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Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral)

Proposed replacement of: TRS 904, Annex 1 and
addendum TRS 910 Annex 1

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be reviewed by a WHO informal consultation on Recommendations to Assure the Quality, Safety and Efficacy of Poliovirus Vaccine, Oral (OPV) to be taken place in 2012. The final draft of the document will be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed WHO Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral) 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. Comments proposing modifications to this text MUST be received by 25 January 2012 and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Quality Safety and Standards (QSS). Comments may also be submitted electronically to the Responsible Officer: Dr Tiegun 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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1	Table of contents
2	Introduction
3	General considerations
4	Part A. Manufacturing recommendations
5	A.1 Definitions
6	A.2 General manufacturing recommendations
7	A.3 Control of source materials
8	A.4 Control of vaccine production
9	A.5 Filling and containers
10	A.6 Control tests on final product
11	A.7 Records
12	A.8 Retained samples
13	A.9 Labelling
14	A.10 Distribution and shipping
15	A.11 Stability, storage and expiry date
16	
17	Part B. Nonclinical evaluation of live attenuated poliomyelitis vaccines (oral)
18	B.1 Characterization of a new virus sub-master seed from the WHO master seed
19	B.2 Characterization of new virus working seed from an established virus master seed
20	B.3 Characterization of virus working seeds from an established master seed where passage level
21	between master and working seed is increased
22	B.4 Characterization following changes in the manufacturing process
23	Part C. Clinical evaluation of live attenuated poliomyelitis vaccines (oral)
24	C.1 General considerations
25	C.2 Safety and immunogenicity studies
26	C.3 Post-marketing studies and surveillance
27	Part D. Recommendations for national regulatory authorities
28	D.1 General
29	D.2 Release and certification

1 **Part E. Recommendations for live attenuated poliomyelitis vaccine (oral) prepared**
2 **in primary cultures of monkey kidney cells**

3 E.4 Control of vaccine production in primary cultures of monkey kidney

4 E.4.1 Control of source materials

5 E.4.2 Production precautions

6 E.4.3 Control of single harvests

7

8 **Authors and Acknowledgements**

9 **References**

10

11 Appendix 1

12 Overview of virus seeds used in OPV production

13 Appendix 2

14 *In vivo* tests for neurovirulence and considerations for the choice of assay

15 Appendix 3

16 Preparation of poliomyelitis vaccine (oral) using cell banks

17 Appendix 4

18 Example, for guidance, of cell-culture techniques for the determination of the virus content of
19 poliomyelitis vaccine (oral)

20 Appendix 5

21 Model summary protocol for manufacturing and control of live attenuated poliomyelitis vaccines
22 (oral)

23 Appendix 6

24 Model certificate for the release of poliomyelitis vaccine (oral) by national regulatory authorities

25 Appendix 7

26 Preparation of poliomyelitis vaccine (oral) using monkey kidney cell cultures

27

28

1 **Introduction**

2 Requirements for poliomyelitis vaccine (oral) (OPV) were first formulated in 1962 (1) and
3 revised in 1966 (2) and 1972 (3) when an appendix detailing the production of OPV in human
4 diploid cells was added. The Requirements were further updated in 1982 (4) following an
5 accumulation of data particularly on the performance and evaluation of the monkey
6 neurovirulence test (MNVT) and tests on the karyology of human diploid cells. The
7 Requirements for Poliomyelitis Vaccine (Oral) were updated in full in 1989 (5) to take account of
8 the general requirements for the characterization of continuous cell lines for the preparation of
9 biologicals which were adopted in 1985 (6) and after a WHO Study Group concluded that, in
10 principle, such cell lines are acceptable as substrates for the production of biologicals (7). An
11 addendum was subsequently adopted (8) that introduced changes in tests for freedom from
12 detectable DNA sequences of Simian virus 40 (SV40), the mutant analysis by PCR and
13 restriction enzyme cleavage (MAPREC) assay as an optional, additional in vitro test of poliovirus
14 type 3, increased levels of laboratory containment of wild polioviruses (9) and guidance on
15 additional antibody screening tests (for foamy viruses) for animals from closed primate colonies
16 used as a source for primary monkey kidney cells.

17
18 The Requirements (now Recommendations) were last revised in full in 1999 (10) when the use of
19 transgenic mice expressing the human poliovirus receptor (TgPVR21 mice) (11) as an alternative
20 to the MNVT for type 3 virus were included in the revision and the MAPREC test was introduced
21 as the in vitro test of preference for evaluation of filtered bulk suspensions for poliovirus type 3
22 (12). The rct40 test then became an optional, additional test. The studies with poliovirus types 1
23 and 2 in TgPVR21 mice were completed by June 2000 and an addendum to the
24 Recommendations for the Production and Control of Poliomyelitis Vaccine (Oral) was adopted in
25 2000 (13) to include the neurovirulence test in TgPVR21 mice as an alternative to the MNVT for
26 all three poliovirus serotypes.

27
28 Since then there have been advances in scientific knowledge, the availability of novel laboratory
29 techniques and the use of new vaccine formulations such as monovalent/bivalent OPV. In 2008,
30 the WHO Expert Committee on Biological Standardization (ECBS) recommended that the

1 requirements for OPV be revised as it is over 10 years since they were published. In addition,
2 various tests are now applicable to all types and their significance needs to be better explained
3 and rationalised. Sections on nonclinical and clinical evaluation for new candidate OPV are also
4 required. To facilitate this process, WHO convened a working group meeting to initiate the
5 revision of the WHO recommendations on the production and control of OPV as presently
6 outlined in the Technical Reports Series (TRS) No. 904 and 910. Experts from academia,
7 National Regulatory Authorities (NRA)/National Control Laboratories (NCL) and industry
8 involved in the research, manufacture, authorization and testing/release of OPV from countries
9 around the world, met on 20-22 July 2010 to discuss and identify the issues to be considered for
10 the revision of the TRS No. 904 and 910 (14).

11 12 **Scope**

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

17
18 At the time of preparation of this document, there is increasing interest in developing, through
19 molecular manipulation, new safer strains for use in OPV production. The poliovirus-specific
20 quality evaluation of such strains e.g. neurovirulence testing, MAPREC etc, may not apply. The
21 tests on such vaccines, which are likely to include extensive pre-clinical and clinical studies to
22 demonstrate attenuation, genetic stability, safety, and transmissibility of the proposed strains, will
23 have to be considered separately on a case by case basis and may be fundamentally different from
24 those described in this document.

25
26 This document should be read in conjunction with the relevant WHO guidelines including those
27 on nonclinical (15) and clinical evaluation (16) of vaccines.

28 29 **General considerations**

30 Poliomyelitis is an acute communicable disease of humans caused by 3 distinct poliovirus
31 serotypes, types 1, 2 and 3, distinguished by neutralization test (17). Poliovirus is a species C

1 human enterovirus of the *Picornaviridae* family and is composed of a single-stranded, positive-
2 sense RNA genome and a protein capsid.

3
4 Where sanitation is poor, these viruses are believed to spread mainly by faecal-to-oral
5 transmission, whereas the oral-to-oral mode of transmission probably dominates in settings with a
6 high standard of sanitation. However, in most settings, mixed patterns of transmission are likely
7 to occur. In the pre-vaccine era, roughly one out of 200 susceptible individuals infected by
8 polioviruses developed paralytic poliomyelitis (17).

9
10 Progress in polio control (and since 1988, polio eradication) has been due mainly to widespread
11 use of vaccines. An inactivated poliovirus vaccine (IPV Salk vaccine) was licensed in 1955; live,
12 attenuated OPV (Sabin vaccine) was licensed as monovalent OPV in 1961 and as trivalent OPV
13 (tOPV) in 1963. The Sabin strains of poliovirus used in the production of OPV were shown to be
14 both immunogenic and highly attenuated when administered orally to susceptible children and
15 adults. Most countries that initially introduced vaccination with IPV later changed to OPV
16 because it provided many advantages including ease of administration, suitability for mass
17 vaccination campaigns, superior induction of intestinal mucosal immunity, and lower production
18 costs. In 1974, OPV was recommended as part of the Expanded Programme on Immunization
19 (EPI) programme, and OPV was again the vaccine of choice in 1988, when the World Health
20 Assembly resolved to eradicate polio globally by the year 2000. By 2010, 3 of the 6 WHO
21 Regions have been certified as free of wild polio viruses, and wild type 2 has not been detected
22 worldwide since 1999 (17).

23
24 In addition to tOPV, which is used in many countries for routine or supplemental vaccination,
25 monovalent OPVs, against type 1 (mOPV1) and against type 3 (mOPV3) and bivalent OPV
26 against type 1 and type 3 (bOPV) (17), used by the Global Polio Eradication Initiative, have been
27 licensed for use in endemic countries or for outbreak control in situations where one or two types
28 can re-emerge. Monovalent OPV against type 2 has been licensed, but is expected to be used
29 primarily for emergency response stockpiles. Recently, the Strategic Advisory Group of Experts

1 (SAGE) on Immunization was asked by WHO to consider the possible replacement of tOPV with
2 bOPV.

3
4 Following the introduction and widespread use of the first mOPV1 and mOPV3 in supplemental
5 immunization activities in 2005, the polio eradication programme has reported substantial
6 reductions in the respective poliovirus types. However, the co-circulation of wild poliovirus types
7 1 and 3 in the four remaining polio-endemic countries has made programmatic decisions difficult
8 regarding which vaccine to use. A clinical trial to evaluate the immunogenicity of different OPV
9 formulations (mOPV1, mOPV3 and bOPV) compared to tOPV in an Indian population was
10 conducted by WHO. The seroconversion rates to poliovirus type 1 and type 3 following
11 immunization with bOPV were significantly higher than those induced by tOPV and not inferior
12 to those induced by immunization with either mOPV1 or mOPV3 respectively (18).

13
14 Live vaccines prepared from the Sabin strains of poliomyelitis viruses of types 1, 2, and 3 were
15 introduced for large scale immunization in 1957. In 1972, Sabin proposed that WHO be the
16 custodian of his poliovirus seed strains. The Director General of WHO agreed to assume
17 responsibility for ensuring the proper use of the strains and established a scientific committee, the
18 Consultative Group on Poliomyelitis Vaccines, to advise WHO on all matters pertaining to their
19 use. Detailed information on the work of the Consultative Group and the preparation of the
20 strains prepared by Behringwerke has been published by Cockburn (19). National regulatory
21 authorities should decide on the use of virus strains and on the detailed procedures applicable to
22 the preparation of virus seed lots for the production of OPV in their own countries.

23
24 The original poliovirus seeds produced by Sabin (SO) (20) were sent to Merck, who generated
25 seeds from them that were designated as SOM (Sabin Original Merck). Aliquots of SOM were
26 supplied to a number of other manufacturers to develop their own seeds. Some seed lots were
27 contaminated with SV40 which was present in the primary Rhesus kidney cells, the preferred cell
28 culture system at that time for virus propagation. OPV manufacturers followed various strategies
29 to reduce the contamination including passage in the presence of specific antibody or treatment
30 with toluidine blue or thermal inactivation of SV40 in the presence of 1M MgCl₂ that stabilizes
31 poliovirus. In 1974 Behringwerke AG, Marburg/Lahn, Germany, generously agreed to produce

1 SO+1 seeds for WHO, free of charge. The Behringwerke type 1 and type 2 seeds have been
2 particularly widely used from the 1970s to date.

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

7
8 The type 3 vaccine was found to be less stable on passage than either type 1 or type 2 which
9 manifested in a higher number of type 3 vaccine lots failing the monkey neurovirulence test. In
10 order to develop a more stable strain, a new seed was prepared by Pfizer by transfecting
11 susceptible cells with viral RNA extracted from poliovirus, one plaque was identified with
12 particularly favorable properties which was designated 457-III (21). Theoretically, vaccine
13 derived from this stock was at passage SO+7. However, the purpose of tracking passage history
14 of seed viruses is to reduce the accumulation of mutations that takes place in the course of their
15 serial propagation. Since plaque purification represents cloning of a single infectious particle, it
16 eliminates heterogeneity of viral population and the passage level is effectively reset to zero.
17 Therefore the cloned stock 457-III was renamed as RSO for RNA derived Sabin Original. Two
18 additional passages were used to prepare virus master (RSO1) and working seeds (RSO2), and
19 vaccines produced from this virus are at RSO3 level. Retrospectively, the sequence of RSO has
20 been shown to be the same as the consensus of SO (22), but it was more homogeneous and
21 contained lower quantities of mutant viruses.

22
23 The RSO seed was not used for production of type 3 vaccine until the 1980s when it became
24 clear that the stocks of material passaged from the SOM and other SO+1 seeds were inadequate.
25 However since then it has been widely used by European and American manufacturers as it is of
26 lower virulence in laboratory tests than the SO+1 type 3 seed. The RSO seeds were bought from
27 Pfizer by Sanofi Pasteur (formerly Merieux, Pasteur Merieux Connaught and subsequently
28 Aventis Pasteur) who have recently donated them to WHO.
29

1 The virus seeds available from WHO (designated ‘the WHO master seeds’) are therefore type 1,
2 2 and 3 at SO+1 level produced by Behringwerke from SO seeds and the type 3 RSO seed
3 donated by Sanofi Pasteur. The seeds are kept at the National Institute for Biological Standards
4 and Control (NIBSC), UK, and include a proportion of the stocks of the SO+1 seeds formerly
5 held at Istituto Superiore di Sanità who has kindly transferred them to NIBSC (19, 21).

6
7 In addition to the RSO type 3 seed a number of manufacturers in China, Japan and Russia used
8 their own purified seed stocks of Sabin 3 strain that were derived by plaque purification (cloning).
9 Sequencing of these seed viruses demonstrated that while they contained low content of
10 neurovirulent mutants, there were differences between these strains and the consensus sequence
11 of Sabin Original virus (22). However, there are no reports of any differences in clinical safety
12 between OPV produced from Pfizer stocks and the alternative seeds of Sabin 3 virus. An
13 overview of virus seeds used in OPV production is given in Appendix 1.

14
15 The MNVT, as described in the 1989 Requirements (5), has been used as a quality control test
16 and is based on the level and the distribution of virus-specific lesions within the central nervous
17 system produced by vaccine virus as compared to an appropriate reference preparation (23).
18 Because non human primates are used, efforts to complement and eventually replace the test are
19 of considerable importance. WHO has encouraged and supported research on various aspects of
20 poliovirus biology, including the development of alternative animal models as part of the WHO
21 initiative to promote the development of new norms and standards for vaccines. Two groups of
22 scientists developed transgenic (TgPVR) mice by introducing into the mouse genome the human
23 gene encoding the cellular receptor for poliovirus (24, 25). This receptor, known as CD155,
24 makes TgPVR mice susceptible to poliovirus infection with clinical signs of flaccid paralysis,
25 and histological lesions in the central nervous system similar to those observed in monkeys.

26
27 In 1992, WHO initiated a project to evaluate the suitability of transgenic mice for testing the
28 neurovirulence of OPV with the aim of replacing monkeys with mice. The advantages of a
29 neurovirulence test in transgenic mice are:

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

- 1 • a reduction in hazards to laboratory personnel through a reduced need to handle primates
- 2 • in some countries, a reduction in the cost of quality control tests for OPV.

