

Review

A liquid hexavalent combined vaccine against diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae* type B and hepatitis B: review of immunogenicity and safety

Eric Mallet^a, Bernd H. Belohradsky^b, Rosanna Lagos^c, Leif Gothefors^d, Patrice Camier^e, Jean-Pierre Carrière^f, Güler Kanra^g, Agnès Hoffenbach^h, Jacques Langue^e, François Undreiner^e, François Roussel^e, Philippe Reinertⁱ, Carl-Erik Flodmark^j, Silvia Stojanov^b, Johannes Liese^b, Myron M. Levine^k, Alma Muñoz^c, Florian Schödel^l, Luc Hessel^{h,*}, On behalf of the Hexavalent Vaccine Trial Study Group^l

^a Charles Nicolle University Hospital, Rouen, France

^b Ludwig Maximilians University, Munich, Germany

^c Centro para Vacunas en Desarrollo, Santiago, Chile

^d University Hospital, Umeå, Sweden

^e Association Française de Pédiatrie Ambulatoire, France

^f Centre Hospitalier du Purpan, Toulouse, France

^g University of Hacettepe, Ankara, Turkey

^h Aventis Pasteur MSD, 8 Rue Jonas Salk, 69367 Lyon Cedex 07, France

ⁱ Creteil, France

^j University Hospital, Malmö, Sweden

^k Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, USA

^l Merck Sharp and Dohme, USA, Blue Bell, PA, USA

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Abstract

To reduce the number of injections needed to comply with paediatric vaccination requirements, a liquid, hexavalent vaccine (DTaP–IPV–PRP–T–HBs; Hexavac[®]; Aventis Pasteur MSD) has been developed for primary and booster vaccination of infants and toddlers. In extensive clinical studies, Hexavac[®] has been shown to be highly immunogenic. Seroconversion or seroprotective titres of antibodies against all antigens were achieved in the majority of infants following a primary series of three doses administered at 1–2-month intervals from 2 months of age. Hexavac[®] also induced immunologic memory, as evidenced by the anamnestic response to booster vaccination at 12–18 months of age. These responses were comparable with those seen following concomitant administration of Pentavac[™] (DTaP–IPV//PRP–T) and monovalent hepatitis B vaccine (H-B-Vax[®] II), and were also within the ranges observed for other relevant licensed vaccines. Clinical studies comparing the immunogenicity of Hexavac[®] administered at either 2, 3 and 4 months or 2, 4 and 6 months demonstrated that it can be used by either vaccination schedule. A further study also supported the use of primary doses of Hexavac[®] at 3 and 5 months with a booster at 12 months of age. Hexavac[®] demonstrated a good reactogenicity and tolerability profile. The most frequently reported adverse events after both primary and booster doses were local reactions of redness and swelling/induration and a systemic response of mild fever, irrespective of the vaccine used for priming. Hexavac[®] provided immunity against six important childhood diseases with a single injection at each visit.

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* Corresponding author. Tel.: +33-4-37-28-40-70; fax: +33-4-37-28-44-11.

E-mail address: lhessel@apmsd.com (L. Hessel).

¹ Members of the Hexavalent Vaccine Trial Study Group are listed in the [Appendix A](#).

1. Introduction

Increasing knowledge and understanding of infectious diseases and the availability of highly effective vaccines have led to a growing number of vaccines being recommended for use in infants. In some countries, at least 13 separate vaccinations are needed to vaccinate a child from birth to 6 years, and during some clinic visits, three or four separate injections can be indicated [1]. Cost, convenience, and compliance have contributed to the use of combination vaccines over monovalent vaccines. The use of combination vaccines reduces the discomfort associated with multiple injections, simplifies vaccination calendars and may enhance compliance. It can also increase the opportunities to vaccinate hard-to-reach patients or populations or frequent non-attenders, and ensure that adequate population coverage is maintained against diseases that may be perceived by some patients (or parents) to be unimportant [2]. A further benefit of combination vaccines is that they can facilitate the introduction of new vaccines into an already crowded calendar [3]. This becomes especially important with the introduction of new vaccines to prevent invasive pneumococcal or meningococcal disease.

As part of its expanded program in immunization (EPI), the World Health Organization (WHO) recommendations include universal childhood vaccination against diphtheria, tetanus, pertussis, poliomyelitis and hepatitis B, while vaccination against *Haemophilus influenzae* type b (Hib) should be included as appropriate to national capacities and priorities. The development of a multivalent vaccine capable of protecting against these six major diseases has been an evolutionary process, which started in the 1950s when diphtheria (D) and tetanus (T) toxoids were first combined with inactivated whole-cell pertussis (wP) vaccine. Concerns regarding the reactogenicity of wP prompted the development of the acellular pertussis (aP) vaccines, composed of pertussis toxin (PT) and typically one or more additional antigens, such as filamentous hemagglutinin (FHA), pertactin and/or fimbriae, purified from *Bordetella pertussis* [4]. A number of studies have demonstrated the superior safety and tolerability profile of these vaccines compared with wP [4,5] along with comparable immunogenicity [6] and effectiveness [4,7–9].

More recently, to further facilitate paediatric vaccination programmes, combination vaccines have been introduced in which inactivated poliovirus (IPV) and/or Hib and hepatitis B antigens are added to the core DTwP or DTaP combination. Although the eradication of poliomyelitis from the Western world, and its global decline, is a result of the successful use of oral polio vaccine (OPV) in most parts of the world [10], the potential risk of vaccine-associated paralytic poliomyelitis (VAPP) with OPV, has led many countries to switch to using IPV for routine immunization.

One of the most striking successes of vaccination in recent years has been the near disappearance of Hib disease following the introduction of the polyribosyl ribitol

phosphate (PRP)-conjugate vaccines in a number of countries. Overall, it has been estimated that, across Europe, current vaccination activity prevents approximately 10,000 cases of invasive Hib disease per year [11].

Vaccination against hepatitis B was added to the EPI in 1992. As infants and children are at highest risk of developing chronic HBV infection, the WHO proposed a strategy of global universal infant immunization [12]. To improve the uptake of universal hepatitis B vaccination, the WHO recommends administering the vaccine at the same time as other common childhood vaccines, such as DTP and Hib [13].

It has previously been shown that simultaneous administration of a pentavalent vaccine against diphtheria, tetanus, pertussis, poliomyelitis and Hib (DTaP–IPV–Hib; PentavacTM; Aventis Pasteur MSD) and a monovalent recombinant hepatitis B vaccine (H-B-Vax[®] II; Aventis Pasteur MSD) at separate sites for primary immunization of infants at 2, 3 and 4 months of age was generally well tolerated and produced good immune responses to all antigens [14]. On this basis, a liquid, hexavalent combination vaccine (DTaP–IPV–Hib–HBs; Hexavac[®]; Aventis Pasteur MSD) has been developed, which allows vaccination against diphtheria, tetanus, pertussis, poliomyelitis, Hib and hepatitis B by single injection at each visit. Here we review the available data on the immunogenicity and safety profile of Hexavac[®] in comparison with the relevant previously licensed vaccines.

