Annex 1

Recommendations for the production and control of poliomyelitis vaccine (oral)\textsuperscript{1}

Recommendations published by WHO are intended to be scientific and advisory. Each of the following sections constitutes guidance for national control authorities and for the manufacturers of biological products. If a national control authority so desires, these Recommendations may be adopted as definitive national requirements, or modifications may be justified and made by a national control authority. It is recommended that modifications to these Recommendations be made only on condition that modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the Recommendations set out below. The parts of each section printed in small type are comments for additional guidance intended for manufacturers and national control authorities, which may benefit from those details.

In these Recommendations, Part A describes the general provisions for the production of poliomyelitis vaccine (oral) (OPV) and is applicable to production of vaccine in all permissible cell substrates, including the use of a cell bank. Part B describes national control requirements, and Part C describes additional or alternative requirements applicable to production from primary monkey kidney-cell cultures.

To facilitate the licensing and international distribution of OPV made in accordance with these Recommendations, a summary protocol for recording the results of tests is provided in Appendix 7.

\textsuperscript{1} Replaces the 1989 Requirements for Poliomyelitis Vaccine (Oral) and Addendum 1998.
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Introduction

The Recommendations (formerly Requirements) for Poliomyelitis Vaccine (Oral) were last revised in full in 1989 (1). An addendum was subsequently adopted (2) that introduced changes in four areas: (1) to meet the need for the working seed virus to be free of detectable DNA sequences of SV40; (2) introducing the MAPREC assay as an optional, additional in vitro test of filtered bulk suspensions of poliovirus type 3; (3) to meet the need for increased levels of laboratory containment of wild polioviruses (3), and (4) introducing guidance on additional antibody screening tests (for foamy viruses) for animals from closed primate colonies used as a source for primary monkey kidney cells.

Since the last full revision, great progress had been made towards the eradication of wild polioviruses, and the goal of eradicating poliomyelitis is in sight. During this time, WHO has worked with the scientific community to improve quality control tests for oral poliomyelitis vaccine (OPV). Two Working Groups were convened by WHO in 1999 to review progress with the new methods. One considered the MAPREC assay and recommended that, for poliovirus type 3, MAPREC should be introduced as the in vitro test of preference for evaluation of filtered bulk suspensions (4). The rect40 tests would then become an optional, additional test. The same changes should be considered for polioviruses types 1 and 2 as soon as reference materials are established and the method validated for these serotypes. The second Working Group considered a neurovirulence test in transgenic mice that express the human poliovirus receptor (TgPVR mice). The Group recommended that, for poliovirus type 3, a neurovirulence test in TgPVR mice be introduced as an alternative to the current monkey neurovirulence test (5). The same changes should be considered for types 1 and 2 as soon as the mouse model is fully validated for these serotypes.

The WHO Secretariat has taken the amendments suggested by the Working Groups and incorporated them in this reformulated document. Additional changes, including a test for retrovirus for vaccines produced in primary monkey kidney cells, have been made to bring the document into line with other WHO Recommendations published since the last revision.

General considerations

The Sabin strains of poliovirus used in the production of OPV have been shown to yield vaccines that are both immunogenic and highly
attenuated when administered orally to susceptible children and adults.

Live vaccines prepared from the Sabin strains of poliomyelitis viruses of types 1, 2, and 3 were introduced for large-scale immunization in 1957. From then until 1972, Sabin himself authorized laboratories to produce vaccines from his strains, if he considered them capable of doing so, and controlled the methods of production in collaboration with the control authorities of the countries concerned. In 1972, Sabin proposed that WHO should accept the responsibility of approving laboratories that wished to produce poliovirus vaccines from the seed materials of the Sabin strains of viruses of types 1, 2, and 3. The Director-General of WHO agreed to assume responsibility for ensuring the proper use of the strains and established a scientific committee, the Consultative Group on Poliomyelitis Vaccines, to advise him on all matters pertaining to their use.

As a few ampoules of the original viruses produced by Sabin were still available, it was decided that they should be used to generate stocks at the Sabin original plus one (SO+1) passage level, and these stocks would be the “master seeds”. Behringwerke AG, Marburg/Lahn, Germany, generously agreed to produce the seeds for WHO, free of charge. The new seeds were produced directly from the original viruses, and producers of OPV can obtain these seeds from WHO free of charge.

Working seed lots at the SO+2 level were also produced by Behringwerke from the WHO master seeds. The Consultative Group agreed that all tests, including the test for neurovirulence, were satisfactory. Although WHO has taken every possible precaution to ensure that these seeds meet the Recommendations for Poliomyelitis Vaccine (Oral), it should be emphasized that, in each country, the national control authority must accept responsibility for the quality of vaccines produced from the seeds and used in that country.

In the meantime, some producers who had found it difficult to meet the neurovirulence requirements for type 3 vaccines made from their established working seeds began to use a Sabin type 3 seed prepared by Pfizer Ltd from a plaque prepared from viral RNA extracted from material at the SO+2 level. This seed gives satisfactory results in the neurovirulence test and has been used safely and effectively in trivalent vaccines in national immunization programmes involving millions of children. The master seed is designated RSO1, so that the working seed passage level is RSO2 and the vaccine RSO3. This master seed is stored by Aventis Pasteur (formerly Pasteur Mérieux Connaught), Marcy l’Etoile, France.
Detailed information on the work of the Consultative Group and the preparation of WHO master seeds and working seed viruses has been published, in the 1989 Requirements (1) and by Cockburn (6). National control authorities should decide on the detailed procedures applicable to the preparation and use of virus seed lots in their own countries.

It has been found that the monkey neurovirulence test described in the 1989 Requirements (1), in which the distribution of virus-specific lesions within the central nervous system is observed, provides the information necessary for a precise comparison of vaccines with the appropriate reference preparation (7). Nevertheless, because primates are used, efforts to complement and eventually replace the test are of considerable importance. As part of its programme on vaccine development, WHO has encouraged and supported research on various aspects of poliovirus biology, including cellular receptors for polioviruses. Two groups of scientists developed transgenic (TgPVR) mice by introducing into the mouse genome the human gene encoding the cellular receptor for poliovirus (8, 9). When infected with poliovirus, TgPVR mice develop flaccid paralysis, followed by death of some animals, and histological lesions in the central nervous system similar to those observed in monkeys.

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

— a reduction in the number of primates used in control of OPV
— the use of animals of highly-defined genetic and microbiological quality standards
— a reduction in hazards to laboratory personnel through a reduced need to handle primates
— in some countries, a reduction in the cost of control tests for OPV.

Studies were carried out initially on type 3 monovalent vaccines using the TgPRV21 mouse line, generously provided free of charge for the study by the Central Institute for Experimental Animals, Kawasaki, Japan. Researchers at the Japan Poliomyelitis Research Institute and at the Center for Biologics Evaluation and Research, Rockville, MD, USA developed an intraspinal inoculation method suitable for tests of vaccine lots. This was evaluated in an international collaborative study on the establishment of a standardized mouse neurovirulence test for OPV. Several laboratories participated in the collaborative study and results were assessed by WHO at meetings held in 1995, 1997 and 1999 in Geneva, Switzerland, in 1997 in Ottawa, Canada,
and in 1998 in Rockville. As a result of these studies, the revised Recommendations for Production and Control of Poliomyelitis Vaccine (Oral) introduce, for type 3 poliovirus, the murine model as an alternative to the monkey neurovirulence test. To be competent to perform the mouse neurovirulence test, laboratories should complete a standard implementation process that is overseen by WHO. Laboratories must also comply with specifications for containment of the transgenic animals (10).

The molecular mechanisms and genetic determinants of attenuation and reversion to virulence of all three types of Sabin polioviruses used for the manufacture of OPV have been studied in several laboratories. Evidence strongly suggests that mutations in the 5’ non-coding region of the poliovirus genome, especially for the Sabin type 3 strain, are critical in determination of the attenuated phenotype (11). A molecular biological test, the mutant analysis by PCR and restriction enzyme cleavage (MAPREC) assay, was developed by researchers at the Center for Biologies Evaluation and Research, Rockville, MD, to quantify reversion at the molecular level (12). Studies showed that all batches of type 3 OPV contained measurable amounts of revertants with C instead of U at nucleotide 472. Batches that failed the monkey neurovirulence test contained significantly higher quantities of 472-C than batches that passed the test. Studies with coded samples at the Center for Biologies Evaluation and research identified 100% of lots that failed the monkey neurovirulence test.

The Biologicals unit of WHO initiated a series of international collaborative studies in 1991 to evaluate the MAPREC assay for all three types of poliovirus and to validate appropriate reference materials. Several laboratories conducted the collaborative studies and results were assessed by WHO at meetings held in 1995 and 1997 in Geneva, Switzerland. It was concluded that the MAPREC assay was a sensitive, robust and standardized molecular biological assay suitable for use by manufacturers and national control authorities for monitoring the consistency of production of type 3 OPV. The revised Recommendations for the Production and Control of Poliomyelitis Vaccine (Oral) introduce, for type 3 poliovirus, the use of MAPREC as the in vitro test of preference in place of the rct40 test.

Neurovirulence and MAPREC reference materials are available and are distributed to national control authorities and manufacturers on request. It is recommended that, before a new virus working seed lot is considered suitable for use, at least four consecutive batches made from the seed are tested for monkey neurovirulence and MAPREC.
profile to establish baseline data for future comparison with new production lots.

In some countries, manufacturing may involve only the filling of final containers with vaccine obtained in bulk form from another manufacturing establishment. In such cases, ensuring conformity with all the recommendations applicable to the final vaccine (Part A, sections A.5–A.11) must be the responsibility of the manufacturer of the final product. In other countries, with the approval of the national control authority, certain production and control tests that are not performed by the manufacturer may be carried out in an independent laboratory. In such cases, the manufacturer must nevertheless assume responsibility for the safety and efficacy of the product.

