**Poliovirus containment**

**Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses**

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# Abbreviations and Acronyms

AFP Acute flaccid paralysis

AME Acute meningoencephalitis

CC Certificate of containment

CCS Containment certification scheme

CCID50 Cell culture infectious dose, 50% endpoint

cDNA complementary deoxyribonucleic acid

CP Certificate of participation in the containment certification process

CPE Cytopathic effect

CSF Cerebrospinal fluid

GAPIII Global Action Plan III

ICC Interim certificate of containment

IPV Inactivated polio vaccine

NAC National authority for containment

NCC National committee for the certification of the eradication of poliomyelitis

NPCC National poliovirus containment coordinator

OPV Oral polio vaccine

OPV-like Oral polio vaccine-like

OPV2 Oral polio vaccine type 2

bOPV Bivalent oral polio vaccine containing type 1 and type 3

mOPV Monovalent oral polio vaccine containing one type only

mOPV2 Monovalent oral polio vaccine type 2

tOPV Trivalent oral polio vaccine containing type 1, type 2 and type 3

PEF Poliovirus-essential facility

dPEF Designated poliovirus-essential facility

PIM Potentially infectious material

PV Poliovirus

PV2 Poliovirus type 2, including WPV2, VDPV2, OPV2, Sabin2

PVR Poliovirus receptor

QC Quality control

RI Routine immunization

RNA Ribonucleic acid

SIA Supplementary immunization activity

VDPV Vaccine-derived poliovirus

VDPV2 Vaccine-derived poliovirus type 2

aVDPV Ambiguous vaccine-derived poliovirus

aVDPV2 Ambiguous vaccine-derived poliovirus type 2

cVDPV Circulating vaccine-derived poliovirus

cVDPV2 Circulating vaccine-derived poliovirus type 2

iVDPV Immunodeficiency-associated vaccine-derived poliovirus

iVDPV2 Immunodeficiency-associated vaccine-derived poliovirus type 2

WHO World Health Organization

WPV Wild poliovirus

WPV2 Wild poliovirus type 2

# Introduction

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

In 2015, the Global Certification Commission (GCC) certified the eradication of wild poliovirus type 2 (WPV2). The eradication of WPV1 and 3 and circulating vaccine-derived polioviruses (cVDPV) is anticipated in the near future. The only remaining poliovirus (PV) reservoirs will be the facilities/laboratories retaining poliovirus infectious or potentially infectious materials. Nations responsible for these facilities must assure the world that these reservoirs do not present a post-eradication risk of re-emerging disease that could undermine this extraordinary humanitarian achievement.

In 2015, WHO published the *Global Action Plan (GAPIII) to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use,* to provide risk reduction guidance for PV facilities/laboratories. As these facilities work with PV, they have the advantage of being aware of the nature of the agents, the operational risks, and effective containment measures to reduce those risks. Non-PV facilities that collect and store clinical and environmental samples for other purposes present a PV transmission risk if samples were collected in a time and place where wild polioviruses/circulating vaccine-derived polioviruses (WPV/cVDPV), or OPV-derived viruses were circulating or oral polio vaccines (OPV/Sabin) were in use. These facilities are at a disadvantage in that the potential presence of an infectious PV in such samples is both undesirable and uncertain.

Non-PV research facilities with a high probability of storing such materials include those working with rotavirus or other enteric agents, hepatitis viruses, influenza/respiratory agents, and measles virus, or those conducting nutritional research.

# Purpose

The purpose of this guidance is to assist non-PV facilities/laboratories to assess the risk of poliovirus potentially infectious materials (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 PVs.

# Rationale

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

* faecal or respiratory secretion samples and their derivatives (extracts, culture isolates) collected in regions endemic for wild poliovirus, that may harbor the pathogen with no indication of symptoms resembling poliovirus or enterovirus infection;
* faecal or respiratory secretion specimens collected for any purpose in a time and geographic area of WPV/VDPV circulation or use of OPV;
* products of such materials (above) from poliovirus-permissive cells or experimentally infected animals;
* 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 handled under conditions conducive to maintaining the viability or enabling the replication of incidental poliovirus;
* environmental samples (i.e. sewage, waste water) collected in areas known or suspected to have circulating WPV/VDPV or use of OPV at the time of collection

The non-PV facility with 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, and
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 mission.
2. PV is encountered only as an incidental, undesirable agent,
3. PV is present in clinical samples at varying rates and moderate titers,
4. PV titers may not be enriched by agent-specific procedures,
5. Historic PIM collections are reserved for special studies.

The inclusion of non-PV facilities with PIM in global PV containment efforts is crucial. Any possible advantage of lower facility-transmission risk of the non-PV facility could be wholly offset by the laboratory worker who is uninformed, unaware, or unconcerned about PIM risks or untrained in procedures to reduce those risks.

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

# Strategy

The global strategy for minimizing risks from the non-PV facility is basically that outlined for the PV facility in GAPIII: 1) risk elimination by PIM destruction or transfer to a “polio-essential facility” (PEF) and 2) biorisk management by those facilities that retain PIM and meet the required safe-handling and containment requirements.

**Risk elimination: The goal is no PIM.**  Non-PV facilities should carefully consider the required resources and set a high bar when deciding on whether to retain PIM collections, particularly those with WPV/cVDPV potential. The scientific value of retaining a specific PIM sample collection should be carefully weighed against the public health value of its destruction.

**Biorisk management:** Non-PV facilities/laboratories electing to retain scientifically valuable PIM collections should be familiar with and prepared to meet biorisk management standards adequate for risk mitigation.

For PIM collections with WPV/cVDPV potential, the requirements are described in GAPIII, Annex 2, *Biorisk management standards for poliovirus-essential facilities wild poliovirus materials.* **These are stringent standards as required for an eradicated agent and should be in place** when working with these potentially infectious collections.

For PIM collections with OPV/Sabin potential, non-PV facilities must meet the risk-appropriate management standards described in this publication.

# Implementation

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

PV2 containment is already in progress. WPV2 was declared eradicated by the Global Commission for the Certification of Poliovirus Eradication in 2015. Trivalent OPV (types 1, 2, and 3) was replaced with bivalent OPV (types 1 and 3) in 2016 to reduce the number of OPV2-associated paralytic poliomyelitis cases and cVDPV2 outbreaks. PV2 is the most transmissible of the three OPV Sabin strains. Monovalent OPV2 has been used in supplemental immunization activities in certain countries to interrupt cVDPV2 outbreaks (Annex 4). At the time of trivalent OPV withdrawal, inactivated poliovirus vaccine (IPV) was introduced in routine immunization programmes in select high-risk countries to maintain immunity for poliovirus type 2. As a consequence of these actions:

Phase I, the inventory, destruction, and preparation for PV2 containment, is nearing completion for PV facilities/laboratories.

Phase II, containment of WPV2/VDPV2 and OPV2/Sabin strains in PV facilities/laboratories, is in progress.

*Implementation of risk reduction actions by non-PV facilities/laboratories for PIM with PV2 potential is a matter of urgency.*

Phase III, final containment of all WPV and OPV/Sabin polioviruses will commence when global WPV transmission has not been detected for three years followed by the planned cessation of bOPV usage, respectively.

