



# Global Polio Laboratory Network

## Guidance Paper 8

## Diagnostic procedures following an accidental exposure to poliovirus

Document version (date)	Description of substantive versions
Version 1 (April 2018)	Comments from SWG processed. Aligned with “Responding to a Poliovirus Containment Breach, Public health management of exposed persons and their contacts”
Version 2/3 (September 2018)	Comments JM, typos
Version 4 (November 2018)	Focus on diagnostic procedures only, PH sections transferred to PH document.
Version 5 (April 2019)	Comments from Italy, France, Canada, Russia processed and aligned with GP lay out.
Version 6 (March 2020)	Comments from SWG-3-2019 processed, GPLN GP format adapted, and referred to WHO documents.
Version 7 (June 2020)	Last rounds of comments of SWG processed, including links to WHO extra information updated.

## 1. Scope

Following the eradication of poliovirus type 2 and Wildtype poliovirus type 3 and the consequential implementation of GAPIII, an updated protocol for “follow up in case of an accidental exposure to poliovirus” is required. This guidance paper relates to the WHO guidance document on “Public Health Management of Facility Related Exposure to Live Polioviruses, 2019”.

## 2. Objectives

The objectives of this document are: To describe the procedures for the collection, shipping and analysis of samples following an accidental exposure (eg following a spill or leakage of tubing containing infectious polioviruses), to poliovirus in a poliovirus facility, to confirm or exclude poliovirus shedding.

## 3. Audience

This document applies to (personnel of) NACs, PEFs, Public Health Authorities and WHO accredited diagnostic laboratories, i.e. all personnel considered to have significant risk of exposure to infectious poliovirus materials, to laboratory staff that might be involved in poliovirus diagnostics, and to personnel involved in responding to an accidental exposure.

## 4. Background

With the successful progression in poliovirus eradication, the subsequent implementation of GAPIII containment requirements, and the change in analytical methods available for poliovirus detection and/or exclusion, we propose an update to the current “follow up protocol in case of an accidental exposure to poliovirus” that dates back to before 2000. At this present time, GAPIII requires different containment for poliovirus type 2 (all types- WPV/VDPV2, and Sabin2) than for poliovirus type 1 and 3. In order to confirm the end of poliovirus type 2 excretion after infection GAPIII requires daily stool sampling from exposure until the stool tests negative for 3 consecutive days. For poliovirus 1 and 3 the requirement is currently 2 negative samples taken at least 24 h apart [WHO Polio laboratory manual 5th edition, 2020]. As a precautionary measure, we propose to follow the GAPIII poliovirus type 2 schedule for polioviruses WPV1-2-3, VDPV1-2-3, but excluding OPV/Sabin 1 and 3 (Any breach involving SL1 or SL3 material are considered minimal or no risk situations and are not considered within the scope of this protocol as at June 2020, but following OPV cessation in the future, will need to be included in a future revision (WHO, Public Health Management of Facility Related Exposure to Live Polioviruses, 2019).

Since GAPIII suggests the possibility of isolation of persons exposed to infectious PV2 (and as mentioned above WPV1-2-3, VDPV1-2-3), a sampling and laboratory analysis schedule that can quickly confirm or exclude poliovirus infection and excretion is desirable. This guideline describes the timing for sample collection and the laboratory procedures used for response to a facility-related exposure to poliovirus.

## 5. Roles and Responsibilities

**NAC:** The NAC should assign a WHO accredited diagnostic laboratory (with virus isolation and ITD capacity) to a PEF. The NAC should inform a WHO accredited diagnostic laboratory on the poliovirus strains that are used by the PEFs for which the lab may be asked to do the diagnostics. Especially if non-standard polioviruses (ie nOPV, GMO or recombinant strains) are being used. If there is no WHO accredited laboratory in the same country we advise to consult the WHO polio Regional Coordinator to assign the most convenient laboratory for testing.

**The facility where a spill may occur (PEF or diagnostic laboratory):** Sample the spill and transport to the designated Diagnostic Laboratory.



The Head of the facility where the accidental exposure happened is responsible for collecting valid spill specimens, timely shipment to the designated reference laboratory, providing specimen information to the diagnostic laboratory, and obtaining feedback of results.

If the spill occurs in a PEF/WHO accredited diagnostic laboratory the laboratory may not be able to analyze their own samples. A contingency plan should be implemented to guarantee continuity for sample processing.

