EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 29 October to 2 November 2018

Guidelines for the safe production and quality control of poliomyelitis vaccine


NOTE:

This draft document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the WHO Expert Committee on Biological Standardization (ECBS). The distribution of this document is intended to bring the proposed revision of the WHO Guidelines for the safe production and quality control of poliomyelitis vaccine to the attention of a broad audience and to improve the transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the ECBS. Written comments proposing modifications to this text MUST be received by 28 September 2018 using the Comment Form available separately and should be addressed to: Department of Essential Medicines and Health Products (EMP), World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Comments may also be submitted electronically to the Responsible Officer: Dr Hye-Na Kang at kangh@who.int.

The outcome of the deliberations of the ECBS will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the second edition of the WHO style guide (KMS/WHP/13.1).
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Guidelines for the safe production and quality control of poliomyelitis vaccine

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References
Guidelines published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRA) and for manufacturers of poliomyelitis vaccines. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. National requirements should be consistent with GAPIII and the Containment Certification Scheme, and should ensure that the risks of reintroducing poliovirus into the community are no greater than would be the case if these WHO Guidelines are followed. This document also sets out a number of guiding principles that might usefully be considered by national authorities for containment (NACs).
### Abbreviations

1. **CAG**  
   Containment Advisory Group

2. **CCID<sub>50</sub>**  
   cell culture infectious dose 50%

3. **CD155**  
   cluster of differentiation 155

4. **cVDPV**  
   circulating vaccine-derived poliovirus

5. **CWG**  
   Containment Working Group

6. **GAPIII**  
   WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use

7. **GCC**  
   Global Certification Commission

8. **GMP**  
   good manufacturing practice(s)

9. **HEPA**  
   high-efficiency particulate air

10. **IPV**  
    inactivated poliomyelitis vaccine

11. **IRES**  
    internal ribosome entry site

12. **NAC**  
    national authority for containment

13. **NRA**  
    national regulatory authority

14. **OPV**  
    oral poliomyelitis vaccine

15. **PCR**  
    polymerase chain reaction

16. **PEF**  
    poliovirus-essential facility

17. **VDPV**  
    vaccine-derived poliovirus
1. Introduction

The WHO Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses (1) were developed in 2003 as an addendum to the previous Recommendations for the production and control of poliomyelitis vaccine (inactivated) (2), and specify the measures to be taken to minimize the risk of accidental reintroduction of wild-type poliovirus from a vaccine manufacturing facility into the community after global certification of polio eradication.

In response to subsequent developments in manufacture and the progress of the polio eradication programme, revised WHO Recommendations for the production of inactivated poliomyelitis vaccine (IPV) (3) and oral poliomyelitis vaccine (OPV) (4) have recently been published. Both of these documents highlight the need for enhanced biorisk management in the production and control of poliomyelitis vaccines after eradication but do not provide detailed guidance on this aspect. Vaccine production must comply with current good manufacturing practices (GMP) requirements to ensure product quality (5, 6). WHO guidance on good manufacturing practices for biological products (6) specifically emphasizes that the production of poliomyelitis vaccines should also comply with the containment requirements outlined in the third revision of the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) (7) to ensure the protection of personnel and the environment.

However, none of the currently available guidelines and recommendations provides sufficient detail concerning strategies or approaches for satisfying the objectives of both current GMP and GAPIII. Furthermore, some of these documents were developed based on different conceptual frameworks and often use incongruent terminology. It was recognized that revision of the 2003 Guidelines (1) was required in order to provide explicit, concise and updated guidance on the biosafety aspects of poliomyelitis vaccine production consistent with current GMP. In October 2015 the need for such a revision was raised at the WHO Expert Committee on Biological Standardization (8) with subsequent progress reported upon. The Committee agreed with the conclusions reached and the proposals made and expressed its support for the revision process (8, 9).

2. Purpose and scope

These revised WHO Guidelines provide information and guidance to vaccine manufacturers and relevant national authorities on the biosafety measures required during poliomyelitis vaccine production and quality control during the final poliovirus containment stage (Phase III) as defined in GAPIII. The current document specifies the steps to be taken to minimize the risk of reintroducing poliovirus from a vaccine manufacturing facility into the community after global certification of polio eradication, and should be read in conjunction with other
relevant WHO guidance (3–7, 10). Detailed and specific guidance on laboratory biosecurity is also available separately (7, 11).

Currently there are three types of poliomyelitis vaccines: OPV made from Sabin strains, IPV made from wild-type strains and IPV made from Sabin strains. IPV derived from wild-type strains and OPV are conventional vaccines developed and introduced in the 1950s and 1960s respectively. Recently, Sabin IPV has been introduced in Japan and China. Manufacturers are also working on alternative versions of poliomyelitis vaccines, with a number of candidate vaccines now in different phases of nonclinical and clinical development. These include: (a) safer and more genetically stable strains of OPV; (b) IPV made from genetically modified strains with improved biological characteristics that may require less stringent biosafety and biosecurity measures; and (c) poliomyelitis vaccines prepared by novel biotechnology processes that do not require the cultivation of live virus (for example, vaccines based on virus-like particles).

The previous version of these WHO Guidelines (1) dealt with the safe production and quality control of IPV based specifically on wild-type strains. These revised Guidelines address the containment measures needed during the production and quality control of:

- IPV produced from wild-type poliovirus strains;
- IPV produced from the live attenuated vaccine (Sabin) strains used in the manufacture of OPV;
- OPV and IPV produced from novel safer strains developed by genetic manipulation.

The document also covers the preparation of all viruses and other biological materials used in poliomyelitis vaccine manufacture and critical quality control tests. Although the production of poliomyelitis vaccines using platforms involving genetic expression systems without virus replication is outside the scope of these current Guidelines, any quality control test using live poliovirus should be carried out in a containment facility following the guidance provided by GAPIII and this document.

The global eradication of wild-type 2 virus was declared in September 2015 (see section 3 below). The use of trivalent OPV then ceased with the withdrawal of the type-2 component in April 2016 and a global switch to bivalent OPV took place. The handling and storage of all type-2 poliovirus materials should now follow the guidance set out in GAPIII (7) and associated GAPIII Containment Certification Scheme (12). Furthermore, the global cessation of OPV use is planned to take place after the declaration of wild-type poliovirus eradication, and OPV will only be used to respond to outbreaks of wild-type poliovirus or vaccine-derived poliovirus (VDPV). As a result, OPV prepared from Sabin strains is also outside the scope of this document (9).
3. Background

In 1988 the Forty-first World Health Assembly adopted a resolution (WHA41.28) to eradicate polio by the year 2000 (13). Although the target date had subsequently to be revised, the number of cases and number of countries with polio cases have both fallen drastically. There are three serotypes of poliovirus (1, 2 and 3). Wild-type 2 poliovirus has not been isolated since 1999, and was officially declared eradicated by the Global Certification Commission (GCC) in September 2015. The last case of polio caused by a wild-type 3 strain occurred in Nigeria on 10 November 2012. To date, wild-type 1 poliovirus remains endemic in several countries. Confirmation of the absence of poliovirus in circulation requires a prolonged period of intense surveillance as these viruses can circulate undetected for several years. This was recently illustrated by two cases of poliomyelitis caused by wild-type 1 poliovirus reported in Nigeria in August 2016. Since 2012 Nigeria had been considered to be free of wild-type polioviruses. In addition, the continued emergence of neurovirulent VDPVs necessitates the replacement of OPV with inactivated vaccines and/or with new further attenuated vaccines requiring appropriate characterization and clinical study to demonstrate their improved safety profile compared to current OPV. A number of efforts are under way to develop and introduce new vaccine products suitable for post-eradication vaccination programmes.

