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Proposed Amendment to "Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)", Annex 3, WHO Technical Report Series No. 993

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed *amendment to Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated), Annex 3, WHO Technical Report Series No.993* to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received by 10 May 2019 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Essential Medicines and Health Products (EMP). Comments may be submitted electronically to the Responsible Officer: **Dr Tiequn Zhou** at email: **zhout@who.int**.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).

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Introduction

The WHO Expert Committee on Biological Standardization (ECBS) adopted Recommendations to Assure the Quality, Safety and Efficacy of Poliomyelitis Vaccines (Inactivated) (1) at its 65th meeting in 2014. This guidance document outlines the recommendations for the manufacture and control, nonclinical and clinical evaluation, and lot release of inactivated poliomyelitis vaccines (IPV). These recommendations included the use of several assays requiring the handling of live poliovirus and were produced at a time when limited data and experience with Sabin-based IPV (sIPV) was available and 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 for the implementation of biosafety and biosecurity measures at the facilities handling poliovirus in the post-eradication era. More recently, the Guidelines for the safe production and quality control of poliomyelitis vaccines (3) were also adopted by ECBS in 2018, which further align the biocontainment requirements for both sIPV and wild-type strains derived IPV (wIPV) production facilities with Good Manufacturing Practice (GMP) and GAPIII requirements. Currently, a step-wise implementation of GAPIII is taking place according to WHO Containment Certification Scheme (4). As a result, global laboratory testing capacity for performing assays using live poliovirus, particularly type 2, is limited. Therefore, the requirement to perform neutralization tests against both Sabin and wild strains of poliovirus may delay the development and availability of new IPV products derived from Sabin and other attenuated strains.

Given the urgent need for sIPV to ensure global IPV supply, as well as recent progress in sIPV development, the ECBS at its meeting in 2018 recommended amending the Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) published in Annex 3 of WHO TRS No.993 (1). Key issues addressed in these revised WHO Recommendations include:

- modifying the requirements for confirmation of the attenuated phenotype of vaccine seeds and monovalent virus pools to provide flexibility for vaccine developers;
- adding cell substrates that can be used for the effective inactivation test based on published studies (5);
- updating the recommendation for evaluation of sIPV vaccine immunogenicity in nonclinical and clinical studies to require the measurement of neutralizing antibodies in serum samples against Sabin viruses, while testing against wild type poliovirus strains only in a subset of clinical serum samples, to reduce the number of tests and thus facilitate development and licensure of new vaccines;
- updating information on WHO International Standards available to support quality control testing.

No attempt was made to review the entire document at this time and only the above issues were addressed. The following amendments are proposed:

In *General considerations* of annex 3, TRS No. 993 (page 95-96), the following two paragraphs should be replaced as indicated below.

~~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:

After the circulation of polioviruses is stopped, vaccine-manufacturing establishments will remain a major source of risk for 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 WHO Guidelines for the safe production and quality control of poliomyelitis vaccine (3). According to GAPIII, containment conditions for wild and Sabin strains are almost identical. sIPV production should therefore be performed under almost the same stringent containment as production from wild strains. It is known that upon cultivation under inappropriate conditions (e.g. higher temperature, multiplicity of infection, etc) Sabin strains can undergo reversion to virulence. Therefore, to prevent it, conditions for growing Sabin viruses for subsequent inactivation should be identical as those used in manufacture of OPV (6).

Additional tests should also be performed on seed viruses and production lots to confirm that attenuated viruses do not undergo reversion during IPV manufacture. This can be demonstrated by determining complete nucleotide sequences of seed viruses and viral harvest and confirming that no mutations occurred during virus growth. Establishing that the consensus sequence of viral harvest is identical to that of the starting seed virus is sufficient. Alternatively, maintenance of attenuated phenotype could be confirmed by performing *in vivo* tests (monkey or transgenic mouse neurovirulence tests) that are described in WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (6).

In addition to Sabin strains, alternative attenuation methods utilizing recombinant DNA technology are being investigated (7-11). 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 the significantly reduced likelihood to convert 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 based on scientific evidence. Recommendation about the appropriate level of containment of such strains will be made by the WHO Containment Advisory Group (CAG), after reviewing detailed information about their biological characteristics (3,4). For example, CAG determined that S19 strains (containing genetically stabilized 5'UTR region) with capsid region P1 of wild-type and Sabin vaccine strain polioviruses of all three serotypes, 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 (12). The containment measures for the new strains should be approved by the NRA and the National Authority for Containment.

