

Development of DTaP-based Hib conjugate combination vaccines:
SmithKline Beecham

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Introduction

In response to the continuing trend for an increase in the number of injections in national childhood immunization schedules, combination vaccines containing multiple antigens have been developed. These vaccines usually include diphtheria and tetanus toxoids (abbreviated here as D and T) and acellular or whole cell pertussis components (aP or wP). Combinations containing inactivated poliovirus (IPV), hepatitis B surface antigen (HBV) and *Haemophilus influenzae* type b (Hib) conjugates have also been evaluated.

Currently the only US-licensed combination for primary vaccination which includes acellular pertussis is DTaP. However, acellular pertussis-containing combinations manufactured by SmithKline Beecham that are licensed elsewhere include DTaP, DTaP/Hib, DTaP-IPV, DTaP-IPV/Hib and DTaP-HBV vaccines. Dossiers concerning the more complex combinations DTaP-HBV-IPV and DTaP-HBV-IPV/Hib are currently under review in many countries. The Hib component in these vaccines is plain polysaccharide conjugated to tetanus toxoid; it is licensed for use as a monovalent vaccine in more than 70 countries worldwide.

Due to the relatively high rates of vaccine coverage, comparative efficacy trials are increasingly difficult to perform. These trials, which would evaluate potential effects that combination would have on vaccine efficacy with respect to monovalent antigens, would need to enroll thousands of children and to ensure their follow-up over relatively long periods of time to generate results which would be statistically valid. There is thus a shift towards demonstration of efficacy using serological correlates, i.e. qualitative and quantitative criteria for the antibody response following vaccination with combination vaccines which permit the conclusion that these combinations will provide adequate protection against disease.

Several criteria have been proposed as immunological surrogates of efficacy for the registration of new conjugate Hib vaccines (1). They constitute the common features of the immune response to monovalent Hib conjugates which have been shown to be efficacious in large field trials. These criteria have been used to characterize the immune

response to SmithKline Beecham's DTaP-based combinations containing a Hib component. The types of data generated will be presented and discussed. Moreover, the fact that two DTaP-based combinations containing Hib have been licensed in 27 countries and are now widely used has permitted the accrual of post-marketing experience, including an assessment of field effectiveness, with this type of vaccine.

Immunologic surrogates of efficacy to be considered in the evaluation of Hib conjugate vaccines

The immunologic surrogates of efficacy for the evaluation of new Hib conjugate vaccines, as discussed at the Vaccines and Related Biological Products Advisory Committee meeting of September 5, 1991 (1), were the following:

1. Randomized comparative immunogenicity studies with currently licensed vaccines in infants
2. Measurement of antibody persistence after the primary immunization series up to age of the recommended booster dose
3. Determination of whether the conjugate vaccine primes infants for a subsequent booster dose to the native polysaccharide given 6 months or more after primary immunization
4. Comparison of IgG, IgM and IgG subclasses following the primary immunization series to those reported for licensed vaccines
5. Demonstration of functional capacity of conjugate-induced antibodies in infants by measurement of opsonic or bactericidal activity

SmithKline Beecham has evaluated each of the above criteria in the context of the development of DTaP-based combination vaccines as follows:

1. Comparative immunogenicity studies involving SmithKline Beecham's DTaP-based Hib conjugate combination vaccines

Lower levels of anti-PRP have been observed in children primed with DTaP-based Hib combinations from multiple manufactureres in comparison with parallel groups of children receiving separately administered, monovalent Hib vaccines. This observation has also been made with the DTaP-based Hib conjugate combination vaccines developed by SmithKline Beecham. As can be seen in Table 1, a lower proportion of children who receive the combination vaccine achieve titers $\geq 1.0 \mu\text{g/ml}$ as compared to separate injection of monovalent products, with a corresponding 2-4-fold decrease in GMC. However, no impact of mixing is observed on the percentage of children with post-primary antibody titers $\geq 0.15 \mu\text{g/ml}$. Indeed, in studies evaluating SmithKline Beecham's DTaP-based Hib conjugate combination vaccines, $\geq 93\%$ of all children administered the combination had antibody titers $\geq 0.15 \mu\text{g/ml}$, irrespective of the primary vaccination schedule used. The GMCs ranged from $1.16 \mu\text{g/ml}$ to $5.06 \mu\text{g/ml}$ (SmithKline Beecham published data given in Figure 1; refs. 2-9).

