

THE LANCET Microbe

Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Sharma AK, Verma H, Estivariz CF, et al. Persistence of immunity following a single dose of inactivated poliovirus vaccine: a phase 4, open label, non-randomised clinical trial. *Lancet Microbe* 2023; published online Sept 26. [https://doi.org/10.1016/S2666-5247\(23\)00215-X](https://doi.org/10.1016/S2666-5247(23)00215-X).

**Cross-sectional study to assess persistence of immunity conferred by a single
IPV dose administered in the EPI routine immunization schedule, Nepal 2019**

Repeated Analysis – 14 March 2022

Figure 1. Study profile

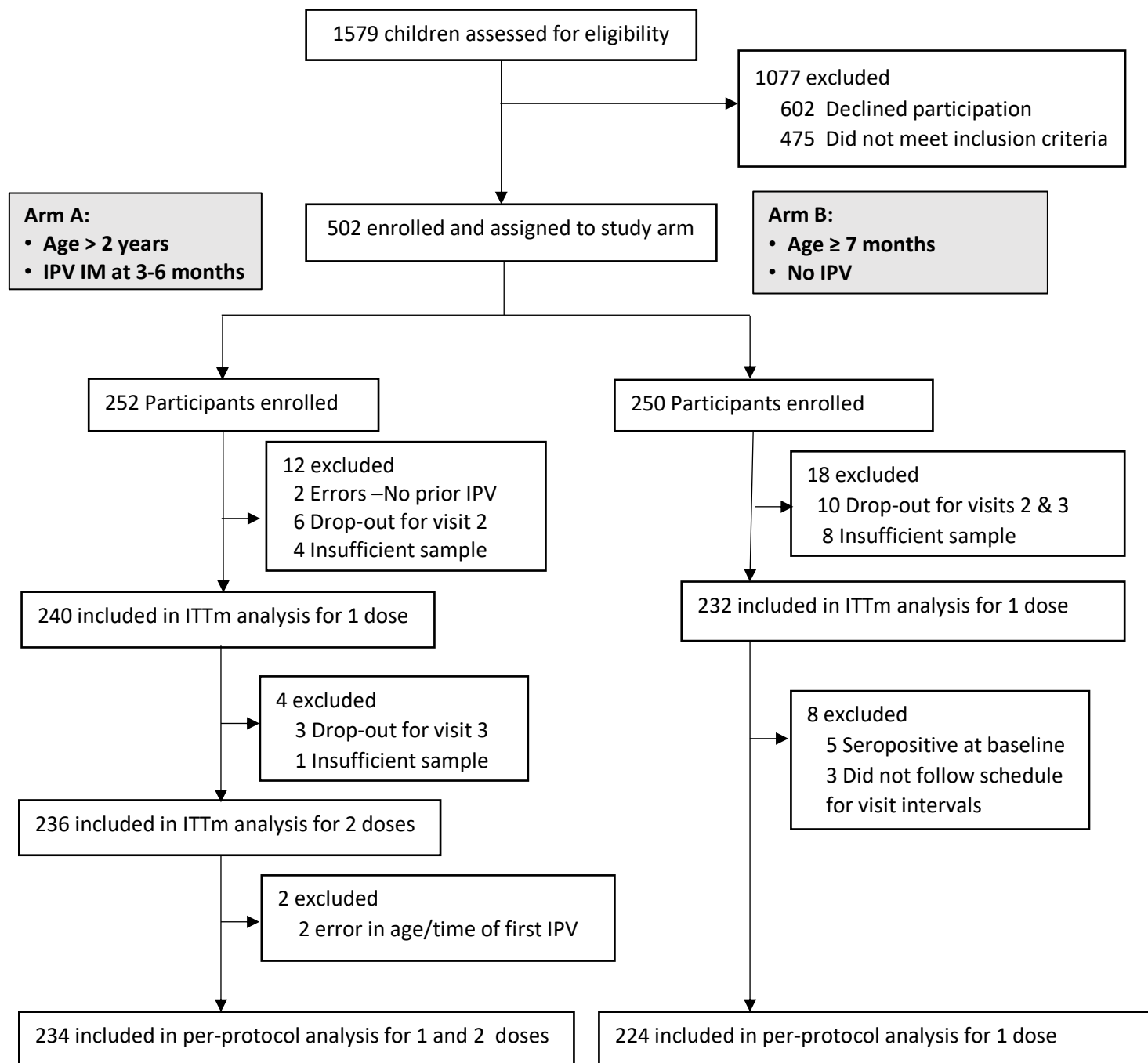


Table 1. Baseline characteristics of study participants

	Modified Intent-to-Treat population						Per-protocol population							
	A. Old IPV dose (N=240)			B. Recent IPV dose (N=232)			p value*	A. Old IPV dose (N=234)			B. Recent IPV dose (N=224)			p value*
	Median (IQR)			Median (IQR)				Median (IQR)			Median (IQR)			
Age at enrolment (months)	32	(29-34)		9	(9-10)		<.0001	32	(30-34)		9	(9-10)		<.0001
Age at IPV receipt (weeks)	16	(15-17)		NA	NA			16	(15-17)		NA	NA		
Time since IPV receipt (months)	28	(26-30)		NA	NA			28	(26-30)		NA	NA		
	n	N	%	n	N	%	p value*	n	N	%	n	N	%	p value*
Gender male	140	240	58	121	232	52	0.195	136	234	58	119	224	53	0.301
female	100	240	42	111	232	48		98	234	42	105	224	47	
Received 3 OPV doses	240	240	100	229	231	99	0.240	234	234	100	221	223	99	0.238
Received Penta3	240	240	100	229	231	99	0.240	234	234	100	221	224	99	0.238
Received MR1	234	238	98	124	229	54	<.0001	228	232	98	100	222	45	<.0001
Received PCV3	220	222	99	135	230	59	<.0001	215	217	99	92	223	41	<.0001
Wasting	2	240	1	1	232	0	1.000	2	234	1	1	224	0	1.000
Stunting	35	240	15	9	232	4	<.0001	34	234	15	8	224	4	<.0001
Mother's education														
