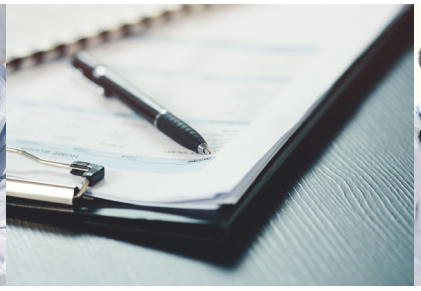
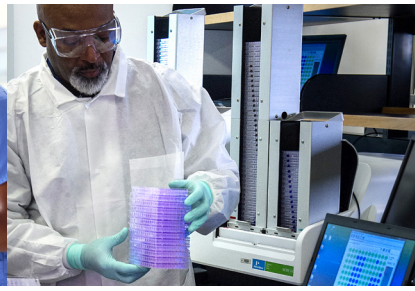
Interim Guidance for U.S. Laboratory Facilities to Store and Work with Poliovirus Potentially Infectious Materials

EFFECTIVE NOVEMBER 22, 2022



NAC
U.S. NATIONAL AUTHORITY
FOR CONTAINMENT OF POLIOVIRUS

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Office of Readiness and Response

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Acronyms

aVDPV	Ambiguous vaccine-derived poliovirus
bOPV	Bivalent oral polio vaccine
BSC	Biosafety cabinet
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CP	Certificate of Participation
cVDPV	Circulating vaccine-derived poliovirus
CWG	Containment Working Group (WHO)
GAPIV	WHO Global Action Plan, Fourth edition
GCC	Global Commission for the Certification of the Eradication of Poliomyelitis (WHO)
IPV	Inactivated polio vaccine
iVDPV	Immunodeficient vaccine-derived poliovirus
mOPV	Monovalent oral polio vaccine
NAC	National Authority for Containment of Poliovirus
nOPV	Novel oral polio vaccine
OPV	Oral polio vaccine
PEF	Poliovirus-essential facility
PIM	Potentially infectious materials
PPE	Personal protective equipment
RNA	Ribonucleic acid
tOPV	Trivalent oral polio vaccine
VDPV	Vaccine-derived poliovirus
WHO	World Health Organization
WPV	Wild poliovirus

Definitions

Certificate of Participation	A certificate required by WHO to retain PV IM. This certificate is endorsed by the GCC-CWG and the U.S. NAC and issued by the U.S. NAC.
Circulating VDPV	"VDPV isolates for which there is evidence of person-to-person transmission in the community, based on evidence from human and/or environmental detections of genetically linked viruses." [i]
Global Action Plan IV	The WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use. The 4th edition of the Global Action Plan (GAPIV) aligns the safe handling and containment of poliovirus infectious and potentially infectious materials with the WHO Endgame Strategy and replaces both the 2014 3rd edition and the 2nd edition of the WHO global action plan for laboratory containment of wild polioviruses.

Inactivated Poliovirus Vaccine	The inactivated poliovirus vaccine was developed in 1955 by Salk and Youngner. IPV is a killed-virus vaccine and is administered by injection.
Infectious materials	WPV/VDPV • "Clinical materials from confirmed wild poliovirus infections; • Environmental sewage or water samples that have tested positive for the presence of wild polioviruses; • Cell culture isolates and reference strains of wild poliovirus; • Seed stocks and infectious materials from IPV production; • Infected animals or samples from such animals, including human poliovirus receptor transgenic mice; • Infectious viruses produced in the laboratory that have capsid sequences from wild polioviruses [1], unless demonstrably proven to be safer than Sabin strains. The safety of new derivatives containing wild poliovirus capsid sequences will be assessed by an expert panel convened by WHO, on the basis of comparison to reference Sabin strains for (i) degree and stability of attenuation; (ii) potential for person-to-person transmission; and (iii) neurovirulence in animal models; • Cells persistently infected with poliovirus strains whose capsid sequences are derived from Sabin/OPV strains [2]." [ii] • "Vaccine-derived polioviruses (VDPVs) are classified with wild polioviruses." [ii] Sabin/OPV • "Cell culture isolates and reference Sabin/OPV strains; • Seed stocks and live virus materials from Sabin/OPV production; • Environmental sewage or water samples that have tested positive for the presence of Sabin/OPV strains; • Fecal or respiratory secretion samples from recent Sabin/OPV recipients; • Infected animals or samples from such animals, including poliovirus receptor transgenic mice; • Derivatives produced in the laboratory that have capsid sequences from Sabin/OPV strains [3]; • Cells persistently infected with poliovirus strains whose capsid sequences are derived from Sabin/OPV strains [4]." [ii]
Novel OPV (nOPV)	Novel OPV strains are more genetically stable and less likely to revert to neurovirulence and induce vaccine-associated paralytic poliomyelitis as compared to previous OPV strains, while producing comparable safety and immunogenicity. [iii, iv]

