Policy for U.S. Facilities to Inactivate Poliovirus Materials

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NAC
U.S. NATIONAL AUTHORITY
FOR CONTAINMENT OF POLIOVIRUS

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

Center for Preparedness and Response

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Contents

Acronyms

BSC	Biosafety cabinet
$CCID_{50}$	Cell culture infectious dose 50%
CDC	Centers for Disease Control and Prevention
cDNA	Complementary deoxyribonucleic acid
CP	Certificate of Participation
CPE	Cytopathic effects
cVDPV	Circulating vaccine-derived poliovirus
ETOH	Ethanol
GAPIII	WHO Global Action Plan, Third edition
GuSCN	Guanidine thiocyanate
HHS	Department of Health and Human Services
IM	Infectious material
IPV	Inactivated polio vaccine
NAC	National Authority for Containment of Poliovirus
OPV	Oral polio vaccine
PEF	Poliovirus-essential facility
PFU	Plaque-forming units
PIM	Potentially infectious material
PPLB	CDC, Polio and Picornavirus Laboratory Branch
PV	Poliovirus
RMS	U.S. NAC *Risk mitigation strategies for working with in vitro and in vivo poliovirus infectious materials*
RNA	Ribonucleic acid
SOP	Standard operating procedures
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.
Global Action Plan III	The WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use (GAPIII). The 3rd edition of the Global Action Plan (GAPIII) aligns the safe handling and containment of poliovirus infectious and potentially infectious materials with the WHO Endgame Strategy and replaces both the 2009 draft version of the 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.
Inactivation	Procedures that render PV non-infectious and unable to grow or replicate in the absence of transfection reagents (*e.g.,* transfection) or cellular manipulation (*e.g.,*

	electroporation). Procedures to inactivate PV may include, but are not limited to, nucleic acid or protein extractions, specimen fixations (*e.g.,* formalin, acetone), irradiation, heat, or enzymes (*e.g.,* lysozymes).
Infectious materials	WPV/VDPV • "Clinical materials from confirmed wild poliovirus (including VDPV) 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; • Derivatives 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 [2], 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 wild poliovirus [3]." [i] OPV/Sabin • "Cell culture isolates and reference OPV/Sabin strains; • Seed stocks and live virus materials from OPV production; • Environmental sewage or water samples that have tested positive for the presence of OPV/Sabin strains; • Fecal or respiratory secretion samples from recent OPV recipients; • Infected animals or samples from such animals, including poliovirus receptor transgenic mice; • Derivatives produced in the laboratory that have capsid sequences from OPV/Sabin strains [4]; • Cells persistently infected with poliovirus strains whose capsid sequences are derived from OPV/Sabin strains [5]." [i]
Oral polio vaccine/Sabin	"Attenuated poliovirus strains (approved for use in oral polio vaccines by national regulatory authorities, principally Sabin strains)." [i] Also called 'Sabin vaccine', 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