The safety of OPV depends on several factors, of which the most important are strict adherence to the virus seed lot system and the period and temperature of incubation of the production cultures. Tests on the working seed viruses and vaccine lots derived from them for consistency of virus characteristics, including tests for neurovirulence and tests in vitro, are of great importance in the production of a safe vaccine. If the quality of consecutive lots of OPV consistently meets these requirements, there is a high level of assurance that the vaccines will be both safe and efficacious.

Oral poliomyelitis vaccines have been in worldwide use for more than 35 years and, although those produced from human diploid cells or continuous cell lines have been used to a lesser extent than those produced in cultures of primary monkey kidney cells, experience has indicated that all three cell substrates produce safe and effective vaccines.

It is generally accepted that cultures of monkey kidney cells can be contaminated with one or more adventitious agents, including simian viruses. The frequency of contamination can be significantly reduced by the use of animals derived from closed colonies and by careful screening of the animals to be used in production for the absence of antibodies to the relevant simian viruses.

The effects of new cell substrates and culture technologies on the properties of the virus call for careful examination, including the monitoring of phenotypic and molecular characteristics following multiple passages of each virus type on the substrate of choice in comparison with those following such passages on currently accepted cell substrates. It is also necessary to monitor the phenotypic and molecular characteristics of stool isolates from samples obtained during initial clinical trials of vaccines made on a new cell substrate compared with those obtained using vaccine made on the previously used cell substrate.
Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 Proper name

The proper name should be “Poliomyelitis vaccine (oral), types 1, 2 and 3” (whichever type or types apply), translated into the language of the country of use.

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

A.1.2 Descriptive definition

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

A.1.3 International reference materials

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

Three monotypic virus suspensions of types 1, 2, and 3 were selected by the WHO Consultative Group on Poliomyelitis Vaccines as international reference materials for the determination of virus titre but have not been formally adopted by WHO.

An International Standard for MAPREC analysis of poliovirus type 3 (Sabin) and international reference materials for control of MAPREC assays of poliovirus type 3 (Sabin) are available.

Reference preparations WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3 virus at the SO+2 passage level are available for the comparison of in vivo neurovirulence with that of homotypic vaccines. The relevant reference materials should be included in each test of vaccine.

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

All reference materials are available from the National Institute for Biological Standards and Control, Potters Bar.

A.1.4 Terminology

Master cell bank: A quantity of well-characterized cells of human or animal origin stored frozen at −100°C or below in aliquots of uniform
composition, one or more of which may be used for the production of a manufacturer's working cell bank.

*Manufacturer's working cell bank (MWCB)*: A quantity of cells of uniform composition derived from one or more ampoules of the master cell bank, which may be used for the production cell culture.

In normal practice, a cell bank is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the MWCB. One or more of the ampoules from such a pool may be used for the production cell culture.

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

*Adventitious agents*: Contaminating microorganisms of the cell substrate or materials used in its culture, which may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses.

*Original vaccine*: A monovalent vaccine, prepared according to stated specifications from the original seed virus, and shown on oral administration to humans in field trials to be immunogenic and highly attenuated.

*Virus master seed lot*: A quantity of virus of uniform composition derived from an original vaccine processed at one time and passaged for a number of times that does not exceed the maximum approved by the national control authority.

*Virus working seed lot*: A quantity of virus of uniform composition derived from the master seed by a single passage by a method approved by the national control authority.

*Single harvest*: A virus suspension of one virus type harvested from cell cultures prepared from a single production run.

*Bulk suspension*: A pool of a number of single harvests of the same virus type.

*Final bulk*: The finished biological preparation present in the container from which the final containers are filled. The final bulk may be prepared from one or more filtered bulk suspensions and may contain more than one virus type.

*Filling lot (final lot)*: A collection of sealed final containers of liquid vaccine that are homogeneous with respect to the risk of contamination during the filling process or the preparation of the finished vaccine. A filling lot must therefore have been filled or prepared in one working session.
Plaque-forming unit (PFU): The smallest quantity of a virus suspension that will produce a plaque in monolayer cell cultures.

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

A.2 General manufacturing recommendations

The general manufacturing recommendations contained in Good Manufacturing Practices for Biological Products (I3) should apply to establishments manufacturing oral poliomyelitis vaccine, with the addition of the following:

Production areas should be decontaminated before they are used for the manufacture of oral poliomyelitis vaccine.

The production of oral poliomyelitis vaccine should be conducted by separate staff who should consist of healthy persons and who should be examined medically at regular intervals. Steps should be taken to ensure that all such persons in the production areas are immune to poliomyelitis and do not excrete poliovirus or other microorganisms of significance from the point of view of the safety of the vaccine. Personnel working in monkey quarters should also be examined for tuberculosis as outlined in Part A, section 2 of the 1988 Requirements for dried BCG vaccine (I4).

Visitors and persons not directly concerned with the production processes should not be permitted to enter the production areas, except with the permission of the national control authority.

A.3 Control of source materials

The general production precautions as formulated in Good Manufacturing Practices for Biological Products (I3) should apply to the manufacture of oral poliomyelitis vaccine, with the addition that, during production, only one type of cell should be introduced or handled in the production area at any one time.

A.3.1 Cell lines

A.3.1.1 Master cell bank and manufacturer's working cell bank

The use of a cell line for the manufacture of oral poliomyelitis vaccines should be based on the cell bank system. The cell bank should be approved by the national control authority. The maximum number of passages (or population doublings) by which the MWC is derived from the master cell bank should be established by the national control authority.

WHO has established a cell bank of Vero cells characterized in accordance with the requirements in the report of the WHO Expert Committee on
A.3.1.2 Identity test

Cell banks should be characterized according to the requirements for continuous cell lines used for production of biologicals or those relating to human diploid cells (15), as appropriate.

The MWCB should be identified by means, inter alia, of biochemical (e.g. isoenzyme analysis), immunological and cytogenetic marker tests, approved by the national control authority.

A.3.1.3 Cell culture medium

Serum used for the propagation of cells should be tested to demonstrate freedom from bacteria, fungi, and mycoplasmas, according to the requirements given in Part A, sections 5.2 and 5.3, of the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16), and from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in Appendix I.

Where appropriate, more sensitive tests for bovine viruses may be used.

As an additional monitor of quality, sera may be examined for freedom from phage and endotoxin.

Irradiation may be used to inactivate potential contaminant viruses.

The source(s) of serum of bovine origin should be approved by the national control authority. The serum should comply with current guidelines in relation to animal transmissible spongiform encephalopathies given in the Report of a WHO Consultation on Medical and Other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies (17).

Human serum should not be used. If human albumin is used it should meet the 1992 Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives (18), as well as current guidelines in relation to human transmissible encephalopathies.

Penicillin and other β-lactams should not be used at any stage of manufacture.

Other antibiotics may be used at any stage in the manufacture provided that the quantity present in the final product is acceptable to the national control authority. Nontoxic pH indicators may be added, e.g. phenol red in a concentration of 0.002%. Only substances that have been approved by the national control authority may be added.
Trypsin used for preparing cell cultures should be tested and found free of cultivable bacteria, fungi, mycoplasmas and infectious viruses, especially bovine or porcine paroviruses, as appropriate. The methods used to ensure this should be approved by the national control authority.

The source(s) of trypsin of bovine origin, if used, should be approved by the national control authority. Bovine trypsin, if used, should comply with current guidelines in relation to animal transmissible spongiform encephalopathies (17).

A.3.2 Virus strains

Strains of poliovirus used in the production of oral poliomyelitis vaccine should be identified by historical records, which should include information on their origin. Only virus strains that are approved by the national control authority should be used (see General considerations).

A.3.2.1 Virus seed lot system

Vaccine production should be based on the virus seed lot system. The virus working seed lot used for the production of vaccine batches should be prepared by a single passage from a master seed lot by a method and at a passage level from the original seed virus approved by the national control authority.

A large working seed lot should be set aside as the basic material that the manufacturer will use for the preparation of batches of vaccine.

All virus seed lots should be stored at a temperature of \(-60^\circ C\) or below.

A.3.2.2 Tests on virus seed lots

The virus working seed lot used for the production of vaccine batches should be free from detectable extraneous viruses and from detectable SV40 sequences 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 and A.4.4.

Sequences of SV40 are widely used as molecular biological reagents, and contamination of polymerase chain reaction (PCR) assays is potentially a major problem. One approach is to identify separate genomic regions of SV40 for amplification, and to use one region for screening purposes and the other for the confirmation of repeatedly positive samples. It is useful if the second genomic region used for confirmation varies between isolates from different sources, as it is then possible to show that it has a unique sequence and that positive results are not due to contamination with laboratory strains of SV40. The sensitivity of the PCR assays for the genomic regions used should be established.
Virus seed lots should not be purified. Each virus working seed lot should have been derived from material tested in accordance with the recommendations set out in Part A, section A.4.4.5.2. In addition, the neurovirulence of consecutive monovalent virus batches prepared from the seed virus should meet the criteria for acceptability given in Appendix 3. A profile of the seed virus using the MAPREC test should be prepared.

The seed virus may continue to be used provided that the frequency with which monovalent virus pools produced with it fail to meet the neurovirulence criteria for monkeys or TgPVR mice is not greater than that predicted from a comparison with the corresponding reference preparation. If the frequency of failure of the monovalent virus pools is greater than predicted, the seed virus should cease to be used in vaccine production.

