

Annex 2

Recommendations for the production and control of poliomyelitis vaccine (inactivated)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 the 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 (inactivated) (IPV) 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. The terms “national control authority” and “national control laboratory”, as used in these Recommendations, always refer to the country in which the vaccine is manufactured.

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

¹ Replaces the 1982 Requirements for Poliomyelitis Vaccine (Inactivated) and Addendum 1987.

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General considerations

Since the Recommendations (formerly Requirements) for Poliomyelitis Vaccine (Inactivated) were last revised in 1981 (1) and amended in 1985 (2), there have been several advances in technology in vaccine production and control that make a further revision of the Recommendations necessary.

At the time of the last revision, the introduction of continuous cells for manufacture of poliomyelitis vaccine (inactivated) (IPV) was a novel development. Since then, the control of products manufactured in continuous cells has been standardized, as reflected in these Recommendations.

The section on production of IPV in primary monkey kidney cells has been deleted in favour of production in continuous cells (Vero), secondary or tertiary monkey kidney cells, or human diploid cells. For vaccines produced in secondary or tertiary monkey kidney cells, a recommendation to use monkeys bred in captivity has been introduced. For these vaccines antibody tests are introduced to exclude animals with antibodies to cercopithecoid herpesvirus 1 (B virus), simian virus 40 (SV40), foamy viruses and simian immunodeficiency virus (SIV) from the production process, and a test for retroviruses is introduced for monovalent pools.

Among the most significant changes in production has been the increasing use of IPV in combination with other vaccines and this introduces considerations that do not apply when IPV is used alone, such as interaction of the poliovirus antigens with other antigens and/or adjuvants. These considerations are not dealt with in the present Recommendations. However, to provide further guidance for control of the vaccine, key tests that may be influenced by other antigens and/or adjuvants in combined vaccines are identified.

As the goal of eradicating poliomyelitis due to wild-type polioviruses is in sight (3), laboratories that use wild-type polioviruses will become an important potential source of accidental reintroduction of such viruses into a community. To minimize this risk, WHO has developed a Global Action Plan that requires increased biosafety containment of wild-type polioviruses (4). In line with the Global Action Plan, these Recommendations introduce increased biosafety containment 1 year after the last wild-type poliovirus is detected, both at the level of production of vaccines using wild-type strains and for the control of such vaccines. It will be important to ensure that the increased biosafety containment levels are applied to all laboratories that need to work with live polioviruses. For example, the tests for bacterial and mycotic sterility on live virus harvests will require the transfer of samples containing live virus from a virology to a bacteriology laboratory. This process should be carried out in such a way as to prevent the escape of live wild-type polioviruses.¹

As an alternative to the use of wild-type polioviruses for production of IPV, recent evidence suggests that production of IPV from the attenuated Sabin poliovirus seed viruses is technically feasible (5–7). New manufacturers are encouraged to explore the production of IPV from the live attenuated polioviruses developed by Sabin as alternative and safer seed viruses than the currently used wild-type viruses. IPV manufacturers that currently use wild-type strains are encouraged to evaluate the potential offered by a Sabin-based IPV versus upgrading production and control facilities to meet the enhanced biosafety requirements.

An *in vivo* potency test is described in these Recommendations in which neutralizing antibodies to each of the three poliovirus types are assayed. The neutralizing antibody test for poliovirus requires the use of live poliovirus. For historical reasons, many laboratories use wild-type strains of poliovirus. The attenuated Sabin vaccine strains of poliovirus, on the other hand, will not require increased laboratory containment until vaccination ceases completely. Therefore, the use

¹ Further detailed guidance is in preparation and will be available from WHO.

of Sabin strains of poliovirus to assay neutralizing antibodies in the *in vivo* test, shown to be suitable for this purpose by a collaborative study (8), is specified. Validation of the use of the Sabin strains by each manufacturer should be provided. It is probable that immunization with poliomyelitis vaccine (oral) should cease at some point in the future, once the disease has been eradicated. After that time the containment levels for use of the Sabin strains for laboratory work will be reviewed. Laboratories are thus encouraged to investigate the use of alternatives to live viruses for assay of poliovirus neutralizing antibodies.

The present Recommendations have been updated with respect to reduction of risk from transmissible spongiform encephalopathies (TSEs).

The tests in animals have been replaced by *in vitro* alternatives wherever possible. For example, the test in monkeys for the detection of virulent virus that may have escaped inactivation and the part it may play in assuring the safety of the vaccine was given as an optional test in the previous Requirements. This has been deleted and replaced by the more sensitive test in cell culture. The cell culture systems that are recommended are considered the gold standard for detection of residual live virus during the inactivation process and laboratories wishing to use other cell cultures will have to demonstrate equivalence with the recommended cells. The use of rabbits for testing for cercopithecoid herpesvirus 1 is replaced by a more sensitive tissue culture test. The general safety (innocuity) test may be deleted for products where validated by historical data and experience, and with the agreement of the national control authority.

In recent investigations the *in vivo* potency assay in rats has been standardized and shown to have advantages over previously described *in vivo* tests for IPV (8). The assay in rats is therefore described in detail and the *in vivo* tests in guinea-pigs and chicks are deleted. The *in vivo* assay should be used to characterize the vaccine after changes in the manufacturing process that may influence the quality of the vaccine, for stability studies of the vaccine, and to establish consistency of production. Some national control authorities may require the potency of each batch of vaccine to be determined by an *in vivo* test. This should be performed at the level of the final bulk.

