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Guidelines for the safe production and quality control of poliomyelitis vaccine

Proposed revision of Annex 2 of WHO Technical Report Series, No. 926

NOTE:

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

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received **by 4 April 2018** in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Essential Medicines and Health Products (EMP). Comments may also be submitted electronically to the Responsible Officer: Dr Hye-Na Kang at email: kangh@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide, second edition" (KMS/WHP/13.1).

The "Polio Eradication and Endgame Strategic Plan 2013-2018" (PEESP), published by the Global Polio Eradication Initiative (GPEI), sets the goal of a polio-free world by 2018. Subsequently, World Health Organization (WHO) published the third edition of the Global Action Plan (i.e. GAPIII) in May 2015, entitled "WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral poliomyelitis vaccine use", which aligns the safe handling and containment of poliovirus infectious and potentially infectious materials with the PEESP. In light of the global updates, the need for revision of the Annex 2 of WHO Technical Report Series (TRS) 926 had been raised. A summary of the review outcomes were reported to the Expert Committee for Biological Standardization in October, 2015 and 2016. The Committee agreed with the conclusions and proposals, expressed its support for the development of a revision of WHO TRS 926 Annex 2, and looked forward to reviewing progress in 2016-2017. This document provides information and guidance on the biosafety measures required during production and quality control of poliomyelitis vaccine at the final poliovirus containment (Phase III) defined in GAPIII. The guidelines specify 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. Global eradication of wild type 2 virus was declared in September 2015. The global switch from trivalent oral poliomyelitis vaccine to bivalent oral poliomyelitis vaccine occurred in April 2016. The handling and storage of all type 2 poliovirus materials should follow GAPIII and Containment Certification Scheme (CCS) as of August 2016.

Guidelines published by the WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRA) and national authorities for containment (NAC), and for the manufacturers of poliomyelitis vaccine. If a national authority so desires, these guidelines may be adopted as definitive national requirements, or modifications may be justified and made by a national authorities. The national requirements must be consistent with GAPIII and CCS and must ensure that the risks of reintroducing poliovirus to the community are no greater than as outlined in the guidelines set out below.

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Abbreviations

CAG	Containment Advisory Group
CAG-ESG	CAG formed Expert Support Group
$CCID_{50}$	50% cell culture infectious dose
CCS	Containment Certification Schemes
CD155	cluster of differentiation 155
cVDPVs	circulating vaccine-derived polioviruses
CWG	Containment Working Group
ECBS	Expert Committee for Biological Standardization
GAP	Global Action Plan
GCC	Global Certification Committee
GMP	good manufacturing practice
HEPA	high efficiency particulate air
IPV	inactivated poliomyelitis vaccine
IRES	internal ribosome entry
NAC	national authorities for containment
NRA	national regulatory authority
OPV	oral poliomyelitis vaccine
PCR	polymerase chain reaction
PEFs	polio essential facilities
TRS	Technical Report Series
VDPVs	vaccine-derived polioviruses
WHA	World Health Assembly

1. Introduction

The *Guidelines for the safe production and quality control of inactivated poliomyelitis vaccine manufactured from wild polioviruses* were developed in 2003 (*1*) as an addendum to the previous *Recommendations for the production and control of poliomyelitis vaccine (inactivated)* (*2*), specifying the measures to be taken to minimize the accidental risk of reintroducing wild-type poliovirus from a vaccine manufacturing facility into the community after global certification of polio eradication. Revised guidelines for the production of inactivated poliomyelitis vaccine (IPV) (*3*) and oral poliomyelitis vaccine (OPV) (*4*) have recently been established in response to developments in manufacture and the rapid pace of the polio eradication program. Both recognize the need for enhanced biorisk management for production and control of poliomyelitis vaccines after eradication while not providing detailed guidance. Polio vaccines production compliant with current Good Manufacturing Practices (GMP) requirements involves only basic safety practices, and virus containment is not ensured. Many other relevant documents have been produced dealing with the eradication plan, general issues in containment and revised Good Manufacturing Practices guidelines (*5, 6*) including the third revision of the Global Action Plan (GAPIII) to minimize polio facility associated risk (*7*). Some of these documents were developed based on different conceptual frameworks and often use incongruent terminology. Therefore there is a need for explicit but concise guidance on the safety aspects of polio vaccine production that would be consistent with current GMP. The previous version of this document dealt with containment of production of IPV based specifically on wild type strains; the current version is intended to deal with different production platforms and products as defined in the scope in line with the application of a number of recent relevant guidelines and policies on containment (*7, 8*).

The need for a revision of Annex 2 of WHO Technical Report Series (TRS) 926 in the light of a range of associated developments was reported to the Expert Committee for Biological Standardization (ECBS) in October, 2015 (*9*); the Committee agreed with the conclusions and proposals and expressed its support for the revision of WHO TRS 926, Annex 2 (*9, 10*).