3
4 Studies were carried out initially on type 3 monovalent polio vaccines using the TgPVR21 mouse
5 line, generously provided free of charge for the study by the Central Institute for Experimental
6 Animals, Kawasaki, Japan. Researchers at the Japan Poliomyelitis Research Institute and at the
7 Center for Biologics Evaluation and Research (CBER), Rockville, MD, USA developed an
8 intraspinal inoculation method suitable for tests of vaccine lots. This was evaluated in an
9 international collaborative study on the establishment of a standardized mouse neurovirulence
10 test (TgmNVT) for OPV (26). Several laboratories participated in the collaborative study and
11 results were assessed by WHO at meetings held in 1995, 1997 and 1999 in Geneva, Switzerland,
12 in 1997 in Ottawa, Canada, and in 1998 in Rockville, Maryland. As a result of these studies, the
13 revised Recommendations for Production and Control of Poliomyelitis Vaccine (Oral) (10)
14 introduced the murine model as an alternative to the MNVT for type 3 poliovirus and further
15 studies demonstrated that this tests was also suitable as an alternative to the MNVT for poliovirus
16 types 1 and 2 (13). Laboratories must comply with specifications for containment of the
17 transgenic animals (27). The MNVT and TgmNVT can provide evidence of consistency of
18 production.

19
20 The molecular mechanisms and genetic determinants of attenuation and reversion to virulence of
21 all three types of Sabin polioviruses used for the manufacture of OPV have been studied in
22 several laboratories. Evidence strongly suggests that mutations in the 5' non-coding region of the
23 poliovirus genome, especially for the Sabin type 3 strain, are critical in determination of the
24 attenuated phenotype (28). A molecular biological test, mutant analysis by PCR and restriction
25 enzyme cleavage (MAPREC) assay, was developed by researchers at CBER, Rockville, MD, to
26 quantify reversion at the molecular level (29). Studies showed that all batches of type 3 OPV
27 contained measurable amounts of revertants with C instead of U at nucleotide 472. Batches that
28 failed the MNVT contained significantly higher quantities of 472-C than batches that passed the
29 test. Studies with coded samples at CBER identified 100% of lots that failed the MNVT (30).

30

1 The Biologicals Unit of WHO initiated in 1991 a series of international collaborative studies to
2 evaluate the MAPREC assay for all three types of poliovirus and to validate appropriate reference
3 materials. Several laboratories participated in the collaborative studies and results were assessed
4 by WHO at meetings held in 1995 and 1997 in Geneva, Switzerland. It was concluded that the
5 MAPREC assay was a sensitive, robust and standardized molecular biological assay suitable for
6 use by manufacturers and national regulatory authorities for monitoring the consistency of
7 production of type 3 OPV. The revised Recommendations for the Production and Control of
8 Poliomyelitis Vaccine (Oral) (10) introduced, for type 3 poliovirus, the use of MAPREC as the in
9 vitro test of preference in place of the rct40 test. Reference materials for MAPREC for
10 comparable positions in type 1 and type 2 have now been established. While the results do not
11 correlate with neurovirulence in the range studied, they provide a measure of production
12 consistency. Quantity of other mutants (such as 2493-U in Sabin 3 virus) can also be used to
13 identify types of seed virus and to monitor consistency of manufacture. After appropriate
14 validation, quantitative profiles of other mutations in stocks of OPV could be used for this
15 purpose.

16
17 The manufacturer of the final lot must be responsible for ensuring conformity with all the
18 recommendations applicable to the final vaccine (Part A, sections A.5—A.11) even where
19 manufacturing involves only the filling of final containers with vaccine obtained in bulk form
20 from another manufacturing establishment. The manufacturer of the final lot must also be
21 responsible for any production and control tests performed by an external contract laboratory, if
22 applicable, with the approval of the national regulatory authority.

23
24 OPV has been in worldwide use since the 1960s and, although those produced from human
25 diploid cells or continuous cell lines have been used to a lesser extent than those produced in
26 cultures of primary monkey kidney cells, experience has indicated that all three cell substrates
27 produce safe and effective vaccines.

28 29 **Part A. Manufacturing recommendations**

30 **A.1 Definitions**

31 **A.1.1 *International name and proper name***

1 The international name should be live attenuated poliomyelitis vaccine (oral) with additions to
2 indicate the virus serotype(s) of the vaccine. The proper name should be equivalent to the
3 international name in the language of the country of origin.

4
5 The use of the international name should be limited to vaccines that satisfy the specifications
6 formulated below.

7
8 **A.1.2 *Descriptive definition***

9 Live attenuated poliomyelitis vaccine (oral) is a preparation of live attenuated poliovirus types 1,
10 2, or 3 grown in vitro cultures of suitable cells, containing any one type or any combination of the
11 three types of the Sabin strains, prepared in a form suitable for oral administration and satisfying
12 all the recommendations formulated in this document.

13
14 **A.1.3 *International reference materials***

15 A trivalent virus mixture is available as an International Reference Reagent for Live Attenuated
16 Poliovirus (Sabin) Types 1, 2, and 3 for determination of virus titre.

17
18 Three monotypic virus suspensions of types 1, 2, and 3 have been established as WHO Reference
19 Reagents to be used in reference laboratories to measure the sensitivity of cell cultures for
20 poliovirus infection.

21
22 International Standards for MAPREC analysis of poliovirus types 1, 2 and 3 (Sabin) and
23 International Reference Reagents for control of MAPREC assays of poliovirus type 1, 2 and 3
24 (Sabin) are available.

25
26 International Standards for anti-poliovirus types 1, 2, and 3 antibodies (human) are available for
27 standardization of neutralizing antibody tests for poliovirus.

28
29 The reference materials listed above are available from the National
30 Institute for Biological Standards and Control (NIBSC), Potters Bar, UK.

1 Reference preparations WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3
2 virus at the SO+2 passage level are available upon request from WHO (Coordinator, Quality,
3 Safety and Standards, World Health Organization, 1211, Geneva 27, Switzerland) for the
4 comparison of in vivo neurovirulence with that of homotypic vaccines. The relevant reference
5 materials should be included in each test of vaccine (see A.4.5 Tests for neurovirulence).

6
7 **A.1.4 Terminology**

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

10
11 **Adventitious agents:** Contaminating microorganisms of the cell substrate or source materials
12 used in their cultures, that may include bacteria, fungi, mycoplasmas, and endogenous and
13 exogenous viruses that have been unintentionally introduced.

14
15 **Cell-culture infective dose 50% (CCID₅₀):** The quantity of a virus suspension that will infect
16 50% of cell cultures.

17
18 **Cell seed:** A quantity of vials containing well-characterized cells derived from a single tissue or
19 cell of human or animal origin stored frozen in liquid nitrogen in aliquots of uniform composition,
20 one or more of which would be used for the production of a master cell bank

21
22 **Comparator vaccine:** An approved vaccine with established efficacy or with traceability to a
23 vaccine with established efficacy that is tested in parallel with an experimental vaccine and
24 serves as an active control in nonclinical or clinical testing.

25
26 **Final bulk:** The finished vaccine from which the final containers are filled. The final bulk may
27 be prepared from one or more monovalent bulks and may contain more than one virus type.

28
29 **Final lot:** A collection of sealed final containers of finished vaccine that is homogeneous with
30 respect to the risk of contamination during the filling process. All of the final containers must
31 therefore have been filled from a single vessel of final bulk in one working session.

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

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

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

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

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

SO: Sabin Original virus as described in Sabin and Boulger1983 (20). All subsequent passages are designated by an additional number eg SO+1 is one passage on from Sabin Original.

Virus master seed lot: A quantity of virus suspension that has been processed at the same time to assure a uniform composition and having been characterized to the extent necessary to support developing the virus working seed lot. The characterized virus master seed lot is used for the preparation of virus working seed lots or a virus sub-master seed (if applicable).

1 *Virus sub-master seed lot (only applicable for master seed supplied by WHO):* A quantity of
2 virus suspension produced by a single passage from the virus master seed supplied by WHO
3 made at the multiplicity of infection ensuring that cytopathic effect (CPE) develops within an
4 appropriate timeframe and that has been processed at the same time to assure a uniform
5 composition and having been characterized to the extent necessary to support developing the
6 virus working seed lot. The characterized virus sub-master seed lot is used for the preparation of
7 virus working seed lots.

8
9 *Virus working seed lot:* A quantity of virus of uniform composition, fully characterized, derived
10 by only one passage made at the multiplicity of infection ensuring that cytopathic effect (CPE)
11 develops within an appropriate timeframe, e.g. three days, from a virus master seed lot or sub-
12 master seed lot by a method approved by the national regulatory authority.

13
14 *Working cell bank (WCB):* A quantity of cells of uniform composition derived from one or more
15 ampoules of the master cell bank at a finite passage level, stored frozen at -70°C or below in
16 aliquots, one or more of which would be used for vaccine production. All containers are treated
17 identically and once removed from storage are not returned to the stock.

18 19 A.2 **General manufacturing recommendations**

20 The general manufacturing recommendations contained in the *Good manufacturing practices for*
21 *pharmaceutical products: main principles (31)* and *Good Manufacturing Practices for Biological*
22 *Products (32)* should apply to establishments manufacturing oral poliomyelitis vaccine, with the
23 addition of the following:

- 24 • The production of oral poliomyelitis vaccine should be conducted by staff who should
25 consist of healthy persons and who should be examined medically at regular intervals.
26 Steps should be taken to ensure that all such persons in the production areas are immune
27 to poliomyelitis. Personnel working in monkey quarters should also be examined for
28 tuberculosis as outlined in Part A, section 2 *Recommendations to Assure the Quality,*
29 *Safety and Efficacy of Freeze-Dried BCG vaccine (33).*
- 30 • In compliance the current containment recommendations for poliovirus (9).

1 A.3 Control of source material

2 The general production precautions as formulated in *Good Manufacturing Practices for*
3 *Biological Products* (32) should apply to the manufacture of oral poliomyelitis vaccine, with the
4 addition that, during production, only one type of cell should be introduced or handled in the
5 production area at any one time.

6 7 A.3.1 Cell lines

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

9 The use of a cell line for the manufacture of oral poliomyelitis vaccines should be based on the
10 cell bank system. The cell seed and cell banks should conform with the *Recommendations for the*
11 *evaluation of animal cell cultures as substrates for the manufacture of biological medicinal*
12 *products and for the characterization of cell banks* (34). The cell bank should be approved by the
13 national regulatory authority. The maximum number of passages (or population doublings)
14 allowed between the cell seed, the MCB, the WCB and the production passage level should be
15 established by the manufacturer and approved by the national regulatory authority. Additional
16 tests include

- 17 • propagation of the MCB or WCB cells or beyond the maximum in vitro age for
18 production; and
- 19 • examination for the presence of retrovirus and tumorigenicity in an animal test system

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

23 The WHO Vero reference cell bank 10-87 is considered suitable for
24 use as a cell seed for generating a MCB (35) and is available to
25 manufacturers on application to the Coordinator, Quality, Safety
26 and Standards, World Health Organization, Geneva, Switzerland.

27 28 A.3.1.2 Identity test

29 Identity tests on the master and working cell banks are performed in accordance with *WHO*
30 *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of*

1 *biological medicinal products and for the characterization of cell banks* (34) and should be
2 approved by the national regulatory authority.

3
4 The WCB should be identified by means, inter alia, of biochemical, (e.g. isoenzyme analysis)
5 immunological, cytogenetic marker tests and DNA fingerprinting or sequencing. The tests shall
6 be approved by the national regulatory authority.

7
8 *A.3.1.3 Cell culture medium*

9 Serum used for the propagation of cells should be tested to demonstrate freedom from bacterial,
10 fungal, and mycoplasma contamination by appropriate tests as specified in Part A, sections 5.2
11 (36) and 5.3 (37) of the *General requirements for the sterility of biological substances*, and from
12 infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix 1 of
13 the *WHO Recommendations for the evaluation of animal cell cultures as substrates for the*
14 *manufacture of biological medicinal products and for the characterization of cell banks* (34).

15
16 Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera if
17 approved by the national regulatory authority. As an additional monitor of quality, sera may be
18 examined for freedom from phage and endotoxin. Irradiation may be used to inactivate potential
19 contaminant viruses, recognizing that some viruses are relatively resistant to irradiation.

20
21 The source(s) of animal components used in culture medium should be approved by the national
22 regulatory authority. These components should comply with current *WHO guidelines on*
23 *transmissible spongiform encephalopathies in relation to biological and pharmaceutical*
24 *products* (38).

25
26 Human serum should not be used. If human serum albumin is used at any stage of product
27 manufacture, the national regulatory authority should be consulted regarding the requirements, as
28 these may differ from country to country. As a minimum, it should meet the *Requirements for the*
29 *Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives*
30 (39). In addition, human albumin and materials of animal origin should comply with current

1 *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and*
2 *pharmaceutical products.* (38).

3
4 Penicillin and other beta-lactams should not be used at any stage of manufacture because of their
5 nature as highly sensitizing substances.

6
7 Other antibiotics may be used at any stage in the manufacture
8 provided that the quantity present in the final lot is acceptable to the
9 national regulatory authority.

10 Nontoxic pH indicators may be added, e.g. phenol red in a
11 concentration of 0.002%.

12 Only substances that have been approved by the national regulatory
13 authority may be added.

14
15 Bovine or porcine trypsin used for preparing cell cultures should be tested and found free of
16 cultivable bacteria, fungi, mycoplasmas and infectious viruses, as appropriate. The methods used
17 to ensure this should be approved by the national regulatory authority. The trypsin should be
18 gamma irradiated.

19
20 The source(s) of trypsin of bovine origin, if used, should be approved by the national regulatory
21 authority and should comply with current *WHO guidelines on transmissible spongiform*
22 *encephalopathies in relation to biological and pharmaceutical products* (38).

23 24 **A.3.2 Virus seeds**

25 *A.3.2.1 Virus strains*

26 Strains of poliovirus used in the production of OPV should be identified by historical records,
27 which should include information on their origin. Producers of OPV can obtain virus master
28 seeds from WHO. Manufacturers receiving this virus may prepare a sub-master seed by a single
29 passage and then their working seed. However, only virus strains that are approved by the
30 national regulatory authority should be used (see General Considerations).

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A.3.2.2 Virus seed lot system

Vaccine production should be based on the seed lot system. Virus seed lots should not be purified. The virus master seed lot and virus working seed lot used for the production of vaccine batches should be prepared by a single passage from the virus strain and the virus master seed lot respectively, by a method and at a passage level from the original seed virus approved by the national regulatory authority. A virus sub-master seed lot may be prepared by a single passage from the WHO master seed and the characterized virus sub-master seed lot used for the preparation of virus working seed lots by a single passage.

Virus master, sub-master and working seed lots should be stored in a dedicated temperature-monitored freezers at a temperature that ensures stability on storage e.g. $\leq -60^{\circ}$. Guidance on additional characterization of master and sub-master seeds is provided in Part B.

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

The virus master, sub-master and working seed lot used for the production of vaccine batches should be free from detectable extraneous viruses and from detectable SV40 DNA as determined by a validated nucleic acid amplification test, and should be in conformity with the recommendations set out in Part A, sections A.4.3 (Single harvests) and A.4.4.1, A.4.4.2, A.4.4.3 and A.4.4.4 (monovalent bulks). The control cell cultures should conform to section A.4.1 (Control of cell cultures).

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

1 laboratory strains of SV40. The sensitivity of the PCR assays for
2 the genomic regions used should be established.

3 4 *A.3.2.4 Test to monitor virus molecular characteristics*

5 *A.3.2.4.1 Tests in vitro*

6 Seed viruses should be tested in MAPREC or temperature sensitivity assays (rct40) (see
7 A.4.4.5.1). At least three consecutive monovalent bulks prepared from the seed virus should meet
8 the criteria for acceptability given in A.4.4.5.1 with agreement of the national regulatory
9 authority.

10 Historically, four consecutive monovalent bulks prepared from the
11 seed virus have been tested for monitoring the virus molecular
12 characteristics and production consistency.

13 14 *A.3.2.4.2 Neurovirulence tests*

15 New virus working seeds should be evaluated for neurovirulence by using either the monkey or
16 the mouse neurovirulence test or both. Summaries of the MNVT and TgmNVT, including
17 pass/fail criteria, are given in Appendix 2 along with considerations on the choice of assay. The
18 test should be approved by the national regulatory authority for the specific product and
19 transgenic mice, non human primates or both may be used.