It is clear that if one country still has circulating poliovirus the world is at risk of reintroduction. This makes the containment of polioviruses during vaccine production and control vital to prevent their release into the environment and the re-establishment of poliovirus circulation. In 2004 polio vaccination was stopped in Nigeria as a result of misinformation about the vaccine, and polio was reintroduced across much of Central Africa as a result. In addition, outbreaks also occurred in Yemen and Indonesia as the virus was exported from Nigeria during the Hajj. In the past, polio was repeatedly introduced into Angola from northern India, and other importations have occurred including from Pakistan into China and from India into Kazakhstan, the Russian Federation, Tajikistan, Turkmenistan and Uzbekistan. More recently, polio was exported from Pakistan to Egypt, Israel and the Syrian Arab Republic in apparently separate events.

The main tool used in the polio eradication programme has been OPV, which has been shown to interrupt transmission by inducing effective intestinal immunity. Eradication has involved the use of OPV during National Immunization Days and other immunization activities which supplement routine programmes where the vaccine is given in association with other childhood vaccines. This strategy has proven to be highly effective in eliminating polio in most of the places where it has been used.

The OPV strains replicate in the gut of the recipient and are shed, potentially infecting contacts. The infection of contacts by excreted vaccine virus boosts immunity in those already immunized. However, where vaccination coverage is suboptimal, it is possible for the OPV viruses to regain both transmissibility and neurovirulence, and to develop into
circulating VDPVs (cVDPVs) leading to polio outbreaks. This has occurred on numerous
occasions and has led to two specific changes to immunization practices. Firstly, a switch
from OPV to IPV for routine immunization was made starting in high-income countries and
more recently expanded to many others. Secondly, as the majority of cVDPVs are derived
from the type 2 Sabin strain, countries using trivalent OPV switched in April 2016 to bivalent
OPV containing only types 1 and 3 Sabin strains (14, 15). Type 2 Sabin strain is also the most
effective in generating immune response in vaccinees and competes with the other vaccine
serotypes thus reducing their effectiveness. Polio was eliminated in India when monovalent
OPV and bivalent OPV (containing types 1 and 3 but not type 2) were used. On rare
occasions, OPV can cause chronic infection in immunodeficient individuals, with the type 2
component being the most common cause. As a risk-mitigation measure, immunization with
bivalent OPV can be supplemented by the use of IPV. Eventually, following the eradication
of any remaining circulating poliovirus, bivalent OPV usage will also be stopped and will be
replaced by the exclusive use of IPV or other products. If there is a need for an emergency
response to a polio outbreak following eradication then stockpiles of monovalent OPV will
be released by the Director-General of WHO.

Once eradication is complete, live polioviruses should be contained or destroyed to prevent
the reintroduction of the disease – a process begun in 2015 with the type 2 strains. Production
of IPV requires the cultivation of large amounts of live poliovirus which is then inactivated to
destroy its infectivity. No polio outbreak has yet been caused by the release of virus from a
production facility even though accidental releases of poliovirus from IPV production plants
have been documented. Biosafety may depend on several factors, and designing and
operating facilities in ways that minimize virus escape while following production practices
that protect workers from infection will be crucially important. Other key elements include
ensuring high levels of immunization against polio among the population in countries hosting
vaccine production facilities and ensuring that adequate sewerage systems are in place.

The need for larger quantities of poliomyelitis vaccine at lower prices to satisfy global
demand has encouraged the development of manufacturing capability in areas of the world
with little previous experience of IPV manufacture, inadequate sewage treatment and greater
potential for poliovirus transmission. Hence, this strategy poses additional risks and one
possible mitigation strategy would be to base vaccine production on the strains used in OPV
production. Their use in eradication, lower infectivity and reduced ability to spread suggest
that they should be safer for production. However, the occurrence of cVDPVs and vaccine-
associated polio cases demonstrate the capacity of OPV strains to revert to a wild-type
phenotype. As a result, despite potentially contributing to increased safety, the use of such
strains cannot be relied upon exclusively. There is clearly a need to recognize, quantify and
mitigate the risks associated with all vaccine production platforms.

The WHO Polio Eradication and Endgame Strategic Plan 2013–2018 (PEESP) (16) was
published by the Global Polio Eradication Initiative. This document discussed and
summarized the complex nature of the end game of polio eradication. The subsequently
developed and published GAPIII aligns the safe handling and containment of infectious and potentially infectious poliovirus materials with the PEESP. Taken together, GAPIII (7) and the GAPIII Containment Certification Scheme (12) provide a framework for the containment of polioviruses by describing the systems and actions required to contain all types of work with polioviruses following their eradication, and should be read in conjunction with these WHO Guidelines. Following the requirement for the containment of wild-type 2 polioviruses and subsequent switch away from the use of Sabin type 2 in 2016, a phased approach to containment is currently under way. At present, only bivalent OPV consisting of type 1 and 3 Sabin strains is used, with the potential use of monovalent Sabin OPV2 reserved for controlling any future outbreak situation (7).

The destruction of unneeded poliovirus materials and containment of the remaining poliovirus stocks will be important considerations in the decision of the GCC regarding the eradication status of individual regions and of the entire world. To coordinate and oversee containment activities GCC is supported by the Containment Working Group (CWG). The GCC and CWG will work with national authorities for containment (NACs) to scrutinize their certification applications and reports as they seek to certify their respective poliovirus-essential facilities (PEFs) based on compliance with GAPIII. According to the GAPIII Containment Certification Scheme (12), the responsibility for PEF containment certification rests with the NACs in coordination with the GCC. Initial certification will result in the issuance of a Certificate of Participation, potentially followed by an Interim Containment Certificate. However, all PEFs intending to retain polioviruses will ultimately require a full Certificate of Containment. The certification of manufacturing facilities as PEFs will be based on compliance with the provisions of GAPIII and all other relevant regulatory requirements, standards and guidelines, including this document.