2. Background

In 1988 the 41st World Health Assembly endorsed a proposal to eradicate polio by the year 2000 (resolution WHA 41-28) (*11*) and while the target date was missed the number of cases and infected countries fell drastically. There are three serotypes of poliovirus (1, 2, and 3). Wild naturally occurring type 2 virus has not been isolated since 1999, and it was officially declared eradicated by the Global Certification Commission in September of 2015. The last case of polio caused by a wild type 3 strain occurred in Nigeria on 10 November 2012. As of this writing, type 1 poliovirus remains endemic in several countries. Ensuring the absence of poliovirus in circulation requires a prolonged period of intense surveillance because viruses can circulate undetected for several years. This was recently illustrated by two cases of poliomyelitis caused by type 1 poliovirus reported in Nigeria in August 2016. Up to that point Nigeria had been thought free of wild type polio since 2012. Continued emergence of

neurovirulent vaccine-derived polioviruses (VDPVs) demands replacement of OPV with inactivated vaccines and/or with new further attenuated vaccines that will need appropriate characterization and validation to demonstrate they are safer than current OPV. A number of efforts are underway to develop and introduce new vaccine products suitable for post-eradication vaccination programs.

There have been many examples of polio being exported from one country to another. In 2004 vaccination 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 occurred in Yemen and Indonesia as the virus was exported from Nigeria through the hajj. In the past polio was repeatedly introduced into Angola from Northern India and other importations have occurred into China from Pakistan and into Tajikistan, Kazakhstan, Russia, Turkmenistan and Uzbekistan from India. More recently polio was exported from Pakistan to Syria, Egypt and Israel in apparently separate events. This is not a comprehensive list. It is clear that if one country still has circulating poliovirus the world is at risk of re-introduction and this makes containment of vaccine production and control vital to prevent release of the virus into the environment and re-establishment of circulation.

The main tool in the eradication program has been OPV which is demonstrated to interrupt transmission by inducing effective intestinal immunity. Eradication has involved the use of OPV in National Immunization Days and supplementary immunization activities which supplement routine programs where the vaccine is given in association with other childhood vaccines. The strategy has proven to be a highly effective means of eliminating polio in most places where it has been used. The OPV strains replicate in the gut of the recipient and are shed, potentially infecting contacts. This contributes to the contact population's immunization. However where vaccination coverage is suboptimal it is possible for the viruses to regain both transmissibility and neurovirulence and develop into circulating vaccine-derived polio viruses (cVDPVs) leading to outbreaks of poliomyelitis. This has occurred on numerous occasions and has contributed to two specific developments. Firstly as the continued use of OPV presents a risk of inducing VDPVs, there has been a change from the use of OPV for routine immunization to the use of IPV beginning in high income countries and more recently extended to many others. Secondly the majority of cVDPVs that were emerging prior to withdrawal of trivalent OPV and its replacement with bivalent (types 1 and 3) vaccine were derived from type Sabin 2 strain. The type 2 component is also most effective in generating a response in vaccinees and competes with the other serotypes reducing their effectiveness. Polio was eliminated in India when monovalent OPVs and bivalent OPV containing type 1 and 3 but not type 2 were used. Finally on rare occasions OPV can cause chronic infection in immunodeficient persons and the type 2 component is the most common cause. Consequently after the official declaration of wild type 2 poliovirus eradication in September 2015, the OPV2 component was removed from OPV and, starting in April 2016, trivalent OPV-using countries switched to bivalent OPV containing only types 1 and 3. As a risk mitigation measure, immunization with bivalent OPV can be supplemented by the use of IPV. Eventually, after eradication of remaining circulating poliovirus bivalent

OPV usage will be globally stopped and be replaced by the exclusive use of IPV, or other products. If there is a need for an emergency response to an outbreak post eradication stockpiles of monovalent OPV will be released by the Director General of WHO. Once eradication is complete, live polioviruses must be contained or destroyed to prevent reintroduction of the disease and this process has begun in 2015 with the type 2 strains. Production of IPV requires the growth of large amounts of live poliovirus, which is subsequently treated to destroy its infectivity. No outbreak of poliomyelitis has yet been caused by virus release from production facility although accidental releases of poliovirus from IPV production plants have been documented. Safety may depend on several factors, including the production practices that protect the workers from infection, the high level of immunization of the populations against polio, and adequate sewage systems in countries hosting the facilities.

The need for more vaccine at lower prices to satisfy global demand has encouraged efforts to develop manufacturing capability in areas of the world with little previous experience of IPV manufacture, inefficient sewage treatment and higher force of poliovirus transmission. The strategy poses risks and one possible mitigation is to base production on the strains used in OPV. Their use in eradication, lower infectivity for human patients and their lower ability to spread (indicated by an R_0 about five fold lower than the wild type strains) suggests that they should be safer for production. However the occurrence of vaccine derived strains and vaccine associated cases demonstrate the ability of OPV strains to revert to a wild type phenotype so that while their use may contribute to safety it cannot be relied upon exclusively.