No formal education	23	240	10	6	232	3	0.003	21	234	9	6	224	3	0.008
Primary	41	240	17	30	232	13		41	234	18	30	224	13	
Middle	78	240	33	72	232	31		75	234	32	69	224	31	
High School	40	240	17	63	232	27		39	234	17	61	224	27	
University graduate	58	240	24	61	232	26		58	234	25	58	224	26	

* Fisher's p-value for categorical variables; Cochran-Armitage trend test for mother's education; Wilcoxon non-parametric for continuous variables

Suppl, Table 1. Differences between participants included in analysis and drop-outs.

A. Old IPV dose (N=240)							A. Old IPV dose (N=232)							
	Complete			Dropouts				Complete			Dropouts			
	Median (IQR)			Median (IQR)			p value	Median (IQR)			Median (IQR)			p value
Age in months	32	(29-34)		29	(25.5-30.5)		0.004	9	(9-10)		9	(9-10)		0.178
Age at IPV receipt (weeks)	16	(15-17)		15.5	(15-18)		0.895							
Time since IPV receipt (months)	28	(26-30)		25	(22-25)		0.001							
	n	N	%	n	N	%		n	N	%	n	N	%	p value
Gender male	140	240	58	6	12	50	0.567	121	232	52	11	18	61	0.625
female	100	240	42	6	12	50		11	232	5	7	18	39	
Received 3 OPV doses	240	240	100	11	11	100	1.000	229	231	99	17	17	100	1.000
Wasting	2	240	1	1	12	8	0.137	1	232	0	0	18	0	1.000
Stunting	35	240	15	3	12	25	0.399	9	232	4	2	18	11	0.183
Mother's education														
No formal education	23	240	10	0	12	0	0.486	6	232	3	1	18	6	0.190
Primary	41	240	17	3	12	25		30	232	13	3	18	17	
Middle	78	240	33	2	12	17		72	232	31	2	18	11	
High School	40	240	17	2	12	17		63	232	27	8	18	44	
University graduate	58	240	24	5	12	42		61	232	26	4	18	22	
Seroprevalence at baseline														
P1 seropositive	239	240	100	10	10	100	1.000	230	232	99	13	13	100	1.000
P2 seropositive	103	240	43	6	10	60	0.339	5	232	2	0	18	0	1.000
P3 seropositive	235	240	98	9	10	90	0.219	226	232	97	13	13	100	1.000

Table 2. Seroprevalence for poliovirus types 1, 2 and 3 by study visit.