2. Pharmaceutical profile

Hexavac[®] contains adjuvant-adsorbed, purified diphtheria (20 IU per dose), tetanus (40 IU per dose) and pertussis toxoids (25 µg per dose), adsorbed, purified FHA (25 µg per dose) and hepatitis B surface antigen (HBs, 5 µg per dose), IPV types 1 (40 D units per dose), 2 (8 D per dose) and 3 (32 D per dose), and Hib polysaccharide conjugated to tetanus protein (PRP–T, 12 µg per dose). Each dose of Hexavac[®] also contains aluminium adjuvant and a phosphate buffered saline solution. No preservative is included in the formulation, conforming to recommendations from the European Agency for the Evaluation of Medicinal Products for infant vaccines [15].

In the final formulation, the diphtheria and tetanus toxoids are prepared from the corresponding toxins by formaldehyde detoxification followed by purification. The acellular pertussis components are extracted from *B. pertussis* and separately purified. PT is detoxified with glutaraldehyde, while FHA is included in its native form. To obtain the IPV vaccine, the three poliovirus types are propagated on Vero cells, then purified by chromatography and inactivated by formaldehyde. The purified polysaccharide of *H. influenzae* type b is conjugated to tetanus protein. The content of PRP–T was increased by 20% compared with the content of the monovalent vaccine, Act-Hib to result in a similar concentration of unadsorbed PRP–T in Hexavac[®], as

approximately 20% of the antigen is adsorbed to aluminium during the formulation process. Finally, the HBsAg is purified from cultures of a recombinant strain of *Saccharomyces cerevisiae*.

3. Clinical profile of Hexavac®

The clinical study program was designed to show that Hexavac® provided protective immune responses that were not inferior to those of two reference vaccines, Pentavac™ and H-B-Vax® II, administered concurrently at separate injection sites, and that these responses were not influenced by the vaccination schedule. Comparisons with separate vaccine components were made during the development of Pentavac™. In addition, the immunogenicity data for Hexavac® were compared with those generated with monovalent vaccines for which protective efficacy against disease had been demonstrated in clinical trials. The seroconversion or seroprotection reference rates and acceptable differences were based on existing data from licensed monovalent and combination vaccines (Table 1) [16].

Seven phase III studies were conducted between 1996 and 1998, involving 3748 infants who received Hexavac® as a complete three-dose primary series between the ages of 2 and 6 months and 4286 toddlers who received a booster dose of Hexavac® during the second year of life. Separate studies of Hexavac® given in a three-dose primary series were performed to investigate its immunogenicity and safety in comparison with separate injections of Pentavac™ and H-B-Vax® II (Study A; pivotal study), the consistency of three different lots of vaccine with respect to immunogenicity (Study B), its safety in a large population cohort (Study C) and its immunogenicity and safety in different vaccination schedules (Study D) (Fig. 1). Studies A and D also investigated the immunogenicity and the safety of a fourth, booster dose of Hexavac®, and the safety of a fourth dose was also assessed in Study C. As a secondary endpoint,

study C also assessed the immunogenicity of the PRP-T component 1 month after the primary series and after the booster vaccination, respectively, in a subset of infants. A further three studies investigated the immunogenicity and safety of Hexavac® for booster vaccination in infants primed with either DTwP-IPV-Hib (Pentacoq®; Aventis Pasteur MSD) plus H-B-Vax® II (Studies F and G) or Pentavac™ plus H-B-Vax® II (Study H). In addition to the phase III program, the immunogenicity and safety of Hexavac® administered according to the 3–5/12 month schedule used in the Nordic countries and Italy has also been investigated (Study E).

3.1. Immunogenicity

3.1.1. Primary series

In the phase III studies, the primary endpoints for the assessment of immunogenicity for each antigen in Hexavac® were the percentage of infants achieving reference value equivalency (demonstrated by non-inferiority testing) for seroprotection or seroconversion rates 1 month after the third vaccine dose, and the percentage of infants who achieved at least a four-fold increase in antibody titres from baseline for pertussis components. The secondary endpoints were geometric mean titres (GMT) before and after vaccination (at 95% confidence intervals). One month after the primary series of three injections of Hexavac®, seroprotective levels of antibodies were present in 89.5–100% of infants, depending upon the antigen (Table 2).

3.1.1.1. Diphtheria–tetanus–IPV (types 1–3). At least 99% of infants in the phase III trials achieved seroprotective titre criteria for diphtheria neutralizing toxoids and tetanus antitoxins (Table 2) [16–18]. The GMT values and detectable antibody levels to poliovirus types 1, 2 or 3 following primary immunization with Hexavac® [16–18] were comparable to those elicited by other licensed vaccines [19–21].

Table 1

Statistical methodology for immunogenicity assessment of Hexavac given to infants as primary series: criteria for seroconversion and seroprotection rates per antigen and pre-defined acceptable differences (Studies A, B and D)

Study code/study no.	Study A		Study D		Study B	
	Pref ^a	δ^b	Pref ^a	δ^b	Pref ^a	δ^b
Criterion for seroconversion and seroprotection rates (%) per antigen						
HBs ≥ 10 mIU/ml	90	15	92	10	92	10 (15%) ^c
PRP ≥ 0.15 μ g/ml	98	10	98	9	99	10
PRP ≥ 1.0 μ g/ml	–	–	65	≥ 15	–	–
D ≥ 0.01 IU/ml	99	10	99	9	99	10
T ≥ 0.01 IU/ml	99	10	99	9	99	10
PT ≥ 4 -fold rise	90	15	92	10	92	10 (15%) ^c
FHA ≥ 4 -fold rise	90	15	92	10	92	10 (15%) ^c
Polio 1, 2, 3, ≥ 5	99	10	99	9	99	10

^a Reference response rate.

^b Pre-defined acceptable difference.

^c For secondary criteria.