A.4 Control of vaccine production
A.4.1 Control of cell cultures

At least 5% or 1000ml of the cell suspension at the concentration and cell passage level employed for seeding vaccine production cultures should be used to prepare control cultures. (See Appendix 5 for an example of a flowsheet of tests in cell cultures.)

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

A.4.1.1 Tests of control cell cultures

The treatment of the cells set aside as control material should be similar to that of the production cell cultures, but they should remain uninoculated for use as control cultures for the detection of extraneous viruses.

These control cell cultures should be incubated under similar conditions to the inoculated cultures for at least 2 weeks, and should be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures should have been discarded for nonspecific, accidental reasons.

At the end of the observation period, the control cell cultures should be examined for degeneration caused by an extraneous agent. If this examination, or any of the tests specified in this section, shows evidence of the presence in a control culture of any adventitious agent, the poliovirus grown in the corresponding inoculated cultures should not be used for vaccine production.
A.4.1.2 Tests for haemadsorbing viruses
At the end of the observation period, 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If these cells have been stored, the duration of storage should not have exceeded 7 days and the storage temperature should have been in the range 2–8°C. In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

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

A reading should be taken after 30 minutes’ incubation at 0–4°C and again after a further incubation for 30 minutes at 20–25°C.

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

A.4.1.3 Tests for other adventitious agents
At the end of the observation period, a sample of the pooled fluid from each group of control cultures should be tested for adventitious agents. For this purpose, 10ml of each pool should be tested in the same cells, but not the same batch of cells, as those used for the production of virus, and additional 10-ml samples of each pool should be tested in human cells sensitive to measles and at least one other sensitive cell system.

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. At least one bottle of each kind of cell culture should remain uninoculated and should serve as a control.

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

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

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

If these tests are not performed immediately, the samples should be kept at a temperature of −60°C or below.
A.4.1.4 Identity test
At the production level, the cells should be identified by means of
tests approved by the national control authority.

Suitable tests are isoenzyme analysis and immunological and cytogenetic
marker tests.

A.4.2 Cell cultures for vaccine production
A.4.2.1 Tests for adventitious agents
On the day of inoculation with the virus working seed lot, each cell
culture or a sample from each culture vessel should be examined for
degeneration caused by infective agents. If such examination shows
evidence of the presence in a cell culture of any adventitious agent,
the culture should not be used for vaccine production.

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

A.4.2.2 Tests for bacteria, fungi, and mycoplasmas
A volume of at least 20ml of the pooled supernatant fluids from the
production cell culture should be tested for bacterial, fungal and
mycoplasmal sterility. The tests should be performed as described in
the 1973 General Requirements for the Sterility of Biological
Substances as amended in 1995 (16).

In some cases the sterility test is performed on the volume of pooled
supernatant fluids after ultracentrifugation, and both the pellet and its
supernatant fluid are tested.

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, neither inoculated nor control cell cultures should at any time be
at a temperature outside the range 33–35°C for the relevant incubation
periods. The temperature should not vary by more than 0.5°C
within that range. 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 control 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.4.2.2, A.4.3.3, A.4.3.4, and
A.4.3.5 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, 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 of neutralized single harvests for adventitious agents
For the purposes of the recommendations set out in this section of Part A, the volume of each single harvest taken for neutralization and testing should be at least 10 ml and should be such that a total of at least 50 ml or the equivalent of 500 doses of final vaccine, whichever is the greater, has been withheld from the corresponding bulk suspension.

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

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

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

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

If adequately justified, lower temperatures may be used.

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

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvest should be discarded.
A.4.3.4 Sterility tests
A volume of at least 10ml of each single harvest should be tested for bacterial, fungal and mycoplasmal sterility according to the requirements given in the 1973 General Requirements for the Sterility of Biological Substances as amended in 1995 (I6).

A.4.3.5 Tests for molecular consistency of production
A test for the molecular consistency of production, the MAPREC assay (see section A.4.4.5.1), has been developed. It should be introduced as soon as a manufacturer has developed acceptance/rejection criteria for each single harvest that demonstrate consistency of production. The criteria should be approved by the national control authority and updated periodically.

A.4.4 Control of bulk suspension
A.4.4.1 Preparation of bulk suspension
The bulk suspension should be filtered through a filter able to retain bacteria and cell debris.

The national control authority may require the 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 bulk suspension 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
The poliovirus in the bulk suspension should be serologically identified.

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 Virus concentration
The amount of infective poliovirus per ml of filtered bulk suspension should be determined in cell cultures in comparison with an existing reference preparation.

The virus concentration as determined by this test should be the basis for the quantity of virus used in the neurovirulence tests in monkeys (Part A, section A.4.4.5.2) or TgPVR mice (Part A, section A.4.4.5.3) and for preparing the final bulk (Part A, section A.4.5).
The detailed procedures for carrying out this test and for interpreting the results should be those approved by the national control authority.

A suitable test is described in Appendix 2.

A.4.4.5 Test for consistency of virus characteristics
The poliovirus in the filtered bulk suspension, prepared as described in section A.4.4.1, should be tested in comparison with the seed lot or a reference virus preparation (see Part A, section A.1.3) with regard to certain characteristics, as described in the following subsection, to ensure that the vaccine virus has not undergone changes during its multiplication in the production cell culture.

From the results of these tests for successive batches of vaccine a critical assessment may be made of the consistency of vaccine quality (see Part B, section B.2).

A.4.4.5.1 Tests in vitro
The virus in the filtered bulk suspension should be tested by at least one in vitro test. For Sabin poliovirus type 3, the MAPREC assay for nucleotide 472C should be used.

MAPREC assays for poliovirus types 1 and 2 are being evaluated.

MAPREC assays should be performed according to the standard operating procedures, “Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) for oral poliovirus (Sabin) vaccine” developed from WHO collaborative studies and available from WHO, or according to a validated alternative procedure. Results should be expressed as ratios relative to the International Standard for MAPREC analysis of poliovirus type 3 (Sabin).

For poliovirus type 3, MAPREC results should be used first to assess consistency of production of filtered bulk suspensions. Acceptance/rejection criteria for assessment of consistency of production should be specific for each manufacturer and each working seed and should be continually updated as each new bulk is prepared. An investigation of consistency should take place if a batch gives results that are inconsistent with previous production batches.

Secondly, MAPREC results for Sabin poliovirus type 3 should be compared with the International Standard for MAPREC analysis of poliovirus type 3 (Sabin). A poliovirus type 3 filtered bulk suspension found to have significantly more 472-C than the International

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1 Coordinator, Quality Assurance and Safety: Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.
Standard for MAPREC assay of poliovirus type 3 (significance determined as specified in the standard operating procedures) fails the MAPREC assay. Since the assay is highly predictive of in vivo neurovirulence, a filtered bulk suspension with significantly more 472-C than the International Standard should not be used to prepare vaccine. The failing in the MAPREC assay of a filtered bulk suspension should trigger an evaluation of the consistency of the manufacturing process, including the suitability of the working seed virus. Filtered bulk suspensions passing the MAPREC assay should be tested subsequently for in vivo neurovirulence.

The MAPREC test is also extremely useful for process development when, for example, an established manufacturer changes production conditions or a new manufacturer starts production. However, in such situations the product also needs to be tested by the monkey neurovirulence test.

Until MAPREC assays for poliovirus types 1 and 2 are validated, the type 1 or 2 virus in the filtered bulk suspension should be tested for the property of reproducing at temperatures of 36°C and 40°C in comparison with the seed lot or a reference virus preparation for the marker tests, and with appropriate rct/40– and rct/40+ strains of poliovirus of the same type. Wild polioviruses, defined as field isolates or reference strains derived from polioviruses known or believed to have circulated persistently in the same community, used as rct/40+ controls in this test, should be contained within the laboratory at progressively higher levels of containment in accordance with the global action plan and timetable for safe handling of wild polioviruses (3). The incubation temperatures used in this test should be controlled to within ±0.1°C.

The use of suitable vaccine-derived strains, defined as progeny of oral poliovirus vaccine strains, as rct/40– controls should be considered.

The filtered bulk suspension passes the test if, for both the virus in the bulk suspension and that in the appropriate reference material, the titre determined at 36°C is at least 5.0log_{10} greater than that determined at 40°C. Unless the titres obtained for all the reference viruses are in line with the expected values, the test should be repeated.

An additional specification that the virus titre must not exceed 10CCID_{50}/ml or 10PFU/ml at the higher temperature may also be applied.

It is desirable that the temperatures used in the test should also include one in the region of 39.0–39.5°C, at which the titre of the reference material should be reduced by a factor in the range of 3.0–5.0log_{10} of its value at 36°C. In one laboratory, a temperature of 39.2°C has been found suitable. The aim is to obtain values of the ratio of the reproductive capacities of the bulk suspension and the reference material over a range of temperatures so that a more accurate comparison can be made.
The national control authority should provide reference virus preparations and appropriate rct/40+ and rct/40− virus strains for this test.

A.4.4.5.2 Neurovirulence tests in monkeys
Monkeys used for neurovirulence tests should satisfy the relevant recommendations in Part C, section C.4.1.1, and weigh not less than 1.5 kg. The pathogenicity of the filtered bulk suspension for *Macaca* or *Cercopithecus* monkeys should be tested in comparison with that of a reference virus preparation for neurovirulence testing (see Part A, section A.1.3) by inoculation into the lumbar region of the central nervous system. A pre-injection serum sample obtained from each monkey should be shown not to contain any neutralizing antibody in a dilution of 1:4 when tested against no more than 1000 CCID₅₀ of each of the three types of poliovirus.

If only the manufacturer performs the neurovirulence test, the histological sections should be made available to the national control authority for evaluation.