Using ITT modified population												Using per-protocol population										
	A. Old IPV dose (N=240)					B. Recent IPV dose (N=232)						A. Old IPV dose (N=234)					B. Recent IPV dose (N=224)					
	n	N	%	LCI	UCI	n	N	%	LCI	UCI	p value*	n	N	%	LCI	UCI	n	N	%	LCI	UCI	p value*
Day 0 visit																						
Type 1	239	240	100%	98%	100%	230	232	99%	97%	100%	0.618 ^a	233	234	100%	98%	100%	222	224	99%	97%	100%	0.616
Type 2	103	240	43%	37%	49%	5	232	2%	1%	5%	<0.0001 ^a	100	234	43%	37%	49%	0	224	0%	98%	100%	<0.0001
Type 3	235	240	98%	95%	99%	226	232	97%	94%	99%	0.768 ^a	229	234	98%	95%	99%	218	224	97%	94%	99%	0.764
Day 7 visit																						
Type 1	240	240	100%	98%	100%	--	--	--	--	--	--	234	234	100%	98%	100%	--	--	--	--	--	--
Type 2	239	240	100%	98%	100%	--	--	--	--	--	--	233	234	100%	98%	100%	--	--	--	--	--	--
Type 3	240	240	100%	98%	100%	--	--	--	--	--	--	234	234	100%	98%	100%	--	--	--	--	--	--
Day 30 visit																						
Type 1	236	236	1.000	98%	100%	232	232	100%	98%	100%	1.000 ^b	234	234	100%	98%	100%	224	224	100%	98%	100%	--
Type 2	235	236	0.996	98%	100%	231	232	100%	98%	100%	<0.0001 ^b	233	234	100%	98%	100%	223	224	100%	98%	100%	--
Type 3	236	236	1.000	98%	100%	231	232	100%	98%	100%	0.216 ^b	234	234	100%	98%	100%	223	224	100%	98%	100%	--
Day 37 visit																						
Type 1	--	--	--	--	--	232	232	100%	98%	100%	--	--	--	--	--	--	224	224	100%	98%	100%	NA
Type 2	--	--	--	--	--	231	232	100%	98%	100%	--	--	--	--	--	--	223	224	100%	98%	100%	1.000
Type 3	--	--	--	--	--	232	232	100%	98%	100%	--	--	--	--	--	--	224	224	100%	98%	100%	NA

* Used Wilcoxon 95% confidence intervals around the proportion. a – Fisher's t test for comparison between Arm A and B at Day 0 visit. B. Fisher's t test for comparison between Arm A at Day 0 (1 IPV dose > 2 years before) and Arm B at Day 30 (1 IPV dose 30 days before).

Table 3. Reciprocal antibody titers for poliovirus types 1, 2 and 3, by study visit (note: children had received 3 bOPV doses and 1 or 0 IPV doses through routine immunization services).

	Using ITT modified population								Using per-protocol population							
	A. Old IPV dose (N=240)				B. Recent IPV dose (N=232)				A. Old IPV dose (N=234)				B. Recent IPV dose (N=224)			
	N	Median	IQR		N	Median	IQR		N	Median	IQR		N	Median	IQR	
Day 0 visit																
Type 1	240	910.2	324.0	1448.2	232	1448.2	1152.1	1448.2	234	910.2	362.0	1448.2	224	1448.2	1152.1	1448.2
Type 2	240	7.1	5.7	14.2	232	5.7	5.7	5.7	234	7.1	5.7	14.2	224	5.7	5.7	5.7
Type 3	240	181.0	72.0	455.1	232	910.2	362.0	1448.2	234	181.0	72.0	455.1	224	910.2	362.0	1448.2
Day 7																
Type 1	240	1448.2	1448.2	1448.2					234	1448.2	1448.2	1448.2	224			
Type 2	240	1448.2	1448.2	1448.2					234	1448.2	1448.2	1448.2	224			
Type 3	240	1448.2	1448.2	1448.2					234	1448.2	1448.2	1448.2	224			
Day 30																
Type 1	236	1448.2	1448.2	1448.2	232	1448.2	1448.2	1448.2	234	1448.2	1448.2	1448.2	224	1448.2	1448.2	1448.2
Type 2	236	1448.2	1448.2	1448.2	232	576.0	181.0	1296.1	234	1448.2	1448.2	1448.2	224	576.0	181.0	1296.1
Type 3	236	1448.2	1448.2	1448.2	232	1448.2	1448.2	1448.2	234	1448.2	1448.2	1448.2	224	1448.2	1448.2	1448.2
Day 37																
Type 1					232	1448.2	1448.2	1448.2					224	1448.2	1448.2	1448.2
Type 2					232	724.1	288.0	1448.2					224	724.1	288.0	1448.2
Type 3					232	1448.2	1448.2	1448.2					224	1448.2	1448.2	1448.2