In 2017, WHO established the Containment Advisory Group (CAG) that includes experts in biosafety and biosecurity. CAG meets on a regular basis to review and provide guidance on issues that are not fully covered in GAPIII. For example, GAPIII describes in detail containment measures for wild-type and Sabin strains of poliovirus, but safer genetically modified strains have recently been developed with limited or no pathogenicity and transmissibility. Such strains, developed using recombinant technology, have been shown to have a better safety profile in laboratory studies, relative to wild-type or Sabin polioviruses, particularly with respect to genetic stability and the ability to infect human subjects. Strains unable to infect humans would be entirely safe and have therefore been proposed for use in the manufacture of poliomyelitis vaccines, and for conducting quality control tests and epidemiological surveillance. These new strains were developed to facilitate the manufacture and quality control of poliomyelitis vaccines by eliminating the need for costly and laborious containment procedures. To determine the appropriate containment measures for working with such strains, CAG formed an Expert Support Group consisting of poliovirus experts who advise CAG on specific aspects of the pathogenicity and transmissibility of the new strains.

In these and all related areas, vaccine manufacturers and NACs are encouraged to submit any questions or requests they may have to CAG.
4. Terminology

The definitions given below apply to the terms as used in these WHO Guidelines. These terms may have different meaning in other contexts.

**Aerosol**: a dispersion of solid or liquid particles of microscopic size in a gaseous medium.

**Air balance**: the necessity to keep air supply and exhaust systems in balance by means of measurements of static pressure, fan and motor performance, and air volumes.

**Airlock**: an enclosed space with two or more doors which is interposed between two or more rooms (for example, of differing classes of cleanliness) for the purpose of controlling the airflow between those rooms when they need to be entered. An airlock is designed for either people (personnel airlock) or goods (material airlock).

**Biological safety cabinet**: both Class II and Class III cabinets are intended to protect the product, worker and environment from contamination. Both the supply and exhaust air are HEPA filtered or incinerated before discharge. Airflow is maintained under negative pressure to protect workers and the environment.

**Biorisk**: the biosafety and biosecurity risk related to a biological agent or material (in this case, poliovirus).

**Biosafety**: the containment principles, technologies and practices used to ensure the prevention of unintentional exposure to, or accidental release of, pathogens and toxins.

**Biosafety manual**: a comprehensive document describing the physical and operational practices of the laboratory facility with particular reference to safe working with biological materials.

**Cell-culture infectious dose 50% (CCID<sub>50</sub>)**: the quantity of a live virus that when inoculated onto a number of susceptible cell cultures will infect 50% of the individual cultures.

**Certification**: a systematic and documented process to ensure that systems perform in accordance with available certification standards or applicable validation guidance.

**Closed system**: a process system with equipment designed and operated such that the product is not exposed to the room environment.

**Containment**: the confining of microorganisms or organisms or other entities within a defined space with controlled access.
Contingency plan: documented procedures for future events or circumstances regarded as likely to occur.

Decontamination: a procedure that eliminates biological agents and toxins or reduces them to a safe level.

Dedicated: denoting a facility, personnel, equipment or a piece of equipment used only in the manufacture of a particular product or group of specified products of similar risk.

Disinfection: the process of reducing the number of microorganisms (but not usually bacterial spores) without necessarily killing or removing them all.

Eyewash station: a dedicated device supplying clean water for emergency cleansing of eyes contaminated with biological or chemical agents.

Good manufacturing practice (GMP): a system which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

High-efficiency particulate air (HEPA) filter: a filter capable of removing at least 99.97% of all particles with a mean aerodynamic diameter of 0.3 µm.

Inactivation: rendering an organism unviable or a virus non-infectious by the application of heat, chemicals or radiation, or by other means.

National authority for containment (NAC): the national authority responsible for GAPIII containment certification. NACs are nominated by the ministry of health or other designated national body or authority.

Penetrations: openings through walls, floors or ceilings to allow access for mechanical services.

Poliovirus-essential facility (PEF): a facility designated by the ministry of health or other designated national body or authority as serving critical national or international functions that involve the handling and storage of needed poliovirus materials post-eradication under the conditions set out in Annex 2 or 3 of GAPIII (7). According to GAPIII, facilities are required to hold a valid certificate to handle and store polioviruses beyond Phase I (7).

Production: the entire set of processes and procedures involved in making vaccines that includes the manufacture of vaccine substances and components, formulation, quality control and filling of final containers.
**Respirator**: a respiratory protective device, with an integral perimeter seal, valves and specialized filtration, used to protect the wearer from toxic fumes or particulates.

**Risk assessment**: a systematic process of organizing information to support a risk decision to be made within a risk-management process. Risk assessment consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.

**Sharps**: laboratory devices capable of cutting or puncturing skin (for example, needles, scissors and glassware).

**Sabin strains**: preparations of polioviruses of types 1, 2 and 3 derived by limited number of passages from stocks developed by Dr Albert Sabin (17) which retain attenuated properties as measured by biological and molecular markers.

**Validation**: the documented act of proving that any procedure, process, equipment, activity or system actually leads to the controlled process.

### 5. General considerations

The production of poliomyelitis vaccines should be carried out in accordance with WHO recommendations for the manufacture and control of IPV (3) and OPV (4), as well as the general requirements outlined in WHO good manufacturing practices for pharmaceutical products: main principles (5) and WHO good manufacturing practices for biological products (6). In addition, the design and operation of poliomyelitis vaccine manufacturing and testing facilities should comply with the poliovirus containment requirements outlined in GAPIII (7). GAPIII describes the containment requirements and procedures developed to minimize the risks of accidental release of poliovirus into the community from laboratories or other facilities that handle or store poliovirus. However, it does not provide guidance on assessing the specific risks associated with poliomyelitis vaccine manufacture. The production of poliomyelitis vaccines from wild-type, Sabin and new genetically modified safer strains raises a set of issues that require additional clarification for proper alignment of the above documents with GAPIII. The applicable containment conditions will depend on the biological characteristics of vaccine strains and production conditions, and should be assessed on a case-by-case basis.

Requirements described in GAPIII should be reconciled with the provisions of current GMP as they apply to the manufacture of poliomyelitis vaccines. Thus, the current document should be read in conjunction with other relevant WHO guidance such as GAPIII (7), GMP for biologicals (5, 6), the GAPIII Containment Certification Scheme (12) and WHO laboratory biosafety and biosecurity manuals (10, 11), as well as other relevant

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1 See also: [http://polioeradication.org/polio-today/preparing-for-a-polio-free-world/containment/containment-resources/](http://polioeradication.org/polio-today/preparing-for-a-polio-free-world/containment/containment-resources/)
documentation and national regulations governing the manufacture and control of these products. Alternative approaches are acceptable if demonstrated to be equivalent and approved by the appropriate national authorities. Contingency plans should be put in place for dealing with potential accidents. In most countries, the regulation of GMP and biosafety are governed by different institutions. Close collaboration between such institutions is particularly important to ensure that both product contamination and environmental contamination levels are controlled within acceptable limits.

