

Poliovirus containment

GUIDANCE TO MINIMIZE RISKS FOR FACILITIES COLLECTING, HANDLING OR STORING MATERIALS POTENTIALLY INFECTIOUS FOR POLIOVIRUSES

NOTE: The original title '*Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses*' was changed to ensure that this guidance is implemented not only by non-poliovirus, but also by poliovirus facilities.

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ABBREVIATIONS AND ACRONYMS

AFP	Acute flaccid paralysis
CAG	Containment Advisory Group
CCID	Cell culture infectious dose
CCID ₅₀	Cell culture infectious dose, 50% endpoint
CCS	GAPIII Containment Certification Scheme
CC	Certificate of containment
CP	Certificate of participation (in the containment certification process)
ICC	Interim certificate of containment
cDNA	Complementary DNA
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent assay
GAPIII	Global Action Plan III for Poliovirus Containment
GCC	Global Commission for the Certification of the Eradication of Poliomyelitis
IPV	Inactivated polio vaccine
MoH	Ministry of Health
NAC	National authority for containment
NCC	National Certification Committee for Poliomyelitis Eradication
OPV	Oral polio vaccine
bOPV	Bivalent oral polio vaccine (containing attenuated Sabin poliovirus type 1 and type 3)
mOPV	Monovalent oral polio vaccine (containing one type of attenuated Sabin poliovirus)
mOPV2	Monovalent oral polio vaccine type 2
OPV2	Oral polio vaccine type 2
tOPV	Trivalent oral polio vaccine (containing attenuated Sabin poliovirus type 1, type 2 and type 3)
PCR	Polymerase chain reaction
PEF	Poliovirus-essential facility
PIM	Potentially infectious material
PV	Poliovirus
PV1	Poliovirus type 1
PV2	Poliovirus type 2
PV3	Poliovirus type 3
RI	Routine immunization
RNA	Ribonucleic acid
SIA	Supplementary immunization activity
VDPV	Vaccine-derived poliovirus
aVDPV	Ambiguous vaccine-derived poliovirus
cVDPV	Circulating vaccine-derived poliovirus
cVDPV2	Circulating vaccine-derived poliovirus type 2
iVDPV	Immunodeficiency-associated vaccine-derived poliovirus
VDPV2	Vaccine-derived poliovirus type 2
WHO	World Health Organization
WPV	Wild poliovirus
WPV1	Wild poliovirus type 1
WPV2	Wild poliovirus type 2
WPV3	Wild Poliovirus type 3

PREFACE

This Guidance intends to facilitate the identification of materials potentially infectious for polioviruses within laboratories that handle human stool specimens, respiratory samples or environmental sewage. Depending on the place and time of collection, these materials may contain infectious polioviruses, which are eradicated (type 2) or nearly eradicated (types 1 and 3) in the wild. Identifying, eliminating the risk through destruction, or mitigating the risk of handling such materials is essential not only to maintain the safety of laboratory workers and their communities but also for the success of the global polio eradication effort.

INTRODUCTION

The Global Polio Eradication Initiative, launched in 1988, has been the largest international public health effort ever undertaken (1). Billions of children have been immunized and millions of paralytic poliomyelitis cases have been prevented through donations from individuals and organizations, the dedicated efforts of governments at all levels, and countless volunteer hours (1).

In 2015, the Global Commission for the Certification of the Eradication of Poliomyelitis (GCC) certified the eradication of wild poliovirus type 2 (WPV2) (2). The eradication of wild poliovirus type 1 (WPV1) and wild poliovirus type 3 (WPV3), and the elimination of circulating vaccine-derived polioviruses (cVDPVs) is anticipated in the near future (3), along with the gradual disappearance of immunodeficiency-associated vaccine-derived poliovirus (iVDPV) excretors. At that point, the only remaining poliovirus (PV) reservoirs will be the facilities retaining PV infectious or potentially infectious materials (PIMs) (2, 4-6). Nations responsible for these facilities must assure the world that these reservoirs do not present a post-eradication risk of re-emerging paralytic disease due to polioviruses that could undermine this extraordinary humanitarian achievement.

In May 2015, the World Health Assembly voted to provide risk reduction guidance for PV facilities by endorsing the *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; 2, 7, 8)*. As these facilities work with PV, they have the advantage of being aware of the nature of the agents, the operational risks, and the effective containment measures to reduce those risks.

Facilities that collected, handle and store clinical and environmental samples for purposes other than polio research present a PV transmission risk if the samples were collected where wild poliovirus (WPV) or vaccine-derived poliovirus (VDPV) was circulating, or oral polio vaccine (OPV) was being used. These facilities are at a disadvantage in that the potential presence of an infectious PV in such samples is both undesirable and uncertain.

Facilities that may possess PV PIM include those working in diarrhoeal and respiratory disease research, nutritional research and other human research areas that involve using faecal and respiratory samples, and environmental research areas using concentrated raw sewage (4, 9-15). Areas of particular risk include (but are not limited to) enterovirus, rotavirus, norovirus, hepatitis A and E, and enteric bacterial agents including *E. coli*, *Shigella*, as well as respiratory agents such as influenza, measles and other respiratory samples.

PURPOSE

The purpose of this Guidance is to assist facilities in assessing the risk of PV PIM in their possession and to implement appropriate risk reduction consistent with GAPIII.

At the time of publication, this Guidance is in effect for all type 2 PV PIMs. Countries and facilities are also encouraged to identify and report poliovirus type 1 (PV1) and poliovirus type 3 (PV3) PIMs in anticipation of eradication completion and bivalent oral polio vaccine (bOPV) cessation, at which time this Guidance will apply to all polioviruses.

RATIONALE

Transmission of the three serotypes of PV is maintained by the person-to-person infection of humans, with no evidence of an extra-human animal reservoir (16). Most PV infections are asymptomatic, with paralytic poliomyelitis occurring in less than 1% of WPV infections (16). A reported community outbreak of 10 paralytic poliomyelitis cases may be the result of 1000-10 000 asymptomatic infections (4). Any faecal, respiratory secretion or concentrated sewage samples collected in the community during that time and stored by a facility for whatever purpose are considered PV PIM, which include:

- faecal or respiratory secretion samples and their derivatives (e.g. stool suspensions, extracted nucleic acids, etc.) collected for any purpose in a geographic area where WPV/cVDPV is present or OPV is being used at the time of collection;
- products of such materials (above) from PV-permissive cells or experimentally infected polio-susceptible animals (17-19);
- uncharacterized enterovirus-like cell culture isolates derived from human specimens from countries known or suspected to have circulating WPV/VDPV or use of OPV at the time of collection;
- respiratory and enteric virus stocks derived from PV PIM and handled under conditions conducive to maintaining the viability or enabling the replication of incidental PV; and
- environmental samples (i.e. concentrated sewage, waste water) collected from areas known or suspected to have circulating WPV/VDPV or use of OPV at the time of collection.

Because no diagnostic test is 100% sensitive, and available tests may differ widely in their sensitivity and degree of validation, it is impossible to exclude the presence of PV in a given sample.