The development of transgenic mice that express the human poliovirus receptor (TgPVR mice) (9, 10) has led to the development of an immunization/challenge model (11) that may be useful for assessment of vaccine efficacy for new strains. This test is not proposed for lot release. Any work with transgenic mice should comply with WHO guidelines (12).

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 *International name and proper name*

The international name should be “*Vaccinum poliomyelitis inactivatum*”. The proper name should be the equivalent of the international name in the language of the country of origin.

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

A.1.2 *Descriptive definition*

Vaccinum poliomyelitis inactivatum should consist of an aqueous suspension of poliovirus types 1–3 grown in cell cultures, concentrated, purified and inactivated. The preparation should satisfy all the recommendations formulated below.

A.1.3 *International standards or reference preparations and International Units*

The first International Reference Preparation of Poliomyelitis Vaccine (Inactivated) (established in 1994) is stored frozen in ampoules containing 1 ml of trivalent inactivated poliomyelitis vaccine (13). This reference preparation is intended for the calibration of secondary reference preparations of poliomyelitis vaccines (inactivated), which in turn are used for determination of relative potencies. The preparation may be used in both D antigen and immunogenicity assays.

The second International Standard for Anti-poliovirus Serum (types 1, 2, 3) (established in 1991) is stored in ampoules, each containing dried human serum with antibodies to all three poliovirus serotypes (14). This standard is intended for calibration of national standards for anti-poliovirus sera.

The above standard and reference preparation are available from the National Institute for Biological Standards and Control, Potters Bar, Herts., England. Samples are distributed free of charge on request to national control laboratories.

A.1.4 *Terminology*

The following definitions are given for the purposes of these recommendations only.

Cell bank: A collection of ampoules containing material of uniform composition derived from a single pool of cells and stored under defined conditions.

Cell seed: A quantity of well-characterized cells derived from a single tissue or cell of human or animal origin and stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which may be used for the production of a master cell bank.

Master cell bank: A quantity of fully characterized cells of human or animal origin derived from the cell seed and stored frozen in liquid nitrogen in aliquots of uniform composition, one or more of which may be used for the production of a manufacturer's working cell bank. The testing performed on a replacement master cell bank (derived from the same clone or from an existing master or working cell bank) is the same as for the initial master cell bank, unless a justified exception is made.

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 kidney tissue used for the production of IPV.

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

Original seed viruses: The monovalent seed viruses from which a vaccine was prepared according to defined specifications and which, after inactivation, was shown on administration to humans in field trials to be suitably safe and immunogenic by induction of neutralizing antibodies.

Virus master seed lot: A quantity of virus of uniform composition derived from the original seed virus 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 not more than two passages 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.

Monovalent pool: A virus suspension of a single virus type processed at the same time.

Purified monovalent pool: A concentrated and purified virus suspension of a single virus type processed at the same time.

Trivalent bulk: A pool of a number of inactivated monovalent pools and containing all three virus types.

Final bulk: The finished biological preparation present in the container from which the final containers are filled.

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.

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

Closed colony: A group of animals sharing a common environment and having their own caretakers who have no contact with other animal colonies. Each animal is tested repeatedly to ensure freedom from specified pathogens and their antibodies. New animals are not admitted to the colony until they have been shown to be free from these specified pathogens.

A.2 **General manufacturing recommendations**

The general manufacturing requirements contained in Good Manufacturing Practices for Biological Products (15) should apply to establishments manufacturing IPV, with the addition of the following:

Production establishments that manufacture IPV should comply with the current version of the WHO Global Action Plan for Laboratory Containment of Wild Polioviruses (4) in both the production and quality control departments.

Recent evidence suggests that production of IPV from the attenuated Sabin poliovirus seed viruses is technically feasible (5–7). New manufacturers are encouraged to explore the production of IPV from the live attenuated polioviruses developed by Sabin as alternative and safer seed viruses than the currently used wild-type viruses. IPV manufacturers that currently use wild-type strains are encouraged to evaluate the potential offered by a Sabin-based IPV versus upgrading production and control facilities to meet enhanced biosafety requirements.

The staff involved in the production and quality control of IPV should be shown to be immune to all three poliomyelitis viruses.

Consideration should be given to regular assessments of serum and mucosal immunity, and freedom from shedding of polioviruses, in all staff involved in the production and quality control of IPV.

A.3 Control of vaccine production¹

A.3.1 Control of source materials

A.3.1.1 Virus strains and seed lot system

Strains of poliovirus used in the production of IPV should be identified by historical records, which should include information on their origin, by infectivity tests and by immunological methods. Only virus strains that are approved by the national control authority and that yield a vaccine meeting the Recommendations set out in the present document should be used.

Vaccine production should be based on the virus seed lot system. The virus working seed lot used for the production of vaccine should not have passed more than 10 subcultures, counted from the original seed virus approved by the national control authority and on which the original laboratory and field tests were done.

Each virus working seed lot used for the production of vaccine batches should be subjected to all tests applicable to a single harvest (in section A.3.2 and certain tests in section A.3.4 (A.3.4.1, A.3.4.2, A.3.4.4 and A.3.4.5)) plus the test for sterility according to the requirements given in sections A.5.1–A.5.3 of the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16). The working seed lot should also be tested in human diploid cells after neutralization with serum (as described in section A.3.4.1) in accordance with the 1998 Requirements for the use of cell substrates (17). The virus working seed lot used for the production of vaccine batches should be free from detectable SV40 sequences as determined by a validated nucleic acid amplification test.

