Global Polio Laboratory Network

Guidance Paper 5

Reporting Vaccine-derived Polioviruses (VDPVs)

Document version (date)	Description of substantive revisions
Version 1 (March 2019)	
Version 2.0 (March 2024)	Revision aim: (i) to clarify the concept and practices on confirming and reporting genetic linkage between isolates, and (ii) add language for novel OPV2 to comprehensively capture sequencing outcomes for all poliovirus type 2 isolates.

1. Purpose

The purpose of this document is to provide a framework for standardized analysis and reporting of VDPVs within the Global Poliovirus Laboratory Network (GPLN). This guidance paper will provide a reporting template and descriptive language for classification and reporting of VDPV sequences from accredited GPLN sequencing laboratories and includes an appendix describing distinct scenarios for VDPV reporting.

2. Applicability

This guide will apply to any sequence report from a WHO-accredited poliovirus sequencing laboratory where a VDPV VP1 sequence has been identified, including from an acute flaccid paralysis (AFP) case, AFP contacts, community sampling, an individual with primary immunodeficiency (with or without AFP), or an environmental sample. The current update of this document also captures the increased availability of expanded sequencing windows including 5'UTR (untranslated region), VP4/VP2, whole capsid sequence and whole genome sequences (WGS) and their application in resolving genetic relationships and origins among polio sequences that are not evident from VP1 data only. Additional updates of this Guidance could be required to incorporate further understanding of genetic characteristics for recently detected viruses.

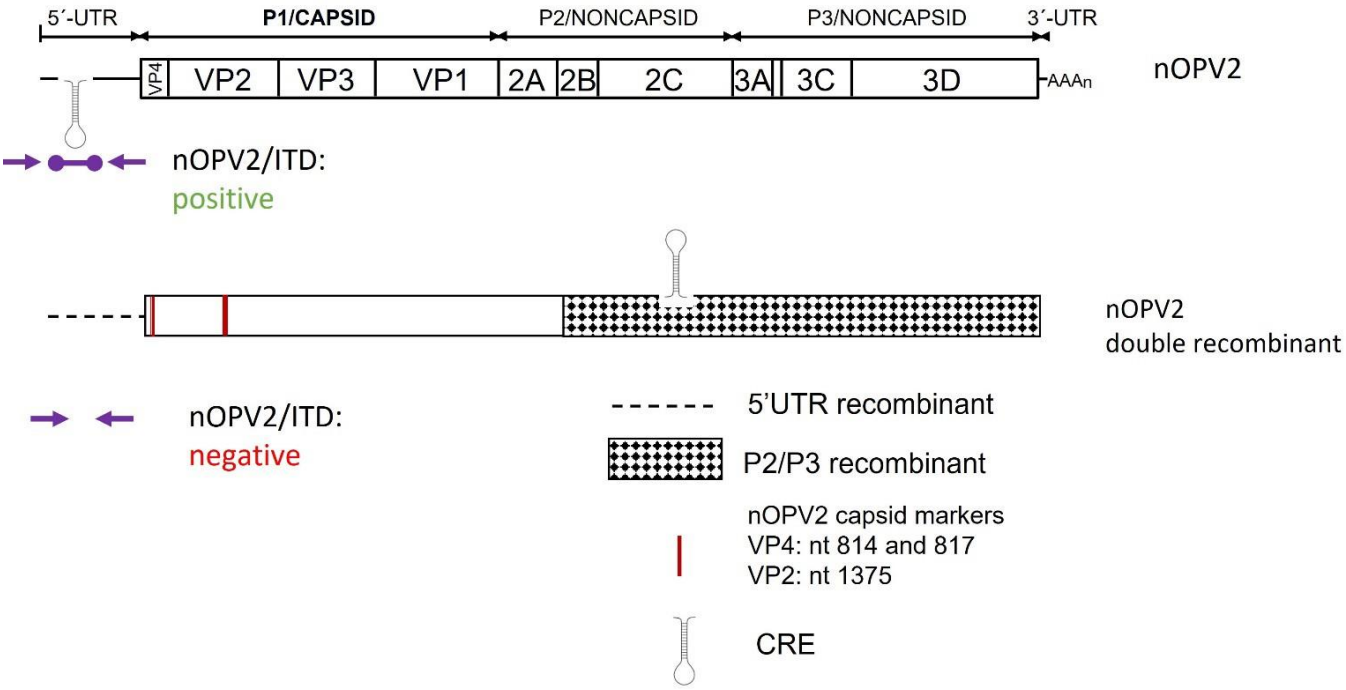
3. Definitions

- **Sabin-like virus (SL) –** Vaccine virus strains that are <1% divergent (<10 nucleotide (nt) differences) for types 1 (SL1) and 3 (SL3) and <0.6% divergent (<6 nt differences) for type 2 (SL2) from the corresponding reference Sabin strain in the VP1 gene region.
- **nOPV2 –** Novel Oral Polio Vaccine type 2. The main differential attenuation site for nOPV2 resides at the 5' UTR (untranslated region), which is the target of the ITD assay (Figure 1) for detection of this unique strain. The complete capsid sequences of nOPV2 isolates differ from Sabin OPV2 in three nucleotide substitutions in the VP4 and VP2 capsid proteins, but the nOPV2 VP3 and VP1 capsid proteins are indistinguishable from those in the regular Sabin OPV2. The 2C protein in nOPV2 contains a short sequence (KO CRE) that differs from that of OPV2 and makes this sequence non-functional for virus replication. Finally, two phenotypic markers were introduced in the nOPV2 in the 3D protein that are not present in OPV2. While replicating in humans, nOPV2 strains are capable of undergoing recombination with polioviruses and members of other species C enteroviruses.
- **nOPV2-like virus (nOPV2-L) –** Novel Oral Polio Vaccine type 2 strains that are <0.6% divergent (<6 nt differences) from the corresponding reference nOPV2 strain in the VP1 gene region.
- **Vaccine-derived Poliovirus (VDPV) –** Vaccine virus strains that are >1% divergent (≥10 nucleotide (nt) differences) for types 1 (VDPV1) and 3 (VDPV3) and >0.6% divergent (≥ 6 nt differences) for type 2 (VDPV2) from the corresponding reference Sabin strain in the VP1 gene region. When vaccine origin is determined to be from novel oral polio vaccine (nOPV), the corresponding type includes -n in its acronym (e.g., VDPV2-n), as per guidance from the GPLN.
- **Circulating VDPV (cVDPV)** – VDPV VP1 sequences that have evidence of circulation or are genetically linked to sequences from previously identified cVDPVs. Please refer to the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses for the exact criteria for cVDPV classification. From the definition of VDPVs, the extent of divergence of VDPVs from parental vaccine strain suggests prolonged replication of the vaccine virus. In some instances, early detection of SL viruses genetically related to VDPVs constitutes a new scenario with the advent of heightened environmental surveillance and obtaining complete genomes using next generation sequencing (NGS) methods. cVDPVs with evidence of nOPV2 origin are classified as cVDPV2-n. This distinction is only possible with additional VP4/VP2, complete capsid, or whole genome sequences.
- **Immune-deficiency associated VDPV (iVDPV)** – VDPVs from individuals that have evidence of primary immunodeficiency (PID). iVDPVs with evidence of nOPV2 origin are classified as iVDPV2-n.
- **Ambiguous VDPV (aVDPV)** – VDPV VP1 sequence that is not genetically linked to other previously identified VDPV sequences and for which there is no evidence of PID if the virus is from an individual. No poliovirus sequencing laboratory can classify a VDPV sequence as ambiguous until there is communication with WHO HQ and the WHO Regional Office, as described in the GPEI Guidelines for Reporting and Classification of Vaccine-

derived Polioviruses. aVDPVs with evidence of nOPV2 origin are classified as aVDPV2-n.