# Categorization of poliovirus potentially infectious materials according to risk

The evidence-based rationale for categorizing sample collections according to relative risks is derived from data provided in *Rationale,* Annex 1 (*Risk assessment for categorization of poliovirus potentially infectious materials*) and Annex 3 (*Country and Territory-specific poliovirus risk*).

The PV transmission risk of a PIM collection is a factor of multiple elements including nature of the sample collection (when, where, and what collected), the PV(s) that may be present (WPV/cVDPV or OPV/Sabin), hazards of the laboratory procedures being used, and worker/community susceptibility (Annexes 1 and 3).

PIM sample collections may be categorized in one of two divergent risk groups based on PV virulence and transmissibility (Annex 1). Of greatest risk are collections with potential for WPV/cVDPV, which are the target viruses of the polio eradication initiative. Of lower risk are collections with potential for only OPV/Sabin and related strains, which have been used for immunization of untold numbers of children for more than 50 years.

Despite the OPV safety record, all three attenuated PV types in the vaccine have been linked with rare vaccine-associated paralytic poliomyelitis. Further, under certain conditions of low immunization rates of populations in high-risk environments, prolonged replication of OPV/Sabin poliovirus can lead to a loss of attenuation, to produce circulating vaccine-derived poliovirus (cVDPV) (Annex 1). cVDPVs pose a public health threat, as outbreaks of paralytic poliomyelitis that clinically were indistinguishable from wild poliovirus infection have occurred due to each poliovirus serotype, with >80% of cVDPV outbreaks associated with type 2. People with primary B-cell immunodeficiencies exposed to OPV can develop a chronic poliovirus infection leading to immunodeficiency-associated VDPV (iVDPV). While iVDPV has not been identified as the source of a polio outbreak, the prolonged shedding of virulent strains of poliovirus represents a threat to the global eradication of poliovirus.

# Biorisk management of poliovirus potentially infectious materials

## Collections with potential for WPV/cVDPV.

WPV2 is an eradicated agent, with WPV1 and WPV3 soon to follow. The retention of samples potentially infectious for WPV2/cVDPV2 **subjects the facility to the approval of the responsible national authority for containment (NAC)** and requires implementation as follows:

1. The responsible national authority agrees to the retention of these materials.
2. The responsible national authority designates the facility as a poliovirus-essential facility (dPEF)
3. The dPEF 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 GAPIII Containment Certification Scheme (CCS).
4. The dPEF holding a CP for the retention of WPV2/VDPV2 materials demonstrates compliance with requirements described in Annex 2 of GAPIII and applies to the NAC for containment certification (ICC/CC) against GAPIII, as described in the (CCS).
5. A dPEF that is NOT granted a CP will have the option to transfer the relevant materials to another PEF or to destroy them.
6. A dPEF that is granted a CP is allowed to continue the retention of relevant materials, as described in the CCS.
7. The validity of a CP/ICC/CC is limited, as described in the CCS.

Facilities that intend to retain WPV2/cVDPV2 samples for a limited period of time within Phase II of GAPIII, e.g. to complete research studies, may wish to consider applying for CP/ICC only, and transfer to a CC-certified PEF or destroy their materials before the end of Phase II of GAPIII. Note that stringent requirements still apply during this period. Facilities that intend to retain materials beyond the end of Phase II of GAPIII are expected to demonstrate full compliance with all GAPIII requirements and be granted a full containment certificate (CC).

## Collections with potential only for OPV/Sabin and related strains

OPV/Sabin materials can be sub-categorized into three risk levels, depending on type of sample and laboratory procedures being used (Table 1).

Table 1. Risk groups for OPV/Sabin poliovirus potentially infectious materials

| Risk Group Level | Type of PIM | Procedures used with PIM |
| --- | --- | --- |
| 1 Moderate | Faecal samples or concentrated sewage | Inoculation into poliovirus-permissive cells |
| Extracted nucleic acid from faecal samples or concentrated sewage | Transfection into poliovirus-permissive cells |
| 2 Low | Faecal samples or concentrated sewage | No cell culture inoculation |
| Respiratory tract samples | Inoculation into polio-permissive cells |
| Extracted nucleic acid from respiratory tract samples | Transfection into poliovirus-permissive cells |
| 3 Lowest | Respiratory tract samples | No cell culture inoculation |
| Extracted nucleic acid from any source | No transfection into polio-permissive cells |
| Non-PIM | CSF, serum/blood, and other clinical materials not listed above; any materials inactivated by a validated method (e.g. formalin) | Not applicable |

All facilities that plan to retain OPV/Sabin PIM must declare their holdings to national authorities and maintain a working inventory of materials in their possession. All PIM and derived materials should be stored securely, with access restricted to staff that are specifically trained to work with such materials.

An algorithm for handling OPV/Sabin PIM is described in Table 2.

Table 2: Algorithm for handling OPV/Sabin poliovirus potentially infectious materials [[1]](#footnote-1)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Risk Mitigation Strategies | Level 1 Moderate | Level 2Low | Level 3 Lowest | Storage Only[[2]](#footnote-2) |
| Declare PIM in National Survey and maintain working inventory |  |  |  |  |
| Biosecurity (locked freezers, limited access) |  |  |  |  |
| Good laboratory/microbiological practices, including documentation and validation of methods/SOPs |  |  |  | n/a |
| Risk assessment for specific procedures being used |  |  |  | n/a |
| Polio immunization of staff required |  |  | n/a | n/a |
| Polio immunization of staff recommended | n/a | n/a |  | n/a |
| Accreditation to a national or international quality standard |  | n/a | n/a | n/a |

### Level 1: Guidance for facilities with collections in the moderate group

In a non-PV facility handling OPV/Sabin PIM, inoculation of fecal samples or sewage concentrates or transfection of nucleic acid derived from such material into PV permissive cell lines (Annex 2) represents the greatest potential risk of inadvertent poliovirus release (Annex 1). Inoculation or transfection of PIM into PV-permissive cells could result in unintentional PV amplification, greatly increasing the risk if undetected.

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

Laboratory staff should provide proof of poliovirus immunization according to the national schedule. If an individual cannot produce proof of polio immunization, they should be immunized according to national recommendations for persons with potential occupational exposure to poliovirus.

### Level 2: Guidance for facilities with collections in the low group

PIM fecal samples or sewage concentrates that will not be inoculated into PV-permissive cell lines (e.g. those that will be handled only for nucleic acid extraction or fixation) pose a lower risk, as procedures will not amplify live virus. Inoculation of respiratory tract specimens or transfection of nucleic acid derived from such material into PV-permissive cells is also of lower risk, largely because of the lower PV incidence.

However, the laboratory should still adhere to accepted standards of good laboratory and microbiological practices, supported by validation/documentation of methods and written standard operating procedures (Table 2). As for Level 1, risk assessments should be conducted for all procedures to identify strategies to minimize and mitigate risks of inadvertent release.

Laboratory staff should provide proof of poliovirus immunization according to the national schedule. If an individual cannot produce proof of polio immunization, they should be immunized according to national recommendations for persons with potential occupational exposure to poliovirus.

