Global Polio Laboratory Network

Guidance Paper 5

Reporting Vaccine Derived Polioviruses (VDPVs)

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Purpose

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

Applicability

This guide will apply to any sequence report from an accredited poliovirus sequencing lab where a VDPV VP1 sequence has been found, 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.

Definitions

The following definitions should be used when reporting VDPVs by sequencing labs:

- Vaccine Derived Poliovirus (VDPV) – Vaccine virus strains that are >1% divergent ($\geqslant$10 nucleotide (nt) differences) for types 1 and 3 and >0.6% divergent ($\geqslant$ 6 nt differences) for type 2 from the corresponding reference Sabin strain in the VP1 gene region.
- Genetic linkage– Genetic relationship between or among poliovirus sequences that suggests a common origin or emergence
- Circulating VDPV (cVDPV) – VDPV VP1 sequences that 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[1]
- Immune-deficiency associated VDPV (iVDPV) – VDPVs from individuals that have evidence of primary immunodeficiency (PID)
- Ambiguous VDPV (aVDPV) – VDPV VP1 sequence that is not genetically linked to other previously identified VDPV sequences and there is no evidence of PID if the virus is from an individual. No poliovirus sequencing lab 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
- Orphan viruses – Viruses with less than or equal to 98.5% VP1 identity from the closest match from the sequence database at that time

VDPV report process

The process for 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, discordant PV1, discordant PV3 or any combination of these three ITD results.
2) The specimen is referred to an accredited GPLN sequence laboratory as a frozen tissue culture isolate or FTA card spotted with tissue culture isolate.
3) RNA is extracted, and Sanger sequence is performed following the WHO Sequence protocol.
4) Raw sequence data are edited and a consensus VP1 sequence is obtained.
5) The consensus VP1 sequence is serotyped and genotyped 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.
   b) If the genotype fits the criteria of a VDPV (as outlined in the Definitions section of this guidance paper), further analysis is needed:
      i) The newly sequenced VDPV is compared to previously identified VDPVs for evidence of genetic linkage
      ii) If needed, collaboration with CDC or another GSL (Global Specialized Laboratory) sequencing lab for questions and consultation concerning genetic linkage of the new VDPV

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[1] http://polioeradication.org/wp-content/uploads/2016/09/Reporting-and-Classification-of-VDPVs_Aug2016_EN.pdf

iii) Forward the sequence to the appropriate laboratories and WHO coordinators for comparison to other databases as needed.

6) Report the sequence as <u>VDPV</u> using the <u>standard report template</u> (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 below.

Genetic analysis

Genetic analysis of the new VDPV sequence (or query sequence) in comparison to other identified VDPV sequences helps to classify the new sequence. Known VDPV sequences for comparisons (or comparison sequences) can be in a local database (such as Bioedit or Geneious), GenBank, and/or PoNS (Poliovirus Nucleotide Sequence database, soon to be available).

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 20nt 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 example below).

	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 newly sequenced VPDVs may not give clear-cut results, especially for highly diverged orphan viruses. 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. Consider the example of a query sequence for ENVID ENV/MTA/SRR/TTT/17/008 that is PV2 with 7nt differences from Sabin2; comparison to other VDPV sequences finds several PV2 VDPVs from different geographic locations with 6nt differences from the query sequence. Closer examination of the relationship between the VDPV sequences (query and comparisons) show that the only shared nucleotide difference among all sequences is the change from T to C at nt428 in the VP1 region, a known "hotspot" for variation from the Sabin2 vaccine strain. The shared change has a higher likelihood of occurring by chance than by common ancestry. Accordingly, the sequence for ENV/MTA/SRR/TTT/17/008 would be reported as not genetically linked to any other VDPV2.

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 IS AROUND 4 SHARED NUCLEOTIDE SUBSTITUTIONS. THIS THRESHOLD IS HIGHER FOR TYPES 1 AND 3.