**Public Health Authority (PH personnel):** Organize, and perform, sampling of employee(s) and employee contacts potentially exposed to poliovirus as per WHO recommendations (WHO, Public Health Management of Facility Related Exposure to Live Polioviruses, 2019), and arrange for sample transport to the diagnostic laboratory.

**WHO accredited diagnostic laboratory:** Receive, assess and record details, and analyze the samples in accordance with validated methods under the appropriate containment. Report the results according to you national guidelines, and at least to the PHA and WHO.

The head of the WHO accredited diagnostic laboratory is responsible for

- maintaining complete documentation of specimens and confidentiality of results and records;
- analyses of the specimen and reporting of results.

## 6. Sampling schedule, specimen collection and shipping

### 6.1. Proposed sampling schedule after a probable accidental exposure to poliovirus (all PV2, WPV1 and WPV3, VDPV1 and VDPV3) at Day 0.

Sample	Day 0 exposure	1	2	3	4	5	6	7	8	9 and onward	15-21
Spill	√										
Serum	√										√
Stool		√	√	√	√	√	√	√	√	√	
Throat swab		√	√	√	√	√	√	√			

### 6.2. Assumptions:

- Persons working with infectious poliovirus will be immunocompetent, fully vaccinated adults.
- For risk assessment on virus transmission, data on virus excretion are crucial: analysis of feces (and throat swabs) is obligatory.
- Data from the serological assays may indicate an infection, even without detectable poliovirus excretion, following an exposure, and are relevant to improve the safety procedures within the facility.
- In vaccinated persons, oral PV excretion as a result of replication in the throat is rare and of short duration, however, if oral excretion does occur this will increase the level of quarantine/isolation required to include droplet isolation.
- For ethical reasons, the duration of quarantine/isolation of a possibly infected employee in should be as limited as possible.
- GAPIII requires daily stool sampling from exposure until the stool tests negative for 3 consecutive days.

### 6.3. Specimen Collection: Make sure to apply the required PPE and safety procedures when sampling.

#### Spill samples

WHY: In PEFs involved in research or vaccine production, the poliovirus type in the spill will be known at the moment of the incident, but the infectivity may be unknown. In a diagnostic PEF the poliovirus type in the spill maybe unknown at the moment of the incident. In order to establish/verify the PV type and/or infectivity, samples of the spill should be collected and sent to the diagnostic laboratory without delay.

HOW: The spilled material can be sampled by pipetting or swabbing. For sampling by swabbing the swab stick and virus transport medium as used for throat swabs (see below) can be used. Swab with the dry swab stick through the spill and put the swab in the virus transport medium.

### **Stool samples**

Sufficient (2-8 g) stool should be sampled to allow for the preparation of two stool suspensions (WHO Laboratory Manual 5<sup>th</sup> Edition, 2020).

### **Throat swab**

WHO/CDS/EPR/ARO/2006.1, Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection Guide for field operations.

[https://www.who.int/csr/resources/publications/surveillance/WHO\\_CDS\\_EPR\\_ARO\\_2006\\_1/en/](https://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1/en/)

## **6.4 Specimen shipping**

For packing and shipping instructions see Guidance on regulations for the transport of infectious substances 2019–2020, Applicable as from 1 January 2019 and PLM 5<sup>th</sup> edition, Chapter 4.

## **7. Laboratory Analysis**

### **7.1 Timing**

First 7 days: employee will be in quarantine/isolation; lab results are nice to know but daily results will not influence the isolation practice in the first week. Most cost efficient (for the diagnostic laboratory) is to test all samples on day 7, but testing at day 4 (day 1-2-3-4 stools and throat swabs) and day 7 (day 5-6-7 stools and throat swabs) is reasonable. If testing needs to be continued (due to a poliovirus detection in the first week), only stool samples will be submitted to the diagnostic laboratory. Daily testing by the diagnostic laboratory is not required but can be considered after a sample tested negative, in order to limit time in isolation for the exposed employee(s).

### **7.2 Containment**

Processing of diagnostic samples should follow GAPIII annex 6 procedures [WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use], and GPLN Guidance paper 1: For safe handling and storage of type 2 poliovirus.

For non-PEF labs samples should not be cultured but analyzed by molecular methods only ( Guidance paper 1]. All diagnostic labs (PEF and non-PEF diagnostic labs) should use RNA extraction procedures that are validated for complete inactivation of poliovirus (GPLN Guidance paper 6: Poliovirus Inactivation) before taking the extracted RNA out of containment.

In the event a technician were to handle an improperly sealed or leaking container of a sample from a suspected or known PV2 incident outside of a BSC and/or without wearing appropriate PPE (including at least FFp2 respiratory protection) , the employee should be considered being exposed.

