

RESEARCH PAPER



Antibody persistence in pre-school children after hexavalent vaccine infant primary and booster administration

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ABSTRACT

Objective: Antibody persistence evaluation for all antigens of a fully liquid DTaP-IPV-HB-PRP~T vaccine at 3.5 and 4.5 y of age following different primary series and booster schedules in South Africa and Latin America. **Methods:** Participants had completed one of two previous studies (Study 1-South Africa; Study 2-Latin America). In Study 1, participants who had not received HB vaccine at birth received a 6–10–14 week primary series of DTaP-IPV-HB-PRP~T or DTwP/PRP~T-Hib+HB+OPV and a third group who had received HB vaccine at birth received a 6–10–14 week primary series of DTaP-IPV-HB-PRP~T; all received a booster (15–18 months) of the primary series vaccine(s) except for HB in the DTwP/PRP~T-Hib group. In Study 2, participants received HB vaccine at birth, a 2–4–6 month primary series of DTaP-IPV-HB-PRP~T or DTaP-HB-IPV//PRP~T, and a DTaP-IPV-HB-PRP~T or DTaP-HB-IPV//PRP~T booster (12–24 months). Participants were followed up at 3.5 and 4.5 y of age for antibody persistence.

Results: Approximately 80% of eligible participants were assessed. In Study 1, a birth dose of HB increased anti-HBs persistence (≥ 10 mIU/mL) following DTaP-IPV-HB-PRP~T primary and booster vaccination from 76.3% to 96.1% at 3.5 y of age and from 73.3% to 96.1% at 4.5 y of age; in Study 2, anti-HBs persistence was high and similar in each group. For the other antigens, there were no differences between groups or studies at 3.5 or 4.5 y.

Conclusion: Good persistence of antibodies to each antigen in the DTaP-IPV-HB-PRP~T vaccine up to pre-school age, irrespective of the vaccination schedule during the first 2 y of life.

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Introduction

Pediatric combination vaccines that include diphtheria (D), tetanus (T), pertussis (acellular [aP] or whole cell [wP]), inactivated poliovirus [IPV], hepatitis B [HB], and *Haemophilus influenzae* type b [Hib] antigens are crucial for the maintenance of high global coverage of protection against these infectious diseases. Commonly such vaccines are co-administered with other age-recommended pediatric vaccines against meningococcal disease, pneumococcal disease, rotavirus, measles, mumps, rubella, and varicella. Combination vaccines facilitate compliance to increasingly crowded pediatric vaccination schedules, usually using a 2- or 3-dose primary infant series followed by a toddler booster in the second year of life, by administering multiple antigens in a single vaccination.¹ While immunogenicity and safety data from primary vaccination series and toddler boosters of hexavalent vaccines have been widely published, few data are available to describe the long-term persistence of antibodies although this is an important aspect when considering continued protection up to pre-school booster age.

A fully liquid DTaP-IPV-HB-PRP~T hexavalent vaccine (Hexaxim™, Hexyon™, or Hexacima™, depending on the country

of sale) was first licensed in 2012, is now approved in more than 110 countries worldwide with >42 million doses distributed, and has been pre-qualified by the World Health Organization.^{2–6} This vaccine builds on the success of established DTaP-IPV tetravalent and DTaP-IPV//PRP~T pentavalent vaccines (Tetraxim and Pentaxim, respectively)^{7,8} by the addition of 10 µg *Hansenula polymorpha*-derived HB surface antigen (HBsAg) with proven immunogenicity and safety.⁹ The only other widely available comparable hexavalent vaccine outside the European Union is a DTaP-IPV-HB//PRP~T vaccine, which is reconstituted prior to use (Infanrix hexa™).^{10–12}

The DTaP-IPV-HB-PRP~T vaccine has undergone an extensive global program of clinical studies both prior to and post-licensure, including demonstration of good and consistent immunogenicity and safety in various infant primary series schedules at 2, 3, 4 months of age,^{13,14} 6, 10, 14 weeks of age,^{15,16} 2, 4, 6 months of age,^{17–22} and 3, 5 months of age²³ both with and without the administration of a standalone HB vaccine at birth and followed by a toddler booster as well as in a mixed hexavalent-pentavalent-hexavalent 3-dose infant schedule. Good antibody persistence for all antigens prior to a booster vaccine administered in the second year of life has

also been consistently demonstrated as well as good immunogenicity and safety following booster vaccination.^{13,17,20,24}

The long-term persistence of HB antibodies at 9–10 y of age and strong anti-HB response to subsequent HB vaccination have recently been reported following administration of the DTaP-IPV-HB-PRP~T vaccine in a 2, 4, 6 month of age primary series with a standalone HB vaccine at birth but no booster.²⁵ However, no long-term immunogenicity data for the other antigens contained in the DTaP-IPV-HB-PRP~T vaccine have previously been published. Two clinical studies from the clinical development program were chosen since they represented two different primary series and booster vaccine regimens, with different comparator vaccines, and were conducted in geographically distinct locations, thereby providing a broad perspective on pre-school antibody persistence to each antigen at 3.5 and 4.5 y of age in two distinct situations.^{16,20,24}

Results

Participants studied

In Study 1, 622 participants received a 6, 10, 14 week primary series of DTaP-IPV-HB-PRP~T (N = 243), DTwP/PRP~T + OPV + HB (N = 242), or DTaP-IPV-HB-PRP~T following

HB at birth (N = 137), of whom 218, 219, and 130 participants received a booster vaccination of DTaP-IPV-HB-PRP~T or DTwP/PRP~T-Hib at 15–18 months of age. A total of 173, 177, and 103 participants were assessed at 3.5 y of age and 167, 167, and 102 participants were assessed at 4.5 y of age (Figure 1).

In Study 2, 1375 participants received a 2, 4, 6 month primary series of DTaP-IPV-HB-PRP~T (N = 1030) or DTaP-IPV-HB//PRP~T (N = 345). Of those who received a primary series of DTaP-IPV-HB-PRP~T, 416 participants received a DTaP-IPV-HB-PRP~T booster and 415 participants received a DTaP-IPV-HB//PRP~T booster at 12–24 months of age. Of those who received a primary series of DTaP-IPV-HB//PRP~T, 275 participants received a DTaP-IPV-HB-PRP~T booster at 12–24 months of age. A total of 219, 206, and 130 participants from the Colombian part of the study were assessed 3.5 y of age and 213, 200, and 125 participants were assessed at 4.5 y of age (Figure 1).

Overall, in Study 1, of the 567 participants who were eligible a total of 453 (79.9%) and 436 (76.9%) participants were assessed at 3.5 y of age and 4.5 y of age, respectively. In Study 2, of the 699 participants who were eligible (participants from Colombia only), a total of 555 (79.4%) and 538 (77.0%) were assessed at 3.5 y of age and 4.5 y of age, respectively.