A number of strains are being developed by recombinant technology that have been shown to have a better safety profile in laboratory studies than wild or Sabin polioviruses particularly with respect to genetic stability and their ability to infect human subjects. Strains unable to infect humans would be entirely safe. They have been proposed for the manufacture of polio vaccines as well as for conducting quality control tests and epidemiological surveillance. There is clearly a need to recognize, quantify and mitigate the risks of all production platforms in order to contain them appropriately.

The complex nature of the end game of polio eradication is discussed and summarized in the Polio Eradication and End Game Strategic Plan 2013-2018 (*12*) and the framework for the containment of polioviruses is given in the GAPIII (*7*) and the Containment Certification Scheme (CSS, *8*). GAPIII covers the systems and actions required to contain all types of work with polio after eradication and should be read in conjunction with this document.

A phased approach to containment is under way, starting with the requirement for containment of wild type 2 polioviruses on 1 January 2016 and ‘the switch’ away from the widespread use of Sabin type 2 on 1 August 2016. Only bivalent OPV, consisting of type 1 and 3 components is currently used in the oral vaccine, other than in outbreak situations when monovalent Sabin OPV2 may be released (Phase II in GAPIII, *7*).

The destruction of unneeded poliovirus materials and the containment of the remaining poliovirus stocks will be an important consideration in the decision by the Global Certification Committee (GCC) about the eradication status of individual regions and the entire world. To coordinate and oversee containment activities GCC is supported by a Containment Working Group (CWG) that will work with National Authorities for Containment (NAC) to scrutinize certification applications and reports submitted by NAC as they seek to certify their respective polio essential facilities (PEF) based on compliance with GAPIII. According to the CCS (*8*) the responsibility for containment certification of PEFs rests with the NACs in individual countries in coordination with the GCC. Initial certification will result in issuance of a Certificate of Participation (CP), potentially followed by an Interim Containment Certificate (ICC), but all PEFs intending to retain polioviruses will ultimately require a full Certificate of Containment (CC). The certification manufacturing facilities as PEFs will be based on compliance with provisions of GAPIII and all other relevant regulatory requirements, standards and guidelines, including this document.

In 2017 WHO also established Containment Advisory Group (CAG) that includes experts in biosafety and biosecurity. It meets on a regular basis to review and provide guidance on issues that are not fully covered in GAPIII. Vaccine manufacturers and NACs are encouraged to submit their questions and requests to CAG. GAPIII describes in detail containment measures for wild and Sabin strains of poliovirus only, while recently new safer genetically modified strains were developed to limit or eliminate their pathogenicity and transmissibility. These strains were developed to facilitate manufacture and quality control of polio 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 (CAG-ESG) consisting of poliovirus experts who will be advising CAG on specific aspects of pathogenicity and transmissibility of the new strains.

3. Scope

Currently there are three types of polio vaccines: OPV made from Sabin strains, IPV made from wild type strains, and IPV made from Sabin strains. OPV and IPV are conventional vaccines developed and introduced in the 1950s and 1960s respectively. Sabin IPV was recently introduced in Japan and China. Additional manufacturers are working on alternative versions of this vaccine. Other polio vaccine candidates are in different phases of non-clinical and clinical development. They include safer and more genetically stable strains of OPV, IPV made from genetically modified strains with improved biosafety and biosecurity characteristics that may require different biosafety and biosecurity measures, and polio vaccines prepared by novel biotechnology processes not requiring cultivation of live virus. Regardless of the nature of viruses/biological materials used in vaccine manufacture, live polioviruses are required for performing critical quality control tests on vaccines including those produced without growing live virus (e.g. vaccines based on virus-like particles). Some

of the genetically modified poliovirus strains described above could also be used for these quality control procedures.

This guidance concerns the containment needed for the production and quality control of polio vaccines by the following platforms:

1. The production of IPV using wild type strains of poliovirus.
2. The production of IPV from the live attenuated vaccine (Sabin) strains used in the manufacture of OPV.
3. The production of OPV and IPV from novel safer strains developed by genetic manipulation
4. The production of polio vaccines by platforms involving genetic expression systems without virus replication.

The production and usage of trivalent OPV ceased with the withdrawal of the type 2 component and will only resume if widespread usage of trivalent OPV is required. Should this happen the containment issues for production will be the same as before the implementation of containment measures, as it will be a live vaccine for use in children in the face of virulent poliovirus circulating in the environment. The manufacture of OPV for use and stockpile is not within the scope of this document.