(1) Number of monkeys

It is recommended that a vaccine and the appropriate homotypic reference virus should, whenever possible, be tested concurrently in a single group of monkeys. Equal numbers of animals should be inoculated with the reference virus and the vaccine being tested. Monkeys should be allocated to vaccine or reference virus and to particular cages using a randomization procedure.

The number of monkeys inoculated should be such that at least 11 positive monkeys are included in the evaluation of the vaccine and at least 11 positive monkeys are included for the reference preparation for virus types 1 and 2. (A “positive” monkey is one in which neuronal lesions characteristic of poliovirus are seen in the central nervous system.) For virus type 3, there should be at least 18 positive monkeys for the reference preparation and a further 18 positive monkeys for the vaccine. More than one vaccine lot may be tested with the same homotypic reference. The monkeys should, when possible, be from the same quarantine group and should be allocated randomly to the preparations. If it is not possible to use monkeys from the same quarantine group for both the homotypic reference and the test vaccine, monkeys from two quarantine groups should undergo tests with each of the preparations (with as close to equal numbers as possible from each quarantine group). If a test is done on two working days, equal numbers of monkeys should be inoculated with the vaccine and the homotypic reference on each working day.
In order to obtain 11 and 18 positive monkeys, it is usual to inoculate 12 and 20 monkeys, respectively.

The monkeys are sedated with ketamine hydrochloride or any other substance that has been shown to be suitable.

(2) Virus content of vaccines and reference preparations inoculated

The virus contents of the vaccine and the homotypic reference preparation should be adjusted to be as similar as possible and should be between $10^{5.5}$ and $10^{6.5}$ CCID$_{50}$/0.1 ml, based on the virus concentration determined as described in Part A, section A.4.4.4. Monkeys should be inoculated with only one concentration of virus.

(3) Observation of monkeys

All monkeys should be observed for 17–22 days for symptoms suggestive of poliomyelitis or other virus infection. Monkeys that survive the first 24 hours but die before the 11th day after inoculation should be autopsied to determine whether poliomyelitis was the cause of death. Those that have died from causes other than poliomyelitis should be excluded from the evaluation.

Animals that become moribund or are severely paralysed should be humanely killed and autopsied.

All monkeys that survive the observation period should be autopsied.

For the test to be valid, no more than 20% of the animals in each group should show signs of an intercurrent infection during the observation period.

(4) Number of sections examined

The lumbar cord, the cervical cord, the lower and upper medulla oblongata, the mesencephalon, the thalamus, and the motor cortex of each monkey, as a minimum, should be subjected to histological examination.

Sections should be cut at a thickness of 15μm and stained with galloycyanin.

If adequately justified, sections may be cut at a thickness of 8–15μm, and Nissl staining may be used as an alternative to galloycyanin.

The minimum number of sections examined should be as follows:
- 12 sections representative of the whole of the lumbar enlargement
- 10 sections representative of the whole of the cervical enlargement
— 2 sections from the medulla oblongata
— 1 section from the pons and cerebellum
— 1 section from the midbrain
— 1 section each from the left and the right of the thalamus and cerebral cortex.

(5) Scoring of virus activity

In the evaluation of virus activity in the hemisections of the spinal cord and brain stem, a method of scoring the severity of the lesions should be used. Since the type of damage, whether cellular infiltration or destruction of neurons, is important, the lesions should be scored as follows:

*Score*
1. Cellular infiltration only (this is not sufficient for the monkey to be considered as positive)
2. Cellular infiltration with minimal neuronal damage
3. Cellular infiltration with extensive neuronal damage
4. Massive neuronal damage with or without cellular infiltration

The scores obtained should be recorded on a standard form (see Appendix 4).

A monkey with neuronal lesions in the sections but which shows no needle tract should be regarded as positive.

A monkey showing a needle tract in the sections but no neuronal lesions should not be regarded as positive.

A section that shows damage due to trauma but no specific virus lesion is not included in the score.

Severity scores are based on hemisection readings of the lumbar (L), cervical (C), and brain (B) histological sections. The lesion score (LS) for each positive monkey is calculated as follows:

\[
LS = \frac{\sum L \text{ scores}}{\text{No. of hemisections}} + \frac{\sum C \text{ scores}}{\text{No. of hemisections}} + \frac{\sum B \text{ scores}}{\text{No. of hemisections}} + 3
\]

A mean lesion score is calculated for each group of positive monkeys.

(6) Evaluation of neurovirulence test

The comparison of the virus activity in the vaccine and the reference preparation should be based on the activity in the lumbar enlargement of the cord and the degree of spread of
activity from this region to the cervical enlargement and the brain.

The acceptance or rejection of the vaccine should be based on the total score of all the test animals. Individual animals showing unusually high activity, either in the lumbar region or as the result of spread from this region, should also be taken into consideration in the final evaluation.

The filtered bulk suspension passes the test if the required number of animals is positive and if none of the clinical and histopathological examinations shows a significant difference in pathogenicity between the vaccine virus and the reference material.

Criteria for the acceptance of vaccines after neurovirulence testing are given in Appendix 3.

A.4.4.5.3 Neurovirulence test in transgenic (TgPVR21) mice for poliovirus type 3

The TgPVR21 transgenic mouse model provides a suitable alternative to the monkey neurovirulence test for the neurovirulence testing of type 3 vaccines. The test should be performed according to the standard operating procedure, “WHO neurovirulence test of type 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus”, available from WHO.¹

The TgPVR21 transgenic mouse model is being evaluated for neurovirulence testing of type 1 and 2 vaccines.

Although the murine model can be used for neurovirulence testing of filtered bulk suspensions, the monkey neurovirulence test should remain the definitive reference test to requalify vaccine production, for example to evaluate any new virus seed materials or vaccines produced on a new substrate and lots prepared to establish consistency from the new seed or substrate.

To qualify as competent to perform the mouse neurovirulence test, laboratories should complete a standard implementation process. Details of the process are available from WHO.²

(1) TgPVR21 mice

Mice used for the neurovirulence test should be aged 6–8 weeks at the time of inoculation and received from a source defined in the standard operating procedure “WHO neurovirulence test of

¹ Coordinator, Quality Assurance & Safety: Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.
type 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus”. Mice should be allowed to recover from shipping for at least 7 days before inoculation. Procedures and standards for maintenance of TgPVR21 mice should follow the WHO guidelines (10).

(2) Number of mice

A vaccine and appropriate homotypic reference virus should be tested concurrently. Equal numbers of animals, with equal numbers of males and females, should be inoculated with the reference virus and the vaccine being tested. Mice should be allocated to vaccine or reference virus and to particular cages using a randomization procedure (defined in the standard operating procedure); 32 mice per test dose should be used for evaluation of the vaccine and 32 mice per test dose for evaluation of the reference. More than one vaccine may be tested with the same homotypic reference virus at the same time. If a test is done on two working days, equal numbers of mice should be inoculated with the vaccine and the reference virus on each working day.

(3) Virus content of vaccines and reference virus inoculated

The virus content of the vaccines and reference preparations should be determined by the assay method described in the standard operating procedure. Groups of mice should be inoculated with two test doses of vaccine and reference. For poliovirus type 3 vaccines tested against the WHO(SO+2)/III reference virus, the doses are 3.5 and 4.5 log_{10} CCID_{50} in 5μl.

If other reference viruses are used, the doses should be determined by the paralysis proportions of the reference: at the high dose <0.95, at the low dose >0.05.

Mice should be sedated appropriately and inoculated into the lumbar region of the spinal cord as described in the standard operating procedure.

(4) Observation of mice

Mice should be observed for occurrence of paralysis for 2 weeks after inoculation. Paralysed mice should be humanely killed as soon as paralysis is confirmed. Other mice should be humanely killed on day 14 after inoculation. Data should be recorded on a standard form (see the standard operating procedure). Mice with traumatic paralysis (appearing 24 hours or less after inoculation and not progressing) and those that die from causes other than poliomyelitis should be excluded from evaluation.
(5) Evaluation of the neurovirulence test

Comparison of the virus neurovirulence activity in the vaccine(s) and reference preparations should be based on the numbers of paralysed animals in both groups of mice, inoculated with two test doses of the vaccine and reference preparation. Validity criteria for each test should be met and are specified in the standard operating procedure.

The filtered bulk suspension passes the test if the numbers of paralysed mice in the groups inoculated with vaccine are not significantly greater than the numbers in the groups inoculated with the reference material. Detailed statistical criteria for acceptance of vaccines after neurovirulence testing in TgPVR21 mice are given in the standard operating procedure.

A.4.5 Final bulk

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

The dilution and mixing procedures involved in preparing the final bulk should be those approved by the national control authority.

A.4.5.1 Stabilizers

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

All the tests described in Part A, sections A.4.3 and A.4.4, should be performed on samples taken before any stabilizers are added.

A.4.5.2 Tests for bacteria and fungi

The final bulk should be tested for bacterial and fungal sterility in accordance with the requirements given in Part A, section 5, of the 1973 General Requirements for the Sterility of Biological Substances as amended in 1995 (16).

A.5 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (13) should apply to vaccine filled in the final form.

Care should be taken that the material of which the container is made does not adversely affect the virus content of the vaccine under the recommended storage conditions.
A.6 **Control tests on final product**

Samples should be taken from each filling lot for the tests described in the following sections.

A.6.1 **Identity test**

The poliovirus type or types should be serologically identified.

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 used for this purpose.

A.6.2 **Tests for bacteria and fungi**

Liquid vaccine should be tested for bacterial and fungal sterility according to the requirements given in Part A, section 5, of the 1973 General Requirements for the Sterility of Biological Substances as amended in 1995 (16).