### Level 3: Guidance for facilities with collections in the lowest risk group

Respiratory tract specimensthat will not be inoculated into PV-permissive cell lines (e.g. those that will be handled only for nucleic acid extraction or fixation) pose the lowest risk, as the PV incidence and titers in clinical materials are low. Nucleic acid extracted from OPV/Sabin PIM that will not be transfected into polio-permissive cell lines is also of the lowest risk. The laboratory should still adhere to accepted standards of good laboratory and microbiological practices, supported by validation/documentation of methods and written standard operating procedures, and risk assessments should be conducted for all procedures to identify strategies to minimize and mitigate risks of inadvertent release (Table 2).

Polio immunization for relevant staff is recommended.

### Guidance for short-term retention of historical collections while final disposition is being determined

Facilities that require a brief period of storage of valuable PIM collections while final disposition is being determined should declare the materials in their National Survey and maintain an accurate inventory of materials in their possession (Table 2). PIM must be segregated from other materials and stored in locked freezers, with access limited to specifically trained 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 for Containment or other regulatory body and should eventually destroy or transfer the materials, or begin the process to become a dPEF (Section 7), if the PIM collection is categorized as WPV/cVDPV.

# Annex 1: Risk Assessment for categorization of poliovirus potentially infectious materials \*\*

The PV transmission risk of a PIM collection is a factor of multiple elements including the nature of the sample collection (when, where, and what), the PV(s) that may be present (WPV/cVDPV or OPV/Sabin), hazards of laboratory procedures, and worker/community susceptibility.

### When and where samples collected

The “when and where” of the collection indicates the likelihood of PV being present. Annex 3 provides country-specific PV2 data for year of last reported WPV, year of last reported cVDPV, and last use of tOPV.

Samples collected as of 3 months after the reported dates in Annex 3 are no longer considered PIM.

Stored samples collected at a time and in a country where WPV was no longer in circulation and OPV not in use are not considered PIM.

### What samples collected

Infection of humans with wild poliovirus is predominantly via the faecal-oral route. OPV is administered orally. Ingestion of either form of poliovirus by a non-immune person leads to an initial brief infection of the throat followed by a more prolonged infection of the gut epithelium. A short period of viremia may occur during the early phase of infection. 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. Poliovirus may replicate in the gut without an initial throat infection. The following describes the relative risk of different sample types.

**Faeces**: PV isolation rates may vary widely in samples collected from asymptomatic subjects in a time and place where WPV/cVDPV or OPV-derived viruses were in circulation or oral polio vaccines (OPV) were in use. A stool survey in Cartagena in 1989 reported a WPV isolation rate of 8%, with the highest rate being reported from Mumbai in 1994 at 19%. A survey of asymptomatic persons of all ages in index households and neighboring households in Uttar Pradesh, India, in 2009 found 4.8% were shedding WPV. Studies undertaken in Bihar, India, by the same group reported a 2.5% stool-positive rate for any PV.

Incidental PV in PIM has been found in the test results (2002) from stool samples stored for more than 20 years in a gastroenteritis laboratory. In the first collection, six wild viruses and one Sabin virus were recovered from 82 samples (9%). In the second, six Sabin viruses were recovered from 183 samples (2%). In 2016, Sabin poliovirus was detected in 5.2% of 241,999 stool samples collected globally for AFP surveillance.

WPV strains present the greatest transmission risk with an estimated human minimum infectious dose of 100-fold less than for OPV strains. Epidemiologic models and field studies estimate transmissibility for WPV/cVDPV to be more than 10-fold greater than for OPV. Secondary spread of WPV was reported to approach 90% among susceptible contacts in family and institutional settings, with secondary spread of OPV strains less than half that.

OPV circulation in the community rarely exceeds three months after an immunization campaign. Immunologically naïve subjects may shed WPV/cVDPV, OPV, or OPV-derived viruses over a range of cell culture infectious doses (CCID) up to 106 CCID50/g (mean ~104 CCID50/g) stool) for 6 weeks to 3 months, although shedding duration sometimes may be less for OPV/Sabin strains. Poliovirus re-infections of the gut may occur, depending on virus challenge dose and length of time since receipt of OPV or natural infection. Virus concentration and duration of fecal shedding is generally lower on re-infection. IPV immunization has little or no effect on the susceptibility of the gut to PV infection.

**Nasopharyngeal, oropharyngeal, and other upper respiratory tract secretions:** Similarly, WPV/cVDPV and OPV/Sabin viruses may be recovered from respiratory secretions of naïve subjects at about the same concentrations for a period 2-6 days, but virus shedding wanes and usually disappears at 7-10 days post-infection, coinciding with the appearance of serum antibodies. Virus is rarely recovered from respiratory secretions after WPV or OPV challenge of persons with measurable serum antibody, including IPV recipients. Based on the limited duration of post-infection virus shedding and absence of shedding on re-infection, the probability of recovering poliovirus from respiratory secretions in surveys is estimated to be <1%, or at least 10-fold less than from stool samples. 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 (2.5%).

**Sewage:** Poliovirus recovery from raw sewage usually involves some form of entrapment or sample concentration. Recovery of WPV or OPV/Sabin has been reported from raw samples, but the concentration of infectious virus is usually <1 CCID50/ml, well below the estimated infectious dose for either OPV strains or WPV. The PV content of sewage concentrates may be several logs higher, depending on the method employed.

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

### Age of subjects

Children <5 years old are the group most often infected during a WPV epidemic and are the target population for routine immunizations and multiple OPV campaigns. Children 6-15 years old are rarely included in OPV campaigns, but may be infected or re-infected by WPV or OPV-derived viruses circulating in the family or community. Re-infection of immunologically experienced adults and older children is less likely, but appears to be a function of virus dosage. Re-infections of older children or adults rarely result in virus recovery from throat samples, and fecal shedding may be greatly reduced in virus content and duration.

### Laboratory hazards

Survival of PV in PIM collections under conventional storage conditions is indefinite under liquid nitrogen or mechanical refrigeration at -70°C. Survival may be slightly reduced at -20°C under conditions of fluctuating temperatures, but viability can be anticipated for many years.

**Inoculating/harvesting poliovirus permissive cells:** Attempts to isolate other infectious agents from PIM collections using poliovirus-permissive cell cultures (Annex 2) may result in an enhanced PV content of up to 108 CCID50 /ml. This possibly >105 increase in virus concentration over the original clinical sample greatly increases the risk to the laboratory worker, particularly if the identity of the enhanced incidental PV remains unrecognized.

Full-length poliovirus RNA can infect permissive cell lines, which can be facilitated by using transfection reagents. Unknown to the laboratory worker, the extraction of nucleic acid from PIM could coincidentally co-purify poliovirus RNA from an incidental poliovirus in the sample. The subsequent transfection of the nucleic acid in poliovirus permissive cell lines may generate infectious poliovirus particles, possibly at high titers.

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

### Facility effluent potential

The risk of community exposure through liquid effluents requires a facility-by-facility assessment and will depend on potential poliovirus content, nature of sewerage system, and potential for human consumption. However, if the non-poliovirus laboratory works with only PIM without further replication of incidental polioviruses and adheres to good laboratory practices, the community risk is very low.