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 for amplification, and to use one 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.

Each virus working seed lot should have been derived from materials that comply with the Recommendations made in sections A.3.1.2–A.3.1.4 and should be approved by the national control authority.

A.3.1.2 Master cell bank and manufacturer's working cell bank

The use of a cell line for the manufacture of IPV should be based on the cell bank system. The master cell bank should be approved by

¹ Information on the production and testing of poliomyelitis vaccine (inactivated) should be summarized in a protocol (an example is given in the Appendix).

the national control authority. The maximum number of passages (or population doublings) by which the MWCB is derived from the master cell bank and the maximum number of passages of the production cultures 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 forty-seventh report of the WHO Expert Committee on Biological Standardization (17).¹

A.3.1.2.1 Identity test

The master cell bank should be characterized according to the requirements for continuous cell lines used for production of biologicals or those relating to human diploid cells (17), 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 *Monkey cell cultures for virus production*

If virus is produced in monkey kidney-cell cultures, animals of a species approved by the national control authority, in good health and not previously used for experimental purposes, should be used. The animals should be bred in captivity.

All procedures on monkeys should be carried out in accordance with relevant national animal care regulations.

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. 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.

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 illnesses among employees and for ensuring that all injuries are properly treated. No worker who has cuts or abrasions on exposed areas of the

¹ Available to manufacturers on application to the Coordinator, Quality Assurance and Safety: Biologicals, World Health Organization, 1211 Geneva 27, Switzerland.

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.

The monkeys should be shown to be free from antibodies to cercopithecid herpesvirus 1 (B virus), SV40 virus, foamy viruses and SIV.

It is desirable that kidney-cell cultures are derived from monkeys shown to be free from antibodies to a range of other potential adventitious agents of monkeys. In one country, monkeys are tested for antibodies to monkey polyoma virus, measles, mumps and rubella.

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

If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it should not be used, 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 monkey kidney cells are passaged in series. The national control authority should establish the number of cell doublings permitted before the cell cultures are used for the growth of the poliomyelitis virus.

It has been shown that a "primary" cell culture may have undergone about five cell doublings, "secondary" cells about 10 cell doublings, and "tertiary" cells about 15 doublings. Records of the cell doublings should be kept.

Experience with tertiary monkey kidney cells shows that the cells at the production level do not exhibit evidence of tumorigenicity. A test for tumorigenicity (17) is not therefore required for previously validated manufacturing processes unless significant changes are made to the cell culture procedures.

In some countries karyology is also required. The extent of such tests should be determined by the national control authority.

A.3.1.4 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 sections A.5.2 and A.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 1 of the Recommendations for the Production and Control of Poliomyelitis Vaccine (Oral) (18).

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, endotoxin and poliovirus inhibitors.

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 Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies (19).

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 (20), as well as current guidelines in relation to human transmissible encephalopathies (19).

Manufacturers are encouraged to explore the possibilities of using serum-free media for production of IPV.

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

Other antibiotics may be used at any stage of 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 at 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 parvoviruses appropriate to the species of animals used. 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 (19).

A.3.2 Control cell cultures

A cell sample equivalent to at least 500ml of the cell suspension, at the concentration employed for seeding vaccine production cultures, should be used to prepare control cultures.

In countries with the technology for large-scale production of vaccine, the national control authority should determine the size of the cell sample to be examined, the time at which it should be taken from the production culture, and the appropriate control vessels.

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 evidence of degeneration caused by an extraneous agent. If continuous cell lines or human diploid cells are used, the control cells should also be tested as described in sections A.3.2.1, A.3.2.2 and A.3.2.5. If subcultured monkey cells are used, the control cells and the supernatant fluid from such cells should also be tested for extraneous agents by the tests described in sections A.3.2.1–A.3.2.4. If the examination for extraneous agents, 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.

Samples not tested immediately should be stored at -60°C or below.

A.3.2.1 Tests for haemadsorbing viruses

At the end of the observation period or at the time the virus is harvested from the production cultures, whichever is the later, at least 25% of the control cells should be tested for the presence of haemadsorbing viruses using guinea-pig red blood cells. If the latter cells have been stored, the duration of storage should not have exceeded 7 days and the storage temperature should have been in the range of $2-8^{\circ}\text{C}$. 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 $0-4^{\circ}\text{C}$ and again after a further incubation for 30 minutes at $20-25^{\circ}\text{C}$.

If a test with monkey red cells is performed, readings should also be taken after a final incubation for 30 minutes at $34-37^{\circ}\text{C}$.

In some countries the sensitivity of each new batch of red blood cells is demonstrated by titration against a haemagglutinin antigen before use in the test for haemadsorbing viruses.

A.3.2.2 Tests for other adventitious agents

At the end of the observation period a sample of the pooled supernatant fluid from each group of control cultures should be tested for other adventitious agents. For this purpose, at least 10 ml of each pool should be tested in the same cells, but not the same batch of cells, as

those used for the production of virus, and additional samples of at least 10ml of each pool should be tested in both human and simian cells.

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.

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

A.3.2.3 Tests in rabbit kidney cell cultures

A sample of at least 10ml of the pooled supernatant fluid from each group of control cultures 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 B virus inhibitors using herpes simplex virus as an indicator 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.

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

For the test 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. The sensitivity of each batch of rabbit kidney cells should be demonstrated by challenge with a validated amount of herpes simplex virus. The challenge test should be approved by the national control authority.

