Annex 3

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

Amendment to Annex 3 of WHO Technical Report Series, No. 993

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Introduction

At its sixty-fifth meeting in 2014, the WHO Expert Committee on Biological Standardization adopted the current WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1). These WHO Recommendations provide guidance on the manufacture and control, nonclinical and clinical evaluation, and lot release of inactivated poliomyelitis vaccines (IPV).11 The Recommendations include guidance on the use of several assays requiring the handling of live poliovirus and were produced at a time when only limited data and experience with Sabin-based IPV (sIPV) were available, and when no specific biocontainment requirements for IPV manufacturing had been adopted. Since then, the third revision of 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) (2) was adopted by the World Health Assembly in 2015 to provide guidance on the implementation of biosafety and biosecurity measures at facilities handling poliovirus in the post-eradication era. In addition, WHO Guidelines for the safe production and quality control of poliomyelitis vaccines (3) were adopted by the Committee in 2018. These Guidelines further align the biocontainment requirements for the production of both sIPV and IPV derived from wild-type strains (wIPV) with good manufacturing practices (GMP) and GAPIII requirements. Currently, a step-wise implementation of GAPIII is taking place according to the WHO GAPIII Containment Certification Scheme (4). As few laboratories have the high-containment facilities required by GAPIII, global capacity for performing assays using live poliovirus, particularly type 2, is limited.

Given the urgent need for sIPV in order to increase global IPV supply, as well as recent progress in sIPV development, the Committee at its meeting in 2018 also recommended that the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1) be amended accordingly. The amendments provided in the current document comprise:

- modified definitions of “virus sub-master seed lot” and “virus working seed lot” to cover all poliovirus strains;
- updated information on the WHO international standards now available to support quality control testing;
- modified requirements for confirming the genetic stability of attenuated vaccine seeds and monovalent virus pools to provide flexibility for vaccine developers;

11 In the current document the use of the abbreviation “IPV” refers to IPV derived from any strain. “wIPV” refers to IPV derived from wild-type strains only and “sIPV” refers to IPV derived from Sabin strains only.
- inclusion of additional cell substrates that can be used for the effective-inactivation test based on published studies (5);
- deletion of the general safety (innocuity) test in line with the decision made by the Committee in 2018 to discontinue the inclusion of this test in all WHO Recommendations, Guidelines and other guidance documents for biological products published in the WHO Technical Report Series (6);
- updated recommendations for the evaluation of sIPV immunogenicity in nonclinical and clinical studies to provide much needed flexibility, and thus facilitate the development and licensure of new vaccines.

No attempt was made at this time to review the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1) in their entirety and only the above issues have been addressed.

**Amendments**

Replace the following two paragraphs which appear under “General considerations” in the current WHO Recommendations (pages 95–96) with the replacement text shown below:

**Original text to be replaced**

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

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

Replacement text

Once the circulation of wild-type polioviruses has been stopped, vaccine-manufacturing establishments will remain a major source of risk for the potential release of virulent polioviruses back into populations. Therefore, enhanced containment measures must be implemented as described in 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) (2) and in the WHO Guidelines for the safe production and quality control of poliomyelitis vaccines (3). According to GAPIII, the required containment conditions for wild and Sabin strains are effectively identical. However, it is still important to prevent the incorporation of mutations during the vaccine production process to ensure that production consistency is achieved. It is known that upon cultivation under inappropriate conditions (for example, at higher temperature or lower multiplicity of infection) Sabin strains can undergo molecular changes resulting in reversion to virulence. Therefore, in order to minimize the likelihood of such changes, the Sabin strains must be propagated under defined and well-controlled conditions – for example, the conditions used to manufacture OPV (7).