[1] For U.S. facilities, PV infectious viruses and derivatives must contain a complete full-length WPV capsid sequence to meet the WPV IM definition.
[2] For U.S. facilities, PV strains must contain a complete full-length WPV capsid sequence to meet the WPV IM definition.
[3] For U.S. facilities, PV derivatives must contain a complete full-length Sabin/OPV capsid sequence to meet the Sabin/OPV IM definition.
[4] For U.S. facilities, PV strains must contain a complete full-length Sabin/OPV capsid sequence to meet the Sabin/OPV IM definition.

Nucleic acids	"Full-length poliovirus RNA, cDNA and total nucleic acid extracted from poliovirus infectious materials (*e.g.,* a virus isolate) or potentially infectious materials (*e.g.,* stool, respiratory specimen, sewage) using methods demonstrated to inactivate poliovirus, or synthesized RNA or cDNA (*e.g.,* cDNA clone, synthetic transcript). Poliovirus nucleic acid can be handled outside of poliovirus containment under the condition that these materials will not be introduced into poliovirus permissive cells or animals (as defined in GAPIV and in the "Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses") with or without a transfection reagent. The use of poliovirus nucleic acids with polio-permissive cells that have been rendered and validated as non-polio permissive by techniques such as genetic engineering, etc. are not subject to these requirements." [ii] **Note: WHO does require that full-length PV nucleic acids be included as part of the facility and national inventories.**
Poliovirus	"A picornavirus consisting of three serotypes: 1, 2 and 3. Poliovirus serotypes are further subdivided into wild (circulating in nature) and Sabin strains (attenuated strains used for oral poliovirus vaccines). Polioviruses use CD155 as the primary cellular receptor." [ii] Protective immunity is type-specific. Poliovirus types 2 and 3 have been eliminated in the wild. In this current stage of polio eradication, only type 1 wild poliovirus continues to circulate in endemic areas. It is highly infectious and causes paralytic polio. Wild: • "Wild polioviruses are naturally occurring isolates known or believed to have circulated persistently in the community. • Vaccine-derived polioviruses (VDPVs) are classified with wild polioviruses. VDPVs are rare strains of poliovirus that have genetically mutated from the strained contained in the oral poliovirus vaccine (OPV). They are >0.6% (type 2) or >1% (types 1 and 3) divergent from the corresponding OPV strain in the complete VP1 genomic region [1]. Some isolates display >15% sequence diversity but are phylogenetically related to parental Sabin strains. They may have circulated in the community (cVDPV) or have replicated for prolonged periods in immunodeficient subjects (iVDPV) or be ambiguous and of unknown origin (aVDPV). • Attenuated strains not licensed for use as live vaccines (Cox/Lederle and Koprowski/Wistar series) are classified with wild polioviruses as their clinical properties are unproven." [ii] Sabin (Sabin/OPV strains): "Attenuated poliovirus strains (approved for use in oral poliovirus vaccines by national regulatory authorities, principally Sabin strains)." [ii]