### Worker/community susceptibility

**The facility/laboratory worker:** For OPV recipients, reinfection of the gut is a function of time since OPV or natural infection and the challenge virus dosage. IPV provides solid pharyngeal protection but little or no immunity to gut infection. 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 poliovirus-contaminated skin or clothing, silent infections of the gut, or work practices that may contribute to contamination of facility effluents**.**

**Community vaccine coverage:** The risk of outbreaks from laboratory-associated transmission isinversely proportional to population immunity. Risk may be assessed by percent vaccine coverage of persons <5 years old.

**Facility location:** Riskassessment of facility location is largely subjective, but should be taken into consideration if the facility is situated near high-risk populations with potentially elevated force of infection.

**Summary:** PIM risk divides naturally into two widely divergent risk groups based on PV virulence and transmissibility. Collections with potential for WPV/cVDPV are highest risk. Collections with potential for only OPV/Sabin and related strains are lower risks. These categories are not overlapping. However, within each category are factors that may raise or lower risk of facility-associated transmission. All non-poliovirus facilities that propose to retain PIM collections should prepare a complete risk assessment of polio-specific risks, with the objective of minimizing facility risks.

# Annex 2. Poliovirus permissive cell lines

Poliovirus grows in nearly all human and monkey cell lines, in addition to mouse L cells (L20B, Lα) that express the human poliovirus receptor (CD155). The list below highlights some, but not all cell lines susceptible to poliovirus infection.

Extracts of faecal specimens, rectal swabs or respiratory specimens that are inoculated onto the poliovirus-susceptible cells listed below will amplify any polioviruses that are present.

|  |  |
| --- | --- |
| Poliovirus-permissive cell lines | origin |
| HeLa | Human |
| Hep-2 | Human |
| HEK | Human |
| MRC-5 | Human |
| RD | Human |
| A549 | Human |
| CaCo-2 | Human |
| WI-38 | Human |
| Various neuroblastoma (e.g. IMR-32, SK-N- MC) | Human |
| PERC-6 | Human |
| BGM | Non-Human Primate |
| LLC-MK2 | Non-Human Primate |
| Vero | Non-Human Primate |
| MA-104 (Vero derivative) | Non-Human Primate |
| Primary monkey kidney cells[[3]](#footnote-3) | Non-Human Primate |
| L20B | Mouse[[4]](#footnote-4) |
| Lα | Mouse |
| Super E-Mix | Hybrid; mixture of cell lines |
| R-Mix | Hybrid; mixture of cell lines |

# Annex 3: Country and Territory-specific poliovirus data

The guidance provided in this document is based on a collection of reported data from 223[[5]](#footnote-5) countries and territories addressing the following parameters:

### To support the completion of the 1st part of Phase I of GAPIII (WPV2/VDPV2)

1. Year of last reported WPV2
2. Month and Year of last reported cVDPV2
3. Year of IPV introduction in routine immunization (RI)

The last detection of WPV2 worldwide was in India in October 1999; however, the month and year of the last detection has not been accurately recorded for all countries. For this reason, the guidance systematically refers to December as the month of last detection of WPV2 for specimens collected during a specific year and assigns 1999 as the year of last detection in a particular country if there was uncertainty surrounding the last reported case of WPV2.

Surveillance activities have detected cVDPVs, iVDPVs and ambiguous VDPVs (aVDPVs); aVDPVs are isolated from people without a known immunodeficiency or from environmental samples (e.g. sewage) with unknown human source, neither of which is genetically linked to another VDPV. This guidance only refers to the date of the last cVDPV2 reported for each country and territory, and does not consider iVDPV and aVDPV.

### To support the completion of the 2nd part of Phase I of GAPIII (OPV2/Sabin2)

1. tOPV use in RI
2. Year of tOPV introduction
3. Month and year of last tOPV use
4. Targeted age groups (tOPV EPI schedule: age at first dose – age at last dose)
5. Pre-tOPV-cessation SIA using tOPV in countries with tOPV in RI
6. Month and year of SIA using tOPV
7. Highest age groups targeted
8. Post-tOPV-cessation SIA using tOPV in countries that previously used tOPV in RI
	1. SIA start – SIA end dates
	2. Target age groups
9. Post-tOPV-cessation SIA using mOPV2 in countries responding to, or at risk of, a PV2 event or outbreak.
	1. SIA start – SIA end dates
	2. Target age groups