This document provides guidance to vaccine manufacturers and relevant national authorities on ensuring biosafety of polio vaccines production and should be read in conjunction with other relevant WHO guidelines (*3-7, 13*).

Detailed and specific guidelines for biosecurity are available in separate guidelines (*7, 14*).

4. Terminology

The definitions given below apply to the terms used in this document. They 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: Areas situated at entrances to or exits from rooms that prevent air in one space from entering another space. Airlocks generally have two doors and a separate exhaust ventilation system. In some cases a multiple-chamber airlock consisting of two interlock doors joined together is used for additional control.

Biological safety cabinet: Both Class II and III cabinets are intended to protect the product, worker and the environment from contamination. Class III cabinets are gas-tight enclosures

with a non-opening view window, with access for materials into the cabinet through a dunk tank or double-door pass-through box that is decontaminated between uses. Both supply and exhaust air are HEPA filtered or incinerated before discharge. Airflow is maintained under negative pressure to protect the workers and environment.

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

Biosafety: Ensuring 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% (CCID50): The quantity of a virus suspension that will infect 50% of cell cultures.

Certification: Systematic, documented process to ensure systems perform in accordance with available certification standards or applicable validation guidance. National certification to this Standard is expected to be performed once a year through responsible national oversight bodies.

Containment: System for confining microorganisms or organisms or other entities within a defined space with controlled access.

Contingency planning: Preparing for future events or circumstances.

Decontamination: Procedure that reduces biological agents and toxins to a safe level.

Disinfection: Process to reduce the number of microorganisms, but not usually of bacterial spores, without necessarily killing or removing all organisms.

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

Good manufacturing practice (GMP): That part of quality assurance which ensures that products are consistently produced as 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 micrometres.

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

Penetrations: Openings through wall, floors, or ceilings to allow access for mechanical services.

Production: The entire set of processes and procedures involved in making of vaccines that includes 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.

Sharps: Devices used in the laboratory, which are capable of cutting or puncturing skin (e.g. needles, scissors and glass).

Sabin strains: Preparations of polioviruses of types 1, 2, and 3 derived by limited number of passages from stocks developed by Dr Albert Sabin (*15*), 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

Production of poliovirus vaccines should be carried out in accordance to WHO recommendations for manufacture and control of IPV (*3*) and OPV (*4*), as well as general requirements outlined in WHO *Good manufacturing practices for pharmaceutical products: main principles* (*5*) and *Good manufacturing practices for biological products* (*6*). In addition, design and operation of polio vaccine manufacturing and testing facilities should comply with poliovirus containment requirements outlined in the WHO Global Action Plan at the time of production to minimize poliovirus facility-associated risk (*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 specific guidance for the assessment of the specific risks associated with vaccine manufacture. Production of IPV and OPV from wild, Sabin, and new genetically modified safer strains raise a set of issues that require additional clarifications for proper alignment of all these documents with GAPIII, serving as in important justification for revising Annex 2 of WHO TRS 926 (*1*). 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 must be reconciled with provisions of current GMP as they apply to manufacture of polio vaccines. Thus, this document should be read in conjunction with other

relevant WHO guidelines such as GAP III (*7*), GMP for biologicals (*5, 6*), Containment Certification Scheme (*8*), WHO laboratory biosafety/biosecurity manual (*13, 14*), and National regulations governing manufacture and control of these products. Any planned deviations should be justified based on risk assessments, and contingency plans should be put in place for dealing with potential accidents. In most countries, the regulation of GMP and biosafety is governed by different institutions. Close collaboration between such institutions is especially important to assure that both product contamination and environmental contamination levels are controlled within acceptable limits.

6. Biosafety implementation within a production facility for IPV derived from the wild-type and the attenuated Sabin strain

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 exposure of personnel by oral and other routes (e.g. via nose or eye) can result in asymptomatic infection and shedding of virus for several weeks. The amount of poliovirus required to infect by oral route is thought to be 1 $CCID_{50}$ for wild type and approximately 10^{1-2} $CCID_{50}$ for attenuated strains (*16, 17*). 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. On these assumptions biosafety procedures may reduce the risk of infection of workers in the plant but cannot remove it altogether. Transmission from the laboratory or vaccine production facility to the community is most likely to result from either equipment failure or human error (*18*). The inadvertent transmission of poliovirus to an immediate contact by an infected vaccine production worker has been documented (*19*).

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

6.1 The polio 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 large-scale polio vaccine production, current GMP and containment, and is independent of production and quality control in his or her reporting structure.

6.2 A detailed and comprehensive risk analysis should be conducted to define possible contamination sources to personnel or the environment that may arise from the production or testing of live poliovirus within the establishment. For each procedure or system, this 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.