	Also called 'Sabin vaccine', Sabin/OPV contains live, attenuated (weakened) poliovirus strains. OPV formulations include: • Trivalent OPV (tOPV) contains all three serotypes of Sabin strains (1 + 2 + 3); use of tOPV ended in April 2016 • Bivalent OPV (bOPV) contains Sabin strains 1 + 3; as of April 2016, only bOPV is used routinely • Monovalent OPV (mOPV) contains only one serotype of Sabin strain Novel OPV (nOPV): See definition above. OPV-like: • "For the laboratory network not involved in manufacture, isolates consistent with a limited period of virus excretion or person-to-person transmission, demonstrating less than 1% difference from parent Sabin/OPV strains for poliovirus types 1 and 3, and less than 0.6% difference from the type 2 parent Sabin/OPV strain by full Viral Protein 1 sequence homology. The phenotype of clinical and environmental OPV-like isolates need not be determined as the great majority are assumed to be of low virulence • Sabin materials may be (a) infectious or (b) potentially infectious. The attenuated phenotype of viruses resulting from manufacture based on the Sabin/OPV seeds must be assured and cannot rely on the lack of sequence drift alone." [ii]
Poliovirus- essential facility	"A facility designated by the ministry of health or designated national body or authority as serving critical national or international functions involving the handling and storage of needed poliovirus materials subject to this standard and as a qualified applicant for national containment certification." [ii] U.S. PEFs will possess or be in pursuit of a CP.
Potentially infectious materials	Unknown/untyped applies to all PIM in which a facility has not tested the material to determine the serotype or cannot determine the collection date or country. WPV [5] • "Faecal or respiratory secretion samples and their derivatives (*e.g.* stool suspensions, extracted nucleic acids, etc.) collected for any purpose in a time and geographic area where wild poliovirus (including VDPV) circulation ; • Products of such materials from poliovirus permissive cells or animals; • Uncharacterized enterovirus-like cell culture isolates derived from countries known or suspected to have circulating wild poliovirus or VDPV at the time of collection; • Respiratory and enteric virus stocks handled under conditions where

[5] WPV includes VDPV per GAPIV WPV definition quoted in "Poliovirus" definition.

	poliovirus contamination or replication is possible; and • Environmental samples (*i.e.* concentrated sewage, wastewater [6]) collected from areas known or suspected to have circulating WPV or VDPV at the time of collection." [ii] Sabin/OPV • "Faecal or respiratory secretion samples and their derivatives collected for any purpose in a time and geographic area of Sabin/OPV use; • Products of such materials from poliovirus permissive cells or animals; • Respiratory and enteric virus stocks handled under conditions where Sabin/OPV contamination or replication is possible; and • Environmental samples (*i.e.* concentrated sewage, wastewater [6]) collected from areas known or suspected to have circulating Sabin/OPV at the time of collection." [ii]
Poliovirus materials	Unless a serotype is specifically identified, PV materials refer to IM and PIM of all three PV serotypes.

Purpose and Scope

The U.S. NAC *Interim Guidance for U.S. Laboratory Facilities to Store and Work with Poliovirus Potentially Infectious Materials* applies to all U.S. laboratory facilities possessing WPV/VDPV and OPV PIM. Please note that WHO classifies VDPV with WPV, as described in the WPV definition above. This U.S. NAC guidance document describes biosafety, security, and other measures to store and handle PV PIM in a safe and secure manner by mitigating the risks PV poses to personnel, the environment, and the global eradication of poliovirus. While not all PV PIM will contain PV, the WHO considers the probability that such material could contain PV to be a significant risk to the environment and community, if not contained properly.

The measures described herein are stratified based on the risks associated with each PIM type, based on the WHO *Guidance to minimize risks for facilities collecting, handling, or storing materials potentially infectious for polioviruses* (WHO PIM Guidance). Any U.S. facility in possession of PIM may adopt additional biosafety and security measures over and above the measures described in this, or other applicable U.S. NAC and WHO documents, to ensure PV PIM safety and security.

The U.S. NAC does not address nOPV in this document as WHO GAPIV considers this material to be usable outside of PV containment at this time. [v] The U.S. NAC recommends that U.S. facilities possessing or working with nOPV PIM review this document and consider implementing elements to prevent exposure to personnel, the environment, or community.

The U.S. NAC does not require U.S. facilities (*i.e.,* non-PEFs) that store and/or work with WPV/VDPV and/or Sabin/OPV PIM to become a PEF at this time. **Non-PEFs that retain VDPV PIM for thirty (30) days or more must**

[6] The U.S. NAC considers unconcentrated and concentrated wastewater and sludge as part of the WPV PIM definition.

report PIM possession to the U.S. NAC by submitting a U.S. NAC Poliovirus Inventory Survey, maintain an accurate inventory, and notify the U.S. NAC of PIM samples transferred to other facilities. Non-PEFs should implement the biosafety and security measures described herein to safely and securely store and work with PV PIM outside of laboratories meeting U.S. NAC and GAPIV containment. The U.S. NAC recommends that non-PEFs also conduct a site-specific risk assessment to identify and mitigate biosafety and security risks associated with these materials. Non-laboratory facilities collecting VDPV PIM should refer to the U.S. NAC *Interim Guidance for Non-Laboratory Facilities that Collect, Handle, Store, and Transport Potentially Infectious Materials in Areas Where Ongoing Poliovirus Positive Samples are Being Detected* (*Pending*).