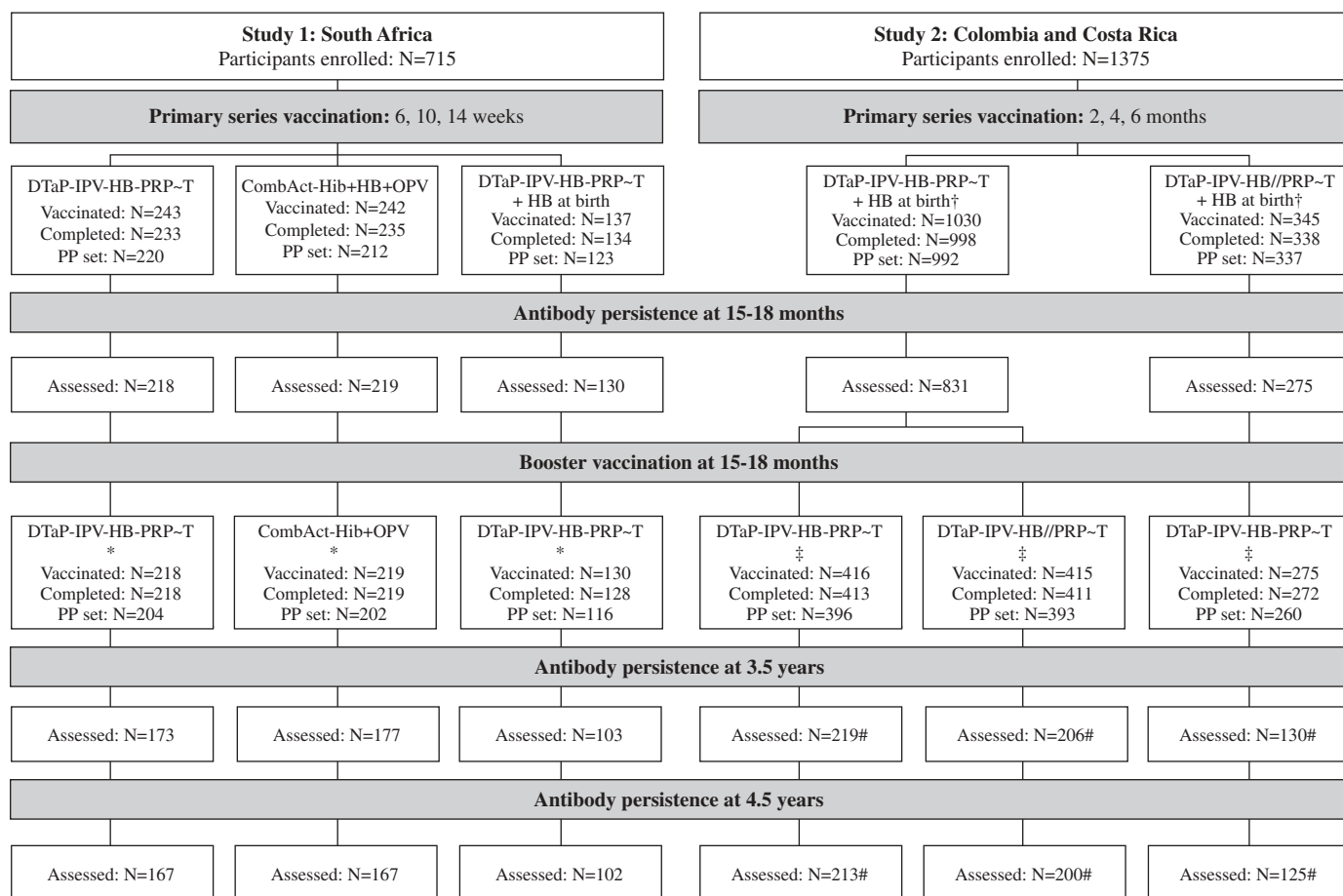


Figure 1. Participant disposition. Primary series and booster data from Madhi et al (Study 1, South Africa)^{16,24} and Lopez et al (Study 2, Colombia)²⁰.

*, coadministered with MMR-V; †, coadministered with PCV7 (2, 4, 6 months) and rotavirus vaccine (2, 4 months); ‡, coadministered with PCV7 and MMR-V; #, data only available for Colombia at 3.5 years and 4.5 years of age in Study 2 (due to closure of site in Costa Rica)

Immunogenicity

Hepatitis b

In Study 1, for participants not vaccinated with a standalone HB vaccine at birth, 76.3% and 73.3% vaccinated with DTaP-IPV-HB-PRP~T and 72.7% and 68.5% vaccinated with DTwP/PRP~T + HB + OPV demonstrated anti-HB ≥ 10 mIU/mL at 3.5 y of age and 4.5 y of age, respectively. The addition of a HB vaccination at birth increased the anti-HB seroprotection (SP) rate (≥ 10 mIU/mL) to 96.1% at both 3.5 and 4.5 y of age following vaccination with DTaP-IPV-HB-PRP~T. Similarly, GMCs persisted at higher levels when HB was given at birth (Table 1).

In Study 2, anti-HB ≥ 10 mIU/mL and GMCs were high and similar in each study group at both 3.5 y of age (>95% of participants with anti-HB ≥ 10 mIU/mL) and 4.5 y of age (>92% of participants with anti-HB ≥ 10 mIU/mL) (Table 1).

Diphtheria

In Study 1, anti-D ≥ 0.01 IU/mL ($\geq 97.0\%$) and ≥ 0.1 IU/mL ($\geq 64.4\%$) was higher at 3.5 and 4.5 y of age following a primary series and booster of DTaP-IPV-HB-PRP~T than following a primary series and booster vaccination of DTwP/PRP~T ($\geq 87.5\%$ [≥ 0.01 IU/mL] and $\geq 33.1\%$ [≥ 0.1 IU/mL]). Similarly, GMCs were higher for DTaP-IPV-HB-PRP~T than for DTwP/PRP~T (Table 2).

In Study 2, anti-D ≥ 0.01 IU/mL ($\geq 99.5\%$ at 3.5 y of age and $\geq 98.0\%$ at 4.5 y of age), ≥ 0.1 IU/mL ($\geq 67.0\%$ at 3.5 y of age and $\geq 48.2\%$ at 4.5 y of age), and GMCs were similar in each group (Table 2).

Tetanus

In both studies, anti-T ≥ 0.01 IU/mL ($\geq 99.0\%$) and ≥ 0.1 IU/mL ($\geq 76.8\%$) was high and similar in each group at 3.5 and 4.5 y of age, and GMCs were in the same range in each group (Table 3).

Poliovirus

Anti-polio 1, 2, and 3 were not assessed in Study 1 due to the occurrence of OPV administrations during National Immunization Days. In Study 2, anti-polio 1, 2, and 3 ≥ 8 (1/dil) was high and similar in each group and at 3.5 y of age ($\geq 98.5\%$) and 4.5 y of age ($\geq 99.0\%$). The GMCs in each group were similar for each poliovirus type and slightly higher at 3.5 y of age than 4.5 y of age (Table 4).