6.3 The biosafety aspects of the production process and quality control activities and 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 area(s) 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 safe containment of the poliovirus strains with which they work. The acceptance by staff that the adequate containment of poliovirus is an individual responsibility is a key factor in its implementation and maintenance as described in GAPIII (*7*).

7.2 Health examinations of personnel should be required before the start of employment and periodically there-after following a predetermined schedule. Any changes in health status that could adversely affect the quality of the product or the containment procedures (e.g. immune deficiency) should preclude the person concerned from working in the production or containment areas.

7.3 Personnel working in poliovirus containment facilities, as well as all visitors to the containment facilities, including representatives of regulatory authorities, or civil inspectors, should be immunized with poliomyelitis vaccines, and adequate blood titres of circulating neutralizing antibodies against all three serotypes should have been confirmed prior to their authorization to enter the containment facilities. The antibody titres should be monitored and a booster immunization given as needed.

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 as it relates to polio vaccine production and poliovirus, as well as biosafety procedures. Attention should be paid to ensure that adequate hygienic precautions are taken to minimize the risk of transmission of poliovirus from personnel to their family members and contacts, and that precautions are ready for implementation in the case that such a danger exists.

7.5 Personal protection

7.5.1 Personnel working in the containment facilities should be trained and deemed to be competent in gowning procedure, all operating practices, standard microbiological practices as outlined in the WHO laboratory biosafety manual (*13*), as well as procedures dealing with emergency, 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 must 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 selected 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. 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 removed when leaving the containment facility, and must be decontaminated by a validated procedure before reuse or disposal.

7.5.4 Impervious gloves must be worn at all times in the containment area and discarded as waste for decontamination when leaving the facility. Double gloving is recommended. Outer gloves must be removed and discarded after handling potentially infectious materials. The inner gloves must be discarded on leaving the facility. Where double gloves are not worn, staff must discard gloves after handling potentially infectious materials, disinfect their hands using an adequate procedure, and put on new gloves.

7.5.5 Hands must be washed and disinfected upon leaving the containment area. Hand-washing sinks equipped with automatic (hands-free) controls should be installed in the personnel air lock. All sinks must be connected with 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. However, a recent study has shown that ethanol combined with 2-propanol, citric acid and urea was effective against poliovirus (*20*).

7.5.6 A full-body shower should be available within the personnel exit airlock from the containment area. The use of shower at exit should follow the established procedure and supported by the risk assessment as recommended by the WHO Containment Advisory Group and GAPIII. Shower drains must be connected with a validated waste decontamination system.

7.5.7 An eyewash station should be available within the personnel exit airlock and at other locations based on a risk assessment by the biosafety committee.

Wastewater from eyewash stations within the containment facility must be connected to the effluent treatment system.

7.5.8 Good microbiological technique should be rigorously enforced (*13*). These include but are not limited to:

- no eating, drinking, smoking and applying of cosmetics in the containment area;
- no mouth-pipetting;
- implementing measures to minimize aerosol when manually transferring or mixing materials containing live poliovirus;
- implementing policies for 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 the personnel and the immediate workspace within the containment areas, and segregating the containment areas with physical barriers, effluent treatments, airlocks and pressure differentials that protect the environment external to it from accidental exposure to infectious materials. These systems should also provide adequate safeguards to protect the product against contamination with extraneous agents, and to preclude 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, polio vaccine production facilities where live poliovirus is processed should be in dedicated buildings. If they are located in multipurpose buildings, the production facility must have dedicated separate entrances and exits for personnel and materials, dedicated biological waste handling systems and dedicated air-handling system. Polio quality control laboratories in multi-purpose buildings should be equipped with dedicated air handling and waste disposal systems that preclude the contamination of other areas with material infected with poliovirus.

8.1.3 Use of the poliovirus facility for production of other organisms on a campaign basis may be acceptable provided that a change-over procedure is validated and implemented.

8.1.4 The containment areas for quality control testing and production should be marked with approved biohazard signs. Signs should be posted in prominent locations at the entry to the facility informing that that poliovirus is contained in the area, that immunization against poliomyelitis is required for entry, 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 and kept up to date at all times.

8.1.5 All exits must be marked. Emergency exit doors from the polio facility must be alarmed and their use treated as a breach of containment.

8.1.6 Windows allow visual monitoring of activities in the laboratory and large scale production areas inside the containment zone. Other devices such as close-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.

8.2.2 Biological safety cabinets must 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 must 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 utilized if approved by the responsible national authorities and 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 must be designed in such a way as to not disturb the air balance of the cabinet or the room in which the cabinet is situated.

8.2.4 Wherever possible, manufacturing process and transfer of intermediates should be carried out in closed systems.

8.2.5 In case of production failure/contamination of product or similar reasons that will necessitate discard of a batch, there must be a predetermined and validated method to inactivate the content of the full tank/container. This procedure must be described for all relevant steps in the production process, and must be trained at regular intervals and documented in training records for relevant employees.