[1] For U.S. facilities, PV derivatives must contain a complete full-length WPV capsid sequence to meet the WPV IM definition.
[2] Expert panel will be determined by WHO.
[3] For U.S. facilities, PV strains must contain a complete full-length WPV capsid sequence to meet the WPV IM definition.
[4] For U.S. facilities, PV derivatives must contain a complete full-length OPV/Sabin capsid sequence to meet the OPV/Sabin IM definition.
[5] For U.S. facilities, PV strains must contain a complete full-length OPV/Sabin capsid sequence to meet the OPV/Sabin 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 GAPIII and in the "Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses") with or without a transfection reagent, except under appropriate containment conditions as described in GAPIII Annex 2 or Annex 3." [ii] **Note: WHO has exempted full-length PV nucleic acids from GAPIII containment. However, WHO does require that full-length PV nucleic acids are included as part of the facility and national inventories.**
Poliovirus	A picornavirus consisting of three serotypes: 1, 2 and 3; protective immunity is type-specific. Poliovirus serotypes are further subdivided into wild (circulating in nature) and Sabin strains (attenuated strains used for oral polio vaccines). 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.
Poliovirus containment area	Poliovirus-essential facility area(s) listed on the PEF CP application. WPV2/3, VDPV2/3, or OPV2 IM cannot leave containment area(s) without a transport container or have been inactivated using a validated method. Access to PV containment area(s) must be limited to essential personnel only.
Poliovirus-essential facility	"A facility designated by the ministry of health or another designated national body or authority as serving critical national or international functions that involve the handling and storage of needed poliovirus infectious materials or potentially infectious materials under conditions set out in this [GAPIII] standard." [i] U.S. PEFs will possess or be in pursuit of a CP.
Poliovirus materials	Unless a serotype is specifically identified, PV materials refer to IM and PIM of all three PV serotypes
Potentially infectious materials	• "Faecal or respiratory secretion samples and their derivatives (*e.g.* stool suspensions, extracted nucleic acids, etc.) collected for any purpose in a geographic area where WPV/cVDPV is present or OPV is being used at the time of collection; • Products of such materials (above) from PV-permissive cells or experimentally infected polio-susceptible animals; • Uncharacterized enterovirus-like cell culture isolates derived from human specimens from countries known or suspected to have circulating WPV/VDPV or use of OPV at the time of collection; • Respiratory and enteric virus stocks derived from PV PIM and handled under conditions conducive to maintaining the viability or enabling the replication of incidental PV; and • Environmental samples (*i.e.* concentrated sewage, wastewater) collected from areas known or suspected to have circulating WPV/VDPV or use of OPV at the time of collection" [iii]

Vaccine derived poliovirus	Classified with wild polioviruses and usually demonstrate 1–15% sequence differences from the parental OPV strain; 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).
Validation	Experiments designed to confirm PV was inactivated effectively. Data generated from validation experiments must demonstrate that the materials do not contain infectious PV before being considered the inactivated PV material.

Version 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
01	New document	06/16/2021

Purpose and Scope

As outlined in GAPIII, "procedures are established and maintained to ensure the complete inactivation of all poliovirus from all materials and solid waste streams leaving the containment perimeter." [*GAPIIIsubelement 14.2.1*] Inactivation procedures must be "validated and shown to be effective against poliovirus prior to their use." [*GAPIII subelement 14.2.1(4)*]

Facilities in the U.S. possessing WPV2/3, VDPV2/3, or OPV2 IM must inactivate PV infectivity prior to use in experimental procedures (*e.g.,* molecular analysis, non-PV work) outside of PV containment area(s). Only U.S. facilities that possess or are in pursuit of a U.S. NAC-issued CP may be in possession of or receive WPV2/3, VDPV2/3, or OPV2 IM [iv, v]. U.S. facilities possessing WPV1, VDPV1, and OPV1/3 IM, as well as all typed and untyped PIM, should consider the implementation of the requirements and recommendations in this policy.

For the purposes of this document, decontamination, disinfection and sterilization of waste, laboratory surfaces and equipment are addressed in the revised U.S. NAC *Risk mitigation strategies for in vitro and in vivo work with poliovirus infectious materials* that applies to facilities that possess or are in pursuit of a CP.
Overview of topics addressed in this policy:

- Facility risk assessment to identify inactivated PV material for experiments [*GAPIII elements 2 and 3*]
- Inactivation using validated methods [*GAPIII subelement 14.2*]
- Inactivation using non-validated methods [*GAPIII subelement 14.2*]
    - Nucleic acid extractions
- Validation conditions [*GAPIII subelement 14.2.1.4*]
- Discovery of inactivation failures [*GAPIII subelement* 14.2.1.3]
- Records [*GAPIII subelement* 14.2.1.6]

Background

The following statements apply to this policy:

- The U.S. NAC evaluates GAPIII biosafety and security requirements and guidance 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 represent the U.S. NAC interpretation of GAPIII and guidance documents.
- U.S. NAC policies are subject to modification depending on external circumstances such as the epidemiological situation, vaccination coverage, new international policies, or final eradication.
- U.S. NAC policies excerpt information from GAPIII, shown in quotations, and/or includes a reference to GAPIII 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.

