



# Global Polio Laboratory Network

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## Guidance Paper 2

Updates on ITD molecular  
assays and testing algorithm

Version 1, August 9<sup>th</sup> 2016

Document version (date)	Description of substantive revisions
	<ul style="list-style-type: none"><li>• NA</li></ul>

## Scope

This document is intended to describe changes on ITD real time RT-PCR assays

## Objectives

The objective of this document is to inform the Global Polio Laboratory Network on:

- the current and new ITD molecular assays and developments: ITD 4.1, ITD 5.0, and
- the selection of an additional real-time RT-PCR kit for the ITD assay.

## 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:

- *GPLN GP2 appendix\_083016.*<sup>1</sup>

## List of acronyms

AFR genotype	African genotype
GPLN	Global Polio Laboratory Network
ITD	Intratypic Differentiation
PV	Poliovirus
rRTPCR	real-time Reverse Transcriptase Polymerase Chain Reaction
SOAS genotype	South Asian genotype
VDPV	vaccine-derived poliovirus
WPV	Wild Poliovirus

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<sup>1</sup> <https://extranet.who.int/gpln/en/Document:>

## **ITD 4.1.**

ITD 4.1 addendum was completed in May 2016 to supplement the ITD 4.0 kit. ITD 4.1 addendum consists of two new assays: WPV1-2015 and AFR WPV3-2015 (appendix 1). Together with the ITD 4.0, the ITD 4.1 addendum is the complete ITD 4.1 kit. Laboratories may be able to request either the ITD 4.1 addendum or the complete ITD 4.1 kit. All ITD 4.0 kits at CDC have been combined with the ITD 4.1 addendum, but it is the laboratories responsibility to remove the ITD 4.0 AFR WPV3 and Duplex WPV1 assays from the ITD 4.0 kit and replace with the 2015 version included in the addendum kit. Both kits are shipped together upon request of a new kit. Please discard the old assays after replacement. Do not store for future use. In addition, any unopened ITD 4.0 kits in stock within the GPLN can be converted to ITD 4.1 by requesting an ITD 4.1 addendum kit. Please send requests to [EVega@cdc.gov](mailto:EVega@cdc.gov).

The new 2015 reactions use different fluorophores than the older versions and contain a single fluorophore for the WPV1 reactions as opposed to two in the ITD 4.0 version (appendix 2). Although the new WPV1 assay will detect all wild type 1 polioviruses, only one fluorophore is used to simplify interpretation. The ITD 4.1 also has new reaction conditions (appendix 1). The new reaction conditions are more stringent leading to less false positives. The new reaction conditions can be used for ITD 4.0, 4.1, and all VDPV real-time RT-PCR reactions.

## **ITD 5.0 and Algorithm Change.**

Starting August 1<sup>st</sup>, poliovirus type 2 containment will take into effect. In order to facilitate type 2 containment CDC will be releasing ITD 5.0 (appendix 3). ITD 5.0 kit will include all ITD 4.1 reactions, as well as an additional poliovirus type 2 assay. The reaction conditions will not change from the ITD 4.1 run method. The major change in the ITD 5.0 will be the addition of a new assay, Poliovirus Type 2, and an algorithm change (appendix 4). Briefly, any PV type 2 positive sample, Sabin 2 or Poliovirus type 2 assay, will be reported and referred to sequencing for verification. To complement the ITD 5.0 reactions, the S2 VDPV assay will be removed in future VDPV kits, starting with VDPV 5.0 (appendix 5). After containment VDPV2 reactions will not be needed even if using VDPV 4.0 kit. Any Sabin 2 positive reactions will be sequenced and it will not be necessary to run an additional S2 VDPV assay. In order to keep adequate

kit levels throughout the network, we will also be releasing a single Poliovirus Type 2 assay to convert ITD 4.1 kits (appendix 6). Together with the ITD 4.1, the Poliovirus type 2 assay is equivalent to the ITD 5.0. The single Poliovirus Type 2 assay can be requested after August 1<sup>st</sup> ([EVega@cdc.gov](mailto:EVega@cdc.gov)). In order to emphasize the changes, the upcoming 2016 panel (to be distributed in October 2016) will be based on ITD 5.0. We encourage all laboratories to become familiarized with the ITD 5.0 algorithm.

**Promega GoTaq® Probe 1-Step RT-qPCR System.**

A second real-time reaction kit, i.e Promega GoTaq® Probe 1-Step RT-qPCR System, was selected for use with the ITD kits. The already validated Quanta Biosciences qScript XLT One-Step RT-qPCR ToughMix (CAT#95132-500), and the Promega GoTaq® Probe 1-Step RT-qPCR System (CAT#A6120) are equivalent and can both be used with the ITD kits. Instructions for use of either kit will be included with ITD 5.0, but either kit can be used starting with ITD 4.1.