| **No**Data in the table that follows is updated as of 4 September 2017 | **Country or territory**  | **Last Reported WPV2 Virus [[6]](#footnote-6)** | **Year of tOPV Introduction** | **IPV Introduction[[7]](#footnote-7)** | **Last Reported cVDPV2 Virus 4,[[8]](#footnote-8)** | **Last Use of tOPV in Routine Immunization5**  | **tOPV in an SIA after tOPV-cessation in Routine Immunization** 6 | **Month and Year of Last Use of mOPV23** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1. 3
 | Afghanistan | 1997 |  | September 2015 | March 2013 | April 2016 | None | None |
|  | Albania | 1985 |  | May 2014 | None | April 2016 | None | None |
|  | Algeria | 1985 |  | December 2015 | None | April 2016 | None | None |
|  | *American Samoa: see under the United States of America*  |
|  | Andorra | none reported |  | 1999 | None | December 2004 | None | None |
|  | Angola | none reported |  | December 2017 | October 2013 | April 2016 | None | None |
|  | *Anguilla: see under the United Kingdom of Great Britain and Northern Ireland*  |
|  | Antigua and Barbuda | 1965 |  | November 2015 | None | April 2016 | None | None |
|  | Argentina | 1982 |  | April 2016 | None | April 2016 | None | None |
|  | Armenia | 1995 |  | July 2016 | None | April 2016 | None | None |
|  | Australia | 1965 |  | 2005 | None | November 2005 | None | None |
|  | Austria | 1995 |  | 2002 | None | December 2001 | None | None |
|  | Azerbaijan | 1996 |  | February 2016 | None | April 2016 | None | None |
|  | Bahamas | 1978 |  | October 2015 | None | April 2016 | None | None |
|  | Bahrain | none reported |  | 2008 | None | April 2016 | None | None |
|  | Bangladesh | 1999 |  | March 2015 | None | April 2016 | None | None |
|  | Barbados | 1967 |  | October 2015 | None | April 2016 | None | None |
|  | Belarus | 1959 |  | 1960 | None | April 2016 | None | None |
|  | Belgium | 1972 |  | 2001 | None | December 2003 | None | None |
|  | Belize | 1981 |  | December 2015 | None | April 2016 | None | None |
|  | Benin | 1997 |  | August 2015 | None | April 2016 | None | None |
|  | *Bermuda: see under the United Kingdom* *of Great Britain and Northern Ireland*  |
|  | Bhutan | 1999 |  | July 2015 | None | April 2016 | None | None |
|  | Bolivia, Plurinational State of  | 1986 |  | February 2016 | None | April 2016 | None | None |
|  | Bosnia and Herzegovina | 1975 |  | 2008 | None | April 2016 | None | None |
|  | Botswana | none reported |  | November 2015 | None | April 2016 | None | None |
|  | Brazil | 1986 |  | 2012 | None | April 2016 | None | None |
|  | *British Virgin Islands: see under the United Kingdom* *of Great Britain and Northern Ireland*  |
|  | Brunei Darussalam | none reported |  | 2011 | None | December 2014 | None | None |
|  | Bulgaria | 1970 |  | 2007 | None | July 2007 | None | None |
|  | Burkina Faso | none reported |  | December 2017 | None | April 2016 | None | None |
|  | *Burma: see Myanmar* |
|  | Burundi | none reported |  | November 2015 | December 2011 | April 2016 | None | None |
|  | Cabo Verde | none reported |  | August 2016 | None | April 2016 | None | None |
|  | Cambodia | 1989 |  | December 2015 | None | April 2016 | None | None |
|  | Cameroon | none reported |  | July 2015 | August 2013 | April 2016 | None | January 2017 |
|  | Canada | 1964 |  | 1955 | None | December 1996 | None | None |
|  | *Cayman Islands: see under the United Kingdom* *of Great Britain and Northern Ireland*  |
|  | Central Africa Republic | 1996 |  | September 2015 | None | April 2016 | None | None |
|  | Chad | none reported |  | August 2015 | May 2013 | April 2016 | None | January 2017 |
|  | Chile | 1971 |  | March 2016 | None | April 2016 | None | None |
|  | China, People's Republic of | none reported |  | December 2014 | February 2012 | April 2016 | None | None |
|  | China, Hong Kong SAR | 1983 |  |  | None | February 2007 | None | None |
|  | China, Macao SAR | 1975?? |  |  | None | December 2008 | None | None |
|  | Taiwan, China | 1979 |  |  | None | April 2016 | None | None |
|  | Colombia | 1982 |  | February 2015 | None | April 2016 | None | None |
|  | Comoros | 1982 |  | January 2015 | None | April 2016 | None | None |
|  | Congo | none reported |  | April 2016 | None | April 2016 | None | None |
|  | Cook Islands | none reported |  | November 2015 | None | April 2016 | None | None |
|  | Costa Rica | 1986 |  | 2010 | None | May 2011 | None | None |
|  | Côte d'Ivoire | 1997 |  | June 2015 | None | April 2016 | None | None |
|  | Croatia | 1989 |  | 1961 | None | December 2007 | None | None |
|  | Cuba | 1962 |  | January 2016 | None | April 2016 | None | None |
|  | Cyprus | 1995 |  | 2003 | None | December 2008 | None | None |
|  | Czech Republic  | 1960 |  | 2007 | None | January 2007 | None | None |
|  | Democratic People's Republic of Korea | 1999 |  | April 2015 | None | April 2016 | None | None |
|  | Democratic Republic of the Congo, the  | none reported |  | April 2015 | Outbreak not declared closed yet | April 2016 | None | September 2017 |
|  | Denmark | 1967 |  | 1955 | None | August 2003 | None | None |
|  | Djibouti | none reported |  | April 2016 | None | April 2016 | None | None |
|  | Dominica | 1981 |  | September 2015 | None | April 2016 | None | None |
|  | Dominican Republic | 1986 |  | December 2015 | None | April 2016 | None | None |
|  | Ecuador | 1987 |  | December 2015 | None | April 2016 | None | None |
|  | Egypt | 1994 |  | December 2017 | None | May 2016 | None | None |
|  | El Salvador | 1987 |  | January 2016 | None | April 2016 | None | None |
|  | Equatorial Guinea | none reported |  | August 2016 | None | April 2016 | None | None |
|  | Eritrea | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Estonia | 1959 |  | 2008 | None | December 2007 | None | None |
|  | Ethiopia | none reported |  | December 2015 | February 2009 | April 2016 | None | None |
|  | Fiji | none reported |  | December 2015 | None | April 2016 | None | None |
|  | Finland | 1981 |  | 1957 | December 2013 | 1956 | March 1985 | None |
|  | France | 1997 |  | 1964 | None | December 1983 | None | None |
|  | French Guiana  | none reported |  |  | None | April 2016 | None | None |
|  | French Polynesia  | none reported |  |  | None | December 1990 | None | None |
|  | Guadeloupe  | none reported |  |  | None | April 2016 | None | None |
|  | Martinique | none reported |  |  | None | April 2016 | None | None |
|  | New Caledonia  | none reported |  |  | None | December 1990 | None | None |
|  | La Réunion | none reported |  |  | None | April 2016 | None | None |
|  | Wallis and Futuna | none reported |  |  | None | December 1990 | None | None |
|  | *French Guiana and French Polynesia: see under France* |
|  | Gabon | none reported |  | December 2015 | None | April 2016 | None | None |
|  | Gambia, Republic of the  | none reported |  | April 2015 | None | April 2016 | None | None |
|  | Georgia | 1987 |  | December 2015 | None | April 2016 | None | None |
|  | Germany | 1990 |  | 1962 | February 2014 | December 1998 | None | None |
|  | Ghana | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Greece | 1995 |  | 2003 | None | December 2004 | None | None |
|  | Grenada | 1956 |  | June 2015 | None | April 2016 | None | None |
|  | *Guadeloupe: see under France**Guam: see under the United States of America* |
|  | Guatemala | 1988 |  | January 2016 | None | April 2016 | None | None |
|  | Guinea | none reported |  | November 2015 | December 2015 | April 2016 | None | None |
|  | Guinea-Bissau | none reported |  | July 2016 | None | April 2016 | None | None |
|  | Guyana | 1963 |  | September 2015 | None | April 2016 | None | None |
|  | Haiti | 1990 |  | January 2016 | None | April 2016 | None | None |
|  | Honduras | 1988 |  | December 2015 | None | April 2016 | None | None |
|  | *Hong Kong, SAR China: see under China* |
|  | Hungary | 1959 |  | 1959 | None | April 2016 | None | None |
|  | Iceland | 1960 |  | 1995 | None | never used | None | None |
|  | India | 1999 |  | November 2015 | January 2010 | April 2016 | None | None |
|  | Indonesia | 1999 |  | July 2016 | None | April 2016 | None | None |
|  | Iran, Islamic Republic of | 1995 |  | September 2015 | None | April 2016 | None | None |
|  | Iraq | none reported |  | January 2016 | None | April 2016 | None | None |
|  | Ireland | 1982 |  | 1957 | None | July 2001 | None | None |
|  | Israel | 1983 |  | 1998 | None | December 2004 | None | None |
|  | *Ivory Coast: see Cote d'Ivoire* |
|  | Italy | 1980 |  | 1958 | None | August 2002 | None | None |
|  | Jamaica | 1983 |  | September 2015 | None | April 2016 | None | None |
|  | Japan | 1962 |  | 2012 | None | September 2012 | None | None |
|  | Jordan | none reported |  | 2005 | None | April 2016 | None | None |
|  | Kazakhstan | 1980 |  | July 2013 | None | April 2016 | None | None |
|  | Kenya | 1984 |  | December 2015 | August 2012 | April 2016 | None | None |
|  | Kiribati | none reported |  | June 2015 | None | April 2016 | None | None |
|  | *Korea: see Democratic People's Republic of Korea or Republic of Korea*  |
|  | Kuwait | none reported |  | 2010 | None | April 2016 | None | None |
|  | Kyrgyzstan | 1992 |  | December 2017 | None | April 2016 | None | None |
|  | Lao People's Democratic Republic | 1993 |  | October 2015 | None | April 2016 | None | None |
|  | Latvia | 1963 |  | 1993 | None | December 2006 | None | None |
|  | Lebanon | none reported |  | 2011 | None | April 2016 | None | None |
|  | Lesotho | none reported |  | April 2016 | None | April 2016 | None | None |
|  | Liberia | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Libya | none reported |  | April 2014 | None | April 2016 | None | None |
|  | Lithuania | 1972 |  | 1995 | None | November 2006 | None | None |
|  | Luxembourg | 1963 |  | 2003 | None | December 1998 | None | None |
|  | *Macao, SAR China: see under China**Macedonia: see the former Yugoslav Republic of Macedonia* |