If the presence of B virus is demonstrated, the manufacture of IPV 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 reappearance of the infection, and then only with the approval of the national control authority.

A.3.2.4 Test in Cercopithecus kidney cell cultures

A sample of at least 10ml of the pooled supernatant fluid from each group of control cultures should be tested for the presence of SV40

virus and other extraneous agents by inoculation on to cell cultures prepared from the kidneys of *Cercopithecus* monkeys by the method described in section A.3.2.3.

The inoculated cultures should be incubated at 37°C and observed for a period of at least 4 weeks. A subculture should be made after 2 weeks and observed for 2 weeks.

A cell culture shown to be equally sensitive to SV40 virus may be used.

For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the observation period. The sensitivity of each batch of *Cercopithecus* kidney cells used should be demonstrated by challenge with a validated amount of SV40 virus. The challenge test should be approved by the national control authority.

If there is any cytopathogenic effect attributable to the supernatant fluid, the virus grown on the same batch of cells should not be used for vaccine production.

A.3.2.5 Identity tests

For vaccines produced in continuous cells or in human diploid cells, the control 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.3.3 Production precautions

The general production precautions called for by Good Manufacturing Practices for Biological Products (15) should apply to the manufacture of IPV. For vaccines prepared using wild-type poliovirus, the current version of the WHO Guidelines for the Safe Production and Quality Control of IPV manufactured from wild polioviruses should also be implemented.¹

If animal serum is used for the growth of cell cultures, the serum protein concentration in the final vaccine should be no more than 50ng/ml. The serum protein concentration should be reduced to this level by rinsing the cell cultures with serum-free medium and/or purification of the virus harvests.

In some countries, control tests are carried out to detect the residual animal serum content in the final vaccine (see section A.5.5).

¹ Editorial Note: These Guidelines were established by the 2003 Expert Committee on Biological Standardisation and are available from WHO.

A.3.4 **Control of single harvests and monovalent pools**

Samples that are not tested immediately should be stored at -60°C or below.

A.3.4.1 *Test in Cercopithecus kidney cell cultures*

Applies to all seeds, irrespective of the cells in which they were prepared, and to all harvests grown in monkey kidney cells, except for continuous cell lines. A sample of at least 40 ml of each single harvest produced in tertiary monkey kidney-cell cultures should be tested for the presence of SV40 virus or other adventitious agents. The single harvest should be neutralized by a high-titred antiserum against the specific type of poliovirus.

The Sabin strains may be used as immunizing antigen. The immunizing antigen used for the preparation of the antiserum should not be the same as the production seed.

The immunizing antigen should be shown to be free from extraneous agents and grown in cell cultures free from extraneous microbial agents that might elicit antibodies that could inhibit the growth of any adventitious agents present in the single harvest.

The sample should be tested in primary *Cercopithecus* kidney cell cultures or cells that have been demonstrated to be of equal susceptibility to SV40 virus. The tissue cultures should be incubated at 37°C and observed for 2 weeks. At the end of this observation period, at least one subculture of supernatant fluid should be made in the same tissue culture system. The sample should be inoculated in such a way that the dilution of the supernatant fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm^2 per ml of supernatant fluid. At least one bottle of the cell cultures should remain uninoculated and should serve as a control.

The inoculated and control cultures should be incubated at 37°C and observed for an additional 2 weeks.

If necessary, serum may be added to the primary cultures at this stage, provided that the serum does not contain SV40 antibody or other inhibitors.

The single harvest passes the test if there is no evidence of the presence of SV40 virus or other adventitious agents attributable to the single harvest. For the test to be valid, not more than 20% of the culture vessels should have been discarded for nonspecific, accidental reasons by the end of the observation period.

A.3.4.2 *Test in primary rabbit kidney cells*

Applies to all seeds, irrespective of the cells in which they were prepared, and to all harvests grown in monkey kidney cells, except for continuous cell lines. A sample of at least 40 ml of each single harvest should be tested in primary rabbit kidney cells as described in section A.3.2.3.

A.3.4.3 Sterility test

Each single harvest should be tested for sterility according to the requirements given in sections A.5.1–A.5.3 of the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16).

If this test is done outside the production facilities, then adequate containment procedures (4) should be used if wild-type polioviruses are used to produce the vaccine.

A.3.4.4 Purification of monovalent pools

Each monovalent pool of virus should be purified before inactivation.

An acceptable method is to clarify the virus suspension by filtration, to concentrate the virus by ultrafiltration and, thereafter, collect the virus peak after passing it through a gel-filtration column. Further purification is achieved by passing the virus through an ion-exchange column. Other purification procedures, such as passing the preparation through an immobilized DNA-ase column, may be used.

The purified monovalent pool should be shown to contain not more than 0.1 µg of protein per D-antigen unit of poliomyelitis virus.

For viruses grown in continuous cells the purified monovalent pool should be tested for residual cellular DNA. By calculation the purification process should be shown to reduce consistently the level of cellular DNA to less than 10ng per human dose. This test may be omitted, with the agreement of the national control authority, if the manufacturing process is validated to achieve this specification.

A.3.4.5 Filtration before inactivation

Each purified monovalent pool should be filtered before inactivation.

The importance of filtration or clarification of the crude virus suspensions as a means of improving the regularity of the inactivation process has been clearly established. Generally, filters are used in series or filtration is performed stepwise through filters of decreasing porosity. Satisfactory results have been reported with several filter types but a final filtration using a 0.22-µm filter should be used.