20
21 The test for neurovirulence in non-human primates should be carried out as summarized in
22 Appendix 2 and following the protocol available from WHO¹ (¹ Coordinator, Quality, Safety and
23 Standards, World Health Organization, 1211, Geneva 27, Switzerland).

24
25 The use of the TgmNVT should be approved by the national regulatory authority and it should be
26 carried out as summarized in Appendix 2 and described in detail in the standard operating
27 procedure (SOP), "WHO neurovirulence test of type 1, 2 or 3 live poliomyelitis vaccines (oral) in
28 transgenic mice susceptible to poliovirus", available from WHO.¹ (¹ Coordinator, Quality, Safety
29 and Standards, World Health Organization, 1211, Geneva 27, Switzerland). Where there is a
30 consistent production process and the TgmNVT has been approved for the release of monovalent

1 bulks, it may also be used for approval of a new virus working seed lot so long as the new seed is
2 derived from the same virus master seed lot or sub-master seed lot as the virus working seed it
3 replaces, and made by a production process used to prepare satisfactory vaccine lots.

4
5 In case of any major changes in the process of production or for a new virus master seed, full
6 characterization in tests in non-human primates and transgenic mice will be required (See Part B).

7
8 The neurovirulence of the virus working seeds and at least three consecutive monovalent bulks
9 prepared from it should meet the criteria for acceptability given in A.4.4.5.2 and the appropriate
10 SOP before being considered suitable for use for the production of OPV, with agreement of the
11 national regulatory authority.

12 Historically, four consecutive monovalent bulks prepared from the
13 seed virus have been tested in monkeys for monitoring the
14 production consistency.

15
16 *A.3.2.5 Genotype characterization*

17 For any new virus working seed, it may be useful to analyze the
18 new virus working seed and the at least three consecutive
19 monovalent bulks for nucleotide sequence changes from the seed
20 virus (deep genome sequence). If such tests are performed, they
21 should be validated and approved by the national regulatory
22 authority.

23
24 **A.4 Control of vaccine production**

25 Part E contains additional or alternative recommendations for oral poliomyelitis vaccine prepared
26 in cultures of primary monkey kidney cells and concerns the testing of the cell substrate used for
27 the production of the vaccine.

28
29 *A.4.1 Control cell cultures*

30 When human diploid or continuous cell lines are used to prepare cultures for production of
31 vaccine, a fraction equivalent to at least 5 % of the total or 500 ml of cell suspension, or 100

1 million cells, at the concentration and cell passage level employed for seeding vaccine production
2 cultures should be used to prepare control cultures. (See Appendix 3 for an example of a
3 flowsheet of tests in cell cultures.

4
5 If fermenter technology is used, the national regulatory authority should determine the size and
6 treatment of the cell sample to be examined.

7 8 *A.4.1.1 Tests of control cell cultures*

9 The treatment of the cells set aside as control material should be similar to that of the production
10 cell cultures, but they should remain uninoculated for use as control cultures for the detection of
11 adventitious agents.

12
13 These control cell cultures should be incubated under conditions as similar as possible to the
14 inoculated cultures for at least 2 weeks, and should be tested for the presence of adventitious
15 agents as described below. For the test to be valid, not more than 20% of the control cell cultures
16 should have been discarded for nonspecific, accidental reasons.

17
18 At the end of the observation period, the control cell cultures should be examined for
19 degeneration caused by an extraneous agent. If this examination, or any of the tests specified in
20 this section, shows evidence of the presence in a control culture of any adventitious agent, the
21 poliovirus grown in the corresponding inoculated cultures should not be used for vaccine
22 production.

23 24 *A.4.1.2 Tests for haemadsorbing viruses*

25 At the end of the observation period, 25% of the control cells should be tested for the presence of
26 haemadsorbing viruses using guinea-pig red blood cells. If these cells have been stored, the
27 duration of storage should not have exceeded 7 days and the storage temperature should have
28 been in the range 2—8 °C. In tests for haemadsorbing viruses, calcium and magnesium ions
29 should be absent from the medium.

30

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

5
6 A reading should be taken after 30 minutes' incubation at 2-8 °C and again after a further
7 incubation for 30 minutes at 20-25 °C.

8
9 If a test with monkey red cells is performed, readings should also be
10 taken after a final incubation for 30 minutes at 34-37°C.

11
12 *A.4.1.3 Tests for other adventitious agents in cell fluids*

13 At the end of the observation period, a sample of the pooled fluid from each group of control
14 cultures should be tested for adventitious agents. For this purpose, 10ml of each pool should be
15 tested in the same cells, but not the same batch of cells, as those used for the production of virus,
16 and additional 10ml samples of each pool should be tested in human cells and at least one other
17 sensitive cell system.

18
19 The pooled fluid should be inoculated into bottles of these cell cultures in such a way that the
20 dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cells
21 should be at least 3 cm² per ml of pooled fluid. At least one bottle of each kind of cell culture
22 should remain uninoculated and should serve as a control.

23
24 The inoculated cultures should be incubated at a temperature of 35-37°C and should be observed
25 for a period of at least 14 days.

26 Some national regulatory authorities require that, at the end of this
27 observation period, a subculture is made in the same culture system
28 and observed for at least an additional 14 days. Furthermore, some
29 national regulatory authorities require that these cells should be
30 tested for the presence of haemadsorbing viruses.

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

3
4 If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus
5 harvests produced from the batch of cells from which the control cells were taken should be
6 discarded.

7
8 Some selected viruses may be screened by using specific validated assays which are approved by
9 the national regulatory authority, such as molecular techniques (e.g., nucleic acid amplification)
10 (34).

11
12 If these tests are not performed immediately, the samples should be kept at a temperature of
13 -60°C or below.

14 15 *A.4.1.4 Identity test*

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

20 21 *A.4.2 Cell cultures for vaccine production*

22 *A.4.2.1 Observation of cultures for adventitious agents*

23 On the day of inoculation with the virus working seed lot, each cell culture or a sample from each
24 culture vessel should be examined for degeneration caused by infective agents. If such
25 examination shows evidence of the presence in a cell culture of any adventitious agent, the
26 culture should not be used for vaccine production (see A.4.1.3).

27
28 If animal serum is used for cell cultures before the inoculation of virus, the medium should be
29 removed and replaced with serum-free maintenance medium, after the cells have been washed
30 with serum- free medium, if appropriate.

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Some selected viruses may be screened by using specific assays, such as molecular techniques (*e.g.*, nucleic acid amplification) (34).

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, shown to be suitable, within the range 33-35°C for the relevant incubation periods. The temperature should not vary by more than 0.5°C from the set temperature. The optimal range for pH, multiplicity of infection, cell density and time of incubation should be established for each manufacturer and be approved by the national regulatory authority.

The virus suspension should be harvested not later than 4 days after virus inoculation.

The inoculated cell cultures should be processed in such a manner 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 Part A sections A.4.1.2, A.4.1.3, A.4.1.4, A.4.3.3.1, A.4.3.3.2, A.4.3.3.3, A.4.3.3.4 and A.4.3.3.5 have been obtained.

A 4.3.2 Sampling

Samples required for the testing of single harvests should be taken immediately on harvesting. If the tests for adventitious agents as described in Part A, 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

1 Each single harvest should be identified as the appropriate poliovirus serotype by immunological
2 assay on cells culture using specific antibodies or by a molecular method which has been
3 validated and approved by the national regulatory authority.
4

5 Care should be taken to ensure that the sera used are monospecific
6 by titrating them against homotypic and heterotypic viruses of
7 known virus titre. Monoclonal antibodies may be useful in this test.
8

9 *A.4.3.3.2 Titration for virus infectivity*

10 The amount of infective poliovirus per ml of single harvest should be determined in cell cultures
11 in comparison with an existing reference preparation (see Appendix 4)
12

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

14 For the purposes of the recommendations set out in this section of Part A, the volume of each
15 single harvest taken for neutralization and testing should be at least 10ml and should be such that
16 a total of at least 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater,
17 has been withheld from the corresponding single harvest.
18

19 The antisera used for neutralization should be of nonhuman origin and should have been prepared
20 in animals other than monkeys, using virus cultured in cells from a species different from that
21 used in the production of the vaccine. Samples of each virus harvest should be tested in human
22 cells sensitive to measles and at least one other sensitive cell system.
23

24 The neutralized suspensions should be inoculated into bottles of these cell cultures in such a way
25 that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the
26 cell sheet should be at least 3 cm² per ml of neutralized suspension. At least one bottle of each
27 kind of cell culture should remain uninoculated and should serve as a control; it should be
28 maintained using nutrient medium containing the same concentration of the specific antiserum
29 used for neutralization.
30

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

5
6 The inoculated cultures should be incubated at a temperature of 35-37°C and should be observed
7 for a period of at least 14 days.

8
9 If adequately justified and validated, lower temperatures may be
10 used.

11
12 For the tests to be valid, not more than 20% of the culture vessels should have been discarded for
13 nonspecific, accidental reasons by the end of the test period.

14
15 If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvest
16 should be discarded.

17
18 New molecular methods with broad detection capabilities are being
19 developed for detection of adventitious agents. These methods
20 include degenerate NAT for whole virus families with analysis of
21 the amplicons by hybridization, sequencing or mass spectrometry;
22 NAT with random primers followed by analysis of the amplicons
23 on large oligonucleotide micro-arrays of conserved viral sequencing
24 or digital subtraction of expressed sequences; and high throughput
25 sequencing. These methods might be used in the future to
26 supplement existing methods or as alternative methods to both *in*
27 *vivo* and *in vitro* tests after appropriate validation and approval of
28 the national regulatory authority (34).

29
30 A.4.3.3.4 Sterility tests for bacteria, fungi and mycoplasmas

1 A volume of at least 10ml of each single harvest should be tested for bacterial, fungal, and
2 mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 (36) and 5.3
3 (37) of the *General requirements for the sterility of biological substances*, or by a method
4 approved by the national regulatory authority.

5
6 Nucleic Acid Amplification Techniques (NAT) alone or in
7 combination with cell culture, with an appropriate detection
8 method, might be used as an alternative to one or both of the
9 compendial mycoplasma detection methods after suitable validation
10 and agreement from national regulatory authority (34).

11 12 *A.4.3.3.5 Test for mycobacteria*

13 Virus harvest should be shown to be free from Mycobacteria by an appropriate method approved
14 by the national regulatory authority.

15
16 Molecular assays may be used as an alternative to mycobacteria
17 microbiological culture method test for the detection of
18 mycobacteria after suitable validation and agreement from national
19 regulatory authority (34).

20 21 *A.4.3.3.6 Tests for molecular consistency of production*

22 Some manufacturers perform a test for the molecular consistency of
23 production, the MAPREC assay (see A.4.4.5.1.1) on single harvests.
24 If performed, the acceptance/rejection criteria should be updated
25 periodically and approved by the national regulatory authority.

26 27 **A.4.4 Control of monovalent bulk**

28 *A.4.4.1 Preparation of monovalent bulk*

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

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

A.4.4.2 Sampling

Samples of the monovalent bulk prepared as described in section A.4.4.1 should be taken immediately and, if not tested immediately, should be kept at 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 as the appropriate poliovirus serotype by immunological assay on cells culture using specific antibodies or by molecular method which has been validated and approved by the national regulatory authority.

Neutralization tests can distinguish the serotypes of poliovirus. Molecular method such as sequencing or deep sequencing, can distinguish Sabin virus from wild type virus.

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.4.4.4 Titration for virus infectivity

The amount of infective poliovirus per ml of filtered monovalent bulk should be determined in cell cultures in comparison with an existing reference preparation (see Appendix 4).

The virus concentration as determined by this test should be the basis for the quantity of virus used in the neurovirulence tests in monkeys or in TgPVR mice (Part A, section A.4.4.5.2) and for formulation of the final bulk (Part A, section A.4.5).

1 The detailed procedures for carrying out this test and for interpreting the results should be
2 approved by the national regulatory authority.

3 4 *A.4.4.5 Tests to monitor virus molecular characteristics (consistency)*

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

9 10 *A.4.4.5.1 Tests in vitro*

11 The virus in the monovalent bulk should be tested by at least one *in vitro* test such as MAPREC
12 or temperature sensitivity (rct40). The test used should be approved by the national regulatory
13 authority.

14 15 *A.4.4.5.1.1 MAPREC*

16 The MAPREC assay is suitable for all three serotypes. Implementation of the assay should be
17 fully validated by each manufacturer and performed according to the SOP, "Mutant Analysis by
18 PCR and Restriction Enzyme Cleavage (MAPREC) for oral poliovirus (Sabin) vaccine",
19 developed from WHO collaborative studies and available from WHO, or according to a validated
20 alternative procedure.

21
22 The MAPREC assay should be used to establish the consistency of the production once the test
23 has been validated and normal values for the standards have been established. The values
24 obtained for the standards should not exceed the established range by more than 2SD.
25 Acceptance/rejection criteria should be specific for each manufacturer and each working seed and
26 should be continually updated as each new bulk is prepared. An investigation of consistency
27 should take place if a batch gives results that are inconsistent with previous production batches.

28
29 Results should be expressed as ratios relative to the relevant type specific International Standard
30 for MAPREC analysis of poliovirus (Sabin). The acceptable variation of mutant content from

1 batch to batch should be agreed with the national regulatory authority in the light of production
2 experience.

3
4 For type 3, (472-C), a batch should be rejected if the level of mutations is above 1.0%. The limits
5 for types 1 and 2 should be approved by the national regulatory authority.

6
7 Levels of mutations obtained by manufacturers who have
8 implemented the test for types 1 and 2 virus have been less than
9 2.0% for Type 1 Sabin (for the sum of both mutations 480-A, 525-C)
10 and 1.5% for Type 2 Sabin (481-G) (14).

11
12 If a filtered monovalent bulk fails in a MAPREC assay, it cannot be used in the manufacturing of
13 finished product and an evaluation of the manufacturing process including the suitability of the
14 virus working seed should be undertaken and discussed with the national regulatory authority.
15 Filtered monovalent bulks that pass the MAPREC assay should be tested subsequently for in vivo
16 neurovirulence.

17
18 The assay for type 3 is highly predictive of in vivo neurovirulence
19 in animal models. No such correlation exists for types 1 and 2 at the
20 level of revertants present in vaccine bulks. For these types the
21 assay results provide a measure of consistency (14).

22
23 Non-radioactive methods for MAPREC are available and may be
24 introduced after validation and approval by the national regulatory
25 authority.

26
27 Alternative molecular biology methods that demonstrate an
28 equivalent or better level of discrimination may be used after
29 validation and approval by the national regulatory authority.

30
31 *A.4.4.5.1.2 Temperature sensitivity*

1 While the MAPREC assay provides a sensitive and quantitative measure for consistency
2 purposes, other assays are acceptable after validation and approval by the national regulatory
3 authority. They include the property of reproducing at temperatures of 36° C and 40° C in
4 comparison with the seed lot or a reference virus preparation for the marker tests with the
5 appropriate rct/40- and rct/40+ strains of poliovirus of the same type. The wild type viruses,
6 defined as field isolates or reference strains from polioviruses known or believed to have
7 circulated persistently in the community, which are used as rct40+ controls in this test, should be
8 contained within the laboratory at progressively higher levels of containment in accordance with
9 the global action plan and timetable for safe handling of wild polioviruses (9). The incubation
10 temperatures used in this test should be controlled to within +/-0.1° C.

11
12 The monovalent bulk passes the test if, for both the virus in the monovalent bulk and that in the
13 appropriate reference material, the titre determined at 36°C is at least 5.0 log₁₀ greater than that
14 determined at 40°C. If the titres obtained for all the reference viruses are not in line with the
15 expected values, the test should be repeated.

