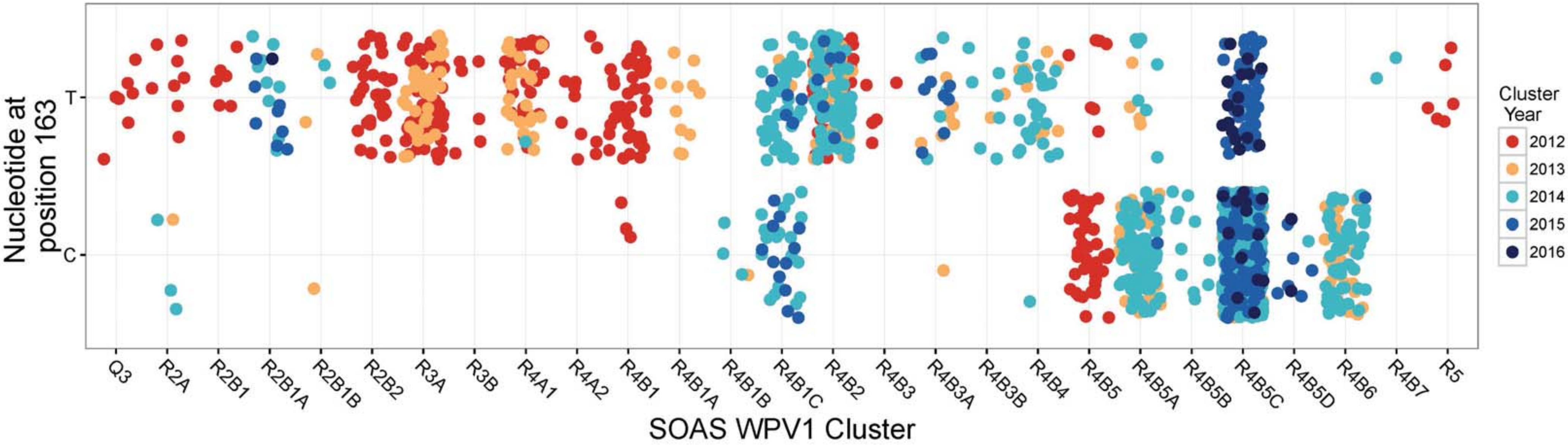

Figure S1. Genetic variation in nucleotide position 163 of the Viral Protein 1, VP1 region of South Asia wildtype poliovirus, WPV1 (no. of sequences = 1433), by year of emergence and by genetic cluster. Each colored dot indicates the year of collection (red = 2012, yellow = 2013, light blue = 2014, blue = 2015, navy = 2016).

