Global Polio Laboratory Network

Guidance Paper 1

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

Document version (date)	Description of substantive revisions
Version 3 (March 2018)	• Adaptation of the Guidance following the Containment Advisory Group (CAG) decision regarding handling of PV2 RNA

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

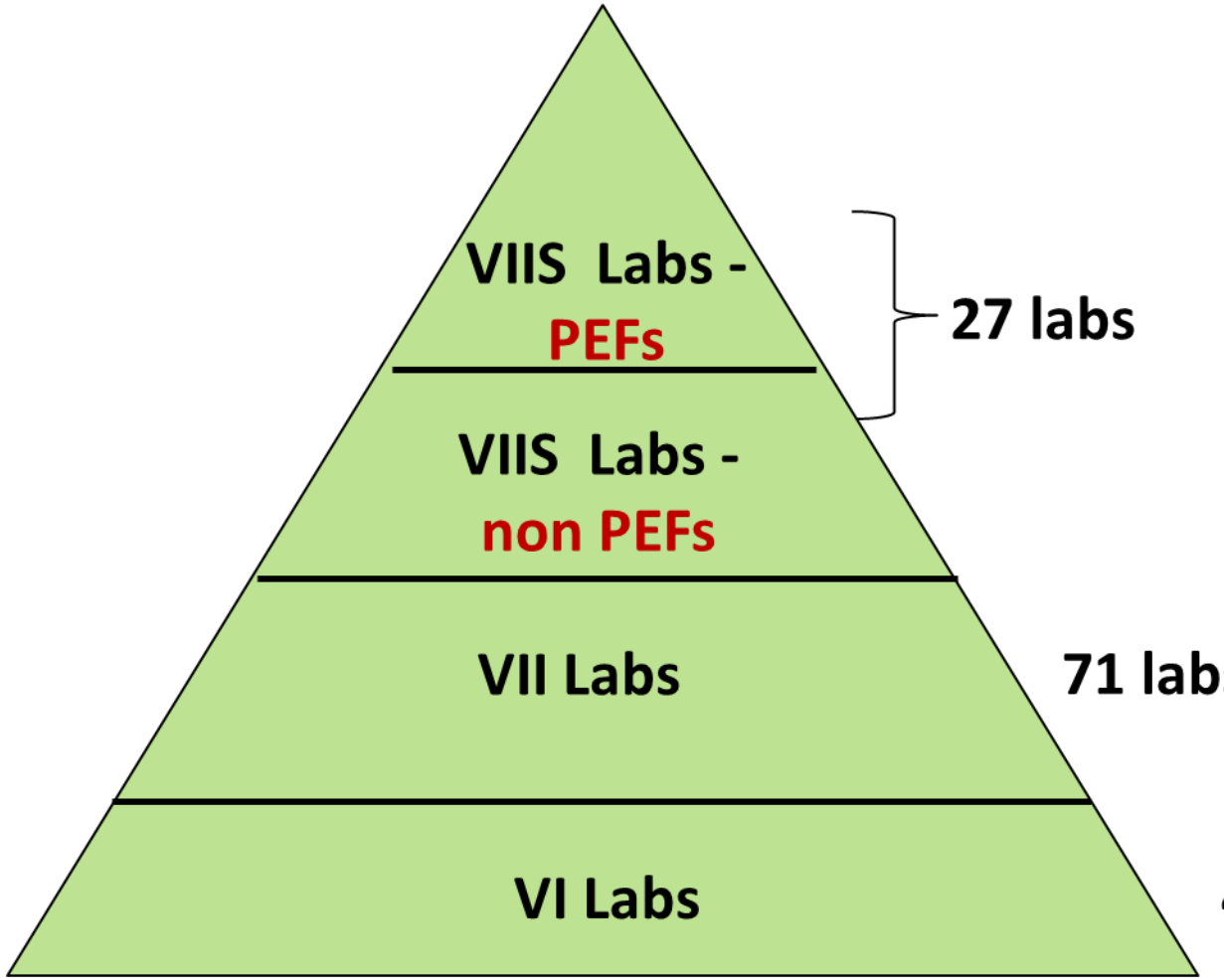
AFP	Acute Flaccid Paralysis
ES	Environmental Surveillance
FTA®	Fast Technology for Analysis
GPEI	Global Polio Eradication Initiative
GPLN	Global Polio Laboratory Network
ITD	Intratypic Differentiation
LC	Laboratory Coordinator
LQC	Laboratory Quality Control
OPV	Oral Polio Vaccine
NEV	Non Enterovirus
NPEV	Non Polio Enterovirus
NSL	Non Sabin like
PEF	Poliovirus Essential Facility (for storage and handling of PV2)
PV	Poliovirus
RNA	Ribonucleic Acid
rRTPCR	real time Reverse Transcriptase Polymerase Chain Reaction
NIBSC	National Institute for Biological Standards and Controls. UK.
SL	Sabin like
SSECI	Stools, Sewage, Extracts, Concentrates and Isolates
SOP	Standard Operating Procedure
VDPV	Ambiguous vaccine-derived poliovirus
VI	Viral Isolation
VII	Viral Isolation and Identification
VIIS	Viral isolation Identification and Sequencing
WHO	World Health Organization