16
17 An additional specification that the virus titre must not exceed 100
18 CCID₅₀/ml at the higher temperature may also be applied.

19
20 It is desirable that the temperatures used in the test should also
21 include one in the region of 39.0-39.5°C, at which the titre of the
22 reference material should be reduced by a factor in the range of 3.0-
23 5.0 log₁₀ of its value at 36°C. In one laboratory, a temperature of
24 39.2°C has been found suitable.

25
26 It is important is to show that the behavior of the monovalent bulk
27 is comparable to that of the Sabin reference strain over a range of
28 temperatures so that a more accurate comparison can be made.

29
30 *A.4.4.5.2 Neurovirulence tests*

1 An appropriate in vivo test should be used to evaluate virus seeds and monovalent bulks.
2 Summaries of the MNVT and TgmNVT, including pass/fail criteria, are given in Appendix 2
3 along with considerations on the choice of assay.
4

5 The test should be approved by the national regulatory authority for the specific product and may
6 use transgenic mice or non human primates or both. The test for neurovirulence in non-human
7 primates should be carried out as summarized in Appendix 2 and described in the protocol
8 available from WHO¹ (¹ Coordinator, Quality, Safety and Standards, World Health Organization,
9 1211, Geneva 27, Switzerland).

10
11 Where the TgmNVT has been approved by the national regulatory authority it should be carried
12 out as summarized in Appendix 2 and described in detail in the SOP: "Neurovirulence test of type
13 1, 2 or 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus", available
14 from WHO.¹ (¹ Coordinator, Quality, Safety and Standards, World Health Organization, 1211,
15 Geneva 27, Switzerland). Its use for batch release purposes should follow the appropriate
16 validation and implementation processes according to national and international regulations. This
17 SOP has been validated for vaccines made from Behringwerke SO-derived seeds (type 1 and 2)
18 and RSO-derived seeds (type 3).

19
20 To qualify as a competent laboratory to perform the TgmNVT, there is a requirement for
21 laboratories to complete a standard implementation process as detailed in the SOP. Once
22 qualified as competent, each laboratory should continue to monitor their performance on a
23 routine basis.

24 The WHO collaborative study had demonstrated that MNVT and TgmNVT are equivalent for
25 testing vaccines prepared from RSO seeds but TgmNVT may fail otherwise acceptable (by
26 MNVT) lots prepared from derivative strains containing additional mutations (26). Therefore
27 TgmNVT can be used as a replacement of MNVT for vaccines made from RSO Sabin 3 strain,
28 while the TgmNVT may require further validation for other derivative strains. This may include
29 development of an appropriate homologous reference.
30

1 **A.4.5 Final bulk**

2 Different final bulks can be formulated.

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

6
7 The operations necessary for preparing the final bulk should be conducted in such a manner as to
8 avoid contamination of the product.

9
10 The dilution and mixing procedures involved in preparing the final
11 vaccine bulk should be approved by the national regulatory
12 authority.

13 14 *A.4.5.1 Stabilizers*

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

18
19 All the tests described in Part A. sections A.4.3.3 and A.4.4, should be performed on samples
20 taken before any stabilizers are added.

21 22 *A.4.5.2 Sterility tests for bacteria and fungi*

23 The final vaccine bulk should be tested for bacterial and fungal sterility as specified in Part A,
24 section 5.2 of the *General requirements for the sterility of biological substances (36)*.

25 26 **A.5 Filling and containers**

27 The requirements concerning filling and containers given in *Good Manufacturing Practices for*
28 *Biological Products (32)* should apply to vaccine filled in the final form.

29

1 Care should be taken that the material of which the container is
2 made does not adversely affect the virus content of the vaccine
3 under the recommended storage conditions.
4

5 A final filtration may be included just before the filling operations.
6

7 The manufacturer should provide the national regulatory authority with adequate data to prove
8 the product is stable under appropriate conditions of storage and shipping
9

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

11 Samples should be taken from each filling lot for the tests described in the following sections.
12 Unless otherwise justified and authorized, the tests should be performed on labeled containers
13 from each final lot by means of validated methods approved by the national regulatory authority.
14 The permissible limits for the different parameters listed under this section, unless otherwise
15 specified, should be approved by the national regulatory authority.
16

17 *A.6.1 Inspection of final containers*

18 Every container in each final lot shall be inspected visually, and those showing abnormalities
19 shall be discarded.

20 *A.6.1.1 Appearance*

21 The appearance of the vaccine should be described with respect to its form and colour.
22
23

24 *A.6.2 pH*

25 The pH of the final lot should be tested in a pool of final containers and an appropriate limit set to
26 guarantee virus stability.
27

28 *A.6.3 Identity*

29 Each final lot should be identified by immunological assay on cells culture using specific
30 antibodies or by molecular method which has been validated and approved by the national
31 regulatory authority.

1 Neutralization tests can distinguish the serotypes of poliovirus.
2 Molecular method such as sequencing or deep sequencing, can
3 distinguish Sabin virus from wild type virus.
4

5 Care should be taken to ensure that the sera used are monospecific
6 by titrating them against homotypic and heterotypic viruses of
7 known virus titre. Monoclonal antibodies may be used for this
8 purpose.
9

10 A. 6.4 Sterility tests for bacteria and fungi

11 Liquid vaccine should be tested for bacterial and fungal sterility as specified in Part A, section
12 5.2 of the *General requirements for the sterility of biological substances* (36), or by the methods
13 approved by the national regulatory authority.
14

15 A.6.5 Potency

16 Three final containers should be selected at random from each final lot and should be individually
17 tested on the same day. The poliovirus content of each serotypes and the total virus content
18 should be determined in an assay as described in Appendix 4 of these Recommendations using
19 assays that include a reference preparation. When the vaccine contains more than one poliovirus
20 type, each type should be titrated separately by using appropriate type-specific antiserum to
21 neutralize each of the other types present. The national regulatory authority should specify the
22 minimum virus titre per human dose.

23 An internal upper limit may be established by each manufacturer to
24 monitor the consistency of production e.g. based on mean titre
25 $CCID_{50} +3$ standard deviations. The upper limit should be approved
26 by the national regulatory authority.
27

28 It is recommended that the estimated mean virus titres for a single
29 human dose of tOPV should be not less than $10^{6.0}$ $CCID_{50}$ for type 1,
30 $10^{5.0}$ $CCID_{50}$ for type 2, and $10^{5.5}$ $CCID_{50}$ for type 3, as determined

1 in an assay described in Appendix 4. The 95% confidence intervals
2 of the assays should not differ by a factor of more than $0.5 \log_{10}$ the
3 estimated number of infectious units in the vaccine.
4

5 In 1986 the Region of the Americas began to use a trivalent
6 formulation with $10^{5.8}$ CCID₅₀ of poliovirus type 3 (40), following a
7 study in Brazil which demonstrated improved immunogenicity
8 when the amount of type 3 virus in the trivalent vaccine was
9 increased (41). The subsequent success in controlling poliomyelitis
10 in the Americas using this formulation led the EPI Global Advisory
11 Group to recommend a formulation of trivalent OPV with $10^{6.0}$,
12 $10^{5.0}$, $10^{5.8}$ CCID₅₀ per dose for types 1, 2, and 3, respectively, on a
13 global basis (18, 42).
14

15 *A.6.6 Thermal stability*

16 Thermal stability should be considered as a vaccine characteristic that provides an indicator of
17 consistency of production. Thermal stability test is not designed to provide a predictive value of
18 real time stability but to evaluate whether the product complies with a defined specification.
19 Additional guidance on evaluation of vaccine stability is provided in the *WHO guidelines on*
20 *stability evaluation of vaccines* (43).
21

22 Three final containers of the vaccine should be incubated at 37°C for 48 hours. The total virus
23 content in both exposed and unexposed containers should be determined concurrently with that of
24 a suitable validated reference preparation. For trivalent vaccines, the vaccine passes the test when
25 the loss on exposure is not greater than a factor of $0.5 \log_{10}$ CCID₅₀ per human dose.
26

27 Several OPV manufacturers have recently demonstrated that the
28 thermal stability test specification applied to tOPV formulations
29 (loss on exposure is not greater than a factor of $10^{0.5}$ CCID₅₀ per
30 human dose) is not applicable to some monovalent and bivalent
31 OPVs. Some manufacturers have shown that mOPV formulations

1 that failed the current specification of 0.5 log₁₀ have an acceptable
2 stability profile throughout the product shelf-life. Therefore, a
3 specification of 0.6 log₁₀ has been accepted by national regulatory
4 authorities and WHO prequalification based on documented
5 evidence that the mOPV1 was stable over 2 years when stored at
6 -20°C and 6 months when stored at 2-8°C.

7 *A.6.7 Residual antibiotics (if applicable)*

8 If any antibiotics are added in the vaccine production, the content of the residual antibiotics
9 should be determined and be within limits approved by the national regulatory authority. This test
10 may be omitted for routine lot release once consistency of production has been established to the
11 satisfaction of the national regulatory authority

12 **A.7 Records**

13 The recommendations given in Section 8 of *Good Manufacturing Practices for Biological*
14 *Products* (32) should apply.

16 **A.8 Retained samples**

17 The requirements given in Section 9.5 of *Good Manufacturing Practices for Biological Products*
18 (32) should apply.

20 **A.9 Labelling**

21 The requirements given in Section 7 of *Good Manufacturing Practices for Biological Products*
22 (32) should apply, with the addition of the following.

23 The label on the container or package should include the following information:

- 24 • the designation(s) of the strain(s) of poliovirus contained in the vaccine
- 25 • the minimum amount of virus of each type contained in one recommended human dose
- 26 • the cell substrate used for the preparation of the vaccine the nature and amount of any
27 stabilizer present in the vaccine
- 28 • the fact that the vaccine is not to be injected.
- 29 • the number of doses in each vial

- volume of the 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 Section 8 of *Good Manufacturing Practices for Biological Products* (32) should apply. Further guidance is provided in the *WHO Model guidance for the storage and transport of time and temperature-sensitive pharmaceutical products* (44).

A.11 Stability, storage and expiry date

A.11.1 Stability testing

Adequate stability studies form an essential part of vaccine development. Current guidance on evaluation of vaccine stability is provided in the *WHO guidelines on stability evaluation of vaccines* (43). Stability testing should be performed at different stages of production, namely on single harvests, monovalent bulk, final bulk, final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. A shelf-life should be assigned to all in-process materials during vaccine production, in particular intermediates such as single harvests, monovalent bulk and final bulk.

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

The formulation of vaccine should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with national regulatory authorities. Following licensure, ongoing monitoring of vaccine stability is recommended to support shelf-life specifications and to refine

1 the stability profile (43). Data should be provided to the national regulatory authority as per local
2 regulatory requirements.

3
4 Where vaccine is to be stockpiled, manufacturers should conduct real time stability studies at
5 -40°C or below on monovalent bulks or at -20°C on finished monovalent, bivalent and trivalent
6 composition.

7
8 Extension of shelf life should be approved by the national regulatory authority.

9
10 The final stability testing program should be approved by the national regulatory authority and
11 should include an agreed set of stability indicating parameters, procedures for the ongoing
12 collection and sharing of stability data and criteria to reject vaccine(s).

13 14 *A.11.2 Storage conditions*

15 Before being released by the manufacturing establishment, all vaccines in final containers should
16 be kept continuously in the frozen state at a temperature below -20°C.

17
18 The manufacturer shall recommend conditions of storage and shipping that will ensure the
19 vaccine conforms to the requirements of potency until the expiry date stated on the label. These
20 shall be approved by the national regulatory authority.

21
22 In the field, the vaccine can be thawed and stored at 2-8°C for a period of time that should not
23 exceed 6 months. Stability data should be generated for each formulation of OPV to support the
24 storage time at 2-8°C following thawing and this should be approved by the national regulatory
25 authority.

26 27 *A.11.3 Expiry date*

28 The expiry date should be defined on the basis of shelf-life and supported by the stability studies
29 with the approval of the national regulatory authority and should relate to the date of the last

1 satisfactory determination, performed in an assay as described in Appendix 4, of virus
2 concentration, i.e. the date on which the test system was inoculated.

3

4 Provided that the vaccine has been stored continuously at a temperature below -20°C, the expiry
5 date should be not more than 2 years after the last titration, except for those that are proven to be
6 stable over this period of time and extension of the shelf life has been approved by their national
7 regulatory authority.

8

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

10

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

The nonclinical evaluation of candidate poliomyelitis vaccines (oral) should be based on *WHO guidelines on nonclinical evaluation of vaccines (15)*. The following specific issues should be considered in addition to the tests described in Part A.3.2.3 and A.3.2.4 in the context of a change in virus seed or manufacturing process for OPV.

The following specific issues should be considered.

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

In the event that a new virus sub-master seed is prepared by a single passage from the WHO master seed, it should be subjected to extensive characterization which should include evaluation of the virus working seeds and at least three monovalent bulks derived from it, as described in Part A.4.4.5. Characterization studies must include evaluation of identity by complete nucleotide sequencing to prove that the new sub-master seed is identical to conventional Sabin master seeds in its nucleotide sequence and MAPREC directed at specific positions. Massively parallel sequencing may also be undertaken to determine the distribution of mutants. These approaches have not been formally validated yet other than the MAPREC tests for base positions in the 5' non-coding region as described in section A.4.4.5.1.1. A new sub-master seed should be tested for neurovirulence in the MNVT or the TgmNVT. Summaries of the MNVT and TgmNVT are given in Appendix 2 along with considerations on the choice of assay.

B.2 Characterization of new virus working seed from an established virus master seed

Under normal circumstances, a new virus working seed will be prepared from the same virus master seed as the one used to prepare the approved working seed using the same production protocol. If the TgmNVT has been approved by the national regulatory authority for the release of vaccine batches and if the virus working seed is generated by the same production process, it can be qualified by use of the mouse test and supporting *in vitro* data alone. This will include testing of the seed and at least three monovalent bulks produced from it, as described in Part A.4.4.5.

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B.3 Characterization of virus working seeds from an established master seed where passage level between master and working seed is increased

The acceptable passage level of live polio vaccines relative to the original seeds is rigidly specified as there is evidence for some seeds that increases in virulence have occurred with increasing passage. However, due to the limited stocks of master seeds available, it may be necessary in the future for some manufacturers to prepare working seed lots by expanding current seed lots with an additional passage. The new virus working seed lots will require careful comparative studies with the previously approved working seed lot and meet the criteria outlined in part A.3.2.3 and A.3.2.4. At least three monovalent bulks produced from it also should be tested and shown to meet the requirements in A.4.4.5.

B.4 Characterization following changes in the manufacturing process

If the OPV manufacturing process is new, or major changes are implemented in vaccine production such as changing from primary monkey cells to cell lines, extensive assessment should be conducted to ensure that the mutational composition is not significantly altered by the new manufacturing process. This evaluation may include nucleotide sequencing and studies of mutant accumulation during passage in production cultures by using MAPREC and other molecular methods such as massively parallel sequencing. The new virus working seed lots will meet the criteria outlined in part A.3.2.3 and A.3.2.4. At least three monovalent bulks produced from it also need to be tested and shown to meet the requirements in A.4.4.5. In addition, based on the results of the genetic characterization and the animal neurovirulence tests, clinical studies may be required (see Part C).

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

Clinical trials should adhere to the principles described in the *WHO guidelines for good clinical practice (GCP) for trials on pharmaceutical products (45)* and to the *WHO guidelines on clinical evaluation of vaccines: regulatory expectations (16)*. All clinical trials should be approved by the relevant national regulatory authorities.

Some of the issues that are specific to the clinical evaluation of oral polio vaccines are discussed in the following sections. These sections should be read in conjunction with the general guidance mentioned above. It is also recommended that manufacturers should consult with relevant national regulatory authorities regarding the overall clinical development program.

