40 antibody or other inhibitors, but the maintenance medium used after inoculation of the test material should contain no added serum except as described below.

The cultures should be incubated at a temperature of 35–37 °C and should be observed for a total period of at least 4 weeks. During this observation period, and after not less than 2 weeks of incubation, at least one subculture of fluid should be made from each of the cultures in the same tissue culture system. The subculture should also be observed for at least 2 weeks.

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

A further sample of at least l0 mL of the pooled fluid should be tested for the presence of herpes B virus and other viruses in rabbit kidney cell cultures. Serum used in the nutrient medium of these cultures should have been shown to be free from inhibitors.[21] The sample should be inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 part in 4. The area of the cell sheet should be at least 3 cm² per mL of pooled fluid. At least one bottle of the cell cultures should remain uninoculated and should serve as a control.

The cultures should be incubated at a temperature of 35–37 °C and should be observed for at least 2 weeks.

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

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

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

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

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

If these tests are not carried out immediately, the samples of pooled cell culture fluid should be kept at a temperature of −60 °C or below, with the exception of the sample to be used for the test for herpes B virus, which may be held at 4 °C provided that the test is done not more than 7 days after the sample has been taken.

E.1.2.3 Test of control cell cultures

[Replacement of section A.4.1 above]

Cultures prepared on the day of inoculation with the virus working seed lot from 25% (but not more than 2.5 L) of the cell suspension obtained from the kidneys of each single monkey, or from not more than 10 near-term monkeys, should remain uninoculated and should serve as controls. These control cell cultures should be incubated under the same conditions as the inoculated cultures for at least 2 weeks, and should be examined during this period for evidence of cytopathic changes. For the tests to be valid, not more than 20% of the control cell cultures should have been discarded for any reason. At the end of the observation period, the control cell cultures should be examined for degeneration caused by an infectious agent. If this examination, or any of the tests required in this section, show evidence of the presence of any adventitious agent in a control culture, the poliovirus grown in the corresponding inoculated cultures from the same group should not be used for vaccine production.

E.1.2.3.1 *Tests for haemadsorbing viruses*

At the time of harvest, or not more than 4 days after the day of inoculation of the production cultures with the virus working seed lot, a sample of 4% of the control cell cultures should be taken and should be tested for haemadsorbing viruses. At the end of the observation period, the remaining control cell cultures should be similarly tested. The tests should be carried out as described above in section A.4.1.2.

E.1.2.3.2 *Tests for other adventitious agents*

At the time of harvest, or no more than 7 days after the day of inoculation of the production cultures with the virus working seed lot, a sample of at least 20 mL of the pooled fluid from each group of control cultures should be taken and tested in two kinds of monkey kidney cell culture, as described in section E.1.2.2 above.

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

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

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

E.1.3 Control of single harvests

[Addition to section A.4.3 above]

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

A sample of at least 10 mL of each single harvest should be neutralized by type-specific poliovirus antiserum prepared in animals other than monkeys. In preparing antisera for this purpose, the immunizing antigens used should be prepared in non-simian cells.

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

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

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

> Animal serum may be used in the propagation of the cells provided that it does not contain inhibitors, but the maintenance medium used after the inoculation of the test material should contain no added serum other than the poliovirus neutralizing antiserum, except as described below.

The cultures should be incubated at a temperature of 35–37 °C and should be observed for a total period of at least 4 weeks. During this observation period, and after no less than 2 weeks of incubation, at least one subculture

of fluid should be made from each of these cultures in the same tissue culture system. The subcultures should also be observed for at least 2 weeks.

> Serum may be added to the original cultures at the time of subculturing provided that the serum does not contain inhibitors. Immunohistochemical techniques may be useful for detecting SV40 and other viruses in the cells.
>
> It is suggested that, in addition to these tests, a further sample of the neutralized single harvest is tested by inoculation of 10 mL into human cell cultures sensitive to measles virus.

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

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

E.1.4 Control of monovalent bulk

[Addition to section A.4 above]

E.1.4.1 Monovalent bulk (before filtration)

E.1.4.1.1 *Tests in rabbits*

A sample of the monovalent bulk should be tested for the presence of herpes B virus and other viruses by injection into at least 10 healthy rabbits each weighing between 1.5 and 2.5 kg. The total sample volume should be at least 100 mL. Each rabbit should receive not less than 10 mL and not more than 20 mL – of which 1 mL should be administered intradermally at multiple sites and the remainder subcutaneously. The rabbits should be observed for between 3 and 5 weeks for death or signs of illness.

> It is suggested that the sample should consist of at least 1% of the total monovalent bulk (provided that this is not less than 100 mL) up to a maximum of 500 mL.

All rabbits that die during the testing period should be examined by autopsy, with the brain and other organs being removed for detailed examination to establish the cause of death. Animals showing signs of illness should be humanely killed and subjected to a similar autopsy.

The monovalent bulk passes the test if no more than 20% of the inoculated rabbits show signs of infection during the observation period and

if none of the rabbits show evidence of infection with herpes B virus or other adventitious agents, or lesions of any kind attributable to the bulk suspension.

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

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

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

Test samples from the filtered monovalent bulk should be examined for the presence of retroviruses using an assay for reverse transcriptase acceptable to the NRA.

Authors and acknowledgements

The first draft of these WHO Recommendations was prepared by a WHO drafting group comprising Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr C. Li, National Institutes for Food and Drug Control, China; Dr L. Mallet, European Directorate for the Quality of Medicines & HealthCare, France; Dr J. Martin, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, World Health Organization, Switzerland. Acknowledgement is also due to Dr P. Minor, St Albans, the United Kingdom for his critical review and valuable input.

The first draft was then reviewed at a WHO informal consultation on the revision of recommendations to assure the quality, safety and efficacy of oral poliomyelitis vaccines, held virtually on 15–17 November 2021, and attended by: Miss P. Agsiri, Ministry of Public Health, Thailand; Dr L.R. Andalucia, Indonesian Food and Drug Authority, Indonesia; Dr M. Arita, National Institute of Infectious Diseases, Japan; Dr A. Bhardwaj, Central Drugs Standard Control Organisation, India; Mr R.M. Bretas, Agência Nacional de Vigilância Sanitária, Brazil; Dr M. Bruysters, National Institute for Public Health and the Environment, Netherlands; Dr K. Chumakov, United States Food and Drug Administration, the USA; Ms G. Cooper, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr V. Dhawan, Ministry of Health and Family Welfare, India; Dr S. Fakhrzadeh, Ministry of Health and Medical Education, the Islamic Republic of Iran; Dr E. Grabski, Paul-Ehrlich-Institut, Germany; Dr N. Gupta, Ministry of Health and Family Welfare, India; Dr P. Haldar, Government of India, India; Mrs T. Jivapaisarnpong, King Mongkut's University of Technology Thonburi, Thailand; Dr J. Konz, PATH, the USA; Dr C. Li, National Institutes for Food and Drug Control, China; Dr K. Mahmood, PATH, the USA; Dr M.

Majumdar, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr L. Mallet, European Directorate for the Quality of Medicines & HealthCare, France; Dr J. Martin, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr C. Milne, European Directorate for the Quality of Medicines & Healthcare, France; Dr P. Minor, St Albans, the United Kingdom; Sr Si Normasari, Indonesian Food and Drug Authority, Indonesia; Dr V. Pithon, Agence nationale de sécurité du médicament et des produits de santé, France; Dr D.E. Putri, Indonesian Food and Drug Authority, Indonesia; Dr S.E. Reddy, Central Drugs Standard Control Organisation, India; Dr L. Stephens, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr A. Macadam, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr S. Pumiamorn, Ministry of Public Health, Thailand; Dr G. Waeterloos, Sciensano, Belgium; and Dr T. Wu, Health Canada, Canada. *Representatives of the Developing Countries Vaccine Manufacturers Network*: Dr R. Cuber, Bio-Manguinhos, Brazil; Dr R. Dhere, Serum Institute of India, India; Ms T.T.B. Hanh, Polyvac, Viet Nam; Mr W. Hidayat, BioFarma, Indonesia; Mr L.Q. Hung, Polyvac, Viet Nam; Dr M. Jain, Panacea Biotec, India; Mr S. Kosaraju, Biological E, India; Dr Y. Li, Institute of Medical Biology, Chinese Academy of Medical Sciences, China; Dr K. Maithal, Zydus-Cadila Healthcare Ltd, India; Dr S.B. Rajan, Zydus-Cadila Healthcare Ltd, India; Mr M.I. Masud, Incepta Vaccine Ltd, Bangladesh; Dr L. Mohanty, Panacea Biotec, India; Mr P. Morgon, Cansino Bio, China; Ms L.T. Nga, Polyvac, Viet Nam; Mr P. Pavuluri, Biological E, India; Mr V. Sivaramakrishnan, Bharat Biotech International, India; Dr V.K. Srinivas, Bharat Biotech International, India; Mr M. Stetsyuk, Nanolek, Russian Federation; Ms J. Tresnabudi, BioFarma, Indonesia; Mr D. Ugiyadi, BioFarma, Indonesia; Dr X.L. Yang, Institute of Medical Biology, Chinese Academy of Medical Sciences, China; Dr Y.C. Zhang, Beijing Institute of Biological Products Co., Ltd, China. *Individual manufacturers*: Dr A. Stefanovic, Bilthoven Biologicals, Netherlands; Dr K. Verheijen, Bilthoven Biologicals, Netherlands; and Dr A. Malkin and Dr A. Sinyugina, Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of the Russian Academy of Sciences, Russian Federation. *WHO Secretariat*: Dr T.Q. Zhou and Dr I. Knezevic, Health Products Policy and Standards Department, Dr M. Eisenhawer, Polio Eradication Initiative, and Dr M. Janssen, Regulation and Prequalification Department, World Health Organization, Switzerland.

Based on the outcomes of the above informal consultation, a second draft was prepared by the above WHO drafting group with input from Dr P. Minor, St Albans, the United Kingdom. The resulting document was then posted on the WHO Biologicals website during February and March 2022 for a first round of public consultation. Feedback and comments were received from: Dr J. Konz, Dr C. Gast and Dr M. Toher, PATH, the USA; Dr M. Ergasheva, Ministry of Health of the Republic of Uzbekistan, Uzbekistan; Dr R-C Guimarães, Bio-

Manguinhos/Fiocruz; Dr I. Fradi, Ministry of Health, Tunisia; Dr C. Milne, European Directorate for the Quality of Medicines & Healthcare, France; Dr V. Pithon, Agence nationale de sécurité du médicament et des produits de santé, France; Dr D. Das and Dr F. Atouf, United States Pharmacopeia, the USA; Dr A.E. de Almeida, Oswaldo Cruz Foundation, Brazil; and Dr A. Malkin, Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of the Russian Academy of Sciences, Russian Federation.

Taking into consideration the comments received, the document WHO/BS/2022.2423 was prepared by the above WHO drafting group and then posted on the WHO Biologicals website for a second round of public consultation during June and August 2022. Written comments were received from: Ms Y. Chen, Beijing Institute of Biological Products Co. Ltd, China; Dr S. Wendel, Hospital Sírio-Libanês Blood Bank, Brazil; Dr B. Zhao, Institute of Medical Biology, Chinese Academy of Medical Sciences, China; Dr A. Muhaidat and Dr M. Malkawy, Jordan Food and Drug Administration, Jordan; Dr A.K. Tahlan, Central Research Institute, India; and Dr M. Mamdouh and Dr A. Fouad, Egyptian Drug Authority, Egypt.