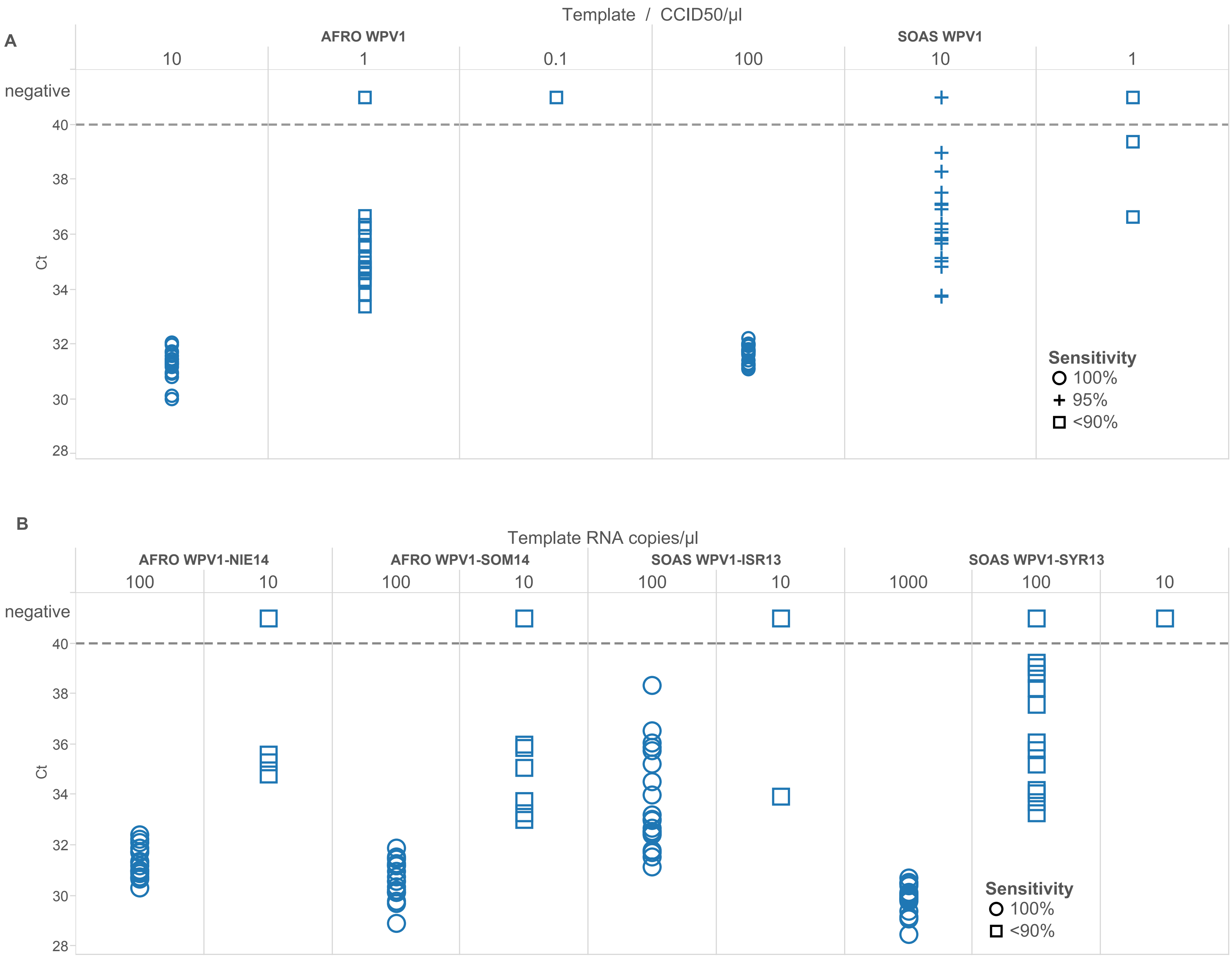

Figure S2 Sensitivity of the WPV1 assay. Dot plot diagram shows Ct values for different template concentrations, ordered by template, then by concentration (CCID50/µl) for virus isolates (A) and stepwise decrease in copy number of RNA transcripts (B). Negative samples (undetected by PCR) are shown above Ct 40 visually divided by a dotted line.