- OBSERVATION 2: WHEN THE QUERY VDPV VP1 SEQUENCE IS HIGHLY DIVERGENT FROM SABIN STRAIN (> 15 NUCLEOTIDE DIFFERENCES), IT IS NECESSARY TO CONSIDER BOTH THE ABSOLUTE NUMBER OF SHARED NUCLEOTIDE DIFFERENCES AND THE PROPORTION OF SHARED NUCLEOTIDE DIFFERENCES WITH RESPECT TO TOTAL SUBSTITUTIONS. IN SOME SITUATION, IT MIGHT BE HELPFUL TO CONSIDER THE PROPORTION OF SHARED NUCLEOTIDE DIFFERENCES AMONG TOTAL VARIABLE SITES.

- 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

GPLN sequencing laboratories with questions concerning genetic linkage should contact CDC or another GSL, as well as their regional lab coordinator for advice.

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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.

Report 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 or ENVID

5. Specimen Collection Date

6. Sequence lab ID

7. Original ITD result of the specimen

8. Date Received in the Sequence lab

9. Sequence Result Serotype

10. Sequence Result Genotype (VDPV or Sabin)

11. Nucleotide Differences (nt diff) from the reference Sabin strain

12. Comments – Include information here as to whether the VDPV is linked or not to other previously reported VDPVs

13. VDPV Classification, if available

14. Sequence report date

15. Onset date, if available

An example of a sequence report template with sample entries is shown in table below.

Formal report template

In addition to the completed sequence report template, a description of each reported VDPV 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 lab, WHO regional office and HQ.

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

Further descriptions and classification of each VDPV sequence will depend on the genetic analysis based on comparison of the sequence to a database of VDPV VP1 sequences. Standard reporting text will be used for each of the circumstances described in **Appendix (Scenarios)** page 9. An example of the standard report text that would accompany the sequence report template is given in figure below. The text should be kept as simple as possible and should adhere to the guidelines in this guidance paper (without extraneous information).

Genetic distinctions between cVDPV and iVDPV sequences

If a query sequence is not clearly linked to a known VDPV sequence, "c versus i VDPVs" analysis can be performed. Genetic variations between cVDPV and iVDPVs may reflect differences in viral micro-environments, host-virus interactions, and selective pressures during person-to-person transmission compared with chronic infections in immunodeficient patients. In some situations, it is possible to predict that a single sporadic VDPV is likely to be a circulating VDPV using genetic information. Genetic characteristics distinguishing between cVDPV and iVDPV include [1,2]:

- A higher proportion of mixed-base nucleotide sites in iVDPV sequences.

- Extensive antigenic divergence from the OPV strains in iVDPV sequences.

- Multiple viral lineages observed in iVDPV sequences.

- Simple genetic measurements of nucleotide and amino acid substitutions in VP1 (or capsid) region are sufficient for distinguishing highly divergent iVDPV2 from cVDPV2 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 cVDPV from iVDPV.