Further changes were made to document WHO/BS/2022.2423 by the WHO Expert Committee on Biological Standardization.

References

1. Requirements for biological substances. 7. Requirements for poliomyelitis vaccine (oral). Report of a Study Group. Geneva: World Health Organization; 1962 (WHO Technical Report Series, No. 237; https://apps.who.int/iris/bitstream/handle/10665/40531/WHO_TRS_237.pdf, accessed 25 November 2022).
2. Requirements for biological substances. Requirements for poliomyelitis vaccine (oral) (Requirements for biological substances No. 7) (revised 1965). Report of a WHO Expert Group. Geneva: World Health Organization; 1966: Annex 3 (WHO Technical Report Series, No. 323; http://apps.who.int/iris/bitstream/handle/10665/39819/WHO_TRS_323.pdf?sequence=1, accessed 25 November 2022).
3. Requirements for poliomyelitis vaccine (oral). In: WHO Expert Committee on Biological Standardization: twenty-fourth report. Geneva: World Health Organization; 1972: Annex 1 (WHO Technical Report Series, No. 486; http://apps.who.int/iris/bitstream/handle/10665/40947/WHO_TRS_486.pdf;jsessionid=C7F88C1ED8AA96F124DFDBBECC39657E?sequence=1, accessed 25 November 2022).
4. Requirements for poliomyelitis vaccine (oral) (revised 1982). In: WHO Expert Committee on Biological Standardization: thirty-third report. Geneva: World Health Organization; 1983: Annex 4 (WHO Technical Report Series, No. 687; https://apps.who.int/iris/bitstream/handle/10665/39217/WHO_TRS_687.pdf, accessed 25 November 2022).
5. Requirements for poliomyelitis vaccine (oral) (revised 1989). In: WHO Expert Committee on Biological Standardization: fortieth report. Geneva: World Health Organization; 1990: Annex 1 (WHO Technical Report Series, No. 800; https://www.who.int/publications/i/item/9241208007, accessed 25 November 2022).

6. Requirements for continuous cell lines used for biologicals production. In: WHO Expert Committee on Biological Standardization: thirty-sixth report. Geneva: World Health Organization; 1987: Annex 3 (WHO Technical Report Series, No. 745; http://apps.who.int/iris/bitstream/handle/10665/41002/WHO_TRS_745.pdf?sequence=1, accessed 25 November 2022).
7. Acceptability of cell substrates for production of biologicals. Report of a WHO Study Group. Geneva: World Health Organization; 1987 (WHO Technical Report Series, No. 747; https://apps.who.int/iris/bitstream/handle/10665/38501/WHO_TRS_747.pdf?sequence=1, accessed 25 November 2022).
8. 1990 Requirements for poliomyelitis vaccine (oral) (Addendum 1998). In: WHO Expert Committee on Biological Standardization: forty-ninth report. Geneva: World Health Organization; 2000; Annex 2 (WHO Technical Report Series, No. 897) (http://apps.who.int/iris/bitstream/handle/10665/42340/WHO_TRS_897.pdf?sequence=1, accessed 25 November 2022).
9. Recommendations for the production and control of poliomyelitis vaccine (oral). In: WHO Expert Committee on Biological Standardization: fiftieth report. Geneva: World Health Organization; 2002: Annex 1 (WHO Technical Report Series, No. 904; https://www.who.int/publications/i/item/9241209046, accessed 25 November 2022).
10. Transgenic mice as an animal model for neurovirulence testing of OPV. Report of a WHO Working Group. Geneva, 28–30 June 1999 (unpublished document BS/99.1908; available on request from: Norms and Standards for Biologicals, World Health Organization, 1211 Geneva 27, Switzerland).
11. Use of the MAPREC assay in the quality control of oral poliovirus vaccine. Report of a WHO Working Group. Geneva, 1–2 July 1999 (unpublished document BS/99.1909; available on request from: Norms and Standards for Biologicals, World Health Organization, 1211 Geneva 27. Switzerland).
12. Recommendations for the production and control of poliomyelitis vaccine (oral) (revised, Addendum 2000). In: WHO Expert Committee on Biological Standardization: fifty-first report. Geneva: World Health Organization; 2002; Annex 1 (WHO Technical Report Series, No. 910; https://www.who.int/publications/i/item/9241209100, accessed 18 November 2022).
13. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated). Replacement of Annex 1 of WHO Technical Report Series, No. 904; and Addendum to Annex 1 of WHO Technical Report Series, No. 910. In: WHO Expert Committee on Biological Standardization: sixty-third report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 980; https://www.who.int/publications/m/item/oral-live-attenuated-poliomyelitis-vaccine-annex-2-trs-no-980, accessed 18 November 2022).
14. nOPV2 [website]. Global Polio Eradication Initiative (https://polioeradication.org/nopv2/, accessed 18 November 2022).
15. WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. GAPIII. Geneva: World Health Organization; 2015 (https://apps.who.int/iris/bitstream/handle/10665/208872/WHO_POLIO_15.05_eng.pdf?sequence=1&isAllowed=y, accessed 18 November 2022).
16. WHO Global Action Plan for Poliovirus Containment. GAPIV. Geneva: World Health Organization; 2022 (unedited version available at: 15 https://polioeradication.org/wp-content/uploads/2022/07/WHO-Global-Action-Plan-for-Poliovirus-Containment-GAPIV.pdf, accessed 10 November 2022).
17. Guidelines for the safe production and quality control of poliomyelitis vaccines. Replacement of Annex 2 of WHO Technical Report Series, No. 926. In: WHO Expert Committee on Biological Standardization: sixty-ninth report. Geneva: World Health Organization; 2019: Annex 4 (WHO Technical Report Series, No. 1016; https://www.who.int/publications/m/item/poliomyelitis-vaccines-annex-4-trs-no-1016, accessed 18 November 2022).

18. Guidelines for the safe production and quality control of poliomyelitis vaccines. Amendment to Annex 4 of WHO Technical Report Series, No. 1016. In: WHO Expert Committee on Biological Standardization: seventieth report. Geneva: World Health Organization; 2020: Annex 3 (WHO Technical Report Series, No. 1028; https://www.who.int/publications/m/item/polio-annex-3-trs-1028, accessed 18 November 2022).
19. WHO informal consultation on revision of recommendations to assure the quality, safety and efficacy of oral poliovirus vaccines, 15–17 November 2021. Meeting report. Geneva: World Health Organization; 2021 (https://www.who.int/publications/m/item/opv-nov-meeting-report, accessed 26 November 2022).
20. WHO guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-fourth report. Geneva: World Health Organization; 2005: Annex 1 (WHO Technical Report Series, No. 927; https://www.who.int/publications/m/item/TRS-987-annex2, accessed 26 November 2022).
21. Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization: sixty-seventh report. Geneva: World Health Organization; 2017: Annex 9 (WHO Technical Report Series, No. 1004; https://www.who.int/publications/m/item/WHO-TRS-1004-web-annex-9, accessed 26 November 2022).
22. WHO good manufacturing practices for biological products. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 2 (WHO Technical Report Series, No. 999; https://www.who.int/publications/m/item/annex-2-trs-no-999-WHO-gmp-for-biological-products, accessed 17 November 2022).
23. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 3 (WHO Technical Report Series, No. 978; https://www.who.int/publications/m/item/animal-cell-culture-trs-no-978-annex3, accessed 11 November 2022).
24. Guidelines for independent lot release of vaccines by regulatory authorities. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 2 (WHO Technical Report Series, No. 978; https://www.who.int/publications/m/item/guidelines-for-independent-lot-release-of-vaccines-annex-2-trs-no-978, accessed 26 November 2022).
25. Stones PB, MacDonald CR, McDougall JK, Ramsbottom PF. Preparation and properties of a derivative of Sabin's type 3 poliovirus strain Leon 12a, b. 10th Symposium of the European Association against Poliomyelitis. Warsaw. 1964;390–397.
26. Sabin AB, Boulger LR. History of Sabin attenuated poliovirus oral live vaccine strains. J Biol Stand. 1973;1(2):115–8 (https://www.sciencedirect.com/science/article/abs/pii/0092115773900486, accessed 11 November 2022).
27. Polio vaccines: WHO position paper – June 2022. Weekly Epidemiological Record. 2022;97: 277–300 (https://apps.who.int/iris/rest/bitstreams/1438202/retrieve, accessed 31 December 2022).
28. Kew OM, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. Ann Rev Microbiol. 2005;59:587–635 (abstract: https://pubmed.ncbi.nlm.nih.gov/16153180/, accessed 26 November 2022).
29. Yeh MT, Bujaki E, Dolan PT, Smith M, Wahid R, Konz J et al. Engineering the live-attenuated polio vaccine to prevent reversion to virulence. Cell Host Microbe. 2020;27(5):736–751.e8 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566161/, accessed 11 November 2022).

30. Konopka-Anstadt JL, Campagnoli R, Vincent A, Shaw J, Wei L, Wynn NT et al. Development of a new oral poliovirus vaccine for the eradication end game using codon deoptimization. NPJ Vaccines. 2020;5:26 (https://www.nature.com/articles/s41541-020-0176-7/, accessed 29 November 2022).

31. Sáez-Llorens X, Bandyopadhyay AS, Gast C, De Leon T, DeAntonio R, Jimeno J et al. Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in children and infants: two clinical trials. Lancet. 2021;397(10268):27–38 (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)32540-X/fulltext, accessed 29 November 2022).

32. De Coster I, Leroux-Roels I, Bandyopadhyay AS, Gast C, Withanage K, Steenackers K et al. Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in healthy adults: two clinical trials. Lancet. 2021;397(10268):39–50 (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)32541-1/fulltext, accessed 29 November 2022).

33. Grassly NC, Wenger J, Durrani S, Bahl S, Deshpande JM, Sutter RW et al. Protective efficacy of a monovalent oral type 1 poliovirus vaccine: a case-control study. Lancet. 2007;369(9570):1356–62 (abstract: https://www.thelancet.com/journals/lancet/article/PIIS0140673607605315/fulltext; erratum in: Lancet. 2007;369(9575):1790 (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(07)60816-2/fulltext, accessed 29 November 2022).

34. Sutter RW, John TJ, Jain H, Agarkhedkar S, Ramanan PV, Verma H et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. Lancet. 2010;376(9753):1682–8 (abstract: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(10)61230-5/fulltext, accessed 29 November 2022).

35. Meeting of the Strategic Advisory Group of Experts on Immunization, 31 March–1 April 2020: conclusions and recommendations. Weekly Epidemiological Record. 2020;95(22):241–56 (https://apps.who.int/iris/handle/10665/332219, accessed 29 November 2022).

36. Cockburn WC. The work of the WHO Consultative Group on Poliomyelitis Vaccines. Bull World Health Organ. 1988;66:143–54 (https://apps.who.int/iris/handle/10665/264497, accessed 11 November 2022).