- **ITD –** Intratypic Differentiation. This WHO-recommended method is a set of real-time molecular detection assays (rRT-PCR) for PV serotyping and genotyping which targets the VP1 region of polioviruses. The most recent ITD version is ITD v6.0, which incorporates an additional assay for detecting nOPV2, compared to previous version. The nOPV2 assay targets one region in the 5'UTR of the virus bearing the unique determinants for nOPV2. It is possible that recombination events remove the 5'UTR erasing the targets for the ITD assay (Figure 1). In such circumstances, nOPV2 will not be identified (negative result) and could be reported as SL2 from the ITD test.
- **Genetic linkage** – Genetic relationship between or among poliovirus sequences that suggests a common origin or emergence. As poliovirus transmits/replicates, it accumulates nucleotide substitutions in the genome. The capsid VP1 region is routinely used to estimate the number of genetic differences among poliovirus sequences. Detection of new cVDPV (from Sabin origin) or cVDPV2-n emergences requires genetic linkage analysis using VP1 sequences. The analysis aims at estimating the number of shared genetic change between or among VP1 sequences; viruses with a common ancestor share a proportion of the total number of changes from the progenitor vaccine dose (linkage). Sequence comparisons among PV complete genomes permits higher resolution analysis of genetic linkage between VDPVs, including the possibility of establishing genetic linkages using complete genomes obtained from SL or nOPV-L isolates of interest.
- **Vaccine origin –** The gradual and country-specific switch from Sabin OPV2 to nOPV2 use for cVDPV2 outbreak response resulted in co-detection of vaccine-like or vaccine-derived polioviruses from both Sabin OPV2 and nOPV2 origin. Determination of vaccine origin in the laboratory is an independent process from determination of genetic linkage. Evidence of prolonged circulation (i.e., cVDPV) constitutes and triggers a programmatic response regardless of vaccine origin as described in the Outbreak Response Guidance.
- **Orphan viruses** – Viruses with ≤98.5% VP1 identity from the closest match virus from the sequence database at the time of detection.

Figure 1. Representation of key nOPV2 genetic traits for the ITD assay



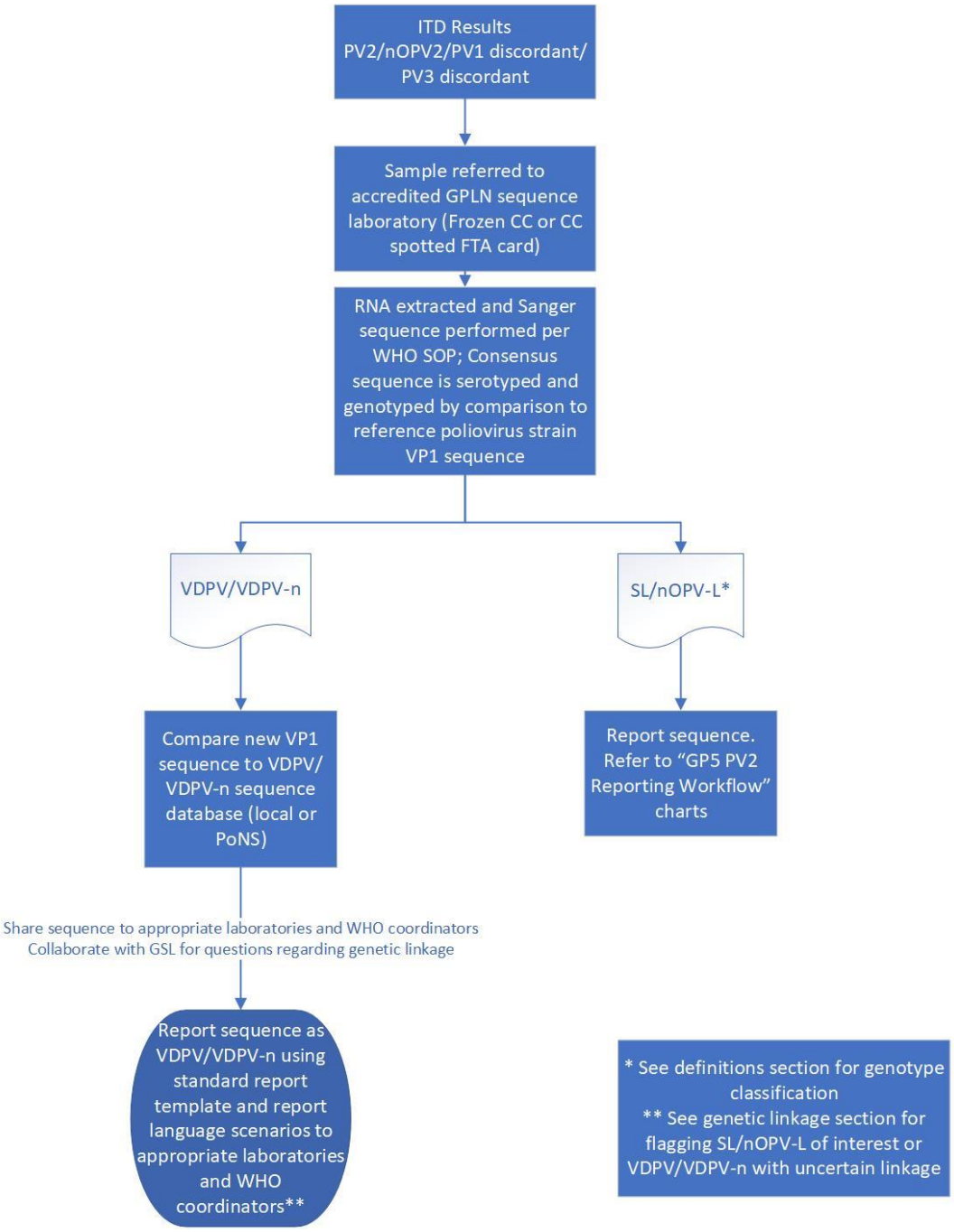
4. VDPV reporting process

The process for referral, sequencing, and analysis of suspected poliovirus VDPV specimens can be subdivided into the following basic steps:

1) An accredited GPLN laboratory tests poliovirus specimens/isolates using ITD and classifies a specimen as either a PV2, PV2/nOPV2, discordant PV1, discordant PV3 or any combination of these three ITD results. A nOPV2 negative result in ITD testing, while PV2 RNA was detected in the PV2 and Sabin 2 assays, does not necessarily indicates absence of nOPV2 and the laboratory should flag the specimen for additional contextualization and identification. The laboratory should consult the WHO Regional and Global Laboratory Coordinators for further guidance.
2) The specimen is referred to an accredited GPLN sequencing laboratory as a frozen cell culture isolate or an FTA card spotted with cell culture isolate. All PV2 and PV2/nOPV2 specimens are referred to the sequencing laboratory as per current GPEI recommendations.
3) RNA is extracted, and VP1 sequencing is performed following the WHO recommended Sequence protocol (Sanger sequencing).
4) Raw sequence data are edited and a consensus VP1 sequence is obtained.
5) The consensus VP1 sequence is used to serotype and genotype by comparison to reference poliovirus strain VP1 sequences. Possible outcomes:
    a) If the genotype of the sequence is determined to be Sabin, it is reported as Sabin-like (SL):
        i) If the specimen is PV2/nOPV2 positive by ITD, it is reported as nOPV2-like (nOPV2-L).
        ii) If the specimen is PV2/nOPV2 negative by ITD, consult the Regional and Global Coordinators for further guidance.
        iii) Refer to genetic linkage section (Section 9) for flagging SL/nOPV-L of interest for further investigation.
    b) If the genotype fits the criteria of a VDPV or VDPV-n (as outlined in the Definitions section of this guidance), further analysis is needed:
        i) The newly sequenced VDPV is compared to previously identified VDPVs for evidence of genetic linkage (see genetic linkage section in this guidance).
        ii) If needed, contact CDC or another GSL (Global Specialized Laboratory) sequencing laboratory for questions and consultation concerning genetic linkage of the new VDPV.
        iii) Forward the sequence (fasta file) to the appropriate laboratories and WHO coordinators for comparison to other databases as needed.
6) Report the sequence as VDPV or VDPV-n using the standard report template (see Standard Report Template section) and suggested text in the report language scenarios (see Standard Report Text section) outlined in this guidance paper.

A flow chart outlining the process is in figure 2 below.

Figure 2. Flow chart for VDPV/VDPV-n and SL/nOPV-L report process



5. Genetic analysis using VP1 sequences

Genetic analysis of the new VDPV/VDPV-n sequence (or query sequence) in comparison to other identified VDPV/VDPV-n sequences help to classify the new sequence. Known VDPV or VDPV-n sequences that can be used for comparisons can be in a local database (such as e.g., Bioedit, Sequencher or Geneious), GenBank, and/or PoNS (Poliovirus Nucleotide Sequence database).

For many sequences, genetic linkage between the new sequence and other sequence(s) is obvious. For example, if the query PV2 sequence for EPID MTA-XYZ-ABC-17-001 has 20 nucleotide differences with Sabin2 but only has 3nt differences with the VDPV sequence for MTA-XYZ-CBA-17-004, the two VDPVs are clearly genetically linked (see matrix of number of nucleotide differences below) with ≥4 shared differences.

	MTA-XYZ-ABC-17-001	MTA-XYZ-CBA-17-004
MTA-XYZ-ABC-17-001		3
MTA-XYZ-CBA-17-004	3	
SABIN	20	21

However, analysis of a newly sequenced VPDV/VDPV-n may not give obvious results, especially for highly diverged orphan viruses or VDPV/VDPV-n with limited divergence from corresponding vaccine strain. In addition, it is critical to be aware that there can be nucleotide substitutions in known VP1 hotspot sites. VP1 "hotspots" are nucleotide positions that change relatively frequently.