A.6.3 **Virus titration**

The poliovirus titre should be determined as described in Part A, section A.4.4.4, of these Recommendations using assays that include a reference preparation. When the vaccine contains more than one poliovirus type, each type should be titrated separately by using appropriate type-specific antisera to neutralize each of the other types present. The national control authority should specify the minimum virus titres per human dose.

It is recommended that, when the Sabin strains are used, the estimated mean virus titres for a single human dose of trivalent oral poliomyelitis vaccine should be not less than \(10^{9.0}\) infectious units for type 1, \(10^{8.0}\) infectious units for type 2, and \(10^{6.5}\) infectious units for type 3, as determined by the procedure described in Appendix 2. The 95% confidence intervals of the assays should not differ by a factor of more than \(10^{0.5}\) from the estimated number of infectious units in the vaccine.

A.6.5 **Accelerated degradation test**

Representative final containers of the vaccine should be incubated at 37 °C for 48 hours. The total virus content in both exposed and unexposed vials should be determined concurrently with that of a trivalent reference preparation. The vaccine passes the test when the loss on exposure is not greater than a factor of \(10^{1.5}\) infectious units per human dose.

A.7 **Records**

The recommendations given in Good Manufacturing Practices for Biological Products (13) should apply.
A.8 **Samples**

The requirements given in Good Manufacturing Practices for Biological Products (I3) should apply.

A.9 **Labelling**

The requirements given in Good Manufacturing Practices for Biological Products (I3) should apply, with the addition of the following.

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

- the designation(s) of the strain(s) of poliovirus contained in the vaccine
- the minimum amount of virus of each type contained in one recommended human dose
- the cell substrate used for the preparation of the vaccine
- the nature and amount of any stabilizer present in the vaccine
- the fact that the vaccine is not to be injected.

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 Good Manufacturing Practices for Biological Products (I3) should apply.

A.11 **Storage and expiry date**

The statements concerning storage temperature and expiry date appearing on the label and in the leaflet, as recommended in Good Manufacturing Practices for Biological Products (I3), should be based on experimental evidence and should be submitted for approval to the national control authority.

A.11.1 **Storage conditions**

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

The maximum duration of storage should be fixed with the approval of the national control authority and should be such as to ensure that the minimum titre of each virus type specified on the label of the container (or package) will still be maintained after release by the manufacturing establishment until the end of the shelf-life, if the conditions under which the vaccine is stored are in accordance with
what is stated on the label. The maximum duration of storage at 2–8°C or below –20°C may be specified.

A.11.2 Expiry date

The expiry date should be fixed with the approval of the national control authority and should relate to the date of the last satisfactory determination, performed in accordance with Part A, section A.4.4.4, of virus concentration, i.e. the date on which the test system was inoculated.

Provided that the vaccine has been stored continuously at a temperature below –20°C, the expiry date should be not more than 2 years after the last titration.

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

Part B. Recommendations for national control authorities

B.1 General

The general recommendations for control laboratories given in Guidelines for National Authorities on Quality Assurance for Biological Products (19), which specify that no new biological substance should be released until consistency of production has been established, should apply.

The detailed production and control procedures and any significant changes in them should be discussed with and approved by the national control authority. The national control authority should obtain the International Reference Reagents for virus titre and, where necessary, establish national working reference preparations by comparison with them.

The national control authority should carry out at least four neurovirulence tests on the reference preparations using the monkey neurovirulence test in order to obtain the necessary baseline data for comparison with the neurovirulence of test vaccines.

Unless the national control authority itself performs the monkey neurovirulence test, it should carry out a second reading of the histological sections provided by the manufacturer for each monovalent bulk.

The national control authority should encourage the use of the standard form for the reporting of data on virus activity in the sections taken from histopathological examination.
If the national control authority itself performs the mouse neurovirulence test, it should complete the standard implementation process.

B.2 Release and certification

A vaccine lot should be released only if it satisfies Part A (and, if applicable, Part C) of the present Recommendations. Before any vaccine lot is released from a manufacturing establishment, the requirements for consistency of production given in Guidelines for National Authorities on Quality Assurance for Biological Products (19) should be met.

A statement signed by the appropriate official of the national control authority should be provided if requested by a manufacturing establishment, and should certify whether or not the lot of vaccine in question meets all national requirements as well as Part A of the present Recommendations. The certificate should further state the date of the last satisfactory determination of virus concentration, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document should be attached.

The purpose of the certificate is to facilitate the exchange of oral poliomyelitis vaccine between countries.

Part C. Recommendations for poliomyelitis vaccine (oral) prepared in primary cultures of monkey kidney cells

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

C.4 Control of vaccine production

C.4.1 Control of source materials

C.4.1.1 Monkeys used for preparation of kidney-cell cultures and for testing of virus

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

Manufacturers are encouraged to use animals from closed or intensively monitored colonies.

The monkeys should be kept in well-constructed and adequately ventilated animal rooms in cages spaced as far apart as possible. Adequate precautions should be taken to prevent cross-infection between cages. Not more than two monkeys should be housed per cage, and cage-mates should not be interchanged. The monkeys should be kept in the country of manufacture of the vaccine in quarantine groups\(^1\) for a period of not less than 6 weeks before use. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5% (excluding deaths from infectious diseases), monkeys from that entire shipment should continue in quarantine for a further period of not less than 6 weeks. The groups should be kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used. After the last monkey of a group has been taken, the room that housed the group should be thoroughly cleaned and decontaminated before being used for a fresh group.

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

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

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

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

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\(^1\) 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.
If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it should not be used, nor should any of the remaining monkeys of the quarantine group concerned be used unless it is evident that their use will not impair the safety of the product.

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

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

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

C.4.2 Production precautions

The general production precautions called for by the Good Manufacturing Practices for Biological Products (15) should apply to the manufacture of vaccine, with the addition of the following.

C.4.2.1 Monkey kidney-cell cultures for vaccine production

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

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

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

C.4.2.2 Tests of cell cultures used for vaccine production

(see Appendix 6)

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

On the day of inoculation with the virus working seed lot, a sample of at least 30 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey or from no more than 10 near-term monkeys should be divided into two equal portions. One portion of the pooled fluid should be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used
for vaccine production. The other portion of the pooled fluid should be tested in kidney-cell cultures from another species of monkey, provided that the tests are done in cell cultures from at least one species known to be sensitive to SV40 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 control 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.

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

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

It is suggested that, in addition to these tests, a further sample of 10 ml of pooled fluid removed from the cell cultures on the day of inoculation with the

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

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

If the presence of the cercopithecoid herpesvirus 1 (B virus) is demonstrated, the manufacture of vaccine should be discontinued and the national control authority should be informed. Manufacturing should not be resumed until a thorough investigation has been completed and precautions have been taken against any reappearance of the infection, and then only with the approval of the national control authority.

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.

C.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.

C.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.

C.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 C, section C.4.2.2.

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

If the presence of cercopithecid herpesvirus 1 (B virus) is demonstrated, the production cell cultures should not be used and the measures concerning vaccine production described in Part C, section C.4.2.2, should be taken.

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

C.4.3 Control of single harvests

C.4.3.1 Single harvest

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

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

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

Half (corresponding to at least 5ml of single harvest) of the neutralized suspension should be tested in monkey kidney-cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other half of the neutralized suspension should be tested in monkey kidney-cell cultures from another species, provided that the tests are done in cell cultures from at least one species known to be sensitive to SV40 virus.
The neutralized suspensions should be inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm² per ml of neutralized suspension. At least one bottle of each kind of cell culture should remain uninoculated, should serve as a control and should be maintained using nutrient medium containing the same concentration of the specific antiserum used for neutralization.

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

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

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

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

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

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

C.4.4 Control of bulk suspension

C.4.4.1 Bulk suspension (before filtration)

C.4.4.1.1 Tests in rabbits

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

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

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

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

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

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

C.4.4.2 Bulk suspension (after filtration)
C.4.4.2.1 Tests for retroviruses
Test samples from the filtered bulk suspension should be examined
for the presence of retroviruses by an assay for reverse transcriptase
(Rtase) acceptable to the national control authority.

Recently developed highly sensitive assays for Rtase may be considered
(20), but the results need to be interpreted with caution because Rtase
activity is not unique to retroviruses and may derive from other sources,
such as retrovirus-like elements which do not encode a complete genome.
Nucleic acid amplification tests for retroviruses may also be used.

Acknowledgements

The first draft of these revised Recommendations was prepared by Dr D.J. Wood,
Scientist, National Institute for Biological Standards and Control, Potters Bar,
Herts., England, with help from Dr J. Furesz, formerly Director, Bureau of Biologics,
Ottawa, Canada.