Facility risk assessment process to identify inactivated PV material for experiments

All PEFs must perform risk assessments to determine if experimental procedures using WPV2/3, VDPV2/3, or OPV2 IM can be replaced with inactivated material (*e.g.,* nucleic acids, inactivated purified virus), attenuated PV strains that are excluded from GAPIII containment for certain experiments (*e.g.,* S19 [6]), or surrogate virus (*e.g.* coxsackie B viruses or echoviruses). [*GAPIII subelement 3.4.2 guidance*] U.S. facilities using WPV1, VDPV1, and OPV1/3 IM, and all typed and untyped PIM, should also consider substituting inactivated material to prepare for final eradication, as appropriate. Please contact the U.S. NAC for additional information regarding attenuated PV strains excluded from GAPIII containment.

Poliovirus-essential facilities should design and perform risk assessments following the U.S. NAC *Biorisk management and risk assessment policy*. Poliovirus-essential facilities should review risk assessments at least periodically (*e.g.,* annually) and when changes are made to inactivation procedures. Risk assessments following changes to inactivation procedures should determine if the changes require a re-validation of the procedure. Risk assessments must be documented, including the risk mitigation decisions made based on the assessment. [*GAPIII subelement 2.5*]

Inactivation using validated methods

All U.S. facilities that possess PV IM or PIM should consider inactivating 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. Unless a facility alters a validated method (*e.g.,* changes in PV titer, reagent concentration, incubation/contact time, equipment, PV strain with demonstrated resistance to inactivation), facilities do not need to perform an in-house validation. Facilities must periodically calibrate and maintain inactivation equipment (*e.g.,* irradiator) to ensure equipment is working properly, according to the manufacturer's specifications. [*GAPIII subelement 13.3.1*] Facilities must

[6] Contact the U.S. NAC for guidance on acquiring and using PV strains excluded from GAPIII.

retain documentation of the validated methods used at their facility. Facilities should integrate validated methods into their SOPs and ensure all personnel performing inactivation steps demonstrate competency with these methods (Please see the U.S. NAC *Security* policy for information on documenting competency).

All inactivation SOPs involving WPV2/3, VDPV2/3, and OPV2 IM must be reviewed by the PEF's institutional biorisk management committee, or facility equivalent.

Nucleic acid extractions

The CDC PPLB observed that some commercial kits do not inactivate PV during the lysis buffer incubation step; however, the final preparations were free of infectious PV (unpublished data). The PPLB tested several commonly used commercial kits to develop modifications (Table 1) and ensure PV inactivation during extraction. The U.S. NAC recommends that facilities review Table 1 to implement the appropriate modifications. In general, PPLB discovered that GuSCN kits must use at least 4M GuSCN and a 20% final ETOH concentration for a 30-minute incubation following the lysis buffer to inactivate PV. Facilities that extract nucleic acids outside of PV containment area(s) without extraction modifications should document the rationale for using original extraction procedures and implement appropriate RMS biosafety, decontamination, and waste disposal measures. Note: Use of trade names and commercial sources is for identification only and does not imply endorsement by CDC or HHS.

At this time, nucleic acids may be extracted from WPV1, VDPV1, and OPV1/3 IM, as well as typed and untyped PIM, in a non-PV laboratory or outside of PEF PV containment area(s) if extraction modifications (Table 1) are implemented. Facilities should consult the WHO *Guidance to minimize risks for facilities collecting, handling, or storing materials potentially infectious for polioviruses* for biosafety and security guidance when using PIM [iii].

Some PEFs may be in possession of nucleic acids from IM that were extracted in accordance with the commercial extraction kit manufacturer's instructions. 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. Facilities do not have to perform a validation on nucleic acid preparations that have been treated with an ethanol wash.