Pertussis

In Studies 1 and 2 for anti-PT GMCs and in Study 1 for anti-FHA, GMCs were slightly higher at 3.5 y of age than 4.5 y of age; in Study 2 anti-FHA GMCs were slightly higher at 4.5 y of age than at 3.5 y of age. Both anti-PT and anti-FHA GMCs were generally similar between groups on each occasion (Table 5).

Haemophilus influenzae type b

In both studies, the majority of children had anti-PRP ≥ 0.15 $\mu\text{g/mL}$ and ≥ 1.0 $\mu\text{g/mL}$ at 3.5 y of age and 4.5 y of age, with no differences between groups (Study 1: $\geq 98.3\%$ and $\geq 98.8\%$ [≥ 0.15 $\mu\text{g/mL}$] and $\geq 87.0\%$ and 78.4% [≥ 0.1 $\mu\text{g/mL}$]; Study 2: $\geq 99.2\%$ and 100.0% [≥ 0.15 $\mu\text{g/mL}$] and $\geq 86.8\%$ and $\geq 84.4\%$ [≥ 0.1 $\mu\text{g/mL}$]). The GMCs were similar in each group

Table 1. Anti-hepatitis B antibody response post-primary vaccination, pre- and post-booster in second year of life, and persistence at 3.5 and 4.5 years of age.

Study	Primary/booster schedule	HB at birth?	Primary/booster vaccine		Post-primary ^a	Pre-booster ^a	Post-booster ^a	3.5 years	4.5 years
			DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV					
Study 1	6, 10, 14 weeks/ 15–18 months	No	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	95.7 (91.6;98.1)	78.9 (92.6;84.3)	98.5 (95.6;99.7)	76.3 (69.3;82.40)	73.3 (65.9;79.9)
					78.8 (72.2;84.5)	39.7 (32.8;46.9)	93.9 (89.6;96.38)	49.1 (41.5;56.8)	40.0 (32.5;47.9)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	330 (259;420)	51.3 (40.0;65.8)	4630 (3402;6302)	76.3 (54.1;108)	54.0 (38.8;75.3)
					95.4 (91.4;97.9)	92.0 (87.3;95.3)	NA	72.7 (65.5;79.2)	68.5 (60.8;75.5)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	65.5 (58.3;72.1)	54.3 (47.1;61.3)	NA	22.2 (16.3;29.0)	17.0 (11.6;23.6)
					148 (120;181)	103 (83.3;127)	NA	30.0 (23.8;37.7)	22.6 (17.7;28.9)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	99.0 (94.4;100.0)	94.7 (88.8;98.0)	100.0 (96.8;100.0)	96.1 (90.4;98.9)	96.1 (90.3;98.8)
					96.9 (91.3;99.4)	78.8 (70.1;85.9)	99.1 (95.2;100.0)	86.4 (78.2;92.4)	84.3 (75.8;90.8)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	1913 (1457;2513)	228 (172;303)	44983 (33652;59890)	1775 (756;1827)	882 (567;1373)
					99.7 (99.1;99.9)	97.5 (95.4;98.8)	99.7 (98.6;100.0)	95.4 (91.8;97.8)	92.3 (87.8;95.5)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	98.5 (97.5;99.2)	82.6 (78.5;86.2)	97.7 (95.7;99.0)	80.4 (74.5;85.4)	74.0 (67.5;79.9)
					3013 (2793;3250)	386 (332;449)	8462 (7154;10010)	500 (379;493)	299 (223;401)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	99.7 (99.1;99.9)	97.7 (95.7;98.9)	99.5 (98.2;99.9)	95.1 (91.3;97.6)	93.0 (88.5;96.1)
					98.5 (97.5;99.2)	85.2 (81.2;88.5)	97.7 (95.7;98.9)	83.5 (77.7;88.3)	71.9 (65.1;78.0)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	3013 (2793;3250)	406 (349;472)	11218 (9482;13272)	475 (364;619)	277 (210;365)
					100.0 (98.8;100.0)	99.2 (97.2;100.0)	100.0 (98.6;100.0)	96.2 (91.3;98.7)	94.4 (88.7;97.7)
Study 2	2, 4, 6 months/ 12–24 months	Yes	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	99.4 (97.7;99.9)	82.2 (77.0;86.7)	99.2 (97.2;99.9)	82.3 (74.6;88.4)	75.0 (66.4;82.3)
					2766 (2466;3102)	336 (284;397)	9688 (7940;11821)	671 (463;971)	399 (271;589)

Data are % (95% CI) participants with concentration above threshold or geometric mean concentration (GMC) (95% CI)

NA, not applicable (no HB booster administered in this group)

^a1 month post-primary series, prior to and 1 month post-booster (from Madhi et al¹⁶ and Madhi et al²⁴ [Study 1]; Lopez et al²⁰ [Study 2])

Table 2. Anti-diphtheria antibody response post-primary vaccination, pre- and post-booster in second year of life, and persistence at 3.5 and 4.5 years of age.