Background

The following statements apply to this guidance document:

- The U.S. NAC interprets WHO containment requirements and guidance from GAPIV, PIM Guidance, *Public Health Management of Facility Related Exposure to Live Polioviruses,* and other documents and, with the assistance of an external working group and feedback from the affected facilities, creates policies for implementing specific aspects of PV containment in the U.S.
- U.S. NAC policies are subject to modification depending on external circumstances such as the epidemiological situation, vaccination coverage, environmental surveillance, new international policies, or changes in eradication status.
- U.S. NAC policies excerpt information from GAPIV or other documents, shown in quotations, and/or include a reference to GAPIV elements or other materials where applicable.
- The terms: a) “shall” or “must” indicate a requirement; b) “should” or “consider” indicate a recommendation; c) “may” indicates a permission; d) “can” indicates a possibility or a capability.

The following statements apply to U.S. facilities possessing PIM:

- U.S. facilities must review materials in their possession including, but not limited to, environmental, stool, and respiratory samples to determine if the material meets the WHO PIM definition. Facilities should use the WHO Web Annex A: Country- and Area-Specific Poliovirus Data to identify PIM. Please review the WHO PIM Guidance for a full list of materials that meet the WHO PIM definition. Once the facility has completed their material review, they must submit a National Inventory for Poliovirus Containment survey, regardless of PIM possession. The U.S. NAC has developed guidance and instructions to complete the survey. Please contact the U.S. NAC for additional questions.
- U.S. facilities possessing PIM should submit a declaration or statement of responsibility to demonstrate commitment from the institution to handle PIM in accordance with U.S. NAC and WHO guidelines. The U.S. NAC will provide guidance for developing this statement.

Potentially Infectious Poliovirus Materials Risk Levels

The U.S. NAC modified Table 1 of the WHO PIM Guidance that includes risk levels for different work procedures and PIM types (Appendix I). U.S. NAC modifications include 1) adding WPV/VDPV PIM, 2) adding material types (*e.g.,* wastewater), 3) adding procedures (*i.e.,* virus propagation, cell culture), and 4) removing inactivated material [7]. The WHO PIM Guidance only addresses Sabin/OPV PIM, which the U.S. NAC did not alter for this document. The U.S. NAC will update this document when additional guidance becomes available from WHO.

The stratified risk levels consider the ability of WPV/VDPV and Sabin/OPV to cause disease (*i.e.*, WPV/VDPV is highest risk), potential PV titers in each sample (*i.e.*, stool contains highest titers), and procedures that could produce high titers (*e.g.*, virus propagation, cell culture). Facilities should consult Appendix II of this document to implement specific biosafety and security mitigations (*e.g.*., PPE, locks) based on each risk level.

High Risk

The U.S. NAC PIM High risk level applies to PV with a high probability to cause disease (*i.e.,* WPV/VDPV) and procedures using materials that may contain high titers (*i.e.,* stool, concentrated sewage/wastewater) and/or produce high titers (*e.g*., PV-permissive cell usage for virus propagation, cell culture, or nucleic acid transfection).

Moderate Risk

The U.S. NAC PIM Moderate risk level applies to

> 1) PV with a high probability to cause disease (*i.e.,* WPV/VDPV) and procedures using materials that may contain high WPV/VDPV titers (*i.e*., stool, concentrated sewage/wastewater) but produce low WPV/VDPV titers (*e.g.*, PV non-permissive cell usage for inoculation or nucleic acid transfection) or
> 2) attenuated PV strains (*i.e*., Sabin/OPV) and procedures using materials that could contain high Sabin/OPV titers (*i.e.*, stool, concentrated sewage/wastewater) and produce high Sabin/OPV titers (*e.g*., PV-permissive cell usage for virus propagation, cell culture, or nucleic acid transfection).