37. Rezapkin GV, Douthitt M, Dragunsky E, Chumakov KM. Reevaluation of nucleotide sequences of wild-type and attenuated polioviruses of type 3. Virus Res. 1999;65(2):111–9 (abstract: https://pubmed.ncbi.nlm.nih.gov/10581384/, accessed 11 November 2022).

38. Furesz J, Contreras G. Some aspects of the monkey neurovirulence test used for the assessment of oral poliovirus vaccines. Dev Biol Stand. 1993;78:61–70 (abstract: https://pubmed.ncbi.nlm.nih.gov/8388832/, accessed 29 November 2022).

39. Ren R, Costantini F, Gorgacz EJ, Lee JJ, Racaniello VR. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. Cell. 1990;63(2):353–62 (abstract: https://www.cell.com/cell/fulltext/0092-8674(90)90168-E, accessed 29 November 2022).

40. Koike S, Taya C, Kurata T, Abe S, Ise I, Yonekawa H et al. Transgenic mice susceptible to poliovirus. Proc Natl Acad Sci U S A. 1991;88(3):951–5 (https://www.pnas.org/doi/epdf/10.1073/pnas.88.3.951, accessed 29 November 2022).

41. Dragunsky E, Nomura T, Karpinski K, Furesz J, Wood DJ, Pervikov Y et al. Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study. Bull World Health Organ. 2003;81(4):251–60 (https://apps.who.int/iris/handle/10665/268915, accessed 29 November 2022).

42. Maintenance and distribution of transgenic mice susceptible to human viruses: memorandum from a WHO meeting. Bull World Health Organ. 1993;71(5):497–502 (https://apps.who.int/iris/handle/10665/261637, accessed 29 November 2022).

43. Wood DJ, Macadam AJ. Laboratory tests for live attenuated poliovirus vaccines. Biologicals. 1997;25(1):3–15 (abstract: https://pubmed.ncbi.nlm.nih.gov/9167004/, accessed 29 November 2022).
44. Chumakov KM, Powers LB, Noonan KE, Roninson IB, Levenbook IS. Correlation between amount of virus with altered nucleotide sequence and the monkey test for acceptability of oral poliovirus vaccine. Proc Natl Acad Sci U S A. 1991;88(1):199–203 (https://www.pnas.org/doi/epdf/10.1073/pnas.88.1.199, accessed 29 November 2022).
45. Chumakov K, Norwood L, Parker M, Dragunsky E, Taffs R, Ran Y et al. Assessment of the viral RNA sequence heterogeneity for control of OPV neurovirulence. Dev Biol Stand. 1993;78:79–89 (abstract: https://pubmed.ncbi.nlm.nih.gov/8388834/, accessed 29 November 2022).
46. Neverov A, Chumakov K. Massively parallel sequencing for monitoring genetic consistency and quality control of live viral vaccines. Proc Natl Acad Sci U S A. 2010;107(46):20063–8 (https://www.pnas.org/doi/10.1073/pnas.1012537107, accessed 29 November 2022).
47. Sarcey E, Serres A, Tindy F, Chareyre A, Ng S, Nicolas M et al. Quantifying low-frequency revertants in oral poliovirus vaccine using next generation sequencing. J Virol Methods. 2017;246: 75–80 (https://www.sciencedirect.com/science/article/pii/S0166093417300654, accessed 29 November 2022).
48. International collaborative study to investigate the utility of NGS as a molecular test of virus stocks used in the manufacture of oral poliomyelitis vaccines. In: WHO Expert Committee on Biological Standardization: seventieth report. Geneva: World Health Organization; 2020 (WHO Technical Report Series, No. 1024, section 3.5.4, pp. 37–38; https://www.who.int/publications/i/item/9789240003736, accessed 18 November 2022).
49. Update on the WHO collaborative study to investigate the utility of next generation sequencing of virus stocks used in the manufacture of oral poliomyelitis vaccines. In: WHO Expert Committee on Biological Standardization: seventy-sixth report. Geneva: World Health Organization; 2023 (WHO Technical Report Series, No. 1045, section 9.2.2; in press).
50. Charlton B, Hockley J, Laassri M, Wilton T, Crawt L, Preston M et al. The use of next-generation sequencing for the quality control of live-attenuated polio vaccines. J Infect Dis. 2022;222(11): 1920–7 (https://academic.oup.com/jid/article/222/11/1920/5850979, accessed 12 November 2022).
51. Israel MA, Chan HW, Hourihan SL, Martin MA. Biological activity of polyoma viral DNA in mice and hamsters. J Virol. 1979;29(3):990–6 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC353259/, accessed 20 November 2022).
52. Lebron JA, Troilol PJ, Pacchione S, Griffiths TG, Harper LB, Mixson LA et al. Adaptation of the WHO guideline for residual DNA in parenteral vaccines produced on continuous cell lines to a limit for oral vaccines. Dev Biol (Basel). 2006;123:35–44 (abstract: https://pubmed.ncbi.nlm.nih.gov/16566435/, accessed 20 November 2022).
53. WHO good manufacturing practices for pharmaceutical products: main principles. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-eighth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 986; https://www.who.int/publications/m/item/who-good-manufacturing-practices-for-pharmaceutical-products-main-principles-a, accessed 17 November 2022).
54. Recommendations to assure the quality, safety and efficacy of BCG vaccines. In: WHO Expert Committee on Biological Standardization: sixty-second report. Geneva: World Health Organization; 2013: Annex 3 (WHO Technical Report Series, No. 979; https://www.who.int/publications/m/item/trs-979-annex-3-bcg-vax, accessed 17 November 2022).

55. Containment Advisory Group [website]. Global Polio Eradication Initiative (https://polioeradication.org/tools-and-library/policy-reports/%20advisory-reports/containment-advisory-group/, accessed 18 November 2022).

56. General requirements for the sterility of biological substances. Requirements for Biological Substances No. 6 (revised 1973). In: WHO Expert Committee on Biological Standardization: twenty-fifth report; Geneva: World Health Organization; 1973: Annex 4 (WHO Technical Report Series, No. 530; https://www.who.int/publications/m/item/sterility-of-biological-substances-annex-4-trs-no-530, accessed 19 November 2022).

57. General requirements for the sterility of biological substances. Requirements for Biological Substances No. 6 (revised 1973, amendment 1995). In: WHO Expert Committee on Biological Standardization: forty-sixth report. Geneva: World Health Organization; 1998: Annex 3 (WHO Technical Report Series, No. 872; https://www.who.int/publications/m/item/sterility-of-biological-substances-annex-3-trs-no-872, accessed 19 November 2022).

58. WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products. Geneva: World Health Organization; 2003 (https://apps.who.int/iris/handle/10665/68932, accessed 18 November 2022).

59. Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (revised 1992). In: WHO Expert Committee on Biological Standardization: forty-third report. Geneva, World Health Organization, 1994: Annex 2 (WHO Technical Report Series, No. 840; https://www.who.int/publications/i/item/9241208406, accessed 20 November 2022).

60. WHO Working group meeting to discuss the revision of the WHO Recommendations for OPV: TRS No. 904 and 910. Geneva, Switzerland 20–22 July 2010. Geneva: World Health Organization; 2010 (https://www.who.int/publications/m/item/who-working-group-meeting-to-discuss-the-revision-of-the-who-recommendations-for-opv-trs-no-904-and-910, accessed 11 November 2022).

61. Wahid R, Mercer L, Macadam A, Carlyle S, Stephens L, Martin J et al. Assessment of genetic changes and neurovirulence of shed Sabin and novel type 2 oral polio vaccine viruses. NPJ Vaccines;2021:6(1):94 (https://www.nature.com/articles/s41541-021-00355-y, accessed 27 November 2022).

62. De Quadros CA, Andrus JK, Olivé JM, Da Silveira CM, Eikhof RM, Carrasco P et al. Eradication of poliomyelitis: progress in the Americas. Pediatr Infect Dis J. 1991;10(3):222–9 (abstract: https://pubmed.ncbi.nlm.nih.gov/2041671/, accessed 27 November 2022).

63. Patriarca PA, Laender F, Palmeira G, Oliveira MJ, Lima Filho J, Dantes MC et al. Randomised trial of alternative formulations of oral poliovaccine in Brazil. Lancet. 1988;331(8583):429–33 (abstract: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(88)91229-9/fulltext, accessed 27 November 2022).

64. Expanded Programme on Immunization. Global Advisory Group. Weekly Epidemiological Record. 1991;66(01–02):3–7 (https://apps.who.int/iris/handle/10665/22787, accessed 31 December 2022).

65. Guidelines on stability evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-seventh report. Geneva: World Health Organization; 2011: Annex 3 (WHO Technical Report Series, No. 962; https://www.who.int/publications/m/item/guidelines-on-stability-evaluation-of-vaccines, accessed 27 November 2022).

66. Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-fifth report. Geneva: World Health Organization; 2011: Annex 9 (WHO Technical Report Series, No. 961; https://www.who.int/publications/m/item/trs961-annex9-modelguidanceforstoragetransport, accessed 27 November 2022).

67. Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 5 (WHO Technical Report Series, No. 999; https://www.who.int/publications/m/item/ectc-annex-5-trs-no-999, accessed 26 November 2022).

68. Guidelines on procedures and data requirements for changes to approved vaccines. In: WHO Expert Committee on Biological Standardization: sixty-fifth report. Geneva: World Health Organization; 2015: Annex 4 (WHO Technical Report Series, No. 993; https://www.who.int/publications/m/item/procedures-and-data-requirements-changes-to-approved-vaccines-annex-4-trs-no-993, accessed 26 November 2022).

69. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: WHO Expert Committee on the Use of Essential Drugs: sixth report. Geneva: World Health Organization; 1995: Annex 3 (WHO Technical Report Series, No. 850; https://www.who.int/publications/i/item/92-4-120850-3, accessed 8 August 2020).

70. El-Sayed N, El-Gamal Y, Abbassy A-A, Seoud I, Salama M, Kandeel A et al. Monovalent type 1 oral poliovirus vaccine in newborns. N Engl J Med. 2008;359:1655–65 (https://www.nejm.org/doi/full/10.1056/NEJMoa0800390, accessed 26 November 2022).

71. Sutter RW, John TJ, Jain H, Agarkhedkar S, Ramanan PV, Verma H et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. Lancet. 2010;376(9753):1682–8 (abstract: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(10)61230-5/fulltext, accessed 26 November 2022).

72. Nathanson N. David Bodian's contribution to the development of poliovirus vaccine. Am J Epidemiol. 2005;161(3): 207–12 (https://academic.oup.com/aje/article/161/3/207/126890, accessed 26 November 2022).

73. Manual for the virological investigation of poliomyelitis. Geneva: World Health Organization; 1990 (Document WHO/EPI/CDS/POLIO/90.1; https://apps.who.int/iris/bitstream/handle/10665/62186/WHO_EPI_CDS_POLIO_90.1.pdf?sequence=1&isAllowed=y, accessed 26 November 2022).

74. Guidelines for national authorities on quality assurance for biological products. In: WHO Expert Committee on Biological Standardization: forty-second report. Geneva: World Health Organization; 1992: Annex 2 (WHO Technical Report Series, No. 822; http://apps.who.int/iris/bitstream/handle/10665/39431/WHO_TRS_822.pdf?sequence=1, accessed 26 November 2022).

75. Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO Expert Committee on Biological Standardization: fifty-fifth report. Geneva: World Health Organization; 2006: Annex 2 (WHO Technical Report Series, No. 932; https://www.who.int/publications/m/item/annex2-trs932, accessed 26 November 2022).

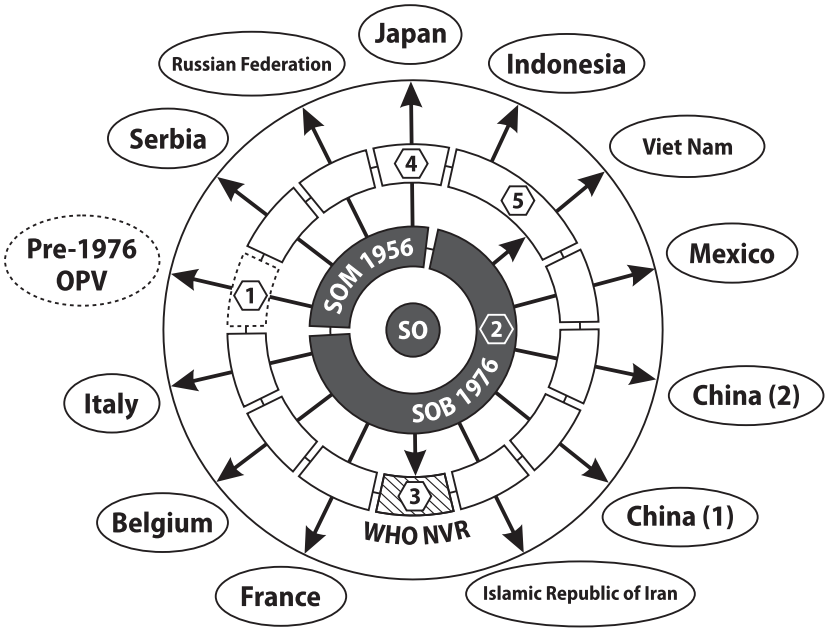
Appendix 1

Overview of virus seeds used in OPV production

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

Fig. 2.1 and Fig. 2.2 show the histories of seed virus and reference materials used in the manufacture of OPV from Sabin 1 and Sabin 2 (Fig. 1) and Sabin 3 (Fig. 2). Concentric circles indicate progressive virus passages made to prepare master seed stocks, working seed stocks and production lots of vaccine. Where relevant, sub-master seed stocks are identified in the notes. Different seed viruses are identified as SO (Sabin Original), SOM (Sabin Original Merck), SOB (Sabin Original Behringwerke), RSO (otherwise known as Pfizer strain), SOJ (Sabin Original Japanese) and SOR (Sabin Original Russian).

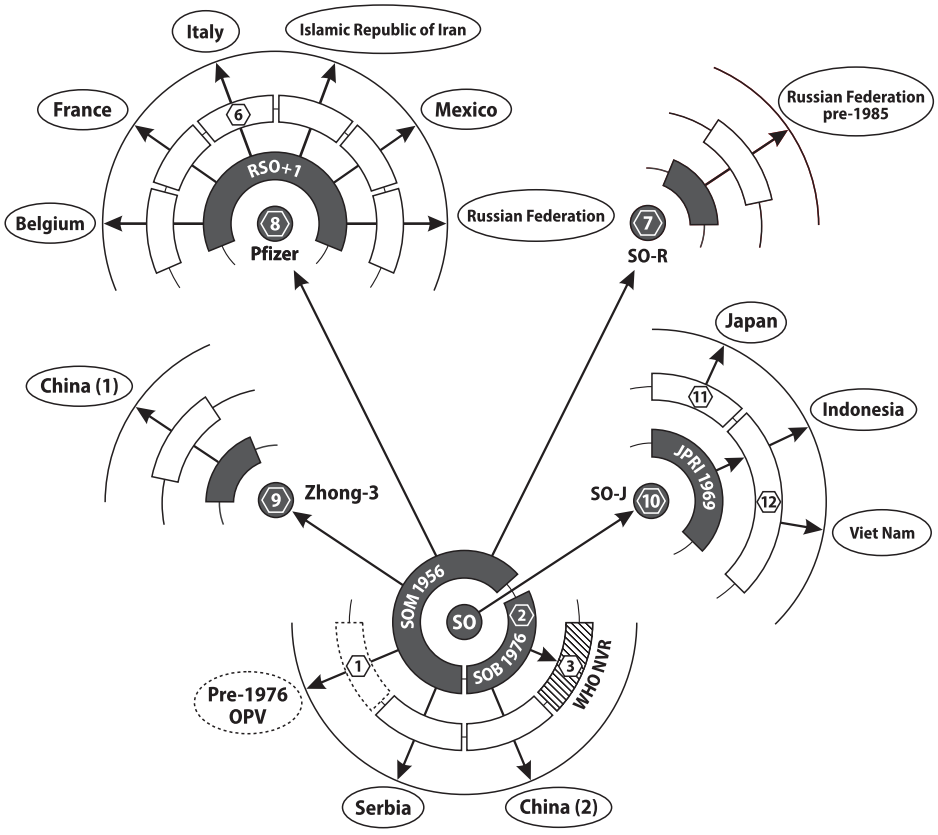
Fig. 2.1
Types 1 and 2 OPV produced from Sabin 1 and Sabin 2



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

The origin of the nOPV given emergency use listing (EUL) by WHO in 2020 has been published (*4*) but this has not yet been provided as a seed by WHO. The design and purpose of the modifications are described in Fig. 3.

Fig. 2.2
Type 3 OPV produced from Sabin 3



The manufacturers corresponding to the countries shown in Fig. 2.1 and Fig. 2.2 are:

Belgium	GlaxoSmithKline Biologicals
China (1)	Institute of Medical Biology, Chinese Academy of Medical Sciences

China (2)	China National Biotec Group, Beijing Tiantan Biological Products Company
France	Sanofi Aventis
Indonesia	PT Bio Farma
Islamic Republic of Iran	Razi Vaccine and Serum Research Institute
Italy	Novartis Vaccines
Japan	Japan Poliomyelitis Research Institute (JPRI)
Mexico	Biologics and Reagents Laboratories of Mexico
Russian Federation	Chumakov Federal Scientific Center for Research & Development of Immune-and-Biological Products of Russian Academy of Sciences
Serbia	Torlak Institute of Virology, Vaccines and Serum
Viet Nam	Center for Research and Production of Vaccines and Biologicals

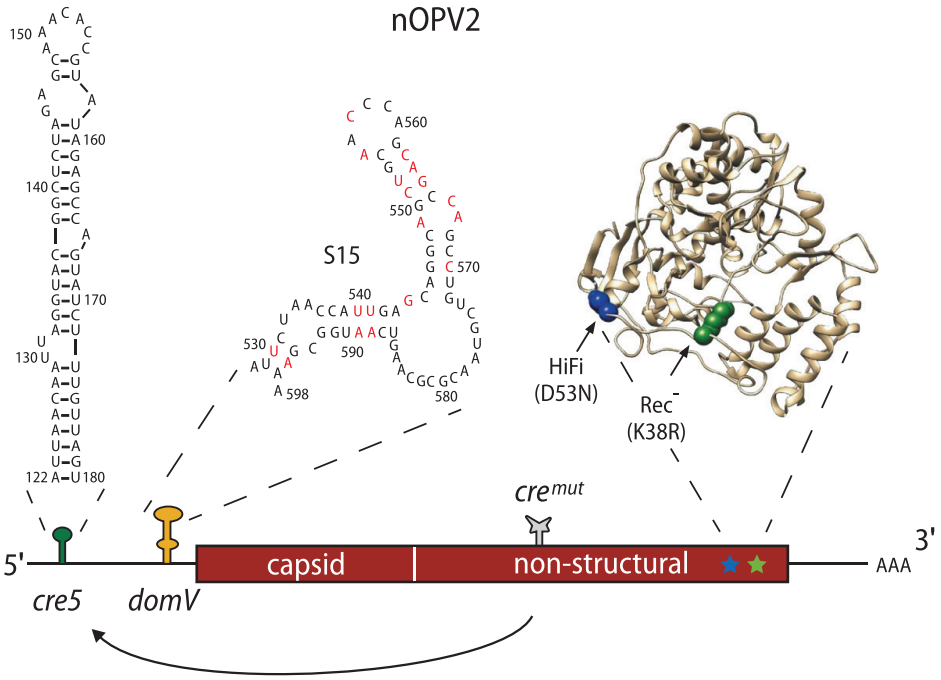
Numbered notes shown in Fig. 2.1 and Fig. 2.2

1. Working seeds produced by different manufacturers before 1976.
2. WHO master seed stock.
3. WHO neurovirulence reference preparation.
4. Type 1 seed stock prepared at JPRI by four passages of SOM, including three terminal dilution passages (passage level SO+5). Type 2 seed stock prepared at JPRI by one passage of SOM (SO+2).
5. Seed stock prepared at JPRI by one passage of SOB (SO+2).
6. Novartis performed an additional passage to prepare sub-master seed stock from which a working seed was produced.
7. Six plaques were selected, pooled and grown to produce seed stock in the Russian Federation.
8. RSO: RNA-plaque-purified Sabin Original.
9. Zhong-3: plaque purification, passage.
10. Produced by JPRI in 1969 from SO stock by one passage (SO+1).
11. Prepared from SOJ by passages in AGMK cells (SOJ+9), including two plaque purifications and three terminal passages (SO+10).
12. Prepared from SOJ by passages in AGMK cells (SOJ+6), including two plaque purifications (SO+7).

Fig. 3 shows a schematic representation of the nOPV2 genome showing modifications and their locations. The sequence of 5′ UTR domain V (S15 domV) prevents an increase in domV thermostability by single point mutations; to prevent replacement of domV attenuation elements by recombination, the cre element, essential for poliovirus replication, was relocated from its original position in the 2C coding region to the 5′ UTR (5′ cre5). The original cre was

inactivated by mutations (cremut); 3Dpol mutations HiFi (D53N) and Rec1 (K38R) reduce overall virus adaptation capacity by reducing mutation and recombination rates, respectively.

Fig. 3
nOPV2 vaccine design



Source: Yeh et al. (2020) (*4*)

References

1. Cockburn WC. The work of the WHO Consultative Group on Poliomyelitis Vaccines. Bull World Health Organ. 1988;66:143–54 (https://apps.who.int/iris/handle/10665/264497, accessed 11 November 2022).
2. Sabin AB, Boulger LR. History of Sabin attenuated poliovirus oral live vaccine strains. J Biol Stand. 1973;1(2):115–8 (https://www.sciencedirect.com/science/article/abs/pii/0092115773900486, accessed 11 November 2022).
3. Stones PB, MacDonald CR, McDougall JK, Ramsbottom PF. Preparation and properties of a derivative of Sabin's type 3 poliovirus strain Leon 12a, b. 10th Symposium of the European Association against Poliomyelitis. Warsaw. 1964;390–397.
4. Yeh MT, Bujaki E, Dolan PT, Smith M, Wahid R, Konz J et al. Engineering the live-attenuated polio vaccine to prevent reversion to virulence. Cell Host Microbe. 2020;27(5):736–751.e8 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566161/, accessed 11 November 2022).