Replace section *A.1.3, International reference materials* with the following text:

An International Standard of IPV is available for use in *in vitro* assays to measure the D-antigen content of IPV containing classical wild-type strains. Several studies revealed differences in the reactivity of antibody reagents in various ELISA methods used by manufacturers and control laboratories with conventional IPV and sIPV products which resulted in high between-laboratory variability of potency results of IPV products when using a heterologous reference. For this reason, a new International Standard specific for sIPV products was established in 2018 and a new antigen unit, Sabin D-Antigen Unit (SDU), was defined. Both conventional IPV and sIPV International Standards are for use in calibrating secondary reference preparations of IPV, which are then used in potency tests to calculate D-antigen or SD-antigen content. International standards and reference reagents for the control of *in vivo* potency assays are under investigation.

> An International Reference Preparation (IRP) of poliomyelitis vaccine (inactivated) was established by the WHO ECBS in 1962 (*13*). 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 ECBS in 1994 as the second WHO International Reference Reagent for in vivo and in vitro assays of IPV (14). Potencies of 430, 95 and 285 D-antigen units per millilitre were assigned, respectively, to poliovirus types 1, 2 and 3 of this preparation. A separate aliquot of the preparation, established by the European Pharmacopoeia Commission as the Biological Reference Preparation (BRP) batch 1, has an identical assigned titre (15). Material from a concentrated trivalent bulk from a commercially available IPV vaccine was established as the BRP batch 2 in 2003, with assigned potencies of 320, 67 and 282 D-antigen units per millilitre for types 1, 2 and 3, respectively (16). 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 ECBS using BRP batch 2 as the reference in the study. Potencies of 277, 65 and 248 D-antigen units per millilitre were assigned to poliovirus types 1, 2 and 3, respectively (17). A collaborative study conducted in 2015/2016 found the International Standard for conventional IPV (12/104) unsuitable for measuring the antigen potency of sIPV as a relatively high proportion of invalid assays and large differences between laboratory potency results were found when using 12/104 as a reference to measure the potency of sIPV products. Assay validity and between-laboratory variability improved when a sIPV sample was used as a reference to determine the potency of sIPV study samples. The decision was to establish a new International Standard specific for sIPV products. The First International Standard for sIPV (17/160) was established by the WHO ECBS in 2018. Potencies of 100 Sabin D-Ag Units (SDU) per millilitre for each of the three poliovirus serotypes were assigned to poliovirus types 1, 2 and 3 (18).

There are still gaps in the scientific knowledge of biological standardization of IPV and some differences have been found between sIPV products which means that assessment of future vaccines, particularly sIPVs, would require further validation of international references.

An International Standard for anti-poliovirus types 1, 2 and 3 antibodies (human) is available for the standardization of neutralizing antibody tests for poliovirus (19).

The 1st International Standards for anti-poliovirus sera types 1, 2 and 3 were established by the WHO ECBS in 1963 from monospecific polyclonal antisera produced by the hyper-immunisation of rhesus monkeys with live virus suspensions (20). Each of the standards was monospecific and were established through a collaborative study (WHO, 1963) and assigned a unitage of 10 IU/vial, for each of the polio serotypes. The 2nd IS (66/202) was established by the WHO ECBS in 1991 to replace the 1st International Standards. Stocks of the 1st ISs ran very low around 1989 (especially for the type 3) and a replacement for the 1st IS was selected through a collaborative study (21). In contrast to the 1st IS the 2nd IS was a single serum that contained activity against each of the three poliovirus serotypes. The following unitage was assigned to the 2nd IS: 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 exhaustion of 66/202, the 3rd International Standard for anti-poliovirus sera (Human) types 1, 2 and 3 (82/585) was established by the WHO ECBS in 2006 with assigned unitage of 11, 32 and 3 IU per vial of neutralising antibody to type 1, 2 and 3 poliovirus respectively (22).