The non-PV facility with PV PIM collections is similar to the PV facility in that:

1. Both are possible sources of facility-associated transmission.
2. Both require facility-specific risk assessments, based on the type of PIM, the procedures used and facility safeguards.
3. Both must implement measures to reduce risks.

The non-PV facility is different from the PV facility in that:

1. PV is not its field of work.
2. PV may be encountered only as an incidental, undesirable agent.
3. PV may be present in clinical samples at varying rates and moderate titres.
4. PV titres are usually not enriched by agent-specific procedures.
5. Historic PIM collections are retained for special studies.

The inclusion of all facilities with PV PIM in global PV containment efforts is crucial. Any possible advantage of a facility's lower facility-transmission risk could be wholly offset by the facility worker who

is uninformed, unaware or unconcerned about PV PIM risks, or untrained in procedures to reduce those risks (4, 20).

Whether originating from a PV or non-PV facility, the global health and economic consequences of facility-associated PV transmission are the same.

Risk is defined in this Guidance as the potential for release of PV from the facility into a polio-free community.

STRATEGY

The global strategy for minimizing risks from the non-PV facility is aligned to the one outlined for the PV facility in GAPIII: 1) risk elimination by PV PIM destruction, inactivation or transfer to a poliovirus-essential facility (PEF) in the same or a different country/region; and 2) biorisk management by those facilities that retain PV PIM and meet the required safe-handling and containment requirements.

Risk elimination: The goal is no PV PIM. Facilities should carefully consider the required resources and set a high bar when deciding on whether to retain PV PIM collections, particularly those with WPV/VPV potential. The scientific value of retaining a specific PV PIM sample collection should be carefully weighed against the public health value of its destruction. Often, the scientific value of PIM collection may be retained via inactivation, fixation or nucleic acid extraction.

Biorisk management: Facilities electing to retain scientifically valuable PV PIM collections should be familiar with and prepared to meet biorisk management standards adequate for risk mitigation, addressing accidental exposure and release, as well as the loss, theft, misuse, diversion, unauthorized access or malicious release of PV PIM.

For PV PIM collections with WPV/VPV potential, the requirements are described in GAPIII, Annex 2, *Biorisk management standard for poliovirus-essential facilities holding wild poliovirus materials*. **These are stringent standards as required for an eradicated agent and should be in place** when working with these PV potentially infectious collections. Alternatively, nucleic acids may be extracted from PV PIM or the materials may be inactivated using an appropriate method (21). However, these procedures must be performed within proper containment.

For PIM collections with OPV/Sabin potential, facilities must meet the biorisk management standards described in this publication.

Responsibility for compliance lies with the facility and its respective national authorities (e.g. the Ministry of Health [MoH]), in coordination with National Certification Committees (NCCs), National Polio Containment Coordinators and other relevant stakeholders where applicable.

IMPLEMENTATION

Containment timelines are described in detail in GAPIII and consist of three phases, leading to the containment of all WPV/VDPV, OPV/Sabin strains, and OPV derivatives, which will occur when PV eradication is complete.

PV type 2 (PV2) containment is already in progress and includes all WPV2 and oral polio vaccine type 2 (OPV2)/Sabin type 2 viruses (2). WPV2 was declared eradicated by the GCC in 2015. Trivalent oral polio vaccine (tOPV, active against PV types 1, 2 and 3) was replaced with bOPV (active against PV types 1 and 3) in 2016 to reduce the number of OPV2-associated paralytic poliomyelitis cases and circulating vaccine-derived poliovirus type 2 (cVDPV2) outbreaks (2). Type 2 is the most transmissible of the three OPV/Sabin strains (4). Monovalent oral polio vaccine type 2 (mOPV2) has been used in supplemental immunization activities (SIAs) in certain countries to interrupt cVDPV2 outbreaks (Annex 2). At the time of tOPV withdrawal, inactivated poliovirus vaccine (IPV) was introduced in routine immunization (RI) programmes in select high-risk countries to maintain immunity for PV2 (22). As a consequence of these actions:

- the inventory to identify facilities with PV2, the destruction of unneeded PV2 material or transfer of the material to a PEF, and the preparation for PV2 containment in facilities retaining such material are nearing completion for PV facilities;
- the containment of WPV2, vaccine-derived poliovirus type 2 (VDPV2) and OPV2/Sabin2 strains in PV facilities is in progress and running in parallel;
- the **implementation of risk reduction actions by facilities for PIM with PV2 potential is a matter of urgency**: the GCC set the deadline for completion of the identification, destruction, transfer or containment (Phase I) for all PV2 at one year after the publication of this Guidance; and
- the final containment of all WPV/VDPV and OPV/Sabin PVs, of all three serotypes, will commence when global WPV transmission has not been detected for a minimum of three years (the standard for certification), followed by the planned cessation of bOPV use.

CATEGORIZATION OF POLIOVIRUS PIM ACCORDING TO RISK

The evidence-based rationale for categorizing sample collections according to relative risks is derived from data provided in this document in *Rationale, Risk factors for the categorization of poliovirus PIM into risk groups* and Annex 2 (*Country- and territory-specific poliovirus data*).

The PV transmission risk of PV PIM collection is a product of multiple elements, including the nature of the sample collection (when, where and what was collected), the PV(s) that may be present (WPV/VDPV or OPV/Sabin), the hazards related to the laboratory procedures being used, and worker/community susceptibility (4).

PV PIM sample collections may be categorized into one of two divergent risk groups based on PV virulence and transmissibility. Of greatest risk are collections with potential for WPV/VDPV, which are the target viruses of the Global Polio Eradication Initiative. Of lower risk are collections with potential only for OPV/Sabin PV and related strains, which have been used to immunize untold numbers of children for more than 50 years (4).

Despite the safety record of OPV in RI programmes, all three attenuated PV types in the vaccine have been linked to rare vaccine-associated paralytic poliomyelitis (23). Further, under certain conditions of low immunization rates of populations in high-risk environments, the prolonged replication of OPV/Sabin PV can lead to a loss of attenuation and the production of VDPV (23, 24). cVDPVs pose a

public health threat, as outbreaks of paralytic poliomyelitis that clinically were indistinguishable from WPV infection have occurred due to each PV serotype, with more than 90% of cVDPV outbreaks associated with VDPV2 (23, 25). People with primary B-cell immunodeficiencies exposed to OPV can develop a chronic PV infection leading to iVDPV (23). While iVDPV has not been identified as the source of a PV outbreak, the prolonged shedding of virulent strains of PV represents a potential threat to the global eradication of PV. Ambiguous vaccine-derived polioviruses (aVDPVs) are isolates from people without a known primary B-cell immunodeficiency or from environmental samples (e.g. concentrated sewage) with an unknown human source, neither of which is genetically linked to another VDPV (23).

RISK FACTORS FOR THE CATEGORIZATION OF POLIOVIRUS PIM INTO RISK GROUPS

The PV transmission risk of PV PIM collection is a product of multiple elements, including the conditions under which the samples were stored, the nature of the sample collection (when, where and what), the PV(s) that may be present (WPV/VDPV or OPV/Sabin), the hazards related to the laboratory procedures, and worker/community susceptibility (4).