Appendix 2

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

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

Summaries of the MNVT and TgmNVT for Sabin OPV are given below, along with the implementation process for the TgmNVT. It is assumed that the in vivo neurovirulence test procedures and acceptance criteria applied to Sabin OPV are suitable for the evaluation of nOPV.

1. Summary of the MNVT

1.1 Key features

Detailed standard operating procedures (SOP) for the MNVT are available from WHO.[22] Between 5.5 and 6.5 $\log_{10}$ $CCID_{50}$ of monovalent virus is delivered in a single dose by intraspinal inoculation into the lumbar cord. A back titration of the inoculum should be carried out after the inoculation step is completed. Residual paralysis, if any, is noted over the following 17–22 days. The animals are sacrificed at the end of the test or earlier on humane grounds and prepared for histological examination of the central nervous system. Regions are scored for damage on a scale from 1 to 4, and a mean lesion score is calculated for each monkey and then for all the monkeys in the test. The clinical signs do not form part of the assessment or of the pass/fail criteria. The homologous WHO/SO+2 reference

[22] See: https://www.who.int/publications/m/item/neurovirulence-test-of-types-1-2-or-3-opv-in-monkeys.

preparation is tested in parallel. For a new laboratory, the implementation process should be agreed with the NRA.

1.2 Number of animals

The number of monkeys has been chosen on statistical grounds, taking into consideration the variability of the test. Valid animals must show some sign of histological damage as evidence of correct placement of active virus. The number of valid monkeys required per virus preparation is 11 for types 1 and 2 and 18 for type 3. Because a reference preparation must be tested at the same time, the total number of monkeys is at least 22 for types 1 and 2 and 36 for type 3.

1.3 Sections examined

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

1.4 Pass/fail criteria

The pass/fail criteria are based on the variation in the test from run to run, established from the scores obtained with the reference preparation and specific to each laboratory and operator. The within-test variance is used to calculate the statistical constants C_1, C_2 and C_3. If the mean lesion score of the test vaccine is greater than that of the concurrently tested reference preparation by more than C_1, the vaccine is not acceptable. If the test vaccine gives a higher score than the reference but the difference in scores lies between C_1 and C_2, the vaccine may be re-tested and the results pooled; if the difference for the pooled test results is greater than C_3, the vaccine fails.

The values for C_1, C_2 and C_3 are initially established on the basis of the data accumulated after four qualifying tests. These values should then be updated after every test until nine tests have been performed. After that, the C values are based on the last 10 tests performed. The C values must be established for each testing laboratory.

2. Summary of the TgmNVT

2.1 Key features

Detailed SOP for the TgmNVT are available from WHO.[23] The test for neurovirulence of polio vaccines in transgenic mice involves the intraspinal inoculation of a defined strain of transgenic mice carrying the human receptor for poliovirus with small volumes of the test vaccine. Two virus concentrations are used and the read-out of the test is based on the clinical dose response. A reference preparation is tested at the same time and a clearly defined process has been established for the implementation of the test in a new laboratory.

2.2 Strain of transgenic mouse

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

2.3 Titration of virus

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

2.4 Inoculation and observation of animals

Animals procured at age 5–6 weeks are randomized to cages and allowed to recover for at least 7 days. They are then appropriately anaesthetized and inoculated with 5 µL of diluted test virus between the last thoracic and first lumbar vertebrae. Animals are observed for clinical signs once a day for the next 14 days and ultimately scored either as normal (slight weakness or no signs) throughout or paralysed (paresis on two consecutive days or paralysis on a single day). For the test to be valid, the lower and higher doses of the reference preparation should result in more than 5% and less than 95% of the animals becoming paralysed, respectively. A test requires 128 mice for one vaccine plus the reference preparation tested concurrently, or 192 for two vaccines and the reference preparation. The reference preparation is the same as that used in the

[23] Available at: https://www.who.int/publications/m/item/neurovirulence-test-sop-of-types-1-2-or-3-opv-in-transgenic-mice-susceptible-to-poliovirus-v8.

MNVT; the use of other reference preparations may be acceptable but should be validated.

The vaccine passes if it is not significantly more virulent than the reference preparation defined in terms of the log odds ratio and statistical constants L1 and L2 which are based on the reproducibility of the test and which define the pass/fail criteria and the grey zone in which a re-test is required. The acceptance and rejection limits, L1 and L2, were selected so that a test vaccine which is equivalent to the reference preparation will have a 0.95 probability of passing and a 0.01 probability of failing, respectively. The constants are regularly updated. Statistical evaluation of test validity includes linearity and dose and gender effects.

3. Implementation process of the TgmNVT

If a manufacturer wishes to use the transgenic mouse test for Sabin OPV, relevant validation data should be available for their specific product to demonstrate the applicability of the test. This may include reference to the extensive collaborative studies through which the test was originally developed. A clear stepwise process for implementing the TgmNVT has been established which involves training in the inoculation technique through the injection of Indian ink, tests with vaccines, and testing of a blinded evaluation panel containing vaccines that pass, fail or marginally fail the test. Competence in clinical scoring is acquired through a standardized training procedure which involves parallel scoring with an experienced scorer, and criteria for declaring a trainee to be competent.

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

4. Considerations in the choice of assay for the evaluation of Sabin OPV

The following issues highlight that care should be taken in the selection of the in vivo test(s) to be performed for neurovirulence, and that the selection should be justified. The report of the WHO working group meeting to discuss the revision of the WHO Recommendations for OPV: TRS No. 904 and 910 provides more detailed discussion of this (*1*).

4.1 Types 1 and 2 Sabin vaccine viruses

The sensitivity of the transgenic mouse and monkey NVTs performed according to WHO procedures with respect to the presence of mutations in the 5′ UTR

in types 1 and 2 appears to be comparable, but significantly lower than that for type 3 (*2*, *3*). It is unknown whether these two models are equally sensitive to other potentially neurovirulent mutations. Most manufacturers use essentially identical seeds of types 1 and 2, in contrast to the situation with type 3.

4.2 Type 3 Sabin vaccine virus

4.2.1 Molecular biology

Studies of the molecular biology of Sabin polio vaccine virus strains have suggested that few mutations are involved in attenuation and that, for the type 3 strain, there may be only two – namely, one base change in the 5′ UTR of the genome at base 472 and one coding change at base 2034 that introduces an amino acid change in the virus protein VP3. A third mutation at position 2493 has also been described (*4*). Growth of Sabin 3 virus in cell culture or in vaccine recipients results in rapid accumulation of U instead of C at nucleotide 2493 (changing Thr to Ile at amino acid 6 of capsid protein VP1), and all Sabin 3 OPV batches contain variable amounts of these mutants. Although this mutation does not affect neurovirulence as determined in the MNVT (*5*), there is evidence that it influences the results obtained in the TgmNVT, as described in the WHO SOP. Variations in the virulence of vaccine batches measured in monkeys correlate well with variations in the base in the 5′ UTR as measured by MAPREC (*5*). Amino acid change in VP3, or changes at other positions that suppress its effect, are not thought to be generated in the course of well-controlled production runs – though this is possible in principle.

4.2.2 Current type 3 seed viruses

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

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

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

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

There is evidence that the TgmNVT, as described in the relevant WHO SOP, is sensitive to the presence of 2493-U, whereas the MNVT is not. Thus, batches produced from RSO seed will pass both types of NVT, whereas batches produced from the alternative seeds that contain 100% 2493-U will pass the MNVT but may fail the TgmNVT – despite still having an acceptable safety profile in clinical use.

The current WHO SOP for the TgmNVT specify the doses and the WHO reference material to be used, and state the proportion of mice that must be affected at the two doses of virus given for the test to be valid. The WHO reference material for the TgmNVT is the same as that used in the MNVT and has approximately 50% 2493-C – and was validated primarily against vaccines made from SO or RSO seeds. However, if used to test vaccines derived from 2493-U-containing seed, it may fail them even if they contain little 472-C and would pass the MNVT. The TgmNVT could be adapted for testing 2493-U-containing bulks – for example, by changing the reference material, the doses and/or the validity criteria. Manufacturers may wish to do this to make it applicable to their product. Any modified test should be validated, and should be approved by the NRA.

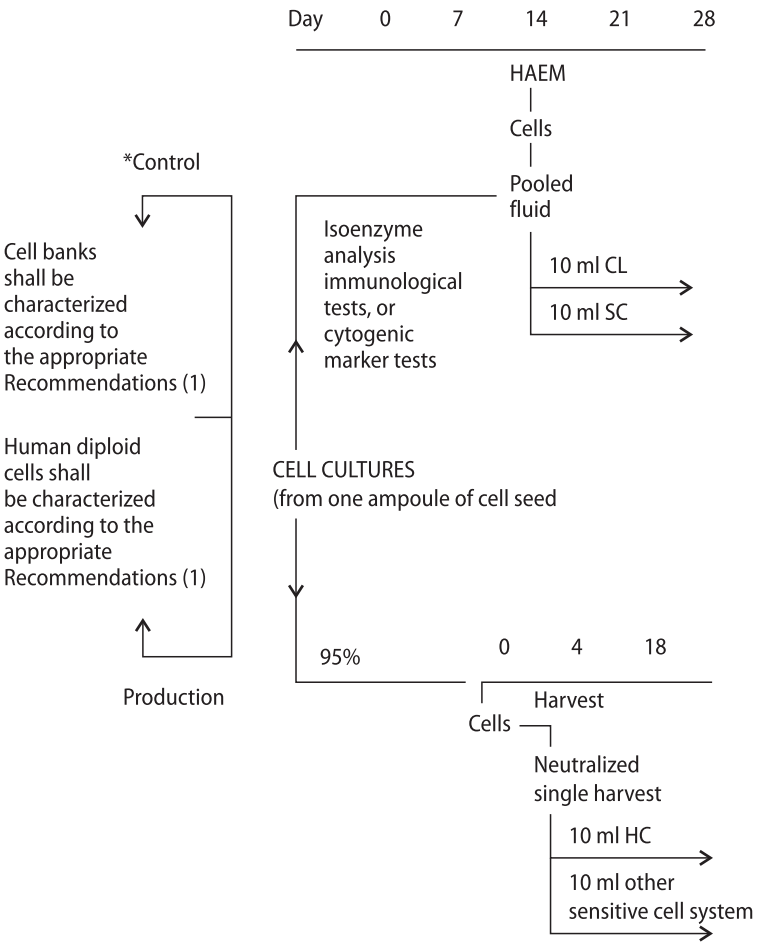
References

1. WHO working group meeting to discuss the revision of the WHO Recommendations for OPV: TRS No. 904 and 910. Geneva, Switzerland 20–22 July 2010. Geneva: World Health Organization; 2010 (https://www.who.int/publications/m/item/who-working-group-meeting-to-discuss-the-revision-of-the-who-recommendations-for-opv-trs-no-904-and-910, accessed 11 November 2022).
2. Rezapkin GV, Alexander W, Dragunsky E, Parker M, Pomeroy K, Asher DM et al. Genetic stability of Sabin 1 strain of poliovirus: implications for quality control of oral poliovirus vaccine. Virology. 1998;245(2):183–7 (abstract: https://pubmed.ncbi.nlm.nih.gov/9636357/, accessed 11 November 2022).
3. Taffs RE, Chumakov KM, Rezapkin GV, Lu Z, Douthitt M, Dragunsky EM et al. Genetic stability and mutant selection in Sabin 2 strain of oral poliovirus vaccine grown under different cell culture conditions. Virology. 1995;209(2):66–73 (abstract: https://pubmed.ncbi.nlm.nih.gov/7778271/, accessed 11 November 2022).
4. Tatem JM, Weeks-Levy C, Georgiu A, DiMichele SJ, Gorgacz EJ, Racaniello VR et al. A mutation present in the amino terminus of Sabin 3 poliovirus VP1 protein is attenuating. J Virol. 1992; 66(5):3194–7 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC241086/pdf/jvirol00037-0606.pdf, accessed 11 November 2022).
5. Chumakov KM Norwood LP, Parker ML, Dragunsky EM, Ran YX, Levenbook IS. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence. J Virol. 1992;66(2):966–70 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC240798/pdf/jvirol00035-0368.pdf, accessed 11 November 2022).