Study	Primary/booster schedule	Primary/booster vaccine	Post-primary ^a	Pre-booster ^a	Post-booster ^a	3.5 years	4.5 years	
Study 1	6, 10, 14 weeks/ 15–18 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	93.4 (89.0;96.4)	100 (98.1;100)	98.8 (95.8;99.9)	98.2 (94.8;99.6)	
			≥0.10 IU/mL	39.8 (33.1;46.8)	30.5 (24.1;37.4)	100 (98.1;100)	81.3 (74.6;86.8)	75.3 (68.0;81.7)
			≥1.0 IU/mL	NC	NC	97.9 (94.8;99.4)	32.7 (25.8;40.3)	18.7 (13.1;25.4)
			GMC	0.074 (0.062;0.088)	0.06 (0.05;0.07)	9.37 (8.05;10.9)	0.437 (0.344;0.556)	0.272 (0.214;0.345)
Study 2	2, 4, 6 months/ 12–24 months	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	≥0.01 IU/mL	86.1 (80.5;90.5)	100 (98.2;100)	91.5 (86.3;95.2)	87.5 (81.4;92.2)	
			≥0.10 IU/mL	13.6 (9.2;19.0)	10.4 (6.6;15.5)	99.0 (96.4;99.9)	47.2 (39.6;54.8)	33.1 (25.9;41.0)
			≥1.0 IU/mL	NC	NC	93.0 (88.5;96.1)	2.8 (0.9;6.5)	0.0 (0.0;2.3)
			GMC	0.040 (0.035;0.046)	0.03 (0.02;0.03)	3.33 (2.92;3.80)	0.086 (0.069;0.107)	0.048 (0.038;0.061)
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	95.1 (89.6;98.2)	100 (96.7;100)	98.1 (93.2;99.8)	97.0 (91.6;99.4)	
			≥0.10 IU/mL	39.3 (30.6;48.6)	32.8 (24.3;42.1)	100 (96.7;100)	68.9 (59.1;77.7)	64.4 (54.2;73.6)
			≥1.0 IU/mL	NC	NC	93.7 (87.4;97.4)	24.3 (16.4;33.7)	24.8 (16.7;34.3)
			GMC	0.074 (0.059;0.094)	0.05 (0.03;0.06)	7.00 (5.61;8.72)	0.244 (0.175;0.342)	0.222 (0.155;0.319)
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	100.0 (99.6;100.0)	100 (99.1;100.0)	100.0 (98.3;100.0)	100.0 (98.2;100.0)	
			≥0.10 IU/mL	76.1 (73.2;78.8)	40.0 (35.1;45.1)	100.0 (99.1;100.0)	72.8 (66.4;78.6)	57.2 (50.2;64.0)
			≥1.0 IU/mL	12.3 (10.3;14.6)	NC	98.0 (96.0;99.1)	12.9 (8.7;18.1)	10.1 (6.4;15.0)
			GMC	0.252 (0.235;0.271)	0.077 (0.069;0.086)	5.55 (5.07;6.08)	0.256 (0.216;0.303)	0.164 (0.136;0.197)
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	100.0 (99.6;100.0)	100 (99.1;100.0)	99.5 (97.3;100.0)	98.0 (94.9;99.4)	
			≥0.10 IU/mL	76.1 (73.2;78.8)	39.2 (34.4;44.3)	99.7 (98.6;100.0)	67.0 (60.1;73.4)	48.2 (41.1;55.4)
			≥1.0 IU/mL	12.3 (10.3;14.6)	NC	95.9 (93.4;97.6)	8.3 (4.9;12.9)	7.0 (3.9;11.5)
			GMC	0.252 (0.235;0.271)	0.074 (0.066;0.083)	4.40 (3.99;4.86)	0.187 (0.159;0.220)	0.119 (0.098;0.145)
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	100.0 (98.8;100.0)	100 (98.6;100.0)	100.0 (97.2;100.0)	99.2 (95.6;100.0)	
			≥0.10 IU/mL	75.2 (70.1;79.9)	27.2 (21.9;33.1)	100.0 (98.6;100.0)	73.1 (64.6;80.5)	59.2 (50.1;67.9)
			≥1.0 IU/mL	9.5 (6.5;13.3)	NC	97.2 (94.4;98.9)	12.3 (7.2;19.2)	10.4 (5.7;17.1)
			GMC	0.240 (0.214;0.269)	0.059 (0.051;0.068)	6.05 (5.41;6.76)	0.231 (0.188;0.284)	0.143 (0.112;0.183)

Data are % (95% CI) participants with concentration above threshold or geometric mean concentration (GMC) (95% CI)

^a1 month post-primary series, prior to and 1 month post-booster (from Madhi et al.¹⁰ and Madhi et al.²⁴ [Study 1]; Lopez et al.²⁰ [Study 2])

NC, not calculated

Table 3. Anti-tetanus antibody response post-primary vaccination, pre- and post-booster in second year of life, and persistence at 3.5 and 4.5 years of age.

Study	Primary/booster schedule	Primary/ booster vaccine	Post-primary ^a	Pre-booster ^a	Post-booster ^a	3.5 years	4.5 years
Study 1	6, 10, 14 weeks/ 15–18 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	100 (98.3;100.0)	100 (98.2;100)	100.0 (97.9;100.0)	100.0 (97.7;100.0)
			≥0.10 IU/mL	100 (98.3;100.0)	75.1 (68.3;81.1)	94.7 (90.2;97.6)	89.5 (83.7;93.8)
Study 2	2, 4, 6 months/ 12–24 months	CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	≥0.01 IU/mL	1.51 (1.37;1.65)	0.22 (0.19;0.25)	10.0 (8.65;11.7)	0.703 (0.594;0.831)
			≥0.10 IU/mL	100 (98.3;100.0)	100 (98.1;100)	100 (98.2;100)	100.0 (97.9;100.0)
		≥1.0 IU/mL	100 (98.3;100.0)	90.3 (85.2;94.0)	100 (98.2;100)	93.7 (89.0;96.8)	
		GMC	NC	NC	99.5 (97.2;100)	8.6 (4.9;13.7)	3.2 (1.1;7.4)
		≥0.01 IU/mL	1.88 (1.70;2.07)	0.31 (0.28;0.35)	8.23 (7.49;9.04)	0.371 (0.329;0.418)	0.246 (0.213;0.283)
		≥0.10 IU/mL	100 (97.0;100.0)	100 (96.9;100)	100 (96.8;100)	100.0 (96.4;100.0)	100.0 (96.3;100.0)
		≥1.0 IU/mL	100 (97.0;100.0)	67.2 (57.9;75.7)	100 (96.8;100)	94.1 (87.5;97.8)	82.8 (73.9;89.7)
		GMC	NC	NC	96.5 (91.3;99.0)	36.6 (27.3;46.8)	20.2 (12.8;29.5)
		≥0.01 IU/mL	1.33 (1.17;1.51)	0.17 (0.14;0.21)	8.13 (6.68;9.89)	0.588 (0.473;0.731)	0.343 (0.273;0.430)
		≥0.10 IU/mL	100.0 (99.6;100.0)	100 (99.1;100.0)	100 (99.1;100.0)	100.0 (98.3;100.0)	100.0 (98.2;100.0)
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.01 IU/mL	99.9 (99.4;100.0)	74.3 (69.6;78.6)	99.7 (98.6;100.0)	80.8 (74.7;85.9)
			≥1.0 IU/mL	73.1 (70.1;75.9)	NC	98.0 (96.0;99.1)	22.6 (17.2;28.7)
		GMC	1.55 (1.48;1.62)	0.208 (0.188;0.231)	5.72 (5.21;6.27)	0.433 (0.372;0.503)	0.297 (0.252;0.350)
		≥0.01 IU/mL	100.0 (99.6;100.0)	99.7 (98.6;100.0)	100.0 (99.0;100.0)	100.0 (98.2;100.0)	99.0 (96.4;99.9)
		≥0.10 IU/mL	99.9 (99.4;100.0)	73.9 (69.2;78.2)	100.0 (99.0;100.0)	86.8 (91.4;91.1)	76.8 (70.3;82.5)
		≥1.0 IU/mL	73.1 (70.1;75.9)	NC	96.6 (94.3;98.2)	14.1 (9.7;19.7)	6.1 (3.2;10.3)
		GMC	1.55 (1.48;1.62)	0.224 (0.200;0.251)	5.21 (4.78;5.68)	0.323 (0.281;0.372)	0.221 (0.189;0.260)
		≥0.01 IU/mL	100.0 (98.8;100.0)	100.0 (98.6;100.0)	100.0 (98.6;100.0)	100.0 (97.2;100.0)	100.0 (97.1;100.0)
		≥0.10 IU/mL	100.0 (98.8;100.0)	76.9 (71.2;81.9)	100.0 (98.6;100.0)	88.5 (81.7;93.4)	81.6 (73.7;88.0)
		GMC	82.5 (77.8;86.5)	NC	96.9 (93.9;98.6)	33.8 (25.8;42.7)	21.6 (14.7;29.8)
≥1.0 IU/mL	1.80 (1.69;1.93)	0.201 (0.180;0.225)	7.52 (6.63;8.52)	0.579 (0.465;0.722)	0.381 (0.302;0.481)		