PIM risk divides naturally into two widely divergent risk groups based on PV virulence and transmissibility. Collections with potential for WPV/VDPV are highest risk and are required to be stored and handled only within PEFs. Collections with potential only for OPV/Sabin PV and related strains present lower risks and may be handled under specific conditions within non-PEFs. These groups do not overlap. However, within each group, factors may raise or lower the risk of facility-associated transmission. **All facilities that propose to retain PV PIM collections should prepare a thorough risk assessment, with the objective of minimizing the risks of PV release back into polio-free communities.**

After eradication, susceptibility may change as immunization policies and coverage change.

What samples were collected

Infection of humans with WPV is predominantly via the faecal-oral route (16). OPV is administered orally. Ingestion of either form of PV by a non-immune person leads to an initial brief infection in the throat followed by a more prolonged infection of the gut epithelium (4). A short period of viraemia may occur during the early phase of infection (4). In rare instances, the virus may cross the blood-brain barrier and lead to meningitis or paralytic poliomyelitis, depending on the site of virus replication (4). PV may replicate in the gut without an initial throat infection (4). The following describes the relative risk of different sample types.

Faeces: PV isolation rates vary widely in samples collected from asymptomatic subjects in a time and place where WPV/VDPV or OPV-derived viruses were in circulation or where OPV was in use. A stool survey of asymptomatic children in Cartagena, Colombia in 1989 reported a WPV isolation rate of 8% (26), while the highest rate reported in a similar survey was 19% in Mumbai, India in 1994 (27). A survey of asymptomatic persons of all ages in index households and neighbouring households in Uttar Pradesh, India in 2009 found 4.8% were shedding WPV. The same study reported a 2.4% stool-positive rate for any PV in Bihar, India (28).

Incidental PV in PIM has been found in stool samples stored for more than 20 years in a gastroenteritis laboratory. In the first collection of 82 samples, viable WPVs were recovered from six samples and Sabin PV was recovered from one sample (9% in total) (29). In the second collection, six Sabin PVs were recovered from 183 samples (3%) (29). Because of extensive immunization campaigns, Sabin PVs may be incidentally detected in stool samples of acute flaccid paralysis (AFP) cases, even though the Sabin

PV is not a cause of the paralysis (4). In 2016, for example, Sabin PV was detected in 5.2% of 241 999 stool samples collected globally for AFP surveillance (30).

WPV strains present the greatest transmission risk, with an estimated human minimum infectious dose of 100-fold less than for OPV strains (~ 10 CCID₅₀ for WPV vs $\sim 10^3$ CCID₅₀ for OPV strains) (4). Epidemiologic models and field studies estimate the transmissibility for WPV/VDPV to be more than 10-fold greater than for OPV (4). The secondary spread of WPV was reported to approach 90% among susceptible contacts in family and institutional settings, with the secondary spread of OPV strains less than half that (4).

OPV circulation in the community rarely exceeds three months after an immunization campaign (31-33). Immunologically naïve subjects may shed WPV/VDPV, OPV or OPV-derived viruses over a range of cell culture infectious doses (CCIDs) up to 10^6 CCID₅₀/g stool (mean $\sim 10^4$ CCID₅₀/g stool) for six weeks to three months, although shedding duration may sometimes be less for OPV/Sabin strains (4). PV reinfections of the gut may occur, depending on the virus challenge dose and the length of time since the receipt of OPV or natural infection. The virus concentration and duration of faecal shedding is generally lower on reinfection (4). IPV immunization has little or no effect on the susceptibility of the gut to PV infection (4, 34).

Nasopharyngeal, oropharyngeal and other upper respiratory tract secretions: Similarly, WPV/VDPV and OPV/Sabin viruses may be recovered from respiratory secretions of naïve subjects at equivalent concentrations for a period of 2-6 days post-infection (4). Virus shedding wanes and usually disappears at 7-10 days post-infection, coinciding with the appearance of serum antibodies (4). The virus is rarely recovered from respiratory secretions after a WPV or OPV challenge of persons with measurable serum antibody, including IPV recipients (4). Based on the limited duration of post-infection virus shedding and the absence of shedding on reinfection, the probability of recovering PV from respiratory secretions in surveys is estimated to be less than 1%, or at least 10-fold less than from stool samples (4). During a community survey in Bihar, India in 2009, PV-positive rates for respiratory samples were 0.1%, ~ 20 -fold less than for stool samples (2.4%) (28).

Sewage: PV recovery from raw sewage usually involves some form of entrapment or sample concentration (e.g. filtration, centrifugation or phase separation). The recovery of WPV or OPV/Sabin has been reported from raw sewage samples, but the concentration of infectious virus is usually <1 CCID₅₀/ml, well below the estimated infectious dose for either OPV strains or WPV (4, 13, 34-37). The PV content of sewage concentrates may be several logs higher, depending on the method employed (4).

Cerebrospinal fluid (CSF), serum and blood: PV is rarely recovered from CSF (4, 38). Blood samples yield WPV in less than 25% of infected persons with levels usually low (<50 CCID₅₀/ml) (4). A similar low-level viraemia pattern in OPV recipients has been observed for Sabin type 2, but no viraemia has been reported for Sabin types 1 and 3 (4). Consequently, collections of CSF, serum and blood samples are not considered PV PIM.

Sample storage conditions

Poliovirus in clinical or environmental specimens survive indefinitely in the laboratory freezer (<-20 °C), for many months in the refrigerator, and for hours to days on the bench top (4).

Who were samples collected from

Age of subjects: Children aged under 5 years are the group most often infected during a WPV epidemic and are the target population for RI programmes and multiple OPV campaigns. Children aged 6-15 years are rarely included in OPV campaigns, but may be infected or reinfected by WPV or OPV-derived viruses circulating in the family or community (4). The reinfection of immunologically experienced adults and older children is less likely, but appears to be a function of virus dosage (4). Reinfections of older

children or adults rarely result in virus recovery from throat samples, and faecal shedding may be greatly reduced in virus content and duration (4).

When and from where samples were collected

The “when and from where” of the collection indicates the likelihood of PV being present. Annex 2 provides country-specific PV data for the time of the last estimated presence of WPV, the time of the last estimated presence of VDPV, and the last use of OPV/Sabin, by PV type, for any given country.

Laboratory hazards

Inoculating/harvesting PV-permissive cells: Attempts to isolate other infectious agents from PV PIM collections using PV-permissive cell cultures (Annex 1) may result in an enhanced PV content of up to 10^8 CCID₅₀/ml (4, 39). This possible greater than 10^5 increase in virus concentration over the original clinical sample greatly increases the risk to the laboratory worker, particularly if the identity of the amplified incidental PV is unrecognized.

Full-length PV ribonucleic acid (RNA) can infect permissive cell lines, facilitated by using transfection reagents (40, 41). Unknown to the laboratory worker, the extraction of nucleic acid from PV PIM could coincidentally co-purify PV RNA. The subsequent transfection of the RNA into PV-permissive cells may generate infectious PV particles, possibly at high titres (41).