Low Risk

The U.S. NAC PIM Low risk level applies to

> 1) PV with a high probability to cause severe disease (*i.e.,* WPV/VDPV) and procedures using materials that could contain low WPV/VDPV titers (*i.e*., respiratory, unconcentrated sewage/wastewater) and produce low WPV titers (*e.g.*, PV non-permissive cell usage for inoculation or transfection) or
> 2) attenuated PV strains (*i.e*., Sabin/OPV) and procedures using materials that could contain high

[7] WHO has determined that material inactivated using a validated method is not PIM.

Sabin/OPV titers (*i.e.*, stool, concentrated sewage/wastewater) and produce low Sabin/OPV titers (*e.g.*, PV non-permissive cell usage for virus propagation, cell culture, or nucleic acid transfection).

Lowest Risk

The U.S. NAC PIM Lowest risk level includes attenuated PV strains (*i.e.*, Sabin/OPV) and procedures using materials that could contain low Sabin/OPV titers (*i.e.*, respiratory, unconcentrated sewage/wastewater) and produce low Sabin/OPV titers (*e.g.*, PV non-permissive cell usage for virus propagation, cell culture, nucleic acid transfection).

Risk Mitigations

For High risk level materials and procedures, facilities should review and implement the U.S. NAC *Risk mitigation strategies for in vitro and in vivo work with poliovirus infectious materials* (RMS) for work and storage. The RMS addresses PV infectious materials; however, the U.S. NAC has determined that these measures are also applicable to High risk level PV PIM materials and procedures. Please contact the U.S. NAC for additional guidance on implementing RMS standards.

For Moderate, Low, and Lowest risk levels, the U.S. NAC has developed strategies to minimize the potential exposure of PV to personnel and the community. Appendix II provides a full summary of the mitigations recommended for each risk level. The NAC has provided descriptions and guidance below for elements that may require explanation.

Biosafety Strategies

Primary containment

For Moderate and Low Risk materials, primary containment (*i.e.,* certified BSC) should be used to open containers and perform work with PIM, particularly work that could produce sprays or splashes, in addition to the other risk-level measures outlined in Appendix II. Poliovirus is very environmentally stable and primary containment will help mitigate personnel and laboratory contamination. If primary containment is unavailable, facilities should still employ all other measures outlined in Appendix II.

Surgical mask

For Moderate and Low Risk materials, personnel should wear fluid-resistant surgical masks (ASTM F2100-20 rated level 2/3) to provide oral mucous membrane protection when handling PV PIM and mitigate oral transmission. [vi] (Refer to U.S. NAC RMS)

Face protection - Face shield

For Moderate and Low Risk materials, personnel should wear face shields that meet ANSI/ISEA Z87.1 (D3) for material manipulations or activities that cannot be performed inside primary containment that may result in splashes or sprays. [vii, viii, ix] Prescription glasses should not substitute for eye or splash protection. [x] Contact lens use in the laboratory should follow NIOSH recommendations. [xi] (Refer to U.S. NAC RMS)

Double gloves

For Moderate Risk material, personnel should double glove to provide an extra layer of protection to reduce fluid leakage while handling PIM. For all risk levels, the cuff length of gloves should be long enough to ensure skin is not exposed between the glove and laboratory coat/gown. (Refer to U.S. NAC RMS)

Segregate materials from other PV types or non-PV materials

Facilities should segregate PIM from other virus isolates, cell lines, cultures or other materials that could be subject to cross-contamination or misidentification. Facilities may store PIM in separate boxes, racks, or shelves in freezers. (Refer to U.S. NAC RMS)

Security Strategies

Identify essential personnel

Facilities should identify individuals who require access to materials, storage units (*e.g.,* refrigerators, freezers, incubators), and PV PIM work area(s). Essential personnel could include laboratorians, facilities staff (*e.g.,* engineering, HVAC), and custodians. (Refer to U.S. NAC *Policy for U.S. poliovirus-essential facilities to control security of poliovirus materials and information*)

Limit access to storage units and works areas to essential personnel only

Facilities should implement measures to ensure essential personnel are the only individuals permitted to access PV PIM materials, work, and storage areas. Facilities may limit access by restricting methods used to access PIM work and storage areas (*e.g.,* passwords, keys, security badges) to essential personnel only. (Refer to U.S. NAC *Policy for U.S. poliovirus-essential facilities to control security of poliovirus materials and information*)