|  | Madagascar | 1995 |  | May 2015 | July 2005 | April 2016 | None | None |
|  | Malawi | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Malaysia | 1974 |  | 2009 | None | December 2015 | None | None |
|  | Maldives | 1999 |  | 2015-03 | None | April 2016 | None | None |
|  | Mali | none reported |  | March 2016 | None | April 2016 | None | None |
|  | Malta | 1964 |  | 2010 | None | September 2010 | None | None |
|  | Marshall Islands | none reported |  | 2010 | None | December 2014 | None | None |
|  | *Martinique: see under France* |
|  | Mauritania | none reported |  | November 2015 | None | April 2016 | None | None |
|  | Mauritius | none reported |  | November 2015 | None | April 2016 | None | None |
|  | Mexico | 1987 |  | 2008 | December 2010 | December 2006 | February 2016 | None |
|  | Micronesia, Federated States of  | none reported |  | August 2013 | None | April 2013 | None | None |
|  | *Moldova: See Republic of Moldova* |
|  | Monaco | 1964 |  | 1964 | None | April 2016 | None | None |
|  | Mongolia | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Montenegro | May 1990 |  | 2011 | None | April 2016 | None | None |
|  | *Montserrat: see under the United Kingdom of Great Britain and Northern Ireland* |
|  | Morocco | none reported |  | 2015-06 | None | April 2016 | None | None |
|  | Mozambique | none reported |  | 2015-11 | None | April 2016 | None | May 20177 |
|  | Myanmar | none reported |  | December 2015 | October 2015 | April 2016 | None | None |
|  | Namibia | none reported |  | 2015-11 | None | April 2016 | None | None |
|  | Nauru | none reported |  | 2015-10 | None | April 2016 | None | None |
|  | Nepal | none reported |  | 2014-09 | None | April 2016 | None | None |
|  | Netherlands | 1983 |  | 1957 | None | Never used | 1993 | None |
|  | Aruba | 1982 |  |  | None | April 2016 | None | None |
|  | Curaçao | 1982 |  |  | None | April 2016 | None | None |
|  | Sint Maarten | 1969 |  |  | None | April 2016 | None | None |
|  | *New Caledonia: see under France* |
|  | New Zealand | none reported |  | 2002 | None | February 2002 | None | None |
|  |  Tokelau | none reported |  |  | None | November 2015 | None | None |
|  | Nicaragua | 1981 |  | 2015-11 | None | April 2016 | None | None |
|  | Niger | 1981 |  | July 2015 | July 2013 | April 2016 | None | January 20177 |
|  | Nigeria | 1998 |  | 2015-02 | Outbreak not declared closed yet4 | April 2016 | None | May 20179 |
|  | Niue | none reported |  | 2002 | None | December 2004 | None | None |
|  | *Northern Mariana Islands, Commonwealth of: see under the United States of America* |
|  | Norway | 1960 |  | 1956 | None | December 1980 | None | None |
|  | Oman | none reported |  | 2010 | None | April 2016 | None | None |
|  | Pakistan | 1997 |  | July 2015 | December 2016 | April 2016 | None | March 20177 |
|  | Palau, Republic of | none reported |  | 2002 | None | December 2004 | None | None |
|  | Panama | 1973 |  | 2014-05 | None | April 2016 | None | None |
|  | Papua New Guinea | none reported |  | August 2015 | None | April 2016 | None | None |
|  | Paraguay | 1986 |  | December 2015 | None | April 2016 | None | None |
|  | Peru | 1989 |  | 2013-07 | None | April 2016 | None | None |
|  | Philippines, the | none reported |  | July 2015 | None | April 2016 | None | None |
|  | *Pitcairn Islands: see under the United Kingdom of Great Britain and Northern Ireland* |
|  | Poland | 1982 |  | 1958 | None | April 2016 | None | None |
|  | Portugal | 1961 |  | 2006 | None | December 2005 | None | None |
|  | *Puerto Rico: see under the United States of America* |
|  | Qatar | none reported |  | 2010 | None | April 2016 | None | None |
|  | Republic of Korea | none reported |  | 2004 | None | December 2004 | None | None |
|  | Republic of Moldova, the  | none reported |  | December 2017 | None | April 2016 | None | None |
|  | *Réunion Island: see under France* |
|  | Romania | 1980 |  | 2008 | None | April 2009 | January 2013 | None |
|  | Russian Federation | 1960 |  | 2008 | None | April 2016 | None | None |
|  | Rwanda | none reported |  | December 2017 | None | April 2016 | None | None |
|  | *Saint Helena: see under the United Kingdom of Great Britain and Northern Ireland* |
|  | Saint Kitts and Nevis | 1969 |  | December 2015 | None | April 2016 | None | None |
|  | Saint Lucia | 1971 |  | 2015-11 | None | April 2016 | None | None |
|  | Saint Vincent and the Grenadines | 1978 |  | 2015-06 | None | April 2016 | None | None |
|  | Samoa  | none reported |  | 2015-10 | None | April 2016 | None | None |
|  | San Marino | 1963 |  | 1960 | None | December 2000 | None | None |
|  | Sao Tome and Principe | none reported |  | April 2016 | None | April 2016 | None | None |
|  | Saudi Arabia | 1995 |  | 2008 | None | April 2016 | None | None |
|  | Senegal | none reported |  | 2015-01 | None | April 2016 | None | None |
|  | Serbia | 1990 |  | 2015-01 | None | April 2016 | None | None |
|  | Seychelles | none reported |  | September 2015 | None | April 2016 | None | None |
|  | Sierra Leone | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Singapore | 1971 |  | 2013-07 | None | April 2016 | None | None |
|  | Slovakia | 1960 |  | 2005 | None | January 2005 | None | None |
|  | Slovenia | 1978 |  | 2004 | None | Autumn 2005 | None | None |
|  | Solomon Islands | none reported |  | September 2015 | None | April 2016 | None | None |
|  | Somalia | 1998 |  | 2015-11 | January 2013 | April 2016 | None | None |
|  | South Africa | none reported |  | 2009 | None | April 2016 | None | None |
|  | South Sudan | none reported |  | December 2015 | October 2014 | April 2016 | None | None |
|  | Spain | 1987 |  | 1963 | None | March 2004 | None | None |
|  | Sri Lanka | 1993 |  | July 2015 | None | April 2016 | None | None |
|  | Sudan  | none reported |  | 2015-06 | None | April 2016 | None | None |
|  | Suriname | 1981 |  | 2015-10 | None | April 2016 | None | None |
|  | Swaziland | none reported |  | 2016-07 | None | April 2016 | None | None |
|  | Sweden | September 1992 |  | 1982 | None | never used | None | None |
|  | Switzerland | 1962 |  | 1957 | None | September 2001 | None | None |
|  | Syrian Arab Republic | none reported |  | 2008 | Outbreak not declared closed yet4 | April 2016 | None | September 20179 |
|  | *Taiwan, China: see under China* |
|  | Tajikistan | none reported |  | December 2017 | None | April 2016 | None | None |
|  | *Tanzania: see United Republic of Tanzania* |
|  | Thailand | 1993 |  | December 2015 | None | April 2016 | None | None |
|  | The former Yugoslav Republic of Macedonia | 1987 |  | August 2015 | None | April 2016 | None | None |
|  | Timor-Leste | none reported |  | 2016-02 | None | April 2016 | None | None |
|  | Togo | none reported |  | December 2017 | None | April 2016 | None | None |
|  | *Tokelau: see under New Zealand*  |
|  | Tonga | none reported |  | December 2015 | None | April 2016 | None | None |
|  | Trinidad and Tobago | 1973 |  | December 2015 | None | April 2016 | None | None |
|  | Tunisia | none reported |  | 2014-09 | None | April 2016 | None | None |
|  | Turkey | July 1991 |  | 2008 | None | April 2016 | None | None |
|  | Turkmenistan | none reported |  | December 2017 | None | April 2016 | None | None |
|  | *Turks and Caicos Islands: see under the United Kingdom of Great Britain and Northern Ireland* |
|  | Tuvalu | none reported |  | 2015-11 | None | November 2015 | None | None |
|  | Uganda | none reported |  | April 2016 | None | April 2016 | None | None |
|  | Ukraine | 1992 |  | 2007 | None | April 2016 | None | None |
|  | United Arab Emirates  | none reported |  | 2010 | None | April 2016 | None | None |
|  | United Kingdom of Great Britain and Northern Ireland | 1977 |  | 2004 | None | August 2004 | None | None |
|  | Anguilla | 1962 |  |  | None | April 2016 | None | None |
|  | Bermuda | 1974 |  |  | None | April 2016 | None | None |
|  | British Virgin Islands | none reported |  |  | None | April 2016 | None | None |
|  | Cayman Islands | 1965 |  |  | None | April 2016 | None | None |
|  | Montserrat | 1977 |  |  | None | April 2016 | None | None |
|  | Pitcairn Islands | 1999 |  |  | None | April 2016 | None | None |
|  | Saint Helena | none reported |  |  | None | April 2016 | None | None |
|  | Turks and Caicos Islands | 1979 |  |  | None | April 2016 | None | None |
|  | United Republic of Tanzania | none reported |  | December 2017 | None | April 2016 | None | None |
|  | United States of America | 1965 |  | 1997 | None | December 1999 | None | None |
|  | American Samoa  | none reported |  |  | None | December 2005 | None | None |
|  | Guam  | none reported |  |  | None | December 1998 | None | None |
|  | Northern Mariana Islands | none reported |  |  | None | December 1998 | None | None |
|  | Puerto Rico | 1975 |  |  | None | April 2016 | None | None |
|  | US Virgin Islands | 1982 |  |  | None | April 2016 | None | None |
|  | Uruguay | 1979 |  | 2012 | None | December 2011 | None | None |
|  | *US Virgin Islands: see under the United States of America* |
|  | Uzbekistan | 1991 |  | December 2017 | None | April 2016 | None | None |
|  | Vanuatu | none reported |  | 2015-11 | None | April 2016 | None | None |
|  | Venezuela, Bolivarian Republic of | none reported |  | January 2016 | None | April 2016 | None | None |
|  | Viet Nam | 1991 |  | December 2017 | February 2012 | April 2016 | None | None |
|  | *Virgin Islands, British: see under the United Kingdom of Great Britain and Northern Ireland;* *Virgin Islands, US: see under the United States of America;* *Wallis and Futuna: see under France* |
|  | Yemen | none reported |  | 2015-11 | October 2011 | April 2016 | None | None |
|  | Zambia | none reported |  | December 2017 | None | April 2016 | None | None |
|  | Zimbabwe | none reported |  | December 2017 | None | April 2016 | None | None |
|  | West Bank and Gaza Strip  | none reported |  |  | None | April 2016 | None | None |

