Global Polio Laboratory Network

Guidance Paper 1

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

<table>
<thead>
<tr>
<th>Document version (date)</th>
<th>Description of substantive revisions</th>
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<tbody>
<tr>
<td>Version 3 (March 2018)</td>
<td>• Adaptation of the Guidance following the Containment Advisory Group (CAG) decision regarding handling of PV2 RNA</td>
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Scope

This document is intended to describe changes in the handling and storage of type 2 viruses within the Global Polio laboratory Network. The rationale is that by 31st of July 2016 all polioviruses type 2 (PV2) and biological materials potentially infectious for PV2 must be destroyed or contained (onsite or after transfer to designated/certified Poliovirus essential facilities).

As diagnostics laboratories, the polio laboratories will continue to receive stool samples from AFP cases and sewage samples from Environmental Surveillance, as well as other biological materials, which may contain polioviruses mainly during the first 3 to 4 months after the last day of the worldwide coordinated OPV2 withdrawal from the trivalent OPV. Therefore specific measures must be implemented to avoid unintentional release of poliovirus in the environment by minimizing polio laboratory-associated risk.

Therefore this guidance paper focuses on:

(i) better definition of the Polio Laboratories duties based on their capacities to perform WHO-recommended testing procedures for polio diagnosis i.e Isolation, Intratypic Differentiation and Sequencing of polioviruses, and

(ii) practical steps to be taken when a Laboratory come across a type 2 poliovirus during the diagnostic process.

It summarizes dispositions, roles and responsibilities of Laboratories and WHO to ensure standard handling and storage practices for PV2 within the GPLN starting 1st August 2016.

This third version of the Guidelines (published in December 2017) aim to adapt the guidance to reflect Containment Advisory Group (CAG) decision regarding handling and storage of PV2 RNA. Indeed, following CAG recommendations, an addendum to GAP-III (containing the chapter below) will be published. The main change brought by decision is that Polio laboratories which have sequencing capacities but are not Polio Essential Facilities, are allowed to inactivate the poliovirus isolates and perform sequencing on extracted nucleic acids.

Addendum to Annex-1 of GAP-III:

Poliovirus nucleic acid: RNA, cDNA and total nucleic acid, extracted/purified 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 poliovirus RNA or cDNA RNA/cDNA (e.g., cDNA clone, synthetic transcript) can be handled outside of poliovirus containment under the condition that these materials will not be introduced into polio-permissive cells or animals as described in this standard and the ‘Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses’ (http://polioeradication.org/wp-content/uploads/2018/04/polio-containment-guidance-for-non-poliovirus-facilities-20180410-en.pdf) with or without a transfection reagent, except under the biorisk management conditions set-out in Annex 2 or Annex 3 of this standard.

Objectives

The objectives of this document are:

- to briefly described the Structure of the GPLN based on laboratory capacities
- to guide GPLN laboratories on handling and storage of biological materials containing type 2 polioviruses
- to establish standards and timeline for response to any polio events and/or outbreaks.
Audience
The proposed audience for this document is the Head and the personnel of laboratories members of the Global Polio Laboratory Network.

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

- Polio Laboratory Manual.¹
- The Global action Plan to minimize poliovirus facility-associated risk after type specific eradication of wild polioviruses and sequential cessation of OPV use (GAP-III)²
- Laboratory Biosafety Manual.³

List of acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AFP</td>
<td>Acute Flaccid Paralysis</td>
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<tr>
<td>ES</td>
<td>Environmental Surveillance</td>
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<tr>
<td>FTA</td>
<td>Fast Technology for Analysis</td>
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<tr>
<td>GPEI</td>
<td>Global Polio Eradication Initiative</td>
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<tr>
<td>GPLN</td>
<td>Global Polio Laboratory Network</td>
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<tr>
<td>ITD</td>
<td>Intratypic Differentiation</td>
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<tr>
<td>LC</td>
<td>Laboratory Coordinator</td>
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<tr>
<td>LQC</td>
<td>Laboratory Quality Control</td>
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<tr>
<td>OPV</td>
<td>Oral Polio Vaccine</td>
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<tr>
<td>NEV</td>
<td>Non Enterovirus</td>
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<tr>
<td>NPEV</td>
<td>Non Polio Enterovirus</td>
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<tr>
<td>NSL</td>
<td>Non Sabin like</td>
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<tr>
<td>PEF</td>
<td>Poliovirus Essential Facility (for storage and handling of PV2)</td>
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<tr>
<td>PV</td>
<td>Polivirus</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>rRTPCR</td>
<td>real time Reverse Transcriptase Polymerase Chain Reaction</td>
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<tr>
<td>NIBSC</td>
<td>National Institute for Biological Standards and Controls. UK.</td>
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<tr>
<td>SL</td>
<td>Sabin like</td>
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<tr>
<td>SSECI</td>
<td>Stools, Sewage, Extracts, Concentrates and Isolates</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
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<tr>
<td>VDPV</td>
<td>Ambiguous vaccine-derived poliovirus</td>
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<tr>
<td>VI</td>
<td>Viral Isolation</td>
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<tr>
<td>VII</td>
<td>Viral Isolation and Identification</td>
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<tr>
<td>VIIS</td>
<td>Viral isolation Identification and Sequencing</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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² GAP-III. So far English and French are available at: [http://www.polioeradication.org/Posteradication/Containment.aspx](http://www.polioeradication.org/Posteradication/Containment.aspx)
## Summary