Locked and dedicated storage unit

Facilities should lock storage units containing PIM (*e.g.,* refrigerators, freezers, incubators) to separate units storing PIM from units storing non-PIM. Appropriate locks include a padlock, combination lock or other unique means to restrict access rather than use of a standard manufacturer installed lock. In addition to an external lock, facilities should also consider dedicating a storage unit or section of a storage unit to separate and secure PIM from other materials to minimize cross-contamination or misidentification. (Refer to U.S. NAC *Policy for U.S. poliovirus-essential facilities to control security of poliovirus materials and information*)

Inventory

Facilities should maintain current inventories of all PIM in their possession. Inventory records should include material characteristics such as date and location of collection, current storage location, and an estimated number of samples for each material type. Inventory records should also document final disposition (*e.g.,* facility no longer possesses material due to consumption, transfer, or destruction). (Refer to U.S. NAC *Policy for U.S. Poliovirus-Essential Facilities to Manage Inventory*)

Transport and transfer

Facilities should establish protocols to transfer PV PIM safely and securely to other laboratories

internal or external to the facility in accordance with institutional policies. Intra-facility transports should protect personnel and the environment during transport including, but not limited to, leak-proof secondary container, gloves and handwashing when handling all material. Inter-facility transfers must be packaged and shipped in accordance with applicable local, state, federal, and international shipping laws. Facilities must notify the U.S. NAC of a transfer prior to a shipment and complete the U.S. NAC Transfer form after the transfer is complete. Facilities should update their records accordingly to maintain an accurate inventory. (Refer to U.S. NAC *Policy for U.S. Facilities to Transfer Poliovirus*)

Personnel Strategies

Occupational health program

Facility occupational health programs (OHP) should monitor essential personnel for PV infection as part of the institutional medical surveillance program. (Refer to U.S. NAC *Policy for poliovirus occupational health programs at U.S. poliovirus-essential facilities (pending)*)

Polio immunization

Facilities should ensure personnel are immunized against all three PV serotypes (*i.e.,* documentation of immunization). Assessing the polio vaccination status of those handling PIM and offering vaccination or adult boosters in accordance with ACIP recommendations, when indicated. (Refer to U.S. NAC *Policy for poliovirus occupational health programs at U.S. poliovirus-essential facilities (pending)*)

Decontamination and Emergency Response Strategies

Decontaminate work surfaces and waste using validated methods

Facility decontamination and waste disposal procedures should include methods validated to inactivate PV. The WHO GAPIV recommends that facilities autoclave or incinerate solid waste and treat liquid waste with sodium hypochlorite. (Refer to U.S. NAC RMS and *Policy to inactivate poliovirus materials*)

Emergency Response

Facilities should develop procedures to contain and disinfect materials, areas, and equipment contaminated following a PV PIM spill or breach outside of primary containment. Procedures should also include medical assessments including administration of applicable first aid and medical treatment as well as notifications to the principal investigator, biosafety officer, occupational health staff, and NAC following an incident or exposure. (Refer to U.S. NAC *Policy for emergency response and exposure management plans at U.S. poliovirus-essential facilities (pending)*)

Inactivate PIM Using Validated Methods

From the U.S. NAC *Policy for U.S. Facilities to Inactivate Poliovirus Materials*:

- "All U.S. facilities that possess poliovirus (PV) infectious material (IM) or potentially infectious material (PIM)" should inactivate PV using validated methods. "Validated methods may include

published validated inactivation protocols (*e.g.,* peer-reviewed journals or protocols, Global Laboratory Network protocols), manufacturer's instructions demonstrated to inactivate PV without modifications, or other procedures demonstrated to be effective in inactivating PV."

- "At this time, nucleic acids may be extracted from typed and untyped PIM, in a non-PV laboratory or outside of PEF PV containment area(s) if extraction modifications validated to inactivate PV are implemented."
    - Guanidine thiocyanate (GuSCN) "kits must use at least 4M GuSCN and a 20% final ethanol (ETOH) concentration for a 30-minute incubation following the lysis buffer to inactivate PV."
    - "Nucleic acids extracted without the modifications must incubate the nucleic acid preparation using a final concentration of at least 90% ETOH for 30 minutes to ensure PV has been inactivated."
    - The U.S. NAC recommends that facilities review the publication by Honeywood et al. to implement inactivation methods effective for poliovirus. [xii]

Revision History

This is a living document subject to ongoing improvement. Feedback or suggestions for improvement are welcomed. Submit comments directly to the U.S. NAC at: poliocontainment@cdc.gov.