# Annex 4. References

World Health Organization. Resolution WHA68.3. Poliomyelitis. In: Sixty-eighth World Health Assembly, Geneva, 18–26 May 2015. Geneva: WHO; 2015 (http://apps.who.int/gb/ebwha/pdf\_files/WHA68/A68\_R3-en.pdf, accessed 24 May 2016).

World Health Organization. Polio Eradication & Endgame Strategic Plan 2013-2018. Geneva:WHO; 2013.

World Health Organization. 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. Geneva: WHO; 2015. (http://www.polioeradication.org/Portals/0/Document/Resources/PostEradication/GAPIII\_2014.pdf)

**Isolation of Sabin viruses in IPV using countries**

Battistone A, Buttinelli G, Fiore S, Amato C, Bonomo P, Patti AM et al. 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. 2014;80(15):4491-501. doi:10.1128/AEM.00108-14.

Zurbriggen S, Tobler K, Abril C, Diedrich S, Ackermann M, Pallansch MA et al.. Isolation of Sabin-like polioviruses from wastewater in a country using inactivated polio vaccine. Appl Environ Microbiol. 2008;74(18):5608-14. doi: 10.1128/AEM.02764-07.

Kew O, Morris-Glasgow V, Landaverde M, Burns C, Shaw J, Garib Z et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science. 2002;296(5566):356-9. doi: 10.1126/science.1068284.

Shulman LM, Martin J, Sofer D, Burns CC, Manor Y et al. Genetic analysis and characterization of wild poliovirus type 1 during sustained transmission in a population with >95% vaccine coverage, Israel 2013. Clin Infect Dis. 2015;60(7):1057-64. doi: 10.1093/cid/ciu1136.

**Use of transgenic mice**

Maintenance and distribution of transgenic mice susceptible to human viruses: memorandum from a WHO meeting. Bull World Health Organ. 1993;71(5):497-502.

**Detection of Polioviruses in waste water, healthy carriers and stored specimens**

Tambini G, Andrus JK, Marques E, Boshell J, Pallansch M, de Quadros CA et al. Direct detection of wild poliovirus circulation by stool surveys of healthy children and analysis of community wastewater. J Infect Dis. 1993;168(6):1510-4. doi: 10.1093/infdis/168.6.1510.

Pallansch M, Staples M. Wild poliovirus found in stored potential infectious materials. World Health Organization Polio Laboratory Network Quarterly Update. 2002;8:1-2.

Savolainen C, Hovi T. Caveat: poliovirus may be hiding under other labels. Lancet. 2003;5;361(9364):1145-6. doi:10.1016/S0140-6736(03)12965-0.

Mach O, Verma H, Khandait DW, Sutter RW, O'Connor PM, Pallansch MA et al. Prevalence of asymptomatic poliovirus infection in older children and adults in northern India: analysis of contact and enhanced community surveillance, 2009. J Infect Dis. 2014 Nov 1;210 Suppl 1:S252-8. doi: 10.1093/infdis/jit234.