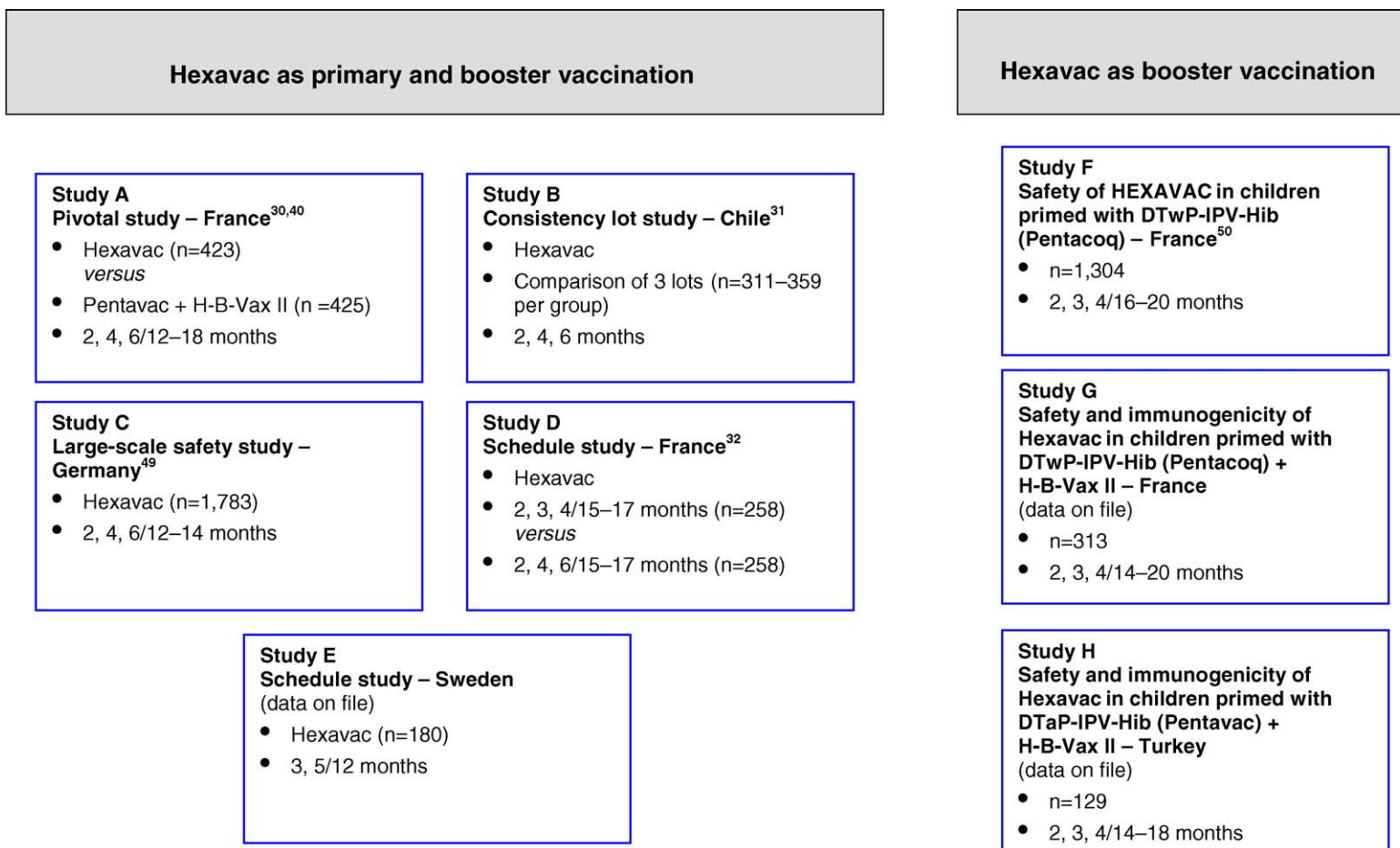


Fig. 1. Overview of phase III clinical development programme for Hexavac®.

Table 2
Immunogenicity of Hexavac® 1 month after the primary vaccination series (per protocol analysis)

Study [vaccination schedule (months)]	N ^a	Percentage of children achieving seroprotection or seroconversion antibody criteria (GMT)									
		Diphtheria EIA (≥0.01 IU/ml)	Tetanus EIA (≥0.01 IU/ml)	PT ^b CHO assay (≥4-fold rise per dilution)	PT ^c ELISA (≥4-fold rise EU/ml)	FHA ^c ELISA (≥4-fold rise EU/ml)	Poliovirus type 1 (≥5 per dilution)	Poliovirus type 2 (≥5 per dilution)	Poliovirus type 3 (≥5 per dilution)	PRP (≥0.15 µg/ml)	HBs (≥10 mIU/ml)
Study A											
[2, 4, 6] [30]	305–331	100 (0.467)	100 (2.07)	100 (50.8)	91.8 (64.7)	90.5 (141)	100 (470)	100 (505)	100 (846)	93.7 (2.06)	96.6 (434)
Study B ^d											
[2, 4, 6] [31]	269–300	100 (1.71)	100 (6.30)	100 (53.4)	93.0 (70.8)	91.3 (190)	100 (2459)	100 (1902)	100 (3010)	97.0 (3.95)	96.3 (458)
	257–289	100 (1.77)	100 (6.49)	100 (54.7)	91.0 (71.5)	90.8 (178)	100 (2007)	100 (1574)	100 (2398)	98.3 (4.37)	95.5 (420)
	236–257	100 (1.59)	100 (6.48)	100 (53.6)	93.0 (72.3)	93.5 (210)	100 (1986)	100 (1679)	100 (2239)	97.3 (4.05)	96.5 (331)
Study C ^e											
[2, 3, 4] (data on file)	112	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	95.5 (2.22)	N/A
Study D											
[2, 3, 4]	180–192	100 (0.291)	100 (1.05)	100 (37.7)	92.9 (58.8)	90.6 (129)	100 (243)	100 (162)	100 (371)	92.7 (1.42)	95.8 (241)
[2, 4, 6] [32]	158–170	100 (0.439)	100 (1.89)	100 (52.3)	89.5 (62.8)	91.8 (141)	99.4 (438)	100 (448)	99.4 (739)	93.5 (2.26)	97.1 (396)

GMT, geometric mean antibody titer; HBs, hepatitis B surface antigen; PRP, polyribosyl ribitol phosphate; PT, pertussis toxin; FHA, filamentous hemagglutinin; N/A, no data available.

^a Number varies according to the number of infants assayed for each antigen.

^b Chinese hamster ovary assay; seroconversion considered as ≥4 (reciprocal dilution in seroneutralization).

^c PT and FHA measured using enzyme-linked immunoassay and seroconversion considered as a four-fold increase in respective antibody titers.

^d The immunogenicity of three different lots of Hexavac® was investigated in this study.

^e Large-scale safety study. The immunogenicity of PRP-T was assessed in a subset of infants.

3.1.1.2. Pertussis. For all phase III studies, the percentage of infants achieving a four-fold rise in antibody titres to PT or FHA was greater than 89%, following a three-dose primary series of Hexavac® (Table 2) [16–18]. GMT values and the rise in PT and FHA antibodies elicited by Hexavac® were comparable with those following separate injections of Pentavac™ plus H-B-Vax® II (Study A) [16].

3.1.1.3. Haemophilus influenzae type b. One month after the third dose of Hexavac®, the seroprotection rate to PRP ($\geq 0.15 \mu\text{g/ml}$) varied between 92.7 and 98.3% (Table 2) [16–18]. The anti-PRP immune response in terms of seroprotection rate ($\geq 0.15 \mu\text{g/ml}$) was comparable with that of Pentavac™ as established by the pivotal study [16]. The GMT values of PRP antibodies varied according to the schedule and the population (1.42–4.37 $\mu\text{g/ml}$ and 62.5–85.1%, for GMTs and seroprotection rates $\geq 1 \mu\text{g/ml}$, respectively), with lower responses seen in infants vaccinated according to the compressed 2–3–4-month schedule and the highest responses occurring in Chilean infants.

3.1.1.4. Hepatitis B. In all phase III studies, more than 95% of infants achieved seroprotective titres ($\geq 10 \text{ mIU/ml}$) of anti-HBs antibodies, 1 month following completion of the primary series (Table 2) [16–18].

In summary, these data showed that the immunogenicity of a primary series of Hexavac® in terms of seroprotection and seroconversion rates was non-inferior to that of reference vaccines. In a direct comparison, Hexavac® met the criteria for clinical equivalence to Pentavac™ and H-B-Vax® II given concomitantly at two separate sites, as it elicited similar seroprotection and seroconversion rates against all antigens (Table 3). Following the three-dose primary immunization series (2–4–6 months), although GMTs for HBs and PRP antibodies were lower in children receiving the

combined vaccine compared with separate administration, seroprotection and seroconversion rates 1 month after the third dose of Hexavac® were $>90\%$ for all antigens [16]. Furthermore, a comparison of three separate lots of Hexavac® found that seroprotection or seroconversion rates and GMT values were equivalent across lots for all components 1 month after the third dose [17].