Evidence supporting genetic linkage between query sequences and known VDPVs include the following observations:

- **Observation 1:** There are several shared VP1 nucleotide substitutions between query VDPV sequence and comparison sequences from the parental Sabin strain in non-hotspot sites. For type 2, the threshold among closely related VP1 sequences is four shared nucleotide substitutions. For all three PV types, inference of genetic linkage based on shared genetic change should be compatible with a high percentage of VP1 sequence identity between query VDPV/VDPV-n and known VDPV/VDPN-n.
- **Observation 2:** Shared genetic change from ancestor-progeny relationships should be distinguished from random shared genetic change. Because of the high rate of PV capsid substitution, random shared changes can become a challenge for inferring genetic linkage. Analytically, this can be tackled by considering both the absolute number of shared nucleotide differences and the proportion of shared nucleotide differences with respect to total substitutions (divergence). In some situation, it might be helpful to consider the proportion of shared nucleotide differences among total variable sites (e.g., 10% in PV2 VP1).
- **Observation 3**: if complete genome sequence is available, analysis of complete capsid sequences and recombination patterns outside the capsid region will further increase the resolution power of genetic linkage analysis. For example, detection of additional shared nucleotide substitutions outside VP1 or identification of shared recombinant blocks (Section 9.1).

Additional factors might rule out genetic linkage, such as collection or onset dates that are clearly incompatible with the molecular clock or geographic regions that do not make sense from an epidemiologic perspective. Further investigation might be needed.

GPLN sequencing laboratories with questions concerning genetic linkage should always contact CDC or another GSL, as well as regional and global lab coordinators for advice.

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6. VDPV line list template

When reporting VDPV sequences, use a tabular format that contains the following information, at a minimum:

1. Sequence Filename
2. Referring LabId
3. Specimen Type
4. EPID
5. Onset date, if relevant/available
6. Specimen Collection Date
7. Sequence lab ID
8. Original ITD result of the specimen (including nOPV2 result)
9. Date Received in the Sequence lab
10. Region of virus sequenced (5'UTR, VP4/VP2, VP1, whole capsid, whole genome)
11. Sequence Result Serotype
12. Sequence Result Genotype: Sabin-like (SL), nOPV-like (nOPV-L), VDPV, VDPV-n
13. Nucleotide Differences (nt diff) from the reference vaccine strain (VP1)
14. Comments – Include information here as to whether the VDPV/VDPV-n is genetically linked or not to other previously reported VDPV/VDPV-n sequences. Give emergence name if linked.
15. VDPV/VDPV-n Classification, if available
16. Sequence report date
17. If referred for further testing, referral date and lab shipped to

Formal VDPV reporting template

In addition to the completed VDPV line list template, a description of each reported VDPV/VDPV-n should accompany the sequence report. This information can be conveyed in the body of the email notification, or it can be included in a separate attachment. Reports should be sent to the referring laboratory, WHO regional office and WHO HQ.

Initially, the report text should reference the referring laboratory ("Sequence results for specimens from referring laboratory XXX are attached"). The next few sentences should highlight the VDPV/VDPV-n listed in the report, stating the EPIDS or Environmental IDS of the sequences, the serotype, the number of nucleotide differences (nt diff) in VP1 from the reference Sabin strain, and the classification (VDPV).

The following are three examples for each VDPV classification:

- MTA-XYZ-ABC-17-001 - The VP1 sequence from MTA-XYZ-ABC-17-001 is genetically linked to the VDPV2 sequence from MTA-XYZ-CBA-17-004 (CBA district, MTA , emergence group XYZ-1). The new virus is classified immediately as cVDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses.
- ENV/MTA/SRR/TTT/17/008 – The VP1 sequence from ENV/MTA/SRR/TTT/17/008 is not genetically linked to known VDPVs. The new virus is reported as VDPV pending further investigations/results as described in the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses.
- 667i - The VP1 sequence from 667i is from a known immune deficient patient who is not an AFP case. The new virus is classified as iVDPV3 and is from sample number 10.

Further descriptions and classification of each VDPV/VDPV-n sequence will depend on the genetic analysis based on comparison of the VP1 sequence to a database of VDPV/VDPV-n VP1 sequences. Standard reporting text will be used for each of the circumstances described in **Appendix 1 (Scenarios)**. The text should be kept as simple as possible and should adhere to the recommendations in this guidance (without extraneous information).

7. VDPV report summary guidance including nOPV/VDPV-n results

- **Types 1 and 3**

The current VDPV classification and reporting remain unchanged. This section will be updated following nOPV1 and nOPV3 use.

- **Type 2**

1. Standard classification and reporting of Sabin-like 2 viruses.

   ITD result: PV2, nOPV2 negative. Sanger VP1 sequencing result: VP1 sequence with <6 nucleotide differences from reference vaccine strain. Genotype: SL2. Refer to Charts 2A and 2B of the 'GP5 PV2 Reporting Workflow' charts (**Appendix 2**) to determine final result. If final result ends up as PV2-SABIN, then this scenario is unlikely, given that current campaigns no longer utilize mOPV2. Any PV2-Sabin would require investigation at every level of processing.

2. Standard classification and reporting of nOPV2-like viruses.

   a. ITD result: PV2, nOPV2 positive. Sanger VP1 sequencing result: VP1 sequence with <6 nucleotide differences from reference vaccine strain. Genotype: nOPV2-L. Final result: nOPV2-L. Refer to Chart 1 of the 'GP5 PV2 Reporting Workflow' charts.

   b. ITD result: PV2, nOPV2 negative. Sanger VP1 sequencing result: VP1 sequence with <6 nucleotide differences from reference vaccine strain. In this case, the laboratory must confirm vaccine origin by either finding a genetic linkage or by detecting nOPV2 markers outside of VP1. Refer to Charts 2A and 2B of the 'GP5 PV2 Reporting Workflow' charts to determine final results.

3. Standard classification and reporting of VDPV2 viruses.

   Classification of sequences genetically related to cVDPV2 emergences originating before nOPV2 release remain unchanged. ITD result: PV2, nOPV2 negative. Sanger VP1 sequencing result: VP1 sequence with a closest match to a VDPV2 linked to a known cVDPV2 emergence. Genotype: VDPV. Final report: PV2-VDPV. Refer to Chart 3A of the 'GP5 PV2 Reporting Workflow' charts to determine final results.

4. Standard classification and reporting of VDPV2-n viruses.

   a. ITD result: PV2, nOPV2 positive. Sanger VP1 sequencing result: VP1 sequence with >=6 nucleotide differences from reference strain. Genotype: VDPV-n. Final report: PV2-VDPV-n. In this case, the laboratory must confirm vaccine origin by either finding a genetic linkage or by detecting nOPV2 markers outside of VP1. Refer to Charts 3A and 3B of the 'GP5 PV2 Reporting Workflow' charts to determine final results.

   b. ITD result: PV2, nOPV2 negative. Sanger VP1 sequencing result: VP1 sequence with >=6 nucleotide differences from reference strain. Genotype: VDPV. In this case, the laboratory must confirm vaccine origin by either finding a genetic linkage or by detecting nOPV2 markers outside of VP1. Refer to Charts 4A and 4B of the 'GP5 PV2 Reporting Workflow' charts to determine final results.

5. See Figure 3 for summary workflow.
6. When reports are not final, further genetic characterization is required to address questions concerning the origin of the vaccine sequence rather than focusing on genetic linkage, as described in the following section.