6. Rezapkin GV, Douthitt M, Dragunsky E, Chumakov KM. Reevaluation of nucleotide sequences of wild-type and attenuated polioviruses of type 3. Virus Res. 1999;65(2):111–9 (abstract: https://pubmed.ncbi.nlm.nih.gov/10581384/, accessed 11 November 2022).

Appendix 3

Example flowsheet of cell culture tests performed during production of poliomyelitis vaccines (oral, live, attenuated) using cell banks



* Control cells – 5% of the total or 500 mL of cell suspension, or 100 million cells.

HAEM = test for haemadsorbing viruses.

CL = cell line used for production, but not the same batch of cells used for production of the virus.

SC = when a human diploid cell line is used for production, a simian kidney cell line should be used as the second indicator cell line. When a simian kidney cell line is used for production, a human diploid cell line should be used as the second indicator cell line (*1*).

HC = human cells.

Note: this example flowsheet includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the NRA, this flowsheet should not be considered as an integral part of such requirements and is provided here solely for guidance. Manufacturers should prepare their own flowsheet in order to clarify the procedures to be used.

Reference

1. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 3 (WHO Technical Report Series, No. 978; https://www.who.int/publications/m/item/animal-cell-culture-trs-no-978-annex3, accessed 11 November 2022).

Appendix 4

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

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

The preparation to be assayed and the reference material are diluted in an appropriate medium. It is convenient to make tenfold dilution steps of the virus suspensions initially but for dilutions that are to be inoculated into Hep-2 (Cincinnati) cell cultures the dilutions should be prepared in 1.0 $\log_{10}$ or smaller steps. A preliminary assay may be required to ensure that, in the test, the dilution range selected encompasses at least three dilutions that will infect between 0% and 100% of the cultures inoculated.

Titrate the vaccine for infectious virus using no fewer than three separate containers of vaccine following the method described below. Titrate one container of an appropriate virus reference preparation in triplicate to validate each assay run. The virus titre of the reference preparation is monitored using a control chart, and a titre is established on an historical basis by each laboratory. If the vaccine contains more than one poliovirus serotype, titration of the individual serotypes is undertaken separately using mixtures of appropriate type-specific antiserum (or preferably a monoclonal antibody) to neutralize each of the other serotypes present.

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

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

The assay is considered valid if:

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

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

The assay is repeated and results are averaged if:

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

The assay should be validated for nOPV.

Appendix 5

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

The following protocol is intended for guidance and indicates the minimum information that should be provided by the manufacturer to the NRA or NCL.

Information and tests may be added or omitted as necessary with the approval of the NRA or NCL. In cases where the testing method is different from the one listed in this model protocol, it should be approved by the NRA. For example, if molecular methods (such as NAT and HTS) are used for the testing of adventitious agents or mycoplasmas, their key parameters and information should be identified and provided, covering, as a minimum, the testing method, date of testing, specification and result.

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 provided 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 (package insert) that accompanies 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 (see Appendix 6 below) from the NRA or NCL of the country in which the vaccine was produced and/or released stating that the product meets the national requirements as well as Part A of these WHO Recommendations.

Summary information on finished product (final vaccine lot)

International name: _____

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

Summary of source 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 is 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)

Cell banks (every submission)

Information on cell banking system: _____

Name and identification of substrate: _____

Origin and short history: _____

Authority that approved the cell bank: _____

Master cell bank (MCB) and working cell bank (WCB) lot numbers and date of preparation: _____

Date the MCB and WCB were established: _____

Date of approval by NRA: _____

Total number of ampoules stored: _____

Passage level (or number of population doublings) of cell bank: _____

Maximum passage approved: _____

Storage conditions: _____

Method of preparation of cell bank in terms of number of freezes, and efforts made to ensure that an homogeneous population is dispersed into the ampoules: _____

Tests on MCB and WCB – first submission only

Percentage of total cell bank ampoules tested: _____

Identification 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: _____
Results of tests for tumorigenicity (if applicable): _____

Virus seed (section A.3.2) – every submission

Vaccine virus strain(s) and serotype(s): _____
Substrates used for preparing seed lots: _____
Origin and short history: _____
Authority that approved virus strains: _____
Date of approval: _____

Information and seed lot preparation (section A.3.2.1) – every submission

Virus master seed (VMS), virus sub-master seed, and virus working seed (VWS)
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, virus sub-master seed and VWS – first submission only

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

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Absence of SV40

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

In vitro tests for molecular characteristics

MAPREC (for Sabin OPV)

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A,
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 % 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 % 472C 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: _____

HTS (for virus seed, if applicable)	Type 1	Type 2	Type 3
Specification:			
Date of test:			
Result:			

In vivo tests for neurovirulence

Neurovirulence test in monkeys

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

Number and species of monkeys inoculated: _____

Quantity ($CCID_{50}$) 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

Strain of mice inoculated: _____

For each dose of the virus 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: _____

Freedom from bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

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

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

Test for mycoplasmas

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

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

Indicator cell culture method (if applicable)

Cell substrate used: _____
Inoculum: _____
Date of test: _____

Passage number: _____
Negative control: _____
Positive controls: _____
Date of staining: _____
Results: _____

Virus titration

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

Genotype characterization

Method used: _____
Date of test: _____
Result _____

Test for mycobacteria

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

Control of vaccine production (section A.4)

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

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 any reason: _____
Results of observation: _____
Date of supernatant fluid collection: _____

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 in cell supernatant fluids

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

Identity test

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

Control of single harvests (section A.4.3)

Lot number(s) _____
Date of inoculation: _____
Temperature of incubation: _____
Date of harvest: _____
Volume harvested: _____
Storage time and approved storage period: _____
Date of sampling: _____

Identity test

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

Virus titration

Method used: _____
Lot number of reference material: _____
Date of start of test: _____
Date of end of test: _____
Result: _____
Result for reference material: _____

Tests of neutralized single harvests for adventitious agents

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

Freedom from bacteria, fungi and mycoplasmas

Tests for bacteria and fungi

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

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

Test for mycoplasmas

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

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

Indicator cell culture method (if applicable)

Cell substrate used: _____

Inoculum: _____

Date of test: _____

Passage number: _____

Negative control: _____

Positive controls: _____

Date of staining: _____

Results: _____

Test for mycobacteria

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Tests for molecular consistency

MAPREC (for Sabin OPV, if applicable)

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A, 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 % 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 % 472C 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: _____

HTS (if applicable)	Type 1	Type 2	Type 3
Specification:			
Date of test:			
Result:			

Control of monovalent bulk (section A.4.4)

Lot number: _____

Date of filtration of bulk: _____

Porosity of filters used: _____

Date of sampling: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Lot number of reference reagents: _____

Virus titration

Date of test: _____

Reference batch number: _____

Result: _____

Tests for bacteria and fungi

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

_____

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

Test for mycobacteria

Method used: _____

Date of start of test: _____

Date of end of test: _____

Result: _____

Tests for consistency of virus characteristics

MAPREC (for Sabin OPV)

Date of test: _____

Type 1

Ratio of % of the sum of both mutations 480-A, 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 % 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 % 472C 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: _____

HTS (if applicable)	Type 1	Type 2	Type 3
Specification:			
Date of test:			
Result:			

Neurovirulence tests for Sabin OPV

Neurovirulence test in monkeys

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

Date of inoculation of monovalent bulk: _____

Number and species of monkeys inoculated: _____

Quantity ($CCID_{50}$) inoculated 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

Strain of mice inoculated: _____

For each dose of the bulk sample:

Number of mice inoculated: _____

Number of mice excluded from evaluation: _____

Number of mice paralysed: _____

Results of validity tests for each dose of the reference virus: _____

_____

Number of mice inoculated: _____

Number of mice excluded from evaluation: _____

Number of mice paralysed: _____

Virus assay results for each dose inoculated (residual 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: _____

Final bulk (section A.4.5)

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

Total volume of blend: _____

Storage time and approved storage period: _____

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:					

Filling and containers (section A.5)

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

Appearance: _____

Date of test: _____

Results: _____

Extractable volume

Extractable volume (mL): _____

The number of drops, using the approved dropper, in a minimum of five individual final containers: _____

pH

Date of test: _____

Result: _____

Identity test

Method used: _____

Date of start of test: _____

Date of end of test: _____

Results: _____

Lot number of reference reagents _____

Tests for bacteria and fungi

Method used: _____

Number of vials tested: _____

Volume of inoculum per vial: _____

Volume of medium per vial: _____

Observation period (specification): _____

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

Virus titration

Date of test: _____

Batch number of reference material: _____
Titre of individual virus types: _____
Batch numbers of antiserum used in test: _____
Date of test: _____

Results	Vaccine	Reference
Type 1:	_____	_____
Type 2:	_____	_____
Type 3:	_____	_____

Thermal stability

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

Results:	Vaccine at 37 °C	Vaccine at storage temperature	Difference
Total virus:	_____	_____	_____

Residual antibiotics (if applicable)

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

Level of stabilizer (if applicable)

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

Additional information for production in monkey kidney cell cultures

Production in monkey kidney cell cultures

Control of vaccine production

Control of monkeys

Monkey species used for production: _____
Quarantine batch number: _____
Percentage of monkeys surviving quarantine period: _____
Nature and concentration of antibiotics used in the production cell culture maintenance medium: _____

_____

Tests for antibodies to simian immunodeficiency virus, SV40, foamy viruses and herpes B virus

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

Production details

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

Cell cultures for vaccine production

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

Tests on pooled supernatant fluids

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

Date of sampling from cell cultures inoculated with the pooled fluid _____

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

Tests in rabbit kidney cell cultures

Volume tested: _____

Date of completion of tests: _____

Results: _____

Control of cell cultures

Ratio of control to production cell cultures or control cell cultures as a proportion of production cell cultures: _____

Period of observation of cultures: _____

Ratio or proportion of cultures discarded for any reason: _____

Results: _____

Tests for haemadsorbing viruses

Methods: _____

Results: _____

Tests for other adventitious agents

Methods: _____

Results: _____

Control of single harvests

Volume harvested: _____

Date of sampling: _____

Tests for bacteria, fungi and mycoplasmas: _____

_____

Results: _____

_____

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

Batch number of antiserum used: _____

Volume tested: _____

Date of starting primary cell culture tests: _____

Period of observation: _____

Date of sampling cell culture fluids: _____

Period of observation: _____

Date of completion of tests: _____

Results: _____

Control of monovalent bulk

Tests in rabbits

Number and weight of animals: _____
Date of inoculation: _____
Results of injection: _____
Quantity injected: _____
Results (survival numbers, etc.): _____

_____

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

Tests for retroviruses

Methods: _____
Date: _____
Results: _____

Certification by the manufacturer

Name of head of production and/or 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 poliomyelitis vaccine (oral, live, attenuated), whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A[24] of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated).[25]

Signature _____
Name (typed) _____
Date _____

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

[25] WHO Technical Report Series, No. 1045, Annex 2.