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 all potential

routes of entry and exit (e.g. air exhausts, waste lines, etc.). Suitable measures for testing and alarms should also be incorporated into the design and operation of such equipment

8.3 Production facilities

All polio vaccine manufacturing steps that process 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 effective segregation between live virus and inactivation stages to prevent cross-contamination, as required by current GMP.

8.3.1 The areas for the storage of viral seed stock must be segregated and fully secured against entry by non-authorized personnel. For secondary (back-up) seed storage locations where stocks are not normally used for production, the national regulatory authority 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. If stored outside of the containment area, storage of polioviruses must be performed under appropriate containment conditions, as determined by a risk assessment approved by the competent authority (NAC), in line with the approach detailed in the Containment Certification Scheme to support the WHO Global Action Plan for Poliovirus Containment (GAPIII-CCS) for a certificate of containment (CC).

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

8.3.2 Containment areas should be separated from access corridors by separate airlocks for personnel and materials. Airlocks should consist of one or more closed chambers and be equipped with interlocking doors or an equivalent system to ensure that both doors cannot be opened simultaneously. Personnel and material airlocks with doors leading to the containment area should be provided with a ducted ventilation system that exhausts air through a HEPA filter. Adequate time should be allowed for the air handling system to flush out contaminants that have entered the airlock from the containment area before opening the door leading to the exterior. To prevent cross contamination within the containment facility, an area of higher contamination risk (e.g. viral culture and purification area) should be segregated from an area of lower contamination risk (e.g. stage 2 inactivation area).

8.3.3 The containment facility should be in negative pressure compared to non-containment areas, and also compared to the outside of the building. Airflow

patterns should not present a contamination risk. Within the containment areas, the air pressure cascade may be used to ensure that air flows do not disturb poliovirus from a zone of higher contamination risk to a zone of lower contamination risk. An adequate pressure differential should be maintained at all times between zones.

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

- The installation of HEPA filters provides a filter efficiency of 99.97% or greater removal of 0.3-micrometre 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 airtight ducts.
- Although not normally recirculated, HEPA filtered exhaust air may be recirculated to the same containment area. A proper system for maintenance and testing of HEPA filters must be in place. Heat exchangers may be utilized to recover warmth from HEPA-filtered exhaust air. HEPA filter housings to be designed to allow in situ filter isolation, decontamination, and testing. Such filters must be tested and certified upon installation and at least annually thereafter.
- Pressure differential monitoring lines penetrating the containment barrier to be provided with HEPA filtration or acceptable alternative [Not required for containment zones with airtight 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 to be provided with automatic mechanical/ electronic interlocks that prevent sustained positive pressurization of the containment zone. HVAC system and controls to be verified during scenarios simulating failure of system components, including exhaust fan(s), supply fan(s), power, and Class II B2 biological safety cabinet (BSC) exhaust fan(s) (where present), as determined by containment zone design. Exhaust air should provide sufficient air changes in both the quality control and production areas to provide an appropriate level of environmental cleanliness. There should be at least 10 air changes per hour.

8.3.5 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 must 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, must be capped, fitted with liquid-tight gaskets, or connected to a waste effluent decontamination system to prevent inadvertent release to the sanitary drain.
- Wherever possible, provisions should be made to contain liquids leaking from bio-reactors or tanks (including waste tanks) for a volume equal to the maximum fluid contained in the vessels plus the disinfectant required for inactivation.
- All liquid and gas services to the containment area must be protected from back flow based on risk assessment. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters or their equivalent.

8.3.6 If circulating water with open taps is used within the containment area, a spill or contamination at the point of use should not result in a breach of containment via the water system. Water loops should be maintained at an appropriate elevated temperature (e.g. 80°C is widely used) and dead legs should be avoided. Heat exchangers may be used to cool water at the point of use provided that water from the exchanger is not returned to the loop leading outside of the containment area. If there is an accidental release of poliovirus in the areas served by the water loop, or if the circulating temperature of the water system drops below its set point to an extent identified by the risk assessment, an alarm should sound and the system temperature should be raised to a temperature and for a time that have been validated for the ability to kill poliovirus before taps can be opened outside or inside the containment area.

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

8.3.8 Emergency lighting and power to the containment area and critical containment devices should be available.

8.3.9 In the event that live poliovirus is to be removed from the facility this will be done through use of a dunk tank, decontamination chamber or other validated mechanism that ensures the exterior surfaces of any packaging materials used is 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 facility prior to decontamination and disposal, they must not be transported through

public areas and must be packaged, labelled and transported in accordance with applicable regulations.

8.4.2 The containment facility must be provided with one or more inter-locking, double door pass-through autoclaves, the performance of each of which is validated at least annually. Autoclave condensate drains located outside the containment barrier to have a closed connection and be 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 to be validated prior to initial use and when significant changes to the processes are implemented or introduced.