Aerosol-generating laboratory procedures: Procedures that may create aerosols through the release of liquids under pressure (sprays), dropping or breaking containers, the mixing of suspensions, mechanical blending, shaking or pouring constitute a high risk (4). The survival of PV in the laboratory environment is favoured by higher initial titre, lower temperatures, a moist environment, and the presence of stabilizing material such as organic matter (4). The laboratory worker may be infected directly through ingestion of droplets or indirectly through contaminated work surfaces or clothing (4). High-content PV materials (high titre and/or high volume) represent the highest risk.

Facility effluents

The risk of community exposure through liquid effluents generated within the facility requires a facility-by-facility assessment and will depend on potential PV content, the nature of the sewage system and the potential for human consumption (4). However, if the laboratory works with only OPV/Sabin PIM without further replication of incidental PV and adheres to good laboratory practices, the community risk is very low (4).

Worker/community susceptibility

The facility/laboratory worker: For OPV recipients, reinfection of the gut is a function of the time between OPV or natural infection and the challenge virus dosage (4). IPV provides a high level of pharyngeal protection but little or no immunity to gut infection (4). IPV recipients are not at risk of paralytic poliomyelitis, but could be at risk of transmitting WPV or OPV/OPV-derived viruses to their family and community through PV-contaminated skin or clothing, silent infections of the gut, or work practices that may contribute to contamination of facility effluents (4).

Community vaccine coverage: The risk of outbreaks from laboratory-associated transmission is inversely proportional to population immunity. Risk may be assessed by percent vaccine coverage of children aged under 5 years (4).

Facility location: Facility location should be taken into consideration if the facility is situated near high-risk populations with a potentially elevated force of infection (high population density, inadequate hygiene standards, high birth rate and suboptimal immunity) (4).

BIORISK MANAGEMENT OF POLIOVIRUS PIM

Faecal, respiratory, concentrated sewage samples or derivatives of such samples may be potentially infectious for PV if they have been **stored under conditions that maintain the viability of PV** (Annex 1). If these samples were collected in/from a place and at a time when WPV or VDPV was in circulation (Annex 2), they are **WPV/VDPV PIM**, and are **subject** to the **full containment** described in GAPIII and **must be stored and handled in a PEF certified by a national authority for containment (NAC)**, as briefly outlined in section A below. If WPV/VDPV were not in circulation, but OPV was in use (Annex 2), these samples are **OPV/Sabin PIM** and may only be handled outside a PEF under the conditions described in section B below.

Samples that are unlabelled, mislabelled or for which the origin, type, date of collection or ownership are unknown, should be inactivated or destroyed following procedures effective against PV.

The retention of PV PIM is subject to the agreement of the responsible national authorities (MoH).

A. Collections with potential for WPV/VDPV

Facilities with WPV/VDPV PIM that do not plan to become a PEF must destroy, inactivate or transfer the materials to a PEF. The retention of samples potentially infectious for WPV/VDPV must be approved by the responsible national authority, and **subjects the facility to the approval of the NAC and GCC, following the GAPIII Containment Certification Scheme (CCS)**. Implementation of the following is required:

1. The responsible national authority (e.g. MoH) agrees to the retention of these materials.
2. The facility engages in the certification process against GAPIII requirements and applies to the NAC for a Certificate of Participation (CP) in the certification process described in the CCS (42).
3. A facility that is granted a CP is expected to continue the certification process as described in the CCS, and is allowed to continue the retention of relevant materials during the certification process, as described in the CCS.
4. The facility holding a CP for the retention of WPV/VDPV materials demonstrates compliance with the requirements described in GAPIII, Annex 2, and applies to the NAC for a certificate of containment (CC) against GAPIII, as described in the CCS. During the PV2 containment period, as described in the CCS, an interim certificate of containment (ICC) will be issued to a CP-holding PEF if the NAC determines the facility does not meet all the requirements for full containment certification but has the ability to address the non-conformities identified. Once an ICC or a CC is obtained, the facility is certified as a PEF.
5. A facility that is not designated to retain PV materials post-eradication has the option to destroy, inactivate or transfer the relevant materials to a PEF.
6. The validity of a CP/ICC/CC is of limited duration and subject to regular reassessments, as described in the CCS.

PV nucleic acid extracted from WPV/VDPV infectious or PIM using methods demonstrated to inactivate poliovirus, or synthesized RNA, or complementary DNA (cDNA) can be handled outside of PV containment under the condition that these materials will not be introduced into PV-permissive cells or animals with or without a transfection agent, except under appropriate containment conditions as recommended by the Containment Advisory Group (CAG) in November 2017 (43).

Facilities that intend to retain WPV/VDPV PIM for a limited period (e.g. to complete research studies) may wish to consider applying for CP/ICC only, as described in the CCS, and transfer to a CC-certified PEF or destroy their materials before their CP/ICC expires. Note that stringent requirements still apply

during this period. Facilities that intend to retain WPV/VDPV PIM long term are expected to demonstrate full compliance with all GAPIII requirements and be granted a CC.

WPV2 is an eradicated agent, with WPV1 and WPV3 soon to follow. **Facilities are required to apply this Guidance to PV2 PIM first:** the GCC set the deadline for completion of the identification, destruction, transfer or containment (Phase I) for all PV2 at one year after the publication of this Guidance, and recommended that countries complete Phase I for WPV1 and WPV3 materials by the end of Phase II of GAPIII.

B. Collections with potential only for OPV/Sabin and related strains

Facilities with OPV/Sabin PIM do not need to become PEFs to retain such materials, as long as the conditions described in this section are followed. OPV/Sabin PIM can be subcategorized into three risk levels, depending on the type of sample and laboratory procedures being used with these materials (Table 1). The risk level is determined by associating the type of PV PIM retained with the procedures to be performed using the PV PIM. In general, procedures introducing PIM into PV-permissive cells (Annex 1) will have a higher risk level than other laboratory procedures (4). For example, inoculation of these materials into PV non-permissive cells, bacterial cultures, polymerase chain reactions (PCRs) (DNA or RNA), mass spectrometry or enzyme-linked immunosorbent assays (ELISAs) would be considered lower risk procedures.

As OPV2 is no longer present in bOPV worldwide, **facilities are required to apply this Guidance to OPV2/Sabin2 PIM now.** This Guidance will apply to all OPV/Sabin strains after the cessation of bOPV use.

All facilities that plan to retain OPV/Sabin PV PIM must declare their holdings to the national authority (e.g. MoH) and maintain an accurate inventory of materials in their possession. All OPV/Sabin PV PIM and derived materials should be stored securely, with access restricted to staff who are eligible and competent to work with such materials. Responsibility for compliance with these measures, summarized in Table 2, lies with the facility and its respective national authorities (e.g. MoH).

Risk mitigation strategies for handling OPV/Sabin PIM are described in Table 2.

