

Supplementary table 1. 50% tissue culture infectious dose (TCID50/mL) of provided echoviruses E1, E18, E30 G2, E30 G6 and E6, determined in the laboratory proper conditions (N.A.=not applicable, ‘-‘=missing data)

Participating laboratory	E1		E18		E30 G2		E30 G6		E6	
	Cell line	TCID50/ml	Cell line	TCID50/ml	Cell line	TCID50/ml	Cell line	TCID50/ml	Cell line	TCID50/ml
1	HT29	1*10 ^{8,94}	HT29	1*10 ^{6,1}	RD	1*10 ^{7,97}	RD	1*10 ^{7,42}	A549	1*10 ^{9,1}
2	RD	1,2*10 ⁷	RD	2,5*10 ⁷	RD	1,2*10 ⁹	RD	1,2*10 ⁸	RD	1,6*10 ¹⁰
3	RD	2*10 ⁷	RD	2*10 ^{7,33}	RD	2*10 ^{8,5}	RD	2*10 ^{8,5}	RD	2*10 ^{9,5}
4	RD	1*10 ^{7,97}	RD	1*10 ^{5,89}	RD	1*10 ^{6,8}	RD	1*10 ^{6,59}	RD	1*10 ^{7,53}
5	RD	1*10 ^{6,8}	RD	1*10 ^{7,3}	RD	1*10 ^{8,18}	RD	1*10 ^{6,87}	RD	1*10 ^{8,3}
6	RD	1*10 ^{7,1}	RD	1*10 ^{4,9}	RD	1*10 ^{5,5}	RD	1*10 ^{5,9}	RD	1*10 ^{6,3}
7	RD	2,4*10 ⁷	RD	2*10 ⁶	RD	1,5*10 ¹⁰	RD	3,2*10 ⁸	RD	1,5*10 ¹⁰
8	N.A.	N.A.	N.A.	N.A.	RD	1*10 ^{7,7}	RD	1*10 ^{6,7}	RD	1*10 ^{7,9}
9	Caco-2	1*10 ^{7,25}	Caco-2	1*10 ^{4,5}	Caco-2	1*10 ^{6,5}	Caco-2	1*10 ^{6,5}	Caco-2	1*10 ⁸
10	RD	1*10 ^{4,39}	RD	1*10 ^{5,54}	RD	1*10 ^{6,66}	RD	1*10 ^{6,03}	RD	1*10 ^{7,39}
11	RD	1*10 ^{4,54}	RD	1*10 ^{4,85}	RD	1*10 ^{6,85}	RD	1*10 ^{6,7}	RD	1*10 ^{8,8}
12	LLC-MK2	-	RD	-	RD	-	RD	-	RD	-

Supplementary table 2. Virus characteristics

Virus	Origin	Country of origin	Year of isolation	Passage	Genbank accession number	Cell line	TCID50/ml
E1	Amsterdam UMC, AMC	the Netherlands	2012	HWE006 p127	KC816737	HT29	7,97
E6	Amsterdam UMC, AMC	the Netherlands	2012	HWE006 p127	Being submitted	A549	7,63
E18	Amsterdam UMC, AMC	the Netherlands	2002	LXB002 p12	Being submitted	HT29	6,80
E30 G2 (genogroup II)	Amsterdam UMC, AMC	the Netherlands	2011	HWE006 p127	Being submitted	RD	6,80
E30 G6 (genogroup III)	RIVM	the Netherlands	2018	HWE006 p127	MK815087	RD	6,59

Supplementary table 3. Neutralising Ab titers and back titration results in sample (S) S1 against E1, E18, E30 G2, E30 G6 and E6 in proper conditions (N.A.=not applicable, ‘-‘=missing data, TCID50=50% tissue culture infectious dose)

Participating lab	nAb titer E1	nAb titer E18	nAb titer E30 G2	nAb titer E30 G6	nAb titer E6	Negative cut-off titer	Back titration performed?	TCID50 /well back titration E1	TCID50 /well back titration E18	TCID50 /well back titration E30 G2	TCID50 /well back titration E30 G6	TCID50 /well back titration E6
1	10	609	609	64	609	<8	Yes	158	257	131	63	56
2	11	<8	512	256	362	<8	Yes	19	631	158	151	190
3	N.A.	N.A.	64	96	128	-	Yes	N.A.	N.A.	200	100	150
4	>512	48	6	8	128	<4	Yes	-	-	-	-	-
5	>1024	>1024	>1024	362	724	<4	Yes	100	100	107	32	110
6	128	64	128	64	96	<8	No	N.A.	N.A.	N.A.	N.A.	N.A.
7	64	256	64	1024	1024	<8	No	N.A.	N.A.	N.A.	N.A.	N.A.
8	N.A.	N.A.	1024	256	256	<8	Yes	N.A.	N.A.	125	158	199
9	48	4	512	512	512	<4	Yes	-	-	-	-	-
10	32	24	1024	128	1024	<4	No	N.A.	N.A.	N.A.	N.A.	N.A.
11	64	256	512	256	256	<8	No	N.A.	N.A.	N.A.	N.A.	N.A.
12	4	64	128	128	64	<2	Yes	-	-	-	-	-