p-values for titer distribution using Wilcoxon non-parametric test: <.0001 for all 3 serotypes comparing A versus B on Day 0 (3bOPV+1 IPV dose > 2 years before versus 3bOPV+ 0 IPV doses 6 months before); <0.001 for all 3 serotypes comparing Arm A on Day 0 (1 IPV dose >2 years before) versus Arm B on day 30 (1 IPV 30 days before). P values for differences in titer distribution using signed rank non-parametric test among participants with titers low enough to detect a change: <0.001 within Arm A for titers on Day 30 (2 IPV doses) versus Day 0 (1 IPV dose) for all 3 serotypes; < 0.001 within Arm A for titers on Day 30 (1 IPV dose) versus Day 0 (0 IPV dose) for all 3 serotypes.

Table 4. Immune response for type 2 poliovirus.

For modified ITT population

	A. Old IPV dose (N=240)					B. Recent IPV dose (N=232)					
	n	N	%	LCI	UCI	n	N	%	LCI	UCI	p value*
Following 1st IPV dose											
Time since receipt of 1st IPV dose, median (IQR), months		240	28	26-30				1	(1 -1)		<.0001 ^a
Seronegative not primed	1	240	0%	0%	0%	0	232	0%			
Seronegative primed	136	240	57%	50%	63%	1	232	0%	0%	2%	<.0001 ^a
Seropositive	103	240	43%	37%	49%	231	232	100%	98%	100%	<.0001 ^a
Cumulative response (primed or seropositive)	239	240	100%	98%	100%	232	232	100%	98%	100%	1.000 ^a
Reciprocal antibody titers, median (IQR) among all participants	240		7.1	(5.7-14.2)			232	576.0	181.0	1296.1	<.0001 ^b
Reciprocal antibody titers, median (IQR) among seropositive	103		18.0	(11.3-45.3)			231	576.0	181.0	1448.2	<.0001 ^b
Following 2nd IPV dose											
Time since receipt of 2nd IPV dose median (IQR), days		236	30	29-30							
Seroconversion among seronegative (including primed)	134	135	99%	96%	100%						
Boosting in titers among seropositive with boostable titers (below 362)	96	96	100%	96%	100%						
Total seropositive	235	236	100%	98%	100%						
Reciprocal antibody titers, median (IQR) among all participants	236		1448.2	(1448.2-1448.2)							<.0001 ^c
Antibody titers, median (IQR) among seropositive	235		1448.2	(1448.2-1448.2)							<.0001 ^c