Data are % (95% CI) participants with concentration above threshold or geometric mean concentration (GMC) (95% CI)

^a1 month post-primary series, prior to and 1 month post-booster (from Madhi et al¹⁶ and Madhi et al²⁴ [Study 1]; Lopez et al²⁰ [Study 2])

Table 4. Anti-poliovirus 1, 2, and 3 antibody response post-primary vaccination, pre- and post-booster in second year of life, and persistence at 3.5 and 4.5 years of age.

Study	Primary/booster schedule	Primary/booster vaccine	Politype	Post-primary ^a		Pre-booster ^a		Post-booster ^a		3.5 years		4.5 years	
				GMT 1/dil	% ≥ 8 1/dil	GMT 1/dil	% ≥ 8 1/dil	GMT 1/dil	% ≥ 8 1/dil	GMT 1/dil	% ≥ 8 1/dil	GMT 1/dil	% ≥ 8 1/dil
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	1	680 (635;729)	100.0 (99.6;100.0)	98.8 (97.0;99.7)	100.0 (98.9;100.0)	2140 (1937;2364)	100.0 (98.3;100.0)	285 (245;332)	100.0 (98.3;100.0)	211 (180;249)	100.0 (98.2;100.0)
			2	1180 (1102;1263)	100.0 (99.6;100.0)	99.4 (97.9;99.9)	100.0 (98.9;100.0)	4232 (3821;4688)	100.0 (98.3;100.0)	694 (587;822)	100.0 (98.3;100.0)	543 (455;647)	100.0 (98.2;100.0)
			3	1106 (1025;1193)	100.0 (99.6;100.0)	95.9 (93.1;97.7)	100.0 (98.9;100.0)	3569 (3164;4027)	100.0 (98.3;100.0)	691 (570;838)	100.0 (98.2;100.0)	408 (338;493)	100.0 (97.2;100.0)
		DTaP-IPV-HB-PRP~T/ DTaP-HB-IPV//PRP~T	1	680 (635;729)	100.0 (99.6;100.0)	98.2 (96.0;99.3)	100.0 (98.9;100.0)	2633 (2363;2933)	100.0 (98.2;100.0)	294 (250;345)	100.0 (98.2;100.0)	207 (173;247)	100.0 (98.2;100.0)
			2	1180 (1102;1263)	100.0 (99.6;100.0)	100.0 (98.9;100.0)	100.0 (98.9;100.0)	4887 (4372;5463)	100.0 (98.2;100.0)	553 (470;652)	100.0 (98.2;100.0)	403 (332;490)	100.0 (98.2;100.0)
			3	1106 (1025;1193)	100.0 (98.8;100.0)	94.8 (91.8;96.9)	100.0 (98.9;100.0)	3322 (2939;3755)	100.0 (98.3;100.0)	98.5 (95.8;99.7)	508 (414;622)	100.0 (97.1;100.0)	307 (254;372)
		DTaP-HB-IPV//PRP~T/ DTaP-IPV-HB-PRP~T	1	1298 (1151;1464)	100.0 (98.8;100.0)	98.6 (95.9;99.7)	100.0 (98.3;100.0)	2978 (2592;3421)	100.0 (97.2;100.0)	508 (410;630)	100.0 (97.2;100.0)	417 (331;525)	100.0 (97.1;100.0)
			2	1981 (1756;2234)	100.0 (98.8;100.0)	224 (188;267)	100.0 (98.3;100.0)	6369 (5569;7283)	100.0 (98.3;100.0)	997 (788;1261)	100.0 (97.2;100.0)	700 (555;883)	100.0 (97.1;100.0)
			3	99.7 (98.2;100.0)	1944 (1680;2249)	207 (173;248)	6015 (5244;6898)	1213 (962;1530)	696 (554;875)				

Data are % (95% CI) participants with concentration above threshold or geometric mean concentration (GMC) (95% CI) Data were not collected in Study 1 since subjects could potentially have received an extra dose of OPV during a national vaccination campaign after the booster vaccination ^a1 month post-primary series, prior to and 1 month post-booster (from Madhi et al¹⁶ and Madhi et al²⁰ [Study 1]; Lopez et al²⁰ [Study 2])

at 3.5 y of age and 4.5 y of age with no difference between groups in each study (Table 6).

Safety

No SAEs occurred in any group since the booster part in either study.

Discussion

A high rate of follow-up of approximately 80% of participants was achieved at 3.5 and 4.5 y of age, which was similar in each study. Good antibody persistence was demonstrated for all antigens in each group in both studies. Due to the differences in study design and vaccines administered (due to the different immunization regimens in South Africa [Study 1] versus Colombia and Costa Rica [Study 2]) a numerical comparison between studies is not valid, and evaluation of anti-PT and anti-FHA was limited to GMCs due to the lack of a correlate of protection for these pertussis antigens.

The results confirm good antibody persistence up to pre-school age following a primary series of the DTaP-IPV-HB-PRP~T vaccine with a booster in the second year of life, even following the less immunogenic 6, 10, 14 week infant primary series schedule. Although it is not possible to fully assess any potential impact of the coadministered vaccines in the two studies, the antibody responses post-primary series, pre-booster, and post-booster^{16,20,24} are aligned with results from a wide range of studies evaluating the immunogenicity of the DTaP-IPV-HB-PRP~T vaccine in a range of schedules, countries, and with and without coadministered vaccines.^{13–15,17–19,21–23} It is therefore unlikely that there would be a clinically important effect of the coadministered vaccines on antibody persistence at 3.5 and 4.5 y of age.

Anti-HBs antibodies are of particular interest since the HB antigen is the new inclusion in the DTaP-IPV-HB-PRP~T vaccine. At 3.5 and 4.5 y of age the percentage of vaccinees with anti-HBs ≥10 mIU/mL was similar for both DTaP-IPV-HB-PRP~T and DTwP/PRP~T + HB + OPV when given in a 6, 10, 14 week infant primary series schedule (with a HB booster at 15–18 months of age in the DTaP-IPV-HB-PRP~T group) if no HB vaccine was administered at birth (Study 1) (≥68.5%). In the DTaP-IPV-HB-PRP~T group the proportion of participants with anti-HBs ≥10 mIU/mL was higher when standalone HB vaccine was given at birth (≥96.1%).