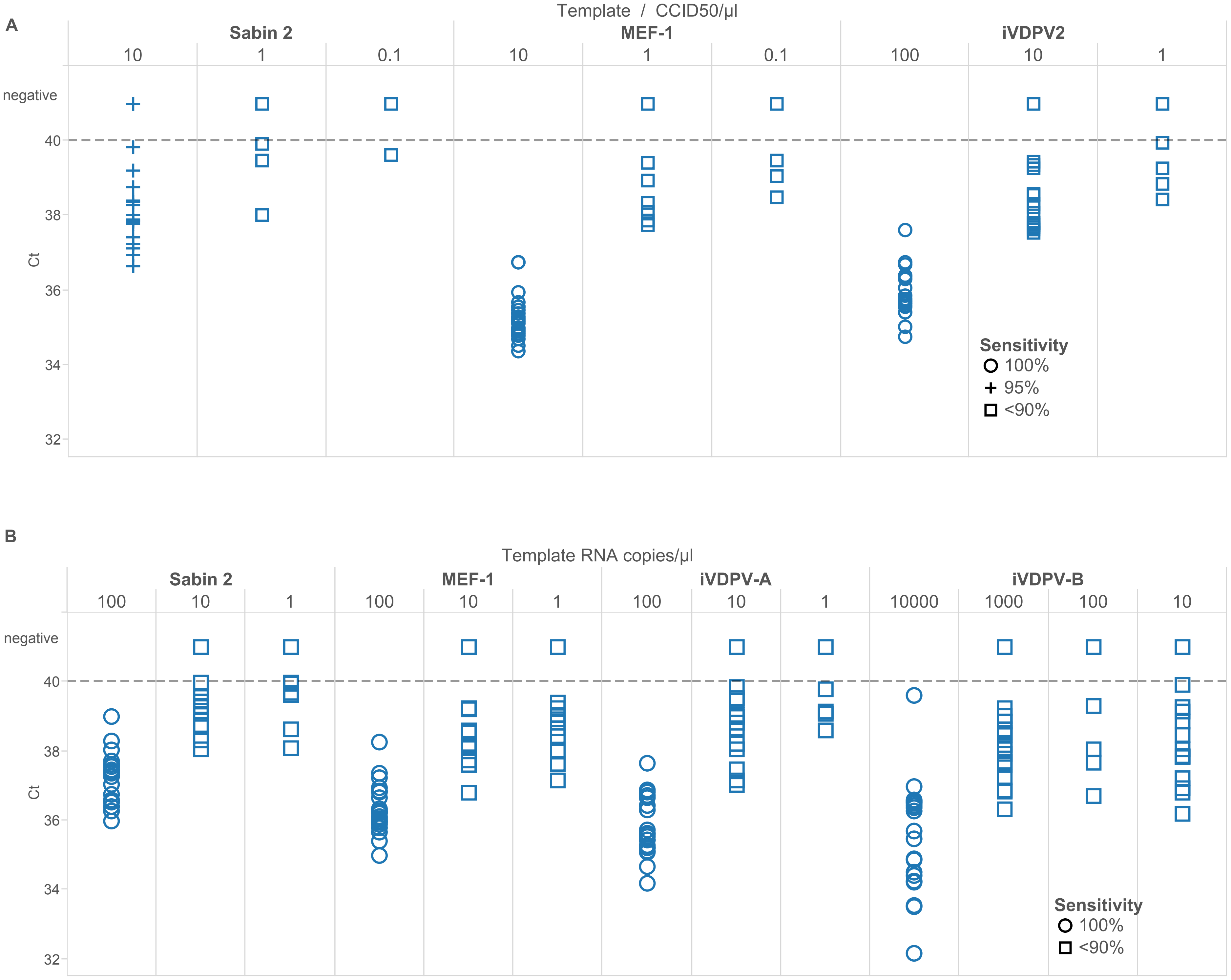

Figure S3. Sensitivity of the PV2 assay. Dot plot diagram shows Ct values for different template concentrations, ordered by template, then by concentration (CCID50/µl) for virus isolates (A) and stepwise decrease in copy number of RNA transcripts (B). Negative samples (undetected by PCR) are shown above Ct 40 visually divided by a dotted line.