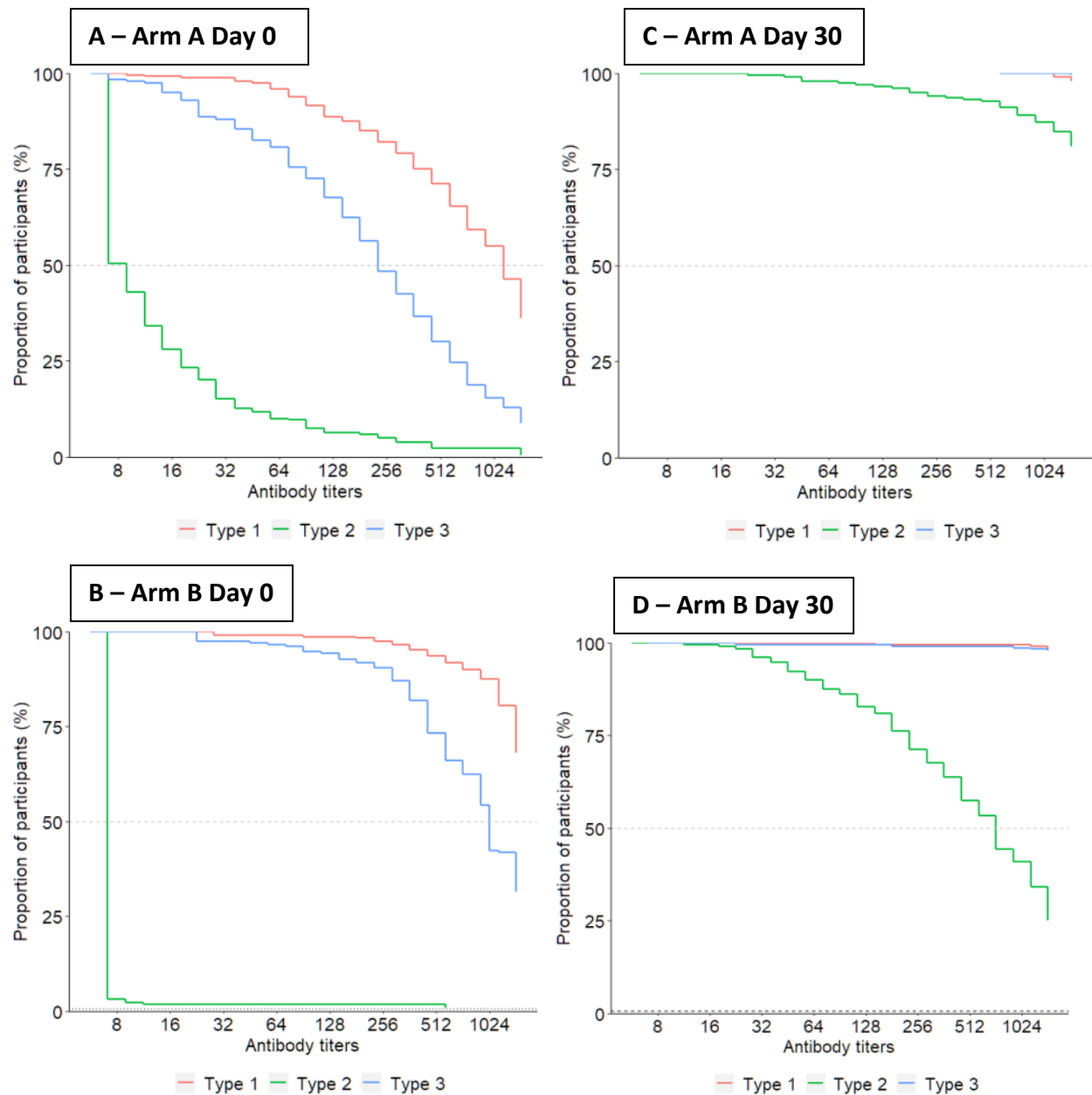
^a Fisher's t test for comparison of Arm A on Day 0 (1 IPV dose > 2 years) versus B on Day 30 ago (1 IPV dose 30 days ago). ^b Wilcoxon non-parametric test for comparison of titers between Arm A on Day 0 (1 IPV dose > 2 years) versus B on Day 30 ago (1 IPV dose 30 days ago). ^c Signed rank test for comparison of titers after first and second IPV dose in Arm A.