Table 1. Risk classification of OPV/Sabin poliovirus potentially infectious material

Type of PV PIM*	Procedure used with PIM	Risk level
Faecal samples or concentrated sewage	Inoculation into PV-permissive cells	Moderate
	Other laboratory procedures**	Low
Extracted nucleic acid from faecal samples or concentrated sewage	Transfection into PV-permissive cells	Moderate
	Other laboratory procedures**	Lowest
Respiratory tract samples	Inoculation into PV-permissive cells	Low
	Other laboratory procedures**	Lowest
Extracted nucleic acid from respiratory tract samples	Transfection into PV-permissive cells	Low
	Other laboratory procedures**	Lowest
Inactivated PV PIM***	Any	Not PIM

* Cerebrospinal fluid, serum/blood and other clinical materials not listed in this table are not considered PV PIM.

** May include, but are not limited to, inoculation into PV non-permissive cells, bacterial cultures, PCRs (DNA or RNA), mass spectrometry or ELISAs.

*** Must be inactivated using a validated method (44).

Table 2. Risk mitigation strategies for handling OPV/Sabin poliovirus potentially infectious material¹

Risk mitigation strategy	Risk level			
	Moderate	Low	Lowest	Storage only ²
Declare PV PIM in National PV Survey and maintain accurate inventory	✓	✓	✓	✓
Biosecurity (including, for example, locked freezers, limited access, staff training)	✓	✓	✓	✓
Biosafety (including, for example, good laboratory/microbiological practices, and documentation and validation of methods/standard operating procedures, as described in GAPIII, Annex 6)	✓	✓	✓	n/a
Risk assessment for specific procedures being used	✓	✓	✓	✓
Required polio immunization of staff	✓	✓	n/a ³	n/a
Certification to a national or international standard that includes biosafety and biosecurity components	✓	n/a	n/a	n/a

¹ ✓: must comply with the risk mitigation strategy; n/a: not applicable.

² For short-term retention only, as determined by the MoH, while the final disposition of the collection is being considered. If “stored” samples are to be handled, the risk mitigation strategies for moderate, low and lowest risk levels must be applied as appropriate for the sample type and procedure (Table 1).

³ Recommended.

Guidance for facilities with collections in the MODERATE risk level

In a facility handling OPV/Sabin PV PIM, the inoculation of faecal samples or sewage concentrates, or the transfection of nucleic acid derived from such material into PV-permissive cells (Annex 1) represents the greatest potential risk of inadvertent PV release (4). The inoculation or transfection of PV PIM into PV-permissive cells could result in unintentional PV amplification, greatly increasing the risk of release from the facility if the production of PV was undetected (4).

If the inoculation of faecal samples or sewage concentrates, or the transfection of nucleic acid from OPV/Sabin PV PIM into PV-permissive cells is deemed essential (e.g. to isolate other viruses of public health importance that replicate in the same cell lines as PV), the laboratory and staff should meet stringent standards of biosafety and biosecurity (Table 2). These include adherence to accepted standards of good laboratory and microbiological practices, supported by the validation/documentation of methods and the implementation of written standard operating procedures, and certification to a national or international biorisk management standard (e.g. GAPIII, Annex 6). Rigorous risk assessments should be conducted and documented for all procedures that will be used with PV PIM faecal samples or sewage concentrates to identify strategies to minimize the risks of inadvertent release.

Laboratory staff should provide proof of PV immunization according to the national schedule. Any individuals who cannot produce proof of polio immunization should be immunized according to national or international recommendations for persons with potential occupational exposure to PV.

Guidance for facilities with collections in the LOW risk level

PV PIM faecal samples or sewage concentrates that will not be inoculated into PV-permissive cells (e.g. samples that will be handled only for nucleic acid extraction or fixation, or inoculation only into PV non-permissive cells) pose a lower risk, as these procedures will not enable live virus to grow (4). The inoculation of respiratory tract specimens, or the transfection of nucleic acid derived from such material into PV-permissive cells is also of lower risk, largely because of the lower PV incidence and titres in these sample types (4).

However, the laboratory should still adhere to nationally or internationally accepted standards of good laboratory and microbiological practices, supported by the validation/documentation of methods and the implementation of written standard operating procedures (Table 2). Similar to the moderate risk level, facilities should conduct and document risk assessments to identify strategies to minimize the risks of inadvertent exposure or release.

As above, laboratory staff should provide proof of PV immunization according to the national schedule. Any individuals who cannot produce proof of polio immunization should be immunized according to national or international recommendations for persons with potential occupational exposure to PV.

Guidance for facilities with collections in the LOWEST risk level

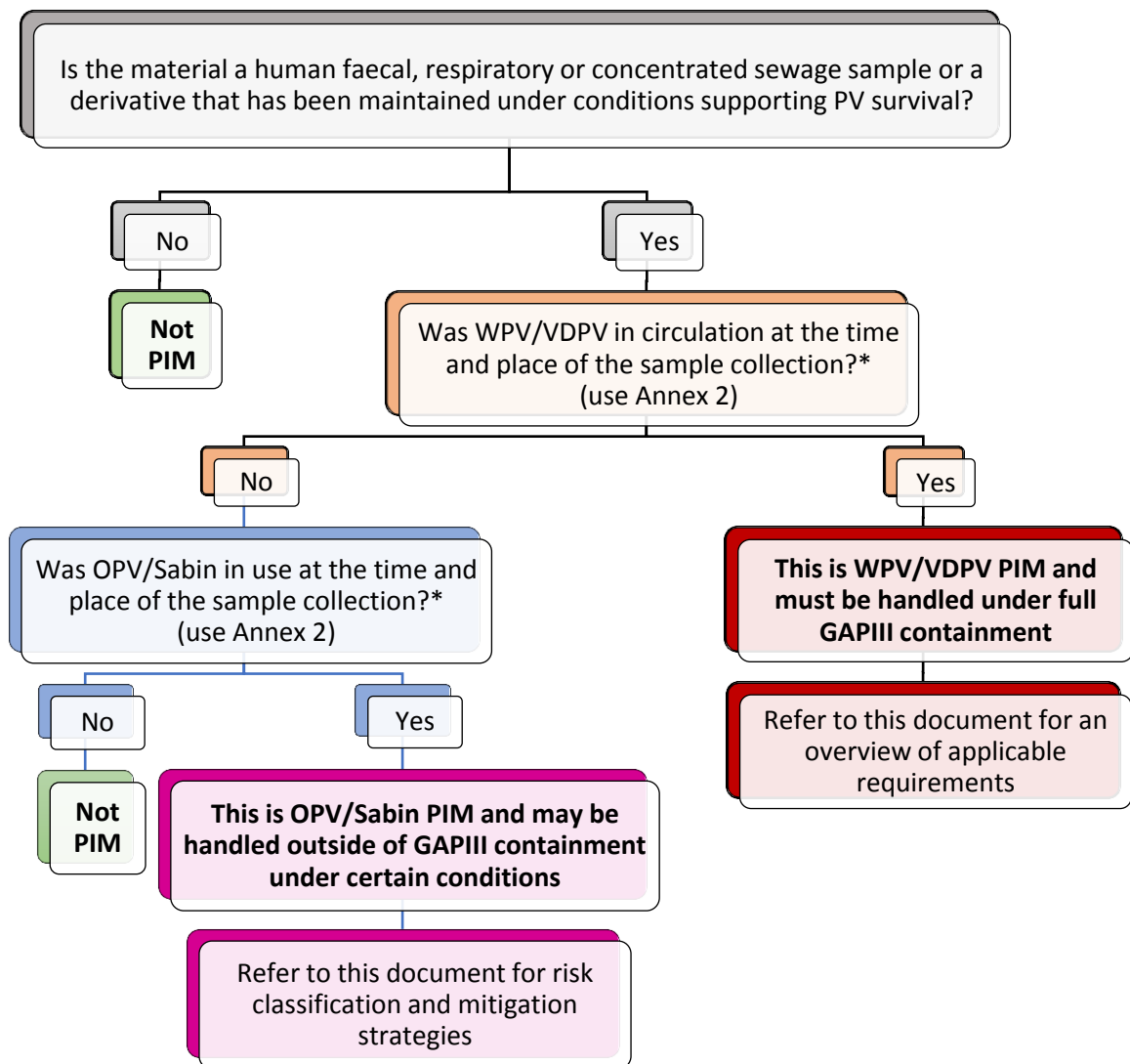
Respiratory tract samples that will not be inoculated into PV-permissive cells (e.g. samples that will be handled only for nucleic acid extraction or fixation, or inoculation only into PV non-permissive cells) pose the lowest risk, as the PV incidence and titres in respiratory materials are low (4). Nucleic acid extracted from OPV/Sabin PV PIM that will not be transfected into PV-permissive cells is also of the lowest risk (4). The laboratory should still adhere to accepted standards of good laboratory and microbiological practices, supported by the validation/documentation of methods and the implementation of written standard operating procedures, and facilities should conduct and document risk assessments to identify strategies to minimize and mitigate the risks of inadvertent release (Table 2).