Certification by the NRA/NCL

If the vaccine is to be exported, attach the model NRA/NCL Lot Release Certificate for poliomyelitis vaccines (oral, live, attenuated) (as shown in Appendix 6), a label from a final container and an instruction leaflet for users.

Appendix 6

Model NRA/NCL Lot Release Certificate for poliomyelitis vaccines (oral, live, attenuated)

This certificate is to be provided by the NRA or NCL of the country in which the vaccine has been manufactured, on request by the manufacturer.

Certificate no. _____

The following lot(s) of poliomyelitis vaccine (oral. live, attenuated) produced by _____[26] in _____,[27] whose numbers appear on the labels of the final containers, meet all national requirements[28] and Part A[29] of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated),[30] and comply with WHO good manufacturing practices for pharmaceutical products: main principles;[31] WHO good manufacturing practices for biological products;[32] and the WHO Guidelines for independent lot release of vaccines by regulatory authorities.[33]

The release decision is based on _____[34]

Final lot number _____

Number of human doses released in this final lot _____

Expiry date _____

[26] Name of manufacturer.

[27] Country of origin.

[28] 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 or NCL.

[29] With the exception of provisions on distribution and transport, which the NRA or NCL may not be in a position to assess.

[30] WHO Technical Report Series, No. 1045, Annex 2.

[31] WHO Technical Report Series, No. 986, Annex 2.

[32] WHO Technical Report Series, No. 999, Annex 2.

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

[34] Evaluation of the product-specific summary protocol, independent laboratory testing and/or specific procedures laid down in a defined document, and so on as appropriate.

The certificate may also include the following information:

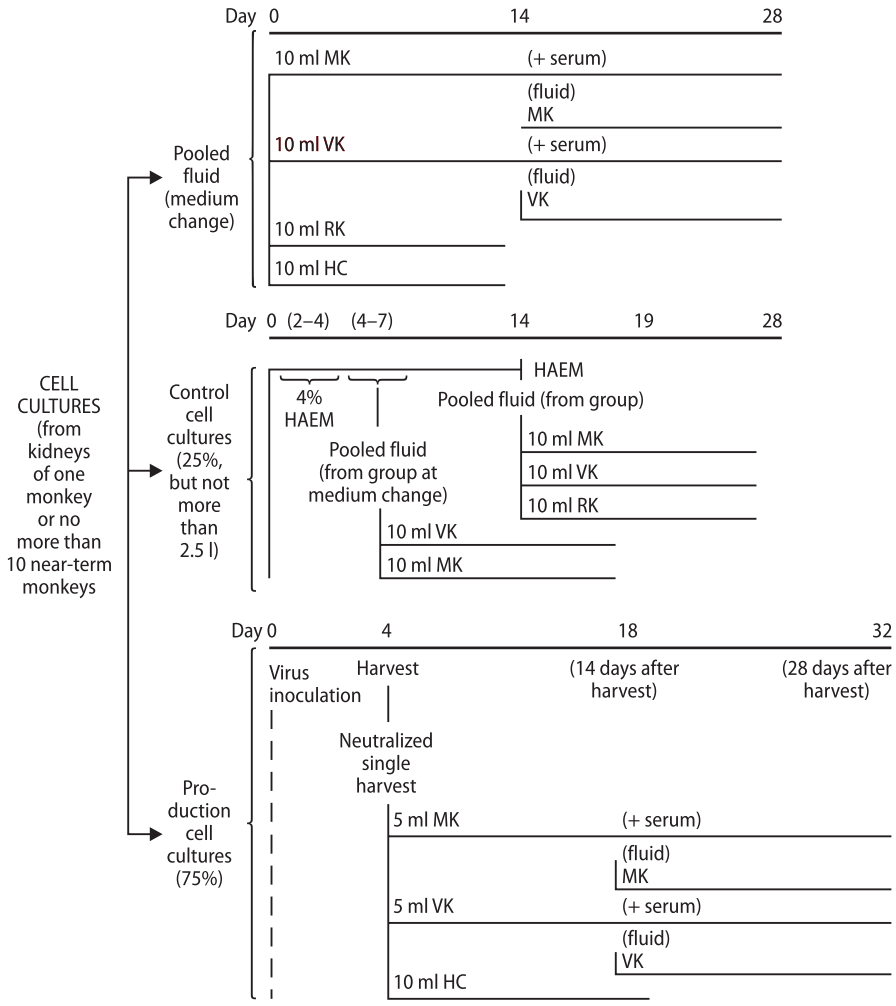
- name and address of manufacturer;
- site(s) of manufacturing;
- trade name and/or common name of product;
- marketing authorization number;
- lot number(s) (including sub-lot numbers and packaging lot numbers if necessary);
- type of container;
- number of doses per container;
- number of containers or lot size;
- date of start of period of validity (for example, manufacturing date) and/or expiry date
- storage conditions;
- signature and function of the person authorized to issue the certificate;
- date of issue of certificate.

The Director of the NRA/NCL (or other appropriate authority):

Signature _____
Name (typed) _____
Date _____

Appendix 7

Example flowsheet of cell culture tests performed during production of poliomyelitis vaccines (oral, live, attenuated) using primary monkey kidney cells



HAEM = test for haemadsorbing viruses.

MK = monkey kidney cells from same species (but not the same animal) used for production.

VK = kidney cells from vervet monkey or one sensitive to SV40.

RK = rabbit kidney cells.

HC = human cells sensitive to measles.

Note: This example flowsheet includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the NRA, this flowsheet should not be considered as an integral part of such requirements and is provided here solely for guidance. Manufacturing establishments should prepare their own flowsheet in order to clarify the procedures to be used.

Appendix 8

International reference materials for poliomyelitis vaccines (oral, live, attenuated)

This appendix describes the currently available international reference materials for OPV developed for Sabin OPV and available through the MHRA[35] and WHO[36] catalogues. International reference materials for nOPV will be needed, particularly WHO international standards for the three serotype versions of nOPV which will likely be required in monovalent and trivalent formulations. Current neurovirulence reference materials used for the MNVT and TgmNVT for Sabin OPV are also suitable for nOPV products. Similarly, international standards for anti-poliovirus antibodies, S19 hyper-attenuated poliovirus strains and anti-polio monoclonal antibody sera are also suitable for nOPV quality control assays. Finally, specific international reference materials for molecular quality control assays based on HTS will be required for Sabin OPV and nOPV products.

WHO international standards and other international reference materials are made available in order to ensure that the manufacture and quality control testing of the different versions of Sabin OPV meet appropriate regulatory requirements.

WHO international standards for the potency testing of tOPV have been available since 1995. More recently, new WHO international standards have been established for bOPV, mOPV1, mOPV2 and mOPV3, with compositions and potencies similar to the vaccines needed for the final phase of the GPEI.

> The WHO International Reference Reagent for the potency estimation of OPV (NIBSC code 85/659) was established by the WHO Expert Committee on Biological Standardization (ECBS) in 1995 as a mixture of three commercially produced monovalent bulks – one of each poliovirus (Sabin) types 1, 2 and 3 (*1*). Following depletion of stocks of this material, the Second WHO International Standard for the potency testing of trivalent OPV (NIBSC code 02/306) was established by the WHO ECBS in 2004 (*2*), calibrated against 85/659. The composition of the Second WHO International Standard was also kept as close as possible to the previous reference material to allow for the direct comparison of both materials – for example, in stability studies. The Second WHO International Standard was prepared by mixing three commercially

[35] https://www.nibsc.org/products.aspx

[36] https://www.who.int/activities/providing-international-biological-reference-preparations

produced and released monovalent bulks – one of each poliovirus (Sabin) types 1, 2 and 3. The passage level of the virus in the bulks was: Sabin Original (SO)+3 for type 1, SO+3 for type 2 and a re-derived SO (RSO)+3 for type 3. All three bulks used in the production of this standard were produced in primary monkey kidney cells. The standard was prepared by blending the three poliovirus serotype monovalent bulks in MEM with 1% w/v bovine albumin and sodium bicarbonate buffer. The assigned potencies for 02/306 were set at: 7.51, 6.51, 6.87 and 7.66 $\log_{10}$ $TCID_{50}$/mL for types 1, 2, 3 and total virus content, respectively. The same bulk materials used to produce the Second WHO International Standard were also used to prepare candidate preparations for bOPV, mOPV1, mOPV2 and mOPV3 in a similar manner, and these were established as international standards by the WHO ECBS in 2017 (*3*). The First WHO International Standard for bOPV 1+3 (NIBSC code 16/164) was assigned potencies of 7.19, 6.36 and 7.32 $\log_{10}$ $TCID_{50}$/mL for types 1, 3 and total poliovirus content, respectively. The First WHO international standards for mOPV1 (16/196), mOPV2 (15/296) and mOPV3 (16/202) were assigned potencies of 7.19, 6.36 and 7.32 $\log_{10}$ TCID50/mL for types 1, 2 and 3 poliovirus, respectively. Monoclonal antibody sera against types 1, 2 and 3 poliovirus, (NIBSC codes 02/256, 02/258 and 02/260, respectively) are available and routinely used globally by a number of manufacturers and NRAs for potency assays of bOPV and tOPV.

Additionally, low-titre monovalent type 1, 2 and 3 poliovirus WHO reference strains are available for use in reference laboratories to measure the sensitivity of cell cultures to poliovirus infection.

Low-titre monovalent Sabin type 1, 2 and 3 poliovirus reference strains were prepared using the same bulk materials used to produce the current WHO international standards for mOPV, bOPV and tOPV with assigned potencies as follows (*4*):

- Type 1 (NIBSC code 01/528): 5.1 $\log_{10}$ $CCID_{50}$/0.1 mL in RD cells and 4.9 $\log_{10}$ $CCID_{50}$/0.1 mL in L20B cells;
- Type 2 (NIBSC code 01/530): 5.1 $\log_{10}$ $CCID_{50}$/0.1 mL in RD cells and 4.8 $\log_{10}$ $CCID_{50}$/0.1 mL in L20B cells; and
- Type 3 (NIBSC code 01/532): 5.3 $\log_{10}$ $CCID_{50}$/0.1 mL in RD cells and 4.9 $\log_{10}$ $CCID_{50}$/0.1 mL in L20B cells.