Additional tests should also be performed on seed viruses and validation lots to confirm production consistency. This can be achieved by comparing the consensus nucleotide sequences of monovalent pools (or viral harvests) with those of the starting seed viruses and confirming that no mutations occurred during virus growth that changed the consensus sequence. Any viral preparation includes molecules that contain mutations that are present in only a small fraction of the entire population. Consensus sequences represent nucleotides that are found in the majority of molecules. Confirming that the consensus sequences of monovalent pools or viral harvests are the same as that of the starting seed virus is sufficient to demonstrate the genetic stability of the virus during production. The seed viruses used by some manufacturers may be different from the original WHO Sabin seeds12 and may have consensus sequences that differ by several nucleotides. Alternatively, instead of nucleotide sequencing analysis, the maintenance of attenuation could be confirmed by performing in vivo tests (monkey or transgenic mouse neurovirulence tests) as described in the WHO

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12 NCBI nucleotide sequence accession numbers for WHO Sabin seed materials are AY184219, AY184220 and AY184221, for type 1, 2 and 3 polioviruses respectively.
Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7). Subsequently, these additional tests may be applied to a proportion of the monovalent pools produced each year in order to ensure production consistency. The number of pools of each serotype tested each year should be justified and agreed to by the NRA.

In addition to Sabin strains, alternative attenuation methods utilizing recombinant DNA technology are being investigated (8–12). Strains derived by such a methodology may have properties specifically designed to be suitable for the safe production of vaccine (for example, the inability to replicate in the human gut and/or significantly reduced likelihood of reverting to virulence). Such strains should be considered as they become available and may require extensive characterization. Biocontainment requirements for such strains and the necessary in-process tests will be determined individually, for each new candidate strain, based on scientific evidence (3). Recommendations on the appropriate level of containment for such strains will be made by the WHO Containment Advisory Group (CAG), after reviewing detailed information on their biological characteristics (3, 4). For example, CAG has previously determined (13) that:

... sufficient data has been provided to conclude the series of S19-poliovirus strains (S19 with capsid region, P1 of wild-type and Sabin vaccine strain polioviruses of all serotypes) and the parallel series of viruses with the substitution of an asparagine by a serine at amino acid 18 in the non-structural protein 2A to allow better growth in Vero cells could be considered for use, outside of the containment requirements of Annex 2 or Annex 3 of GAPIII, as applicable for IPV production, rat neutralization IPV potency assays, human serum neutralization test for poliovirus antibody determination and potency testing for immunoglobulin (human) lot control and release.

As indicated above, there are S19 versions containing Sabin capsid sequences and S19 versions containing wild-type capsid sequences (S19-Sabin and S19-wild respectively) for the three poliovirus serotypes. The immunological properties of S19-Sabin and S19-wild are identical to the respective Sabin and wild strains. Therefore they can be used instead of Sabin and wild strains in the tests recommended in this current document (3, 13).

Replace the definition of “Virus sub-master seed lot” in the “Terminology” section with the following text:

**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 time frame,
and that has been processed at the same time to ensure its uniform composition. The virus sub-master seed lot should be characterized to the extent necessary to support the development of the virus working seed lot. The characterized virus sub-master seed lot is used for the preparation of virus working seed lots (see section A.3.1).

Replace the definition of “Virus working seed lot” in the “Terminology” section with the following text:

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

Replace section A.1.3 “International reference materials” with the following text:

**A.1.3 International reference materials**

A WHO International Standard is available for use in in vitro assays to measure the D-antigen content of wIPV. However, several studies have revealed differences in the reactivity of antibody reagents used in various ELISA methods used by manufacturers and control laboratories to measure the potency of wIPV and sIPV products. This has resulted in high inter-laboratory variability in the potency results obtained for IPV products when using a heterologous reference – that is, a wild-type reference for sIPV or a Sabin reference for wIPV. For this reason, a new WHO International Standard specific to sIPV products was established in 2018 and a new sIPV antigen unit – the Sabin D-Antigen Unit (SDU) – was defined (14). This new unit is independent of the D-antigen unit (DU) used to express the potency of wIPV. Both wIPV and sIPV International Standards are intended for use in calibrating secondary reference preparations of IPV, which are then used in potency tests to calculate the D-antigen content of IPV products. The D-antigen content of wIPV and sIPV should be expressed in DU or SDU respectively. However, manufacturers of existing sIPV products, including those already licensed and those in late-stage development, that use potency values expressed in DU measured against their internal standards can continue to use these values with the approval of the NRA. It is recommended that these manufacturers also determine the potency of their sIPV products in SDU using the sIPV International Standard, and establish the correlation between “SDU” and “DU”. This can serve as a useful quality characteristic to ensure product comparability.
At present there is no standardized ELISA assay available as a resource for laboratories, and efforts are under way to develop standardized reagents and protocols. International standards and reference reagents for the control of in vivo potency assays in rats are also currently being evaluated.