6. Biosafety implementation within a production facility for IPV

A breach of containment of poliovirus used in a vaccine production or testing facility could occur in a variety of ways, including through contact with contaminated equipment, clothing, skin and hair, or inadequate decontamination and disposal of liquid effluents, air emissions and other waste. In addition, inappropriate manipulation with live poliovirus leading to the exposure of personnel by oral and other routes (for example, via nose or eye) can result in asymptomatic infection and shedding of virus for several weeks. The amount of poliovirus required to cause infection by the oral route is thought to be 1 CCID$_{50}$ for wild-type and approximately $10^{1-2}$ CCID$_{50}$ for attenuated strains (18, 19). In production facilities, viral culture fluid before concentration contains poliovirus in the order of $10^8$ CCID$_{50}$ per ml and bulk concentrates $10^{12}$ CCID$_{50}$ per ml. Based on these assumptions biosafety procedures may reduce the risk of infection of workers in the plant but cannot remove it altogether.

Accidental release and transmission from the laboratory or vaccine production facility to the community is most likely to result from either equipment failure or human error (20, 21). The inadvertent transmission of poliovirus to an immediate contact by an infected vaccine production worker has been documented (22).

Although Sabin vaccine strains are considered to be less transmissible than the wild-type poliovirus they can establish population infections, as the existence of cVDPVs demonstrates. There is also the possibility that a failure to either adequately identify an emergency situation or manage the risk associated with it could lead to the release of infectious materials into the community. The provisions in this document seek to minimize the risk of such occurrences.

6.1 The poliomyelitis vaccine manufacturer should employ one or more biorisk management advisers and establish a biorisk management committee as described in GAPIII. The biorisk management adviser(s) should be knowledgeable in poliomyelitis vaccine production, current GMP and containment, and be independent of production and quality control in their reporting structure.

6.2 A detailed and comprehensive risk analysis should be conducted to identify possible sources of contamination of personnel or the environment that may arise from the production or testing of live poliomyelitis vaccine within the establishment. For each procedure or system this risk analysis should take into account the volume, concentration and stability of the poliovirus at the site, the potential for inhalation, ingestion or injection that could result
from accidents, and the potential results of a major or minor system failure. The procedural
and technical measures to be taken to reduce the risk to workers and the environment should
be considered as part of this analysis. The analysis should be documented and lead to the
implementation of appropriate risk-mitigation strategies.

6.3 The biosafety aspects of the production process and quality control activities,
including response to biosafety emergencies and accidents, waste disposal and the
requirements for safe practices and procedures as identified in the risk analysis, should also
be documented, and reviewed and updated following a predetermined schedule.

7. Personnel

7.1 Personnel required to work in the poliovirus containment facilities, both
manufacturing and testing, should be selected with care to ensure that they may be relied
upon to observe the appropriate codes of practice, and are not subject to any disease or
condition that could compromise the integrity of the product or the containment of the
poliovirus strains with which they work. The acceptance by staff that the containment of
poliovirus is an individual responsibility is a key factor in its implementation and
maintenance as described in GAPIII (7).

7.2 Procedures should be in place to ensure that all staff working at the containment
facilities are of suitable health status before the start of employment and periodically
thereafter following a predetermined schedule. Any changes in health status that could
adversely affect the quality of the product or the containment procedures (for example,
immune deficiency) should preclude the person concerned from working in the production or
containment areas.

7.3 Personnel working in poliovirus containment facilities should be immunized with
poliomyelitis vaccines, and adequate blood titres of circulating neutralizing antibodies against
all three serotypes should be confirmed, prior to their authorization to enter the containment
facilities unless it is decontaminated following a validated process. The antibody titres should
be monitored and a booster immunization given as needed. Temporary workers, contractors
and visitors should also have protective immunity against poliovirus. Personnel entering the
containment facilities only after decontamination of the facilities using a validated procedure
may be exempted from the immunization requirement.

7.4 All personnel (including those concerned with cleaning, maintenance or quality
control) employed in areas where live poliovirus is manufactured or tested should receive
additional training and periodic retraining specific to their work with poliovirus. This should
include relevant information and training in hygiene and microbiology in relation to
poliomyelitis vaccine production and poliovirus, as well as biosafety procedures. Attention
should also be given to ensuring that hygiene precautions are taken to minimize the risk of
transmission of poliovirus from personnel to their family members and contacts.
7.5 Personal protection:

7.5.1 Personnel working in the containment facilities should be trained and deemed to be competent in all operating practices and standard microbiological practices – as outlined in the WHO Laboratory biosafety manual (10) – including gowning procedures and procedures for dealing with emergencies, biohazards and other hazards associated with the work.

7.5.2 Personnel should be provided with the facilities and equipment required to minimize potential exposure. Provision should be made for the changing of clothing and emergency decontamination of personnel in the event of a spill or other release of infectious materials.

7.5.3 Appropriate protective clothing and equipment should be provided based on risk assessment. For example, solid-front or wrap-around gowns, scrub suits or coveralls with head and shoe covers should be worn at all times by operators while in the containment facility. The use of eye protection or full-face masks should be required when there is a potential for generating aerosols. Respirators should be used when conducting procedures with a high probability of aerosol generation. Protective clothing is to be removed when leaving the containment facility, and should be decontaminated using a validated procedure before re-use or disposal.

7.5.4 Double gloves should be worn at all times in the containment area and discarded as waste for decontamination in the dirty area of the personnel exit airlock. Outer gloves should be removed and discarded after handling potentially infectious materials.

7.5.5 Hands should be disinfected and washed upon leaving the containment area. Hand-washing sinks equipped with automatic (hands-free) controls should be installed in the personnel exit airlock. All sinks should be connected to a validated waste decontamination system. The use of validated water-free (chemical) hand-washing systems with decontamination features is an acceptable alternative.

- Poliovirus is known to be resistant to many common disinfectants (23, 24). However, a recent study has shown that ethanol combined with 2-propanol, citric acid and urea is effective against poliovirus (25).

7.5.6 A full-body shower should be available within the personnel exit airlock from the containment area. The use of a shower upon exit should follow an established procedure supported by the risk assessment and consistent with policies established by GAPIII and CAG2 (26, 27).

2 See: http://polioeradication.org/tools-and-library/policy-reports/advisory-reports/containment-advisory-group/
7.5.7 Based on a risk assessment, an eyewash station should be available within the personnel exit airlock or at other locations.

7.5.8 Good microbiological techniques should be followed (10). These include but are not limited to:

- no eating, drinking, smoking or applying of cosmetics in the containment area;
- no mouth pipetting;
- implementing measures to minimize aerosol generation when manually transferring or mixing materials containing live poliovirus;
- implementing policies on the safe handling of sharps;
- decontaminating work surfaces after handling materials containing live poliovirus and after any spill of viable material; and
- decontaminating equipment before removing it from the facility for repair or maintenance.

