Risk Mitigation Strategies for *In Vitro* and *In Vivo* Work with Poliovirus Infectious Materials

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NAC
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CONTENTS

Acronyms

ABSL	Animal biosafety level
ACIP	Advisory Committee for Immunization Practices
BMBL	*Biosafety in Microbiological and Biomedical Laboratories*
BSC	Biosafety cabinet
BSL2	Biosafety level
CAG	Containment Advisory Group
CDC	Centers for Disease Control and Prevention
CC	Certificate of Containment
cDNA	Complimentary deoxyribonucleic acid
cVDPV	Circulating vaccine-derived poliovirus
CP	Certificate of Participation
EOC	Emergency Operations Center
ELISA	Enzyme-linked immunosorbent assay
GAPIV	Global Action Plan, Fourth Edition
GCC-CWG	Global Certification Commission - Containment Working Group
HEPA	High efficiency particulate air
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
ICC	Interim Certificate of Containment
IM	Infectious materials
IPV	Inactivated polio vaccine
NAC	National Authority for Containment of Poliovirus
OHP	Occupational Health Program
OPV	Oral polio vaccine
PBS	Phosphate-buffered saline
PEF	Poliovirus-essential facility
PI	Principal investigator
PIM	Potentially infectious materials
PIN	Personal identification number
PPE	Personal protective equipment
PPM	Parts per million
PV	Poliovirus
RMS	U.S. NAC *Risk mitigation strategies for in vitro and in vivo work with poliovirus infectious materials*
RNA	Ribonucleic acid
VDPV	Vaccine-derived poliovirus
WHO	World Health Organization
WPV	Wild poliovirus

Definitions

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] 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 ." [ii]

[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.

Novel OPV (nOPV)	Novel OPV candidates 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]
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]

Poliovirus *cont*.	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 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/VDPV • "Fecal 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 poliovirus contamination or replication is possible; and • Environmental samples (*i.e.,* concentrated sewage, wastewater) collected from areas known or suspected to have circulating WPV or VDPV at the time of collection." [ii] Sabin/OPV • "Fecal 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) 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

To assist facilities in the transition to GAPIII implementation, the U.S. NAC developed *in vitro* and *in vivo* risk mitigation strategies for work with WPV, VDPV (including cVDPV), and OPV type 2 IM and WPV and VDPV type 3 IM as the U.S. works toward that goal *(RMS_0001)*. The RMS applies to U.S. facilities that possess or are in pursuit of a U.S. NAC-issued CP to become a PEF. U.S. facilities in possession of WPV1, VDPV1, and OPV1/3 IM, and all WPV/VDPV PIM should consider developing these procedures. For complete guidance and information on working and storing PV PIM, please see the U.S. NAC and WHO PIM Guidance documents.

Note: *The RMS number in parentheses indicates the U.S. NAC Current Containment Conditions checklist number (e.g., RMS_XXXX)*

Background

World Health Organization GAPIII GAPIV outlines containment requirements for facilities working with PV that WHO has declared eradicated [4]. The U.S. NAC expects PEFs to implement the RMS until their CP expires, or the PEF withdraws from the certification process. The strategies are not a substitute for GAPIV and PEFs are encouraged to implement additional mitigations as identified in their site-specific risk assessments.

Note: The U.S. NAC consulted WHO GAPIV, WHO CAG reports, external subject matter experts, and the BMBL in developing this document. The U.S. NAC will review this document at least annually to make appropriate revisions as the world progresses to PV eradication, or as additional information becomes available (*e.g.,* epidemiological, declarations of eradication).

Risk Mitigation Strategies

Biosafety standards and notifications

Poliovirus-essential facilities must implement, at minimum, BMBL BSL2 and ABSL2 standards for *in vitro* and/or *in vivo* work with PV IM covered under the RMS, as determined by risk assessment *(RMS_0002)*. Poliovirus-essential facilities seeking an ICC/CC must also follow the U.S. NAC *Biorisk management and risk assessment policy* when performing risk assessments *(RMS_0002.a)*. In addition, PEFs should perform a detailed risk assessment with mitigation strategies targeted specifically to the details of their own facility and must implement, at minimum, the risk mitigation strategies outlined in this document. Poliovirus-essential facilities must maintain documentation of risk assessment used to assess BMBL standards and U.S. NAC RMS *(RMS_0003)*.