8. Genetic distinctions between cVDPV/cVDPV-n and iVDPV/iVDPV-n sequences

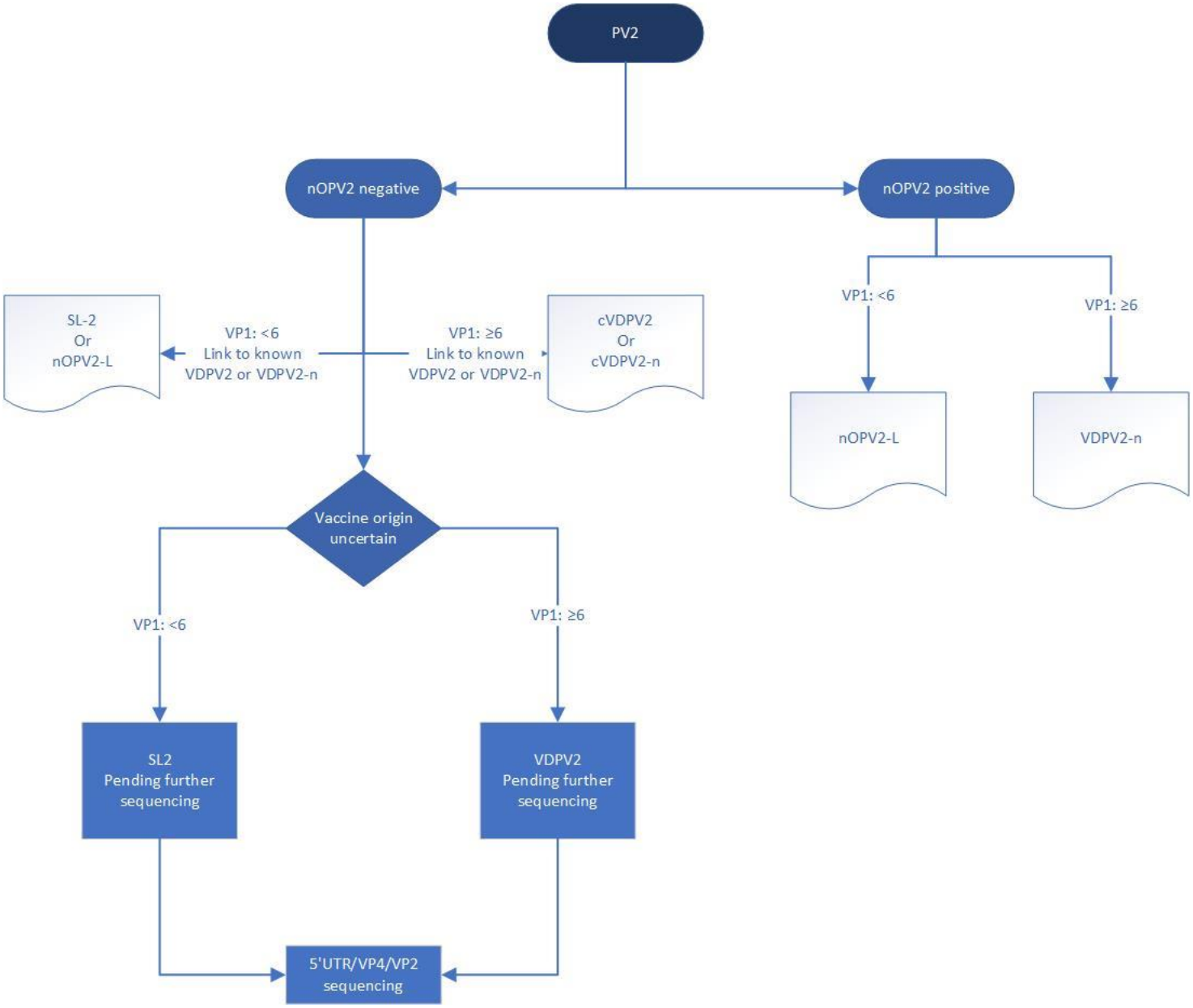
Differential genetic characteristics between circulating and immunodeficient related VDPV/VDPV-n sequences include:

- A higher proportion of mixed-base nucleotide sites in iVDPV/iVDPV-n sequences.
- Extensive antigenic divergence from originating vaccine strain in iVDPV/iVDPV-n sequences.
- Multiple viral lineages observed in iVDPV/iVDPV-n sequences.
- Simple genetic measurements of nucleotide and amino acid substitutions in VP1 (or capsid) region are sufficient for distinguishing highly divergent iVDPV2/iVDPV2-n from cVDPV2/cVDPV2-n sequences (e.g. nt substitutions ≥22) but may be insufficient to make a clear distinction between the two categories among less divergent sequences.
- No single nucleotide or amino acid position can be used to differentiate circulating from immunodeficiency-related viruses.

Genetic distinctions between the two groups of type 2 VDPVs have been extensively studied. Distinctions between the two groups of type 1 and 3 VDPVs are ongoing. GPLN sequencing laboratories with questions concerning these analyses should contact CDC for advice.

Please note that a high number of mixed bases could indicate cross-contamination of one sample with another, or it could be an indicator of possible VDPV from an immunodeficient person. Sequencing laboratories should perform the editing exercise of removing the Sabin bases from a mixed sequence to determine the underlying hypothetical non-Sabin sequence. This sequence should then be compared to recently sequenced viruses to determine if the sequence is identical to other recently sequenced viruses or viruses handled recently in the lab. If so, then cross-contamination might explain the mixed bases. Consensus sequence files and sequence chromatographs can be referred to a Global Specialized Laboratory for confirmatory analysis.

Figure 3. Guidance for PV2 classification following ITD and VP1 sequencing (excluding mixtures for simplicity)*



* Refer to GPEI Guidelines for Reporting and Classification of vaccine-derived polioviruses.

9. Genetic analysis using genomic regions outside VP1

While VP1 sequencing is the standard method for resolving genetic relationships, complete or partial genomes beyond VP1 can a) increase the resolution power for establishing genetic linkages among vaccine strains and b) identify nOPV vaccine origin. Partial or complete genome sequences can be obtained using Sanger and/or Next Generation Sequencing (NGS). NGS (Illumina or nanopore platforms) allows rapid obtention of complete or partial genomes while substantially increasing sample throughput. This section provides guidance on referring isolates for complete or partial genome sequencing depending on the questions to be addressed, particularly for type 2 vaccine-related or vaccine-derived viruses.

9.1 Using additional sequence data beyond VP1 for establishing genetic linkage using PV2 as an example

The standard definition for VDPV2/VDPV2-n classification establishes a threshold value of 6 or more VP1 nucleotide substitutions from parental vaccine strain. However, it has been observed that establishing genetic linkages at or near the threshold value (6-12 nucleotide differences) among VDPV2/VDPV2-n can sometimes pose an analytical challenge. In addition, detection of extreme orphan viruses can pose a similar analytical challenge (see Scenario 9). Figure 4a shows a flow diagram for guiding sequencing laboratories in referrals for widening the sequencing region. Similar approach applies to consideration of PV1 and PV3 without the need to differentiate vaccine origin.

Limited VP1 genetic signal of sequences with <6 nucleotide differences from corresponding vaccine strain leaves potential genetic linkages (SL2/SL2, SL2/VDPV2, nOPV2-L/nOPV2-L, nOPV2-L/VDPV2-n) unresolved and flagged for further sequencing. Figure 4b shows a flow diagram for guiding sequencing laboratories in referrals for widening the sequencing region using the following screening criteria:

1. Sequence-based screening
    a. VP1 sequencing identifies SL2/nOPV2-L of interest (SL2*/nOPV2-L*: 4-5 nucleotide differences) and/or VDPV2/VDPV2-n with limited divergence (6-12 nucleotide differences) with no genetic linkage to any known VDPV2/VDPV2-n (circulation status unresolved).
    b. Two or more SL2*/nOPV2-L* share ≥2 VP1 substitutions (excluding hotspots) from parental strain AND
        i. SL2*/nOPV2-L* detected from ≥2 AFP cases in geographically related locations OR
        ii. SL2*/nOPV2-L* from ≥1 ES and from ≥1 AFP cases in geographically related locations OR
        iii. SL2*/nOPV2-L* from ES collected at the same environmental site at least one month apart or from separate ES collection sites in geographically related locations.
2. Surveillance-based screening
    a. Increased frequency of ES detections in a particular region and in multiple environmental sites with possible links to a case
    b. Immunization activities preceding detections during last 12 months in the area of detections and in adjacent regions/countries.