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.
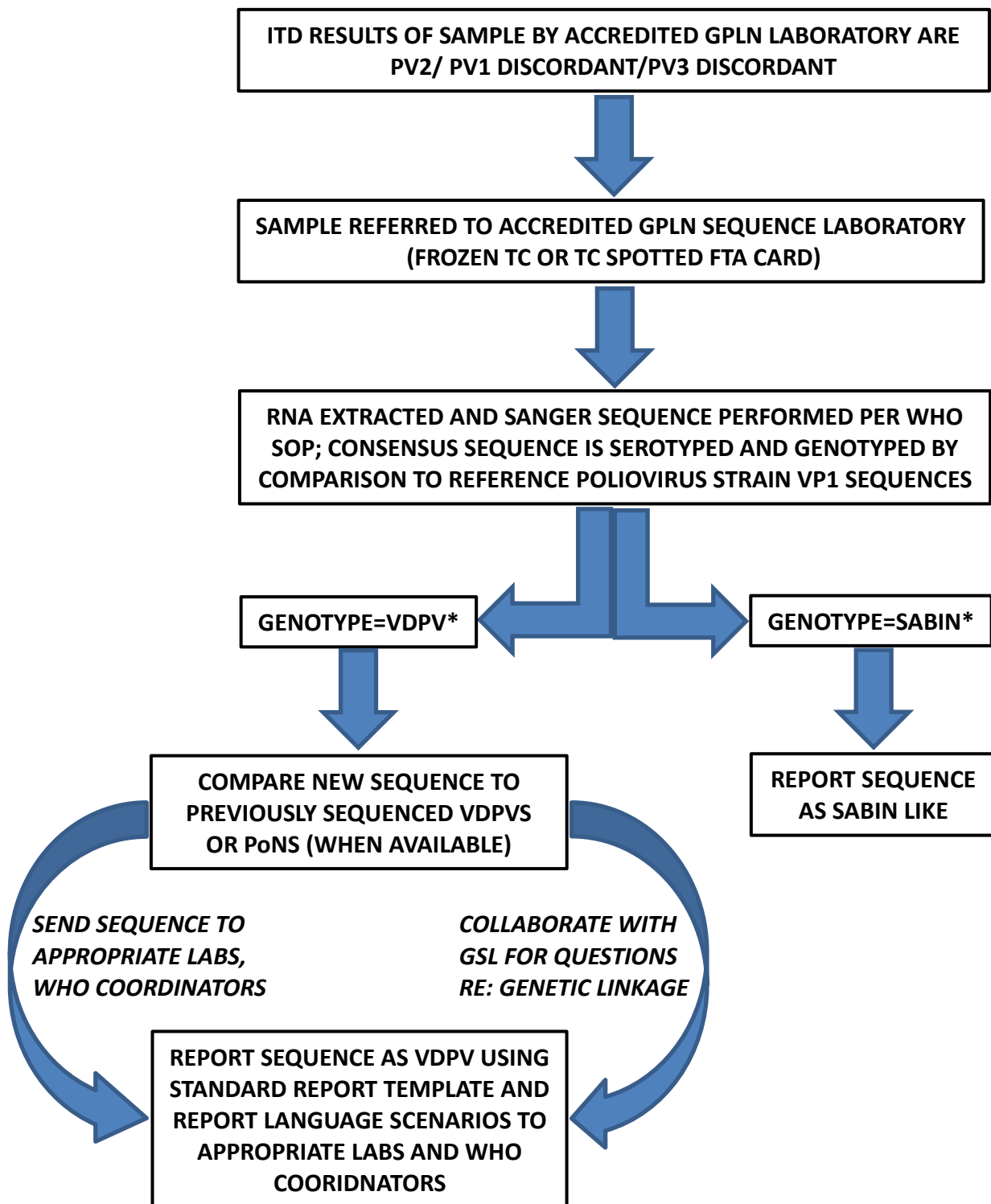

Please note that a high number of mixed bases could indicate cross-contamination of one sample with another, or it could be an indicator of possible immunodeficient VDPV. Sequencing laboratories should perform the virtual exercise of removing the Sabin bases from a mixed sequence to determine the underlying 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.

References

1. Burns, C.C., et al., *Vaccine-derived polioviruses.* J Infect Dis, 2014. **210 Suppl 1**: p. S283-93.
2. Zhao, K., et al., *Are Circulating Type 2 Vaccine-derived Polioviruses (VDPVs) Genetically Distinguishable from Immunodeficiency-associated VDPVs?* Comput Struct Biotechnol J, 2017. **15**: p. 456-462.