Poliovirus-essential facilities must notify appropriate state (*e.g.,* state health department) and local agencies (*e.g.,* local health, police, and fire departments; private security and commercial waste disposal companies, as appropriate) of possession of PV materials *(RMS_0004)*. Poliovirus-essential facilities must address regulatory statutes or concerns expressed by these agencies *(RMS_0004.a)*.

[4] WHO declared eradication of wild type 2 in September 2015; eradication of wild type 3 was declared in October 2019.

Biorisk management

Poliovirus-essential facilities must ensure that *in vitro* and *in vivo* work are reviewed and approved by the facility IBC and the IACUC, respectively *(RMS_0005)*. PEFs should engage equivalent authorities if these committees are not part of their institution.

Poliovirus-essential facilities must perform risk assessments to identify procedures in which the risks associated with *in vitro* (*e.g.,* high titer PV cultures) and *in vivo* (*e.g.,* accidental self-inoculation, PV-infected animals shedding virus) work can be reduced by using inactivated material *(RMS_0006)*. Facilities that reduce the identified risks through inactivation, fixation, or nucleic acid extraction of PV IM must comply with the requirements and guidance outlined in the U.S. NAC *Inactivation* policy *(RMS_0006.a)*.

Inventory

Poliovirus-essential facilities must destroy unneeded PV IM (*i.e.,* material that will not be used experimentally) *(RMS_0007)*.

Poliovirus-essential facility inventory must maintain records that include all PV IM covered under the RMS *(RMS_0008)*. Poliovirus-essential facilities must ensure that on-site inventory records are current, accurate, and reflect the qualitative estimates (*i.e.,* 1-99, 100-999, 1,000-9,999, 10,000-49,999, > 50,000), including sample types (*e.g.,* seed stocks/cell culture isolates; human fecal, respiratory, tissue; PV-infected animal tissue; environmental; extracted nucleic acids) *(RMS_0008.a).* Poliovirus-essential facilities must report their inventories to the U.S. NAC annually by April 30 via the NAC Inventory Update Form [5]. Poliovirus-essential facilities must maintain records that document characteristics (*e.g.,* serotype, strain, date of collection if known, disposition) associated with each sample *(RMS_0008.b)*. If the PEF inventory of PV IM has changed qualitatively, include the updated estimates and/or specimen descriptions in the annual inventory update.

Poliovirus-essential facilities performing *in vivo* experiments must establish animal tracking procedures and maintain records to account for all PV-infected animals and infected tissues through final disposition *(RMS_0008.c)*. Poliovirus-essential facilities that possess or are pursuing a CP must follow the U.S. NAC *Inventory* and *Transfer* policies *(RMS_0008.d).*

PV IM and PIM inventory changes, destruction and transfer of material must be reported to the U.S. NAC using the NAC Inventory Update, Material Destruction Attestation, and Material Transfer forms, respectively. These forms can be obtained from the NAC website or upon request to poliocontainment@cdc.gov.

Poliovirus-essential facilities must develop procedures to investigate missing, lost, or stolen PV IM covered under the RMS including, but not limited to, viral stocks and PV-infected animals. Investigations should determine the cause and extent of the incident including, as necessary, assessment of the surrounding laboratory area(s) and storage area(s) to locate missing materials, and review access records in case of a potential theft. All inventory investigations must be documented and, if the material cannot be found within 72 hours or person(s) were exposed, the PEF must notify the U.S. NAC *(RMS_0008.e)* and submit the NAC

[5] Poliovirus material that is consumed while performing experiments would not be reported as destroyed. U.S. NAC defines destroyed PV material as material that is determined to be no longer essential and is subsequently inactivated via autoclaving, incineration, or using another validated method (*e.g.,* sodium hypochlorite).