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

Replace section *A.3.1.3.3, Additional tests on seeds from Sabin strains and other attenuated strains derived by recombinant DNA technology,* by the following:

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 is available in Appendix 2 of TRS 980 (6). The conditions used during working seed virus growth, including the temperature and the duration of incubation, the multiplicity of infection, etc., should be identical to those for OPV as described in WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (6). In addition, tests should be performed to ensure that viruses grown in cell culture retained markers of attenuation present in virus seeds. Complete consensus nucleotide sequences of master seed Sabin virus and working seed used in manufacture of IPV should be determined and demonstrated to be identical. Alternatively, other *in vitro* (MAPREC or deep sequencing) or *in vivo* tests (monkey or transgenic-mouse neurovirulence tests) could be used to confirm attenuated phenotype, as described in section A.3.2.4 of the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated) (6) and section A.4.4.2.7 of this document (1). The specifications for these tests must be established in agreement with the NRA.

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

The need for testing virus master seed lots of attenuated strains derived by recombinant DNA technology in *in vivo* neurovirulence tests should be considered and scientifically justified, in agreement with the NRA. Any new virus working seed derived from an established master seed, including Sabin strains and other attenuated strains derived by recombinant DNA technology, and at least three consecutive purified monovalent pools should be analysed in tests to monitor virus molecular characteristics such as nucleotide sequence analysis, when relevant (see section A.4.4.2.7.1).

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:

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 the Sabin virus growth conditions (e.g. the temperature and the duration of incubation, the temperature, multiplicity of infection, etc.), should be the same as used for manufacture of OPV, and be validated by testing a sufficient number of consecutive

purified monovalent virus pools (the number to be approved by the NRA) using suitable methods, 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 type tested each year should be justified and should be agreed with the NRA.

The suitable tests for Sabin strains include comparing complete consensus nucleotide sequences of the seed virus and purified monovalent pools and confirming that they are identical. Alternatively, 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) (6), could be used to confirm stability of attenuated properties of the virus and to monitor consistency of virus molecular characteristics. They include *in vitro* and *in vivo* tests described in sections A.4.4.2.7.1 and A.4.4.2.7.2 of this document.

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

In section *A.4.4.2.7.1, Tests to monitor virus molecular characteristics (consistency)*, replace the first paragraph:

~~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).~~

with the following:

Characterization of molecular characteristics of Sabin virus grown for IPV production may provide an additional tool for monitoring consistency of manufacture. If IPV manufacturers choose to implement this approach, consistency of molecular characteristics of Sabin virus could be monitored by determining quantities of mutations present at different genomic 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) (6). The specifications for interpreting the tests should be established and approved by the NRA.

Replace the first paragraph of *section A.4.5.2, Test for effective inactivation*, with the following:

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* sps, as well as mouse L20B cells expressing human poliovirus receptor 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.

Replace section *B.4, Evaluation of immunogenicity in animal models,* with the following:

B.4 Evaluation of immunogenicity in animal models

Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in suitable animal models (for example, rats). Proof-of-concept nonclinical studies should include the comparison of immunogenicity between a candidate IPV and a licensed IPV based on type-specific serum neutralizing antibody titres against 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 (23,24). *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 useful to assess the neutralizing antibody titres induced by a candidate sIPV against both attenuated Sabin strains and wild type strains (i.e. strains used in manufacture of wIPV). 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 strain (i.e. Sabin strains used for vaccine production) is higher than that measured against heterologous strains (i.e. strains used for wIPV production). It is critical that the D-antigen content selected for further clinical studies is sufficient to induce protective immunity against heterologous strains.

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 (25).

Replace section *C.2.1, Assessment of the immune response,* with the following:

C.2.1 Assessment of the immune response

The presence of neutralizing antibody against polioviruses is considered a reliable correlate of protection against poliomyelitis. However, immunity induced by one serotype does not provide protection against the other two serotypes. A serum neutralizing antibody titre of ≥ 8 is considered to be a marker of clinical protection against poliomyelitis (26). The demonstration of an immune response to IPV vaccination should be based upon the measurement of neutralizing antibody titres at pre- and post-vaccination time points. Seroconversion for polio antigen is defined as:

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

The assay used to assess serum neutralizing antibodies of the clinical samples should follow the procedure described in the WHO Manual for the virological investigation of polio (27), with the exception of the challenge poliovirus strains. 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) (28,29), should be used to control the assay performance.