An International Reference Preparation (IRP) of poliomyelitis vaccine (inactivated) was established by the WHO Expert Committee on Biological Standardization in 1962 (15). This preparation was a trivalent blend prepared in 1959 in primary monkey kidney cells from type-1 (Mahoney), type-2 (MEF) and type-3 (Saukett) strains of poliovirus.

After preparation of the IRP, significant advances in the production and control of IPV occurred and vaccines of increased potency and purity were developed. An enhanced potency IPV (PU78-02) from the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) was widely used as a reference preparation for control purposes. When stocks of this reagent were almost exhausted, a new reference material (91/574) was established by the WHO Expert Committee on Biological Standardization in 1994 as the second WHO International Reference Reagent for in vivo and in vitro assays of IPV (16). Potencies of 430, 95 and 285 DU/mL were assigned, respectively, to poliovirus types 1, 2 and 3 of this preparation. A separate aliquot of the preparation, established by the European Pharmacopoeia Commission as the Biological Reference Preparation (BRP) batch 1, has an identical assigned titre (17). Material from a concentrated trivalent bulk from a commercially available IPV vaccine was established as the BRP batch 2 in 2003, with assigned potencies of 320, 67 and 282 DU/mL for types 1, 2 and 3 respectively (18). Following inconsistency in the performance of some vials of 91/574, the use of this reference was discontinued in 2010. In 2013, the Third WHO International Standard for inactivated poliomyelitis vaccine (12/104) was established by the WHO Expert Committee on Biological Standardization using BRP batch 2 as the reference in the study. Potencies of 277, 65 and 248 DU/mL were assigned to poliovirus types 1, 2 and 3 respectively (19).

A collaborative study conducted in 2015–2016 found the International Standard for conventional IPV (12/104) unsuitable for measuring the antigen potency of sIPV. A relatively high proportion of invalid assays and large differences between laboratory potency results were observed when using 12/104 as a reference to measure the potency of sIPV products. Assay validity and inter-laboratory variability improved when an sIPV sample was used as a reference to determine the potency of sIPV study samples. The decision was made to establish a new WHO International Standard specific to sIPV products. The First WHO International Standard for Sabin inactivated poliomyelitis vaccine (17/160) was established by the WHO Expert Committee on Biological Standardization in 2018.
A unitage of 100 SDU/mL was assigned to each of the three poliovirus serotypes (14). The value 100 is an arbitrary unit.

There are still gaps in the scientific knowledge required for the further standardization of IPV products. Some differences have been noted in the antigenic profile of different IPV products, highlighting the importance of product-specific assessment of future IPV products, particularly sIPV products, against current international standards (20–24).

A WHO International Standard for anti-poliovirus sera, types 1, 2 and 3 is available for the standardization of neutralizing antibody tests for poliovirus (25).

The First WHO International Standards for anti-poliovirus sera of types 1, 2 and 3 were established by the WHO Expert Committee on Biological Standardization in 1963 from serotype-specific polyclonal antisera produced by the hyper-immunization of rhesus monkeys with live virus suspensions. Each of the standards was specific to one serotype only. They were established through a collaborative study (26) and assigned a unitage of 10 IU/ampoule for each of the three serotypes.

In around 1989, stocks of these international standards ran very low (especially for type 3) and a replacement was selected following a collaborative study (27). This Second WHO International Standard for anti-poliovirus sera types 1, 2 and 3 (66/202) was established by the WHO Expert Committee on Biological Standardization in 1991 (28). In contrast to the previous standards, the replacement standard was a single serum that exhibited activity against each of the three poliovirus serotypes. The following unitages were assigned: 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. Following the exhaustion of stocks of 66/202, the Third WHO International Standard for anti-poliovirus sera, types 1, 2 and 3 (82/585) was established by the WHO Expert Committee on Biological Standardization in 2006 with assigned unitages of 11, 32 and 3 IU/vial of neutralizing antibody to types 1, 2 and 3 polioviruses respectively (25).