Theft/Loss Incident Reporting Form (available upon request). The U.S. NAC will review the investigation documentation and may notify the WHO, if necessary.

Biosafety

All *in vitro* and *in vivo* work with PV IM covered under the RMS should be performed in a dedicated PV laboratory (*e.g*., an isolation room within a larger laboratory) *(RMS_0009)*. If a dedicated laboratory is unavailable, PEFs may perform work in a laboratory room that maintains spatial and temporal separation (*i.e.,* no non-PV agents present when PV work is performed) from non-PV agents and a validated decontamination procedure (*e.g.,* 10,000 ppm sodium hypochlorite [6] for 5 minutes at 21°C [v] prepared each day of use) before and after PV is used *(RMS_0010)*. Ethyl or isopropyl alcohol does not inactivate PV effectively [vi] and should not be used as a disinfectant. However, a 60-70% alcohol solution can be used after disinfection with sodium hypochlorite, followed by removal of residual hypochlorite with water, to mitigate damage to work surfaces and equipment. Poliovirus-essential facilities that cannot dedicate laboratory area(s) and equipment to PV IM must implement measures to prevent cross-contamination as described in the U.S. NAC *Policy for Shared Use Space within Poliovirus-Essential Facilities (RMS_0010.a).*

All *in vitro* and *in vivo* work with PV IM covered under the RMS must be performed in primary containment devices such as a certified, dedicated BSC (*e.g.,* annually certified class II) including, but not limited to, inoculations of PV-permissive cells and animals, PV-infected animal necropsies and sample collections, PV-infected animal anesthesia and euthanasia, viral titrations and dilutions, and any procedures that could generate aerosols (*e.g.,* opening containers, vortex) *(RMS_0011)*. Manipulations of PV-infected small animals must be performed in primary containment and as appropriate, should employ restraint devices *(RMS_0011.a)*. The U.S. NAC does not recommend using downdraft tables to perform necropsies on PV-infected large animals. Poliovirus-essential facilities performing experiments with large animals should contact the U.S. NAC for additional guidance. Facilities must perform risk assessments for all *in vivo* work and implement measures to mitigate the identified risks *(RMS_0011.b)*.

Poliovirus-essential facilities must implement the following containment measures when PV IM covered under the RMS is removed from primary containment:

- Safety cups or sealed rotors for centrifugation *(RMS_0012)*
    - Cups or rotors are loaded and unloaded in a BSC or other primary containment device *(RMS_0012.a)*
- Durable leak proof transport containers to transport material from primary containment *(RMS_0013)*
- Containment caging (*e.g,* open cages placed in inward flow ventilated enclosures) for small animal housing and transport *(RMS_0014)*
- Cell culture containers (*e.g.,* flasks, plates), animal cages, and specimen containers (*e.g.,* freezer boxes, vials) are closed properly
- Negative airflow cabinets with HEPA filtered exhaust for equipment that generate aerosols but cannot be placed in a BSC (*e.g,* cell sorter, ELISA plate washer) *(RMS_0015)*

Based on a risk modeling study performed by Gryphon Scientific (unpublished, manuscript in preparation), PEFs must implement the following PPE and maintain strict hand hygiene and PPE strategies for everyone entering PV

[6] Undiluted bleach contains 52,500-61,500 ppm sodium hypochlorite. Facilities should make a solution of 1-part bleach to 4-parts water to ensure 10,000 ppm is the final concentration.

containment area(s) performing *in vitro* and *in vivo* work with PV IM. The PPE requirements are applicable to essential personnel and individuals not handling PV materials such as the institution's non-essential personnel (*e.g.,* HVAC or plumbing services specialists associated with PV containment area(s)) and individuals not associated with the institution (*e.g.,* visitors, auditors, contractors) *(RMS_0058)*.