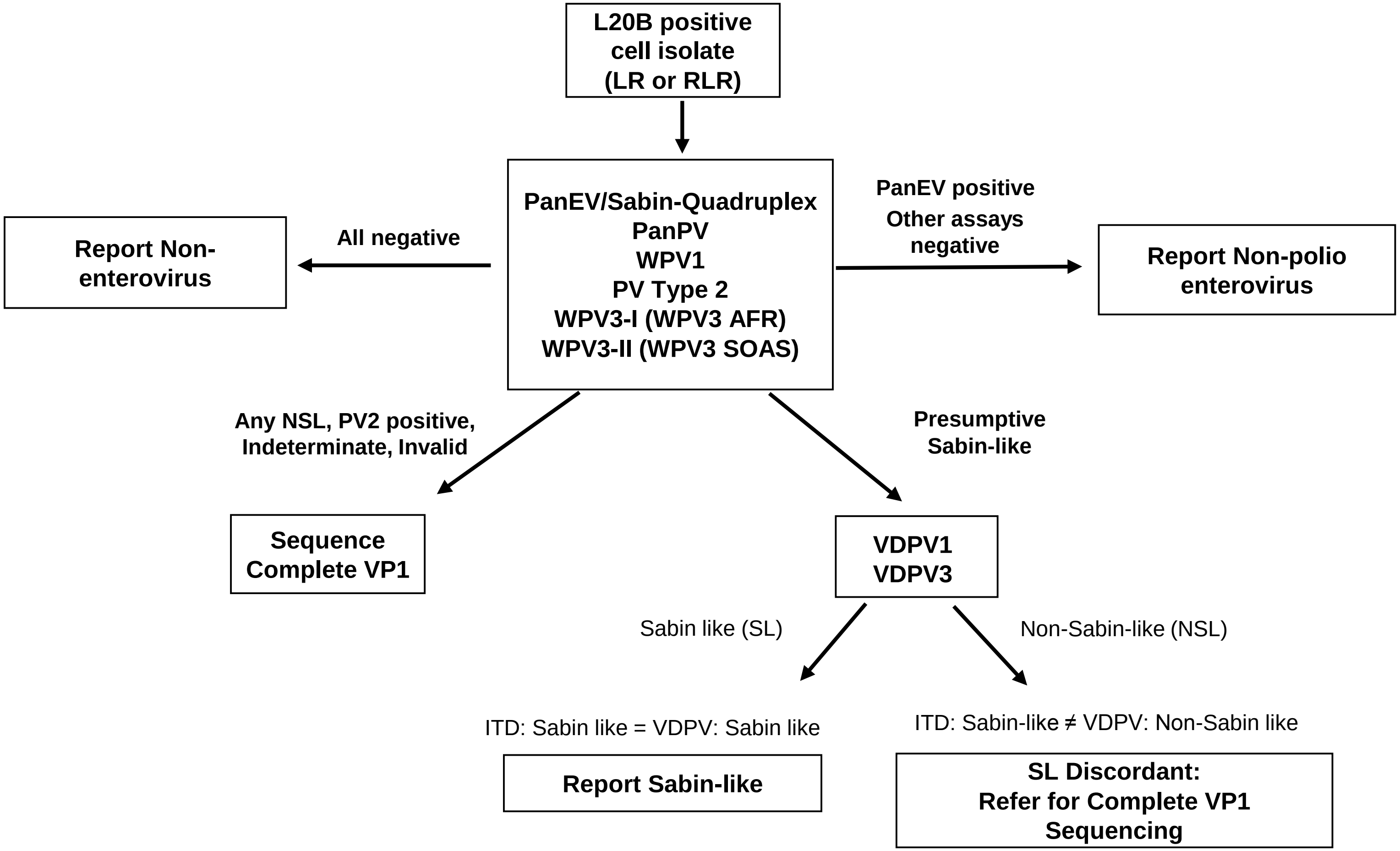

Figure S4 Flowchart of the ITD 5.0 algorithm for testing virus isolates with the rRT-PCR assay

Table S1. Statistical probability of positivity in each rRT-PCR assay by target, template and concentration of virus (titer is CCID$_{50}$µl^{-1}).