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

8.4.5 Large equipment in the containment areas, such as bioreactors, stainless steel vessels, and transfer lines, should be decontaminated, cleaned and sterilized in-place.

9. Documentation and validation

9.1 Detailed records of operating parameters for the containment facility should be produced and maintained for conducting assessments of the 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 facility and applicable operating procedures in a scheduled and verified manor.

9.3 The production facility and equipment must be designed and constructed in such a manner as to allow for full validation and verification of containment processes. It is the responsibility of the polio vaccine manufacturers to ensure that these facilities and equipment meet acceptable standards that will ensure containment of poliovirus as well as the protection of the staff and the environment. Tests should be carried out at the completion of 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 polio 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 not limited, to the following:

- integrity of containment perimeter, including penetrations through floors, walls and ceilings;
- integrity of the vessels, transfer pipes and other production equipment to prevent the exposure of poliovirus to the room environment;
- air-tightness of supply and exhaust ductwork between incoming and first outgoing HEPA filter ducting in the air handling systems. The duct should be considered to be part of the room up to the point of the disinfecting filter or incinerator;
- integrity of all HEPA filters and high efficiency filters and filter housings;
- directional inward air-flow from non-contained areas to containment areas;
- biological safety cabinets and all primary containment devices;
- autoclaves for decontamination, including heat distribution and penetration studies, and biological challenge reduction studies if appropriate. Validations studies should consider the worst case for load configurations;
- waste effluent systems and holding tanks;
- air, liquid and gas back-flow prevention devices;
- alarm systems for air system failures, room pressure failures, electrical failures and failures of waste treatment systems;
- fire suppression devices and alarms; and
- communication systems.

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. Data generated will facilitate the biosafety management within the production and testing facilities.

9.5 Data sheets and associated materials that have been used in containment areas must 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

Production of polio 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, must be in compliance with each of the relevant sections of these 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 (e.g. viral culture and purification area)

to an area of lower contamination risk (e.g. 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, prior to be placed in secondary packaging materials. The packaging procedure, with or without a disinfection procedure, should ensure that the outside of the packaging materials are free of infectious poliovirus. If a disinfection procedure is used, 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 the complete inactivation using a validated test approved by the national regulatory authority, 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 the complete inactivation, comply with the specifications approved by the NRA. The battery of tests should include the integrity test of the 0.2 micrometer filters used before and during the inactivation, formaldehyde content and poliovirus loads at one or more time points during the inactivation. The specifications for these tests should be adequately justified scientifically and statistically.
- A formal risk assessment is performed to identify all materials that may be contaminated in case of an incomplete inactivation, and a formal procedure is in place to decontaminate all potential contaminated materials in case of an incomplete inactivation.
- A procedure is in place to quarantine the IPV monovalent bulk transferred out of the containment facility until the completion of all quality control testing required in Annex 3 of WHO Technical Report Series (*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:

11.2.1 The non-dedicated quality control laboratories are within the containment facility.

11.2.2 All non-poliovirus related activities performed within the containment laboratories and all personnel admitted into the containment laboratories adhere to GAPIII requirements.

11.3 If quality control laboratories are housed within the production facility to enhance containment control, they must 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 unwrapped using validated 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 in 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 (EM), taken from the containment areas are unlikely to contain live poliovirus, and may be tested outside the containment laboratories. However, a risk assessment should be performed to identify the likelihood of exposure of the samples to live poliovirus at the sampling locations, and to recommend necessary precautions covering sample handling, transportation and disposal.

11.6 The Absence of Infective Poliovirus Test performed on the IPV monovalent bulk (*3*), which has been transferred out of the containment production facility as described in section 10.2.2 of these guidelines, may be performed outside of the containment laboratories. However, the positive control of the test or 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. Extensive investigation and revalidation of the inactivation process are required if infective poliovirus is detected in this test, 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. Special care should be taken in laboratories working with animals in line with GAPIII recommendations. Species susceptible to poliovirus infection, including transgenic mice expressing the human poliovirus receptor, should be treated as infectious or potentially infectious materials following infection with virus samples. This affects all aspects of work including handling, transporting, storage, inventory, etc. and includes all animal materials such as tissues, blood, carcasses, stools, etc. The risk of infected animals escaping the facility should be assessed and managed as this represents a potential threat of exposure to infectious agents for community members. An animal-care manager should be designated with responsibilities conforming to requirements set out in these guidelines and other relevant documents. The animal-care manager would normally have an in-depth knowledge of animal handling, and zoonotic and animal diseases. The animal-care manager should liaise with other personnel (e.g. biorisk management adviser, 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 guided by risk assessments and will meet all poliovirus containment criteria set out in this document.