- Don PPE prior to entering the PV laboratory in an order specified by the facility that minimizes potential personnel exposure *(RMS_0016)*
    - Protective laboratory clothing with a fluid-resistant solid-front (*e.g.*, disposable wrap-around gown, scrubs, coverall) *(RMS_0017)*
    - Double gloves. Wrist area should be covered so that hands are not contaminated during glove changes. *(RMS_0018)*
        - Facilities may consider disposable sleeve covers to reduce the risk of skin exposure.
        - Glove selection should consider strategies to reduce permeability, contamination of hands and maintain dexterity (*e.g.,* appropriate glove size, longer cuff length for inner gloves, different glove color options, assessment of chemicals being used in conjunction with virus).
        - Care should be taken when handling materials.
    - Face or surgical mask (to prevent oral mucous membrane exposure) *(RMS_0019)*
    - Full face shield to protect personnel from splashes *(RMS_0020)*
    - Fluid-resistant shoe covers or dedicated shoes *(RMS_0021)*
- Doff PPE in an order specified by the facility that minimizes potential contamination of personnel prior to exiting the laboratory *(RMS_0022)*
- Wash hands prior to exit of the laboratory. Personnel to follow procedures based on site-specific risk assessment that considers the location of the handwashing sink *(RMS_0023)*.
- Discard disposable PPE as biohazardous waste *(RMS_0024)*
- Decontaminate reusable PPE (*e.g.,* safety goggles, face shield, scrubs (if used)) using a validated method (*e.g.,* 10,000 ppm sodium hypochlorite for 5 minutes at 21°C for surface decontamination [iv] or autoclave sterilization at 121^{0}C) prior to storage and reuse *(RMS_0025)*
    - Facilities should load test decontamination methodology (*e.g.,* spores) prior to autoclaving reusable PPE

Poliovirus-essential facilities must implement the following measures to prevent cross-contamination or misidentification during *in vitro* and *in vivo* work with PV IM covered under the RMS.

- Segregate PV IM from all non-PV materials (*e.g.*, in own clearly labeled freezer box) *(RMS_0026)*
- Perform work with one PV serotype at a time to prevent cross-contamination [7] *(RMS_0027)*
- Decontaminate all work surfaces as described above before and after work with each PV IM serotype using a validated method (*e.g.,* sodium hypochlorite) *(RMS_0028)*
- Dedicate reagents for PV IM use only *(e.g.*, media, PBS, trypsin). Dedicated reagents may be aliquoted for single use. *(RMS_0029)*
- Segregate PV-infected animals in a separate containment caging system (*e.g,* open cages placed in inward flow ventilated enclosures) *(RMS_0030)*

[7] Serotype segregation is not possible for samples that contain more than one PV serotype.

- Caging system exhaust must be HEPA-filtered *(RMS_0031)*

Access requirements

Poliovirus-essential facilities must identify essential personnel that require access to PV IM covered under the RMS as well as the associated *in vitro* and *in vivo* containment area(s). **Only essential personnel that are authorized to work with PV IM can access and handle PV material covered under the RMS.** When identifying institutional essential personnel, PEF must include:

- Personnel that directly handle PV IM or PV-infected animals and non-laboratorians that require access to the PV containment area(s) (*e.g.,* housekeepers, engineers, security staff, HVAC or plumbing services specialists associated with PV containment area(s)) *(RMS_0032)*
- Personnel who have contact with PV-infected animals waste or caging prior to disinfection with a validated decontamination method, if *in vivo* work is performed *(RMS_0032.a)*

Prior to granting access and working with PV IM covered under the RMS, essential personnel must:

- Be enrolled in the institution's OHP *(RMS_0033)*
- Provide proof of immunization or antibody levels demonstrating PV immunity *(RMS_0034)*. For essential personnel unable to provide proof of immunization or immunity, adult boosters must be administered in accordance with ACIP recommendations.
- Complete general laboratory safety (*e.g.,* applicable OSHA, institutional safety standards) and PV-specific (*e.g.,* asymptomatic infections, signs and symptoms of infection, routes of transmission associated with PV infection and disease, biosafety, emergency response and security procedures specific to the PV IM covered under the RMS) trainings *(RMS_0035)*
- Receive annual refresher training *(RMS_0036)*
- Demonstrate competency with applicable *in vitro* and *in vivo* laboratory techniques *(RMS_0037)*

Poliovirus-essential facilities must document training (*e.g.,* certificates of completion, post-training quizzes to demonstrate understanding) and demonstrated *in vitro* and/or *in vivo* competence for all essential personnel, as applicable to their work duties.