The draft was reviewed by the following participants at an Informal WHO
Consultation held at the National Institute for Biological Standards and Control,
Potters Bar, from 14 to 15 October 1999:

Dr M. Ban, Torlak Institute of Immunology and Virology, Belgrade, Yugoslavia;
Dr J. Beals, Cranbrook, Kent, England; Dr K. Chumakov, Center for Biologics
Research and Evaluation, Rockville, MD, USA; Dr E. Dragunsky, Center for Biologics Research and Evaluation, Rockville, MD, USA; Dr M. Fibi, Chiron Behring, Marburg, Germany; Dr J. Furesz, Ottawa, Canada; Dr K. Goran, Torlak Institute of Immunology and Virology, Belgrade, Yugoslavia; Dr V. Grachev, Deputy Director, MP Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russian Federation; Dr E. Griffiths, Quality Assurance and Safety: Biologicals, World Health Organization, Geneva, Switzerland; Dr A. Heath, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr Nguyen Dang Hien, Poliomyelitis Vaccine Research and Production Centre, Hanoi, Viet Nam; Dr G. Kado-Boll, Director, National Institute of Virology, Mexico City, Mexico; Dr I. Knezovic, Head of Virology Division, Institute of Pharmacy, Belgrade, Yugoslavia; Dr I. Levenbrook, Northbrook, IL, USA; Mrs. A. F. Macq, Assistant Manager, QC Virology, SmithKline Beecham Biologicals, Rixensart, Belgium; Dr N. Medunitsin, Director, Tarasievic Institute for the Standardization and Control of Medical Biological Preparations, Moscow, Russian Federation; Dr P. Minor, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr T. Nomura, Director, Central Institute for Experimental Animals, Kawasaki, Japan; Dr I. Parent, Assistant Manager, QC New Products, SmithKline Beecham Biologicals, Rixensart, Belgium; Dr V. Pellegrini, Chiron SpA, Siena, Italy; Dr F. Pelloquin, Pasteur Mérieux Connaught, Marcy l'Etoile, France; Dr L. Petini, Chiron SpA, Siena, Italy; Dr Y. Pervikov, Department of Vaccines and Biologicals, World Health Organization, Geneva, Switzerland; Dr M. Roumiantzeff, Lyon, France; Dr O. Saidate Castaneda, Director, National Laboratory of Public Health, Mexico City, Mexico; Dr G. Schili, Director, National Institute for Biological Standards and Control, Potters Bar, Herts., England; Dr J.-C. Vincent-Falquet, Pasteur Mérieux Connaught, Marcy l'Etoile, France; Dr D.J. Wood, National Institute for Biological Standards and Control, Potters Bar, Herts., England.

For his comments and advice on the first draft of these revised Recommendations, acknowledgements are also due to:

Dr S.C. Arya, Centre for Logistical Research and Innovation, New Delhi, India.

References


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17. Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives. In: WHO Expert Committee on


Appendix 1
Tests for bovine viruses in serum

The serum to be tested is used in a growth medium to cultivate primary bovine testis cells or cells of a continuous bovine kidney-cell line known to be sensitive to bovine viruses.

The method, in which primary bovine testis cells are used as a suspension in Eagle’s Minimum Essential Medium (MEM) containing 10^5 cells/ml, is as follows:

A 90-ml sample of the cell suspension is mixed with 10ml of the test serum and dispensed into two 75-cm² plastic tissue-culture flasks. The process is repeated with a further 90ml to which 10ml of control serum (pretested for freedom from bovine viruses) is added. The cultures are incubated at 36.5°C ± 1°C for a period of 28 days with frequent microscopic examination for evidence of extraneous viruses. The growth medium may be replaced, as required, by fresh medium containing the relevant serum.

At least one subculture of the cells is carried out for both the test and control sera during the observation period in order to provide not less than 300 cm² (e.g. six bottles each of 25 cm²) of cells of each group for further examination at 28 days after initiation.

At the end of the observation period, each of the following tests is applied to at least one 25-cm² culture each of cells grown on test serum and on control serum, appropriate controls being retained in each case:

(a) a test to show that the cells are sensitive to a suitable challenge virus,
(b) microscopic examination for abnormalities following staining by the May Grunwald–Giemsa technique,
(c) a test for haemadsorbing agents, using a mixed suspension of 0.2% chick, 0.2% guinea-pig, and 0.2% human group O erythrocytes,
(d) examination by electron microscopy of the negatively stained extract produced after disruption and clarification of the cell culture,
(e) examination for bovine viruses by direct or indirect immuno-fluorescence tests.
Appendix 2

Assay method for the determination of the virus content of poliomyelitis vaccine (oral)

The preparation to be assayed and the reference preparation are diluted in an appropriate medium.

It is convenient to make tenfold dilution steps of the virus suspensions initially, but for dilutions that are to be inoculated into cell cultures the dilutions should be prepared in $0.5 \log_{10}$ or smaller steps. A preliminary assay may be required to ensure that, in the test, the dilution range selected encompasses at least three dilutions that will infect between 10% and 90% of the cultures inoculated.

Groups of 8–12 flat-bottomed wells in a microtitre plate are inoculated with 0.1 ml of each of the selected dilutions of virus followed by 0.1 ml of a suitable cell suspension of the Hep-2 (Cincinnati) line. The plates are incubated at 35–36°C for 7 days.

The cultures are examined for the presence of a specific viral cytopathic effect on days 3–5 and again on day 7. The observations are recorded and the $\text{CCID}_{50}$ is calculated on the basis of the observation on day 7. The assay should be repeated on at least two occasions and a mean taken. Results should be presented for the test preparation and the reference. For the estimated titre to be accepted as valid, the observed mean for the reference should be within $0.5 \log_{10}$ of the established mean for this preparation and the 95% confidence intervals of the assay should be within $0.5 \log_{10}$ of the estimated number of infectious units in the vaccine.
Appendix 3
Criteria for the acceptance of vaccines after neurovirulence testing

It is recommended that each laboratory should perform a minimum of four neurovirulence tests (referred to here as “qualifying” tests) on each reference vaccine (Types 1, 2, and 3) to provide sufficient data on the activity of such vaccines for the development of criteria for the acceptability of test vaccines. On practical grounds, each of these tests should include a homotypic lot of production vaccine tested concurrently with the reference so that the results of the tests may be used in assessing vaccines in addition to providing information on the reference. The minimum number of animals in each of these tests is as specified on page 50 for each poliovirus type. The overall mean Lesion Score ($M$) for the replicate tests on each reference virus is calculated together with the pooled estimate ($s^2$) of the within-test variance and the within-test deviation ($s$).

Criteria for the validity of the results of a test of a reference preparation can be determined by each laboratory only on the basis of the data accumulated after the four qualifying tests. No generally applicable criteria can therefore be given. For laboratories with limited experience with neurovirulence testing, the following empirical method of establishing acceptable limits for the mean Lesion Score for the reference ($\bar{X}_{\text{ref}}$) may be helpful:

<table>
<thead>
<tr>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M - s$</td>
<td>$M + s$</td>
</tr>
</tbody>
</table>

For Types 1 and 2

For Type 3

$$M - \frac{s}{2}$$

$$M + s$$

If the mean Lesion Score for the test is $\bar{X}_{\text{test}}$, and $C_1$, $C_2$, and $C_3$ are constants, then:

The vaccine is not acceptable if:

$$\bar{X}_{\text{test}} - \bar{X}_{\text{ref}} > C_1.$$  

The vaccine may be retested once at the discretion of the national control authority if:

$$C_1 < \bar{X}_{\text{test}} - \bar{X}_{\text{ref}} < C_2.$$  

If the vaccine is retested, the means of the Lesion Scores for the test and reference vaccines are recalculated, and the vaccine is rejected if:
\[ \frac{\bar{X}_{\text{test}} - \bar{X}_{\text{ref}}}{{\sigma}} > C_3 \]

The constants \(C_1, C_2,\) and \(C_3\) are calculated as follows:

\[ C_1 = 2.3 \sqrt{\frac{\sigma^2}{N}} \]
\[ C_2 = 2.6 \sqrt{\frac{\sigma^2}{N_1}} \]
\[ C_3 = 1.6 \sqrt{\frac{\sigma^2}{N_2}} \]

Where \(N_1\) = number of positive monkeys per vaccine test,
\(N_2\) = number of positive monkeys for the two tests,
2.3 = normal deviate at the 1% level,
2.6 = normal deviate at the 0.5% level,
1.6 = normal deviate at the 5% level.

In some countries, the national control authority may permit an experienced manufacturer to accumulate data on the qualifying tests of the Types 1 and 2 International preparations for neurovirulence as serial batches of vaccine are tested and released, rather than wait until the data are available from the four qualifying tests before the release of any future vaccine.

In some countries, however, the qualifying test may be required on only two occasions for a seed (Type 3) that has been extensively tested and used.

A neurovirulence test in which the mean Lesion Score for the reference (\(\bar{X}_{\text{ref}}\)) is not compatible with previous experience should not be used for assessing a test vaccine.

If the test is valid, the mean Lesion Score for the test vaccine (\(\bar{X}_{\text{test}}\)) is calculated and compared with that of the homotypic reference vaccine.

It is assumed that the values of the constants \(C_1, C_2,\) and \(C_3\) will be calculated by each laboratory for each reference vaccine. As experience with the reference accumulates, it is recommended that laboratories should review the values of \(\sigma^2\) and \(M\).

It is expected that, for a single test, the analysis recommended above will result in the rejection of approximately 1% of test vaccines that are identical to the homotypic reference, on the assumption that, in each laboratory, the within-test variation is similar to that observed in the qualifying tests with that reference.

Estimates of the probability that test vaccines with a true Lesion Score double that of the reference vaccine will be rejected are given in Table 1 for different coefficients of variation.
Table 1
**Estimated probability that a test vaccine for which the true Lesion Score is double that of the homotypic reference will be rejected**

<table>
<thead>
<tr>
<th>Total number of positive animals per test</th>
<th>Coefficient of variation&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99%</td>
</tr>
<tr>
<td>40</td>
<td>99%</td>
</tr>
</tbody>
</table>

<sup>a</sup> The coefficient of variation is defined as the within-test standard deviation divided by the mean Lesion Score. Boxes show acceptable coefficients of variation.

<sup>b</sup> Divided equally between tests on Type 1 and 2 vaccines.

<sup>c</sup> Corresponds to tests on Type 1 and 2 vaccines.

<sup>d</sup> Corresponds to tests on Type 3 vaccines.