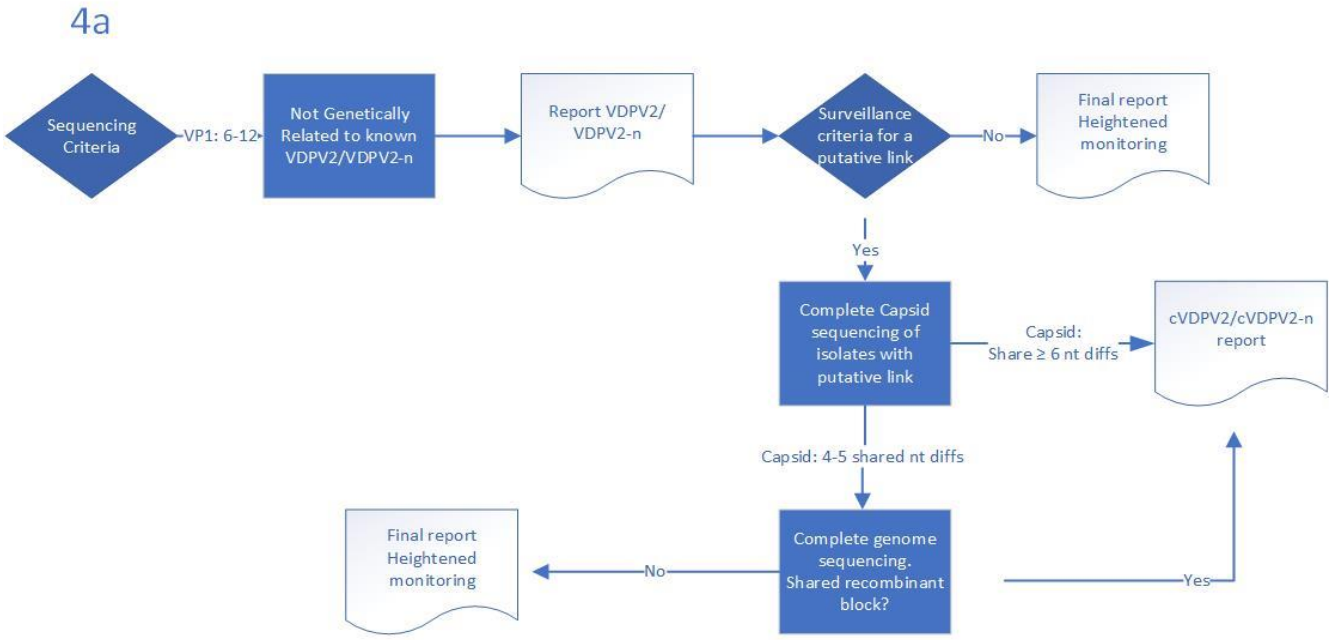
There are two main approaches for genetic linkage analysis of expanded sequencing windows:

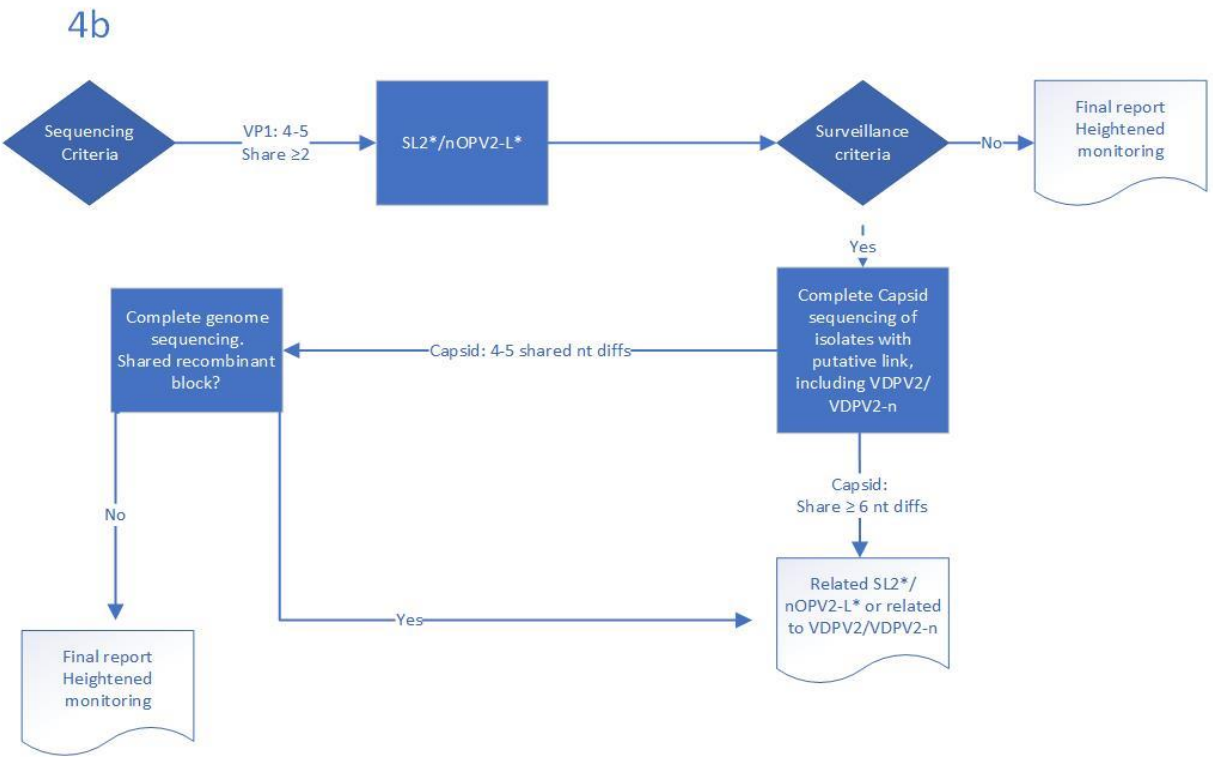
1. Establishing genetic linkage using complete capsid sequences (~2,600 nucleotides). Laboratories should flag samples that show two or more shared nucleotide substitutions outside VP1 in addition to the shared VP1 changes.
2. Establishing common ancestry using detection of common recombinant blocks linking SL2*/nOPV2-L* and/or VDPV2/VDPV2-n. The 5'UTR and P2 and P3 regions should be scanned for putative shared recombination junctions including near sequence identity of the introduced genome segments. If there is uncertainty of the specificity of the recombination junction, or unrelated introduced genome segments, the viruses should be referred.

Relevant SL/nOPV-L viruses that show evidence of genetic linkage with VDPV following extended genome sequence analysis should be referred as “SL/nOPV-L genetically related to VDPV with EPID X”. If there is evidence of genetic linkage to a known cVDPV emergence, the SL/nOPV-L should be referred as “SL/nOPV-L genetically related to cVDPV emergence XXX-YYY-Z”.

Guidelines for referrals to expanded sequencing is independent of suspected vaccine origin.

Figure 4. Guidance for extended sequencing in a) VDPV2/VDPV2-n and b) SL2/nOPV2-L



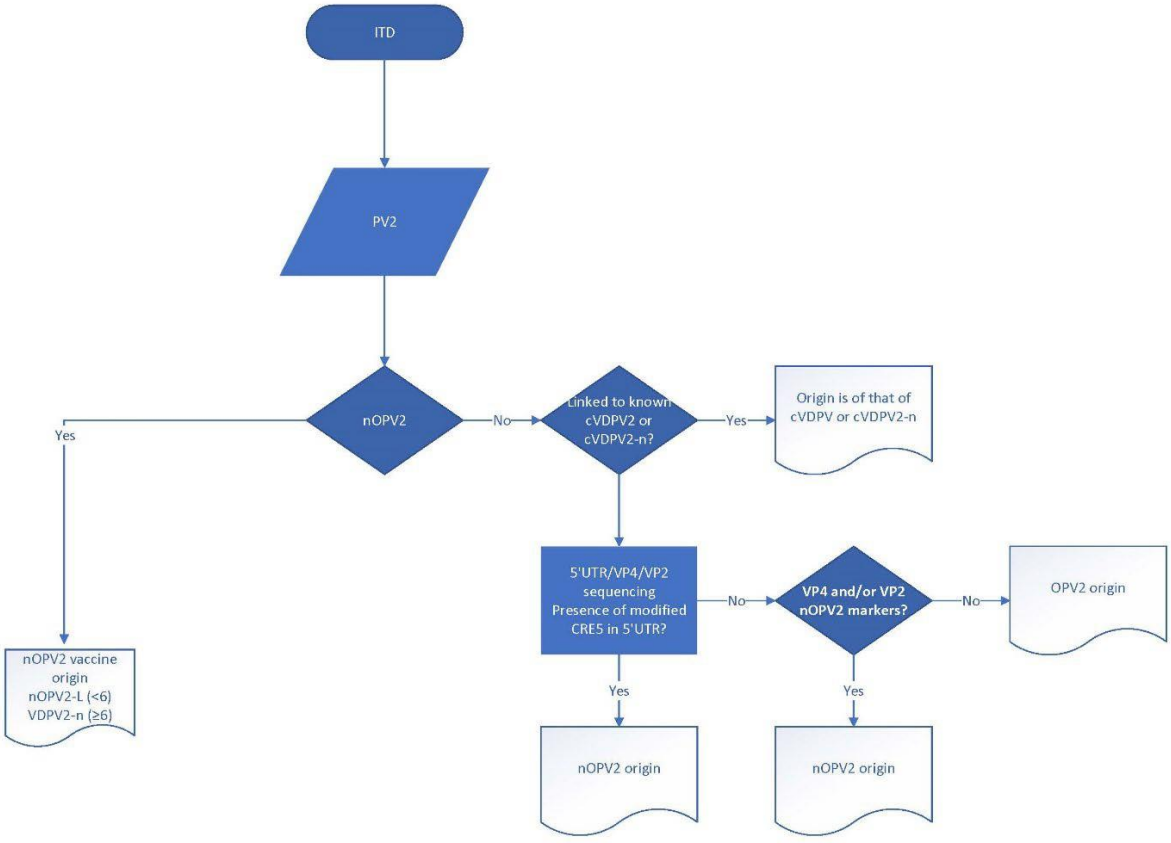


9.2 Genetic characterization of nOPV2 isolates

Since VP1 sequences from nOPV2 isolates are indistinguishable from regular Sabin2 isolates, sequencing of the 5'UTR and VP4/VP2 (or complete capsid region) is recommended when detections of PV2 isolates suspected of nOPV2 origin yield a negative result in the nOPV2 ITD test (Figure 1). Recombination at the 5'UTR region can erase specific ITD sites for the nOPV2 assay resulting in a negative test result. However, there are three capsid nOPV2 markers that can help establish nOPV2 origin if present. In addition, there are two phenotypic markers at the nOPV2 3Dpol region. However, the 3Dpol markers are subject to elimination by recombination as well.