Poliovirus-essential facilities must provide training to these non-essential individuals prior to entering PV containment area(s) including, but not limited to, risks associated with entry into the laboratory, asymptomatic infections, signs and symptoms of infection, work done in the facility, and emergency procedures. *(RMS_0059)* Poliovirus-essential facilities must ensure that non-essential individuals entering PV containment area(s), including institutional non-essential personnel (*e.g.,* housekeepers, engineers, security staff, HVAC or plumbing services specialists associated with PV containment area(s)) and individuals not associated with the institution (*e.g.,* visitors, auditors, contractors), provide either proof of immunization or antibody levels demonstrating PV immunity prior to entering the area(s) *(RMS_0059.a)*. In addition, PEFs must document training and confirmation of proof of immunization or immunity for all non-essential personnel entering the PV containment area(s) *(RMS_0059.b)*.

Poliovirus-essential facilities must not grant access to any individual that does not meet these requirements.

Security

Poliovirus-essential facilities must ensure at least two doors are present between public spaces and the PV containment area(s) *(RMS_0038)*.

Poliovirus-essential facilities must develop and implement security mitigations and procedures to limit access to PV covered under the RMS *in vitro* and *in vivo* containment area(s) and materials to personnel that require access *(RMS_0060)*:

- Restrict access to PV laboratory and storage unit (*e.g*., freezer) to essential personnel only *(RMS_0039)*
- Ensure PV containment area(s) are secure (*e.g.,* locked door) *(RMS_0040)*
- Lock storage unit (*e.g.,* freezer) if the unit is located outside the PV laboratory in an area that is shared with other laboratories or an area that is not dedicated to PV *(RMS_0041)*
- Limit personnel with keys, combinations, and other methods (*e.g.,* PIN codes, biometrics) used to access containment area(s) or storage unit(s) (*e.g.,* freezers) to staff that require access. Essential personnel must not share any method used to access containment area(s) or storage unit(s) with non-essential individuals. Facilities must develop procedures to address security of PV material following personnel changes *(RMS_0042)*
- Record access of essential personnel to PV containment and storage area(s) manually or electronically *(RMS_0043)*
- Escort visitors (*i.e.,* all institutional non-essential personnel and individuals not associated with the institution) who have not been granted access to PV containment area(s) (*e.g.,* physical facility staff, housekeeping staff, guests of the facility) to ensure PV IM covered under the RMS are handled only by laboratory staff approved by the PEF. *(RMS_0044)*
    - Visitors must be escorted by essential personnel
    - Visitor entrance into PV containment area(s) must be documented in the PEF records of access, including the name of the escort
    - Visitor access can be recorded once daily

Poliovirus-essential facilities that are seeking an ICC/CC must follow the U.S. NAC *Security* policy *(RMS_0045)*.

Decontamination and waste disposal

Poliovirus-essential facilities must coordinate decontamination and waste disposal procedures with state and local health departments, environmental companies, and comply with federal, state and local regulations *(RMS_0046)*.

Poliovirus-essential facilities must establish procedures for on-site autoclaves that include periodic validation (*e.g.,* annual). Autoclave procedures must include autoclave cycle parameters (*e.g.,* pounds per square inch, temperature, and time [vii]) and inclusion of an appropriate indicator (*e.g.,* spore-based biological indicators) for all autoclaved PV IM waste and reusable materials *(RMS_0047)*.