The section considers the provision of clinical data required for: 1) new formulations based on licensed OPV containing one or two Sabin poliovirus strains and, 2) when there have been major changes to the manufacturing process of an established vaccine e.g. changing from primary monkey kidney cells to a cell line. Clinical evaluation of vaccine manufactured using a new virus working seed lot is not required provided that the passage level is not more than one from the master seed lot, the working seed has been characterized and consistency of the manufacturing process has been demonstrated (see section A.3.2.3 and A.3.2.4). Generation of a sub-master seed will require full characterization (see section B1). Based on the results of the genetic characterization and the animal neurovirulence tests, clinical studies may be required.

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 have demonstrated that the efficacy of mOPV1 is superior to trivalent OPV in inducing immunity against poliovirus type 1 (18, 46). Health authorities have recommended widespread use of this vaccine to eliminate poliovirus type 1 transmission in India. In addition, studies on bOPV containing type 1 and type 3 have demonstrated that bOPV is ‘non-inferior’ to mOPV1 and mOPV3 individually, and ‘superior’ to tOPV. Therefore, based on these results the Advisory Committee on Poliomyelitis Eradication (ACPE) recommended that bOPV should be used to

1 complement tOPV in routine immunization and to complement tOPV and mOPVs in
2 supplementary immunization activities.

3
4 **C.1 General considerations**

5 The success of the poliomyelitis eradication initiative following the World Health Assembly
6 resolution in 1988 has led to the dramatic decrease in poliomyelitis cases globally (17).
7 Therefore, efficacy studies for poliovirus vaccines are not feasible and the clinical evaluation and
8 seroprevalence studies should be based on the comparative assessment of safety and
9 immunogenicity of new candidate vaccines with a licensed vaccine. The assessment of
10 seroconversion should be based on the elicitation of neutralizing antibodies, which are
11 established to be the basis of protection (17). The basis of approval of a new candidate OPV
12 should be based on a clear demonstration of non-inferiority compared to current licensed OPV.
13 The relative risk of poliomyelitis cases due to vaccine derived poliovirus (VDPV) for a new
14 candidate vaccine versus approved vaccines cannot be estimated from pre-approval studies but
15 should be addressed as part of post-marketing surveillance.

16
17 **C.2 Safety and immunogenicity studies**

18
19 **C.2.1 Assessment of the immune response**

20 A serum neutralising antibody titre of 1/4–1/8 is considered to be a marker of protection against
21 poliovirus (47). The demonstration of an immune response to OPV vaccination should be based
22 on the measurement of neutralizing antibody titres pre- and post-vaccination. Geometric mean
23 titers (GMTs), seroconversion rates and reverse cumulative distributions (RCD) should be
24 provided. Seroconversion for polio antigen is defined as:

- 25 - For subjects seronegative at the pre-vaccination time point: antibody
26 titres above the cut-off
- 27 - For subjects seropositive at pre-vaccination timepoint: antibody titres 4-
28 fold above the expected titre of maternal antibodies based on the pre-
29 vaccination titre declining with a half-life of 28 days.
- 30 - A change from below the highest dilution tested (<8192) to the highest
31 dilution tested (>8192) will also indicate seroconversion.

1
2 The half-life of antibody decay may be assumed to be 28 days (48). It is desirable to consider
3 these two parameters separately in the comparison between a new oral poliovirus vaccine and a
4 licensed one used as control.

5
6 WHO has made effort to standardize polio virology methods which led to the publication in 1990
7 of a WHO Manual for Virological Investigation of Poliomyelitis (49). It is recommended that a
8 standardized technique for measurement of neutralizing antibodies, involving standard cell lines,
9 and other standard reagents. International standard anti-poliovirus sera for types 1, 2, and 3
10 should be used. Results should be expressed in international units of neutralizing antibody
11 (50,51).

12 13 **C.2.2 Immunogenicity studies**

14 New candidate oral poliovirus vaccines manufactured using a different vaccine composition (e.g.
15 monovalent or bivalent) should be compared with the licensed trivalent formulation. New
16 candidate vaccines should be compared to at least one well-established and licensed oral
17 poliovirus vaccine. The comparator vaccine(s) selected should have been in use for some years so
18 that some data on effectiveness are available as well as a reliable description of the safety profile.

19 20 **C.2.3 Population**

21 The evaluation of mOPV and bOPV formulations based on a licensed OPV may be conducted in
22 infants and newborns, since safety profiles in these populations have already been established.

23
24 The study exclusion criteria should reflect the current contraindications to administration of oral
25 poliovirus vaccines.

26 27 **C.2.4 Endpoints and analyses**

28 The clinical study protocol should state the primary objective(s) of the study. The neutralizing
29 antibody response to the candidate vaccine should be demonstrated to be non-inferior versus an
30 appropriate licensed oral poliovirus vaccine based primarily on GMTs and/or seroconversion

1 rates. The primary endpoint should be selected according to the study population and the
2 anticipated immune response. For example, very high seroprevalence rates are expected in highly
3 immunized populations which have implications for the selection of the non-inferiority margin
4 and therefore the sample size calculation. Further details on demonstrating non-inferiority are
5 described in the *WHO guidelines on clinical evaluation of vaccines: regulatory expectations (16)*.

6
7 Other immunological parameters should be compared in planned secondary analyses (e.g.
8 percentages reaching predefined titres).

9 10 **C.2.5 Dose ranging studies**

11 Dose ranging studies may be undertaken for new poliovirus attenuated vaccines to determine the
12 minimum dose of virus in CCID₅₀ required to provide adequate immune responses. These data
13 could also be used to support the derivation of the minimum viral titre that should be present in
14 the vaccine at the end of shelf-life.

15 16 **C.2.6 Vaccine virus shedding and transmission**

17 Manufacturers should undertake studies to determine the amounts of the vaccine virus (if
18 applicable, by serotype) excreted in the stools of vaccinees and the duration of shedding.
19 Evaluation of virus excretion of new vaccine formulations containing one or two serotypes (e.g.
20 bivalent or monovalent) should be evaluated in comparison to the trivalent licensed formulation
21 (18).

22
23 A low level viraemia is known to occur after vaccination with OPV (52, 53). Assessment of
24 viraemia is not routinely required for a vaccine derived from Sabin strains.

25 26 **C.2.7 Challenge studies with attenuated Sabin poliovirus**

27 Induction of mucosal immunity by the candidate and the control vaccines should be determined
28 by the assessment of virus excretion after the administration of a challenge dose of mOPV.
29 Excretion of poliovirus in stool specimens is determined at various intervals immediately before
30 the challenge (day 0) and on days 7, 14, 21, 28 thereafter (46).

1 **C.2.8 Concomitant administration with other vaccines**

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

5
6 When oral poliovirus vaccines are used in EPI programme simultaneously with other vaccines, it
7 is particularly important that the effects of co-administration should be evaluated. For example,
8 co-administration studies with rotavirus vaccines which are also administered by the oral route.

9
10 Immune responses to all other antigens co-administered with the new oral poliovirus vaccine
11 should be measured at least in subsets. While the study will usually be powered only to
12 demonstrate non-inferiority with respect to neutralizing antibody against the different poliovirus
13 types used in the vaccine the protocols should at least include planned secondary analyses of
14 antigen-specific responses. If these analyses indicate that immune responses are lower on co-
15 administration with a new oral poliovirus vaccine compared to the licensed vaccine(s) national
16 regulatory authorities will need to consider the potential clinical consequences on a case by case
17 basis.

18 19 **C.2.9 Pre-licensure safety data**

20 The general approach to the assessment of safety of a new oral poliovirus vaccine during clinical
21 studies should be in accordance with the *WHO guidelines on clinical evaluation of vaccines:
22 regulatory expectations* (16). Planned safety studies should be supported by a clear scientific
23 rationale. Given the long history of the use of vaccines based on Sabin strains, the national
24 regulatory authority may decide that additional pre-licensure safety studies are not required.
25 Where a new attenuated poliovirus vaccine, which has not been used previously, is investigated,
26 larger scale studies will be needed.

27
28 An appropriate pharmacovigilance plan should be developed and approved by the national
29 regulatory authority prior to licensure.

30

1 **C.3 Post-marketing studies and surveillance**

2 Enhanced safety surveillance particularly for detection of vaccine associated polio paralysis
3 (VAPP) should be undertaken during the initial post-approval years in collaboration with national
4 regulatory authorities. Manufacturers and health authorities should work in collaboration with the
5 global polio surveillance laboratory network to monitor new vaccines once introduced in
6 immunization programs. These laboratories have extensive experience in poliovirus surveillance
7 and may provide excellent surveillance and post-marketing support.

8
9 The total duration of enhanced surveillance should be regularly reviewed by the national
10 regulatory authority. If particular issues arise during pre-licensure studies or during post-licensure
11 safety surveillance then it may be necessary to conduct specific post-licensure safety studies.

12
13 **Part D. Recommendations for national regulatory authorities**

14
15 **D.1 General**

16 The general recommendations for control laboratories given in the *Guidelines for national*
17 *authorities on quality assurance for biological products (54)* and *Guidelines for Independent Lot*
18 *Release of Vaccines by Regulatory Authorities (55)* should apply.. These guidelines specify that
19 no new biological substance should be released until consistency of manufacturing and quality as
20 demonstrated by a consistent release of batches has been established. The detailed production and
21 control procedures and any significant changes in them that may affect quality, safety and
22 efficacy of poliomyelitis vaccine (oral) should be discussed with and approved by the national
23 regulatory authority. For control purposes, the national control laboratory should obtain the
24 International Reference Reagents for virus titre for potency testing and, where necessary,
25 establish national working reference preparation(s) calibrated against the International Standard.

26
27 If the national control laboratory does not perform the monkey
28 neurovirulence test itself, it should carry out a second reading of the
29 histological sections provided by the manufacturer for each
30 monovalent bulk.

1 In addition, the national control laboratory should perform a second
2 reading of at least four neurovirulence tests on the reference
3 preparations using the monkey neurovirulence test in order to obtain
4 the necessary baseline data for comparison with the neurovirulence
5 of test vaccines.

6
7 The national control laboratory should encourage the use of the
8 standard form for the reporting of data on virus activity in the
9 sections taken from histopathological examination.

10
11 If the national control laboratory itself performs the mouse
12 neurovirulence test, it should complete the standard implementation
13 process.

14
15 Unless the national control laboratory itself performs the transgenic
16 mouse neurovirulence test, it should carry out a clinical scoring of
17 mice in parallel with the manufacturer at least at days 3/4 and 14 for
18 each monovalent bulk. Moreover once a year, the injection of mice
19 should be followed by the national control laboratory.

20
21 Only appropriately trained staff from a competent national control
22 laboratory can carry out a clinical scoring of mice in parallel with
23 the manufacturer.

24
25 Only a monovalent bulk approved by the national regulatory authority regarding the
26 neurovirulence test can be used by the manufacturer for the formulation of a final bulk.

27 28 **D.2 Release and certification**

29 A vaccine lot should be released only if it fulfils the national requirements and Part A of the
30 present Recommendations. A protocol based on the model given in Appendix 5, signed by the

1 responsible official of the manufacturing establishment, should be prepared and submitted to the
2 national regulatory authority in support of a request for release of vaccine for use. A statement
3 signed by the appropriate official of the national regulatory authority should be provided if
4 requested by a manufacturing establishment and should certify whether or not the lot of vaccine
5 in question meets all national requirements, as well as Part A of these Recommendations. The
6 certificate should also state the lot number, the number under which the lot was released, and the
7 number appearing on the labels of the containers. In addition, the date of the last satisfactory
8 potency test as well as assigned expiry date on the basis of shelf life should be stated. A copy of
9 the official national release document should be attached. The certificate should be based on the
10 model given in Appendix 6. The purpose of the certificate is to facilitate the exchange of vaccines
11 between countries.

12

13

1 **Part E. Recommendations for live attenuated poliomyelitis vaccine (oral)**
2 **prepared in primary cultures of monkey kidney**

3 The following additional or alternative recommendations are for oral poliomyelitis vaccine
4 prepared in cultures of primary monkey kidney cells and concern the testing of the cell substrate
5 used for the production of the vaccine (Part A, section A.4) they should therefore be added to or
6 substituted for the appropriate sections in Part A. Recommendations E.4.1, E.4.3.1.1, E.4.4.1 and
7 E.4.4.2.1 are additions; recommendations E.4.2.1 and E.4.2.2 together replace A.4.2.1, and
8 E.4.2.3 replaces A.4.1. All the other recommendations given in Parts A and B of the document
9 are also applicable to this vaccine.
10

11 **E.4 Control of vaccine production**

12 **E.4.1 *Control of source materials***

13 E.4.1.1 Monkeys used for preparation of kidney cell cultures and for testing of virus.

14 If vaccine is prepared in monkey kidney cell cultures, animals of a species approved by the
15 national regulatory authority, in good health and not previously employed for experimental
16 purposes, should be used.

17
18 Manufacturers are encouraged to use animals from closed or
19 intensively monitored colonies.
20

21 The monkeys should be kept in well-constructed and adequately ventilated animal rooms in cages
22 spaced as far apart as possible. Adequate precautions should be taken to prevent cross-infection
23 between cages. Cage-mates should not be interchanged. The monkeys should be kept in the
24 country of manufacture of the vaccine in quarantine groups¹ for a period of not less than 6 weeks
25 before use. If at any time during the quarantine period the overall death rate of a shipment
26 consisting of one or more groups reaches 5% (excluding deaths from accidents or where the
27 cause was specifically determined not to be an infectious disease), monkeys from that entire
28 shipment should continue in quarantine for a further period of not less than 6 weeks. The groups

¹ 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.

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

5
6 In countries in which the kidneys from near-term monkeys are used,
7 the mother should be quarantined for the term of pregnancy.

8
9 All actions taken by working personnel should be based on the assumption that a great potential
10 hazard exists at all times in the quarantine area. Personnel should be provided with protective
11 clothing, including gloves, footwear and masks or visors. Street clothes should not be permitted
12 in the animal rooms. Smoking, eating, and drinking should be forbidden while personnel are in
13 the animal rooms.

14
15 A supervisor should be made responsible for reporting unusual illness among employees and for
16 ensuring that all injuries are properly treated. No worker who has cuts or abrasions on exposed
17 areas of the body should enter the animal area. Any unexplained febrile illness, even while off
18 duty, should be considered as potentially related to the employee's occupation.

19
20 Monkeys from which kidneys are to be removed should be anaesthetized and thoroughly
21 examined, particularly for evidence of tuberculosis and cercopithecoid herpesvirus 1 (B virus)
22 infection.

23
24 If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of
25 a seed lot or vaccine, it should not be used, nor should any of the remaining monkeys of the
26 quarantine group concerned be used unless it is evident that their use will not impair the safety of
27 the product.

28
29 All the operations described in this section should be conducted outside the areas where vaccine
30 is made.

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

3
4 It is desirable that kidney-cell cultures are derived from monkeys
5 shown to be free from antibodies to foamy viruses. In some
6 countries, monkeys are tested for antibodies to cercopithecoid
7 herpesvirus 1 (B virus).

8 9 E.4.2 **Production precautions**

10 The general production precautions called for by the *Good Manufacturing Practices for*
11 *Biological Products* (32) should apply to the manufacture of vaccine, with the addition of the
12 following.

13 14 E.4.2.1 *Monkey kidney-cell cultures for vaccine production*

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

19
20 To reduce animal use, the virus may be grown in serially passaged
21 monkey kidney cell cultures from primary monkey kidney cells.

22
23 Each group of cell cultures derived from a single monkey or from no more than 10 near-term
24 monkeys should be prepared and tested as an individual group.