### **7.3 Laboratory procedures**

#### **7.3.1 Stool Suspension preparation**

As RNA extraction requires specific suspension preparation, two stool suspensions will be prepared: one for virus isolation and one for RNA extraction and RT-PCR.

#### **Suspension preparation according to WHO protocol Virus Isolation protocol**

1. Label centrifuge tubes with sample numbers.
2. Add 10 ml PBS, 1 g of glass beads and 1 ml chloroform to each tube.



3. Working in a BSC, transfer approximately 2 g of each faecal sample to a labelled centrifuge tube (ensure that the number of the original sample matches the number on the centrifuge tube).
4. Retain the remaining original sample, preferably in its original container, for storage at -20°C.
5. Close centrifuge tubes securely and shake vigorously for 20 minutes using a mechanical shaker.
6. Spin for 20 minutes at 1500 g in a refrigerated centrifuge ensuring that centrifuge caps are securely in place and centrifuge buckets are sealed.
7. Working in a BSC, transfer supernate from each sample into two labelled externally threaded screw-capped storage vials (If supernate is not clear, repeat chloroform treatment).
8. Store one faecal suspension at -20°C as a back up and store the other at 4 to 8°C.
9. Inoculate 2 RD and 2 L20B tubes with 200 µL.

### 7.3.2 Suspension preparation and RNA isolation for RT-PCR

A recommended method for suspension preparation and RNA isolation for RT-PCR will be published on <https://extranet.who.int/gpln/en/Document> when available. Until then, the WHO accredited diagnostic laboratories will use the molecular method for which non-inferiority to virus isolation is shown.

### 7.3.3 Throat swab suspension preparation

1. make sure the swab is in >2 mL medium and the tube is closed (if the swab is dry or volume < 2 mL: add medium to obtain a volume of 2 mL)
2. Vortex mix for 1 min
3. Label a 2 mL microcentrifuge tube with LIMS number
4. Transfer 1.3 mL medium from the vortexed mixed cotton swab into the labelled 2 mL tube
5. Add 0.5 mL chloroform
6. Vortex mix 1 min
7. Centrifuge 5 min 14,000 rpm
8. Transfer 1 mL of the clarified supernatant into a new labeled 2 mL tube
9. Inoculate 2 RD and 2 L20B tubes with 200 µL

### 7.3.4 RT-PCR

The preferred method for RT-PCR on poliovirus suspected samples is the ITD [Poliovirus Laboratory Manual 5th Edition, 2020. Chapter 7: Molecular Identification and Intratypic Differentiation of Polioviruses], however, other methods for which non-inferiority is shown maybe used.

Note: Direct detection on RNA extracted from stool and/or throat swab will give a good indication on poliovirus excretion, and thus risk of transmission. Molecular methods may be less sensitive, as sensitive or more sensitive as virus isolation methods, depending on virus type and quality of the sample. Therefore, ideally, both methods should be initiated simultaneously without delay.

### 7.3.5 Serological methods

Perform the poliovirus serum neutralization test to all three PVs according to the method as described by Weldon et al., 2016. Laboratories that do not perform serological testing can forward these samples to another laboratory (Eg. CDC, RIVM) [GPLN Guidance paper 3 – Poliovirus antibody testing for GPLN personnel using Dried Blood Spot].

Note: Results for serological testing will not be relevant for the PH response (they come too late), but signs of infections missed by virus detection but detected by seroconversion may help facilities to recognize the need to improve their safety measures and risk assessments.

## 8. Laboratory data Interpretation and reporting

### 8.1 RT-PCR and virus isolation

1/ All stools and throat swabs collected day 1-7 **negative** by RT-PCR (and virus isolation): The probable exposure has not led to measurable virus excretion. There is no risk for virus transmission, and quarantine/isolation of the employee(s) can be discontinued.

2/ Any stools and/or throat swabs collected day 1-7 **positive** by molecular detection (or virus isolation): Exposed person is shedding poliovirus and there is a risk for virus transmission. The isolation and sampling of the employee will be continued. Report the detection of a poliovirus immediately and according to national and international regulations.

The end of viral shedding is confirmed when daily stool samples test negative for 3 consecutive days. Fully vaccinated (at least 3 doses of IPV or tOPV or any combination, or 3 doses of bOPV and at least one dose of IPV) immunocompetent persons are likely to stop shedding soon after day 10-14 [Duintjer Tebbens et al., 2013], but shedding may continue for several weeks [Duintjer Tebbens et al., 2013; Duizer et al., 2017].