In Study 2 (2, 4, 6 month primary series with HB vaccination at birth and a booster in the second year of life), anti-HBs SP rates were high (>92%) in each group, with no difference between participants who received DTaP-IPV-HB-PRP~T/DTaP-IPV-HB-PRP~T, DTaP-IPV-HB-PRP~T/DTaP-IPV-HB-PRP~T, or DTaP-IPV-HB-PRP~T/DTaP-IPV-HB-PRP~T infant/toddler series. The high persisting anti-HB SP rate in Study 2 is consistent with that from Study 1 when a primary series and booster of DTaP-IPV-HB-PRP~T was given following HB vaccination at birth, and higher than the DTaP-IPV-HB-PRP~T group in Study 1 that did not receive HB vaccine at birth.

Anti-HBs antibody persistence, in terms of antibody titers ≥10 mIU/mL, after a 2, 4, 6 month primary series of DTaP-IPV-HB-PRP~T with HB vaccine administered at birth and with no booster vaccination has recently been shown to be maintained at

Table 5. Anti-pertussis (anti-PT and anti-FHA) antibody geometric mean concentrations post-primary vaccination, pre- and post-booster in second year of life, and persistence at 3.5 and 4.5 years of age.

Antibody	Study	Primary/booster schedule	Primary/booster vaccine		Post-primary ^a	Pre-booster ^a	Post-booster ^a	3.5 years	4.5 years
(a) Anti-PT	Study 1	6, 10, 14 weeks/	DTaP-IPV-HB-PRP~T/	GMC	332 (304;362)	11.6 (9.88;13.6)	288 (260;318)	10.8 (9.17;12.7)	6.68 (5.43;8.21)
		15–18 months	DTaP-IPV-HB-PRP~T CombAct-Hib+ HB	GMC	191 (147;249)	10.4 (80.3;13.6)	110 (88.7;137)	8.82 (7.34;10.6)	6.09 (5.02;7.39)
			+ OPV/ CombAct-Hib+ OPV DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	GMC	288 (256;323)	12.0 (9.62;14.9)	235 (206;268)	7.09 (5.73;8.76)	4.27 (3.38;5.41)
	Study 2	2, 4, 6 months/	DTaP-IPV-HB-PRP~T/	GMC	102 (98.5;107)	7.43 (6.63;8.32)	154 (143;166)	4.75 (4.07;5.56)	3.16 (2.71;3.69)
		12–24 months	DTaP-IPV-HB-PRP~T DTaP-IPV-HB-PRP~T/ DTaP-HB-IPV//PRP~T DTaP-HB-IPV//PRP~T/ DTaP-IPV-HB-PRP~T	GMC	102 (98.5;107)	8.47 (7.52;9.56)	191 (178;206)	5.09 (4.32;5.98)	3.13 (2.67;3.68)
				GMC	98.9 (92.3;106)	7.41 (6.38;8.61)	140 (127;153)	4.62 (3.78;5.65)	3.06 (2.45;3.82)
(b) Anti-FHA	Study 1	6, 10, 14 weeks/	DTaP-IPV-HB-PRP~T/	GMC	207 (190;226)	30.5 (25.4;36.7)	570 (514;630)	68.4 (58.0;80.7)	46.3 (39.3;54.5)
		15–18 months	DTaP-IPV-HB-PRP~T CombAct-Hib+HB+OPV/ CombAct-Hib+OPV	GMC	37.4 (33.4;41.9)	5.43 (4.52;6.53)	211 (193;231)	17.0 (14.0;20.7)	14.1 (11.2;17.7)
			DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	GMC	188 (166;212)	25.1 (19.7;31.9)	472 (419;533)	60.4 (46.8;78.0)	33.3 (26.8;41.4)
	Study 2	2, 4, 6 months/	DTaP-IPV-HB-PRP~T/	GMC	3.56 (3.19;3.97)	0.482 (0.406;0.573)	42.4 (37.0;48.6)	26.1 (21.8;31.1)	33.8 (28.5;40.1)
		12–24 months	DTaP-IPV-HB-PRP~T DTaP-IPV-HB-PRP~T/ DTaP-HB-IPV//PRP~T DTaP-HB-IPV//PRP~T/ DTaP-IPV-HB-PRP~T	GMC	3.56 (3.19;3.97)	0.556 (0.472;0.656)	41.5 (36.6;47.0)	26.3 (22.2;31.1)	35.1 (29.2;42.1)
				GMC	2.24 (1.90;2.64)	0.455 (0.375;0.553)	56.5 (48.4;65.9)	19.9 (15.9;24.9)	27.3 (21.3;34.9)

Data are % (95% CI) participants with concentration above threshold or geometric mean concentration (GMC) (95% CI)

^a1 month post-primary series, prior to and 1 month post-booster (from Madhi et al¹⁶ and Madhi et al²⁴ [Study 1]; Lopez et al²⁰ [Study 2])

NC, not calculated

9–10 y of age in approximately 50% of participants.²⁵ Furthermore, a strong anamnestic response was reported following HB vaccine challenge re-vaccination at 9–10 y of age despite the reduced SP rate. These data are consistent with previous studies that have shown the persistence of anti-HB T-cell and B-cell immune memory for decades following vaccination with HB-containing combination vaccines, even without regular booster vaccination.^{26–30} As such, a strong anti-HBs response would be expected following subsequent HB exposure even in individuals with an anti-HBs antibody titer <10 mIU/mL, and the WHO have stated that *‘the substantial body of evidence does not provide a compelling basis for recommending a booster dose of hepatitis B vaccine after completion of the primary vaccination series for persons with normal immune status’*.³¹

Overall, very good antibody persistence was demonstrated in two different vaccination schedules and in two different populations (South Africa and Latin America) with a variety of primary series/booster sequences, indicating that mixed primary series/booster schedules are equally immunogenic as the administration of the same vaccine in a primary series and as a booster. These data add to those recently reported that show good primary series and booster immunogenicity following a mixed infant primary series and booster schedule (hexavalent-pentavalent-hexavalent primary series and pentavalent booster). There was no clinically important difference in

antibody persistence between the two populations, irrespective of the different environmental factors in the different regions.