Table 1: Anti-PRP immune response following primary vaccination with SmithKline Beecham combinations - PRP-T given either separately (+ PRP-T) or mixed (/PRP-T)

Schedule (Reference)	Vaccine	N	% $\geq 0.15 \mu\text{g/ml}$	% $\geq 1.00 \mu\text{g/ml}$	GMC ($\mu\text{g/ml}$) Value	(95% CI)
3-4-5 months (2)	DTaP + PRP-T	185	97	91	7.2	(5.9–8.7)
	“ / PRP-T	387	96	72	2.0	(1.7–2.3)
3-4-5 months (3)	DTaP-HBV + PRP-T	51	100	92	7.9	(1.0–6.5)
	“ / PRP-T	42	100	71	2.1	(1.4–2.0)
2-4-6 months (4)	DTaP-IPV + PRP-T	90	95	76	3.2	(4.0–7.4)
	“ / PRP-T	90	96	66	1.6	(0.9–1.6)
3-4-5 months (5)	DTaP-HBV-IPV + PRP-T	140	100	87	4.4	(3.6–5.5)
	“ / PRP-T	145	99	77	2.6	(2.1–3.2)

N = number of subjects

The “Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Disease” (April 1997) states :

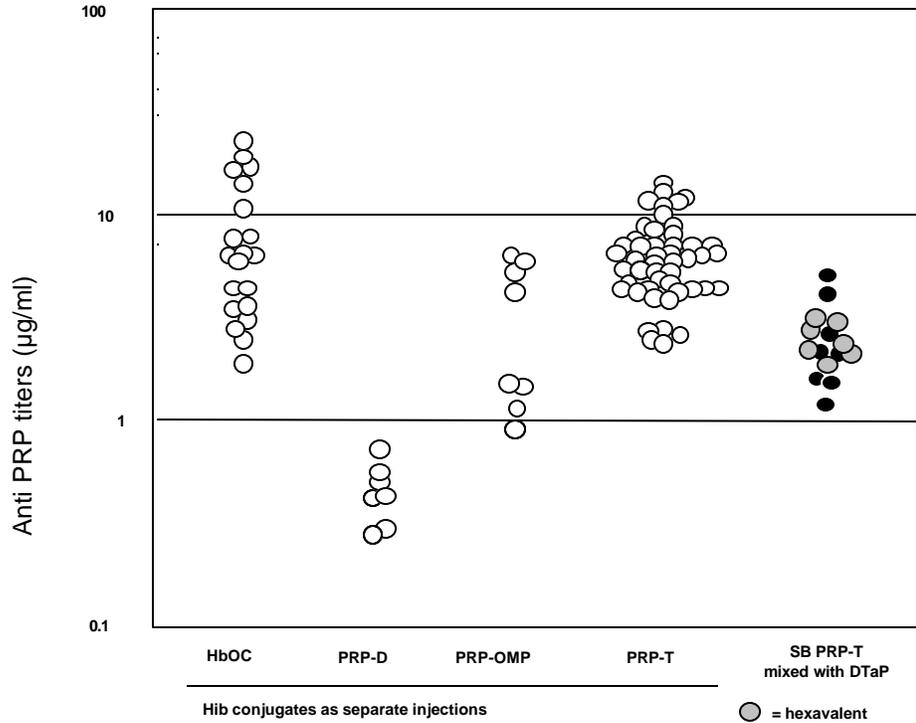
“If antibody levels induced by the combination vaccines are lower than those induced by the component vaccines, a “protective” antibody level might still be attained. In such case, the manufacturer should provide data or information to support the premise that the lower response will not affect the protective efficacy of the product.”

Therefore, in order to put the lower antibody titers into perspective, an extensive review of the published data on the use of monovalent Hib conjugate vaccines was performed. It is noteworthy that the GMCs following primary vaccination with SmithKline Beecham’s DTaP-based Hib conjugate combination vaccines are within the broad range of published values for monovalent Hib conjugate vaccines which are licensed for use in infants in the US (HbOC, PRP-OMP, PRP-T) i.e. 0.89-22.14 µg/ml (Figure 1). Further, values following vaccination with SmithKline Beecham’s DTaP-based Hib conjugate combination vaccines are above those published following primary vaccination with PRP-D (0.28-0.73 µg/ml), a Hib conjugate product which is not licensed for infants in the US due to demonstrated lower efficacy in a Native American population (Table 2).

Furthermore, the antibody levels considered to correlate with short-term and long-term protection against Hib disease (≥ 0.15 and 1.0 µg/ml, respectively) were established based on efficacy trials with immune globulin or plain polysaccharide vaccines. The large body of data now available on the efficacy of Hib conjugate vaccines, and their proposed mechanisms of conferring protection, has led to discussion on the relevance of these levels.