Supplementary table 4. 50% tissue culture infectious dose (TCID50) results of provided echoviruses E30 G2 and E30 G6 with proper and provided RD99 cell line passage (N.A.=not applicable)

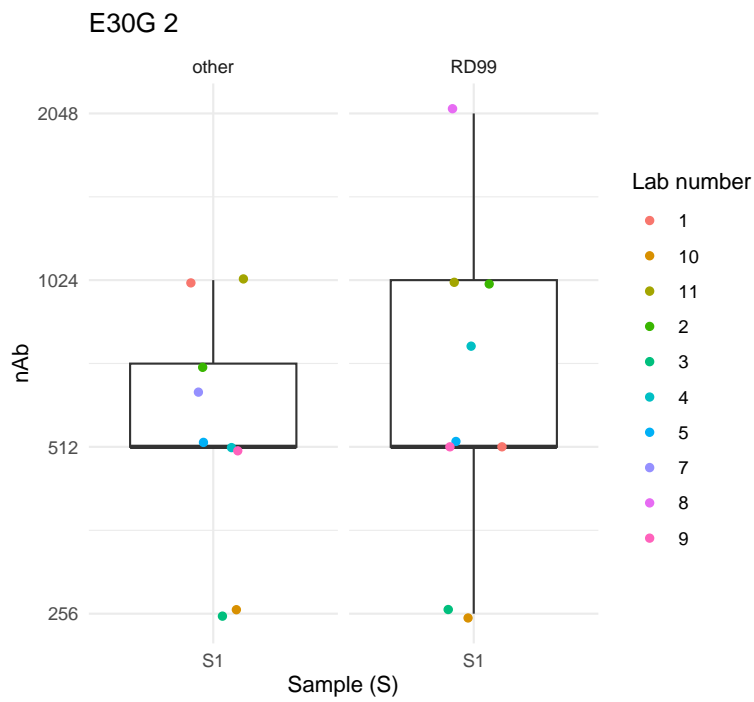
La b	E30 G2				E30 G6			
	Own cell line*	TCID50/ml	Cell line	TCID50/ml	Own cell line	TCID50/ml	Cell line	TCID50/ml
1	RD99p258	1*10 ^{6,87}	RD99	1*10 ^{6,1}	RD99p258	1*10 ^{4,57}	RD99	1*10 ^{4,57}
2	RD	1,3*10 ⁷	RD99	1,3*10 ⁷	RD	3,2*10 ⁶	RD99	1,6*10 ⁶
3	RD	2*10 ^{8,83}	RD99	2*10 ^{8,5}	RD	2*10 ^{8,67}	RD99	2*10 ^{8,5}
4	RD	1*10 ^{6,4}	RD99	1*10 ⁶	RD	1*10 ⁶	RD99	1*10 ^{6,25}
5	RD	1*10 ⁹	RD99	1*10 ^{9,3}	RD	1*10 ^{7,8}	RD99	1*10 ⁸
7	RD	3,2*10 ⁷	RD99	N.A.	RD	2,8*10 ⁷	RD99	N.A.
8	RD	N.A.	RD99	1*10 ^{7,9}	RD	N.A.	RD99	1*10 ^{7,1}
9	Caco-2	1*10 ^{6,16}	RD99	1*10 ^{4,75}	Caco-2	1*10 ^{5,16}	RD99	1*10 ^{5,16}
10	RD	1*10 ^{3,5}	RD99	1*10 ^{4,2}	RD	1*10 ^{3,1}	RD99	1*10 ^{3,9}
11	RD	1*10 ^{6,0}	RD99	1*10 ^{6,1}	RD	1*10 ^{5,5}	RD99	1*10 ^{6,9}

* Origin of cell lines: RD=Rhabdomyosarcoma, HT29=Human B cell lymphoma cell line, A549=Alveolar carcinoma epithelial cell line, Caco-2=Colorectal adenocarcinoma cell line, LLC-MK=Rhesus Monkey Kidney Epithelial Cell line