3.1.2. Persistence of immunogenicity

Persistence of immunogenicity was evaluated in the pivotal phase III study (Study A) and in a study comparing different primary immunization schedules (Study D). Antibodies to D, T, PT, FHA and poliovirus types 1–3 remained at satisfactory levels from the end of the primary vaccination series to the time of the booster injection at 12–18 months of age (Table 4) [18,22].

Between 75.9 and 85.2% of children primed with Hexavac® had seroprotective titres of PRP antibody ($\geq 0.15 \mu\text{g/ml}$) at the time of booster vaccination, irrespective of the primary immunization schedule [18]. The GMT values and seroprotection rates at the $\geq 1 \mu\text{g/ml}$ level were lower than those seen after priming with Pentavac™ and H-B-Vax® II [22].

Seroprotection rates for HBs antibodies at the time of booster vaccination ranged between 72.8 and 85.7%, regardless of the immunization regimen of the primary series [18,22].

3.1.3. Response to booster vaccination

Hexavac® induced an immunologic memory for all antigens, as demonstrated by the good anamnestic response for all components following a booster injection at 12–18 months of age (Table 4).

One month after the administration of a booster dose of Hexavac®, all but one of the 807 children included in the per protocol analysis had anti-PRP titres $\geq 0.15 \mu\text{g/ml}$ and 97.4% of children achieved titres $\geq 1 \mu\text{g/ml}$. The priming effect for PRP afforded by Hexavac® was strongly supported

Table 3
Immunogenicity of a three-dose primary series of Hexavac® compared with Pentavac™ plus H-B-Vax® II (per protocol analysis (Study A) [30])

Antigen	Primary endpoint	Pentavac™ + H-B-Vax® II ($n = 301\text{--}332$) ^a		Hexavac® ($n = 305\text{--}331$) ^a		[95% CI] ^b Reference-Hexavac®
		Seroprotection/ seroconversion rate	GMT	Seroprotection/ seroconversion rate	GMT	
Diphtheria	$\geq 0.01 \text{ IU/ml}$	99.7	0.48	100	0.47	[−0.91; 0.30]
Tetanus	$\geq 0.01 \text{ IU/ml}$	100	1.65	100	2.07	
PT	$\geq 4\text{-fold increase}$	93.7	87.4	91.8	64.7	[−2.24; 6.01]
FHA	$\geq 4\text{-fold increase}$	88.6	118	90.5	141	[−6.72; 2.94]
Polio virus type 1	$\geq 5 \text{ (1/dil)}$	100	343	100	470	
Polio virus type 2	$\geq 5 \text{ (1/dil)}$	100	315	100	505	
Polio virus type 3	$\geq 5 \text{ (1/dil)}$	100	707	100	846	
PRP	$\geq 0.15 \mu\text{g/ml}$	99.7	3.69	93.7	2.06	[3.35; 8.74]
HBs	$\geq 10 \text{ mIU/ml}$	100	983	96.6	434	[1.41; 5.30]

^a Number varies according to the number of infants assayed for each antigen.

^b 95% confidence interval of the difference in seroprotection/seroconversion rates between the reference vaccines (Pentavac™ and H-B-Vax® II) and Hexavac®; criterion for success = upper bound below δ (pre-established acceptable difference [16]). HBs, hepatitis B surface antigen; PRP, polyribosyl ribitol phosphate; PT, pertussis toxin; FHA, filamentous hemagglutinin.

Table 4

Persistence of antibodies and immunogenicity of a booster injection of Hexavac® in toddlers aged 12–18 months who completed a primary immunisation series with Hexavac® (per protocol analysis)

Study [vaccination schedule (months)]	N ^a	Percentage of children with seroprotective antibody levels ^b (GMT)											
		Diphtheria (IU/ml) EIA	Tetanus (IU/ml)	PT ^c CHO assay (per dilution)	PT ^d (EU/ml)	FHA ^d (EU/ml)	Poliovirus type 1	Poliovirus type 2 (per dilution)	Poliovirus type 3 (per dilution)	PRP (μg/ml)		HBs (mIU/ml)	
										≥0.15	≥1.0		
Study A													
[2, 4, 6/12–15.5]													
Pre-booster	145–162	41.4 (0.067)	93.9 (0.447)	15.4 (14.3)	8.9 (14.0)	N/A (56.4)	96.8 (77.9)	96.8 (91.5)	98.1 (121)	79.0 (0.46)	30.9 (0.46)	85.7 (62.3)	
Post-booster	153–165	100 (1.22)	100 (5.52)	93.7 (68.1)	94.5 (74.6)	29.8 (176)	100 (2226)	100 (2704)	100 (3644)	100 (13.7)	95.8 (13.7)	98.2 (967)	
GMTR	143–162	19.1	12.9	4.80	5.37	3.11	29.4	28.8	28.9	29.3	29.3	15.6	
[2, 4, 6/15.5–18]													
Pre-booster	127–158	38.6 (0.054)	82.2 (0.327)	13.2 (12.6)	4.3 (7.99)	N/A (30.9)	95.8 (81.2)	96.5 (91.0)	97.9 (121)	75.9 (0.34)	18.4 (0.34)	77.5 (35.3)	
Post-booster	149–162	97.5 (1.87)	100 (7.17)	96.2 (87.7)	92.6 (81.3)	69.1 (184)	100 (3132)	100 (3178)	100 (4577)	100 (20.5)	97.5 (20.5)	95.0 (898)	
GMTR [40]	126–158	34.2	21.9	7.06	10.6	6.00	36.7	36.1	37.5	61.3	61.3	27.3	
Study C ^e													
[2, 4, 6/12–14]													
Pre-booster	109	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	29.4 (0.44)	29.4 (0.44)	N/A	
Post-booster	108									100 (11.9)	95.4 (11.9)		
GMTR (data on file)										26.8	26.8		
Study D													
[2, 3, 4/15–17]													
Pre-booster	164–176	30.5 (0.050)	84.0 (0.279)	13.6 (14.1)	6.9 (8.77)	N/A (30.5)	89.4 (46.2)	88.2 (40.5)	90.5 (63.4)	85.2 (0.62)	39.8 (0.62)	85.5 (51.4)	
Post-booster	172–176	99.4 (1.78)	100 (9.23)	98.3 (111)	96.0 (99.9)	75.4 (205)	100 (3036)	100 (3217)	100 (4716)	99.4 (45.7)	98.3 (45.7)	94.9 (1465)	
GMTR	164–176	35.0	33.0	7.86	11.4	6.70	66.7	78.9	74.0	73.3	73.3	29.1	
[2, 4, 6/15–17]													
Pre-booster	155–173	31.0 (0.056)	88.6 (0.314)	9.4 (12.5)	4.1 (8.50)	N/A (32.2)	95.8 (75.4)	92.8 (72.0)	99.4 (112)	77.5 (0.42)	26.0 (0.42)	72.8 (36.7)	
Post-booster	167–175	99.4 (1.72)	100 (8.09)	95.3 (92.4)	94.3 (81.6)	67.8 (192)	100 (2802)	100 (2985)	100 (4793)	100 (22.5)	98.3 (22.5)	97.7 (1089)	
GMTR [32]	153–172	29.6	25.5	7.35	9.49	5.89	39.2	42.5	43.2	54.4	54.4	28.0	

GMT, geometric mean antibody titer; GMTR, geometric mean of post-dose to pre-dose titer ratio; HBs, hepatitis B surface antigen; PRP, polyribosyl ribitol phosphate; PT, pertussis toxin; FHA, filamentous hemagglutinin; N/A, not available.