For per-protocol population

	A. Old IPV dose (N=234)					B. Recent IPV dose (N=224)					
	n	N	%	LCI	UCI	n	N	%	LCI	UCI	p value*
Following 1st IPV dose											
Time since receipt of 1st IPV dose, median (IQR), months		234	28	(26-30)			224	1	(1 -1)		<.0001 ^a
Seronegative not primed	1	234	0%	0%	0%	0	224	0%			
Seronegative primed	133	234	57%	50%	63%	1	224	0%	0%	2%	<.0001 ^a
Seropositive	100	234	43%	37%	49%	223	224	100%	98%	100%	<.0001 ^a
Cumulative response (primed or seropositive)	233	234	100%	98%	100%	224	224	100%	98%	100%	1.000 ^a
Reciprocal antibody titers, median (IQR) among all participants	240		7	5.7	14.2		232	576.0	181.0	1296.1	<.0001 ^{b,c}
Reciprocal antibody titers, median (IQR) among seropositive	103		18	11.3	45.3		231	576.0	181.0	1448.2	<.0001 ^{b,c}
Following 2nd IPV dose (30 days after 2nd dose)											
Time since receipt of 2nd IPV dose median (IQR), days		236	30	(30-30)							
Seroconversion among seronegative (including primed)	133	134	99%	96%	100%						
Boosting in titers among seropositive with boostable titers (below 362)	95	95	100%	96%	100%						
Total seropositive	233	234	100%	98%	100%						
Reciprocal antibody titers, median (IQR) among all participants	234		1448.2	(1448.2-1448.2)							<.0001 ^d
Antibody titers, median (IQR) among seropositive	229		1448.2	(1448.2-1448.2)							<.0001 ^d

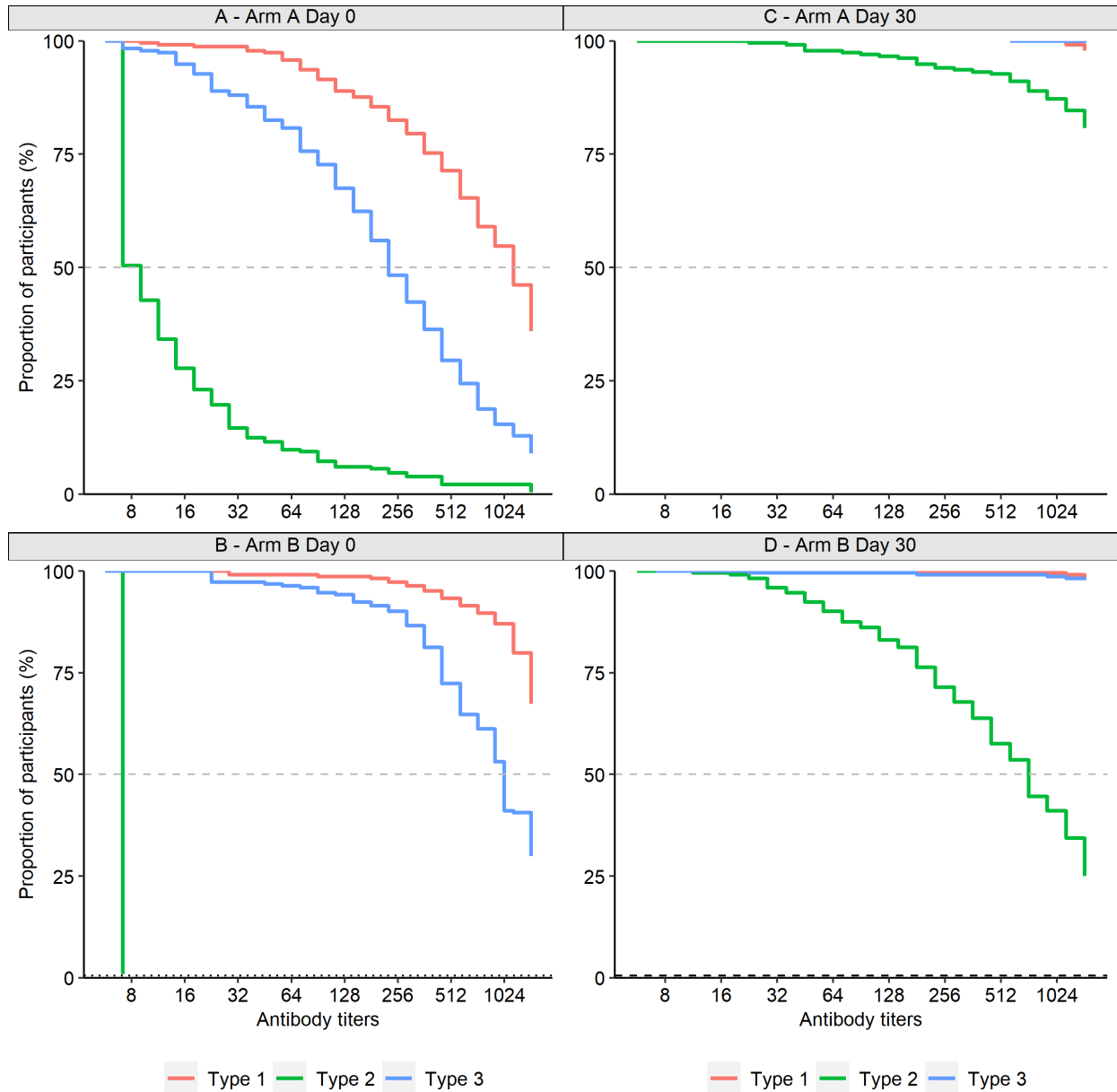
^a Fisher's t test for comparison of Arm A on Day 0 (1 IPV dose > 2 years) versus B on Day 30 ago (1 IPV dose 30 days ago). ^b Wilcoxon non-parametric test for comparison of titers between Arm A on Day 0 (1 IPV dose > 2 years) versus B on Day 30 ago (1 IPV dose 30 days ago). ^c Signed rank test for comparison of titers between Day 0 and Day 30 within Arm B; ^d Signed rank test for comparison of titers after first and second IPV dose within Arm A.