8. Premises and equipment

Premises should be designed in such a way as to control the risks to the product, the personnel and the environment. This is accomplished by using appropriate primary containment devices such as biological safety cabinets, isolators, vessels and transfer pipes to protect personnel and the immediate workspace within the containment areas, and by segregating the containment areas with physical barriers, effluent treatments, airlocks and pressure differentials that protect the environment external to them from accidental exposure to infectious materials. These systems should also provide adequate safeguards to protect the product against contamination with extraneous agents and to prevent the cross-contamination of intermediates that have undergone viral inactivation.

8.1 General requirements:

8.1.1 Live poliovirus and materials in which live poliovirus may be present should be handled in contained areas. Contaminated materials, including equipment for repair or maintenance, should be decontaminated by a validated method prior to removal from the containment area.

8.1.2 Whenever possible, poliomyelitis vaccine production facilities where live poliovirus is processed should be housed in dedicated buildings. If they are located in multipurpose buildings then the production facility should be separated by a physical barrier, and should have separate entrances and exits, dedicated biological waste handling systems and a dedicated air handling system. Poliomyelitis vaccine quality control laboratories located in multipurpose buildings should be equipped with dedicated air handling and waste
disposal systems that prevent the contamination of other areas with material infected with poliovirus.

8.1.3 Use of the poliovirus facility for the production of other microorganisms on a campaign basis may be acceptable provided that a changeover procedure is validated and implemented as outlined in Annexes 2 and 3 of GAPIII.

8.1.4 The containment areas for production and quality control testing should be marked with approved biohazard signs. Signs should be posted in prominent locations at the entry to the facility clearly stating that poliovirus is contained in the area and that only personnel authorized to work with poliovirus are permitted to enter. The name(s) and contact information of persons to be contacted in the event of an emergency should be displayed at all times and kept up to date.

8.1.5 All exits should be marked. Emergency exit doors from the poliovirus facility should be alarmed and their use treated as a breach of containment unless a closed system is maintained.

8.1.6 Windows may be used to allow visual monitoring of activities in the laboratory and production areas inside the containment zone. Other devices such as closed-circuit television cameras may be effective alternatives where windows are not appropriate.

8.2 Equipment:

8.2.1 Biological safety cabinets or equivalent equipment should be provided and used within the production and quality control areas where live poliovirus or infected cell cultures are handled or manipulated. Only a Class III biological safety cabinet is considered to be a closed system as described in GAPIII.

8.2.2 Biological safety cabinets should be constructed and manufactured in accordance with national regulations or standards, such as EN12469, British Standards Institution (BSI), Deutsche Industries Norm (DIN) or National Sanitation Foundation (NSF). They should be tested and certified on a regular schedule as meeting those standards. Cabinets with design modifications to meet the requirements of large-scale operations, but providing equivalent containment levels, may be used if approved by the responsible national authorities and if they also meet the manufacturer’s specifications.

8.2.3 When exhaust air from biological safety cabinets is to be discharged through the building exhaust air system, the air handling system should be designed in such a way as to not disturb the air balance of the cabinet or of the room in which the cabinet is situated.

8.2.4 Whenever possible, manufacturing process and transfer of intermediates should be carried out in closed systems.
8.2.5 In situations where production failure, product contamination or similar reason necessitates the discarding of a batch, there should be a predetermined and validated procedure for inactivating the contents of the full tank/container. This procedure should be described in sufficient detail and all relevant staff should be trained in it with such training documented.

8.2.6 All equipment used to handle and store live poliovirus should be designed and operated in such a manner as to prevent uncontrolled release through any potential route of entry and exit (for example, air exhausts, waste lines, etc.). Suitable measures for testing, as well as alarms, should also be incorporated into the design and operation of such equipment.

8.3 Production facilities:

All poliomyelitis vaccine manufacturing steps that involve the processing of live poliovirus, including viral culture, viral purification and viral inactivation (3), should be performed within the containment facility. The design of the containment facility should also permit the effective segregation of the live virus and inactivation stages to prevent cross-contamination, as required by current GMP.

8.3.1 Areas used for the storage of poliovirus seed stock should be fully secured against entry by non-authorized personnel. For secondary (back-up) seed storage locations where stocks are not normally used for production, the NRA may approve storage in leak-proof containment containers within a dedicated freezer that is subject to security and access restrictions appropriate for the storage of poliovirus. Outside the containment area, polioviruses should be stored under appropriate containment conditions, as determined by a risk assessment approved by the competent authority (for example, the NAC) and in line with the approach detailed in the GAPIII Containment Certification Scheme (12).

- The viral seed stock should be inventoried. The addition or removal of material should only be undertaken by authorized personnel following the approval of two authorized signatories on record, or the electronic equivalent of this approval. Records of the addition or removal of viral seed should be securely stored.
- The viral seed storage area should be equipped with a back-up emergency power source and with recording and alarm systems to monitor freezers.

8.3.2 The air flow system of the containment facility (including personnel and material airlocks) should be designed to prevent a breach of virus containment. The containment facility should be appropriately designed to facilitate the maintenance of negative pressure relative to the environment and of the required pressure cascades as described in the WHO good manufacturing practices for pharmaceutical products containing hazardous substances (28). To prevent cross-contamination within the containment facility, an area of higher contamination risk (for example, viral culture and purification area) should
be segregated from an area of lower contamination risk (for example, stage 2 inactivation area).

8.3.3 An air handling system should maintain a negative pressure (inward directional air flows) in areas where live poliovirus is handled or where there is a potential for room contamination (for example, spills).

- The installation of high-efficiency particulate air (HEPA) filters provides a filter efficiency of 99.97% or greater removal of 0.3 µm particles. Air from areas where live poliovirus is handled or where there is a potential for contamination should be extracted through HEPA filters at the point of air removal from the chamber or sealable ducts.
- HEPA-filtered exhaust air may be recirculated to the same poliovirus containment area – as described in the WHO Supplementary guidelines on good manufacturing practices for heating, ventilation and air-conditioning systems for non-sterile pharmaceutical dosage forms (29). A proper system for the maintenance and testing of HEPA filters should be in place. Heat exchangers may be used to recover warmth from HEPA-filtered exhaust air. HEPA filter housings should be designed to allow for in situ filter isolation, decontamination and testing. Such filters should be tested and certified upon installation and at least annually thereafter.
- Pressure differential monitoring lines penetrating the containment barrier should be provided with HEPA filtration or acceptable alternative. This is not required for containment zones with sealable pressure differential monitoring devices.
- Pressure difference readings for rooms should be monitored and recorded regularly. A warning system consisting of an audible or visual signal that can be readily perceived by personnel in the containment facility should be installed to indicate any failure in the air handling system.
- Supply and exhaust air systems should be provided with automatic mechanical/electronic interlocks or other appropriate devices that prevent sustained positive pressurization of the containment zone. The heating, ventilation and air conditioning (HVAC) system and controls should be verified during scenarios simulating the failure of system components, including exhaust fan(s), supply fan(s), power and Class II B2 biological safety cabinet exhaust fan(s) (where present), as determined by containment zone design. The rate of removal of exhaust air should result in sufficient air changes in both the production and quality control areas to provide an appropriate level of environmental cleanliness.