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FIGURE – Flow chart for VDPV report process

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┌─────────────────────────────────────────────────┐
│  ITD RESULTS OF SAMPLE BY ACCREDITED GPLN         │
│  LABORATORY ARE PV2/ PV1 DISCORDANT/PV3 DISCORDANT│
└─────────────────────────────────────────────────┘
                        │
                        ▼
┌─────────────────────────────────────────────────┐
│  SAMPLE REFERRED TO ACCREDITED GPLN SEQUENCE      │
│  LABORATORY (FROZEN TC OR TC SPOTTED FTA CARD)    │
└─────────────────────────────────────────────────┘
                        │
                        ▼
┌─────────────────────────────────────────────────┐
│  RNA EXTRACTED AND SANGER SEQUENCE PERFORMED      │
│  PER WHO SOP; CONSENSUS SEQUENCE IS SEROTYPED     │
│  AND GENOTYPED BY COMPARISON TO REFERENCE         │
│  POLIOVIRUS STRAIN VP1 SEQUENCES                  │
└─────────────────────────────────────────────────┘
            │                           │
            ▼                           ▼
  ┌──────────────────┐        ┌──────────────────┐
  │ GENOTYPE=VDPV*   │◄──────►│ GENOTYPE=SABIN*  │
  └──────────────────┘        └──────────────────┘
            │                           │
            ▼                           ▼
  ┌──────────────────┐        ┌──────────────────┐
  │ COMPARE NEW      │        │ REPORT SEQUENCE  │
  │ SEQUENCE TO      │        │ AS SABIN LIKE    │
  │ PREVIOUSLY       │        └──────────────────┘
  │ SEQUENCED VDPVS  │
  │ OR PoNS (WHEN    │
  │ AVAILABLE)       │
  └──────────────────┘
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SEND SEQUENCE TO APPROPRIATE LABS, WHO COORDINATORS

COLLABORATE WITH GSL FOR QUESTIONS RE: GENETIC LINKAGE

┌─────────────────────────────────────┐
│ REPORT SEQUENCE AS VDPV USING   │
│ STANDARD REPORT TEMPLATE AND   │
│ REPORT LANGUAGE SCENARIOS TO   │
│ APPROPRIATE LABS AND WHO   │
│ COORIDNATORS   │
└─────────────────────────────────────┘

*See Definitions section for genotype classification

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TABLE – Example Reporting Template for VDPV VP1 Sequences

SEQFILENAME	REFERRINGLABID	SPECTYPE	EPIDorENVID	SPECDATE	SEQLABID	ITDRESULT	DATERECVD	SEROTYPE	GENOTYPE	NTDIFFfromSABIN	COMMENTS	CLASSIFICATION	SEQREPORTDATE
MTA17-1050	MTA-17-6674	AFP- FTA	MTA-XYZ-ABC-17-001	12/20/2017	1050	PV2	1/15/2018	2	VDPV	22	MTA patient specimen, sequence from FTA card. Genetically linked to VP1 sequence from MTA-XYZ-CBA-17-004	cVDPV2	1/19/2018
MTA17-1051	MTA/17/0454-F1	ENV-TC	ENV/MTA/SRR/TTT/17/008	12/5/2017	1051	PV2	1/15/2018	2	VDPV	7	MTA environmental specimen, sequence from ENV-F1. Not genetically linked to any other VDPV2.	VDPV2	1/19/2018
MTA17-1052	MTA-17-667i-10	FTA	667i	12/7/2017	1052	PV1SL,PV3Dis	1/15/2018	3	VDPV	15	MTA patient specimen with known PID (no AFP). Serial sample number 10	iVDPV3	1/19/2018

Standard report text

Sequence results for specimens from referring lab MTA are attached. VDPV VP1 sequences for this report have the following nucleotide differences (nt diff) from Sabin:

- MTA-XYZ-ABC-17-001 – PV2, 22 nt diff, VDPV2
- ENV/MTA/SRR/TTT/17/008 – PV2, 7 nt diff, VDPV2
- MTA-17-667i-10 – PV3, 15 nt diff, VDPV3

The three VDPV VP1 sequences described in this report fall into the following categories:

- 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, 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.

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Appendix

- *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: <u>"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."</u>

- *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: <u>"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."</u>
    2. Environmental sample (VDPV)- The VDPV cannot be classified by the sequencing lab at this time. The standard report text will state: <u>"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.."</u>

- *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: <u>"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>"</u>

- *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: <u>"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."</u>

- *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/ambigous 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."

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- *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 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 , all 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#s> . 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."