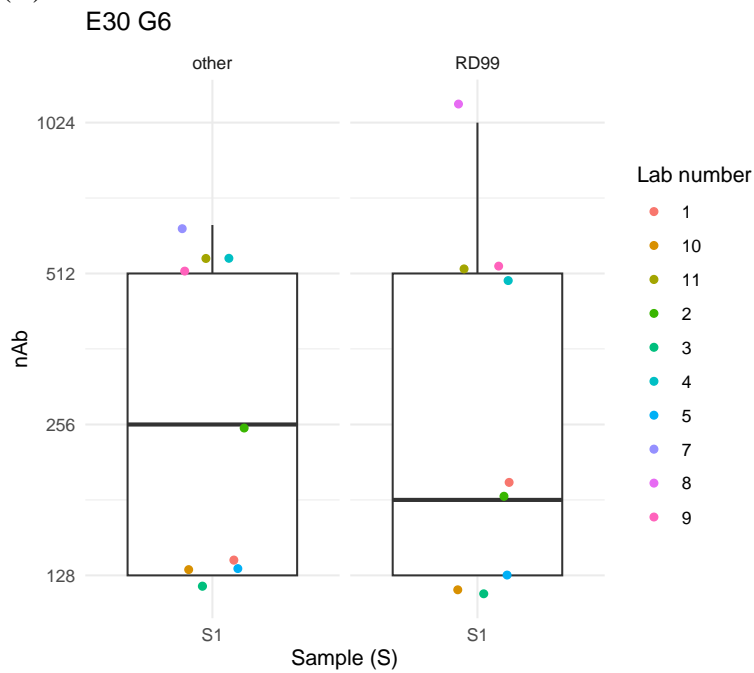
Supplementary table 5. Neutralising Ab titers and back titration results in sample (S) S1 against E30 G2 and E30 G6 with proper and provided RD99 cell line passage (N.A.=not applicable, TCID50=50% tissue culture infectious dose)

La b	Sample	E30 G2				E30 G6			
		Lab cell line		RD99		Lab cell line		RD99	
		nAb titer	Back titration TCID50/well	nAb titer	Back titration TCID50/well	nAb titer	Back titration TCID50/well	nAb titer	Back titration TCID50/well
1	S1	1024	46	512	131	128	215	181	316
	S2	128		128		45		32	
	S3	45		23		<8		11	
2	S1	724	45	1024	79	256	89	181	100
	S2	181		256		64		45	
	S3	45		45		16		22	
3	S1	256	47	256	100	128	7	128	316
	S2	64		64		64		64	
	S3	8		16		8		32	
4	S1	512	40	768	100	512	89	512	89
	S2	32		32		32		64	
	S3	32		16		32		22	
5	S1	512	1995	512	1995	128	3162	128	3162
	S2	128		128		64		64	
	S3	64		64		32		64	
7	S1	64	1150	N.A.	N.A.	64	210	N.A.	N.A.
	S2	64		N.A.		64		N.A.	
	S3	16		N.A.		16		N.A.	
8	S1	N.A.	N.A.	2048	20	N.A.	N.A.	1024	40
	S2	N.A.		262		N.A.		256	
	S3	N.A.		90		N.A.		45	
9	S1	>512	31	512	794	512	83	>512	20
	S2	512		128		64		256	
	S3	192		48		32		128	
10	S1	256	251	256	200	128	501	128	1000
	S2	64		64		32		16	
	S3	32		16		<8		<8	
11	S1	1024	10	1024	8	512	79	512	1
	S2	256		128		128		128	
	S3	128		64		64		32	