Filters containing asbestos should not be used.

Inactivation should be initiated as soon as possible and not later than 72 h after filtration.

It is preferable to start inactivation within 24 h of filtration. Since the purpose of the filtration step is to remove particulate matter and other interfering substances that may diminish the effectiveness of the inactivation process, and since aggregates tend to increase on standing after filtration, efforts should be made to keep within this time limit.

A.3.4.6 Identity test

The poliovirus in the filtered purified monovalent pool should be tested for identity by neutralization with specific antiserum.

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.3.4.7 Virus titration

Before any inactivating agent is added, a sample should be taken of each filtered purified monovalent pool for titration of infective polio-virus using tissue culture methods. This titration should be carried out in not more than 10-fold dilution steps and using 10 cultures per dilution, or any other arrangement yielding equal precision.

Each filtered purified monovalent pool should show a titre of not less than that known to yield inactivated vaccine of suitable potency.

The use of Hep-2C cells in microtitre plates is suitable for this purpose (18). The same cells should be used for virus titrations before and after the inactivation process.

The main purposes of determining the titre of virus pools destined for inactivation are to provide the starting titre to monitor the kinetics of inactivation and to select pools that can be expected to meet potency requirements after inactivation.

A.3.4.8 Test for retroviruses

For vaccines produced in secondary or tertiary monkey kidney cells, test samples from the filtered purified monovalent pool 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 (21), but the results need to be interpreted with caution because RTase activity may be derived from sources other than retroviruses, such as retrovirus-like elements which do not represent a complete genome. Nucleic acid amplification tests for retroviruses may also be used.

The test for retroviruses is not required if the closed monkey colony used as the source of kidneys is certified to be free of retroviruses.

A.3.4.9 Inactivation procedure

The virus in the filtered purified monovalent pools should be inactivated through the use of a method approved by the national control authority. The method of inactivation should be shown to give consistent inactivation for the production of acceptable vaccine. A record of consistency should be established by the production of five consecutive lots and if broken a further five monovalent lots should be prepared and shown to be satisfactory for re-establishing this record.

The progress of inactivation should be followed by suitably spaced determinations of virus titres. The inactivation period should exceed the time taken to reduce the titre of live virus to undetectable amounts by a factor of at least 2.

Formaldehyde has been used for over 40 years as the inactivating agent in the production of IPV. Most manufacturers have encountered some

irregularities in the inactivation process that have not been fully explained. On the basis of the observation that a decrease in the concentration of free formaldehyde occurs in the course of the process, it has been recommended that tests for free formaldehyde should be performed at intervals and the concentration maintained at the desired level by, if necessary, intermittent readjustments.

A second filtration during the process of inactivation should be made.

This step is made after the virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

A.3.4.10 Test for effective inactivation

Two samples of a volume equivalent to at least 1500 human doses of each monovalent pool should be taken and, after removal or neutralization of the inactivating agent, should be tested by inoculation into tissue cultures for the absence of infective poliovirus. Kidney cells from some monkey species, for instance those of the genera *Macaca*, *Cercopithecus* and *Papio*, appear to be more sensitive than others. If other tissue culture systems are used, they should have been shown to possess at least the same sensitivity as those specified above.

The kinetics of inactivation should be established by each manufacturer and approved by the national control authority. One sample should be taken at the end of the inactivation period and the other not later than three-quarters of the way through this period. When primary monkey kidney cells are used for this test, the two samples should be inoculated into bottles of tissue cultures derived from different batches of cells. The dilution of the sample in the nutrient fluid should not exceed 1 in 4 and the area of the cell sheet should be at least 3cm² per ml of sample. One or more bottles of each batch of cultures should be set aside to serve as uninoculated control bottles with the same medium.

The formaldehyde in samples of vaccine for tissue culture tests is generally neutralized at the time of sampling by the addition of bisulfite. Usually, the samples are subsequently dialysed.

It is possible to conduct tissue culture tests on nondialysed material; however, this is often found to be toxic to cells, even with a dilution of 1 in 4. If in such tests nonspecific degeneration of cells occurs, or if the sensitivity of the tissue culture system is reduced, the test should be repeated on dialysed material.

In some countries this test is performed using tissue cultures that are also sensitive to SV40 virus, as an additional measure for detecting this extraneous agent.

The tissue culture bottles should be observed for at least 3 weeks. Not less than two subcultures should be made from each original bottle, one at the end of the observation period and the other 1 week earlier. The subcultures should be observed for at least 2 weeks.

If infectious poliovirus is isolated, the monovalent pool should not be used. The isolation of active poliovirus from a monovalent pool must be regarded as a break in the consistency record.

If primary monkey kidney cells are used in this test, they may contain adventitious agents that could interfere with the test result. It is important to demonstrate that each test retains sensitivity to detect partially inactivated polioviruses.

At the end of the observation period, the cell culture used for the detection of residual live virus should be challenged with a validated amount of live Sabin virus of the same type as that of the monovalent pool. The details of the challenge procedure should be approved by the national control authority.

The problem of detecting residual active poliovirus in a vaccine is not the same as that of measuring infective virus in untreated suspensions. Poliovirus that has been exposed to the action of formaldehyde without becoming inactivated has been shown to require a much longer time to produce cytopathogenic changes than does untreated virus. For this reason it is desirable that tissue cultures in tests for the presence of residual active virus be observed for as long a time as is technically possible. A satisfactory tissue culture system for this purpose therefore depends not only on the sensitivity of the cells used for the preparation of the cultures but also on the nutrient fluid.