<table>
<thead>
<tr>
<th>Viral isolation procedure</th>
<th>ITD procedure</th>
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<tbody>
<tr>
<td>Follow standard protocol.</td>
<td>Follow standard protocol (on L+R+ or R+L+R+)</td>
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<tr>
<td>Timeline: 14 days</td>
<td>Timeline: 7 days</td>
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</table>

### Suspected polioviruses (L+R+ or R+L+R+)
Refer for ITD

### PV2 negative:
Proceed following standard algorithm.

### PV2 positive:
1. Report to Ministry of Health (MoH) and WHO in 24 hours
2. All original stool samples, stool extracts and cell-culture harvests to be packed, sealed and kept under lock and key at -20°C
3. Sent sample for sequencing and track.
4. When sequencing results are received (=day 0), immediate notification is sent to the Ministry of Health and WHO (acknowledgement of receipt needed).
5. Obtain green-light (at day 3 at the latest) and destroy the sealed package, document and share with MoH and WHO.
Capacity & Facility-based structure of the GPLN

- VIIS Labs - PEFs: 27 labs
- VIIS Labs - non PEFs
- VII Labs: 71 labs
- VI Labs: 48 labs

- Viral Isolation
- ITD and Sequencing
- Viral Isolation and ITD
- Viral Isolation
If PV2 detected, All SSECI i.e Stools, Extracts, Sewage, Concentrates and Isolates (all passages, both arms) in VI lab are:

A. destroyed:
1. 72 hours following final results communicated by VIIS lab
2. Documentation* of destruction is shared with the Regional and Global LCs, and MoH.

B. Transferred to a PEF if necessary:
Under the guidance of Regional and Global LCs
When sequencing results available, Stools, Sewage Extracts, Concentrates and Isolates (first passage, both arms) in VII lab are:

A. destroyed:
   1. 72 hours following final sequencing results communicated by VIIIS
   2. Documentation of destruction is shared with the Regional and Global LCs, and MoH.

B. Transferred to a PEF for long term storage:
   Under the guidance of the Regional LC

* No VDPV2 screening if ITD rRTPCR assay reveal PV2
VIIS Lab and PEF

Stools
Sewage

Viral isolation

L20B pos
L20B neg

Report

rRT-PCR
ITD and VDPV

NPEV
NEV
SL1, SL3

Any NSL
Any disc.
All PV2

Report

VP1 SEQ

Long-term storage if needed

VI LAB

L20B pos

VII LAB*

1) PV2 isolates or original specimens
2) PV2 RNA and all other PV1 and PV3 to be sequenced

Transfer

VIIS Lab (not PEF)

Transfer

VP1 SEQ (including PV2 RNA)

Temporary storage and destruction of Stools, Sewage, Extracts, concentrates, Isolates, (SSECI) when final results available

Temporary storage and destruction of SSECI when final results available.

- PV2 RNA can be stored under conditions defined in GAP-III Annex-1 (definitions)

* No VDPV2 screening if ITD rRTPCR assay reveal PV2
### Summary: handling and storing materials containing PV2

<table>
<thead>
<tr>
<th></th>
<th>Poliovirus Isolation and Identification (VI) Laboratory</th>
<th>Poliovirus Isolation and Identification and Sequencing (VIIS) Laboratory - Non PEF</th>
<th>Poliovirus Isolation, Identification and Sequencing (VIIS) Laboratory - PEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool samples extracts</td>
<td>Extraction + cell c.</td>
<td>Extraction + cell culture</td>
<td>Extraction + cell culture</td>
</tr>
<tr>
<td>Raw sewage samples</td>
<td>Concentration</td>
<td>Concentration</td>
<td>Concentration</td>
</tr>
<tr>
<td>Concentrates of sewage samples</td>
<td>Cell-culture</td>
<td>Cell-culture</td>
<td>Cell-culture</td>
</tr>
<tr>
<td>Suspected Poliovirus isolates (L+R+)</td>
<td>ITD rRT-PCR</td>
<td>ITD rRT-PCR</td>
<td>ITD rRT-PCR</td>
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<tr>
<td>Poliovirus isolates</td>
<td></td>
<td>PV inactivation and RNA extraction</td>
<td>RNA extraction</td>
</tr>
<tr>
<td>ITD result on L+R+ = PV2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PV2 RNA from Polio isolates (L+R+)</td>
<td></td>
<td>sequencing</td>
<td>sequencing</td>
</tr>
</tbody>
</table>

**NIBSC and LQC Sabin 2 Strains**  
Destroy (and document) by end of July 2016  
CST, Serology

**process**
- Short-term storage until sequencing results available
- Long-term storage
  - Destroy or transfer to an PV Essential Facility for storage when sequencing results available (PV2)
Practical handling of biological materials containing PV2
Practical handling scheme (1)
Aliquoting and testing

Frozen and thawed L+R+

1. Heat inactivation (using validated GPLN protocol i.e. GP6) and storage

2. ITD testing

3. If no PV2: Follow current Work practices
   PV2
   Spot FTA card and refer to a VIIS Laboratory
Practical handling scheme (2)
Temporary storage and destruction

Original specimens and all derivatives are individually wrapped in ziplock bags and placed in an autoclavable container/bag.

Autoclaved within 72 hours following final results communicated by VIIS laboratory (unless otherwise advised by WHO).