Figure 5 shows a flow diagram guiding sequencing laboratories for deciding referrals for extended sequencing while communicating with regional and global coordinators and GSLs when needed. Sequencing laboratories should follow guidance in charts 2B, 3B, and 4B included in the 'GP5 PV2 Reporting Workflow' (Appendix 2).

Figure 5. Guidance for extended sequencing in establishing nOPV vaccine origin.



Appendix 1

- ***Scenario 1 (AFP case, and contacts)***
  The newly reported VDPV sequence shows genetic linkage to other previously reported VDPVs. The VDPV is classified as a cVDPV(x), where x refers to serotype. The standard report text for this VDPV would state: "The VP1 sequence from <EPID> is genetically linked to the VDPV(x) sequence from <related sequence EPID/ENVID> (< District/Country> , <Name of Emergence Group>). The new virus is classified immediately as cVDPV(x), as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."

- ***Scenario 2 (Environmental isolate)***
  The newly reported VDPV sequence shows genetic linkage to other previously reported VDPVs. Classification of the VDPV will depend on factors outlined by the GPEI Guidelines for Reporting and Classification of VDPVs requiring two or more genetically linked VDPVs from environmental isolates, detected for a period of ≥60 days at a single collection site or for any length of time at more than one collection site, as long as the catchment areas do not overlap. The standard report text will depend upon these factors:
  1. Environmental sample (cVDPV) – The VDPV is classified as a cVDPV(x) where x refers to serotype. The standard report text for this VDPV would state: "The VP1 sequence from <ENVID> is genetically linked to the VDPV(x) sequence from <related sequence EPID/ENVID> (< District/Country> , <Name of Emergence Group>). The new virus is classified immediately as cVDPV(x), as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."
  2. Environmental sample (VDPV)- The VDPV cannot be classified by the sequencing lab at this time. The standard report text will state: "The VP1 sequence from <ENVID> is genetically linked to VDPV sequence from <related sequence ENVID>. It cannot be classified as cVDPV at this time because it does not meet the GPEI guidelines for classification of VDPVs from environmental samples. GPEI guidelines specify that two environmental isolates from a single collection site can be classified as cVDPV only if detection occurs for a period of ≥ 60 days."

- ***Scenario 3 (PID patient)***
  The newly reported VDPV sequence is from a known PID patient and will be classified as an iVDPV(x), where x refers to the serotype. The standard report text for this VDPV would state: "The VP1 sequence from <PID identifier> is from a known immune deficient patient who <is/is not> an AFP case. The new virus is classified as iVDPV(x) and is from sample number <if this is a serial sample>"

- ***Scenario 4 (community contacts, household contacts)***
  The newly reported VDPV sequence shows no genetic linkage to previously sequenced VDPVs but is genetically related to other VDPV sequences that are part of the same batch. An example might be community contact samples that are collected on the same date and processed together. The sequencing lab would need guidance in the classification of these VDPVs, and the standard report text would state: "The VP1 sequence for <EPID> is genetically linked to other VDPV sequences from community sampling in <Country> on collection date <date>. EPI investigation should be carried out to determine if these contacts are non-household contacts."

- ***Scenario 5***
  The newly reported VDPV sequence shows no genetic linkage to other previously reported VDPVs. The VDPV cannot be classified by the sequencing lab, and the standard report text will depend on the specimen source:
  1. AFP case/contacts, community contact samples – The standard report text will state: "The VP1

sequence for <EPID > is not genetically linked to known VDPVs. Clinical examination and immunological tests should be carried out to investigate the case, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses".

2. Environmental samples – The standard report text will state: "The VP1 sequence for isolate <ENVID> is not genetically linked to known VDPVs, as described in the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."

- ***Scenario 6***

  The newly reported VDPV sequence shows no genetic linkage to other previously reported VDPVs and additionally has a high number of amino acid changes, a high number of mixed/ambiguous base calls, or a mixture of both in the VP1 sequence. The VDPV cannot be classified by the sequencing lab, and the standard report text will depend on the specimen source:

  1. AFP case/contacts, community contact samples – The standard report text will state: "The VP1 sequence for isolate <EPID> is not genetically linked to known VDPVs. However, a high number of amino acid changes and mixed bases observed in the VP1 sequence suggest the possibility that the virus might be an iVDPV. Clinical examination and immunological tests should be carried out to investigate the <case or contact>, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."
  2. Environmental samples – The standard report text will state: "The VP1 sequence for isolate <ENVID> is not genetically linked to known VDPVs. However, a high number of amino acid changes and mixed bases observed in the VP1 sequencing suggest the possibility that the virus might have been excreted by a person with primary immunodeficiency. Because it is an environmental isolate, clinical investigation is not possible. Refer to GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses for appropriate actions."

- ***Scenario 7 (environmental specimens)***

The newly reported VDPV sequence has ≥6nt differences from Sabin2 and shows no genetic linkage to other previously reported VDPVs but is closely related to other Sabin2 sequences from the same environmental sample. The sequencing lab will need guidance in the classification of these VDPVs, and the standard report text would state: "The VDPV2 sequence for <ENVID, Flask #> is not genetically linked to known VDPVs. The sequence is genetically linked to Sabin 2 isolates from the same environmental sample <flask #s>, which have <#> nt differences from Sabin 2 VP1. Please note that sequences from environmental isolates are composite sequences from viruses present in the environmental sample, which usually includes virus mixtures. The new VDPV2 cannot be classified under the current GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."

- ***Scenario 8 (environmental specimens)***

The newly reported VDPV sequence has ≥6nt differences from Sabin2 but most or all of the differences are due to ambiguous base calls in the VP1 sequence. The other sequences from other flasks for this environmental specimen are all Sabin2; where the Sabin-like sequences have nucleotide differences from Sabin2 is equivalent to an ambiguous base call in the VDPV sequence. The VP1 sequence for the VDPV *most likely represents a mixture of different Sabin2 viruses* that have no genetic linkage to previously sequenced cVDPV2 viruses. The sequencing lab will need guidance in the classification of these VDPVs, and the standard report text would state: "The VDPV2 sequence for <ENVID, Flask #> has <#> nt differences from Sabin2, and all are ambiguous base calls; it is not genetically linked to known VDPVs but appears to be a mixture of the Sabin2 sequences found in the same environmental sample < flask#> . The new VDPV2 virus cannot be classified under the current GPEI Guidelines for

Reporting and Classification of Vaccine-derived Polioviruses."