Table 1. Modifications for nucleic acid extraction kits to achieve poliovirus inactivation tested by PPLB[7]

Name of reagent/kit	Modification
MagMax Pathogen RNA/DNA Kit™ and MagMAX-96 Viral RNA Isolation Kit™	No modifications needed.
Qiagen Qiamp Viral mRNA Mini Kit™	Move step 5 to step 3 (Add 560µL of 96-100% ETOH) and incubate for 5 minutes. Final ETOH concentration should be 44%.
TRIzol™ Reagent User Guide	Extend incubation Step 5 in "Lyse samples and separate phases" section from 2-3 minutes to 5 minutes or longer.
UNEX™	Add 200µL of 96-100% ETOH to Step 3 and incubate for 10 minutes. Final ETOH concentration should be 50%.
Zymo Quick-RNA Viral Kit™	Increase buffer:sample ratio in Step 1 from 2:1 to 3:1 and incubate for 5 minutes.

[7] Use of trade names and commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, or the U.S. Department of Health and Human Services.

Nucleic acids extracted from WPV2/3, VDPV2/3, and OPV2 infectious materials

WPV2/3, VDPV2/3, and OPV2 IM nucleic acids must be extracted in PV containment area(s) using validated methods, if the material is outside of PEF PV containment area(s). Experiments with WPV2/3, VDPV2/3, and OPV2 IM nucleic acids can occur outside of PEF PV containment area(s) if the material is extracted from using modifications (Table 1) and is not introduced into PV-permissive cells or animals with or without a transfection reagent [ii]. Prior to relocating to non-PV containment area(s), WPV2/3, VDPV2/3, and OPV2 IM nucleic acids must be extracted in area(s) listed on the PEF CP. If PEFs use WPV2/3, VDPV2/3, and OPV2 IM nucleic acids within PV containment area(s), the manufacturer's instructions can be followed without implementing the modifications in Table 1.

Note: Some extraction modifications could interfere with the generation of high-quality RNA required for some experiments and, because of this, facilities may need to use the original extraction procedures. Please contact the U.S. NAC at poliocontainment@cdc.gov for more information if these extractions involve WPV2/3, VDPV2/3, and OPV2 IM.

Inactivation using novel methods

Inactivation of WPV2/3, VDPV2/3, and OPV2 IM must be performed within PEF PV containment area(s) meeting the RMS. All inactivation SOPs involving WPV2/3, VDPV2/3, and OPV2 IM must be reviewed by the PEF's institutional biorisk management committee, or facility equivalent. [*GAPIII subelement 1.8.1 guidance*] Inactivation procedures for WPV2/3, VDPV2/3, and OPV2 IM must be designed in accordance with the conditions and requirements outlined in this policy.

All facilities inactivating PV materials using novel methods must develop inactivation SOPs that demonstrate effectiveness of the inactivation. Inactivation SOPs must outline each inactivation step and condition before inactivating material to be used outside of PV containment area(s). [*GAPIII subelement 14.2.1*] When developing novel inactivation methods, facilities must determine applicable inactivation conditions empirically including, but not limited to, specimen type, PV titers, inactivation treatment dosage and volume, multiple time points, distance from source, and safety margins. To develop novel inactivation procedures, facilities must consider "the nature of the material being treated (*e.g.* volume, presence of protein/other potentially inhibitory substances); contact times, material compatibility issues (*e.g.* interaction with stainless steel or rubber seals); potential health hazards associated with the disinfectant; the need to maintain the required level of active compound, including deterioration over time." [*GAPIII 14.2.1 guidance*] Facilities using surrogates should maintain documentation describing the surrogate selection. Facilities must periodically calibrate and maintain inactivation equipment (*e.g.,* irradiator) to ensure equipment is working properly, according to the manufacturer's specifications. [*GAPIII subelement 13.3.1*]

Validation conditions

U.S. facilities must validate PV materials that are inactivated using a novel method including, but not limited to, modifications to commercial kits not outlined in Table 1 or non-commercial methods that have not been validated previously. Facilities validating PV materials inactivated using novel methods must develop a validation SOP that outlines each validation step. Inactivated WPV2/3, VDPV2/3, and OPV2 IM must be validated and maintained inside PEF PV containment area(s) until the inactivation has been validated. Novel inactivation methods at non-PEFs could be validated in collaboration with a PEF so long as validations using WPV2/3,

VDPV2/3, and OPV2 IM is performed within a PEF containment area and the PEF must follow the non-PEF protocol.