8.3.4 The containment zone should have the following physical characteristics:

- Surfaces and interior coatings within the containment zone – including, but not limited to, floors, ceilings, walls, doors, frames, casework, benchtops and furniture – to be cleanable, non-absorbent and resistant to scratches, stains,
moisture, chemicals, heat, impact, repeated decontamination and high-pressure washing, in accordance with function.

- There should be no windows that can be opened or any direct venting to the outside. Windows should be constructed of break-resistant safety glass with strength characteristics conforming to those required for the purpose for which they are used.
- Passageways for pipes, tubes and ducts passing through the wall between the containment area and surrounding areas should be completely sealed with materials resistant to contaminants and capable of withstanding disinfectants.
- Floor drains, where installed, should be capped, fitted with liquid-tight gaskets or connected to a waste effluent decontamination system to prevent inadvertent release into the sanitary drain.
- Provisions should be made to contain liquids leaking from bioreactors or tanks (including waste tanks) for a volume equal to the maximum amount of fluid contained in the vessels plus the amount of disinfectant required for inactivation.
- Liquid and gas services to the containment area in which backflow may occur should be protected to prevent it. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters or their equivalent.

8.3.5 If circulating water (for example, Purified Water or Water for Injection) is supplied in the containment facilities then the water treatment, storage and distribution systems should be designed, constructed and maintained with features for microbial control as described in WHO good manufacturing practices: water for pharmaceutical use (30). The method selected for microbial control should also be effective for inactivating poliovirus (for example, elevated temperature). An adequate monitoring system should be in place to ensure that the microbial control method works properly.

8.3.6 A communication system consistent with the facility containment conditions should be maintained between the support or administrative area and the containment area and should be kept in working order at all times.

8.3.7 Emergency lighting and power to the containment area and critical containment devices identified by risk assessment should be available.

8.3.8 In the event that any item is to be removed from the containment facilities it should be decontaminated as described in section 8.4 of this document or be sealed in an appropriate unbreakable leak-proof container (or containers) followed by a decontamination procedure to ensure that the exterior surfaces of the container(s) are free of infectious poliovirus.

8.4 Decontamination and waste disposal systems:
8.4.1 Decontamination of solid, liquid and gaseous wastes should take place within the containment area. Should any wastes have to be transported out of the containment area prior to decontamination and disposal they should be packaged, labelled and transported in accordance with applicable regulations.

8.4.2 The containment facility should be equipped with one or more interlocking double door pass-through autoclaves – the performance of each of which is validated prior to its initial use and then periodically following a predetermined schedule. Autoclave condensate drains located outside the containment barrier should have a closed connection directly connected to the drain piping servicing areas inside the containment barrier, unless condensate is effectively decontaminated prior to release.

8.4.3 Decontamination technologies and processes should be validated prior to initial use and revalidation should be performed when significant changes to the processes are introduced.

8.4.4 Effluents from equipment, showers and sinks within the containment area should be decontaminated by autoclaving or by discharge into a liquid effluent decontamination system. Such a system should be fully validated to ensure efficacy and be located in the containment area. The effluent treatment tanks should be situated in an area with floor dams or other measures capable of containing the full tank volume and allowing for the full inactivation of its contents.

9. Documentation and validation

9.1 Detailed records of operating parameters for the containment facility should be produced and maintained for conducting assessments of facility performance.

9.2 All spills or accidental release of infected materials and the response to such events should be properly investigated and documented. The results of these investigations should be used to review and revise the applicable facility operating procedures.

9.3 The production facility and equipment should be designed and constructed in such a way as to allow for full validation and verification of containment processes. It is the responsibility of the poliomyelitis vaccine manufacturer to ensure that these facilities and equipment meet acceptable standards that will ensure the containment of poliovirus as well as the protection of staff and the environment. Tests should be conducted following completion of facility construction or renovation. Regular maintenance should be carried out to ensure that the facility and equipment continue to meet the containment conditions. Records of the qualification and maintenance of the containment facility and equipment should be kept throughout the lifetime of the poliomyelitis vaccine production facility and for at least 5 years after the facility stops production. The containment features concerning biosafety to be assessed should include, but are not limited to, the following:
9.4 Cleaning and disinfecting procedures should be validated and documented. Manufacturers are urged to develop and implement assays for monitoring the poliovirus on work surfaces. The data generated will facilitate biosafety management within the vaccine production and testing facilities.

9.5 Data sheets and associated materials that have been used in containment areas should be decontaminated upon removal from the containment facility, or an electronic data gathering and transmission system implemented to transfer data from the containment area.

10. Production

The production of poliomyelitis vaccine involves handling large volumes of concentrated live poliovirus. The majority of operations are carried out in closed systems. Nevertheless, leaks can occur from valves or during procedures such as taking samples for testing purposes. Effective containment therefore requires that all aspects of production – from the specifications for the facility and equipment through to personnel and working procedures – should be in compliance with each of the relevant sections of these WHO Guidelines.

10.1 The movement of all personnel involved in production and quality control testing should be controlled to avoid cross-contamination. In general, personnel should not pass from an area of higher contamination risk (for example, viral culture and purification area) to an
area of lower contamination risk (for example, stage 2 inactivation area) within the containment facility per work day.

10.2 Material flow:

10.2.1 The flow of materials and equipment within the containment facility should be controlled to avoid cross-contamination.

10.2.2 Samples for quality control testing, and environment and water monitoring, should be sealed in appropriate unbreakable leak-proof containers and transported as described in section 8.3.8 above. If a disinfection procedure is used for the external container surfaces it should be validated and shown to have no impact on sample integrity. All samples should be handled safely and transported in accordance with applicable regulations.

10.2.3 Following a validated inactivation procedure, and prior to the confirmation of complete inactivation using a validated test approved by the NRA, the IPV monovalent bulk may be transferred out of the containment facility if the following conditions are met:

- The results of a battery of tests, which are predictive of complete inactivation, comply with the predetermined specifications. The battery of tests should include integrity testing of the 0.2 µm filters used to remove aggregates at the beginning and middle of the inactivation process, formaldehyde content and poliovirus loads at one or more time points during the inactivation.
- A formal risk assessment is performed to estimate the likelihood of the occurrence of an incomplete inactivation, as well as the residual virus level in the case of an incomplete inactivation. Precautionary measures may be recommended based on the outcome of the assessment.
- A procedure is in place at the manufacturing facility to quarantine the IPV monovalent bulk transferred out of the containment facility until the completion of all quality control testing recommended in Annex 3 of WHO Technical Report Series No. 993 (3), including the test for complete inactivation of poliovirus.