- ***Scenario 9 (extreme orphans, environmental and AFP)***

The newly reported VDPV sequence is detected in a region experiencing a known cVDPV outbreak but does not appear to be genetically linked to any previously reported VDPV sequences reported in the outbreak. Deeper genetic analysis is required to confirm if the newly sequenced VDPV is a separate emergence or if it is related to the ongoing outbreak but is an extreme orphan (>1.5% divergence); if necessary, a GSL (global specialized laboratory) may have to be consulted. The standard report text would state: 'The VDPV2 sequence for <ENVID or EPI> has no close genetic link to previously sequenced cVDPV2s that are part of the ongoing outbreak in <Region>. The new VDPV2 virus cannot be classified at this time. Sample referred for additional sequencing"

- **Scenario 10 (limited VP1 divergence, flagged for WGS)**

The newly reported VDPV2 sequence (an AFP case) is detected in an IPV country, and it has 10 nucleotide differences from Sabin 2. An environmental sample collected in an IPV country different from the original case identification yields an SL2 with 5 nucleotide differences from Sabin 2. The case and environmental sample share four nucleotide differences in VP1. A third sample from an environmental detection in a third IPV country yields a VDPV2 with 8 nucleotide differences from Sabin 2, sharing two VP1 nucleotide substitutions with the AFP and ENV sample from the other countries. Complete capsid analysis shows six additional shared substitutions outside VP1. In addition, the three samples show a common P3/Non-capsid region sequence from an unknown species C enterovirus recombination donor. The two VDPV2s can be classified as cVDPV2 from a single emergence and the SL2 can be classified as SL2 genetically related to that cVDPV2 emergence. [Provide sample report language for each virus or refer to Scenario above]

Appendix 2

PV2 Reporting Workflow

CHART 1

ITD = PV2, nOPV2-POS

SANGER VP1 = Sanger detected one PV2 sequence with <6 nucleotide differences from reference strain

****Assumption that ITD has been confirmed by repeat, if necessary***

Step 1 of analysis	***Step 2 of analysis***	***Final Results***			
VP1 Genetic Linkage (utilizing PoNS)	***Detection of nOPV2 markers by Sanger or NGS***	***Final Sanger VP1 Sequencing Result***	***Final Result of Sample (ITD+VP1)***	***Final Report Standard Text***	***Final Line Listing***
No need to perform.	No need to perform.	PV2-SL	PV2-nOPV	No need to produce.	Include on nOPV2-L line list (see appendix 'nOPV2LList' for expected format).

CHART 2A -- (Part A using genetic linkage to confirm vaccine origin)

ITD = PV2, nOPV2-NEG

SANGER VP1 = Sanger detected one PV2 sequence with <6 nucleotide differences from reference strain

****Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin***

****Assumption that ITD has been confirmed by repeat, if necessary***

Step 1 of analysis	***Step 2 of analysis***	***Final Results***			
VP1 Genetic Linkage (utilizing PoNS)	***Detection of nOPV2 markers by Sanger or NGS***	***Final Sanger VP1 Sequencing Result***	***Final Result of Sample (ITD+VP1)***	***Final Result Standard Text***	***Final Line Listing***
Option 1: The sequence is genetically linked with at least 4 shared changes to a sequence of a known VDPV emergence group (circulating).	No need to perform.	PV2-SL	PV2-SABIN	No need to produce. Send an email to WHO regional and global coordinators because this scenario should not occur now.	No need to produce.
Option 2: The sequence is genetically linked with at least 4 shared changes to a known VDPV sequence that is not part of an existing emergence group (not circulating).	No need to perform.	PV2-SL	PV2-SABIN	No need to produce. Send an email to WHO regional and global coordinators because this scenario should not occur now.	No need to produce.
Option 3: The sequence is genetically linked with at least 4 shared changes to a sequence of a known VDPV-n emergence group (circulating).	No need to perform.	PV2-SL	PV2-nOPV	**"The sequence for <EPID> (<labid>) is genetically linked to the cVDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The sequence is classified as a PV2-nOPV."**	Include on nOPV2-L line list (see appendix 'nOPV2LList' for expected format).
Option 4: The sequence is genetically linked with at least 4 shared changes to a known VDPV-n sequence that is not part of an existing emergence group (not circulating).	No need to perform.	PV2-SL	PV2-nOPV	**"The sequence for <EPID> (<labid>) is genetically linked to the VDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The sequence is classified as a PV2-nOPV."**	Include on nOPV2-L line list (see appendix 'nOPV2LList' for expected format).
Option 5: The sequence is not genetically linked to any known VDPV-n/VDPV sequence or has less than 4 shared changes to a known VDPV-n/VDPV sequence.	Need to perform. Go to CHART 2B for next set of options.				

CHART 2B -- (Part B detecting nOPV2 markers to confirm vaccine origin)

ITD = PV2, nOPV2-NEG

SANGER VP1 = Sanger detected one PV2 sequence with <6 nucleotide differences from reference strain

**Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin*

**Assumption that ITD has been confirmed by repeat, if necessary*

Step 1 of analysis	***Step 2 of analysis***	***Final Results***			
VP1 Genetic Linkage (utilizing PoNS)	***Detection of nOPV2 markers by Sanger or NGS***	***Final Sanger VP1 Sequencing Result***	***Final Result of Sample (ITD+VP1+nOPV2 markers)***	***Final Report Standard Text***	***Final Line Listing***
Already attempted in CHART 2A.	**Option 1:** The CRE present in 5'utr. There is no need to check VP4. The sequence is guaranteed to be nOPV2 based on 5'utr.	PV2-SL	PV2-nOPV	*This is an unlikely scenario indicating conflicting results. Please discuss with WHO coordinators.*	*This is an unlikely scenario indicating conflicting results. Please discuss with WHO coordinators.*
Already attempted in CHART 2A.	**Option 2:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, but the sequence indicates a suspected recombinant. Both nOPV2 markers are detected in VP4.	PV2-SL	PV2-nOPV	**"The nOPV2 markers were detected outside of the VP1 region of sequence for <EPID> (<labid>). The sequence is classified as a PV2-nOPV."**	Include on nOPV2-L line list (see appendix 'nOPV2LList' for expected format).
Already attempted in CHART 2A.	**Option 3:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, but the sequence indicates a suspected recombinant. nOPV2 markers are detected in VP2 (other markers in VP4 are assumed to be obscured by recombinant).	PV2-SL	PV2-nOPV	**"The nOPV2 markers were detected outside of the VP1 region of sequence for <EPID> (<labid>). The sequence is classified as a PV2-nOPV."**	Include on nOPV2-L line list (see appendix 'nOPV2LList' for expected format).
Already attempted in CHART 2A.	**Option 4:** The CRE is NOT present in 5'UTR. There are no nOPV2 markers in 5'utr, and the sequence resembles Sabin2. No other nOPV2 markers are detected.	PV2-SL	PV2-SABIN	*This is an unlikely scenario indicating a Sabin result. Please discuss with WHO coordinators.*	*This is an unlikely scenario indicating a Sabin result. Please discuss with WHO coordinators.*
Already attempted in CHART 2A.	**Option 5:** No results obtainable outside of VP1.	PV2-SL	PV2-SL-UNSETTLED	**"The vaccine origin of the sequence for <EPID> (<labid>) could not be determined. The sequence is classified as a PV2-SL-UNSETTLED."** *Please discuss with WHO coordinators.*	No need to produce.

CHART 3A -- (Part A using genetic linkage to confirm vaccine origin)

ITD = PV2, nOPV2-POS

SANGER VP1 = Sanger detected one PV2 sequence with >=6 nucleotide differences from reference strain

**Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin*

**Assumption that ITD has been confirmed by repeat, if necessary*

Step 1 of analysis	*Step 2 of analysis*	*Final Results*			
VP1 Genetic Linkage (utilizing PoNS)	*Detection of nOPV2 markers by Sanger or NGS*	*Final Sanger VP1 Sequencing Result*	*Final Result of Sample (ITD+VP1)*	*Final Report Standard Text*	*Final Line Listing*
Option 1: The sequence is genetically linked, with at least 4 shared changes, to a sequence of a known VDPV emergence group (circulating).	No need to perform.	PV2-VDPV	(suspected?) Mixture of PV2-nOPV2 PV2-VDPV	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2 sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Option 2: The sequence is genetically linked, with at least 4 shared changes, to a known VDPV sequence that is not part of an existing emergence group (not circulating).	No need to perform.	PV2-VDPV	Mixture of PV2-nOPV2 PV2-VDPV	*If this new genetic linkage creates a new emergence group, contact WHO coordinators to confirm and assign name. If so:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2 sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. These sequences have been assigned to a new emergence group <name>. "** *Otherwise:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the VDPV2 sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as VDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Option 3: The sequence is genetically linked, with at least 4 shared changes, to a sequence of a known VDPV- n emergence group (circulating).	No need to perform.	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2-n, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format). In Comments field, include statement "ITD result= nOPV2+"
Option 4: The sequence is genetically linked, with at least 4 shared changes, to a known VDPV-n sequence that is not part of an existing emergence group (not circulating).	No need to perform.	PV2-VDPV	PV2-VDPV-n	*If this new genetic linkage creates a new emergence group, contact the* WHO regional and global coordinators *to confirm and assign name. If so:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2-n, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. These sequences have been assigned to a new emergence group <name>. "** *Otherwise:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the VDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as VDPV2-n, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format). In Comments field, include statement "ITD result = nOPV2+"
Option 5: The VDPV sequence is not genetically linked to any known VDPV-n/VDPV sequence or has less than 4 shared changes to a known VDPV-n/VDPV sequence. The number of nucleotide changes don't indicate orphan status (<14 nt from Sabin2 in VP1).	No need to perform.	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2-n."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format). In Comments field, include statement "ITD result = nOPV2+"

Option 6: The VDPV sequence is not genetically linked to any known VDPV-n/VDPV sequence or has less than 4 shared changes to a known VDPV-n/VDPV sequence. The nucleotide changes do indicate orphan status (>=14 nts from Sabin2 in VP1).	Need to perform. Go to CHART 3B for next set of options.