In tests on vaccines which satisfy the above criteria of acceptability, individual animals may occasionally develop extremely high Lesion Scores. Such findings should be taken into consideration in evaluating the acceptability of vaccines, but precise criteria for use in making a decision are difficult to define.

Sample calculations are shown in Tables 2 and 3.
Table 2
Sample calculations of results of qualifying tests of reference vaccines

<table>
<thead>
<tr>
<th>Basic data</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mean Lesion Score ( M ) (initial four tests)</td>
<td>1.110</td>
<td>0.678</td>
<td>1.043</td>
</tr>
<tr>
<td>Within-test pooled variance ( s^2 ) of ( M )</td>
<td>0.444</td>
<td>0.236</td>
<td>0.686</td>
</tr>
<tr>
<td>Within-test pooled standard deviation ( s ) (square root of ( s^2 ))</td>
<td>0.666</td>
<td>0.486</td>
<td>0.828</td>
</tr>
<tr>
<td>Coefficient of variation (CV) ( CV = \frac{s}{M} )</td>
<td>( \frac{0.666}{1.110} = 0.60 )</td>
<td>( \frac{0.486}{0.878} = 0.55 )</td>
<td>( \frac{0.828}{1.043} = 0.79 )</td>
</tr>
<tr>
<td>Upper and lower limits for satisfactory test result. Mean Lesion Score of reference vaccine ( \bar{X}_{ref} )</td>
<td>( M + s = 1.110 + 0.666 ) = 1.776</td>
<td>( M + s = 0.878 + 0.486 ) = 1.364</td>
<td>( M + s = 1.043 + 0.828 ) = 1.871</td>
</tr>
<tr>
<td>( M - s = 1.110 - 0.666 ) = 0.444</td>
<td>( M - s = 0.878 - 0.486 ) = 0.392</td>
<td>( M - s = 1.043 - 0.828 ) = 0.215</td>
<td></td>
</tr>
</tbody>
</table>

Constants for assessing acceptability of difference between mean Lesion Score of test vaccine \( \bar{X}_{test} \) and mean Lesion Score of reference vaccine \( \bar{X}_{ref} \)

\[
\begin{align*}
C_1 &= \frac{2\sqrt{2}}{N_0} = 0.626 \\
C_2 &= \frac{2\sqrt{2}}{N_0} = 0.456 \\
C_3 &= \frac{2\sqrt{2}}{N_0} = 0.516 \\
C_4 &= \frac{2\sqrt{2}}{N_0} = 0.308 \\
\end{align*}
\]

\[
\begin{align*}
M + s &= 1.110 + 0.666 = 1.776 \\
M - s &= 1.110 - 0.666 = 0.444 \\
\end{align*}
\]

\[
\begin{align*}
M + s &= 0.878 + 0.486 = 1.364 \\
M - s &= 0.878 - 0.486 = 0.392 \\
\end{align*}
\]

\[
\begin{align*}
M + s &= 1.043 + 0.828 = 1.871 \\
M - s &= 1.043 - 0.828 = 0.215 \\
\end{align*}
\]

\[
\begin{align*}
\frac{2\sqrt{2}}{N_0} &= 0.626 \\
\frac{2\sqrt{2}}{N_0} &= 0.456 \\
\frac{2\sqrt{2}}{N_0} &= 0.516 \\
\frac{2\sqrt{2}}{N_0} &= 0.308 \\
\end{align*}
\]

\[
\begin{align*}
\frac{2\sqrt{2}}{N_0} &= 0.626 \\
\frac{2\sqrt{2}}{N_0} &= 0.456 \\
\frac{2\sqrt{2}}{N_0} &= 0.516 \\
\frac{2\sqrt{2}}{N_0} &= 0.308 \\
\end{align*}
\]
### Table 3

**Examples of tests with Type 1 reference and vaccine**

<table>
<thead>
<tr>
<th>Example</th>
<th>Mean Lesion Scores</th>
<th>Difference between vaccine and reference</th>
<th>Conclusion/Action*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference ($\bar{X}_{ref}$)</td>
<td>Vaccine ($\bar{X}_{test}$)</td>
<td>($\bar{X}<em>{test} - \bar{X}</em>{ref}$)</td>
</tr>
<tr>
<td>One test</td>
<td>0.826</td>
<td>1.188</td>
<td>0.362</td>
</tr>
<tr>
<td>Two tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.826</td>
<td>1.493</td>
<td>0.667</td>
</tr>
<tr>
<td>2nd</td>
<td>1.285</td>
<td>1.209</td>
<td>-0.076</td>
</tr>
<tr>
<td>Combined tests</td>
<td>1.056</td>
<td>1.351</td>
<td>0.295</td>
</tr>
<tr>
<td>Two tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.826</td>
<td>1.493</td>
<td>0.667</td>
</tr>
<tr>
<td>2nd</td>
<td>1.285</td>
<td>1.405</td>
<td>0.120</td>
</tr>
<tr>
<td>Combined tests</td>
<td>1.056</td>
<td>1.449</td>
<td>0.393</td>
</tr>
</tbody>
</table>

* For values of $C$, see Table 2.
Appendix 4

Form on which to report the score of virus activity for each histological section from all monkeys included in the neurovirulence test

Although the test requires that at least 11 monkeys should be positive after inoculation with the vaccine and reference virus for Types 1 and Types 2, provision is made on the following form (pp. 78-79) for recording results for 12 monkeys that may be inoculated and survive the test. A separate form to record the lesions in 20 monkeys will be required for Type 3. Records for each vaccine and reference preparation must be on separate forms.

On the forms, the method of scoring the lesions used for all sections from all areas is that already indicated on p. 52, namely:

1. Cellular infiltration only.
2. Cellular infiltration with minimal neuronal damage.
3. Cellular infiltration with extensive neuronal damage.
4. Massive neuronal damage with or without cellular infiltration.

A model of the certificate of compliance with the international requirements for the neurovirulence testing of final OPV bulks in monkeys is given below.

Certificate of compliance with the recommendations for the neurovirulence testing of final OPV bulks in monkeys

Final bulk no. ______________________________________________

Date of certification _______________________________________

I certify that the above final bulk complies with the recommendations for tests in monkeys for neurovirulence published in WHO Technical Report Series, No. 904.

Signature ______________________________

Name (typed) ____________________________

Date ________________________________
All data from all monkeys must be recorded, which may require a larger form.
Clinical signs of paralysis must be recorded on a separate form.

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Type</th>
<th>Final bulk no.</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post-inoculation titre: log₁₀ CCID₅₀/ml

<table>
<thead>
<tr>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

Date of inoculation

<table>
<thead>
<tr>
<th>Date of end of test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

### Histological lesions due to poliomyelitis

<table>
<thead>
<tr>
<th>N</th>
<th>V</th>
<th>Lumbar enlargement</th>
<th>Corvical enlargement</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>Av Med 1 2 Cb P M T Co Av</td>
</tr>
<tr>
<td>L</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>R</td>
<td></td>
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<tr>
<td>L</td>
<td>R</td>
<td></td>
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<td></td>
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</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>N</th>
<th>V</th>
<th>Lumbar enlargement</th>
<th>Cervical enlargement</th>
<th>Brain</th>
<th>Lesion Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
<td>Av 1 2 3 4 5 6 7 8 9 10</td>
<td>Av Med</td>
<td>Cb P M T Co Av</td>
</tr>
<tr>
<td>L</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>R</td>
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<td>L</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>R</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

**KEY**

N = Monkey number  
V = Virus titre  
L = Left  
R = Right  
Av = Average  
Med = Medulla  
Cb = Cerebellum  
P = Pons  
M = Midbrain  
T = Thalamus  
Co = Cortex  

Signature ___________________________ Date ___________________________

Name (typed) ___________________________
Appendix 5

Preparation of poliomyelitis vaccine (oral) using cell banks

Example of a flowsheet of tests in cell cultures

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAEM Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pooled fluid</td>
<td>10 ml CL</td>
<td>10 ml HC</td>
<td>10 ml other sensitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoenzyme analysis, immunological tests, or cytogenic marker tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% or 1000 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cell banks shall be characterized according to the appropriate Requirements (1)

Human diploid cells shall be characterized according to the appropriate Requirements (1)

Production 95%

Harvest Cells Neutralized single harvest

10 ml HC

10 ml other sensitive cell system

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 sensitive to measles.

Note. This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the national control 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.

Reference

Appendix 6
Preparation of poliomyelitis vaccine (oral) using monkey kidney-cell cultures

Example of a flowsheet of tests in cell cultures

<table>
<thead>
<tr>
<th>Day 0</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ml MK</td>
<td>(+ serum)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fluid) MK</td>
<td></td>
</tr>
<tr>
<td>10 ml VK</td>
<td>(+ serum)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fluid) VK</td>
<td></td>
</tr>
<tr>
<td>10 ml RK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ml HC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 0 (2–4)</th>
<th>(4–7)</th>
<th>14</th>
<th>19</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cell cultures (25%, but not more than 2.5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled fluid (from group)</td>
<td>HAEM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4% HAEM</td>
<td>Pooled fluid (from group)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ml MK</td>
<td>10 ml VK</td>
<td>10 ml RK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ml VK</td>
<td>10 ml MK</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 0</th>
<th>4</th>
<th>18</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus inoculation</td>
<td>Harvest (14 days after harvest)</td>
<td>(28 days after harvest)</td>
<td></td>
</tr>
<tr>
<td>Neutralized single harvest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ml MK</td>
<td>(+ serum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fluid) MK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ml VK</td>
<td>(+ serum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(fluid) VK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ml HC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Note. This example includes all tests, whether obligatory or not. Since the requirements applicable in a particular place are those authorized by the national control 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.
Appendix 7

**Summary protocol for poliomyelitis vaccine (oral)**

<table>
<thead>
<tr>
<th>Name and address of manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proprietary name of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lot no. of vaccine trivalent blend</th>
</tr>
</thead>
<tbody>
<tr>
<td>-----------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Filling lot no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>-----------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of filled containers</th>
</tr>
</thead>
<tbody>
<tr>
<td>--------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date on which last determination of virus concentration was started</th>
</tr>
</thead>
<tbody>
<tr>
<td>---------------------------------------------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shelf-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expiry date</th>
</tr>
</thead>
<tbody>
<tr>
<td>-------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nature and concentration of stabilizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>----------------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume of vaccine container</th>
</tr>
</thead>
<tbody>
<tr>
<td>-------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume of human dose (in drops and/or ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-------------------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prescribed virus concentration per human dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
</tr>
<tr>
<td>Type 2</td>
</tr>
<tr>
<td>Type 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nature of any antibiotics present in vaccine and amount per human dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>-----------------------------------------------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Production cell tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bulk nos of monovalent bulk suspensions blended in trivalent vaccine</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date of approval of protocol indicating compliance with Requirements for Biological Substances No. 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>-----------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
The following sections are intended for the recording of the results of the tests performed during the production of the vaccine, so that the complete document will provide evidence of consistency of production; thus if any test has to be repeated, this must be indicated. Any abnormal result must be recorded on a separate sheet.