**Detection of Polioviruses in CSF, respiratory secretion and other biological specimens**

Davies M, Bruce C, Bewley K, Outlaw M, Mioulet V, Lloyd G et al. Poliovirus type 1 in working stocks of typed human rhinoviruses. Lancet. 2003;5;361(9364):1187-8. doi:10.1016/S0140-6736(03)12919-4.

Routine tests of CSF for poliovirus surveillance give low yield. World Health Organization Polio Laboratory Network Quarterly Update. 1998;4(3):1-2.

Leparc-Goffart I, Julien J, Fuchs F, Janatova I, Aymard M, Kopecka H. Evidence of presence of poliovirus genomic sequences in cerebrospinal fluid from patients with postpolio syndrome. J Clin Microbiol. 1996;34(8):2023-6.

Centers for Disease Control and Prevention. Polio Laboratory Diagnostic Methods. (https://www.cdc.gov/polio/us/lab-testing/diagnostic.html, accessed 24 May 2016)

Portes SA, Da Silva EE, Siqueira MM, De Filippis AM, Krawczuk MM, Nascimento JP. Enteroviruses isolated from patients with acute respiratory infections during seven years in Rio de Janeiro (1985-1991). Rev Inst Med Trop Sao Paulo. 1998;40(6):337-42. http://dx.doi.org/10.1590/S0036-46651998000600001.

Grard G, Drexler JF, Lekana-Douki S, Caron M, Lukashev A, Nkoghe D et al. Type 1 wild poliovirus and putative enterovirus 109 in an outbreak of acute flaccid paralysis in Congo, October-November 2010. Euro Surveill. 2010;15(47):pii: 19723.

Nakamura T, Hamasaki M, Yoshitomi H, Ishibashi T, Yoshiyama C et al. Environmental surveillance of poliovirus in sewage water around the introduction period for inactivated polio vaccine in Japan. Appl Environ Microbiol. 2015;81(5):1859-64. doi: 10.1128/AEM.03575-14.

Esteves-Jaramillo A, Estívariz CF, Peñaranda S, Richardson VL, Reyna J et al. Detection of vaccine-derived polioviruses in Mexico using environmental surveillance. J Infect Dis. 2014;210(suppl. 1):S315-23. doi: 10.1093/infdis/jiu183.

Khan S, Peng X, Yin J, Zhang P, Wimmer E. Characterization of the New World monkey homologues of human poliovirus receptor CD155. J Virol. 2008;82(14):7167-7179.

Racaniello V. One hundred years of poliovirus pathogenesis. Virology. 2005;344(2006):9-16.

Burns C, Diop O, Sutter R, Kew O. Vaccine-derived polioviruses. J Infect Dis. 2014;210(S1):S283-293.

Aylward R. 2015. Poliomyelitis. In: Heymann D. ed. Control of communicable diseases manual. (20th ed.). Washington, DC: APHA Press, pp. 477-484.

Diop O. Overview of the performance of the Global Polio Laboratory Network. Presented at 22nd Informal Consultation on the Global Polio Laboratory Network, Geneva, Switzerland, 10 March 2016.

**Molecular detection and manipulation of PV**

Lee-Montiel FT, Reynolds KA, Riley MR. Detection and quantification of poliovirus infection using FTIR spectroscopy and cell culture. J Biol Eng. 2011;5:16. doi: 10.1186/1754-1611-5-16.

Cello J, Paul AV, Wimmer E. Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. Science. 2002;297(5583):1016-8. dOI: 10.1126/science.1072266.

Wimmer E, Paul AV. Synthetic poliovirus and other designer viruses: what have we learned from them? Annu Rev Microbiol. 2011;65:583-609. doi: 10.1146/annurev-micro-090110-102957.

Nijhuis M, van Maarseveen N, Schuurman R, Verkuijlen S, de Vos M, Hendriksen K et al. Rapid and sensitive routine detection of all members of the genus enterovirus in different clinical specimens by real-time PCR. J Clin Microbiol. 2002;40(10):3666-70. doi: 10.1128/JCM.40.10.3666-3670.2002.

Macadam AJ, Ferguson G, Stone DM, Meredith J, Knowlson S, Auda G et al. Rational design of genetically stable, live-attenuated poliovirus vaccines of all three serotypes: relevance to poliomyelitis eradication. J Virol. 2006;80(17):8653-63. doi: 10.1128/JVI.00370-06.

Knowlson S, Burlison J, Giles E, Fox H, Macadam AJ, Minor PD. New Strains Intended for the Production of Inactivated Polio Vaccine at Low-Containment After Eradication. PLoS Pathog. 2015;31;11(12):e1005316. doi: 10.1371/journal.ppat.1005316.

Dowdle WR, Birmingham ME. The biologic principles of poliovirus eradication. J Infect Dis. 1997;175(suppl. 1):S286-92. doi:10.1093/infdis/175.Supplement\_1.S286

**Risk Analysis and containment:**

Thompson KM. Poliomyelitis and the role of risk analysis in global infectious disease policy and management. Risk Anal. 2006;26(6):1419-21. doi: 10.1111/j.1539-6924.2006.00853.x.

Dowdle W, van der Avoort H, de Gourville E, Delpeyroux F, Desphande J, Hovi T et al. Containment of polioviruses after eradication and OPV cessation: characterizing risks to improve management. Risk Anal. 2006;26(6):1449-69. doi: 10.1111/j.1539-

Fine, PEM, Ritchie, S. (2006) Perspective: Determinants of the Severity of Poliovirus Outbreaks in the Post Eradication Era. Risk Analysis, 26, 1533-1540.Mach, O, et al (2014) Prevalence of asymptomatic Poliovirus infection in older children and adults in Northern India: Analysis of contact and enhanced community surveillance, 2009. J. Inf. Dis, 210, 252-258

1. : must comply with the risk mitigation strategy; n/a: not applicable. [↑](#footnote-ref-1)
2. For short-term retention only, while the final disposition of the collection is being considered. If “stored” samples are to be handled, the risk mitigation strategies for Level 1, 2, or 3 must be applied as appropriate for the sample type and procedure (Table 1). [↑](#footnote-ref-2)
3. Old World Monkeys [↑](#footnote-ref-3)
4. Transgenic mouse cell lines [↑](#footnote-ref-4)
5. Calculated as the sum of 194 Member States, 4 territories, 1 dependent territory, 15 overseas territories, 2 special administrative regions, 4 constituent countries of other countries, 1 overseas collectivity and 2 countries with WHO observer status, that may have different routine immunization and SIA schedules, and different epidemiological situations as a result of their geographic location than the sovereign states that administer them. [↑](#footnote-ref-5)
6. Data available at WHO HQ including WHO Polio Information System (POLIS). Data sources for WPV2 virus may include: AFP case, environmental sampling, enterovirus surveillance or other sources. Data sources for cVDPV2 virus include AFP case or environmental sampling. [↑](#footnote-ref-6)
7. WHO/UNICEF Joint Reporting form [↑](#footnote-ref-7)
8. WHO Polio - Supplementary Immunization Activity (POLSIA) on Immunization [↑](#footnote-ref-8)