25 26 E.4.2.2 *Tests of cell cultures used for vaccine production (see Appendix 7)*

27
28 On the day of inoculation with virus working seed lot, each cell culture should be examined for
29 degeneration caused by an infective agent. If, in this examination, evidence is found of the
30 presence in a cell culture of any adventitious agent, the entire group of cultures concerned should
31 not be used for vaccine production.

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On the day of inoculation with the virus working seed lot, a sample of at least 30ml 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 virus. 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 in 4. The area of the cell sheet should be at least 3 cm² 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 virus, a test in a second species may be omitted with the approval of the national regulatory authority.

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' incubation, from each of these cultures at least one subculture of fluid should be made in the same tissue culture system. The subculture should also be observed for at least 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.

1
2 A further sample of at least 10 ml of the pooled fluid should be tested for the presence of
3 cercopithecoid herpesvirus 1 (B virus) and other viruses in rabbit kidney cell cultures. Serum used
4 in the nutrient medium of these cultures should have been shown to be free from inhibitors¹. The
5 sample should be inoculated into bottles of these cell cultures in such a way that the dilution of
6 the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should
7 be at least 3 cm² per ml of pooled fluid. At least one bottle of the cell cultures should remain
8 uninoculated and should serve as a control.

9
10 The cultures should be incubated at a temperature of 35-37 °C and should be observed for a
11 period of at least 2 weeks.

12
13 It is suggested that, in addition to these tests, a further sample of
14 10ml of pooled fluid removed from the cell cultures on the day of
15 inoculation with the seed lot virus should be tested for the presence
16 of adventitious agents by inoculation into cell cultures sensitive to
17 measles virus.

18
19 For the tests to be valid, not more than 20% of the culture vessels should have been discarded for
20 nonspecific, accidental reasons by the end of the respective test periods.

21
22 If, in these tests, evidence is found of the presence of an adventitious agent, the single harvest
23 from the whole group of cell cultures concerned should not be used for vaccine production.

24
25 If the presence of the cercopithecoid herpesvirus 1 (B virus) is demonstrated, the manufacture of
26 vaccine should be discontinued and the national regulatory authority should be informed.

27 Manufacturing should not be resumed until a thorough investigation has been completed and
28 precautions have been taken against any reappearance of the infection, and then only with the
29 approval of the national regulatory authority.

¹ Human herpesvirus (herpes simplex) has been used as an indicator for freedom from B virus inhibitors because of the danger of handling cercopithecoid herpesvirus 1 (B virus).

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If these tests are not done 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 for the test for B virus, which may be held at 4°C provided that the test is done not more than 7 days after it has been taken.

E.4.2.3 Test of control cell cultures

Cultures prepared on the day of inoculation with the virus working seed lot from 25%, but not more than 2.5 litres, 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 nonspecific, accidental reasons. 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 shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures from the same group should not be used for vaccine production.

E.4.2.3.1 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 made as described in Part A, section A.4.1.2.

E.4.2.3.2 Tests for other adventitious agents

At the time of harvest, or not more than 7 days after the day of inoculation of the production cultures with the virus working seed lot, a sample of at least 20ml 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 Part E, section E.4.2.2.

1 At the end of the observation period for the original control cell cultures, similar samples of the
2 pooled fluid should be taken and the tests referred to in this section in the two kinds of monkey
3 kidney cell culture and in the rabbit cell culture should be repeated, as described in Part E, section
4 E.4.2.2.

5
6 If the presence of cercopithecoid herpesvirus 1 (B virus) is demonstrated, the production cell
7 cultures should not be used and the measures concerning vaccine production described in Part E,
8 section E.4.2.2, should be taken.

9
10 In some countries, fluids are collected from the control cell cultures
11 at the time of virus harvest and at the end of the observation period.
12 Such fluids may then be pooled before testing for adventitious
13 agents.

14 15 E.4.3 *Control of single harvests*

16 E.4.3. 1 *Single harvest*

17 E.4.3.1.1 Tests for neutralized single harvests in monkey kidney-cell cultures

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

21
22 Care should be taken to ensure that the antiserum used is
23 monospecific. This may be demonstrated by titration of the
24 antiserum against homotypic and heterotypic virus of known virus
25 titre using the same dilution of the antiserum as that used for
26 neutralization.

27
28 Half (corresponding to at least 5ml of single harvest) of the neutralized suspension should be
29 tested in monkey kidney-cell cultures prepared from the same species, but not the same animal,
30 as that used for vaccine production. The other half of the neutralized suspension should be tested

1 in monkey kidney-cell cultures from another species, provided that the tests are done in cell
2 cultures from at least one species known to be sensitive to SV40 virus.

3
4 The neutralized suspensions should be inoculated into bottles of these cell cultures in such a way
5 that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the
6 cell sheet should be at least 3cm² per ml of neutralized suspension. At least one bottle of each
7 kind of cell culture should remain uninoculated, should serve as a control and should be
8 maintained using nutrient medium containing the same concentration of the specific antiserum
9 used for neutralization.

10
11 Animal serum may be used in the propagation of the cells provided
12 that it does not contain inhibitors, but the maintenance medium used
13 after the inoculation of the test material should contain no added
14 serum other than the poliovirus neutralizing antiserum, except as
15 described below.

16
17 The cultures should be incubated at a temperature of 35-37 °C and should be observed for a total
18 period of at least 4 weeks. During this observation period and after no less than 2 weeks'
19 incubation, at least one subculture of fluid should be made from each of these cultures in the
20 same tissue culture system. The subcultures should also be observed for at least 2 weeks.

21
22 Serum may be added to the original cultures at the time of
23 subculturing provided that the serum does not contain inhibitors.
24 Immunohistochemical techniques may be useful for detecting SV40
25 and other viruses in the cells.

26
27 It is suggested that, in addition to these tests, a further sample of the
28 neutralized single harvest is tested by inoculation of 10ml into
29 human cell cultures sensitive to measles virus.
30

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

3
4 If any cytopathic changes occur in any of the cultures, the causes of these changes should be
5 investigated. If the cytopathic changes are shown to be due to unneutralized poliovirus, the test
6 should be repeated. If there is evidence of the presence of SV40 virus or other adventitious agents
7 attributable to the single harvest, that single harvest should not be used for vaccine production.

8 9 **E.4.4 Control of monovalent bulk**

10 *E.4.4.1 Monovalent bulk (before filtration)*

11 *E.4.4.1.1 Tests in rabbits*

12 A sample of the monovalent bulk should be tested for the presence of cercopithecoid herpesvirus 1
13 (B virus) and other viruses by injection in at least 10 healthy rabbits each weighing between 1.5
14 and 2.5 kg. The sample should consist of at least 100 ml. Each rabbit should receive not less than
15 10ml or more than 20ml, of which 1ml is given intradermally at multiple sites, and the remainder
16 subcutaneously. The rabbits should be observed for between 3 and 5 weeks for death or signs of
17 illness.

18
19 It is suggested that the sample consists of at least 1% of monovalent
20 bulk, provided that this is not less than 100ml, up to a maximum of
21 500ml.

22
23 All rabbits that die after the first 24 hours of the test should be examined by autopsy, the brain
24 and organs being removed for detailed examination to establish the cause of death. Animals
25 showing signs of illness should be humanely killed and subjected to a similar autopsy.

26
27 The monovalent bulk passes the test if no more than 20% of the inoculated rabbits show signs of
28 intercurrent infection during the observation period and if none of the rabbits shows evidence of
29 infection with B virus or other adventitious agents or lesions of any kind attributable to the bulk
30 suspension.

31

1 If the presence of B virus is demonstrated, the measures concerning vaccine production described
2 in Part E, section E.4.2.2, should be taken.

3
4 A test for the presence of Marburg virus may be carried out in
5 guinea-pigs.
6

7 **E.4.4.2 Monovalent bulk (after filtration)**

8 **E.4.4.2.1 Tests for retroviruses**

9 Test samples from the filtered bulk suspension should be examined for the presence of
10 retroviruses by an assay for reverse transcriptase (RTase) acceptable to the national regulatory
11 authority (34).
12

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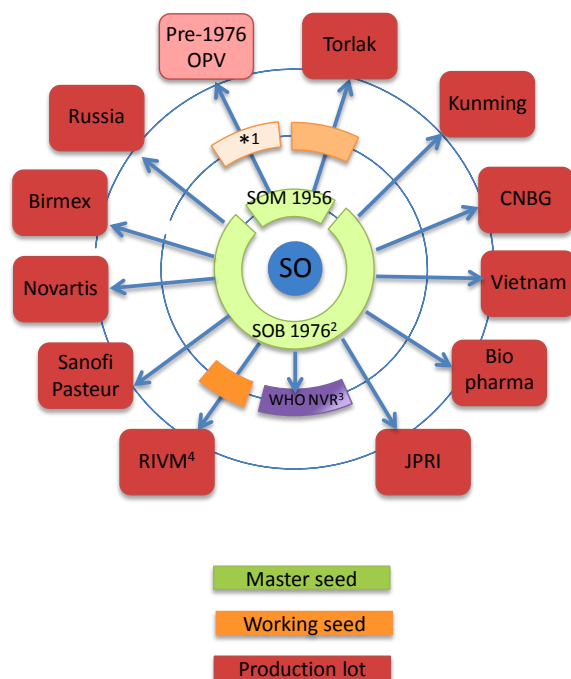
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22

23

- 1 **Appendix 1:**
- 2 **Overview of virus seeds used in OPV production**

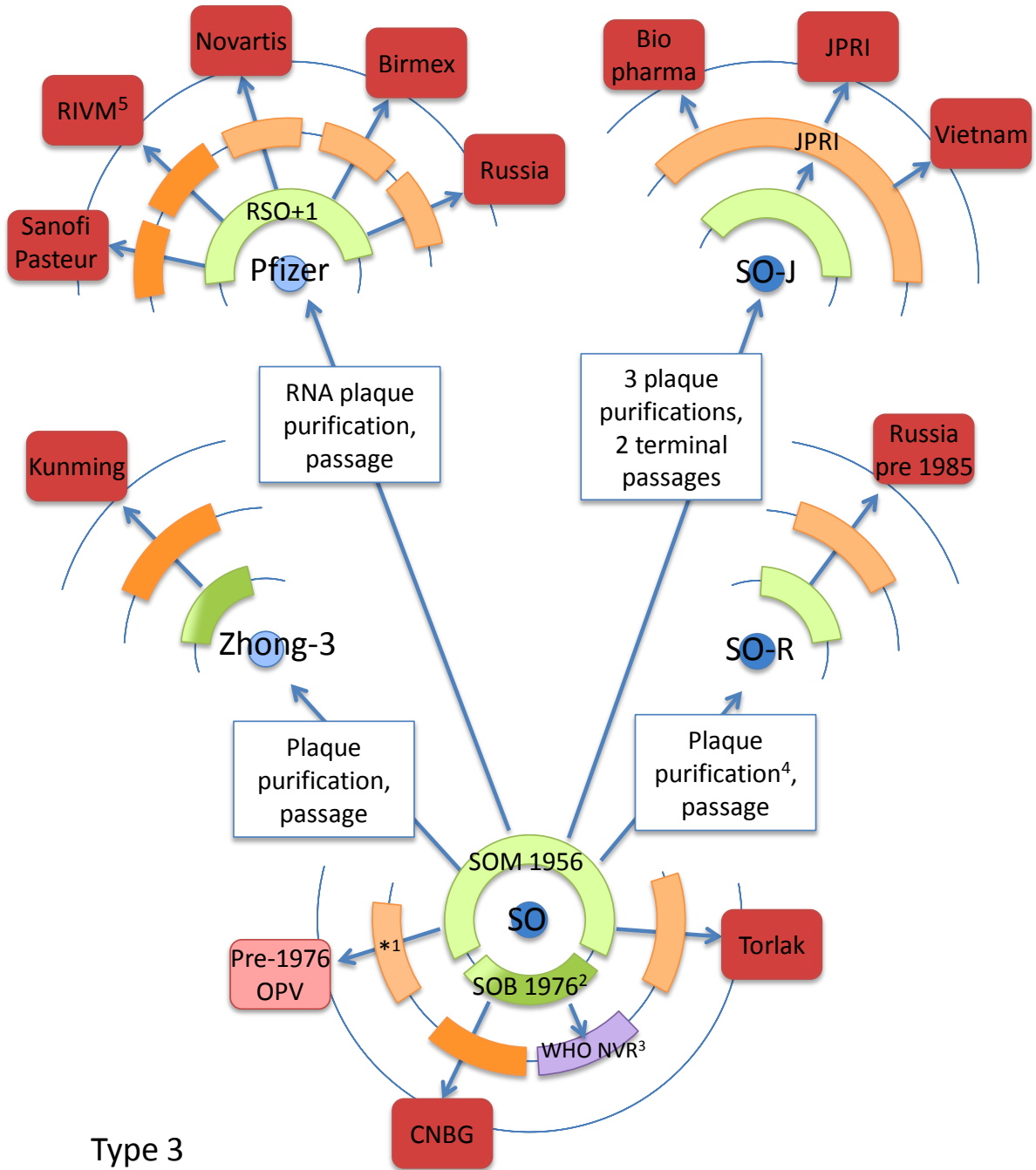


Type 1 and 2

- 1 Working Seeds produced by different manufacturers before 1976
- 2 WHO Master seed stock
- 3 WHO Neurovirulence reference preparation.
- 4 Stock used to produce Sabin IPV

3

4 Note: This diagram only provides information on a historical overview of the use of different seeds
 5 derived from Sabin vaccine strain in OPV production (as of November 2011). It does not indicate
 6 any WHO "qualification" or "approval" of the strains or vaccines in the context of this document.



Type 3

- 1 Working Seeds produced by different manufacturers before 1976
- 2 WHO Master seed stock
- 3 WHO Neurovirulence reference preparation
- 4 Six plaques were selected, pooled together, and grown to produce seed stock
- 5 Stock used to produce Sabin IPV

1

2 **Appendix 2**

3 ***In vivo* tests for neurovirulence and considerations for the choice of assay**

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

14

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

17

18 **1. Summary of the monkey neurovirulence test (MNVT)**

19 **1.1 Key features**

20 A detailed protocol is available from WHO. Between 5.5 and 6.5 log₁₀ CCID₅₀ of monovalent virus is
21 delivered in a single dose by intraspinal inoculation into the lumbar cord. A back titration of the
22 inoculum should be done after the inoculation step is completed. Residual paralysis if any is noted over
23 the following 17-22 days. The animals are sacrificed at the end of the test or earlier on humane grounds
24 and prepared for histological examination of the central nervous system (CNS). Regions are scored for
25 damage on a scale from 1 to 4 and a mean lesion score is calculated for each monkey and then for all
26 the monkeys in the test. The clinical signs do not form part of the assessment or the pass/fail criteria.
27 The homologous WHO/SO+2 reference is tested in parallel.

28

29 **1.2 Number of animals**

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

7 8 **1.3 Sections examined**

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

14 15 **1.4 Pass/fail criteria**

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

23
24 The values for C_1 , C_2 and C_3 are initially established on the basis of the data accumulated after four
25 qualifying tests. These values should then be updated after every test until nine tests have been
26 performed. After that the C values are based on the last ten tests performed. They must be established
27 for each testing laboratory.

28 29 **2. Summary of the transgenic mouse neurovirulence test (TgmNVT)**

30 **2.1 Key features**

1 The detailed SOP for the TgmNVT, "WHO neurovirulence test of type 1, 2 or 3 live poliomyelitis
2 vaccines (oral) in transgenic mice susceptible to poliovirus" (1), is available from WHO¹.⁽¹Coordinator,
3 Quality, Safety and Standards, World Health Organization, 1211, Geneva 27, Switzerland). The test
4 for neurovirulence of polio vaccines in transgenic mice involves the intraspinal inoculation of a defined
5 strain of transgenic mice carrying the human receptor for poliovirus with small volumes of the test
6 vaccine. Two virus concentrations are used and the read-out of the test is based on the clinical dose
7 response. A reference preparation is tested at the same time and a clearly defined process has been
8 established for implementation of the test in a new laboratory.