Figure 2. Reverse cumulative antibody titers for poliovirus types 1, 2 and 3 at different study visits.



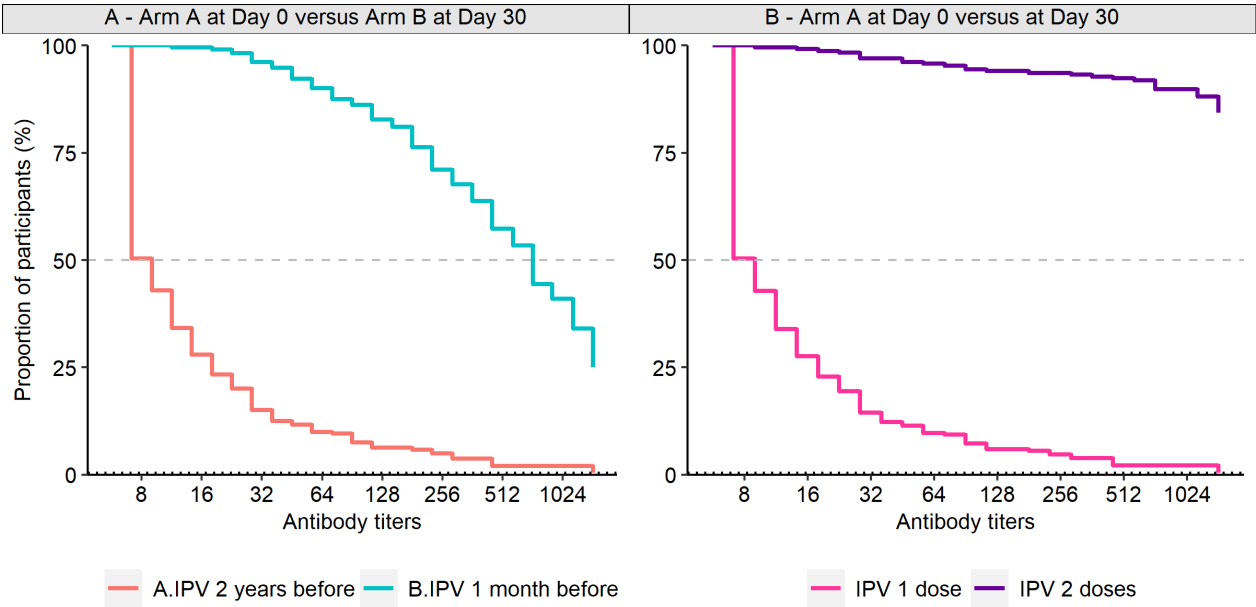
A. Day 0 visit in Arm A - ~2 years after receiving 1IPV and the last of 3 bOPV doses; B. Day 0 visit in Arm B - ~ 6 months after receiving the last of 3 bOPV doses and 0 IPV doses. C. Day 30 visit in Arm A - after receiving a booster dose of IPV; D- Day 30 visit in Arm B - after receiving the first dose of IPV

Fig 2 (Suppl – in PP population) Reverse cumulative antibody titers for poliovirus types 1, 2 and 3 at different study visits in per protocol population.