The serum added to the nutrient fluid should be tested for inhibitors to poliovirus. Only serum free from inhibitors to all three types of poliovirus should be used.

Maintenance of the cultures in good condition may require frequent changes of culture medium. However, it should be borne in mind that by early changes of fluid unadsorbed virus might be removed and the validity of the test thus impaired; therefore, the fluid should be changed no earlier than 5–7 days after inoculation.

A.3.4.11 Sterility test

Each purified monovalent pool should be tested for sterility after inactivation, according to the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16).

A.3.5 Control of trivalent bulk

Only those inactivated monovalent pools that have been shown to be satisfactory should be blended to form a trivalent bulk.

A.3.5.1 Test for absence of infective poliovirus

A sample of at least 1500 ml or, if purified and concentrated vaccine is prepared, the equivalent of at least 1500 doses of each trivalent bulk should be tested in cell cultures for the absence of infective poliovirus by the procedure described in section A.3.4.10 of these Recommendations. If infective poliovirus is isolated, this batch of trivalent bulk product should not be used.

A.3.5.2 Sterility test

The trivalent bulk should be tested for sterility according to the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16).

A.3.5.3 Residual formaldehyde

The content of free residual formaldehyde in the trivalent bulk should be determined by a method approved by the national control authority. The limits should be approved by the national control authority.

A.3.6 Control of final bulk

Preservatives or other substances that might be added to or combined with the trivalent bulk to form the final bulk should have been shown to have no deleterious effect on the immunizing potency of the polio-virus antigens.

A.3.6.1 Sterility test

The final bulk should be tested for sterility according to the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16).

A.3.6.2 Potency tests

Each final bulk should be tested for immunizing potency by tests approved by the national control authority. Such tests should include an *in vitro* assay for antigen content and may include an *in vivo* assay for immune response. In both tests the results obtained with the test sample should be compared with those obtained with a reference preparation calibrated by comparison with the WHO International Reference Preparation (see section A.1.3).

The *in vitro* assay that has been found most suitable for measuring the antigen content is the D-antigen enzyme-linked immunosorbent assay (ELISA). Although this assay is widely used, particular attention is required for its standardization. Some national control authorities accept the use of polyclonal antisera whereas others accept the use of monoclonal antibodies in the test. The D-antigen specificity of the antibodies should be demonstrated. Whichever types of antisera are used, the validation studies should show that the assay can determine consistency of production.

If IPV is formulated with other antigens into a combination vaccine, then the suitability of performing the D-antigen ELISA on the final bulk will have to be determined. If the D-antigen ELISA is not suitable for a particular combination, an *in vivo* assay should be used.

A suitable *in vivo* assay method consists of intramuscular injection into the hind limb(s) of rats of four dilutions of the vaccine to be examined and a reference vaccine, using for each dilution a group of not fewer than 10 rats of a suitable strain, and which are specific pathogen-free. The number of dilutions used and the number of animals used may be different from that specified here, provided that any alternative scheme gives the same sensitivity in the test. The weight of the individual animals should not vary by

more than 20% from the group mean. An inoculum of 0.5 ml is used per rat. The dose range is chosen such that a dose response to all three poliovirus types is obtained. The animals are bled after 20–22 days. Neutralizing titres against all three poliovirus types are measured separately using 100 CCID₅₀ of the Sabin strains as challenge viruses, Vero or Hep-2C as indicator cells, and neutralization conditions of 3 h at 35–37°C followed by 18 h at 2–8°C. Results should be read after fixation and staining after 7 days of incubation at 35°C. For the antibody assay to be valid, the titre of each challenge virus must be shown to be within the range of 30–300 CCID₅₀ and the neutralizing antibody titre of a control serum must be within two 2-fold dilutions of the geometric mean titre of the serum. The potency is calculated by comparison of the proportion of animals defined as responders to the test vaccine and the reference vaccine by the probit method. To define an animal as a responder, it is necessary to establish a cut-off neutralizing antibody titre for each poliovirus type. Owing to between-laboratory variation, it is not possible to define cut-off values that could be applied by all laboratories. Rather, the cut-off values should be determined by each laboratory, based on a minimum series of three tests with the reference vaccine. The mid-point on a log₂ scale of the minimum and maximum geometric mean titres of the series of three or more tests is used as the cut-off value. For each of the three poliovirus types, the potency of the vaccine should not be statistically significantly less than that of the reference preparation. The test is not valid unless:

- the median effective dose (ED₅₀) for both the test and reference vaccines lies between the smallest and the largest doses given to the animals;
- the statistical analysis shows no significant deviation from linearity or parallelism;
- the fiducial limits of the estimated relative potency fall between 25% and 400% of the estimated potency.

Laboratories that have established the parallel line method of analysis of antibody titres for the rat test may use it instead of converting titres to proportions of responders as in the probit method of analysis.

Laboratories are encouraged to validate alternatives to the neutralizing antibody test to reduce the use of live polioviruses in laboratories.

If IPV is formulated with other antigens into a combination vaccine, then the suitability of performing the rat immunogenicity test will have to be determined.

If the immunogenicity test is performed, the potency of the final bulk for each virus type should be approved by the national control authority.

A.3.6.3 Preservative content

If preservative is added, the content in the final bulk (or final lot) should be determined by a method approved by the national control authority. The preservative used and content permitted should be approved by the national control authority.