11. Quality control

The risks from live poliovirus in testing facilities are different from those in the production facilities. Although the volumes of poliovirus are smaller than those in the production facilities there are many more manual manipulations of samples and infected cell cultures containing viable polioviruses in testing facilities. The risk assessment should reflect these important differences.

11.1 Quality control testing laboratories should maintain containment conditions for all areas where materials containing live poliovirus are manipulated.
11.2 The use of non-dedicated quality control laboratories may be permissible under the following conditions:

- The non-dedicated quality control laboratories are located within the containment facility.
- All non-poliovirus-related activities performed within the containment laboratories and all personnel admitted into the containment laboratories adhere to all applicable containment procedures.

11.3 If quality control laboratories are housed within the production facility to enhance containment control then they should be kept separate from the production rooms, with separate air handling systems and dedicated airlocks for personnel and material provided from access corridors.

11.4 Quality control laboratories for poliovirus should be equipped with facilities for hand washing and disinfection. If sinks are used, the waste water should be collected in a waste disposal tank and disinfected prior to disposal. The use of validated water-free (chemical) hand-washing systems with decontamination features is an acceptable alternative. All solid, liquid and gaseous waste materials from the containment laboratories should be decontaminated prior to disposal.

11.5 Samples received from the containment areas should be handled using established procedures to prevent the release of live poliovirus. Procedures used to decontaminate sample containers or packaging materials should be validated and shown to have no impact on sample integrity. The packaging materials should be decontaminated prior to disposal. All samples received from the containment production facilities, with the exception described below in section 11.5.1, should be tested in containment laboratories. All test procedures using reagents containing live poliovirus should also be performed within the containment laboratories.

11.5.1 Certain samples (such as those for water and environment monitoring) taken from the containment areas may be tested outside the containment laboratories if a risk assessment concludes that they are unlikely to contain live poliovirus, based on facility design, equipment used (especially closed system) and sampling locations. However, necessary precautions covering sample handling, transportation and disposal may be recommended based on the risk assessment.

11.6 The Absence of Infective Poliovirus Test performed on the IPV monovalent bulk (3) – when transferred out of the containment production facility as described in section 10.2.2 above – may be performed outside the containment laboratories. However, the positive control of the test – along with the steps performed to demonstrate the sensitivity of the cells – require the use of live poliovirus and should be performed within the containment laboratories.
11.6.1 If the Absence of Infective Poliovirus Test is performed within the containment laboratories, care should be taken to prevent cross-contamination from the live poliovirus handled in the same area. If infective poliovirus is detected in this test then extensive investigation and revalidation of the inactivation process are required— which will interrupt routine manufacturing and product release.

11.7 Test procedures involving the inoculation of animals with live poliovirus (such as neurovirulence tests) should be performed within containment laboratories and special care taken in line with GAPIII recommendations. Species susceptible to poliovirus infection, including transgenic mice expressing the human poliovirus receptor, should be treated as infectious materials following infection with virus samples. Non-susceptible animals inoculated with live poliovirus should also be considered as potentially infectious materials. This affects all aspects of work (including handling, transporting, storage, inventory, etc.) and also includes all animal-derived materials (including tissues, blood, carcasses, stools, etc.). The risk of infected animals escaping the facility should be assessed and managed as this represents a potential threat to the community. An animal-care manager should be designated with responsibilities conforming to the requirements set out in these WHO Guidelines and other relevant documents. The animal-care manager should have an in-depth knowledge of animal handling, as well as of zoonotic and animal diseases. The animal-care manager should liaise with relevant personnel (for example, biorisk management adviser and occupational health professional) to implement effective and proportionate laboratory biosafety and biosecurity measures. A qualified veterinarian should be available for additional advice. The role should include providing input into risk assessment and management from an animal-care perspective. The poliovirus animal facility should incorporate features based on risk assessments and should accord with all poliovirus containment principles set out in this document.

12. Emergency procedures

Production and quality control testing of poliomyelitis vaccine using live polioviruses under containment conditions require planning for emergencies that could result in the release of live poliovirus within the facility or into the surrounding environment. The failure of containment systems within the facility as well as external events not under the control of the manufacturer could result in the exposure of plant personnel or the public to infectious poliovirus. Emergency response and contingency plans should be established based on risk assessment and should comply with the requirements outlined in GAPIII to minimize the impact and consequences of such incidents.

12.1 The response to an uncontrolled release of poliovirus resulting from a failure in containment systems should be planned and rapidly implemented to limit the exposure of persons to poliovirus.
12.1.1 The immediate response to a spill due to equipment failure (such as failure of vessels or transfer pipes) should be to evacuate the premises, followed by the deployment of clean-up personnel no sooner than 30 minutes after the incident to allow time for any aerosols to settle.

12.1.2 Staff and emergency personnel should be supplied with protective equipment (for example, respirators, coveralls and gloves) prior to re-entering containment areas for production or quality control. This equipment should be available in sufficient quantities at the entrance to the containment facilities and kept in good working order, with personnel having received prior instruction in its use.

12.1.3 The response should also include actions to limit the volume of the spill, as well as the use of validated methods for the inactivation of poliovirus and decontamination.

12.2 Emergency equipment such as disinfectants and other clean-up materials for spills should be available in sufficient quantities for use in responding to the release of infected material equivalent to the maximum capacity of the facility.

12.3 Personnel in the containment area at the time of the spill, emergency response personnel, law enforcement, medical or fire-fighting personnel, and those involved in the risk assessment, clean-up and disinfection of the area all present a risk for a further breach in containment and subsequent poliovirus dissemination into the environment. Emergency personnel should be immunized against poliomyelitis and have adequate training to enable them to understand the need for the containment measures in place. Whenever these precautions are not possible, emergency personnel should be supplied with adequate protective clothing and equipment to reduce the risk of them becoming infected with poliovirus in the course of their duties. Such protective clothing and equipment should be adequately disinfected before removal from the containment facility.

12.4 Appropriate medical evaluation, surveillance and treatment should be provided following spills. Infected or potentially infected personnel should be monitored following the protocol currently under development by WHO [reference TBP].

12.5 A full evaluation should be carried out after any emergency involving a breach of containment. The incident and all aspects of the response to it should be fully investigated and documented, and revisions made to existing procedures, contingency plans and staff training as necessary to minimize the risk of a repeat incident.

12.6 Any spill or accident that results in a breach of containment, as well as any suspected or confirmed poliovirus infection occurring within or surrounding the containment facility, should be investigated and documented as described above in section 9.2. The institutional biorisk management committee should be notified without delay. The investigation and
communication with appropriate national and international authorities should follow the
WHO protocol currently under development [reference TBP].