Version	Change Summary	Effective Date
001	New document	11/22/2022

Appendix I. Poliovirus potentially infectious material risk levels

Material [8]	Procedure	PV Type	Risk Level
Stool, concentrated sewage/wastewater	Inoculation of cells (including all propagation activities, cell culture, etc.)	WPV/VDPV [9], Unknown/untyped	High
		OPV/OPV Unknown	Moderate
	Other procedures [10]	WPV/VPDV [9], Unknown/untyped	Moderate
		OPV/OPV Unknown	Low
Extracted nucleic acids from stool, concentrated sewage/wastewater	Transfection of PV-permissible cells (propagation)	WPV/VDPV [9], Unknown/untyped	High
		OPV/OPV Unknown	Moderate
	Other procedures [10]	WPV/VDPV [9], Unknown/untyped	Low
		OPV/OPV Unknown	Lowest
Respiratory tract samples, unconcentrated sewage/wastewater	Inoculation of cells (propagation)	WPV/VDPV [9], Unknown/untyped	Moderate
		OPV/OPV Unknown	Low
	Other procedures [10]	WPV/VDPV [9], Unknown/untyped	Low
		OPV/OPV Unknown	Lowest
Extracted nucleic acids from respiratory samples, unconcentrated sewage/wastewater	Transfection of PV-permissible cells (propagation)	WPV/VDPV [9], Unknown/untyped	Moderate
		OPV/OPV Unknown	Low
	Other procedures [10]	WPV/VDPV [9], Unknown/untyped	Low
		OPV/OPV Unknown	Lowest

[8] Cerebrospinal fluid, serum/blood and other clinical materials not listed in this table are not considered PV PIM.
[9] WPV/VDPV or OPV terminology used without a specific serotype refers to all three PV serotypes (*i.e.,* 1, 2, and 3).
[10] May include, but are not limited to, PCRs (DNA or RNA), inoculation into PV non-permissive cells, bacterial cultures, mass spectrometry or ELISAs.

Appendix II. Risk mitigations for PIM work and storage [11]

Risk Mitigations	Appendix I Risk Levels			
	Moderate	Low	Lowest	Storage
Biosafety				
Open containers and perform PV PIM work in primary containment (e.g., BSC)	Yes	Yes	No	Yes [12]
Front covering gown	Yes	Yes	No	No
Lab coat	No	No	Yes	Yes
Surgical Mask	Yes	Yes	No	No
Face protection (*i.e.*, face shield)	Yes	Yes	No	No
Gloves	Yes	Yes	Yes	Yes
Double gloves	Yes	No	No	No
Shoe covers	Yes	No	No	No
Segregate materials from other PV types or non-PV materials	Yes	Yes	Yes	Yes
Practice good microbiology techniques and demonstrate competency	Yes	Yes	Yes	Yes [12]
Security				
Identify essential personnel	Yes	Yes	Yes	Yes
Limit access to storage units and work areas to essential personnel only	Yes	Yes	Yes	Yes
Locked and dedicated storage unit	Yes	Yes	Yes	Yes
Inventory	Yes	Yes	Yes	Yes
Transport and transfer protocols	Yes	Yes	Yes	Yes
Personnel				
Essential personnel and visitors trained on U.S. NAC PIM Guidance including PPE, safe handling and storage of PV, risks, decontamination and disposal, etc., as well as general safety procedures (e.g., fire, chemical, radiation, as appropriate)	Yes	Yes	Yes	Yes
Enrolled in OHP	Yes	Yes	Yes [13]	Yes [13]
Immunization	Yes	Yes	Yes [14]	Yes [14]

[11] High risk materials and procedures should follow the U.S. NAC RMS, which contains additional mitigations.
[12] Primary containment should be used to open boxes containing Moderate or Low risk materials. Primary containment is not required for Lowest risk materials.
[13] Unknown PIM could contain High or Moderate risk level materials.
[14] Recommended by NAC and WHO PIM Guidance. [xiii]

Decontamination and emergency response				
Decontaminate work surfaces and waste using validated methods	Yes	Yes	Yes	Yes
Emergency response	Yes	Yes	Yes	Yes
Inactivate PIM using validated methods	Yes	Yes	Yes	Yes

References

[i] Standard operating procedures: responding to a poliovirus event or outbreak (who.int)

[ii] WHO Global Action Plan IV (polioeradication.org)

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