Limitations of this analysis include the evaluation of only two primary series schedules and no available data for HB immunogenicity after a 2, 4, 6 month primary series and booster without a HB vaccination at birth. However, good primary series and booster immunogenicity has been shown consistently for the DTaP-IPV-HB-PRP~T vaccine, as well as good long-term HB immunogenicity with an anamnestic response on challenge re-vaccination,²⁵ and so it would be expected that the data described can be extrapolated to other primary series and booster schedules. Second, in Study 2, the window for booster administration was 12–24 months of age, which meant that subjects assessed at 3.5 and 4.5 y of age could have received the booster from 1.5–2.5 y prior to the assessment at 3.5 y of age and from 2.5–3.5 y prior to the assessment at 4.5 y of age. However, for a descriptive analysis such as that reported this was considered acceptable. Third, in Study 2, due to the closure of the study site in Costa Rica, it was only possible to collect follow-up data at 3.5 and 4.5 y of age from participants in the Colombian part of the study. It is, however, appropriate to consider these data in the context of primary and booster series data from both Colombia and Costa Rica due to the similar ethnicity and due to the descriptive nature of the analysis at 3.5 and 4.5 y of age.

These results are the first to describe antibody persistence for the fully liquid DTaP-IPV-HB-PRP~T vaccine at 3.5 and 4.5 y of age following primary series and booster vaccination. These encouraging results add to a growing body of literature describing the good immunogenicity of this vaccine in a variety of primary series and booster schedules.

Materials and methods

Study design and participants

Phase III, randomized, observer-blind, controlled, primary vaccination and booster clinical studies that are reported elsewhere were conducted separately in South Africa (Study 1)^{16,24} and Colombia and Costa Rica (Study 2).²⁰

In Study 1, participants in South Africa who had not received standalone HB vaccine at birth were randomized to receive a 6, 10, 14 week primary series of either DTaP-IPV-HB-PRP~T or DTwP/PRP~T + HB + OPV, and a third group of participants who had received a standalone HB vaccine at birth received a 6, 10, 14 week primary series of DTaP-IPV-HB-PRP~T;¹⁶ all participants were to receive a booster vaccination of the same vaccine(s) administered in the primary series, co-administered with measles, mumps, rubella, and varicella vaccines (MMR-V).²⁴

In Study 2, all participants in Colombia and Costa Rica received a standalone HB vaccination at birth and were randomized to receive a primary series of either DTaP-IPV-HB-PRP~T or DTaP-HB-IPV//PRP-T at 2, 4, 6 months of age co-administered with pneumococcal (PCV) 7 vaccine (2, 4, 6 months) and rotavirus vaccine (2 and 4 months); in the second year of life, participants who had received DTaP-IPV-HB-PRP~T in the primary series were randomized to receive a booster of DTaP-IPV-HB-PRP~T or DTaP-HB-IPV//PRP-T, and those who had received a primary series of DTaP-HB-IPV//PRP-T were to receive a booster of DTaP-IPV-HB-PRP~T (booster vaccines were co-administered with PCV7; additionally, MMR-V and PCV13 vaccines were available at 15 months of age in Costa Rica, but not in Colombia, in accordance with the national recommendations).²⁰

Participants in each study were followed up at 3.5 y of age and 4.5 y of age to assess antibody persistence (UTNs: U1111-1111-5789 and NCT01105559 [South Africa] and U1111-1122-2457 and NCT01983540 [Colombia and Costa Rica]). These antibody persistence data are reported in this article. For the study in Colombia and Costa Rica (Study 2) only participants in Colombia were followed up at 3.5 y of age and 4.5 y of age due to the closure of the study site in Costa Rica (due to a lack of proposed clinical studies), so only data from Colombian participants are presented at these time points for Study 2.

All study protocols and amendments were approved by independent ethics committees and studies were performed according to local regulations, Good Clinical Practice, and the ethical principles of Declaration of Helsinki (Edinburgh revision, October 2000). Prior to enrolment an informed consent form was signed by at least one parent or legally acceptable representative and an independent witness if applicable according to the appropriate local regulations. Participants attended the study sites to provide blood samples for 3.5 year and 4.5 year antibody persistence between

April 2010 and September 2011 (Study 1) and between October 2013 and April 2015 (Study 2).

Participants were included in the primary series and booster parts according to standard inclusion and exclusion criteria described elsewhere,^{16,20,24} and all were healthy infants born at full term (≥ 37 weeks) with birth weight ≥ 2.5 kg. For the 3.5 and 4.5 y of age follow-up assessments, participants were 3.5 y of age at enrolment and had received a complete 3-dose primary series and booster vaccination according to the study protocols. Exclusion criteria at 3.5 y of age included participation in another clinical trial in the previous 4 weeks; history of diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae*, or hepatitis B, or vaccination against any of these since the booster vaccination; any vaccination within the previous 30 d (except measles, monovalent polio, of pandemic influenza vaccines that were prohibited within 2 weeks of inclusion); receipt of blood products or immunosuppressant drugs in the previous 3 months; acquired immunodeficiency or hepatitis C infection since the booster vaccination; chronic illness following the booster vaccination (e.g. leukemia, lymphoma, Crohn's disease); and any acute illness or febrile illness.

Vaccines and vaccine administration

The composition and batch numbers of the vaccines administered in the primary series and booster parts are described elsewhere for Study 1 (DTaP-IPV-HB-PRP~T [Hexaxim; Sanofi Pasteur], DTwP/PRP~T [CombAct-Hib; Sanofi Pasteur], HB [Engerix B; GlaxoSmithKline], OPV [Sanofi Pasteur], MMR [ROR, Sanofi Pasteur], and V [Varilrix, GlaxoSmithKline])^{16,24} and Study 2 (DTaP-IPV-HB-PRP~T [Hexaxim; Sanofi Pasteur], DTaP-IPV-HB//PRP~T [Infanrix hexa; GlaxoSmithKline], PCV7 [Prevenar; Pfizer], and rotavirus [Rotarix; GlaxoSmithKline]).²⁰

The DTaP-IPV-HB-PRP~T, DTaP-IPV-HB//PRP~T, PCV7, and HB vaccines were administered intramuscularly, MMR was administered either intramuscularly or subcutaneously, V was administered subcutaneously, and rotavirus vaccine was administered orally (see Figure 1 for subjects disposition).

Serology

Blood samples post-primary series, pre-booster and post-booster were collected and analyzed as reported elsewhere.^{16,20,24}

Blood samples were collected at 3.5 y of age (5 mL) and 4.5 y of age (5 mL) for determination of anti-HB, anti-D, anti-T, anti-polio 1, 2 and 3, anti-PT, anti-FHA, and anti-PRP antibodies. Anti-polio 1, 2, and 3 were not evaluated in Study 1 due to the possibility that participants could have received an extra dose of OPV since the booster vaccination as part of a national vaccination campaign against polio.

All assays were performed at either the Sponsor's Global Clinical Immunology (GCI) laboratory (Swiftwater, PA, USA) or at qualified contract laboratories approved by GCI. Anti-D antibody concentrations (IU/mL) and anti-polio 1, 2, 3 antibody titers (1/dil) were measured by a microneutralization assay (with an assay against Mahoney, MEF-1 and Saukett poliovirus strains), anti-T (IU/mL), anti-PT (EU/mL), and anti-FHA (EU/mL) concentrations by an enzyme linked

Table 6. Anti-PRP antibody response post-primary vaccination, pre- and post-booster in second year of life, and persistence at 3.5 and 4.5 years of age.