All of the above WHO international standards are available from the National Institute for Biological Standards and Control, Potters Bar, the United Kingdom. For the latest list of appropriate WHO international standards and reference materials, the WHO Catalogue of International Reference Preparations (29) should be consulted.
Replace section A.3.1.3.3 “Additional tests on seeds from Sabin strains and other attenuated strains derived by recombinant DNA technology” with the following text:

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

If live-attenuated Sabin strains are used for vaccine production, established master seeds validated for OPV production should be used as starting materials. A list of seeds used for OPV production is provided in Appendix 1 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7). In order to maintain production consistency, the conditions used for virus seed propagation – including the temperature and duration of incubation, the multiplicity of infection, and so on – should be well defined and controlled. The conditions used to manufacture OPV described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) are suitable for sIPV production. In addition, tests should be performed to ensure that viruses grown in cell culture do not undergo major genetic changes. Complete consensus nucleotide sequences of Sabin virus master seed and working seed used in the manufacture of IPV should be determined and demonstrated to be identical, as detailed in General considerations above. Alternatively, instead of nucleotide sequence analysis, other in vitro tests (MAPREC or deep sequencing) or in vivo tests (monkey or transgenic mouse neurovirulence tests) could be used to confirm genetic stability, as described in sections A.3.2.4 and A.3.2.5 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) and below in section A.4.4.2.7 of this amendment document. The specifications for these tests must be established in agreement with the NRA.

Suitable in vitro tests should be performed on the master seed produced from attenuated strains derived by recombinant DNA technology. Such tests may include full genome characterization by determining consensus nucleotide sequences, or deep sequencing techniques and demonstration of genetic or phenotypic stability on passage under production conditions. Tests should be validated for this purpose using appropriate standards and materials, and should be approved by the NRA. The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology using in vivo neurovirulence tests should be considered and scientifically justified, in agreement with the NRA.

Any new virus working seed derived from an established master seed, including Sabin strains and other attenuated strains derived by recombinant DNA technology, along with at least three consecutive purified monovalent pools, should be analysed to monitor virus molecular characteristics – for example, through nucleotide sequence analysis (see section A.4.4.2.7.1 of this amendment document).
Replace section A.4.4.2.7 “Additional tests for purified monovalent pools produced from Sabin vaccine seeds or from other attenuated seeds derived by recombinant DNA technology” with the following text:

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

Production conditions – particularly Sabin virus growth conditions (for example, the temperature and duration of incubation, the multiplicity of infection, and so on) – should be well defined and controlled in order to maintain the genetic stability of the virus. The conditions used to manufacture OPV described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) are suitable for this purpose. The production conditions should be validated by testing a sufficient number of consecutive purified monovalent virus pools using suitable methods (with the number of pools used and tests selected to be approved by the NRA) to confirm the maintenance of consistent properties. Such tests may be applied to a proportion of the monovalent virus pools produced each year in order to ensure production consistency. The number of pools of each serotype tested each year should be justified and should be agreed with the NRA.

Suitable tests for Sabin strains include nucleotide sequencing to demonstrate that there is no difference between the complete consensus nucleotide sequences of the seed virus and purified monovalent pools, as detailed in General considerations above. Alternatively, instead of nucleotide sequence analysis, the tests described in section A.3.2.4 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7) could be used to confirm the stability of the attenuated properties of the virus and to monitor the consistency of virus molecular characteristics. Suitable tests include the in vitro and in vivo tests described in section A.4.4.2.7.1 of this amendment document and in section A.4.4.2.7.2 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1).

The use of the rct40 test to confirm attenuated phenotype is discouraged as it is insufficiently sensitive.