11.8 Manual manipulations of live poliovirus on growth-permissive cell substrates should be considered as high- risk activities, and contained within the biological safety cabinet.

11.9 Special consideration should be given to providing adequate space within the containment area for storage of samples that may contain live poliovirus.

12. Emergency procedures

Production of polio vaccine using live polioviruses under containment requires planning for emergencies that could result in the release of live poliovirus within the facility or into the surrounding environment. Failures 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. Response to emergency and

contingency plans must be established based on risk assessment and comply with requirements outlined in GAPIII to minimize the impact and consequences of such accidents.

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 exposure of persons to poliovirus.

12.1.1 The immediate response to a spill due to equipment failure, such as vessels or transfer pipes, should be to evacuate the premises and return with clean-up personnel no sooner than 30 minutes after the incident, to allow time for aerosols to settle.

12.1.2 Staff and emergency personnel should be supplied with protective equipment (e.g. respirators, coveralls and gloves) prior to entering containment areas within the production and quality control units. This equipment should be available in sufficient quantities at the entrance to the facilities, kept in good working order, and personnel should be instructed in its use.

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

12.2 Emergency equipment such as disinfectants and other clean-up materials for spills should be available in sufficient quantities for use in response 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 persons 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 to 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 must be supplied with adequate protective clothing and equipment to ensure that they do not become infected with poliovirus in the course of their duties. Such protective clothing and equipment must 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.

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 that incident should be fully investigated and documented, and revisions made to existing procedures, contingency plans and staff training as necessary to minimize its repetition.

12.6 Any major spill, accident, or suspected or confirmed poliovirus infection occurring in the area surrounding a polio vaccine testing or manufacturing facility should be considered an urgent international public health emergency. NAC and WHO should be notified without delay.

13. Risk assessment of new safer strains of poliovirus

Biosafety and containment measures described above and contained in GAPIII guidance were developed based on well-known biological characteristics of wild and attenuated (Sabin) strains used in manufacture and control of polio vaccines. They include their ability to induce disease and to transmit from person to person. Since attenuated viruses used for production of oral vaccine can revert to virulence and regain the ability to transmit and cause outbreaks of paralytic disease, these measures reduce risks but not eliminate them completely. This is why new strains were developed by genetic manipulation based on detailed knowledge of poliovirus biology with the specific purpose to stabilize their attenuated phenotype and to limit their ability to infect humans and spread in populations. These strains were proposed for making new genetically stable OPV, for manufacture of IPV, and for performing quality control tests to minimize or eliminate risks of restarting poliovirus circulation. Introduction of these strains would not only significantly mitigate consequences of accidental release of poliovirus, but also could simplify handling of virus stocks and ultimately reduce the cost of vaccine manufacture and increase vaccine supply.

The containment measures appropriate for the new strains must be defined based on risk analysis performed on a case by case basis. If proven to be considerably safer than attenuated Sabin strains they can, after approval by the NAC, be handled under containment conditions less stringent than the ones described above or in GAPIII. WHO CAG with its constituent Expert Support Group (CAG-ESG) will review 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 vaccine production and quality control.

The evaluation may consider the following elements:

13.1 Vaccine manufacturer proposing to handle strains with reduced virulence and transmissibility under less stringent containment conditions must perform risk analysis and present it to the appropriate National and International authorities (NAC, CAG/CWG, etc.) for approval. Risk analysis should be based on biological properties of the strains in conjunction with the intended use and the design of the facility it will be used in and the proposed handling procedures.

13.2 Evaluation of pathogenicity can be done based on known in vitro markers as well as experiments in laboratory animals. Several molecular structures within poliovirus genome (e.g. in IRES element) were shown to be good predictors of neurovirulence.

Animal models that could be used for evaluation of pathogenicity include primates (Rhesus and Cynomolgus macaques) and transgenic mice expressing human poliovirus receptor CD155 (*21, 22*). There are validated tests in both animal models that were recommended by WHO Expert Committee on Biological Standardization for lot release of OPV (*4*) and that could also be used to demonstrate superior safety of new poliovirus strains. Such experiments should include attenuated Sabin strains as the benchmark.

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

13.3 Genetic stability is an important indicator of safety of vaccine strains, because replication in vitro and in vivo usually leads to reversion of attenuated phenotype and 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. Molecular approach is based on 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 (*23, 24*).

13.4 There are no validated tests for transmissibility of poliovirus. However, it could 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, its inability to grow at higher temperatures all could suggest that virus transmission will be restricted. It is possible to develop virus derivatives unable to replicate in normal cells, but that could grow in engineered cell cultures expressing factors enabling virus replication. The 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 on no virulence in susceptible animal models, genetically stable upon passage, unable to transmit or replicating only in specially designed cell cultures could be handled at conditions less strict than those described above for wild and Sabin strains.

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