Polio immunization for relevant staff is recommended.

C. Guidance for the short-term retention of historical collections while final disposition is being determined

Facilities that require a brief period of storage of valuable PV PIM collections while their final disposition is being determined should declare the materials in their National PV Survey and maintain an accurate inventory of materials in their possession (Table 2). PV PIM must be segregated from other materials and stored in locked freezers, with access limited to specifically trained and competent staff. It must be emphasized that this is a short-term measure only, while the final disposition of the collection is being considered. During this time, the facility is still subject to oversight by the national authority (e.g. MoH) and should eventually destroy, inactivate or transfer the materials, adopt the biorisk management strategies described above if the PIM collection is Sabin/OPV material, or begin the process to become a PEF if the PV PIM collection is categorized as WPV/VDPV and the facility is designated to become a PEF.

Figure 1. Determination process of poliovirus potentially infectious material



* If a sample has a missing or damaged label or the type, country of origin or date of collection is unknown, the sample should be destroyed or inactivated using a method known to inactivate poliovirus.

ANNEX 1: POLIOVIRUS-PERMISSIVE CELL LINES

PV grows in nearly all human and monkey cell lines, in addition to mouse L cells (L20B, L α) that were engineered to express the human PV receptor (CD155) (18). **Table A1.1 highlights some, but not all cell lines susceptible to PV infection.**

Extracts of faecal specimens, rectal swabs, respiratory specimens or concentrated sewage that are inoculated onto the PV-susceptible cells listed below will enable growth of any PVs present.

Table A1.1. Examples of cell lines susceptible to poliovirus infection

PV-permissive cell lines	Origin
A549 (45)	Human
CaCo-2 (46)	Human
HeLa (45)	Human
HEp-2 (47)	Human
HEK (48)	Human
MRC-5 (49)	Human
PERC-6 (50)	Human
RD (47)	Human
WI-38 (51)	Human
Various neuroblastoma (e.g. IMR-32, SK-N-MC) (52)	Human
BGMK (sometimes referred to as BGM or GMK) (19)	Non-human primate
LLC-MK2 (53)	Non-human primate
MA-104 (Vero derivative) (45)	Non-human primate
Primary monkey kidney cells ¹ (49)	Non-human primate
Vero (45)	Non-human primate
L20B (54)	Mouse ²
L α (55)	Mouse ²
Super E-Mix (56)	Hybrid; mixture of cell lines
R-Mix (57)	Hybrid; mixture of cell lines

¹ Old World monkeys.

² Transgenic mouse cell lines.

ANNEX 2: COUNTRY- AND TERRITORY-SPECIFIC POLIOVIRUS DATA

Facilities are encouraged to assess the risk of PV PIM in their collections using the data provided in this Annex. The data address the following parameters:

In support of the identification of WPV2/VDPV2 PIM

The following information in Table A2.1 of Annex 2 can help determine whether a facility has WPV2/VDPV2 PIM:

1. year of last detection of WPV2¹
2. period of detection of VDPV2²

The last detection of WPV2 worldwide was in India in October 1999. However, the month and year of the last detection was not accurately recorded for all countries. Table A2.1 of Annex 2 systematically refers to December as the month of last detection of WPV2 for specimens collected during a specific year, and assigns 31 October 1999 as the date of last detection in any country or territory where uncertainty surrounds the last reported case of WPV2. Samples collected up to the indicated dates of last detected WPV2 virus presence are considered WPV2 PIM.

Surveillance activities have detected cVDPVs, iVDPVs and aVDPVs. This Guidance refers to the date of any first and last detected VDPV2 with evidence of circulation for each country or territory.

Samples are considered VDPV2 PIM if collected between the time of the first reported VDPV2 and the last detection in any given country or territory.

As indicated in Table A2.1 of Annex 2, the inventories and destruction of unneeded cVDPV2 PIM must be completed after a VDPV2 outbreak is declared closed.

In support of the identification of OPV2/Sabin2 PIM

The following information in Table A2.1 of Annex 2 can help determine whether a facility has OPV2/Sabin2 PIM:

1. tOPV use in RI
 - a. year of tOPV introduction³
 - b. month and year of last tOPV use⁴
2. post-tOPV-cessation SIA using mOPV2 in countries responding to, or at risk of, a PV2 event or outbreak
 - a. SIA start and end dates.

In countries showing evidence of continued use of tOPV post-switch, the last date of tOPV use was adjusted to the latest detection. In the absence of evidence showing otherwise, samples collected as of three months after the reported last use of tOPV are no longer considered OPV2/Sabin2 PIM.

In the absence of evidence showing otherwise, samples collected as of three months after the reported last use of mOPV2 are no longer considered OPV2/Sabin2 PIM.

¹ Source of virus: acute flaccid paralysis (AFP), environmental sampling (i.e. waste water, sewage), enterovirus surveillance or any other source, including contact sampling, healthy children and special studies.

² VDPV: OPV virus strains that are >1% divergent (or ≥10 nucleotide changes for types 1 and 3) or >0.6% divergent (≥6 nucleotide changes for type 2) from the corresponding OPV strain in the complete VP1 genomic region (see "Classification and reporting of vaccine-derived polioviruses (VDPV), GPEI guidelines", August 2016, available at http://polioeradication.org/wp-content/uploads/2016/09/Reporting-and-Classification-of-VDPVs_Aug2016_EN.pdf).