9

10 **2.2 Strain of Transgenic mouse**

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

15

16 **2.3 Titration of virus**

17 Two doses of virus are inoculated in a volume of 5 microlitres: for type 1, 1.75 and 2.75 CCID₅₀; for
18 type 2, 5.0 and 6.0 CCID₅₀ and for type 3, 3.5 and 4.5 CCID₅₀. The inocula must be prepared and
19 titrated accurately to ensure that these doses are given; the precision of the determinations should be
20 better than $\pm 0.3 \log_{10}$. A back titration of the inoculum should be done after the inoculation step is
21 completed.

22

23 **2.4 Inoculation and observation of animals**

24 Animals procured at age 5-6 weeks are randomised to cages and allowed to recover for at least 7 days.
25 They are then appropriately anaesthetised and inoculated with 5 microlitres of diluted test virus
26 between the last thoracic and first lumbar vertebrae. Animals are observed for clinical signs once a day
27 for the next 14 days and ultimately scored either as normal (slight weakness or no signs) throughout or
28 paralysed (paresis or paralysis) on two consecutive days. The lower and higher doses of the reference
29 should give more than 5% and less than 95% of animals paralysed, respectively, for the test to be valid.
30 A test requires 128 mice for one vaccine plus concurrently tested reference or 192 for two vaccines and

1 the reference. The reference is the same as that used in the monkey test; the use of other references may
2 be acceptable but should be validated.

3
4 The vaccine passes if it is not significantly more virulent than the reference defined in terms of the log
5 odds ratio and statistical constants L1 and L2 which are based on the reproducibility of the test and
6 define the pass fail criteria and the grey zone in which a retest is required. The acceptance and rejection
7 limits, L1 and L2, were selected so that a test vaccine which is equivalent to the reference will have a
8 0.95 probability of passing and a 0.01 probability of failing, respectively. The constants are regularly
9 updated. Statistical evaluation of test validity includes linearity and dose and gender effects.

10

11 **3. Implementation process of the TgmNVT**

12 If a manufacturer wishes to use the mouse test, relevant validation data should be available for their
13 specific product to demonstrate its applicability. This may include reference to the extensive
14 collaborative studies by which the test was originally developed. A clear stepwise process for
15 implementing the TgmNVT has been established, involving evaluation of the inoculation through
16 injection of Indian ink, tests with vaccines and testing of a blinded evaluation panel containing vaccines
17 that pass, fail or marginally fail the test. Competence in clinical scoring is also acquired through a
18 standardized training procedure. Alternatively, manufacturers may wish to test only in the monkey
19 model.

20

21 In either case, testing should be performed according to procedures specified in WHO SOP (1), using
22 appropriate WHO reference materials unless modified procedures have been validated and shown to be
23 suitable. The test chosen should be used to test virus seeds and bulks as described in Sections A.3.2.4.2
24 and A.4.4.5.2 respectively.

25

26 **4. Considerations for the choice of assay**

27 The following specific issues suggest that care should be taken in the selection of the *in vivo* tests for
28 neurovirulence to be performed and that the selection should be justified. The report of the WHO
29 Working Group Meeting to Discuss the Revision of the WHO Recommendations for OPV: TRS Nos.
30 904 and 910, provides more detailed discussions (2).

31

1 **4.1 Types 1 and 2 viruses**

2 The relative sensitivity of the mouse and monkey tests performed according to WHO procedures with
3 respect to the presence of mutations in the 5'- Untranslated Region (UTR) in types 1 and 2 appears to
4 be comparable but significantly lower than that in type 3 (3, 4). It is unknown whether these two
5 models are equally sensitive to other potential neurovirulent mutations. Most manufacturers use
6 essentially identical type 1 and 2 seeds, in contrast to the situation with type 3.

7

8 **4.2 Type 3 Sabin vaccine virus**

9 *4.2.1 Molecular biology*

10 Studies of the molecular biology of the Sabin polio vaccine virus strains have suggested that few
11 mutations are involved in attenuation and that, for the type 3 strain, there may be only two: one base
12 change in the 5' noncoding region of the genome at base 472 and one coding change at base 2034 that
13 introduces an amino acid change in the virus protein VP3. A third mutation at position 2493 has been
14 described (5). Growth of Sabin 3 virus in cell culture or in vaccine recipients results in rapid
15 accumulation of U instead of C at nucleotide 2493 (changing Thr to Ile at amino acid 6 of capsid
16 protein VP1), and all Sabin 3 OPV batches contain variable amounts of these mutants. This mutation
17 does not affect neurovirulence as determined in monkey test but there is evidence that it influences the
18 results obtained in the mouse test as described in the WHO SOP (6). Variations in the virulence of
19 vaccine batches measured in monkeys correlate well with variations in the base in the 5' non coding
20 region as measured by MAPREC. Changes in the amino acid in VP3 or changes at other positions that
21 suppress its effect are not thought to be generated in the course of well controlled production runs,
22 although this is possible in principle.

23

24 *4.2.2 Current type 3 seed viruses*

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

- 27 • The original WHO reference material (passage level SO+2) for neurovirulence testing contained
28 about equal mixture of both forms (2493 C or U).
- 29 • Batches prepared from RSO, the seed most commonly used in production in Europe, typically
30 contain about 5% or less of 2493-U (mutant).

- 1 • A seed used in production in much of Asia (a plaque purified from SO) contains 100% of mutant
2 form (2493-U).

3 Other seeds may differ slightly in sequence from the SO (7).

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

5

6 **5. Experience from the use of MNVT and TgmNVT with type 3 seeds and vaccines**

7 There is evidence that the transgenic mouse test as described in the WHO SOP (1) is sensitive to the
8 presence of 2493-U, whereas the monkey test is not sensitive to this mutation. Thus, batches produced
9 from RSO seed will pass both the monkey and mouse tests, whereas batches produced from the
10 alternative seeds that contain 100% 2493-U will pass the monkey test but may fail the mouse test,
11 although still being of an acceptable safety profile in clinical use.

12

13 The current WHO SOP for the TgmNVT specifies the doses and the WHO reference material to be
14 used and includes the proportion of mice affected at the two doses of virus given for the test to be valid.

15 The WHO reference material for TgmNVT is the same as that used in the monkey test and has
16 approximately 50% 2493-C, and was validated primarily against vaccines made from SO or RSO seeds.

17 However if used to test vaccines derived from 2493-U containing seed, it may fail them even if they
18 contain little 472-C and would pass MNVT. The TgmNVT could be adapted for testing 2493-U
19 containing bulks for example by changing the reference material, the doses and/or the validity criteria.

20 Manufacturers may wish to do so to make it applicable to their product. Any modified test should be
21 validated and approved by the national regulatory authority.

22

23 The relative sensitivity of the mouse and monkey tests performed according to WHO procedures to
24 other attenuating mutations in type 3 is not known. So far as is known, the other mutations do not vary
25 to the same extent in the course of existing production processes and they are therefore of lesser
26 concern.

27

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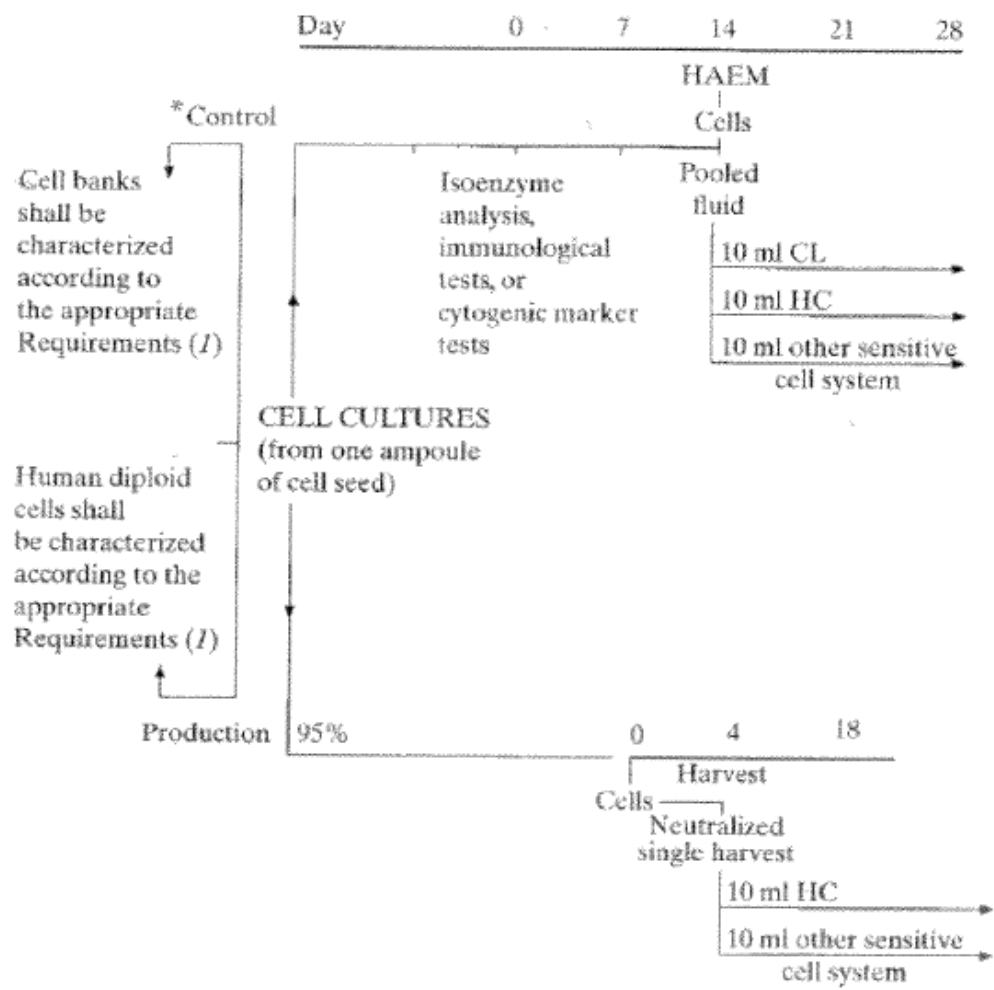
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21

Appendix 3

Preparation of poliomyelitis vaccine (oral) using cell banks

Example of a flowsheet of tests in cell cultures



* 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 virus; HC = human cells.

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

1

2

Reference

3

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4

5

1 **Appendix 4**

2 **Example, for guidance, of cell-culture techniques for assay method**
3 **for the determination of the virus content of poliomyelitis vaccine**
4 **(oral)**

5
6 The preparation to be assayed and the reference preparation are diluted in an appropriate medium.
7 It is convenient to make tenfold dilution steps of the virus suspensions initially, but for dilutions
8 that are to be inoculated into cell cultures the dilutions should be prepared in 0.5 log₁₀ or smaller
9 steps. A preliminary assay may be required to ensure that, in the test, the dilution range selected
10 encompasses at least three dilutions that will infect between 0% and 100% of the cultures
11 inoculated.

12
13 Titrate the vaccine for infectious virus using not fewer than 3 separate containers of vaccine
14 following the method described below. Titrate one container of an appropriate virus reference
15 preparation in triplicate to validate each assay. The virus concentration of the reference
16 preparation is monitored using a control chart and a titre is established on a historical basis by
17 each laboratory.

18
19 If the vaccine contains more than one poliovirus type, titration of the individual serotypes is
20 undertaken separately using mixtures of appropriate type specific antiserum (or preferably a
21 monoclonal antibody) to neutralize each of the other types present.

22
23 For titration of individual serotypes inoculate a suitable number of wells (ideally 8 to 10) in a flat
24 bottomed microtitre plate with equal volumes of the selected dilutions of virus and the
25 appropriate antisera mixture. Total virus content is determined without any prior incubation, by
26 directly diluting the vaccine in the assay medium. The assay is then incubated for 1-3 hours at
27 34—36 °C followed by the addition of an appropriate volume of a suitable cell. The plates are
28 further incubated at 34-36 °C and examined between days 5-9 for the presence of viral
29 cytopathic effect.

1 The cytopathic effect can be observed by direct reading or after an appropriate staining (vital or
2 fixed staining).The individual virus concentration for each of the polio serotypes and reference
3 preparation is then calculated using an appropriate method.
4

5 The assay is considered valid if:
6

- 7 • the observed titre for the reference is within $0.3 \log_{10}$ of the established mean for this
8 preparation.
- 9 • the 95% confidence intervals of the assay should be within $0.3 \log_{10}$ of the estimated
10 number of infectious units in the vaccine.
11

12 The assay is not valid if the confidence interval ($p=0.95$) of the estimated virus concentration of
13 the reference preparation for the 3 replicates combined is greater than $\pm 0.3 \log \text{CCID}_{50}$
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Appendix 5

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

The following protocol is intended for guidance, and indicates the information that should be provided as a minimum by the manufacturer to the national regulatory authority.

Information and tests may be added or deleted as required by the national regulatory authority, if applicable.

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

The section concerning the final lot must be accompanied by a sample of the label and a copy of the leaflet 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 from the national regulatory authority of the country in which the vaccine was produced/released stating that the product meets the national requirements as well as Part A recommendations of this document published by WHO.

Summary information on the 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: _____

Composition (antigen concentration) / volume of single human dose

Date of filling of final lot: _____

Date on which last determination of virus concentration was started or date of start of period of validity: _____

Shelf-life approved (months): _____

Expiry date: _____

Storage conditions: _____

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 concentration was started or date of start of period of validity: _____

Cell banks	
3.1.1 Information on cell banking system	
Name and identification of substrate	
Origin and short history	_____
Authority that approved cell bank	_____
Master cell bank (MCB) and working cell bank (WCB) lots number and date of preparation	_____
Date MCB and WCB were established	_____
Date of approval by national regulatory authority	_____
Total number of ampoules stored	_____
Passage level (or no. of population doublings) of cell bank	_____
Maximum passage approved	
Storage conditions	
Method of preparation of cell bank in terms of no. of freezes and efforts made to ensure that a homogeneous population is dispersed into the ampoules	_____
3.1.2 Tests on MCB and WCB	
Percentage of total cell-bank ampoules tested	_____
Identification of cell substrate	_____
Method	_____
Specification	_____
Date of test	_____
Result	_____
Growth characteristics	_____
Morphological characteristics	_____
Immunological marker	_____
Cytogenetic data	_____

Biochemical data						_____
Results of other identity tests						_____
Tests for adventitious agents						_____
Method used						_____
Number of vials tested						_____
Volume of inoculum per vial						_____
Date test on						_____
Date test off						_____
Result						_____
Tests for bacteria, fungi and mycoplasma						
Method used						_____
Number of vials tested						_____
Volume of inoculum per vial						_____
Volume of medium per vial						_____
Observation period (specification)						_____
Incubation	Media used	Inoculum	Date test began	Date test ended	Results	
20–25 °	_____	_____	_____	_____	_____	
30–36 °	_____	_____	_____	_____	_____	
Negative control	_____	_____	_____	_____	_____	
Test for mycoplasma						
Method used						_____
Volume tested						_____
Media used						_____
Temperature of incubation						_____
Observation period (specification)						_____
Positive controls (list of species used and results)						
			Date test began	Date test ended	Results	

Sub cultures at 3 rd day	_____	_____	_____
Sub cultures at 7 th day	_____	_____	_____
Sub cultures at 14th day	_____	_____	_____
Sub cultures at 21th day	_____	_____	_____
Results of tests for tumorigenicity (if applicable)	_____		
3.2 Virus seed			
Vaccine virus strain(s) and serotype(s):	_____		
Substrates used for preparing seed lots:	_____		
Origin and short history:	_____		
Authority data approved virus strains	_____		
Date of approval	_____		