^a Number varies according to the number of infants assayed for each antigen.

^b Antibody levels considered to be seroprotective are ≥10 mIU/ml for HBs, ≥1.0 µg/ml for PRP (for the *Haemophilus influenzae* type b component), GMT ≥32 for PT, a four-fold increase in FHA antibody titer relative to pre-booster levels, ≥0.1 IU/ml for diphtheria and tetanus antigens and ≥5 (reciprocal dilution in seroneutralization) for poliovirus types 1–3.

^c PT measured using Chinese hamster ovary assay; antibody levels considered to be seroprotective are ≥32 (reciprocal dilution in seroneutralization).

^d PT and FHA measured using enzyme-linked immunoassay.

^e Large-scale safety study. Immunogenicity of PRP-T assessed in a subset of children.

by the high GMT values (13.7–45.7 µg/ml) and the marked 29.3–73.3-fold increase in mean antibody titres following administration of the booster dose.

Between 94.9 and 98.2% of children who received four doses of Hexavac[®] achieved seroprotective levels of anti-HBs antibodies at the ≥ 10 mIU/ml level 1 month after a booster dose of Hexavac[®] [18,22]. The anamnestic response to HBs was marked for both primary immunization regimens, as evidenced by values of 15.6–29.1 for the geometric mean of post-dose to pre-dose titre ratios (Table 4).

The immune response to a booster dose of Hexavac[®] following priming with pentavalent vaccines containing either aP (Pentavac[™]; Study H) or wP (Pentacoq[®]; Study G) plus H-B-Vax[®] II has been examined in two studies. Both studies showed a good anamnestic response to the booster dose of Hexavac[®]. GMT levels and seroconversion rates following the booster dose of Hexavac were similar to those reported for Pentavac[™] following priming with Pentacoq[®] [23].

3.1.4. The effect of dosing schedule on immunogenicity

The immunogenicity of Hexavac[®] in different dosing schedules was investigated in a study that compared the effects of primary immunization at 2–4 months with 2, 4 and 6 months (Study D). Hexavac[®] administered according to either schedule provided antibody titres that were within a clinically acceptable range and consistent with protection throughout the first 15 months of life [18]. Although the GMT for PRP was higher in children vaccinated at 2, 4 and 6 months compared with the 2–3–4-month schedule, the percentage of infants that achieved at least 0.15 µg/ml was similar in the two groups after the primary series (93.5% vs. 92.7%). The 2–4–6-month schedule also elicited higher GMT levels for anti-HBs antibodies after the primary series. In contrast, both the pre- and post-booster GMTs for PRP and HBs appeared higher among children who were primed using the 2–3–4-month schedule [18].

The immunogenicity of Hexavac[®] administered according to the 3–5/12-month immunization schedule used by the Nordic countries and Italy has also been investigated in a study involving 180 Swedish infants (Study E). One month after the third dose, 100 and 95.9% of infants had seroprotective levels of anti-PRP antibodies at the ≥ 0.15 and ≥ 1 µg/ml levels, respectively. In addition, 97.2% of infants were seroprotected against HBs at the ≥ 10 mIU/ml level. The immune responses to the D, T, aP and IPV components post-dose three all met the pre-defined criteria for seroprotection or seroconversion (Table 5).

3.2. Safety

During the course of the clinical development of Hexavac[®], 3905 infants received at least one dose of vaccine for primary immunization, representing a total of 11,525 doses. Of these, 11,507 (99.8%) doses completed

Table 5
Immunogenicity of Hexavac[®] 1 month after the third dose of a three-dose series at 3, 5 and 12 months of age (per protocol analysis; Study E)

N ^a	Percentage of children achieving seroprotection or seroconversion antibody criteria ^b (GMT)									
	Diphtheria EIA (IU/ml)	Tetanus (IU/ml)	PT ^c CHO assay (per dilution)	PT ^d (EU/ml)	FHA ^d (EU/ml)	Poliovirus type 1 (per dilution)	Poliovirus type 2 (per dilution)	Poliovirus type 3 (per dilution)	PRP (µg/ml) ≥ 0.15 ≥ 1.0	HBs (mIU/ml)
118–146	100 (4.44)	100 (7.58)	97.8 (94.1)	100 (141)	72.3 (256)	100 (3808)	100 (2807)	100 (3300)	100 (12.3)	97.2 (894)

GMT, geometric mean antibody titer; HBs, hepatitis B surface antigen; PRP, polynitrosyl ribitol phosphate; PT, pertussis toxin; FHA, filamentous hemagglutinin.

^a Number varies according to the number of infants assayed for each antigen.

^b Antibody levels considered to be seroprotective are ≥ 10 mIU/ml for HBs, ≥ 0.1 IU/ml for diphtheria and tetanus antigens, GMT ≥ 32 for PT, a four-fold increase in FHA antibody titer relative to pre-dose 3 levels and ≥ 8 (reciprocal dilution in seroneutralization) for poliovirus types 1–3.

^c PT measured using Chinese hamster ovary assay; antibody levels considered to be seroprotective are ≥ 32 (reciprocal dilution in seroneutralization).

^d PT and FHA measured using enzyme-linked immunoassay.

Table 6

Solicited local and systemic reactions (%) within 3 days of each dose of Hexavac® during the primary immunisation series (2, 3, 4 months or 2, 4, 6 months) in French, German and Chilean infants (Studies A–D plus phase II study in 157 French infants)

	First dose	Second dose	Third dose	All doses
Number of infants	3897	3826	3784	11507
Local reactions				
Any local reaction	14.1	17.9	19.6	17.2
Redness ≥ 2 cm	6.08	9.96	12.2	9.37
Induration and/or swelling ≥ 2 cm	10.2	12.6	13.4	12.0
Systemic reactions				
Irritability and/or unusual crying	25.7	25.3	22.0	24.4
Drowsiness	15.2	8.76	6.29	10.1
Inconsolable crying >3 h	0.23	0.16	0.08	0.16
Fever $\geq 38^\circ\text{C}$				
38–38.9°C	9.06	18.0	18.0	15.0
39–39.9°C	0.44	1.62	2.67	1.56
$\geq 40^\circ\text{C}$	0.03	0.13	0.16	0.10

safety and reactogenicity assessments. In addition, 4437 children received a booster dose of Hexavac® during the second year of life.

In all studies, evaluation of safety included:

1. Immediate reactions within the first 30 min after each dose.
2. Local reactions and systemic events from 30 min to 72 h following each dose.
3. Any adverse event that resulted in a visit to a physician 4 and 30 days after each injection.
4. Any serious adverse event (SAE) occurring throughout the trial.