Study	Primary/booster schedule	Primary/booster vaccine	Primary	Post-primary ^a	Pre-booster ^a	Post-booster ^a	3.5 years	4.5 years		
Study 1	6, 10, 14 weeks/ 15–18 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.15 µg/mL	95.4 (91.8;97.8)	81.4 (75.3;86.5)	100 (98.2;100)	98.3 (95.0;99.6)	98.8 (95.6;99.9)		
			≥1.0 µg/mL	79.5 (73.5;84.6)	45.6 (38.6;52.7)	98.5 (95.7;99.7)	87.9 (82.0;92.3)	84.7 (78.2;89.8)		
		CombAct-Hib+ HB+ OPV/ CombAct-Hib+ OPV	GMC	3.31 (2.69;4.08)	0.76 (0.59;0.98)	68.5 (55.7;84.2)	4.96 (3.98;6.18)	4.14 (3.34;5.13)	4.14 (3.34;5.13)	
			≥0.15 µg/mL	100.0 (98.3;100.0)	92.5 (87.9;95.7)	100 (98.2;100)	99.4 (96.9;100.0)	98.8 (95.7;99.9)		
		Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥1.0 µg/mL	92.5 (88.0;95.6)	54.0 (46.8;61.1)	98.5 (95.3;99.7)	87.0 (81.1;91.6)	84.1 (77.6;89.4)
					GMC	5.18 (4.47;6.00)	1.2 (0.95;1.48)	52.2 (43.9;62.2)	4.33 (3.59;5.22)	3.48 (2.83;4.30)
DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.15 µg/mL			97.5 (93.0;99.5)	75.9 (67.0;83.3)	100 (96.8;100)	99.0 (94.7;100.0)	100.0 (96.4;100.0)		
	≥1.0 µg/mL			79.5 (71.3;86.3)	37.1 (28.3;46.5)	100 (96.8;100)	89.3 (81.7;94.5)	78.4 (69.2;86.0)		
DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	GMC			3.83 (2.92;5.02)	0.63 (0.45;0.89)	63.1 (47.6;83.8)	4.44 (3.31;5.95)	3.34 (2.56;4.37)		
	≥1.0 µg/mL			94.6 (93.0;96.0)	73.4 (68.8;77.7)	99.7 (98.6;100.0)	100.0 (98.3;100.0)	100.0 (98.2;100.0)		
Study 2	2, 4, 6 months/ 12–24 months	DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.15 µg/mL	77.8 (75.0;80.5)	27.8 (23.5;32.6)	98.7 (97.1;99.6)	86.8 (81.5;89.9)	85.6 (80.1;90.1)		
			GMC	3.56 (3.19;3.97)	0.482 (0.406;0.573)	42.4 (37.0;48.6)	4.55 (3.84;5.40)	4.02 (3.39;4.78)		
		DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥0.15 µg/mL	94.6 (93.0;96.0)	77.7 (73.3;81.8)	100.0 (98.1;100.0)	100.0 (98.2;100.0)	100.0 (98.2;100.0)		
			≥1.0 µg/mL	77.8 (75.0;80.5)	33.0 (28.3;37.9)	99.0 (97.4;99.7)	89.8 (84.8;93.6)	84.4 (78.6;89.2)		
		DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	GMC	3.56 (3.19;3.97)	0.556 (0.472;0.656)	41.5 (36.6;47.0)	5.22 (4.37;6.23)	4.34 (3.59;5.26)		
			≥0.15 µg/mL	95.9 (93.1;97.8)	76.4 (70.7;81.4)	100.0 (98.6;100.0)	99.2 (95.8;100.0)	100.0 (97.1;100.0)		
DTaP-IPV-HB-PRP~T/ DTaP-IPV-HB-PRP~T	≥1.0 µg/mL	71.5 (66.2;76.4)	28.3 (22.9;34.2)	100.0 (98.6;100.0)	90.8 (84.4;95.1)	90.4 (83.8;94.9)				
	GMC	2.24 (1.90;2.64)	0.455 (0.375;0.553)	56.5 (48.4;65.9)	5.37 (4.32;6.69)	4.87 (3.83;6.19)				

Data are % (95% CI) participants with concentration above threshold or geometric mean concentration (GMC) (95% CI) at 1 month post-primary series, prior to and 1 month post-booster (from Madhi et al.¹⁶ and Madhi et al.²⁴ [Study 1]; Lopez et al.²⁰ [Study 2]).

immunosorbent assay (ELISA), anti-PRP-T (µg/mL) concentrations by a radioimmunoassay, and anti-HB concentrations (mIU/mL) by a commercially available chemiluminescence assay (VITROS ECi/ECiQ).

Safety

Since the 3.5 year of age and 4.5 year of age assessments were non-interventional no safety data were recorded (other than ongoing SAE long-term monitoring after the primary series and booster vaccinations).

Statistical analyses

The objective of these two studies was to describe the long-term antibody persistence and 3.5 y of age and 4.5 y of age following primary series and booster vaccinations that are described elsewhere.^{16,20,24} There were no formal statistical tests performed for this assessment of long-term antibody persistence and all analyses were descriptive.

Antibody thresholds and criteria used to define the SP rates are presented for anti-HB, anti-D, anti-T, anti-polio 1, 2 and 3, and anti-PRP antibodies in Table 1, Table 2, Table 3, Tables 4, and 6, respectively. Additionally, geometric mean titers (GMTs) are presented for IPV and geometric mean concentrations (GMCs) are presented for HB, PRP, D, T, PT, and FHA (Table 1 to Table 6). Previously published data are presented for the primary series and booster vaccinations in order to provide the full profile for each antigen.^{16,20,24} All data are presented with their 95% confidence intervals (CIs) calculated using the exact binomial distribution (Clopper-Pearson)³² for proportions and the normal approximation method for GMCs and GMTs.

No calculation for sample size was performed since these were descriptive studies. The sample size in each study was based on the available participants who had completed the prior primary series and booster vaccinations, i.e. a maximum of 567 participants for Study 1 and a maximum of 699 participants in Study 2 (see Figure 1).

All statistical analyses were performed under the responsibility of the Sponsor's Biostatistics

Platform using SAS® software, Version 9.1 or 9.2 (SAS Institute, Cary, North Carolina, USA).

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Authors Contributions

SM, SB'C, EJ, and EF contributed to the conception, design, and clinical conduct of Study 1.

PL, BZ, SB'C, EJ, and FN contributed to the conception, design, and clinical conduct of Study 2.

All authors contributed to the analysis and interpretation of study data presented in this article and all approved the final version of this article.

Disclosure of potential conflicts of interest

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BZ, EJ, SB'C, FN, and EF are employees of Sanofi Pasteur.

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