13. Risk assessment of new safer strains of poliovirus

The biosafety and containment measures described above and set out in GAPIII were
developed based on well-known biological characteristics of wild-type and attenuated Sabin
strains used in the manufacture and control of poliomyelitis vaccines – including their ability
to induce disease and to be transmitted from person to person. Since attenuated polioviruses
used for the production of OPV can revert to virulence and regain the ability to be transmitted
and cause outbreaks of paralytic disease, these measures reduce risks but do not eliminate
them completely. New strains have therefore been developed by genetic manipulation based
on detailed knowledge of poliovirus biology, with the specific purpose of stabilizing their
attenuated phenotype and limiting their ability to infect humans and spread among
populations. These strains have now been proposed for use in the manufacturing of new
genetically stable OPV and IPV, and for performing quality control tests, to minimize or
eliminate the risks of restarting poliovirus circulation. The introduction of these strains would
not only significantly mitigate the consequences of accidental release of poliovirus but could
also simplify the handling of virus stocks, and ultimately reduce the cost of vaccine
manufacture thus increasing vaccine supply.

The containment measures appropriate for the new strains should be defined based on risk
analysis performed on a case-by-case basis. If proven to be considerably safer than attenuated
Sabin strains then such strains could, following NAC approval, be handled under containment
conditions less stringent than those described in these WHO Guidelines or in GAPIII. WHO
CAG along with its affiliated Expert Support Group will review and evaluate the available
scientific evidence and risk assessment, and advise on the appropriate level of containment
for each new strain and new process proposed for implementation in poliomyelitis vaccine
production and quality control.

The evaluation may consider the following elements:

13.1 Vaccine manufacturers proposing to handle strains with reduced virulence and
transmissibility under less stringent containment conditions should perform a risk analysis
and present it for approval to the appropriate national authorities. Risk analysis should be
based on the biological properties of the specific strain, the intended use and design of the
facility in which it will be used, and the proposed handling procedures.

13.2 An evaluation of pathogenicity can be carried out based on known in vitro markers as
well as on experiments in laboratory animals. Several molecular structures within the
poliovirus genome (for example, in the IRES element) have been shown to be good
predictors of neurovirulence. Animal models that could be used to evaluate pathogenicity
include primates (rhesus and cynomolgus macaques) and transgenic mice expressing human
poliovirus receptor CD155 (31, 32). Validated tests in both animal models were
recommended by the WHO Expert Committee on Biological Standardization for use in the
lot release of OPV (4) and could also be used to demonstrate the superior safety of new
poliovirus strains. Such tests should include attenuated Sabin strains as the benchmark.

Another marker indicative of pathogenicity is the ability of viruses to replicate at higher
temperature. Attenuated strains tend to grow better at sub-physiological temperatures, while
pathogenic strains can grow at temperatures of up to 40 °C. Viruses producing lower yields
of live virus can be expected to exhibit lower pathogenicity and transmissibility (see section
13.4 below).

13.3 Genetic stability is an important indicator of the safety of vaccine strains because
replication in vitro and in vivo usually leads to reversion of the attenuated phenotype and the
regaining of virulent properties. Genetic stability can be evaluated by both biological and
molecular methods. Biological methods include passage in vitro and in vivo followed by
neurovirulence testing in transgenic mice or monkeys. The molecular approach is based upon
the quantification of mutants accumulated during virus growth by using direct methods such
as nucleotide sequencing, mutant analysis by PCR and restriction enzyme cleavage
(MAPREC), and deep sequencing (33, 34).

13.4 There are no validated tests for the transmissibility of poliovirus. However, it can be
inferred from a number of indirect markers. Lower stability of virus particles in the
environment, lower yield of infectious virus (including shedding by susceptible animals
infected orally) and an inability to grow at higher temperatures can all indicate that virus
transmission will likely be restricted. It is possible to develop virus derivatives unable to
replicate in normal cells, but which could grow in engineered cell cultures expressing factors
enabling virus replication. Such viruses (that cannot grow in vivo) can be expected to be
highly safe.

13.5 Appropriate containment conditions should be selected based on the above properties
to minimize the risk of accidental virus release into circulation. Polioviruses shown to have
significantly lower or no virulence in susceptible animal models, to be genetically stable upon
passage and capable of replication only in specially designed cell cultures could be handled
under conditions less stringent than those described above for wild-type and Sabin strains.

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The second draft of these Guidelines was prepared by a WHO Drafting Group comprising Ms A. Bonhomme, Public Health Agency of Canada, Canada; Dr K. Chumakov, United States Food and Drug Administration, the USA; Dr J. Martin, National Institute for Biological Standards and Control, the United Kingdom; Dr T. Wu, Health Canada, Canada; and Dr H-N. Kang, World Health Organization, Switzerland, taking into consideration the comments received from the second Working Group Meeting and from Dr N. Previsani (provided the consolidated comments of the WHO Containment Advisory Group), World Health Organization, Switzerland.

The resulting draft document was then posted on the WHO Biologics website for a first round of public consultation from 6 March to 4 April 2018 and comments were received from the following reviewers: Dr B.D. Akanmori, World Health Organization, Gabon; Dr P. Barbosa (provided the consolidated comments of IFPMA and of the non-IFPMA manufacturers Intravacc, Biological E. Limited and AJ Vaccines), Switzerland; C. Cahill (provided the consolidated comments of sIPV manufacturers: I. Rudebeck and A. Thomas, AJ Vaccines; Dr C. Breda, Biological E. Limited; Dr I. Knott, J-B. Mayet and C. Pierret, GlaxoSmithKline Vaccines; W. Bakker and J. Boes, Intravacc; C. Cahill, N. Papic and X.B. Vinken, Janssen Vaccines; J. Hanselaer and J.M. Malby, Sanofi Pasteur), Netherlands; Ms E. Febrina, National Agency of Drug and Food Control, Indonesia; Dr J. Fournier-Caruana, World Health Organization, Switzerland; Dr P.J. Huntly, Riskren PTE Ltd, Singapore; Dr A. Malkin (provided the consolidated comments of the Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products), Russian Federation; Mr W. Meng (provided the consolidated comments of Sinovac Biotech Co.), China; Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr P. Minor, London, the United Kingdom; Dr H. Shimizu (provided the consolidated comments of the sIPV Working Group in Japan), National Institute of Infectious Diseases, Japan; Mr G. Singh, Bharat Biotech International Ltd, India; and Professor Y. Yao, Institute of Medical Biology, China.

The document WHO/BS/2018.2350 was prepared by the above WHO Drafting Group, taking into consideration the comments received from the first round of public consultation as well as from the Third WHO Working Group meeting on developing WHO Guidelines on the safe production of polio vaccines held in Geneva, Switzerland, 7–8 May 2018 and attended by: Ms A. Bonhomme, Public Health Agency of Canada, Canada; Dr X. Bouwstra, Bilthoven.
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The document WHO/BS/2018.2350 is now being posted on the WHO Biologicals website for a second round of public consultation from 25 July to 28 September 2018.

References


33. Standard Operating Procedure. Mutant analysis by PCR and restriction enzyme cleavage (MAPREC) for oral poliovirus (Sabin) vaccine types 1, 2 or 3. Geneva: World Health Organization; 2012