Tolerability data for each country were considered separately, as well as globally, because of possible local differences in the reporting of reactogenicity.

3.2.1. Primary series

Overall, the reactogenicity of Hexavac® administered between 2 and 6 months of age was low, with the most frequently reported adverse reactions after primary vaccinations being local reactions (redness; induration and/or swelling) ≥ 2 cm and irritability and/or crying within the first 72 h after each injection. These reactions were generally transient and resolved without sequelae.

At least one local reaction during the first 3 days after vaccination was reported for 17.2% of doses of Hexavac® (Table 6). Redness ≥ 2 cm was reported following 9.37% of all doses and induration and/or swelling ≥ 2 cm was reported for 12.0% of doses. The rate of large (≥ 5 cm) local reactions was low, however. Redness and induration ≥ 5 cm were observed following 1.39 and 0.96% of doses, respectively, while 0.12 and 0.10% of doses were associated with redness and induration >7 cm, respectively. These large local reactions were transient and resolved within 2–9 days.

Irritability and/or unusual crying was the most frequently reported systemic reaction, and was associated with 24.4% of all doses of Hexavac® during the primary vaccination series. However, inconsolable crying lasting for more than 3 h occurred after only 0.16% of doses (Table 6). The rate of mild fever (38.0–38.9°C) tended to be lowest after the first dose of Hexavac®, and the overall rate of mild fever was 15.0%. Fever above 39°C was reported infrequently (39.0–39.9°C: 1.56% of doses; $\geq 40^\circ\text{C}$: 0.1% of doses), with an expected trend towards an increased rate with successive doses of vaccine.

In the comparative study of Hexavac® and Pentavac™ plus H-B-Vax® II (Study A), the incidence of local reactions following the primary series with Hexavac was similar to that seen with Pentavac™, while the incidence of systemic

Table 7

Solicited local and systemic reactions (%) within 3 days of a booster dose of Hexavac® at age 12–20 months

Vaccine for primary immunisation	N	Any local reaction	Redness ≥ 2 cm	Induration ≥ 2 cm	Fever $\geq 38^\circ\text{C}$			Irritability/unusual crying	Drowsiness	Inconsolable crying >3 h
					38–38.9°C	39–39.9°C	$\geq 40^\circ\text{C}$			
Hexavac® (Studies A, C and D plus phase II study)	2688	21.3	17.4	15.6	24.9	3.61	0.74	15.6	5.73	0.19
Pentacoq® (Studies F and G)	1608	40.5	28.8	26.2	35.3	3.79	0.31	21.3	7.03	0.25
Pentavac™ (Study H)	125	34.1	30.2	31.7	21.6	4.0	0	21.6	3.2	0.80

events was comparable following vaccination with either Hexavac[®] or PentavacTM plus H-B-Vax[®] II [16].

The lowest incidence of adverse reactions was seen in the large-scale safety study of 1783 German infants who received Hexavac[®] at 2, 4 and 6 months of age (Study C). Redness ≥ 2 cm and induration/swelling ≥ 2 cm were reported after 6.7 and 7.1% of doses in the primary series, while the most frequent systemic adverse events were irritability (19.3% of doses) and fever ≥ 38.0 °C (15.4% of doses). Fever ≥ 40.0 °C was reported following 0.1% of doses and inconsolable crying for more than 3 h was reported for 0.2% of doses [24].

A total of 247 SAEs were reported throughout the clinical development program, of which five were considered by the investigators to be related to vaccination with Hexavac[®]. There was one case of high-pitched crying prolonged for 4 h and one hypotonic–hypo-responsive episode (HHE) 1 day after the first dose. In addition, one case of intussusception 1 day after the second dose, one case of pyelonephritis due to *Escherichia coli* infection 5 days after the third dose, and a case of influenza-like syndrome associated with gastroenteritis 2 days after the second dose were considered to be probably, possibly and doubtfully related to vaccination, respectively.

3.2.2. Booster vaccination

Rates of local and systemic adverse events were higher when Hexavac[®] was given as a booster vaccination compared with rates observed following the primary series. The overall rate for any local reaction increased from 17.2% of all doses for the primary series (Table 6) to 21.3% for the booster injection among children primed with Hexavac[®] (Table 7), with redness ≥ 2 cm, induration ≥ 2 cm and fever being more common after booster vaccination. However, fever ≥ 40 °C occurred in 0.55% of children who received a booster dose of Hexavac[®].

The overall incidence of large local reactions in French and German children following booster vaccination with Hexavac[®] was low, with 2.6% of doses associated with redness ≥ 5 cm and 2.16% associated with induration ≥ 5 cm. Redness and induration > 7 cm were associated with 0.22 and 0.26% of doses, respectively.

In the pivotal study, the incidence of local reactions was comparable for Hexavac[®] and PentavacTM, while the rates of systemic reactions with Hexavac[®] were comparable with those following booster doses of PentavacTM and H-B-Vax[®] II [22]. In the schedule study (Study D), the incidence of local and systemic reactions following a booster dose of Hexavac[®] was unaffected by the schedule used for the primary vaccination series [18].

The rates of local and systemic adverse events did not differ markedly with the type of vaccine used for the primary series. The reactogenicity profiles of Hexavac in children primed with either Pentacoq[®] [25] or PentavacTM were comparable with those for children who received a primary series of Hexavac[®] (Table 7).

Overall, 33 SAEs were reported following the total of 4437 booster doses of Hexavac and only three of these events (febrile convulsions, which appeared a few hours after vaccination) were considered to be probably associated with the vaccine. No cases of HHE were reported.

4. Discussion

A number of issues have been raised by the development of combination vaccines, including the possibility of interference in the immune responses to PRP and HBs and the degree of protective efficacy conferred by acellular pertussis vaccines comprising different pertussis antigens. The clinical data presented in this review show that the level of immune response to all antigens elicited by a three-dose primary series plus booster of Hexavac[®] was comparable to that following immunization with PentavacTM alone [20,26] and with PentavacTM plus H-B-Vax[®] II [22]. Furthermore, the immunogenicity of Hexavac[®] was shown to be acceptable when it was administered according to the main vaccination schedules (2–3–4/12–18; 2–4–6/12–18; 3–5/12) currently used in Europe. Hexavac[®] was approved by the European Agency for the Evaluation of Medicinal Products in October 2000. It is already available in Germany, Austria, Italy, Spain and Switzerland, and will be progressively introduced throughout the European Union.

The overall immune response to PRP following a primary series with Hexavac[®] was within the range reported for PentavacTM [20], although the GMT values for anti-PRP antibodies were approximately twice as high in the Chilean population as in the French infants. This is consistent with previous reports of a vigorous response to PRP among Chilean infants [19,27].

The results of the phase III studies with Hexavac[®] were also consistent with observations from studies of other licensed combination vaccines with a Hib component that have provided evidence of interference in the PRP response [28–30]. Although GMTs were somewhat lower in the hexavalent vaccine group compared with the pentavalent group, anti-PRP antibody rates ≥ 0.15 µg/ml elicited by Hexavac[®] were consistent with protection against Hib disease. The primary criterion for the evaluation of the immune response to PRP was a seroconversion rate above the antibody threshold generally considered to confer short-term protection (≥ 0.15 µg/ml) [31,32]. Hexavac[®] surpassed this criterion in $> 92\%$ of children following a three-dose primary series. Furthermore, the seroprotection rates obtained with Hexavac[®] were above those reported for the monovalent PRP-D vaccine that was shown to be protective against invasive Hib disease during efficacy trials in Finland [33,34] and were within the same range as that seen in native American Navajos and which was associated with a dramatic reduction in the incidence of invasive disease in that population [35].