Once an inactivation procedure has been validated, facilities will not need to repeat the validation process unless the procedure is modified significantly (*e.g.,* cell lines, exposure time, reagents). Facilities must ensure validation experiments are temporally separated from experimentation involving live PV or other agents to mitigate the potential for cross-contamination. As part of the temporal separation, facilities must decontaminate the work area and equipment prior to work with live PV or other agents.

Non-PEFs may develop validation methods in collaboration with a PEF; however, the method would still need to be validated by the non-PEF. If a non-PEF chooses this approach, please contact the U.S. NAC at poliocontainment@cdc.gov for guidance.

To ensure the greatest probability of discovering infectious virus, facilities must use 100% of the inactivated material from each treatment, time point, and any other pertinent parameters, until the inactivation procedure has been validated[8]. Experiments must include conditions that produce a gradient to demonstrate inactivation effectiveness (*e.g.,* kill curve). Facilities that cannot use 100% of the inactivated material should document the rationale for using less than 100%.

Validation procedures must be designed in accordance with the conditions and requirements outlined in this policy. Experiments to validate novel methods must first consider the starting material (*e.g.,* stool, purified virus) and the virus titers that are likely to be present. Facilities must validate novel inactivations in PV-permissive cells [vi] to measure infectivity (*e.g.,* CPE), an assay that can detect low levels of virus (*e.g.*, 10^{0-1} virus (either or $CCID_{50}$) per gram [vii]). Validation procedures will vary depending on the specimen type, PV-permissive cell line, and PV strain. Facilities that use surrogates should maintain documentation describing the surrogate selection. All validation experiments must include appropriate positive, negative, and process controls for the variables listed below.

1. Specimen type (*e.g.,* stool, purified virus)
2. Virus titer in specimen, known or estimated. In general, stool PV titers range between $10^3 – 10^7$ PFU/ml. Due to the lack of available data for respiratory secretions, facilities should establish a titer range between $10^0 – 10^4$. Titrations should be performed for virus preparations (*i.e.,* purified virus, cell culture supernatants) to determine the titer.
3. Cell culture. Facilities should use a PV-permissive cell line (*i.e.,* L20B, RD, Hep2C, Vero) and appropriate incubator temperature (*i.e.,* 34°C - 36°C). Incubate for at least 5 days and perform at least 2 blind passages of the inactivated material. (*Refer to the WHO Guidance to minimize risks for facilities collecting, handling, or storing materials potentially infectious for polioviruses for a full list of PV-permissive cell lines. Please note that cell culture conditions (e.g., temperature, PV strain or serotype) will differ for each cell line.*)
4. Biological/experimental replicates. Virus titer range should include data points 10-fold higher than expected PV titers in the sample (See Number 2 for titer ranges). Facilities should use at least 5 independent samples of the same specimen type.
5. Technical replicates. Facilities should use at least 5 replicates of the same sample.

[8] Some chemical inactivation methods (*e.g.,* formaldehyde) may be too toxic to perform validation experiments on live cells. For these chemicals, facilities can treat the inactivated material with a neutralizing media (*e.g.,* Letheen Broth®, Dey-Engley Neutralizing Broth®). A facility may also dialyze, wash, or dilute inactivated material prior to performing validation experiments.

6. Repeat validation at least two times.
7. Virus strain. Facilities should select a virus strain (*i.e.,* Sabin type 1 or 3, surrogate) that is appropriate for the specimen type and cell culture conditions (see validation condition variable number 3).

Inactivation failure

As part of the facility emergency response SOP, poliovirus-essential facilities must develop procedures to respond to failed inactivation of WPV2/3, VDPV2/3, and OPV2 IM including, but not limited to, the steps listed below. [*GAPIII Element 10, subelement 14.2.1(3)*] U.S. facilities using WPV1, VDPV1, and OPV1/3 IM, and all PV PIM should consider developing these procedures.

1. Activate biosafety and security measures to contain the material, as necessary.
2. Identify personnel who used the material, determine if personnel were exposed and refer to occupational health as necessary.
3. Determine if the material was shared with other facilities. If so, contact the facility to alert them of the presence of infectious PV.
4. Contact U.S. NAC, PI, facility BSO and local authorities (*e.g.,* state or local department of health).
5. Include steps to identify the root cause of a failed inactivation in the facility SOP.
6. Report to the U.S. NAC about any data that may indicate material received was inactivated improperly.