³ The year of tOPV introduction is generally not known. For this reason, the table assumes that materials collected between the listed last WPV2 case and three months after the last use of tOPV, excluding periods with VDPVs, would fall under the category of OPV2/Sabin2 PIMs.

⁴ In countries and territories where only the year is known, the date of the last tOPV use was arbitrarily set at 31 December.

Countries using mOPV2 are expected to repeat and submit their inventories for OPV2/Sabin2 materials once the use of mOPV2 is discontinued.

Annex 2 of the *Guidance to minimize risks for facilities collecting, handling or storing materials potentially infectious for polioviruses* is available as a separate document [here](#) or by visiting <http://polioeradication.org/polio-today/preparing-for-a-polio-free-world/containment/containment-resources/>

ANNEX 3: REFERENCES

1. **Cochi SL, Freeman A, Guirguis S, Jafari H, Aylward B.** 2014. Global polio eradication initiative: lessons learned and legacy. *J Infect Dis* **210 Suppl 1**:S540-546.
2. **Previsani N, Tangermann RH, Tallis G, Jafari HS.** 2015. World Health Organization Guidelines for Containment of Poliovirus Following Type-Specific Polio Eradication - Worldwide, 2015. *MMWR Morb Mortal Wkly Rep* **64**:913-917.
3. **WHO.** 2013. Polio Eradication & Endgame Strategic Plan 2013–2018.
4. **Dowdle W, van der Avoort H, de Gourville E, Delpeyroux F, Desphande J, Hovi T, Martin J, Pallansch M, Kew O, Wolff C.** 2006. Containment of polioviruses after eradication and OPV cessation: characterizing risks to improve management. *Risk Anal* **26**:1449-1469.
5. **Thompson KM.** 2006. Poliomyelitis and the role of risk analysis in global infectious disease policy and management. *Risk Anal* **26**:1419-1421.
6. **Fine PE, Ritchie S.** 2006. Perspective: determinants of the severity of poliovirus outbreaks in the post eradication era. *Risk Anal* **26**:1533-1540.
7. **WHO.** 2015. 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). **Third Edition.**
8. **WHO.** 2015. World Health Assembly Resolution WHA68.3. Poliomyelitis. Sixty-eighth World Health Assembly, Geneva.
9. **Davies M, Bruce C, Bewley K, Outlaw M, Mioulet V, Lloyd G, Clegg C.** 2003. Poliovirus type 1 in working stocks of typed human rhinoviruses. *The Lancet* **361**:1187-1188.
10. **Arya SC.** 2003. Hiding polioviruses. *The Lancet* **361**:2156-2157.
11. **de Gourville E, Wolff C.** 2003. Hiding polioviruses. *The Lancet* **361**:2157.
12. **Savolainen C, Hovi T.** 2003. Caveat: poliovirus may be hiding under other labels. *The Lancet* **361**:1145-1146.
13. **Esteves-Jaramillo A, Estivariz CF, Penaranda S, Richardson VL, Reyna J, Coronel DL, Carrion V, Landaverde JM, Wassilak SG, Perez-Sanchez EE, Lopez-Martinez I, Burns CC, Pallansch MA.** 2014. Detection of vaccine-derived polioviruses in Mexico using environmental surveillance. *J Infect Dis* **210 Suppl 1**:S315-323.
14. **Portes SA, Da Silva EE, Siqueira MM, De Filippis AM, Krawczuk MM, Nascimento JP.** 1998. Enteroviruses isolated from patients with acute respiratory infections during seven years in Rio de Janeiro (1985-1991). *Rev Inst Med Trop Sao Paulo* **40**:337-342.
15. **Grard G, Drexler JF, Lekana-Douki S, Caron M, Lukashev A, Nkoghe D, Gonzalez JP, Drosten C, Leroy E.** 2010. Type 1 wild poliovirus and putative enterovirus 109 in an outbreak of acute flaccid paralysis in Congo, October-November 2010. *Euro Surveill* **15**.
16. **Racaniello VR.** 2006. One hundred years of poliovirus pathogenesis. *Virology* **344**:9-16.
17. **WHO.** 1993. Maintenance and distribution of transgenic mice susceptible to human viruses: Memorandum from a WHO meeting. *Bull World Health Organ* **71**:497-502.
18. **Khan S, Peng X, Yin J, Zhang P, Wimmer E.** 2008. Characterization of the New World Monkey Homologues of Human Poliovirus Receptor CD155. *J Virol* **82**:7167-7179.
19. **Lee-Montiel FT, Reynolds KA, Riley MR.** 2011. Detection and quantification of poliovirus infection using FTIR spectroscopy and cell culture. *J Biol Eng* **5**:16.
20. **Aylward RB, Sutter RW, Cochi SL, Thompson KM, Jafari H, Heymann D.** 2006. Risk management in a polio-free world. *Risk Anal* **26**:1441-1448.
21. **WHO.** 2017. CAG June 2017 Report. Report of the First Meeting of the Containment Advisory Group, 19-20 June 2017.
22. **Morales M, Tangermann RH, Wassilak SG.** 2016. Progress Toward Polio Eradication - Worldwide, 2015-2016. *MMWR Morb Mortal Wkly Rep* **65**:470-473.
23. **Burns CC, Diop OM, Sutter RW, Kew OM.** 2014. Vaccine-derived polioviruses. *J Infect Dis* **210 Suppl 1**:S283-293.