There has recently been discussion of the levels of anti-PRP antibodies that are associated with protection after immunization with conjugate vaccines. The thresholds of $\geq 0.15 \mu\text{g/ml}$ for short-term protection and $\geq 1 \mu\text{g/ml}$ for long-term protection were derived from passive immunization or immunization with unconjugated Hib polysaccharide vaccines [36]. However, efficacy and effectiveness data from infants immunized with Hib conjugate vaccines suggest that protective antibody titres are closer to the $\geq 0.15 \mu\text{g/ml}$ level [35,37]. For example, a study performed in the UK, where immunization is provided by PRP-T at 2–4 months of age without booster in the second year of life, found that at age 1 year less than 62% of children had PRP antibody titres $\geq 0.15 \mu\text{g/ml}$ [38]. Nevertheless, the observed efficacy to age 4 years was 94.7–99.1% [37]. In addition, controlled efficacy and effectiveness trials of Hib conjugate vaccines have shown greater long-term protective efficacy than would have been predicted by the application of either a $1.0 \mu\text{g/ml}$ or even a $\geq 0.15 \mu\text{g/ml}$ threshold for anti-PRP antibody titres [32,33,35,39,40].

In the present series of studies with Hexavac[®], persistence of anti-PRP antibodies up to the time of booster vaccination was within the same range as has been reported previously for a three-dose primary series of PRP-T combined with DTaP [26,28,41] or DTaP–IPV [20]. It was also comparable with the levels seen in double-blind efficacy studies with the PRP-D [34] and PRP–OMP [35] vaccines, which have demonstrated protective efficacy against invasive Hib disease. Furthermore, the seroprotection rates for PRP $\geq 0.15 \mu\text{g/ml}$ were greater than the rate of 62% reported in the effectiveness study performed with PRP-T in the UK [38].

In the study that compared Hexavac[®] administered at 2–4 months with the 2–4–6-month schedule, the percentage of infants with PRP antibody levels $\geq 1.0 \mu\text{g/ml}$ after the primary series was not predictive of protective antibody levels $\geq 0.15 \mu\text{g/ml}$ throughout the first 15 months of life. Nevertheless, children in both study arms achieved comparable and clinically acceptable GMT levels and seroprotection rates following a booster dose of Hexavac[®] at 15–17 months. Both vaccination schedules, therefore, appeared similarly effective, with any apparent advantage of the 2–4–6-month schedule for the primary series being counterbalanced by the higher responses to booster vaccination seen in children who received the primary series at 2–4 months.

Overall, when Hexavac[®] was administered as either a 2–3–4-month schedule or 2–4–6-month schedule with booster vaccination at 15–17 months, the post-primary, pre-booster and post-booster anti-PRP responses seen with either schedule were consistent with those previously reported for both monovalent Hib vaccines for which controlled efficacy data are available, as well as other Hib-containing combined vaccines such as Pentavac[™] (Fig. 2).

The anti-PRP level after primary vaccination is known to reflect only part of the immune response to the conjugated vaccines. A major protective role may be played by

cell-based immunologic memory, as indicated by booster response elicited by either unconjugated or conjugated PRP. The seroprotection rates and GMT values for anti-PRP antibodies following a booster dose of Hexavac[®] were similar to those reported after primary and booster vaccination with Pentavac[™] [20,26], and with other Hib-containing vaccines [26,36,41–44]. This strong anamnestic response demonstrated that Hexavac[®] effectively primes the immune system. Active Hib disease surveillance in Germany under the auspices of the national paediatric surveillance system (ESPED) has shown that vaccines with anti-PRP responses within the same range as those seen with Hexavac[®] are associated with excellent control of the disease [45]. Active surveillance of Hexavac[®] vaccinees for 3 years will form part of the on-going ESPED programme.

The inclusion of HBs in Hexavac[®] is intended to increase vaccination coverage and thereby increase protection against hepatitis B later in life. Several controlled clinical studies have established that a level of anti-HBs antibodies $\geq 10 \text{ mIU/ml}$ is indicative of seroprotection against hepatitis B [46–48]. It is important to note that despite a trend to lower GMTs following Hexavac[®] compared to the separate administration of H-B-Vax[®] II, the seroprotection rates achieved after primary immunization with Hexavac[®] were within the range (87.7–100%) that has been reported for other DTaP combination vaccines containing hepatitis B given as a three-dose primary series between 2 and 6 months of age [49,50], and also for a combination vaccine against Hib and hepatitis B (92.1%) given at 2 and 4 months of age [51]. The antibody titres against HBs, and particularly the seroprotection rates at the 10 mIU/ml level, were also comparable to those seen with Pentavac[™] plus H-B-Vax[®] II [16] and with other licensed hepatitis B vaccines [52]. Furthermore, GMT values and seroprotection rates for HBs antibodies at the time of booster vaccination were within the range observed following primary immunization with monovalent hepatitis B vaccine [53] or the bivalent Hib-HBs vaccine [51].

The key to long-term protection against hepatitis B is the persistence of a strong immunologic memory [54]. A number of studies have demonstrated that previously vaccinated subjects retain the ability to mount a rapid anamnestic response to booster vaccination [55–58], even where anti-HBs antibodies have declined to low or undetectable levels. In addition, it has been found that vaccinated subjects continued to be protected against symptomatic and chronic HBV infections although their antibody titres had declined below the 10 mIU/ml level [59,60]. The studies in which children received a primary series and booster dose of Hexavac[®] provided evidence of a strong anamnestic response to the booster vaccination [18,22].

An important issue in the development of aP vaccines is the absence of positive serological correlates of protection against pertussis for the various different vaccines [61]. Whilst it is recognized that the presence of antibodies against PT is necessary for protection against *B. pertussis*