Facilities possessing inactivated WPV2/3, VDPV2/3, and OPV2 materials

Non-PEFs may possess WPV2/3, VDPV2/3, and OPV2 inactivated materials that have been validated by a PEF. Any facility possessing such materials will not have to validate the material received from the PEF. A non-PEF in possession of inactivated WPV2/3, VDPV2/3, and OPV2 IM should retain documentation (*e.g.,* email) showing the inactivated material has been validated. Poliovirus-essential facilities that transfer inactivated material must notify the U.S. NAC within 7 days, per the U.S. NAC *Transfer policy*. Material should be shipped in accordance with all applicable local, state, federal, and international laws.

Records

Data collected from novel inactivation methods and validation procedures must be documented as described below. [*GAPIII subelement 14.2.1*] Records may be manual or electronic. Inactivation, validation, and transfer records must include:

1. Updated inventory that includes inactivated PV materials. Please see the U.S. NAC *Inventory* policy for inventory records requirements.
2. The name(s) of the individual(s) performing inactivation, method used for inactivation, locations(s), and date(s) of the inactivation and/or validation when the procedure is tested
3. SOP of the facility inactivation, validation, and failure identification procedures
    a. PV titers used for inactivation
    b. Volume of PV
    c. Concentration/dosage of inactivation agent
    d. Exposure time
    e. Distance from inactivation source, as appropriate
    f. Maintenance of inactivation equipment to ensure effectivity (*e.g.,* irradiator)

   g. Decontamination of work surfaces and potentially contaminated equipment used during the inactivation procedures using a validated method.
4. Validation data for each inactivation procedure
   a. Cell culture conditions (*i.e.,* PV-permissive cell type(s) used, temperatures, virus strain) (See Validation Conditions, Number 3, Appendix A)
   b. Metrics used to determine PV growth and replication (*e.g.,* CPE)
   c. Appropriate positive (*e.g.,* untreated PV) and negative (*e.g.,* no PV) controls
   d. PV titer range
   e. Concentrations/dosages of inactivation agent to demonstrate dose-dependent effect
   f. Number of biological/experimental replicates (See Validation Conditions, Number 4)
   g. Number of technical replicates (See Validation Conditions, Number 5)
   h. Number of inactivated material passages and length of cell culture time (*e.g.,* days) (See Validation Conditions, Number 3)
   i. Number of repeats
5. Inactivation failures
   a. Data collected that demonstrate that inactivation failed
   b. Documentation of actions taken (*e.g.,* after action report) (*See Inactivation failure section above*)
   c. Data demonstrating validation if the material has been inactivated multiple times
6. Removal of validated material from PEF PV containment area(s)
   a. Validation should be documented by a representative (*e.g.,* PI) at the facility performing the inactivation, as well as the inactivation date and the method of inactivation.
   b. For inactivation material that is shipped, documentation must be retained by both the facility performing the inactivation and the facility receiving the material.
   c. Documentation should also be included with the shipment of the material.

Please contact the U.S. NAC (poliocontainment@cdc.gov) for additional information and guidance.

References

[i] WHO Global Action Plan III

[ii] WHO Containment Activity Group, Report of the Second Meeting of the Containment Advisory Group, November 2017

[iii] WHO *Guidance to minimize risks for facilities collecting, handling, or storing materials potentially infectious for polioviruses*

[iv] Seventy-first World Health Assembly. Poliomyelitis – containment of polioviruses. May 2018.

[v] Poliovirus Type 3 (PV3) Containment after Declaration of Wild Poliovirus Type 3 (WPV3) Eradication, September 2019

[vi] Annex 1, *Guidance to minimize risks for facilities collecting, handling or storing materials potentially infectious for polioviruses*

[vii] Koopmans M, Duizer E. Foodborne viruses: an emerging problem. *International Journal of Food Microbiology* 2004, 90: 23-41. 10.1016/S0168-1605(03)00169-7.