If any cell lot or virus harvest intended for production was rejected during the control testing, this should also be recorded either in the following sections or on a separate sheet.

### Production in cell lines

**Control of source materials**

*Master cell bank*

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin and short history</td>
<td></td>
</tr>
<tr>
<td>Authority that approved cell bank</td>
<td></td>
</tr>
<tr>
<td>Total no. of ampoules stored</td>
<td></td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Passage level (or no. of population doublings) of cell bank</td>
<td></td>
</tr>
<tr>
<td>Storage conditions</td>
<td></td>
</tr>
<tr>
<td>Percentage of total cell-bank ampoules tested</td>
<td></td>
</tr>
<tr>
<td>Growth characteristics</td>
<td></td>
</tr>
<tr>
<td>Morphological characteristics</td>
<td></td>
</tr>
<tr>
<td>Immunological markers</td>
<td></td>
</tr>
<tr>
<td>Cytogenetic data</td>
<td></td>
</tr>
<tr>
<td>Biochemical data</td>
<td></td>
</tr>
<tr>
<td>Results of other identity tests</td>
<td></td>
</tr>
<tr>
<td>Results of tests for adventitious agents</td>
<td></td>
</tr>
<tr>
<td>Results of tests for tumorigenicity (if applicable)</td>
<td></td>
</tr>
</tbody>
</table>
Capacity for interferon production
(if determined)

Viral susceptibility

**Manufacturer's working cell bank (MWCB)**

Date MWCB was established

Quantity of cells stored

Passage level of MWCB

Storage conditions

Percentage of total MWCB
ampoules tested

Results of identity tests

Results of tests for adventitious
agents

Results of tests for tumorigenicity
(if applicable)

**Virus strains**

Reference no. of seed lot

Seed virus strain

Substrate used for preparing seed lot

Date(s) of satisfactory test(s) for
freedom from adventitious agents

Sterility test

Identity test

**Test for consistency of virus characteristics**

Neurovirulence test in monkeys

Result of blood serum test in
monkeys prior to inoculation

Date of inoculation of seed lot

No. and species of monkeys
inoculated: control and test

Quantity (CCID<sub>50</sub>) inoculated in
each test monkey
No. of monkeys surviving (without specific symptoms): control and test

Result of histopathological examination (specify any abnormal findings): control and test

Test in vitro

**Control of vaccine production**

**Control of cell cultures**

Ratio of control to production cell cultures or control cultures as proportion of production cell cultures

Period of observation of cultures

Ratio or proportion of cultures discarded for nonspecific reasons

Results of observation

Tests for haemadsorbing viruses:

  - Methods
  - Results

Tests for adventitious agents:

  - Methods
  - Results

Identity test

**Cell cultures for vaccine production**

Tests for adventitious agents:

  - Methods
  - Results

Tests for bacteria, fungi, and mycoplasmas:

  - Methods
  - Results
**Control of single harvests**

<table>
<thead>
<tr>
<th>Volume harvested</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of sampling</td>
<td></td>
</tr>
<tr>
<td>Tests of neutralized single harvests for adventitious agents:</td>
<td></td>
</tr>
<tr>
<td>Methods</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>Sterility tests:</td>
<td></td>
</tr>
<tr>
<td>Methods</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>Tests for molecular consistency of production:</td>
<td></td>
</tr>
<tr>
<td>Methods</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

**Control of bulk suspension**

<table>
<thead>
<tr>
<th>Date of filtration of bulk</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity of filters used</td>
<td></td>
</tr>
<tr>
<td>Date of sampling</td>
<td></td>
</tr>
<tr>
<td>Identity test:</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>Virus concentration:</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td></td>
</tr>
</tbody>
</table>

**Tests for consistency of virus characteristics**

<table>
<thead>
<tr>
<th>Neurovirulence tests in monkeys:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Species of monkey inoculated</td>
<td></td>
</tr>
<tr>
<td>Dose of vaccine virus injected</td>
<td></td>
</tr>
<tr>
<td>No. of “valid” monkeys inoculated with test sample</td>
<td></td>
</tr>
<tr>
<td>No. of positive monkeys observed</td>
<td></td>
</tr>
<tr>
<td>Reference preparation</td>
<td></td>
</tr>
<tr>
<td>No. of “valid” monkeys inoculated with reference</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>No. of positive monkeys observed</td>
<td></td>
</tr>
<tr>
<td>Mean Lesion Score of test sample</td>
<td></td>
</tr>
<tr>
<td>Mean Lesion Score of reference</td>
<td></td>
</tr>
<tr>
<td>(see also attached forms giving details of histological observations and assessment)(^1)</td>
<td></td>
</tr>
<tr>
<td>Neurovirulence test in transgenic mice</td>
<td></td>
</tr>
<tr>
<td>applicable to type 3):</td>
<td></td>
</tr>
<tr>
<td>Strain of mice inoculated</td>
<td></td>
</tr>
<tr>
<td>For each dose of the bulk sample:</td>
<td></td>
</tr>
<tr>
<td>No. of mice inoculated</td>
<td></td>
</tr>
<tr>
<td>No. of mice excluded from evaluation</td>
<td></td>
</tr>
<tr>
<td>No. of mice paralysed</td>
<td></td>
</tr>
<tr>
<td>For each dose of the reference virus:</td>
<td></td>
</tr>
<tr>
<td>No. of mice inoculated</td>
<td></td>
</tr>
<tr>
<td>No. of mice excluded from evaluation</td>
<td></td>
</tr>
<tr>
<td>No. of mice paralysed</td>
<td></td>
</tr>
<tr>
<td>Results of validity tests:</td>
<td></td>
</tr>
<tr>
<td>Virus assay results for each dose inoculated</td>
<td></td>
</tr>
<tr>
<td>Paralysis rates for reference virus at each dose</td>
<td></td>
</tr>
<tr>
<td>Results:</td>
<td></td>
</tr>
<tr>
<td>Log odds ratio</td>
<td></td>
</tr>
<tr>
<td>L1 and L2 values</td>
<td></td>
</tr>
<tr>
<td>Pass/fail decision</td>
<td></td>
</tr>
<tr>
<td>In vitro rc/40 marker test</td>
<td></td>
</tr>
<tr>
<td>(applicable to types 1 and 2):</td>
<td></td>
</tr>
<tr>
<td>Reduction of titre of bulk sample</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Completed forms in the format given in Appendix 4, pages 77–78, should be attached.
Reduction of titre of negative reference
Reduction of titre of positive reference
Result

MAPREC (applicable to type 3):
Date of test
Ratio of %472C of bulk sample to the International Standard
Result of test of consistency of production
Result of test of comparison with the International Standard

**Final bulk**

<table>
<thead>
<tr>
<th>Preparation of trivalent bulk:</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot no. of trivalent blend</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monovalent bulks in blend</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume in blend</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nature and volume of stabilizer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nature and volume of diluent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total volume of blend</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sterility test:
Date
Media used
Result

**Filling and containers**

| Total volume of final filling |        |        |
| Date of final filling         |        |        |
| No. of vials filled           |        |        |
| Test on final filling         |        |        |

**Control tests on final product**

Identity test:
Method
Result
Tests for bacteria and fungi:

No. of containers examined
Methods
Results

Virus titration:

Identity of reference preparations
Titre of individual virus types
Batch nos of antiserum used in test
Date of test
Results:

Vaccine Reference

Type 1
Type 2
Type 3

Accelerated degradation test:

Method
Result

Production in monkey kidney-cell cultures

Control of vaccine production

Control of monkeys

Monkey species used for production
Quarantine batch no.
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
Results
Production details:

Production monkey no. ____________________________
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 bulk suspension

Tests in rabbits:

No. and weight of animals

Date of inoculation

Results of injection

Quantity injected

Results (survival nos, etc.)

Date of filtration of bulk

Porosity of filters used

Date of sampling

Tests for retroviruses:

Methods

Date

Results

Certification by the manufacturer

Name of head of production (typed)

Certification by 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 satisfies Part A of the Requirements for Biological Substances No. 7, revised 1999, and (if applicable) Part C for poliomyelitis vaccine (oral).

Signature

Name (typed)

Date
Certification by the national control authority

If the vaccine is to be exported, attach a certificate from the national control authority as described in Part B, section 2, a label from a final container, and an instruction leaflet for users.

If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.