Suitable tests should be performed on purified monovalent pools produced from attenuated strains derived by recombinant DNA technology. Tests may include full genome characterization by nucleotide sequencing or deep sequencing techniques. Such tests should be validated for this purpose by the use of appropriate standards and materials, and should be approved by the NRA.
Replace the following opening paragraph of section A.4.4.2.7.1 “Tests to monitor virus molecular characteristics (consistency)” with the replacement text shown below:

Original text to be replaced

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

Replacement text

Characterization of the molecular properties of Sabin virus and strains derived by recombinant DNA technology grown for IPV production may provide an additional tool for monitoring consistency of manufacture. If IPV manufacturers choose to implement this approach, the consistency of the molecular characteristics of Sabin virus and strains derived by recombinant DNA technology can be monitored by determining the proportion of mutations present at specific nucleotide positions (mutational profiles) using deep sequencing or MAPREC as described in the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (7). The specifications for interpreting these tests should be established and approved by the NRA.

Replace the following opening paragraph of section A.4.5.2 “Test for effective inactivation” with the replacement text shown below:

Original text to be replaced

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

Replacement text

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

Two samples should be taken of a volume equivalent to at least 1500 human doses of each inactivated purified monovalent pool. One sample should be taken at the end of the inactivation period and the other not later than three quarters of the way through this period. After removal or neutralization of the inactivating agent, the samples should be tested for the absence of infective poliovirus by inoculation into tissue cultures. Kidney cells from some monkey species, such as those of the genera Macaca, Cercopithecus and Papio spp. – as well as mouse L20B cells expressing human poliovirus receptor, and HEp-2 (Cincinnati) cells – all appear to possess adequate sensitivity. If other tissue culture systems are used, they should have been shown to possess at least the same sensitivity to poliovirus as those specified above by inoculating with partially formalin-inactivated virus (as opposed to infectious, untreated virus) as formalin treatment changes the biological properties of poliovirus (see below). When primary monkey kidney cells are used for this test, the two samples should be inoculated into culture vessels of tissue cultures derived from different batches of cells.

Delete section A.6.4 “General safety test (innocuity)” – see section 3.1.3 in WHO Technical Report Series No. 1016 (6)

Replace section B.4 “Evaluation of immunogenicity in animal models” with the following text:

B.4 Evaluation of immunogenicity in animal models

Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in suitable animal models (for example, rats). Proof-of-concept nonclinical studies should include the comparison of immunogenicity between a candidate IPV and a licensed IPV based on serotype-specific serum neutralizing antibody titres against suitable poliovirus strains. These studies may also assist in the selection of D-antigen content to be tested in the dose-finding studies in humans. However, it is important to note that immunogenicity data in animals do not reliably predict the antigen content that might be appropriate for inclusion as a single human dose in the final vaccine formulation. An assay using transgenic mice may be performed to compare the immune response and protection against virulent challenge induced by a candidate IPV to that induced by a licensed IPV (30, 31). In vivo tests are also important tools to be used as characterization tests to demonstrate comparable manufacturing processes when major changes are introduced.

In view of antigenic differences between different poliovirus strains of the same serotype and limited clinical experience with IPV derived from
Sabin strains or other attenuated strains, it is important to assess the neutralizing antibody titres induced by a candidate IPV derived from Sabin or other attenuated strains against heterologous poliovirus strains that differ from the production strains in antigenicity. For practical reasons, wild-type poliovirus strains used in the manufacture of wIPV (or their S19-wild equivalents) are considered suitable heterologous challenge strains for a candidate sIPV. Any other heterologous strain, demonstrated to be antigenically different from production strains, may be used if justified and approved by NRA. When comparing serum neutralizing antibody titres between a candidate sIPV and a licensed IPV for the purpose of selecting D-antigen content for clinical studies, it is important to bear in mind that the titre of sIPV-induced neutralizing antibody measured against homologous production strains or their S19 equivalents will most likely be higher than that measured against heterologous strains (for example, strains used for wIPV production or their S19 equivalents). It is vital that the D-antigen content selected for further clinical studies is sufficient to induce protective immunity against heterologous strains. Adequate antigen content is required for better cross-protection against heterologous strains (32).

When an adjuvant is included in the formulation, manufacturers should provide a rationale and immunogenicity data to support the use of an adjuvant in the vaccine (33).