Poliovirus-essential facilities must decontaminate all *in vitro* and *in vivo* work surfaces (*e.g.,* benchtops, BSC, transport containers) and equipment (*e.g,* centrifuges, microscopes) using a validated method (*e.g.,* 10,000 ppm sodium hypochlorite for 5 minutes at 21°C for surface decontamination [v]) before and after completing work with PV IM covered under the RMS *(RMS_0048)*. Cell culture and specimen containers (*e.g.,* flasks, plates, freezer boxes, vials) and equipment (*e.g.,* pipettes, centrifuges, animal cages, animal restraint and anesthesia devices)

must be surface decontaminated prior to removal from primary containment and *in vitro* and *in vivo* containment area(s) using a validated method *(RMS_0049)*.

Reusable contaminated sharps (*e.g.,* pipettes, scalpels, needles, necropsy tools) can be placed in puncture-resistant containers filled with validated disinfectant, sealed with a lid, and surface decontaminated prior to removal from primary containment. After appropriate contact time (see instructions provided with the disinfectant), drain the disinfectant according to state and local regulations and autoclave the sharps prior to final cleaning and reuse. Alternatively, PEFs may use single-use disposable sharps to mitigate the risks of decontaminating sharps. Disposable sharps must be disposed of using a validated method (*e.g.,* sharps container that is autoclaved) *(RMS_0050)*. For a review of disinfectants effective against PV, please see the CDC Guideline for Disinfection and Sterilization in Healthcare Facilities.

Poliovirus-essential facilities must treat all solid biohazardous waste prior to removal from the containment area(s) using a validated method (*e.g.,* sodium hypochlorite, autoclave). Poliovirus-essential facilities may autoclave solid biohazardous waste (*e.g.,* cell culture flasks, sample vials, pipette tips, PPE, PV-infected animal products and bedding) within the containment area(s) if an autoclave is available. If an autoclave is not available within the containment area(s), PEFs should treat solid biohazardous waste chemically using a validated method (*e.g.,* sodium hypochlorite) prior to removal from the containment area(s). Once treated, solid biohazardous waste can be removed from the containment area(s). Solid biohazardous waste must be disposed of according to local and state regulations following autoclave or incineration. Autoclaving chemically treated waste (*e.g.,* sodium hypochlorite) may be hazardous, depending on the amount and nature of the chemical [viii]. Poliovirus-essential facility should contact their Biosafety Officer to confirm safety procedures for autoclaving chemically treated waste. *(RMS_0051)*

Poliovirus-essential facilities must also treat all liquid biohazardous waste (*e.g.,* floor drains, culture supernatants, water collected from emergency showers of contaminated workers) prior to disposal using a validated method (*e.g.,* sodium hypochlorite). Following treatment, liquid biohazardous waste must be disposed of according to local and state regulations. *(RMS_0052).*

Destruction of material must be reported to the U.S. NAC using the NAC Material Destruction Attestation (NAC.AUDIT.FORM.005). The form may be obtained from the NAC website or upon request to poliocontainment@cdc.gov.

Emergency Response

Laboratory personnel must report incidents (*e.g.,* containment breach) and potential exposures to appropriate facility personnel (*e.g.,* Principal Investigator, Biosafety Officer, Occupational Health staff, as appropriate) per institutional policy. In addition, each PEF must develop post-exposure procedures including, but not limited to, medical consultation with occupational health professionals to determine exposure, diagnostic testing, and follow-up medical appointments *(RMS_0053).* Poliovirus-essential facility incident reporting procedures must include contacting the CDC Emergency Operations Center (EOC) (770-488-7100) and all appropriate federal, state, and local agencies (*e.g.,* local health, police, and fire departments; private security and commercial waste disposal companies, as appropriate) within 12 hours of the incident *(RMS_0054)*. Facilities must submit a U.S. NAC *Facility Incident Reporting Form* to poliocontainment@cdc.gov within the same timeframe. Please see the WHO Public Health Management of Facility Related Exposure to Live Polioviruses for information on developing

procedures for exposed personnel including isolation and quarantine, specimen collection and testing, and education.