## 8.2 Serology

If the polio SN test shows a >4-fold increase in titer between the day 0 and day 15-21 sample: the person has recently been exposed to poliovirus, even if no shedding was found, the biosafety/containment procedures within the facility need to be reviewed critically to prevent exposure to PV in the future.

## 9. Time to results for poliovirus detection

If the accident and expected shipping of specimen is reported to the diagnostic laboratory, the molecular poliovirus detection protocol can usually be finalized within 12 hours after receipt of the specimen (either in house type specific RT-PCR or ITD). Virus isolation usually takes several days and further processing of isolates is not required if poliovirus is already detected by RT-PCR, in the original sample. Sequencing will usually not be required since the virus in the spill will be known and confirmed by the specific in house test or ITD.

	Detection RT-PCR (ITD or specific in house test)	Virus isolation	sequencing (emergency)	sequencing standard
Stool sample /Throat swab	<12 h	2-10 days	<24 h	< 7 days

## Serology

Day 0 and day 10-15 sample will have to be tested at the same time. The polio SN assay takes 5 days. Alternative tests as the PoBi and the MIA [Herremans et al., 1997; Schepp et al., 2017] can be performed in < 12 h but are not yet fully validated for human diagnosis purposes. Results on poliovirus antibody titer increase will not be available within 10-15 days.

## 10. Reference documents

Additional information that may be useful to users of this document includes:

### WHO documentation

- WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. WHO/POLIO/15.05. [http://polioeradication.org/wp-content/uploads/2016/12/GAPIII\\_2014.pdf](http://polioeradication.org/wp-content/uploads/2016/12/GAPIII_2014.pdf)
- WHO Polio laboratory manual 5th edition, 2020.
- Public Health Management of Facility Related Exposure to Live Polioviruses - Guidance in managing exposed persons for countries hosting facilities that maintain live polioviruses, 18 October 2019. <http://polioeradication.org/wp-content/uploads/2016/07/Public-Health-Management-of-a-facility-PV-exposure.pdf>
- Guidance on regulations for the transport of infectious substances 2019–2020, Applicable as from 1 January 2019. <https://www.who.int/ihr/publications/WHO-WHE-CPI-2019.20/en/>
- Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection Guide for field operations. WHO/CDS/EPR/ARO/2006.1, ([https://www.who.int/csr/resources/publications/surveillance/WHO\\_CDS\\_EPR\\_ARO\\_2006\\_1/en/](https://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1/en/))



## **GPLN Guidance Papers (GP)**

GP1 – For safe handling and storage of type 2 poliovirus (PV2) in GPLN laboratories.

GP2 – Updates on ITD molecular assays and testing algorithm

GP3 – Poliovirus antibody testing for GPLN personnel using Dried Blood Spot

GP6 - GPLN's PV Inactivation Protocol.

## **Other references**

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Weldon William, C., M. Steven Oberste, and Mark A. Pallansch. Standardized Methods for Detection of Poliovirus Antibodies. Javier Martín (ed.), *Poliovirus: Methods and Protocols, Methods in Molecular Biology*, vol. 1387, DOI 10.1007/978-1-4939-3292-4\_8, © Springer Science+Business Media New York 2016.

Wright PF, Wieland-Alter W, Ilyushina NA, Hoen AG, Arita M, Boesch AW, Ackerman ME, van der Avoort H, Oberste MS, Pallansch MA, Burton AH, Jaffar MA, Sutter RW. Intestinal immunity is a determinant of clearance of poliovirus after oral vaccination. *J Infect Dis.* 2014 May 15;209(10):1628-34. doi: 10.1093/infdis/jit671.

## **11. List of acronyms**

**BSC** BioSafety Cabinet

**GAPIII** Global Action Plan III

**GPLN** Global Polio Laboratory Network

**ITD** Intratypic Differentiation

**LIMS** Laboratory Information Management System

**NAC** National Authority for Containment (of poliovirus)

**nOPV** novel OPV (S15 Sabin strains)

**OPV** Oral Polio Vaccine

**PEF** Poliovirus Essential Facility (for storage and handling of PV2)

**PH** Public Health

**PLM** Polio Laboratory Manual

**PV** Poliovirus

**RT-PCR** Reverse Transcriptase Polymerase Chain Reaction

**SL** Sabin like

**SOP** Standard Operating Procedure

**SWG** Small Working Group of the GPLN

**WHO** World Health Organization