Replace section C.2.1 “Assessment of the immune response” with the following text:

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 (34). The demonstration of an immune response to IPV vaccination should be based upon the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for polio antigen is defined as:

- for subjects seronegative at the pre-vaccination time point, post-vaccination antibody titres of ≥ 8;
- for subjects seropositive at the pre-vaccination time point, a ≥ 4-fold rise in post-vaccination antibody titres. In the event that the pre-vaccination titre is due to maternal antibodies, a 4-fold rise above the expected titre of maternal antibodies based
on the pre-vaccination titre declining with a half-life of 28 days indicates seroconversion, or post-vaccination antibody titres of $\geq 8$, 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 (35), with the exception of the challenge poliovirus strains. The challenge poliovirus strains should be carefully selected, as this may affect the results (and the interpretation of the results) of clinical studies as further discussed below in section C.2.4 of this amendment document. IPV developers are encouraged to use appropriate 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 International Standard (see section A.1.3 of this amendment document) should be used to control assay performance (36, 37).

Replace section C.2.2 “Comparative immunogenicity studies” with the following text:

**C.2.2 Comparative immunogenicity studies**

A candidate IPV should be directly compared with at least one licensed IPV in prospective controlled studies. The choice of comparator vaccine is crucial for interpreting the results of a non-inferiority study, and should take into account the available evidence supporting its efficacy. Currently licensed wIPV products are better suited as comparator vaccines as they are similar to IPV products shown to be efficacious against paralytic poliomyelitis both in clinical studies and during more than 60 years of use in controlling poliomyelitis (38, 39). This recommendation is in line with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (40).

Non-inferiority studies to evaluate immunogenicity after completion of the primary vaccination series in the target population (for example, naive infants) are required for regulatory approval of a candidate IPV. Persistence of the serum neutralizing antibodies after completion of the primary series should also be investigated to determine whether and when a booster dose might be required. However, data on long-term antibody persistence might not be available prior to regulatory approval. The waning of antibodies over time is inevitable and should not be interpreted as indicating the need for a booster dose per se.
Replace section C.2.4 “End-points and analyses” with the following text:

C.2.4 End-points and analyses

The primary study analysis should be based on the rate of seroconversion (see section C.2.1 of this amendment document) measured at approximately 4 weeks following completion of the primary infant immunization. The primary study objectives should be based on the demonstration of the non-inferiority of the seroconversion rates achieved with the candidate IPV versus the comparator vaccine.

It is well recognized that the antigenic properties of different poliovirus strains of the same serotype are different, and that the titre of IPV-induced neutralizing antibody measured against homologous strains is generally higher than that measured against heterologous strains (32). Therefore, the level of neutralizing antibody present in clinical samples should in principle be assessed against heterologous poliovirus strains that differ in antigenicity when compared to the production strains to ensure that the conclusions of the clinical studies are applicable to different poliovirus strains. However, if the serum-neutralizing antibodies induced by a candidate sIPV are measured against the Sabin strains for the entire study population then, at a minimum, a subset of the clinical samples should be assessed against heterologous strains to ensure that the adequate seroconversion rate is reached. For practical reasons, wild-type poliovirus strains used in the manufacture of wIPV (or their S19-wild equivalents) are considered to be suitable heterologous challenge strains for assessing a candidate sIPV. In order to minimize bias, the subset should be randomly assigned from the total randomized test vaccine (candidate) and control vaccine (comparator) groups according to study protocol.

The clinical study protocol, including the sample size of the subset, should be discussed and agreed upon with the NRA prior to commencement of the study.

The requirement for assessing neutralizing antibody titres against heterologous poliovirus strains may be waived for technology-transfer products if such data have already been generated at a different site, and the comparability of the products used at these sites has also been demonstrated. However, such a waiver should be discussed with, and approved by, the NRA.

Other strategies may also be acceptable, provided that the data and analysis demonstrate adequate seroconversion rates, induced by a candidate IPV derived from attenuated strains, against heterologous poliovirus strains.