Target Assay	Template	1000	100 CCID$_{50}$µl^{-1}	10 CCID$_{50}$µl^{-1}	1 CCID$_{50}$µl^{-1}	0.1 CCID$_{50}$µl^{-1}	0.01 CCID$_{50}$µl^{-1}
Quadruplex EV	Sabin 1	NA	NA	p< .001** (100%, 83-100%)	p= .12 (70%, 46-88%)	p< .001†	NA
	Sabin 2	NA	NA	p< .001** (100%, 83-100%)	p= .12 (70%, 46-88%)	p< .001†	NA
	Sabin 3	NA	NA	NA	p< .001** (100%, 83-100%)	p> .82 (45%, 23-68%)	p< .001† (5%,
	AFRO WPV1	NA	p< .001** (100%, 83-100%)	p< .001** (95%, 83-100%)	p= .041 (25%, 9-49%)	NA	NA
	SOAS WPV1	NA	p< .001** (100%, 83-100%)	p= .012 (80%, 56-94%)	p= .003 (15%, 3-38%)	NA	NA
Quadruplex-Sabin 1	Sabin 1	NA	NA	NA	p< .001** (100%, 83-100%)	p= .5 (40%, 19%-64%)	p< .001† (10%, 0.1-
Quadruplex-Sabin 2	Sabin 2	NA	NA	NA	p< .001** (95%, 83-100%)	p< .001† (5%, 0.1%-	p< .001† (5%,
Quadruplex-Sabin 3	Sabin 3	NA	NA	NA	p< .001** (100%, 83-100%)	p= .044 (75%, 51-91%)	p= .003 (15%, 3-
PanPV	Sabin 1	NA	p< .001** (100%, 83-100%)	p< .001** (100%, 83-100%)	p= .5 (40%, 19-64%)	NA	NA
	Sabin 2	NA	NA	NA	p< .001** (100%, 83-100%)	p> .82 (45%, 23-68%)	p< .001
	Sabin 3	NA	NA	p< .001** (100%, 83-100%)	p< .001** (100%, 83-100%)	p=1 (50%, 27-	NA
	AFRO WPV1	NA	p< .001** (100%, 83-100%)	p= .12 (30%, 12-54%)	p< .001† (0-17%)	NA	NA
	SOAS WPV1	p< .001** (100%, 83-	p< .001** (100%, 83-100%)	p= .26 (35%, 15-60%)	NA	NA	NA
PV2	Sabin 2	NA	NA	p< .001** (95%, 83-100%)	p= .003 (15%, 3-38%)	p< .001† (5%, 0.1-	NA
	iVDPV2	NA	p< .001** (100%, 83-100%)	p= .12 (70%, 46-88%)	p= .012 (20%, 6-44%)	NA	NA
	MEF-1	NA	NA	p< .001** (100%, 83-100%)	p= .12 (30%, 12-54%)	p= .003 (15%, 3-38%)	NA
WPV1	AFRO WPV1	NA	NA	p< .001** (100%, 83-100%)	p= .003 (85%, 62-96%)	p< .001†	NA
	SOAS WPV1	NA	p< .001** (100%, 83-100%)	p< .001** (95%, 83-100%)	p< .001† (10%, 0.1-32%)	NA	NA
WPV3 AFR	AFRO WPV3	NA	NA	p< .001** (100%, 83-100%)	p< .001** (95%, 83-100%)	p> .82 (45%, 23-68%)	NA
WPV3 SOAS	SOAS WPV3	NA	p< .001** (100%, 83-100%)	p< .001** (100%, 83-100%)	p= .041 (25%, 9-49%)	NA	NA

*Probability of sample residing within the 95% Confidence Interval

**Significantly greater than 99% probability for positivity

†Significantly less than 99% probability for positivity

Table S2. Statistical probability of positivity for each rRT-PCR assay by target, template and concentration of RNA transcripts.