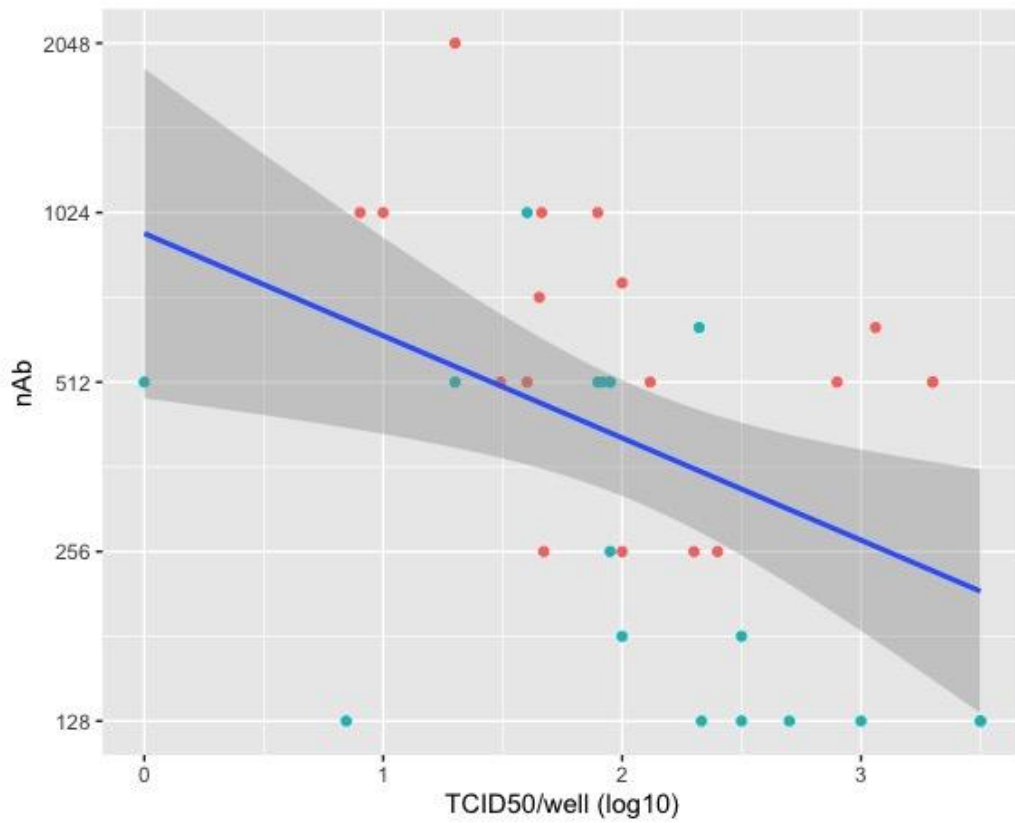
(A)



(B)

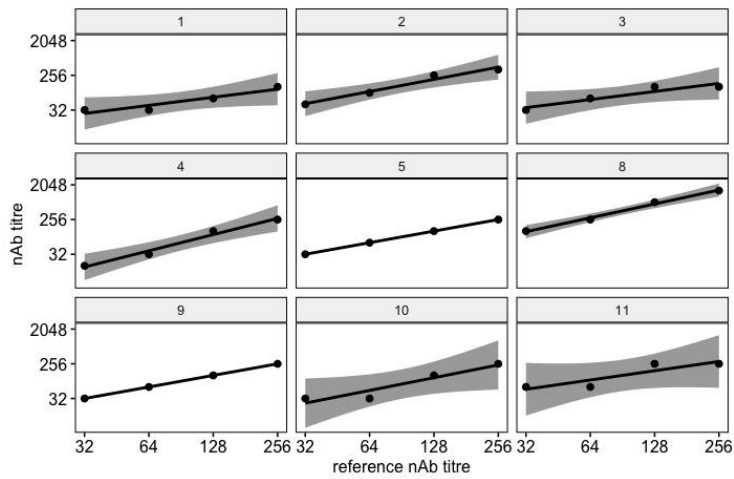


Supplementary figure 1 Comparison of nAb titers in sample (S) S1 between RD99 and laboratories' own cell lines against (A) E30 G2 and (B) E30 G6

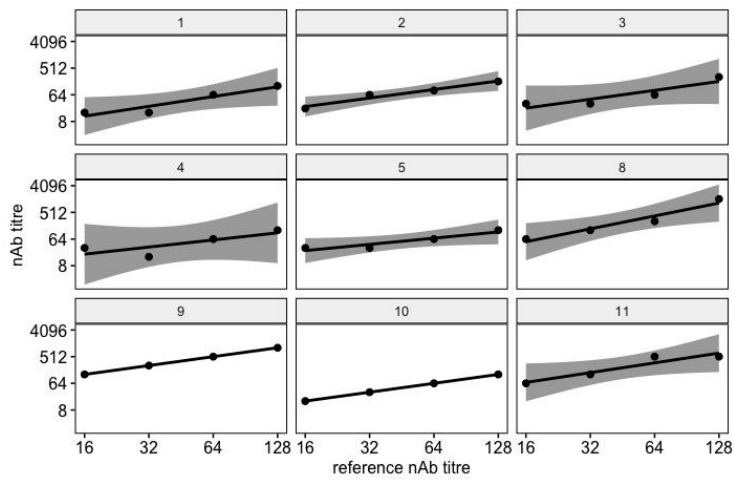


Supplementary figure 2. Investigation of the correlation between back titration results (50% tissue culture infectious dose (TCID50)/well) and nAb titers against E30 G2 (red) and E30 G6 (green)

(A)



(B)



Supplementary figure 3. Linear regression of reference serum dilution series against (A) E30 G2 and (B) E30 G6 in the RD99 cell line