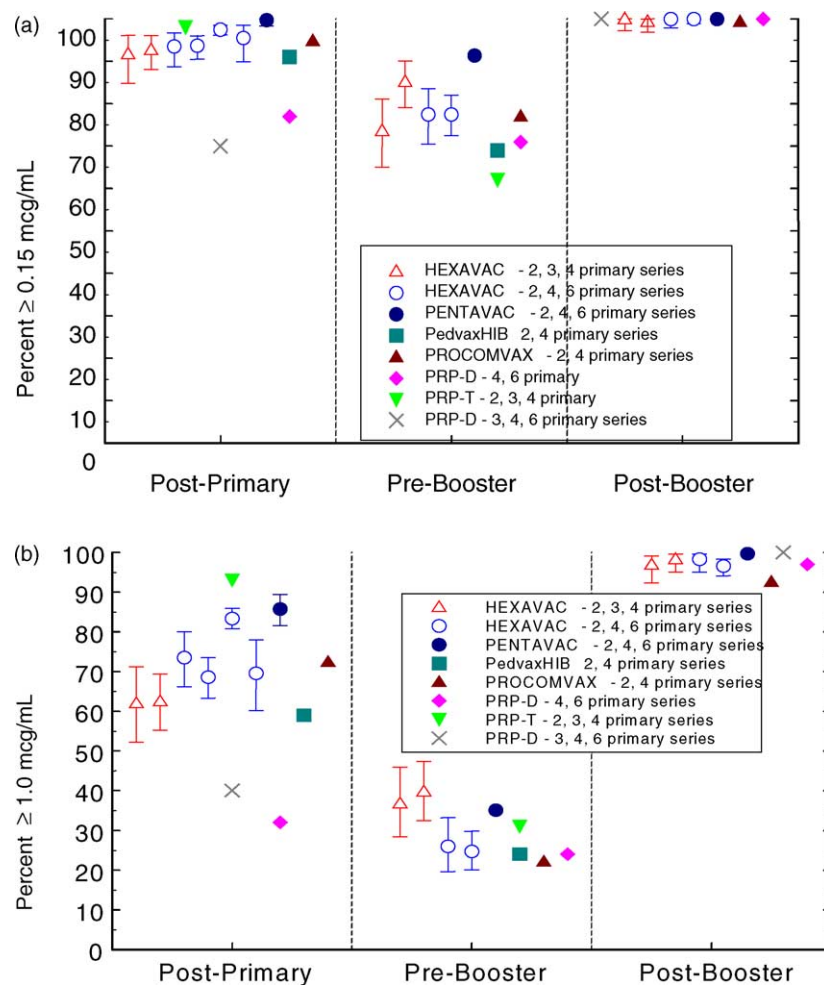


Fig. 2. (a) Anti-PRP responses by bleed interval; % ≥ 0.15 μ g/mL (with 95% CI). (b) Anti-PRP responses by bleed interval; % ≥ 1.0 μ g/mL (with 95% CI). (△) Hexavac[®] 2–3–4 primary series; (○) Hexavac[®] 2–4–6 primary series; (●) Pentavac[™] 2–4–6 primary series; (■) PedvaxHIB[®] 2–4 primary series; (▲) PROCOMVAX[®] 2–4 primary series; (◆) PRP-D 4–6 primary series; (▼) PRP-T 2–3–4 primary series; (×) PRP-D 3–4–6 primary series.

infection [9], and a vaccine containing only PT was shown to be clinically effective [62], this does not appear to be related to antibody titre or duration of antibody response. However, studies suggest that the additional presence of FHA is associated with a greater degree of protection, particularly against mild or asymptomatic disease, compared with the monocomponent vaccine [63,64]. Furthermore, during an outbreak of pertussis in Finland, schoolchildren with higher anti-FHA antibody titres remained healthy while those with lower titres contracted the disease [65].

In the absence of serological correlates of protection, clinical efficacy and effectiveness remain the most important criteria for evaluating protection conferred by pertussis vaccines. Evidence for the effectiveness of the aP component used in Hexavac[®] has been obtained both in a randomized clinical trial and in routine use. In a comparative study of a DTaP vaccine with DTwP performed in Senegal, the protective efficacy of the DTaP vaccine over the 12-month period following the third dose was within the same range as that conferred by the DTwP vaccine [66]. Analysis of data from

a 6-year follow-up period has suggested that the relative protective efficacy of the two vaccines was maintained over this time (Simondon, unpublished observations). In addition, a surveillance programme to assess the effectiveness of universal immunization with aP vaccine, which began in 1996, is being undertaken in Sweden. Preliminary results show a dramatic reduction in the incidence of pertussis, especially in children under 5 years of age. Pentavac[™], which contains the same aP components as Hexavac[®], has been used in approximately two-thirds of the Swedish birth cohort (according to the 3–5/12-month schedule), so this should provide definitive information on the field effectiveness of the aP component.

The clinical study programme for Hexavac[®] has demonstrated that it has a good safety and tolerability profile. Although the number of local reactions following the primary series varied across the different populations, the overall incidence of such reactions was within the range reported for existing vaccines containing the same antigens [16]. Similarly, the overall occurrence of systemic adverse

events fell within the range reported for PentavacTM plus H-B-Vax[®] II [16]. Furthermore, the overall reactogenicity profile of booster immunization with Hexavac[®] was comparable with that previously seen following booster injections of PentavacTM [20].

5. Conclusion

The clinical study programme with the liquid hexavalent DTaP–IPV–PRP–T–HBs vaccine, Hexavac[®], has demonstrated that it was generally well tolerated and induced seroprotection and seroconversion rates that were within the range for other licensed vaccines containing aP. In particular, although the immune responses to the PRP and HBs components were lower than those obtained with separate administration of Hib or hepatitis B components, there were comparable to those that have been reported with vaccines that have been shown to be efficacious in the prevention of Hib disease and hepatitis B, respectively. Furthermore, Hexavac[®] conferred excellent priming for all the component antigens, as evidenced by the good responses to booster vaccination.

The data presented here suggest that concerns regarding the potential for immune interference with combination vaccines remain theoretical and, for Hexavac[®], are outweighed by the benefits it can bring to paediatric vaccination schedules and immunization programmes by permitting vaccination against six important diseases with a single ready-to-use formulation.

Appendix A

The Hexavalent Vaccine Trial Study Group comprised Dr. A. Abeille, Dr. R. Amar, Dr. J-P. Arsene, Dr J-M. Aurand, Dr. L. Azoulay, Dr. E. Badescou, Dr. S. Barrois, Dr. N. Baudino, Dr. M. Béal, Dr. V. Beaudé-Chervet, Dr. P. Berlier, Dr. E. Billard, Dr. L. Billet, Dr. B. Blanc, Dr. J-P. Blanc, Dr. D. Bohu, Dr. C. Bonardo, Dr. C. Bossu, Dr. G. Bovero, Dr. M. Brieve, Dr. G. Brun, Dr. B. Collet, Dr. G. Danjou, Dr. T. David, Dr. A. Defour, Dr. C. Dispa, Dr. P. Dudognon, Dr. J-P. Feit, Dr. P. Feurgard, Dr. J-C. Franceschini, Dr. A. Gerbay, Dr. R. Ghez, Dr. J. Ghipponi, Dr. M-F. Grancher, Dr. G. Guerrier, Dr. J-L. Guillon, Dr. F. Haagen-Tanani, Dr. J-F. Hassan, Dr. V. Jaccard, Dr. P. Kahil, Dr. I. Kornbaun, Dr. B. Kugener, Dr. E. Lacroix, Dr. M. Lamy, Dr. X. Lanse, Dr. C. Lecointre, Dr. F. Lecruit, Dr. D. Lepetit, Dr. F. Levavasseur, Dr. L. Levesque, Dr. E. Lezmy, Dr. S. Liberas, Dr. D. Livon, Dr. J-C. Menage, Dr. E. Mothe, Dr. E. Nicolas, Dr. P. Pacoret, Dr. P. Pelletier, Dr. C. Perreard, Dr. E. Pichot, Dr. H-P. Pujol, Dr. M-T. Pujol, Dr. J. Richard, Dr. M. Robert, Dr. J. Saint-Paul, Dr. C. Talbot, Dr. J-M. Thiron, Dr. A. Valancogne and Dr. H. Volkman who are acknowledged for their contribution.

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