Target Assay	Template (copy number/µl)	1000 RNA copies 99% p value	100 RNA copies 99% p value	10 RNA copies 99% p value	1 RNA copy 99% p value	0.1 RNA copy 99% p value
Quadruplex EV	SOAS WPV3 + EV	NA	NA	p< .001** (100%, 83-100%)	p< .001** (75%, 51-91%)	p< .001† (5%, 0.1-25%)
Quadruplex-Sabin 1	Sabin 1	NA	NA	p< .001** (100%, 83-100%)	p=1 (50%, 27-73%)	p< .001† (10%, 0.1-32%)
Quadruplex-Sabin 2	Sabin 2	NA	NA	p< .001** (100%, 83-100%)	p= .012 (20%, 6-44%)	p< .001† (0-17%)
Quadruplex-Sabin 3	Sabin 3	NA	NA	p< .001** (100%, 83-100%)	p= .5 (40%, 19-64%)	p< .001† (5%, 0.1-25%)
PanPV	Sabin 1	p< .001** (100%, 83-100%)	p= .26 (35%, 15-60%)	p< .001† (0-17%)	NA	NA
	Sabin 3	NA	p< .001** (100%, 83-100%)	p> .5 (55%, 32-77%)	p< .001† (0-17%)	NA
	AFRO WPV1-SOM14	NA	p< .001** (100%, 83-100%)	p= .012 (20%, 6-44%)	p< .001† (0-17%)	NA
PV2	PV2-VDPV2-NIE	NA	p< .001** (100%, 83-100%)	p> .5 (55%, 32-77%)	p= .041 (25%, 9-49%)	NA
	PV2-MEF-1	NA	p< .001** (100%, 83-100%)	p= .26 (65%, 41-85%)	p=1 (5%, 27-73%)	NA
	iVDPV-A	NA	p< .001** (100%, 83-100%)	p>0.5 (60%, 36-81%)	p= .012 (20%, 6-44%)	NA
	iVDPV-B^	p= .012 (80%, 56-94%)	p= .012 (20%, 6-44%)	p= .26 (65%, 41-85%)	NA	NA
WPV1	AFRO WPV1-SOM14	NA	p< .001** (100%, 83-100%)	p= .12 (30%, 12-54%)	p< .001† (0-17%)	NA
	AFRO WPV1-NIE14	NA	p< .001** (100%, 83-100%)	p=0.003 (15%, 3-38%)	p< .001† (0-17%)	NA
	SOAS WPV1-SYR13	p< .001** (100%, 83-100%)	p= .26 (65%, 41-85%)	p< .001† (0-17%)	NA	NA
	SOAS WPV1-ISR13	NA	p< .001** (100%, 83-100%)	p< .001† (5%, 0.1-25%)	p< .001† (0-17%)	NA
WPV3 AFR	AFRO WPV3-NIE12	NA	NA	p< .001** (100%, 83-100%)	p= .12 (70%, 46-88%)	p= .012 (20%, 6-44%)
	AFRO WPV3-NIE12_2	NA	p< .001** (100%, 83-100%)	p= .12 (30%, 12-54%)	p< .001† (5%, 0.1-25%)	NA
WPV3 SOAS	SOAS WPV3-PAK1	NA	p< .001** (100%, 83-100%)	p= .26 (35%, 15-60%)	p< .001† (0-17%)	NA
	SOAS WPV3-PAK2	NA	p< .001** (100%, 83-100%)	p> .5 (55%, 32-77%)	p< .001† (5%, 0.1-25%)	NA

*Probability of sample residing within the 95% Confidence Interval (CI)

**Significantly greater than 99% probability

†Significantly less than 99% probability

^iVDPV (iVDPV-B: most diverse from Sabin 2 VP1 sequence) tested with 10,000 copies/µl with p< 0.001 (100%, 83-100%).

Table S3. Sequences of primers and components used for RNA transcript library.

Sequence name	**Name**	**Position in transcript (bp)**	**Sequence (5’ to 3’)**
T7 promotor		1-20	TAATACGACTCACTATAGGG
2A PVA	Primer	1353 -1378	ATGGARGCHAAYRAHTAYTAYCCRGC
5’ UTR	(Sabin 1-like)	1379-1541	TCCTCCGGCCCCTGAATGCGGCTAATCCCAACCACGGAGCAGGT
			AGTAATGTGATTGTTAGTGACCTGTCGTAACGCGCAAGTCCGTG
			GCGGAACCGACTACTTTGGGTGTCCGTGTTTCCTTTTATCTTTATT
			CTGGCTGCTTATGGTGACAATCGCAGATT
Mlu1 and *NotI*	Restriction sites	1542-1555	ACGCGTGCGGCCGC

Table S4 Primers and primer sequences used for whole-genome amplification of polioviruses serotypes 1, 2 and 3