Poliovirus-essential facilities must develop emergency response procedures for breaches of containment outside of primary containment (*e.g.,* outside of a BSC) such as a release (*e.g.,* spills) or an escape of PV-infected animals. Spill procedures must ensure that spills are immediately contained and disinfected using a validated method to inactivate poliovirus (*e.g.,* sodium hypochlorite) *(RMS_0055)*. Procedures for escaped PV-infected animals must include methods to contain the animal, identify personnel or animals exposed to the infected animal(s), and decontaminate all areas *(RMS_0056)*.

Poliovirus-essential facilities should perform drills and exercises periodically (*e.g.,* annually) to test and evaluate the effectiveness of PV emergency response procedures and revise procedures as necessary.

Poliovirus-essential facility procedures must be developed and coordinated with first responders (*e.g.,* local health, police, and fire departments; private security and commercial waste disposal companies, as appropriate) to protect personnel, environment, and the general public *(RMS_0057)*. Poliovirus-essential facility coordination with first responders can include first responders participating in PEF drills and exercises, assisting in the PEF in the development of emergency response plans, training first responders on PPE locations and donning procedures, identifying responders with documented PV immunization [8], or requesting concurrence with previously developed procedures.

For additional information, please visit the U.S. NAC website or email the U.S. NAC at poliocontainment@cdc.gov.

References

[i] Standard operating procedures: responding to a poliovirus event or outbreak (who.int)
[ii] WHO Global Action Plan IV
[iii] Van Damme, P., et al., The safety and immunogenicity of two novel live attenuated monovalent (serotype 2) oral poliovirus vaccines in healthy adults: a double-blind, single-centre phase 1 study. Lancet, 2019. 394(10193): p. 148-158.
[iv] De Coster, I., et al., Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in healthy adults: two clinical trials. Lancet, 2021. 397(10268): p. 39-50.
[v] Chiossone C, Fanuel S, Gedge LM, Nims RW, Suchmann DB, Zhou SS. 2018. Inactivation and Disinfection of Poliovirus Type 1 on Nonporous Carriers. Adv Biotech & Micro. 9(5): 96-102.
[vi] Klein M, DeForest A. 1963. The inactivation of viruses by germicides. Chem. Specialists Manuf. Assoc. Proc. 49:116-8.
[vii] WHO Polio Laboratory Manual
[viii] Fleming D., Richardson J. H., Tulis J. J., Vesley D. Laboratory Safety: Principles and Practices. 2nd ed. Washington, ASM Press, 1995

[8] First responders do not need to provide proof of immunization at the time of an emergency. The PEF must determine first responder PV immunization and potential exposure after the emergency. If a non-immunized first responder is exposed to PV, the PEF must notify federal, state, and local agencies and provide medical evaluation as described above. In addition, first responder quarantine and isolation must be considered as outlined in WHO Public Health Management of Facility Related Exposure to Live Polioviruses. Poliovirus-essential facilities should encourage local emergency agencies to identify first responder with documented PV immunizations to participate in PV-related responses.

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	06/01/2021
002	Modified RMS elements RMS_0008, RMS_0010, RMS_0032, RMS_0047 significantly to clarify requirements. Updated verb tense throughout document. Added recommendation to implement RMS at facilities possessing WPV1, VDPV1, OPV1, and OPV3 IM and all WPV PIM; new RMS elements RMS_0058, RMS_0059, and RMS_0060 that address PPE requirements, visitor entry requirements and post-exposure procedures, respectively.	10/14/2021
003	Modified definitions to align with GAPIV. Replaced references to GAPIII with GAPIV. Added cVDPV as example of VDPV material; VDPV to PIM materials included in facility mitigations; 12-hour notification deadline to submit U.S. NAC Incident Reporting Form and notify agencies; report PV IM and PIM inventory changes, destruction and transfers to U.S. NAC and CDC EOC as point of contact for reporting incidents to U.S. NAC.	10/26/2022