It is well recognized that the antigenic properties are different between different poliovirus strains of the same serotype and the titre of IPV-induced neutralizing antibody measured against homologous strain is generally higher than that measured against heterologous strains (30). Therefore, the level of neutralizing antibody present in clinical samples should ideally be assessed against a heterologous poliovirus strain to ensure that the conclusions of the clinical studies are applicable to different poliovirus strains. If the serum-neutralizing antibodies induced by a candidate IPV derived from Sabin or other attenuated strains are measured against the Sabin strains per WHO Manual for the virological investigation of polio (27), at a minimum, a subset of the clinical samples should be assessed against heterologous strains (e.g. the production strains for wIPV) to ensure protective neutralizing antibody titres.

Replace section *C.2.2, Comparative immunogenicity studies*, with the following:

C.2.2 Comparative immunogenicity studies

A candidate IPV should be directly compared with at least one well established and licensed IPV in prospective controlled studies. The choice of comparator is critical for interpreting the results of a non-inferiority study, and should takes into account the available evidence supporting its efficacy. Based on considerations outlined in WHO Guidelines on clinical

evaluation of vaccines: regulatory expectations (31), the current licensed wIPV are better suited as comparator vaccines.

Non-inferiority studies to evaluate immunogenicity after completion of the primary vaccination series in the target population (for example, naive infants) are required for regulatory approval of a candidate IPV. Persistence of the serum neutralizing antibodies after the primary series should also be investigated to recommend whether and when a booster dose is required. However, data concerning long-term antibody persistence might not be available prior to regulatory approval. The waning of antibodies over time is inevitable and should not be interpreted as indicating the need for a booster dose per se, as available data suggest that persistent immune memory may be sufficient to protect against poliomyelitis (32,33).

Replace section *C.2.4, Endpoints and analyses*, with the following:

C.2.4 Endpoints and analyses

The primary study analysis should be based on the rate of seroconversion (as described in section C.2.1) 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.

When the candidate IPV is prepared from Sabin strains or other attenuated strains and the rate of seroconversion is based on neutralizing antibody titres against Sabin strains, additional tests to measure neutralizing antibody titres against heterologous strains (e.g. the production strains for wIPV) should also be performed and data analysed to ensure that the candidate IPV induces protective neutralizing antibody titres against heterologous poliovirus strains. Genetically modified poliovirus strains that can be manipulated outside of the containment facilities (e.g. S19), but retain identical antigenicity to Sabin or wild types poliovirus may be used for serum neutralizing tests, with the approval of NRA. Serum neutralization tests against homologous strains (from which the candidate vaccine is made) should be performed for the entire cohort of subjects. Further guidance on demonstrating non-inferiority trials, such as non-inferiority margin and sample size, is described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (31).

Tests against the heterologous strains (e.g. the strains used to prepare wIPV) may be performed in a subset. In order to minimise bias, the subset should be randomly assigned from the total randomized test vaccine (candidate) and control vaccine (comparator) groups according to study protocol. The number of subjects included in the subset should be sufficient to represent the entire study cohort, so that the analysis and conclusion from the subset can provide an indication as to whether the seroconversion rate against Sabin strains represent an overestimate of the immune response against wild type strains. The clinical study protocol, including the sample size of the subset, should be discussed and agreed upon with the NRA prior to the commencement of the study.

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

Other strategies may also be acceptable, as long as the data and analysis demonstrate adequate seroconversion rates, induced by a candidate IPV derived from attenuated strains, against both Sabin and wild type poliovirus.

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 as a secondary analysis. While it may be that the GMT(s) for one or more poliovirus types induced by the candidate IPV derived from attenuated strains is lower than that induced by the comparator, it is not clear if a lower GMT at 4 weeks post-primary affects long-term antibody persistence. Consequently, any significant differences in observed GMT (for example, not meeting pre-specified criteria) should be carefully considered by the NRA and a decision should be supported by additional studies of antibody persistence (as described in section C.2.2) and by a commitment to post-marketing studies (described in section C.5).

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

Authors and acknowledgements

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