24. **Kew O, Morris-Glasgow V, Landaverde M, Burns C, Shaw J, Garib Z, Andre J, Blackman E, Freeman CJ, Jorba J, Sutter R, Tambini G, Venczel L, Pedreira C, Laender F, Shimizu H, Yoneyama T, Miyamura T, van Der Avoort H, Oberste MS, Kilpatrick D, Cochi S, Pallansch M, de Quadros C.** 2002. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science* **296**:356-359.
25. **Diop OM, Burns CC, Sutter RW, Wassilak SG, Kew OM.** 2015. Update on Vaccine-Derived Polioviruses - Worldwide, January 2014–March 2015. *MMWR Morb Mortal Wkly Rep* **64**:640-646.
26. **Tambini G, Andrus JK, Marques E, Boshell J, Pallansch M, de Quadros CA, Kew O.** 1993. Direct detection of wild poliovirus circulation by stool surveys of healthy children and analysis of community wastewater. *J Infect Dis* **168**:1510-1514.
27. **Deshpande JM, Kamat JR, Rao VK, Nadkarni SS, Kher AS, Salgaokar SD, Rodrigues JJ.** 1995. Prevalence of antibodies to polioviruses & enteroviruses excreted by healthy children in Bombay. *Indian J Med Res* **101**:50-54.
28. **Mach O, Verma H, Khandait DW, Sutter RW, O'Connor PM, Pallansch MA, Cochi SL, Linkins RW, Chu SY, Wolff C, Jafari HS.** 2014. Prevalence of asymptomatic poliovirus infection in older children and adults in northern India: analysis of contact and enhanced community surveillance, 2009. *J Infect Dis* **210 Suppl 1**:S252-258.
29. **Pallansch M, Staples M.** 2002. Wild poliovirus found in stored potential infectious materials. *World Health Organization Polio Laboratory Network Quarterly Update* **8**:1-2.
30. **Maes EF, Diop OM, Jorba J, Chavan S, Tangermann RH, Wassilak SG.** 2017. Surveillance Systems to Track Progress Toward Polio Eradication - Worldwide, 2015–2016. *MMWR Morb Mortal Wkly Rep* **66**:359-365.
31. **Alexander JP Jr, Gary HE Jr, Pallansch MA.** 1997. Duration of poliovirus excretion and its implications for acute flaccid paralysis surveillance: a review of the literature. *J Infect Dis* **175 Suppl 1**:S176-182.
32. **Dowdle WR, Birmingham ME.** 1997. The Biologic Principles of Poliovirus Eradication. *J Infect Dis* **175 Suppl 1**:S286-292.
33. **Mas Lago P, Gary HE Jr, Perez LS, Caceres V, Olivera JB, Puentes RP, Corredor MB, Jimenez P, Pallansch MA, Cruz RG.** 2003. Poliovirus detection in wastewater and stools following an immunization campaign in Havana, Cuba. *Int J Epidemiol* **32**:772-777.
34. **Shulman LM, Martin J, Sofer D, Burns CC, Manor Y, Hindiyeh M, Gavrilin E, Wilton T, Moran-Gilad J, Gamzo R, Mendelson E, Grotto I, Group GPI, Group GPIG-PI.** 2015. Genetic Analysis and Characterization of Wild Poliovirus Type 1 During Sustained Transmission in a Population With >95% Vaccine Coverage, Israel 2013. *Clin Infect Dis* **60**:1057-1064.
35. **Nakamura T, Hamasaki M, Yoshitomi H, Ishibashi T, Yoshiyama C, Maeda E, Sera N, Yoshida H.** 2015. Environmental Surveillance of Poliovirus in Sewage Water around the Introduction Period for Inactivated Polio Vaccine in Japan. *Appl Environ Microbiol* **81**:1859-1864.
36. **Zurbriggen S, Tobler K, Abril C, Diedrich S, Ackermann M, Pallansch MA, Metzler A.** 2008. Isolation of sabin-Like Polioviruses from Wastewater in a country Using Inactivated Polio Vaccine. *Appl Environ Microbiol* **74**:5608-5614.
37. **Battistone A, Buttinelli G, Fiore S, Amato C, Bonomo P, Patti AM, Vulcano A, Barbi M, Binda S, Pellegrinelli L, Tanzi ML, Affanni P, Castiglia P, Germinario C, Mercurio P, Cicala A, Triassi M, Pennino F, Fiore L.** 2014. Sporadic Isolation of Sabin-Like Polioviruses and High-Level Detection of non-Polio Enteroviruses during Sewage Surveillance in Seven Italian Cities, after Several Years of Inactivated Poliovirus Vaccination. *Appl Environ Microbiol* **80**:4491-4501.
38. **Leparc-Goffart I, Julien J, Fuchs F, Janatova I, Aymard M, Kopecka H.** 1996. Evidence of Presence of Poliovirus Genomic Sequences in Cerebrospinal Fluid from Patients with Postpolio Syndrome. *J Clin Microbiol* **34**:2023-2026.
39. **WHO.** 2004. Polio laboratory manual. **4th Edition.**
40. **van der Werf S, Bradley J, Wimmer E, Studier FW, Dunn JJ.** 1986. Synthesis of infectious poliovirus RNA by purified T7 RNA polymerase. *Proc Natl Acad Sci U S A* **83**:2330-2334.
41. **Wimmer E, Paul AV.** 2011. Synthetic poliovirus and other designer viruses: what have we learned from them? *Annu Rev Microbiol* **65**:583-609.

42. **WHO.** 2017. Containment Certification Scheme to support the WHO Global Action Plan for Poliovirus Containment (GAPIII-CCS).
43. **WHO.** 2017. CAG2 November 2017 Meeting report. Report of the Second Meeting of the Containment Advisory Group, 28-30 November 2017.
44. **Salk JE, Gori JB.** 1960. A review of theoretical, experimental, and practical considerations in the use of formaldehyde for the inactivation of poliovirus. *Ann N Y Acad Sci* **83**:609-637.
45. **Lee JH, Lee GC, Kim JI, Yi HA, Lee CH.** 2013. Development of a new cell culture-based method and optimized protocol for the detection of enteric viruses. *J Virol Methods* **191**:16-23.
46. **Ammendolia MG, Tinari A, Calcabrini A, Superti F.** 1999. Poliovirus infection induces apoptosis in CaCo-2 cells. *J Med Virol* **59**:122-129.
47. **Thorley BR, Roberts JA.** 2016. Isolation and Characterization of Poliovirus in Cell Culture Systems. *Methods Mol Biol* **1387**:29-53.
48. **Campbell SA, Lin J, Dobrikova EY, Gromeier M.** 2005. Genetic determinants of cell type-specific poliovirus propagation in HEK 293 cells. *J Virol* **79**:6281-6290.
49. **Chonmaitree T, Ford C, Sanders C, Lucia HL.** 1988. Comparison of cell cultures for rapid isolation of enteroviruses. *J Clin Microbiol* **26**:2576-2580.
50. **Minor PD, Lane B, Mimms S, Bar P.** 2017. Scientific consultation on the safety and containment of new poliovirus strains for vaccine production, clinical/regulatory testing and research. Report of a meeting held at NIBSC, Potters Bar, Hertfordshire, UK, 6/7th July 2016. *Biologicals* **48**:92-100.
51. **Stones PB.** 1976. Production and control of live oral poliovirus vaccine in WI-38 human diploid cells. *Dev Biol Stand* **37**:251-253.
52. **Colbere-Garapin F, Christodoulou C, Crainic R, Pelletier I.** 1989. Persistent poliovirus infection of human neuroblastoma cells. *Proc Natl Acad Sci U S A* **86**:7590-7594.
53. **Hull RN, Cherry WR, Tritch OJ.** 1962. Growth characteristics of monkey kidney cell strains LLC-MK1, LLC-MK2, and LLC-MK2(NCTC-3196) and their utility in virus research. *J Exp Med* **115**:903-918.
54. **Pipkin PA, Wood DJ, Racaniello VR, Minor PD.** 1993. Characterisation of L cells expressing the human poliovirus receptor for the specific detection of polioviruses in vitro. *J Virol Methods* **41**:333-340.
55. **Arita M, Ohka S, Sasaki Y, Nomoto A.** 1999. Multiple pathways for establishment of poliovirus infection. *Virus Res* **62**:97-105.
56. **Buck GE, Wiesemann M, Stewart L.** 2002. Comparison of mixed cell culture containing genetically engineered BGMK and CaCo-2 cells (Super E-Mix) with RT-PCR and conventional cell culture for the diagnosis of enterovirus meningitis. *J Clin Virol* **25 Suppl 1**:S13-18.
57. **Leland DS, Ginocchio CC.** 2007. Role of cell culture for virus detection in the age of technology. *Clin Microbiol Rev* **20**:49-78.