Primer name	Concentration used	Target Gene	Orientation	Reference virus	Start	End	Sequence	Target virus
PPV/PCR-1A	80uM	VP1	A	Sabin 1 AY184219	2956	2933	AYRTACATIATYTGRTAIAC	Pan Polio
EV/PCR-2	80uM	5 UTR	S	Sabin 1 AY184219	446	470	TCCGGCCCCTGAATGCGGCTAATCC	Pan EV
EV/PCR-1	10uM	5 UTR	A	Sabin 1 AY184219	559	533	ACACGGACACCCAAAGTAGTCGGTTCC	Pan EV
PV2S	80uM	VP1	S	Sabin 1 AY184219	2856	2875	CITAITCIMGITTYGAYATG	Pan Polio
239S	80uM	2A	S	Sabin 1 AY184219	3389	3408	TTPGGICAPCARAAYAARGC	Pan Polio
153A	40uM	2B	A	Sabin 1 AY184219	3839	3821	TIAYICCITGYTCCATNGC	Pan Polio
4934S	40uM	2C	S	Sabin 1 AY184219	4926	4950	TGTGYAARAACTGYCAYCAACCAGC	Species C
4474S	10uM	CRE	S	Sabin 1 AY184219	4463	4485	TTCAAGAGCAAACACCGTATTGA	Species C
4474A	10uM	CRE	A	Sabin 1 AY184219	4485	4463	TCAATACGGTGTTTGCTCTTGAA	Species C
5107A	40uM	2C-3A	A	Sabin 1 AY184219	5112	5093	CCYTGRAAIAGIGCYTCCAT	Species C
7450A	40uM	UTR-polyA	A	Sabin 2 AY184220	7446	7425	TTTTTTCYCCGAATYAAAGAA	Species C
S2-1197A	10uM	VP2	A	Sabin 2 AY184220	1197	1179	TTTCCACCACCACCCTCTG	Sabin 2
188S	40uM	2A	S	Sabin 1 AY184219	3698	3717	TCICCIGGIGAdPTGdPGGNGG	Pan Polio
5761A	20uM	3C	A	Sabin 1 AY184219	5766	5747	GGGTACTTRCTAGTGTTCAC	Species C
5918S	20uM	3C	S	Sabin 1 AY184219	5912	5937	GGGATGCATGTYGGIGGGAACGGTTC	Species C
6007A	60uM	3C-3D	A	Sabin 1 AY184219	6005	5984	TCATCCAYTGRATYTCACCTTG	Species C
237A	3uM	3D	A	Sabin 1 AY184219	7065	7046	GAYTTRTCIGCIGGIGTCAT	Pan Polio
6573A	20uM	3D	A	Sabin 1 AY184219	6564	6545	TTICCAAAIGCCATYCTCAT	Species C
6643S	40uM	3D	S	Sabin 1 AY184219	6635	6653	TTYTGGAGYAARATACCAG	Species C
7024S	10uM	3D	S	Sabin 1 AY184219	7013	7035	CTAGCCCAATCAGGAAAAGACTA	Species C
0023S	10uM	5 UTR	S	Sabin 1 AY184219	23	39	CCCACCCCAGAGGCCCA	Species C
116A	40uM	VP1	A	Sabin 1 AY184219	2640	2621	GGRTTIGTIGCICCIGTYTC	Pan Polio
204A	80uM	VP2	A	Sabin 1 AY184219	1044	1025	ACISARTTIGCIGCYTCYTG	Pan Polio
206A	40uM	VP2	A	Sabin 1 AY184219	1638	1619	GCIATICCCCARTTRTTRTG	Pan Polio
207S	40uM	VP2	S	Sabin 1 AY184219	1541	1560	ATIAAYCTIMGIACIAAYAA	Pan Polio
210S	80uM	VP2	S	Sabin 1 AY184219	1169	1189	GARTCIMRIGGITGGTGGTGG	Pan Polio
208A	20uM	VP3	A	Sabin 1 AY184219	2154	2135	AGYTTICCIGTIGCCATCAT	Pan Polio
227S	40uM	VP3	S	Sabin 1 AY184219	2078	2097	AAITAYTAYACICAYTGGGC	Pan Polio
5446S	80uM	VPg	S	Sabin 1 AY184219	5432	5451	GTICARGGYCCIGGRTTTGA	Species C

Table S5 Reference viruses by sequence ID, strain, serotype and accession number in GenBank (Acc. no.)

Sequence_ID	Strain	Serotype	Acc. no.
ETH1319533	ETH1319533	Human poliovirus 1	KY941931
PAK1519534	PAK1519534	Human poliovirus 1	KY941934
NIE1219535	NIE1219535	Human poliovirus 3	KY941933
PAK1019536	PAK1019536	Human poliovirus 3	KY941935
IRQ1219537	IRQ1219537	Human poliovirus 2	KY941932