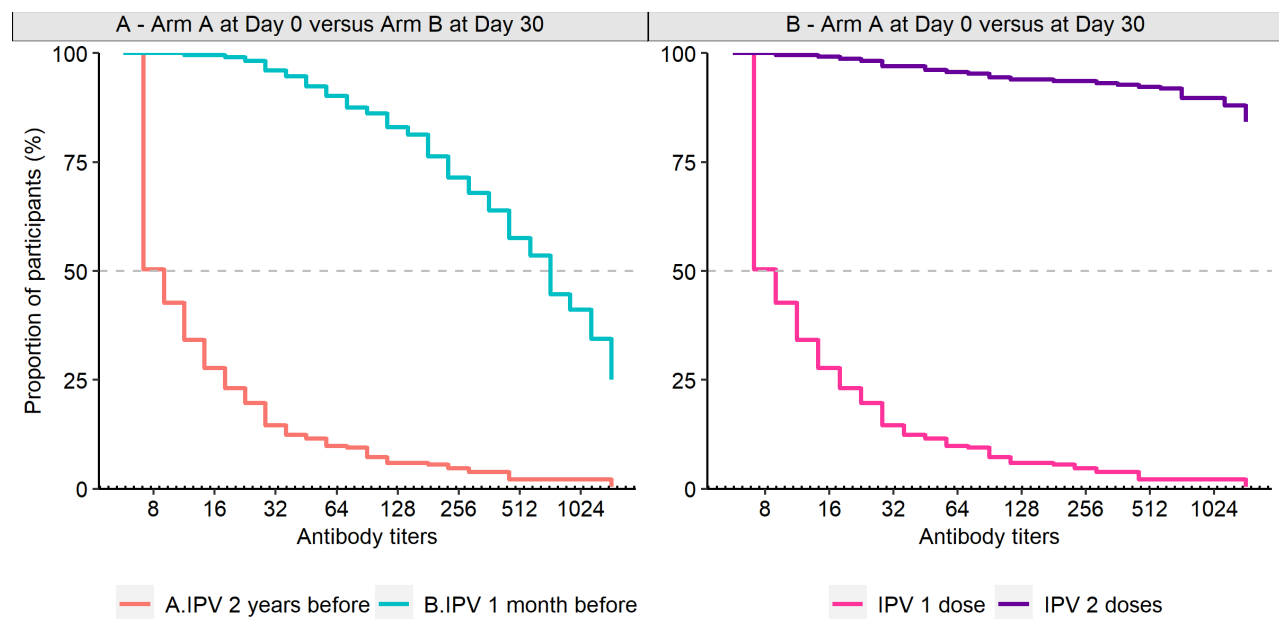
A. Day 0 visit in Arm A - ~2 years after receiving 1IPV and the last of 3 bOPV doses; B. Day 0 visit in Arm B – ~ 6 months after receiving the last of 3 bOPV doses and 0 IPV doses. C. Day 30 visit in Arm A – after receiving a booster dose of IPV; D- Day 30 visit in Arm B – after receiving the first dose of IPV

Figure 3. Type 2 antibody titers following 1 dose or 2 doses of IPV.



A. Day 0 in Arm A (1 IPV dose received > 2 years before) compared with Day 30 in Arm B (1 IPV dose received 30 days before). B. Day 0 (after 1 IPV dose) and Day 30 (after a second IPV dose) within Arm A.

Figure 3 (suppl in PP population). Type 2 antibody titers following 1 dose or 2 doses of IPV.



A. Day 0 in Arm A (1 IPV dose received > 2 years before) compared with Day 30 in Arm B (1 IPV dose received 30 days before). B. Day 0 (after 1 IPV dose) and Day 30 (after a second IPV dose) within Arm A.