Comparison of geometric mean titres (GMTs) and reverse cumulative distributions of individual titres against all poliovirus strains tested at 4 weeks...
post-primary should also be performed. While it may be that the GMT(s) for one or more poliovirus serotypes induced by the candidate IPV derived from attenuated strains is lower than that induced by the comparator vaccine, it is not clear if a lower GMT at 4 weeks post-primary affects long-term antibody persistence. Consequently, any significant differences in observed GMT should be carefully considered by the NRA and a decision should be supported by additional studies of antibody persistence (see section C.2.2 of this amendment document) and/or by a commitment to post-marketing studies – see section C.5 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) (1).

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

Further guidance on demonstrating non-inferiority is provided in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (40).

Authors and acknowledgements

The first draft of this document was prepared by Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, Technologies, Standards and Norms, World Health Organization, Switzerland. In addition, Dr R. Sutter, Dr M. Eisenhawer and Dr C. Sein, Department of Polio Operations and Research (WHO/POL), World Health Organization, Switzerland and Dr G. Enwere, Dr M. Janssen and Dr O. Lapujade, Vaccine Prequalification Team, World Health Organization, Switzerland contributed to the discussions and provided inputs into the first draft.

The draft document was then posted on the WHO Biologics website during April and May 2019 for a first round of public consultation and comments were received from: Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Ms Q. Guan, Beijing Institute of Biological Products, China; Dr H-S Kim, LG Chem Ltd, Republic of Korea; Dr J. Li and Dr M. Sun, Institute of Medical Biology at the Chinese Academy of Medical Sciences, China; Dr K. Mahmood, PATH, the USA; Dr K. Maithal, Cadila Healthcare Ltd (Zydus Cadila), India; Dr W. Meng, Sinovac Biotech Ltd, China; Dr P. Minor, London, the United Kingdom; Dr J. Modlin, Dr D. Vaughn, Dr A. Chakrabarti and Dr A. Bandyopahiyay, Bill & Melinda Gates Foundation, the USA; Dr M. Pallansch, Centers for Disease Control and Prevention, the USA; Dr M. Song, National Institute of Food and Drug Safety Evaluation, Republic of Korea; Dr R. Sutter, Dr M. Eisenhawer and Dr C. Sein (WHO/POL); Dr T. Wu and Dr M. Baca-Estrada, Health Canada, Canada; Dr W. Wulandari, National
Agency of Drug and Food Control, Indonesia; Dr H. Zheng, Beijing Minhai Biotechnology Co., Ltd, China; and Dr C. Cahill, (on behalf of the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) provided the consolidated comments of subject matter expert reviewers from Janssen Pharmaceuticals, Sanofi Pasteur, GSK and Takeda Pharmaceutical Company Ltd).

Taking into consideration the comments received, a second draft document was prepared by Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, Technologies, Standards and Norms, World Health Organization, Switzerland.

Acknowledgments are also due to the following experts who provided written comments in response to the posting of the resulting document WHO/BS/2019.2354 on the WHO Biologicals website for a second round of public consultation between July and September 2019: Dr M. Eisenhawer, WHO/POL; Dr I. Feavers, National Institute for Biological Standards and Control, the United Kingdom; Dr H-S Kim, LG Chem Ltd, Republic of Korea; Dr W. Meng, Dr T. Wang, Dr J. Li, Dr G. Zeng, Dr D. Jiang and Dr Z. Yao, Sinovac Biotech Ltd, China; Dr V. Pithon, Agence nationale de sécurité du médicament et des produits de santé, France; Mr S.D. Prasad, Bharat Biotech International Ltd, India; Dr J. Southern, Advisor to the South African Health Products Regulatory Authority, South Africa; Dr A.K. Tahlan and Dr R.P. Roy, Central Research Institute, India; Ms L. Li, Wuhan Institute of Biological Products Co., Ltd, China; and Dr I. Knott (on behalf of IFPMA provided the consolidated comments of GSK Vaccines, Belgium and Sanofi Pasteur, France).

In response to the comments received during the second round of public consultation, a number of proposed amendments were presented by Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin and Dr M. Majumdar, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr T.Q. Zhou, Technologies, Standards and Norms, World Health Organization, Switzerland to the WHO Expert Committee on Biological Standardization which made further changes to document WHO/BS/2019.2354.

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