Following depletion of stocks of these reagents, new virus reference stocks were prepared from the same original material. The new monovalent reference reagents were established with assigned potencies as follows:

- Type 1 (NIBSC code 10/164): 5.5 $\log_{10}$ $CCID_{50}$/0.1 mL in RD cells and 5.3 $\log_{10}$ $CCID_{50}$/0.1 mL in L20B cells;

- Type 2 (NIBSC code 10/166): 5.1 $\log_{10}$ $CCID_{50}$/0.1 mL in RD cells and 4.8 $\log_{10}$ $CCID_{50}$/0.1 mL in L20B cells; and
- Type 3 (NIBSC code 10/168): 5.3 $\log_{10}$ $CCID_{50}$/0.1 mL in RD cells and 4.8 $\log_{10}$ $CCID_{50}$/0.1 mL in L20B cells.

WHO international standards for anti-poliovirus types 1, 2 and 3 antibodies (human) are also available for the standardization of neutralizing antibody tests for poliovirus.

> The First WHO international standards for anti-poliovirus sera types 1, 2 and 3 were established by the WHO ECBS in 1963 from serotype-specific polyclonal antisera produced by hyper-immunization of rhesus monkeys with live virus suspensions (*5*). Each of the standards was specific to one serotype only. They were established through a collaborative study and assigned a unitage of 10 IU/vial for each of the polio serotypes (*5*). The Second WHO International Standard (NIBSC code 66/202) was established by the WHO ECBS in 1991 to replace the depleted original international standards (*6*). In contrast to the original international standards, the Second WHO International Standard was a single serum that exhibited activity against each of the three poliovirus serotypes (*7*). Unitages of 25 IU of anti-poliovirus serum (type 1) human; 50 IU of anti-poliovirus serum (type 2) human; and 5 IU of anti-poliovirus serum (type 3) human were assigned. Following exhaustion of 66/202, the Third WHO International Standard for anti-poliovirus sera (human) types 1, 2 and 3 (NIBSC code 82/585) was established by the WHO ECBS in 2006 with assigned unitages of 11, 32 and 3 IU/vial of neutralizing antibody to poliovirus types 1, 2 and 3, respectively (*8*).

WHO international standards for MAPREC analysis of poliovirus types 1, 2 and 3 (Sabin) and WHO international reference reagents for the control of MAPREC assays of poliovirus types 1, 2 and 3 (Sabin) are available (*9*). Some of these reference materials might also be useful for HTS assays (*10*) or, alternatively, new reference materials might be needed for this purpose.

> The WHO international standards and international reference reagents were prepared from commercial vaccines and viruses generated by cell culture infection. The full list of WHO MAPREC reference materials currently available is as follows (*11–13*):
>
> - NIBSC code 00/410 – MAPREC assay of poliovirus type 1 (Sabin); 100% 480-A, 525-C DNA (WHO International Reference Reagent).
> - NIBSC code 00/416 – MAPREC assay of poliovirus type 1 (Sabin); low mutant virus reference (WHO International Reference Reagent).
> - NIBSC code 00/418 – MAPREC assay of poliovirus type 1 (Sabin); (First WHO International Standard).

- NIBSC code 00/422 – MAPREC assay of poliovirus type 1 (Sabin); high mutant virus reference (WHO International Reference Reagent).
- NIBSC code 97/758 – MAPREC analysis of poliovirus type 2 (Sabin); synthetic DNA, 0.67%481-G (First WHO International Standard).
- NIBSC code 98/524 – MAPREC analysis of poliovirus type 2 (Sabin); synthetic DNA, 100% 481-G (First WHO International Standard).
- NIBSC code 98/596 – MAPREC analysis of poliovirus type 2 (Sabin); high virus reference, 1.21% 481-G (WHO International Reference Reagent).
- NIBSC code 94/790 – MAPREC analysis of poliovirus type 3 (Sabin); synthetic DNA, 100% 472-C (First WHO International Standard).
- NIBSC code 95/542 – MAPREC analysis of poliovirus type 3 (Sabin); synthetic DNA, 0.9% 472-C (First WHO International Standard).
- NIBSC code 96/572 – MAPREC analysis of poliovirus type 3 (Sabin); low virus reference, 0.7% 472-C (WHO International Reference Reagent).
- NIBSC code 96/578 – MAPREC analysis of poliovirus type 3 (Sabin); high virus reference, 1.1% 472-C (WHO International Reference Reagent).
- NIBSC code 97/756 – MAPREC analysis of poliovirus type 2 (Sabin); low virus reference, 0.65% 481-G (WHO International Reference Reagent).

Reference materials at the SO+2 passage level (designated WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3 virus) are available upon request through WHO. These reference materials are intended for use in the in vivo neurovirulence testing of vaccines. The relevant reference materials should be included in each such test (see section A.4.4.7.2 above). Virus panels for validation and implementation of the TgmNVT, as specified in the WHO SOP (*14*), are also available.

New non-pathogenic hyper-attenuated poliovirus strains (S19) are available for use in OPV quality control assays (*15*). S19 strains are polioviruses that replicate in tissue culture but are unlikely to replicate at all in humans exposed even to large amounts. For this reason, they can be used outside GAPIV containment requirements.

> The strains are genetically stable and include a portfolio of strains containing the capsid proteins, (and thus possessing the antigenic properties) of the Sabin OPV strains or wild-type strains used most commonly in the production of inactivated polio vaccine. In December 2018, the WHO Containment Advisory Group concluded that S19 strains can be used outside the containment requirements of GAPIV for neutralization assays (*16*). Organizations wishing to use S19 poliovirus

strains should follow a detailed validation process to ensure that the genetic properties of S19 strains are maintained and can be used to replace current original poliovirus strains. There is a seed lot system for producing banks of highly characterized S19 strains that resembles the vaccine production system. MHRA advises that S19 strains should be tested on a seed lot basis to minimize the risks of reversion and will work with any suitable facility to help generate and validate further banks.

The reference materials listed above are available from MHRA.[37]

References

1. Live attenuated poliovirus. In: WHO Expert Committee on Biological Standardization: forty-sixth report. Geneva: World Health Organization; 1998 (WHO Technical Report Series, No. 872, pp. 20–21; http://apps.who.int/iris/bitstream/handle/10665/42058/WHO_TRS_872.pdf?sequence=1, accessed 11 November 2022).
2. Poliomyelitis vaccine, oral – second International Standard In: WHO Expert Committee on Biological Standardization: fifty-fifth report. Geneva: World Health Organization; 2005 (WHO Technical Report Series, No. 932, pp. 30–31; https://www.who.int/publications/i/item/9241209321, accessed 11 November 2022).
3. First WHO international standards for oral poliomyelitis vaccines. In: WHO Expert Committee on Biological Standardization: sixty-eighth report. Geneva: World Health Organization; 2018 (WHO Technical Report Series, No. 1011, section 8.1.1, pp. 68–69; https://www.who.int/publications/i/item/9789241210201, accessed 11 November 2022).
4. Polio laboratory manual – 4th edition. Evaluating cell-line sensitivity; pp. 73–80. Geneva: World Health Organization; 2004 (WHO/IVB/04.10; https://apps.who.int/iris/bitstream/handle/10665/68762/WHO_IVB_04.10.pdf?sequence=1&isAllowed=y, accessed 12 November 2022).
5. Lyng J, Bentzon MW. International standards for anti-poliovirus sera types 1, 2 and 3. Bull World Health Organ. 1963;29:711–20 (http://apps.who.int/iris/bitstream/handle/10665/267076/PMC2555101.pdf?sequence=1&isAllowed=y, accessed 12 November 2022).
6. Wood DJ, Heath AB. The Second International Standard for anti-poliovirus sera types 1, 2 and 3. Biologicals. 1992;20(3):203–11 (abstract: https://pubmed.ncbi.nlm.nih.gov/1333776/, accessed 12 November 2022).
7. Anti-poliovirus serum. In: WHO Expert Committee on Biological Standardization: forty-second report. Geneva: World Health Organization; 1992 (WHO Technical Report Series, No. 822, pp. 7–8; https://www.who.int/publications/i/item/9241208228, accessed 12 November 2022).
8. Anti-poliovirus sera, types 1, 2 and 3 – Third International Standard. In: WHO Expert Committee on Biological Standardization: fifty-seventh report. Geneva: World Health Organization; 2011 (WHO Technical Report Series, No. 962, pp. 31–32; https://www.who.int/publications/i/item/9789241209625, accessed 12 November 2022).
9. Standard operating procedure. Mutant analysis by PCR and restriction enzyme cleavage (MAPREC) for oral poliovirus (Sabin) vaccine types 1, 2 or 3. Version 5. Geneva: World Health Organization; 2012 (https://www.who.int/publications/m/item/maprec-sop-for-opv-types-1-2-or-3, accessed 12 November 2022).

[37] Medicines and Healthcare products Regulatory Agency, Potters Bar, United Kingdom: https://www.nibsc.org/.

10. Charlton B, Hockley J, Laassri M, Wilton T, Crawt L, Preston M et al. The use of next-generation sequencing for the quality control of live-attenuated polio vaccines. J Infect Dis. 2022;222(11): 1920–7 (https://academic.oup.com/jid/article/222/11/1920/5850979, accessed 12 November 2022).

11. Poliovirus type 1 (Sabin). In: WHO Expert Committee on Biological Standardization: sixtieth report. Geneva: World Health Organization; 2013 (WHO Technical Report Series, No. 977, pp. 14–15; https://www.who.int/publications/i/item/9789241209779, accessed 12 November 2022).

12. Mutant analysis by polymerase chain reaction and restriction enzyme cleavage assays for poliovirus. In: WHO Expert Committee on Biological Standardization: fifty-third report. Geneva: World Health Organization; 2004 (WHO Technical Report Series, No. 926, pp. 25–26; https://www.who.int/publications/i/item/9241209267, accessed 12 November 2022).

13. MAPREC analysis of poliovirus type 3 (Sabin). In: WHO Expert Committee on Biological Standardization: forty-eighth report. Geneva: World Health Organization; 1999 (WHO Technical Report Series, No. 889, pp. 22–23; https://www.who.int/publications/i/item/9241208899, accessed 12 November 2022).

14. Standard operating procedure. Neurovirulence test of types 1, 2 or 3 live attenuated poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus. Version 6. Geneva: World Health Organization; 2021 (https://www.who.int/publications/m/item/neurovirulence-test-sop-of-types-1-2-or-3-opv-in-transgenic-mice-susceptible-to-poliovirus-v8, accessed 12 November 2022).

15. Knowlson S, Burlison J, Giles E, Fox H, Macadam AJ, Minor PD. New strains intended for the production of inactivated polio vaccine at low-containment after eradication. PLoS Pathog. 2015;11(12):e1005316 (https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005316, accessed 12 November 2022).

16. Third meeting of the Containment Advisory Group, 13–14 December 2018, Geneva, Switzerland. Note for the record. Geneva: World Health Organization (https://polioeradication.org/wp-content/uploads/2017/08/CAG3-Dec-2018-Report-EN-20181213-14.pdf, accessed 12 November 2022).