CHART 3B -- (Part B detecting nOPV2 markers to confirm vaccine origin)

ITD = PV2, nOPV2-POS

SANGER VP1 = Sanger detected one PV2 sequence with >=6 nucleotide differences from reference strain

****Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin***

****Assumption that ITD has been confirmed by repeat, if necessary***

Step 1 of analysis	*Step 2 of analysis*	*Final Results*			
VP1 Genetic Linkage (utilizing PoNS)	*Detection of nOPV2 markers by Sanger or NGS*	*Final Sanger VP1 Sequencing Result*	*Final Result of Sample (ITD+VP1+nOPV2 markers)*	*Final Report Standard Text*	*Final Line Listing*
Already attempted in CHART 3A.	**Option 1:** The CRE present in 5'utr. There is no need to check VP4. The sequence is guaranteed to be nOPV2 based on 5'utr.	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2-n."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Already attempted in CHART 3A.	**Option 2:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, but the sequence indicates a suspected recombinant. Both nOPV2 markers are detected in VP4.	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2-n."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Already attempted in CHART 3A.	**Option 3:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, but the sequence indicates a suspected recombinant. nOPV2 markers are detected in VP2 (other markers in VP4 are assumed to be obscured by recombinant).	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2-n."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Already attempted in CHART 3A.	**Option 4:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, and the sequence resembles Sabin2. No other nOPV2 markers are detected.	PV2-VDPV	Assume mixture of nOPV2 and PV2-VDPV	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed there are NO nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2."** *Do not mention the ITD result in the standard text.* *This is an unlikely scenario. Please discuss with* WHO regional and global coordinators.	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).

Already attempted in CHART 3A.	**Option 5:** No results obtainable outside of VP1.	PV2-VDPV	Assume mixture of PV2-nOPV2 and PV2-VDPV-UNSETTLED	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. All attempts to determine vaccine origin via whole genome sequencing (WGS) have been exhausted without resolution. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as PV2-VDPV-UNSETTLED."** *Do not mention the ITD result in the standard text. Please discuss with WHO regional and global coordinators.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).

CHART 4A -- (Part A using genetic linkage to confirm vaccine origin)

ITD = PV2, nOPV2-NEG

SANGER VP1 = Sanger detected one PV2 sequence with >=6 nucleotide differences from reference strain

****Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin***

****Assumption that ITD has been confirmed by repeat, if necessary***

Step 1 of analysis	***Step 2 of analysis***	***Final Results***			
VP1 Genetic Linkage (utilizing PoNS)	***Detection of nOPV2 markers by Sanger or NGS***	***Final Sanger VP1 Sequencing Result***	***Final Result of Sample (ITD+VP1)***	***Final Report Standard Text***	***Final Line Listing***
Option 1: The sequence is genetically linked with at least 4 shared changes to a sequence of a known VDPV emergence group (circulating).	No need to perform.	PV2-VDPV	PV2-VDPV	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2 sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Option 2: The sequence is genetically linked with at least 4 shared changes to a known VDPV sequence that is not part of an existing emergence group (not circulating).	No need to perform.	PV2-VDPV	PV2-VDPV	*If this new genetic linkage creates a new emergence group, contact the WHO regional and global coordinators to confirm and assign name. If so:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2 sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. These sequences have been assigned to a new emergence group <name>. "** *Otherwise:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the VDPV2 sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as VDPV2, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Option 3: The sequence is genetically linked with at least 4 shared changes to a sequence of a known VDPV-n emergence group (circulating).	No need to perform.	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1and is genetically linked to the cVDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2-n, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV ' for expected format).
Option 4: The sequence is genetically linked with at least 4 shared changes to a known VDPV-n sequence that is not part of an existing emergence group (not circulating).	No need to perform.	PV2-VDPV	PV2-VDPV-n	*If this new genetic linkage creates a new emergence group, contact the WHO regional and global coordinators to confirm and assign name. If so:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the cVDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as cVDPV2-n, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. These sequences have been assigned to a new emergence group <name>. "** *Otherwise:* **"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <#> nucleotide differences (nt diff) from Sabin 2 VP1 and is genetically linked to the VDPV2-n sequence for <EPID> (<labid>) (ntdiff=<#>) (<locality> district, emergence group <name>). The new PV2 virus is classified immediately as VDPV2-n, as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).

Option 5: (need to assess joining option 5 and 6) The VDPV sequence is not genetically linked to any known VDPV-n/VDPV sequence or has less than 4 shared changes to a known VDPV-n/VDPV sequence. The number of nucleotide changes don't indicate orphan status (<14 nts from Sabin2 in VP1).	Need to perform. Go to CHART 4B for next set of options.
Option 6: The VDPV sequence is not genetically linked to any known VDPV-n/VDPV sequence or has less than 4 shared changes to a known VDPV-n/VDPV sequence. The nucleotide changes do indicate orphan status (>=14 nt from Sabin2 in VP1).	Need to perform. Go to CHART 4B for next set of options.

CHART 4B -- (Part B detecting nOPV2 markers to confirm vaccine origin)

ITD = PV2, nOPV2-NEG

SANGER VP1 = Sanger detected one PV2 sequence with >=6 nucleotide differences from reference strain

**Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin*

**Assumption that ITD has been confirmed by repeat, if necessary*

Step 1 of analysis	*Step 2 of analysis*	*Final Results*			
VP1 Genetic Linkage (utilizing PoNS)	*Detection of nOPV2 markers by Sanger or NGS*	*Final Sanger VP1 Sequencing Result*	*Final Result of Sample (ITD+VP1+nOPV2 markers)*	*Final Report Standard Text*	*Final Line Listing*
Already attempted in CHART 4A.	**Option 1:** The CRE present in 5'utr. There is no need to check VP4. The sequence is guaranteed to be nOPV2 based on 5'utr.	PV2-VDPV	PV2-VDPV-n	*This is an unlikely scenario indicating conflicting results. Please discuss with WHO regional and global coordinators.*	*This is an unlikely scenario indicating conflicting results. Please discuss with WHO regional and global coordinators.*
Already attempted in CHART 4A.	**Option 2:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, but the sequence indicates a suspected recombinant. Both nOPV2 markers are detected in VP4.	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2-n."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Already attempted in CHART 4A.	**Option 3:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, but the sequence indicates a suspected recombinant. nOPV2 markers are detected in VP2 (other markes in VP4 are assumed to be obscured by recombinant).	PV2-VDPV	PV2-VDPV-n	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2-n."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Already attempted in CHART 4A.	**Option 4:** The CRE is NOT present in 5'utr. There are no nOPV2 markers in 5'utr, and the sequence ressembles Sabin2. No other nOPV2 markers are detected.	PV2-VDPV	PV2-VDPV	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) from Sabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. The Sanger sequence for <EPID> was further characterized by the whole genome sequencing (WGS), which confirmed there are no nOPV2 markers. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as VDPV2."** *Unlikely scenario, contact the WHO regional and global coordinators. Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).
Already attempted in CHART 4A.	**Option 5:** No results obtainable outside of VP1.	PV2-VDPV	PV2-VDPV-UNSETTLED	**"The VDPV sequence for <EPID> (<labid>) is serotype 2, has <nt> nucleotide differences (nt diff) fromSabin 2 VP1 and is not genetically linked to any previously sequenced VDPV2(-n)s. The new PV2 virus cannot be classified as circulating by the laboratory as described in GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses. All attempts to determine vaccine origin via whole genome sequencing (WGS) have been exhausted without resolution. In conclusion, following the GPEI Guidelines for Reporting and Classification of Vaccine-derived Polioviruses, this isolate is classified as PV2-VDPV-UNSETTLED."** *Do not mention the ITD result in the standard text.*	Include on VDPV line list (see appendix 'VDPV Line list' for expected format).

CHART 5.1

ITD = mixture of different serotypes

SANGER VP1 = mixture of multiple serotypes, but only one PV2 sequence

****Assumption is that there are no more mOPV2 campaigns, so any Sabin-like needs to \be checked for nOPV2 origin***

****Assumption that ITD has been confirmed by repeat, if necessary***

Step 1 of analysis
See CHARTS 1-4B for appropriate course of action.

CHART 5.2

ITD = mixture of different serotypes

SANGER VP1 = homotypic mixture

Step 1 of analysis	***Step 2 of analysis***
Use WGS to tease apart mixtures.	See CHARTS 1-4B for appropriate course of action.