3.2.1 Information and seed lot preparation	
Virus Master seed (VMS) and virus working seed (VWS) (<i>to be provided upon first submission only and whenever a change has been introduced</i>)	
Source of VMS	_____
VMS and VWS lot number	_____
Name and address of manufacturer	_____
VWS passage level from VMS	_____
Dates of inoculation	_____
Dates of harvest	_____
Numbers of containers	_____
Conditions of storage	_____
Dates of preparation	_____
Maximum passages levels authorized	_____
Tests on Virus Master seed (VMS) and virus working seed (VWS)	
Test for adventitious agents	
Date(s) of satisfactory test(s) for freedom from adventitious agent	_____

Volume of virus seed samples for neutralization and testing	
Batch number of antisera used for neutralization virus seed	
Method used	
Date test on	
Date test off	
Result	
Identity test	_____
Method used	_____
Date test on	
Date test off	_____
Result	_____
Absence of SV40	
Method used	_____
Date test on	_____
Date test off	_____
Results	_____
In vitro tests MAPREC or rct/40 marker test	
<i>MAPREC</i>	
Date of test	_____
Type 1 Ratio of % of the sum of both mutations 480-A, 525-C of bulk sample to the International Standard 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 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 Level of mutations	_____	
Result of test of consistency of production	_____	
Result of test of comparison with the International Standard	_____	
In vitro rct/40 marker test		
Date of test	_____	
	36° - 40°C	36° - 39°C (39,2°C)
Reduction of titre of bulk sample	_____	_____
Reduction of titre of negative reference	_____	_____
Reduction of titre of positive reference	_____	_____
Result	_____	
Result of test of consistency of production	_____	
A.3.2.4.4. 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	_____	
Number and species of monkeys inoculated	_____	
Quantity (CCID50) inoculated in each test monkey	_____	
No. of "valid" monkeys inoculated with test sample	_____	
No. of positive monkeys observed	_____	
Reference preparation	_____	
No. of "valid" monkeys inoculated with reference	_____	
No. 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	_____
No. of mice inoculated	_____
No. of mice excluded from evaluation	_____
No. of mice paralysed	_____
Results of validity tests for each dose of the reference virus	_____
No. of mice inoculated	_____
No. of mice excluded from evaluation	_____
No. of mice paralysed	_____
Virus assay results for each dose inoculated (residual inoculums)	_____
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 test began	Date test ended	Results
20–25 °	_____	_____	_____	_____	_____
30–36 °	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____
Test for mycoplasma					
Method used			_____		
Volume tested			_____		
Media used			_____		
Temperature of incubation			_____		
Observation period (specification)			_____		
Positive controls (list of species used and results)					
	Date test began	Date test ended	Results		
Sub cultures at 3 rd day	_____	_____	_____		
Sub cultures at 7 th day	_____	_____	_____		
Sub cultures at 14th day	_____	_____	_____		
Sub cultures at 21th day	_____	_____	_____		
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 for infectivity	
Date of test	_____
Reference batch number	_____
Date of test	_____
Result	_____
Genotype characterisation	
Method used	_____
Date of test	_____
Result	_____
Test for mycobacteria	
Method used	_____
Date test on	_____
Date test off	_____
Result	_____

Control of vaccine production A.4.1	
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	
4.1.2 Tests on Control cell cultures	
Ratio of control to production cell cultures	_____
Incubation conditions	
Period of observation of cultures	_____
Date started/ended	
Ratio or proportion of cultures discarded for nonspecific reasons	_____

Results of observation	_____
Date of supernatant fluid collected	_____
Tests for haemadsorbing viruses:	
Quantity of cell tested	_____
Method used	_____
Date test on	_____
Date test off	_____
Results	_____
Tests for adventitious agents on supernatant culture fluids	
Method used	_____
Date test on	_____
Date test off	_____
Result	_____
Identity test	
Method used	_____
Date test on	_____
Date test off	_____
Result	_____
Control of single harvests A.4.3.3	
Volume harvested	_____
Date of sampling	_____
Identity test	
Method used	_____
Date test on	_____

Date test off						_____
Result						_____
Virus titration for infectivity						
Date of test						_____
Reference batch number						_____
Date of test						_____
Result						_____
Tests of neutralized single harvests for adventitious agents						
Method used						_____
Date test on						_____
Date test off						_____
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 test began	Date test ended	Results	
20–25 °C	_____	_____	_____	_____	_____	
30–36 °C	_____	_____	_____	_____	_____	
Negative control	_____	_____	_____	_____	_____	
Test for mycoplasma						
Method used						_____
Volume tested						_____

Media used		_____	
Temperature of incubation		_____	
Observation period (specification)		_____	
Positive controls (list of species used and results)		_____	
	Date test began	Date test ended	Results
Sub cultures at 3 rd day	_____	_____	_____
Sub cultures at 7 th day	_____	_____	_____
Sub cultures at 14th day	_____	_____	_____
Sub cultures at 21th day	_____	_____	_____
<i>Indicator cell-culture method (if applicable)</i>			
Cell substrate used		_____	
Inoculum		_____	
Date of test		_____	
Passage number		_____	
Negative control		_____	
Positive controls		_____	
Date of staining		_____	
Results		_____	
Test for mycobacteria			
Method used		_____	
Date test on		_____	
Date test off		_____	
Result		_____	
Control of monovalent bulk A.4.4			
Date of filtration of bulk		_____	

Porosity of filters used	_____
Date of sampling	_____
Identity test	
Method used	_____
Date test on	_____
Date test off	_____
Results	_____
Lot number of reference reagents	_____
Titration for virus infectivity	
Date of test	_____
Reference batch number	_____
Result	_____
Tests for consistency of virus characteristics	
In vitro rct/40 marker test	
Date of test	_____
Reference used	_____
Reduction or titre of negative reference	_____
Reduction of titre of positive reference	_____
Result	_____
MAPREC	
Date of test	_____
Type 1 Ratio of % f the sum of both mutations 480-A, 525-C of bulk sample to the International Standard 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 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 Level of mutations	_____
Result of test of consistency of production	_____
Result of test of comparison with the International Standard	_____
<i>Neurovirulence tests in monkeys</i>	
Result of blood serum test in monkeys prior to inoculation	_____
Date of inoculation of monovalent bulk	_____
Number and species of monkeys inoculated	_____
Quantity (CCID50) inoculated in each test monkey	_____
No. of "valid" monkeys inoculated with test sample	_____
No. of positive monkeys observed	_____
Reference preparation	_____
No. of "valid" monkeys inoculated with reference	_____
No. 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	
<i>Neurovirulence test in transgenic mice</i>	
Strain of mice inoculated	_____
For each dose of the bulk sample	
No. of mice inoculated	_____

No. of mice excluded from evaluation		_____	
No. of mice paralysed		_____	
Results of validity tests for each dose of the reference virus		_____	
No. of mice inoculated		_____	
No. of mice excluded from evaluation		_____	
No. of mice paralysed		_____	
Virus assay results for each dose inoculated (residual inoculums)		_____	
Paralysis rates for reference virus at each dose		_____	
Results		_____	
Log odds ratio		_____	
L1 and L2 values		_____	
Pass/fail decision		_____	
Final bulk A.4.5			
Preparation of bulk (types as appropriate)	Type 1	Type 2	Type 3
Monovalent bulks in blend	_____	_____	_____
Volume in blend	_____	_____	_____
Nature and volume of stabilizer	_____	_____	_____
Nature and volume of diluent	_____	_____	_____
Total volume of blend		_____	
Tests for bacteria and fungi			
Method used		_____	
Number of vials tested		_____	
Volume of inoculum per vial		_____	
Volume of medium per vial		_____	

Observation period (specification)			_____		
Incubation	Media used	Inoculum	Date test began	Date test ended	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____
Filling and containers A.5					
Total volume for final filling			_____		
Date of filling			_____		
No. of vials after inspection			_____		
No. of vials filled			_____		
Control tests on final lot A.6					
Inspection of final containers					
Appearance			_____		
Date of test			_____		
Results			_____		
pH					
Date of test			_____		
Result			_____		
Identity test					
Method used			_____		
Date test on			_____		
Date test off			_____		
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 test began	Date test ended	Results
20–25 °C	_____	_____	_____	_____	_____
30–36 °C	_____	_____	_____	_____	_____
Negative control	_____	_____	_____	_____	_____
Virus titration					
Date of test		_____			
Reference batch number		_____			
Titre of individual virus types		_____			
Batch numbers of antiserum used in test		_____			
Date of test		_____			
Results	Vaccine			Reference	
Type 1	_____			_____	
Type 2	_____			_____	
Type 3	_____			_____	
Thermal stability					
Date of test		_____			
Batch numbers of antiserum used in test		_____			
Results	Vaccine 37°	Vaccine 2-8°	Difference		
Total virus	_____	_____	_____		
Residual antibiotics (if applicable)			_____		
Date test on			_____		
Date test off			_____		
Results			_____		

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2 **Additional information for Production in monkey kidney-cell cultures**
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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 production cell culture maintenance medium	_____
Tests for antibodies to simian immunodeficiency virus, SV40, foamy viruses and B virus	
Methods used	_____
Date test on	_____
Date test off	_____
Results	_____
Production details	
Production monkey number	_____
Date of trypsinizing	_____
No. of cultures prepared	_____
Cell cultures for vaccine production	
Virus seed lot no.	_____
Virus infectivity/cell ratio	_____
No. of cultures inoculated	_____
Date of inoculation	_____
Date of harvest	_____

Temperature of incubation	_____
Period of incubation	_____
No. 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 proportion of production cell cultures	_____
Period of observation of cultures	_____
Ratio or proportion of cultures discarded for nonspecific reasons	_____
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 no. 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	
No. 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	_____

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Submission addressed to national regulatory authority

Name of Head of Production (typed) _____

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

I certify that lot no. _____ of poliomyelitis vaccine (oral), whose number appears on the label of the final container, meets all national requirements and/or satisfies Part A of the Recommendations for Biological Substances No. 7 (Recommendations for poliomyelitis vaccine (oral) vaccine, revised xxxx) and (if applicable) Part E for poliomyelitis vaccine (oral)

Signature: _____

Name (typed): _____

Date: _____

Appendix 6

Model certificate for the release of live attenuated poliomyelitis vaccine (oral) by national regulatory authorities

Lot release certificate

Certificate no. _____

The following lot(s) of acellular pertussis vaccine produced by _____¹ in _____² whose numbers appear on the labels of the final containers, complies with the relevant specification in the marketing authorization³ and provisions for the release of biological products and Parts A⁴, of WHO recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral) (_____)⁵ and comply with Good Manufacturing Practices for Pharmaceutical Products⁶, Good Manufacturing Practices for Biological Products⁷ and Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities⁸.

The release decision is based on _____⁹.

The certificate may include the following information:

- Name and address of manufacturer;
- Site(s) of manufacturing;
- Trade name and/common name of product;
- Marketing authorization number;
- Lot number(s) (including sub-lot numbers, packaging lot numbers if necessary);
- Type of container;
- Number of doses per container;
- Number of containers/lot size;
- Date of start of period of validity (e.g. manufacturing date) and/or expiry date;
- Storage condition;
- Signature and function of the authorized person and authorized agent to issue the certificate;

1 • Date of issue of certificate; and

2 • Certificate number.

3

4 The director of the National Regulatory Authority (or authority as appropriate):

5 Name (typed) _____

6 Signature _____

7 Date _____

8

9 **Footnote**

10 1 Name of manufacturer.

11 2 Country of origin.

12 3 If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has
13 nevertheless been authorized by the National Regulatory Authority.

14 4 With the exception of provisions on distribution and shipping, which the National Regulatory Authority may not be
15 in a position to assess.

16 5 WHO Technical Report Series, No. (xx, xxxx).

17 6 WHO Technical Report Series, No. 961,2011, Annex 3.

18 7 WHO Technical Report Series, No. 822, 1992, Annex 1.

19 8 WHO Technical Report Series, 2011 (in press).

20 9. Evaluation of summary protocol, independent laboratory testing, and/or specific procedures laid down in defined
21 document etc. as appropriate.

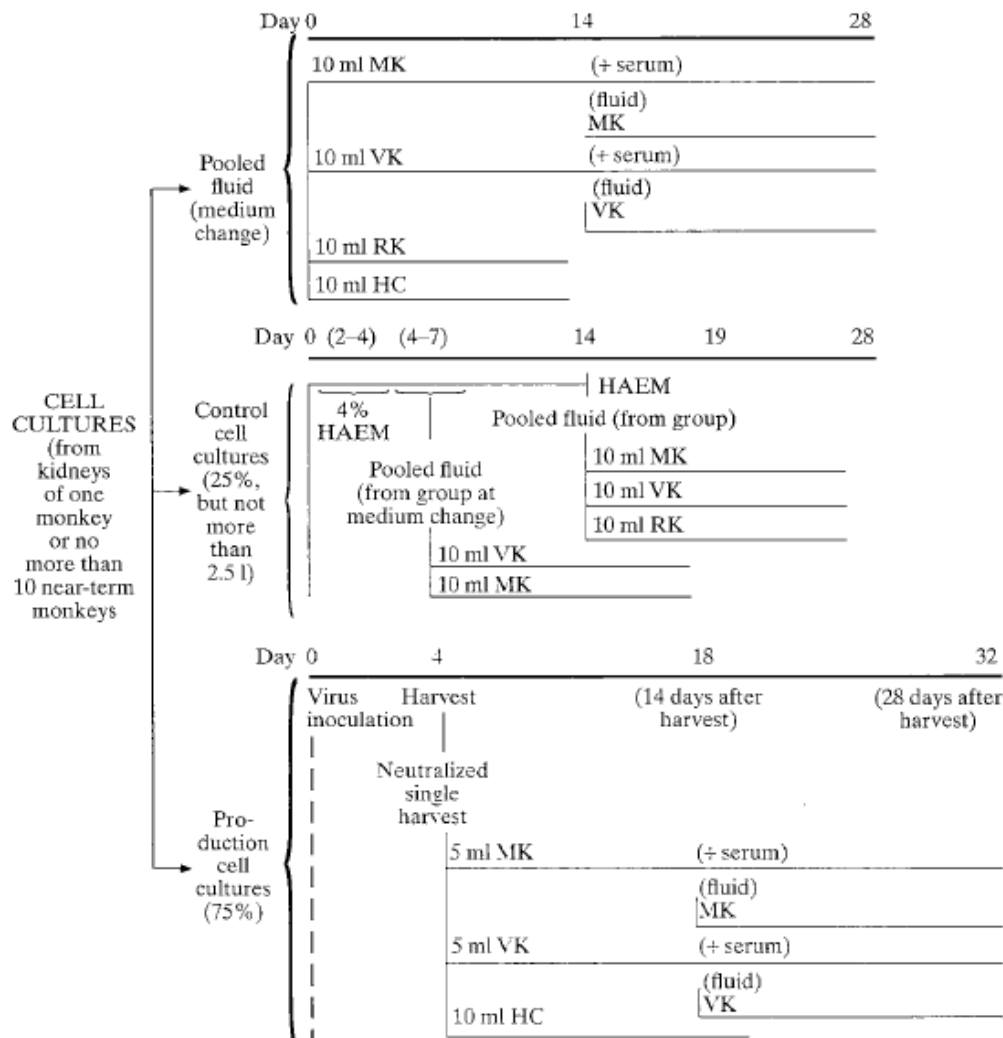
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1 **Appendix 7**

2 **Preparation of poliomyelitis vaccine (oral) using monkey kidney-cell cultures**

3 **Example of a flowsheet of tests in cell cultures**



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

9
10 Note. This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those
11 authorized by the national regulatory authority, this flowsheet should not be considered as an integral part of the requirements and
12 has been included solely for guidance. Manufacturing establishments should prepare their own flowsheet in order to clarify the
13 procedures used.