[1] Polio Laboratory Manual and supplement at:
http://www.polioeradication.org/ResourceLibrary/GPLNpublications.aspx
[2] GAP-III. So far English and French are available at:
http://www.polioeradication.org/Posteradication/Containment.aspx .
[3] Laboratory Biosafety Manual.
http://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/

Summary

Viral isolation procedure **Follow standard protocol.** **Timeline: 14 days**	**ITD procedure** **Follow standard protocol (on L+R+ or R+L+R+)** **Timeline: 7 days**
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



27 labs

71 labs

48 labs

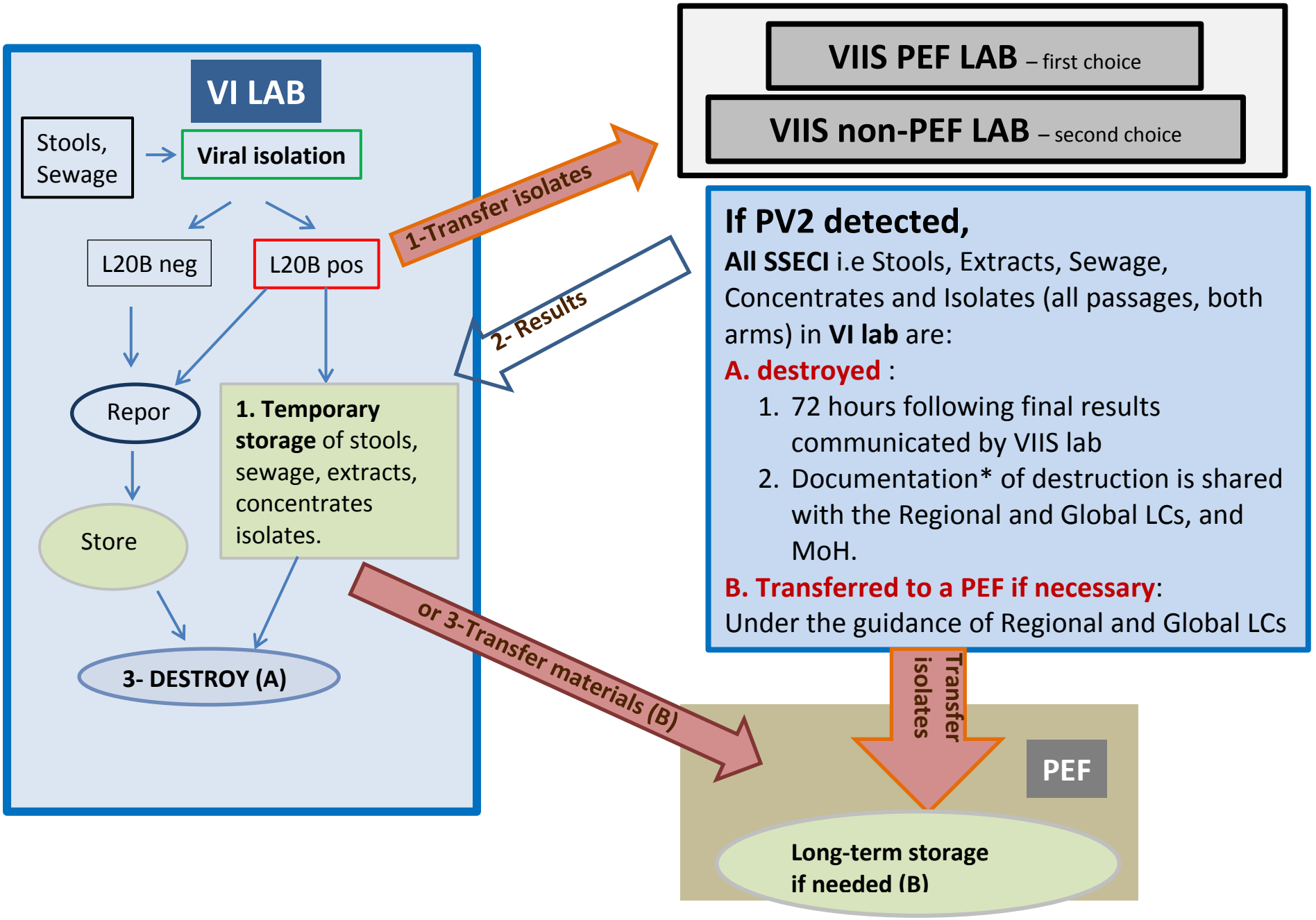
Viral Isolation ITD and Sequencing

Viral Isolation and ITD

Viral Isolation

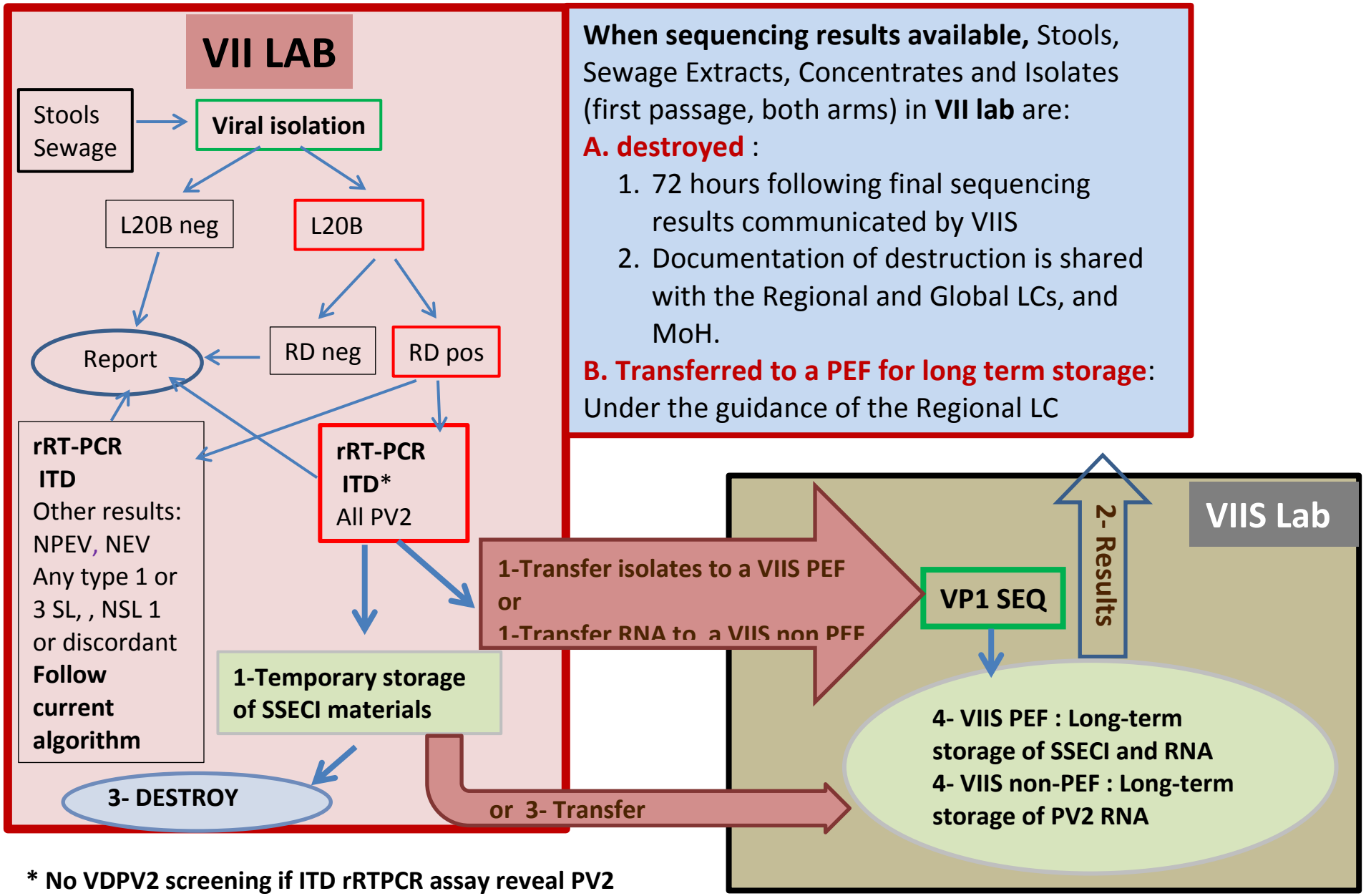
VIRAL ISOLATION (VI) LAB

SCHEME FOR BIOLOGICAL MATERIALS REFERRAL AND HANDLING



VIRUS ISOLATION AND IDENTIFICATION (VII) LAB

SCHEME FOR BIOLOGICAL MATERIALS REFERRAL AND HANDLING



When sequencing results available, Stools, Sewage Extracts, Concentrates and Isolates (first passage, both arms) in **VII lab** are:

A. destroyed :

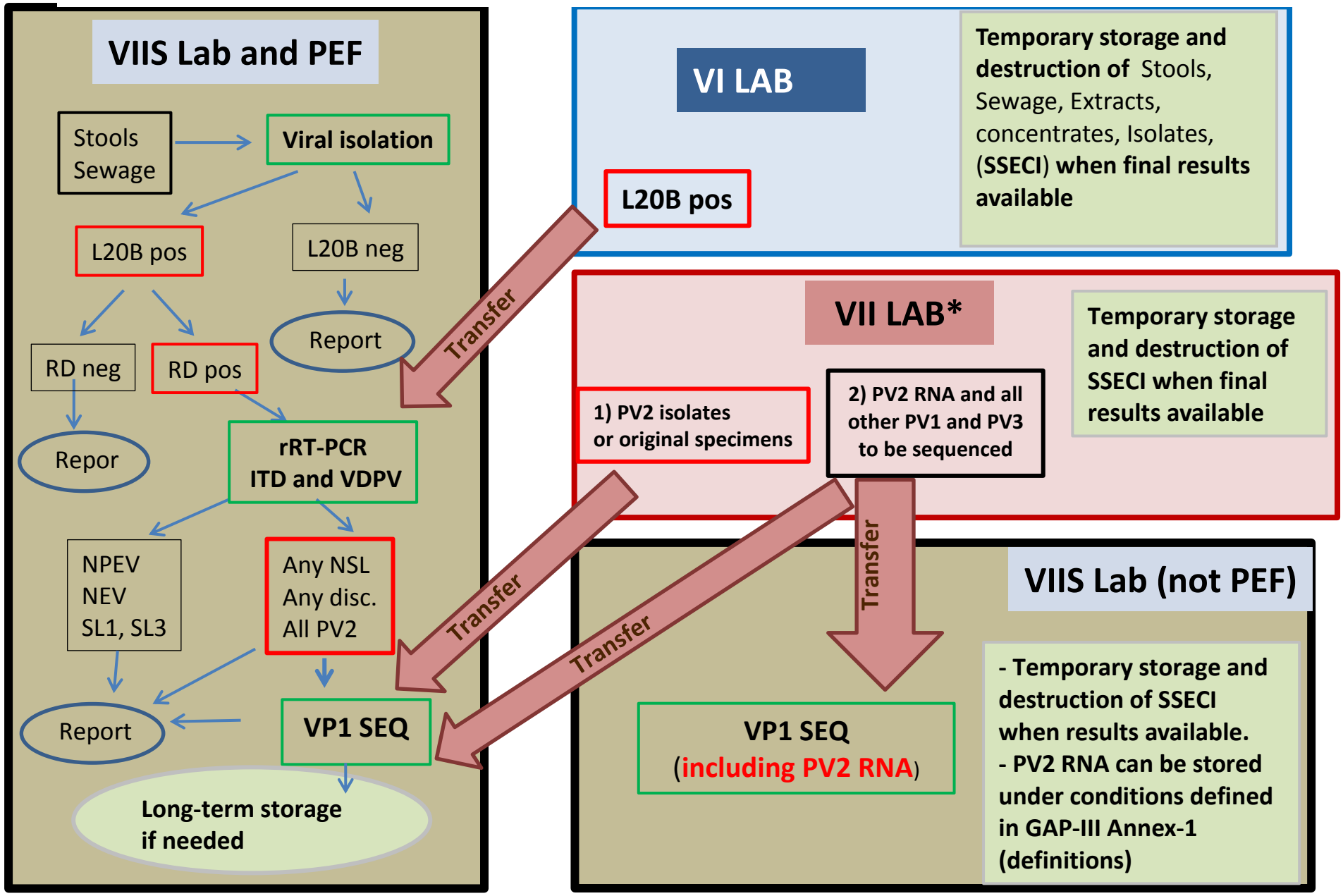
1. 72 hours following final sequencing results communicated by VIIS
2. Documentation of destruction is shared with the Regional and Global LCs, and MoH.

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**

VIRUS ISOLATION, IDENTIFICATION AND SEQUENCING (VIIS) LAB

SCHEME FOR BIOLOGICAL MATERIALS REFERRAL AND HANDLING



*** No VDPV2 screening if ITD rRTPCR assay reveal PV2**

Summary: handling and storing materials containing PV2

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

2

Heat inactivation

(using validated GPLN protocol i.e GP6)

and storage

2

ITD testing

If no PV2:
Follow current
Work practices

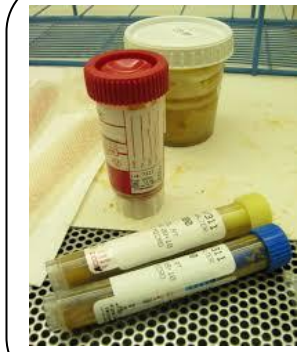
PV2

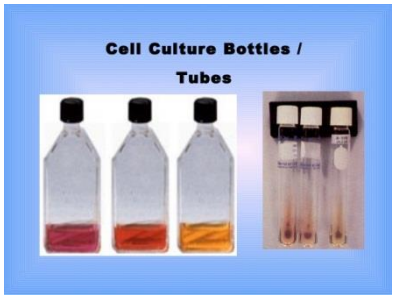
3

Spot FTA card
and refer to a
VIIS Laboratory

Practical handling scheme (2)

Temporary storage and destruction





4

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

5

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