A.3.6.4 Endotoxin content

The endotoxin content in the final bulk (or final lot) should be determined by a method approved by the national control authority. The endotoxin limit should be approved by the national control authority.

A.4 **Filling and containers**

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (15) should apply.

Single- and multiple-dose containers may be used.

A.5 **Control tests on final product¹**

A.5.1 ***Identity test***

An identity test should be done on at least one labelled container from each filling lot by an appropriate method.

The potency test described in section A.5.4 of these Recommendations may serve as the identity test.

A.5.2 ***Sterility test***

Each filling lot should be tested for sterility according to the 1973 General Requirements for the Sterility of Biological Substances, as amended in 1995 (16).

A.5.3 ***General safety (innocuity) test***

Each filling lot should be tested for unexpected toxicity (sometimes called abnormal toxicity) using a general safety (innocuity) test approved by the national control authority.

This test may be omitted for routine release of filling lots if consistency of production has been well established to the satisfaction of the national control authority and good manufacturing practices are in place. Each lot, if tested, should pass a test for abnormal toxicity.

A.5.4 ***Potency test***

Each filling lot should be tested by an in vitro assay for antigen content. The results obtained with the filling lot should be compared with those obtained with a reference vaccine calibrated by comparison with the WHO International Reference Preparation (see section A.1.3).

The D-antigen ELISA is sufficiently sensitive to measure the antigen content of the final vaccine. Although this assay is widely used, particular attention is required for its standardization. Some national control authorities accept the use of polyclonal antisera, whereas others accept the use of monoclonal antibodies in the test. The D-antigen specificity of the antibodies should be demonstrated. Whichever types of antisera are used, the validation studies should show that the assay can determine consistency of production. For D-antigen ELISAs to be valid, they should comply with specified criteria of linearity and parallelism. The effect of a change in the method of calculation of the D-antigen content on registered specifications should also be taken into account.

¹ Information on the production and testing of poliomyelitis vaccine (inactivated) should be summarized in a protocol (an example is given in the Appendix).

In general, vaccines manufactured from wild-type poliovirus strains that have been formulated to contain 40, 8 and 32 D-antigen units or more per dose for types 1, 2 and 3, respectively, are effective (22). Vaccines with lower D-antigen contents may be acceptable, where supported by clinical data. Vaccines produced from other seed viruses (e.g. Sabin viruses) may also be licensed with a different antigenic composition.

If IPV is formulated with other antigens into a combination vaccine, then the suitability of performing the D-antigen ELISA on the final lot will have to be determined. If the D-antigen ELISA is not suitable for a particular combination, an in vivo assay such as that described in A.3.6.2 should be used.

The potency of the vaccines for each virus type should be approved by the national control authority.

A.5.5 *Protein content*

Poliomyelitis vaccine (inactivated) should not contain more than 10µg of protein per human dose.

In some countries in vitro tests for residual serum proteins are included.

A.5.6 *Preservative content*

Where appropriate, the preservative content of each filling lot should be determined by a method approved by the national control authority, if this has not been done for the final bulk. The method used and content permitted should be approved by the national control authority.

A.5.7 *Endotoxin content*

The endotoxin content of each filling lot should be determined by a method approved by the national control authority, if this has not been done for the final bulk. The content permitted should be approved by the national control authority.

A.5.8 *Test for residual formaldehyde*

The content of free residual formaldehyde in each filling lot should be determined by a method approved by the national control authority, if this has not been done for the trivalent bulk. The limit should be approved by the national control authority.

A.5.9 *Test for pH*

The pH of each filling lot should be determined and be within limits approved by the national control authority.

A.6 *Records*

The requirements given in Good Manufacturing Practices for Biological Products (15) should apply.

A.7 **Samples**

The requirements given in Good Manufacturing Practices for Biological Products (15) should apply.

A.8 **Labelling**

The requirements given in Good Manufacturing Practices for Biological Products (15) 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 cell substrate used for the preparation of vaccine;
- the D-antigen content of each poliovirus type;
- the method used for inactivating the virus;
- the nature and amount of any stabilizer and preservative present in the vaccine.

A.9 **Distribution and shipping**

The requirements given in Good Manufacturing Practices for Biological Products (15) should apply.

A.10 **Stability testing, storage and expiry date**

A.10.1 **Stability testing**

Adequate stability studies form an essential part of vaccine development. The stability of the vaccine in its final containers, maintained at the recommended storage temperature up to the expiry date, should be demonstrated to the satisfaction of the national control authority.

As a guide, containers from at least three consecutive final lots, and derived from different antigen production lots, may be tested.

A.10.2 **Storage and expiry date**

The statements concerning storage temperature and expiry date appearing on the label and the leaflet, as recommended in Good Manufacturing Practices for Biological Products (15), should be based on experimental evidence and should be submitted for approval to the national control authority.

Poliomyelitis vaccine (inactivated) should be stored at all times at a temperature between 2°C and 8°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 approved antigenic content 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 should be specified.

The date of the potency test may be used to determine the start of the shelf-life period. Where an in vivo potency test is used, the date of the potency test is the date on which the test animals were inoculated with the final bulk. Other defined events may be used to specify the start of the shelf-life period with the approval of the national control authority.

Part B. Recommendations for national control authorities

B.1 General

The general requirements for control laboratories given in Guidelines for National Authorities on Quality Assurance for Biological Products (23) should apply, with the addition of the following:

- The national control authority should approve the strains used.
- The national control authority should approve the cell substrate used.
- The national control authority should specify the potency requirements.
- The national control authority should approve the challenge tests used to validate the sensitivity of tests for adventitious agents and to detect residual live virus in inactivated samples.

The national control authority should be satisfied that the results of all tests, including those done on monovalent pools during the process of manufacture, are satisfactory and that consistency has been established.

National control authorities should progressively move towards implementation of a quality system so that, in time, all tests carried out by the national control authority are performed under a suitable quality assurance system that is based on ISO 17025 and is fully documented and maintained.

B.2 Release and certification

A vaccine lot should be released only if it fulfils Part A of the present Recommendations.

A statement signed by the appropriate official of the national control laboratory should be provided if requested by a manufacturing establishment, and should certify whether or not the vaccine lot 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 potency test, 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 IPV between countries.

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Appendix

Summary protocol for the production and testing of poliomyelitis vaccine (inactivated)

Identification of final lot

Name and address of manufacturer _____

Lot no. of final product _____
Date of manufacture of final lot _____
(namely, date of last potency test) _____
Expiry date _____
Total volume of final lot _____

Information on source materials

Virus seed lots

Type (1, 2 or 3)^a _____
Strain used _____
Origin and source of strain _____
Authority that approved virus seed _____
No. of subcultures between primary
seed lot and production _____

Cell cultures (complete only the relevant part, i.e. A, B or C)

Type of cell cultures used _____

A. Continuous cell cultures
Origin and short history of cell seed _____
Authority that approved cell seed _____
Amount of cell culture inoculated _____
Amount of control cell culture
investigated _____
Tests performed on control cultures _____
Result _____

^a A separate protocol should be completed for each type.

B. Monkey secondary or tertiary cell cultures

Type of cells (kidney, fetal kidney, etc.)

Species of monkey from which the cells originated

Controls performed on animals from which the cells originated

No. of cell doublings

Result

Amount of cell culture inoculated

Amount of control cell culture investigated

Tests performed on control cultures

Results

C. Human diploid cell cultures

Origin and short history of cell seed

Authority that approved cell seed

Amount of cell culture inoculated

Amount of control cell culture investigated

Tests performed on control cultures

Results

Serum for cell cultures

Origin of serum used

Tests performed on serum

Results

Information on production

Virus type (1, 2 or 3)^a

Single harvests and monovalent pools

Type of cells used

Date of inoculation of cells with virus

^a A separate protocol should be completed for each type.

Date of virus harvest	_____
Special observations on single harvests	_____
Date of pooling	_____
<i>Tests on pooled single harvests</i>	
Tests in <i>Cercopithecus</i> cell cultures:	
Method used	_____
Results	_____
Test in rabbit kidney cell cultures:	
No. of cell cultures	_____
Total volume inoculated	_____
Period of observation	_____
Result	_____
<i>Sterility test</i>	
Method used	_____
Result	_____
<i>Tests on control cells</i>	
Test for extraneous agents:	
Method used	_____
Result	_____
Test for haemadsorbing agents:	
Method used	_____
Result	_____
Tests in <i>Cercopithecus</i> cell cultures (if applicable):	
Method used	_____
Results	_____
Test in rabbit kidney cell cultures (if applicable):	
No. of cell cultures	_____
Total volume inoculated	_____
Period of observation	_____
Result	_____

*Monovalent product before
inactivation:*

Details of filtration and/or
clarification and/or purification
(if applied)

Date

Tests on monovalent pool

Identity test:

Method

Result

Virus titration:

TCID₅₀ per ml

Details on filtration and/or
clarification and/or purification
(if applied)

Date(s)

Test for retroviruses (if applicable):

Method

Result

Inactivation of monovalent product:

Agent(s) and concentration

Date of start of inactivation

Date of taking first sample

Date of completion of inactivation

Test for effective inactivation (after
removal/neutralization of inactivating agent):

Sample size tested

Date of first sample

Date of second sample

Details of testing procedure

Period of observation of cell
cultures

Period of observation of subcultures

Result	_____
Result of challenge of used culture with live virus	_____
Sterility tests:	
Method (media)	_____
Results	_____
<i>Trivalent bulk product (monovalent pools incorporated)</i>	
Date of preparation	_____
Preservative (if added, type and concentration)	_____
<i>Tests on trivalent bulk</i>	
Test for absence of infective poliovirus:	
Sample size tested	_____
Details of testing procedure	_____
Period of observation of cell cultures	_____
Period of observation of subcultures	_____
Result	_____
Sterility test:	
Method (media)	_____
Result	_____
<i>Tests on final product</i>	
Identity test:	
Method used	_____
Result	_____
Sterility test:	
Method (media)	_____
Result	_____
Innoccuity test (if applicable):	
No. of animals	_____

Dose	_____
Period of observation	_____
Result	_____
Potency test:	
Results (and date) of in vitro tests (D-antigen)	_____
Results (and date) of in vivo tests, (in rats) if performed	_____
Protein content:	
Content of protein in µg per human dose	_____
Serum protein tests (if applicable):	
Result	_____
Signature of head of laboratory	_____
Certification by person taking overall responsibility for production of the vaccine.	
I certify that lot no. _____ of trivalent poliomyelitis vaccine (inacti- vated) satisfied Part A of the WHO Requirements for Poliomyelitis Vaccine (Inactivated).	
Date	_____
Signature	_____
Name typed	_____
The protocol must be accompanied by a sample of the vaccine and a copy of the patient information leaflet.	