Several large field trials were designed to evaluate the efficacy of monovalent Hib conjugate vaccines over periods of approximately 6 months to 2 years after primary vaccination (10-17). The trials listed in Table 2 demonstrated, in general, a greater efficacy for the Hib conjugate vaccines than would have been predicted by application of the 1.0 µg/ml cut-off.

Figure 1. Anti-PRP titers one month after a primary series of the four types of Hib conjugate vaccines (○), or PRP-T given as a mix with SmithKline Beecham's DTaP-based combinations (●) administered in the first six months of life.



Each symbol represents the geometric mean concentration of one study group. (adapted from reference 18)

Table 2. Protective efficacy and serum antibody responses in infants after primary immunization with Hib conjugate vaccines

Country (refs.)	Vaccine	Recommended Schedules	Protective efficacy (95% CI)	Anti-PRP (mg/ml) post primary vaccination			
				N	GMC	% [≥] 0.15	% [≥] 1.0
Finland (10)	PRP-D	3,4,6 months	90% (70–96)	113	0.53	68	40
	PRP-D	4,6 months	87% (69–96)	71	0.63	73	32
Finland (11)	HbOC	4,6 months	95% (76–99)	45	4.32	100	78
US (12, 13)	HbOC	2,4,6 months	100% (68–100)	144	18.9	100	97
US (Navajo) (14)	PRP-OMP	2,4 months	95% (72–99)	735	1.35	91	59
US (Alaska) (15)	PRP-D	2,4,6 months	35% (-57–73)	88	0.18	48*	15
UK (16, 17)	PRP-T	2,3,4 months	94.7–99.1%	107	5.01	98	90

N = number of subjects

* % > 0.1 µg/ml shown.

The lower efficacy observed in the trial conducted in Alaska with PRP-D merits comment. The reason for this lower efficacy is likely to be multi-factorial (i.e. frequency and intensity of exposure of infants at a much younger age with respect to other populations, highest incidence of disease early in the first year of life). However, the low proportion of children who achieved an antibody level of 0.15 µg/ml cannot be ignored. Indeed, the application of a level of 0.15 µg/ml may be a more sensitive indicator of efficacy when one examines the data in Table 2.

2. Persistence of antibodies up age of a recommended booster dose after primary immunization with SmithKline Beecham's DTaP-based Hib conjugate combination vaccines

Field experience data from the UK indicate that a high level of protection is maintained despite waning antibody levels after priming according to a 2, 3, 4 month schedule without subsequent administration of a booster. Nevertheless, SmithKline Beecham has assessed the persistence of antibodies up until the time of the booster dose.

The Radiolabelled Antigen Binding Assay (RABA) is the gold standard for the measurement of total antibody to *Haemophilus influenzae* type b capsular polysaccharide PRP. Based on the performance characteristics and validation of the assay at SmithKline Beecham, the reporting limit is 0.15 µg/ml.

Lower levels of PRP antibody can be measured with the RABA method. However, the technical validity of the titers below 0.15 µg/ml cannot always be guaranteed because often extrapolation from the linear regression line is used to calculate the titers.

To ensure that all PRP titers below 0.15 µg/ml are technically valid, an alternative calculation based on a 4-parameter curve fitting method can be used instead of the classic linear regression analysis. This allows assessment of PRP titers down to 0.05 µg/ml *without modification of the RABA methodology.*

To demonstrate the feasibility of this approach, two studies with Hib conjugate combination vaccines have been analyzed. The results, shown in Table 5, indicate that circulating PRP antibodies can be detected in a very high percentage of children ($\geq 89\%$) at the time of booster following separate or mixed injection of Hib vaccine.

Table 5: Proportion of children with detectable antibodies pre-booster (15-20 months) after 3 doses of Hib conjugate vaccine (3, 4, 5 months of age) given as a separate injection or as part of a DTaP-based Hib conjugate combination vaccine

	N	% $\geq 0.15 \mu\text{g/ml}$	% $\geq 0.05 \mu\text{g/ml}$
Study A		(RABA)	(RABA 4 param)
DTaP/Hib	83	64%	89%
DTaP+Hib	36	94%	97%
Study B		(ELISA)	(RABA 4 param)
DTaP-HBV-IPV/Hib	103	89%	98%
DTaP-HBV-IPV+Hib	109	91%	97%

N = number of subjects

3. Determination of whether SmithKline Beecham's DTaP-based Hib conjugate combination vaccines prime infants for a subsequent booster response to native polysaccharide

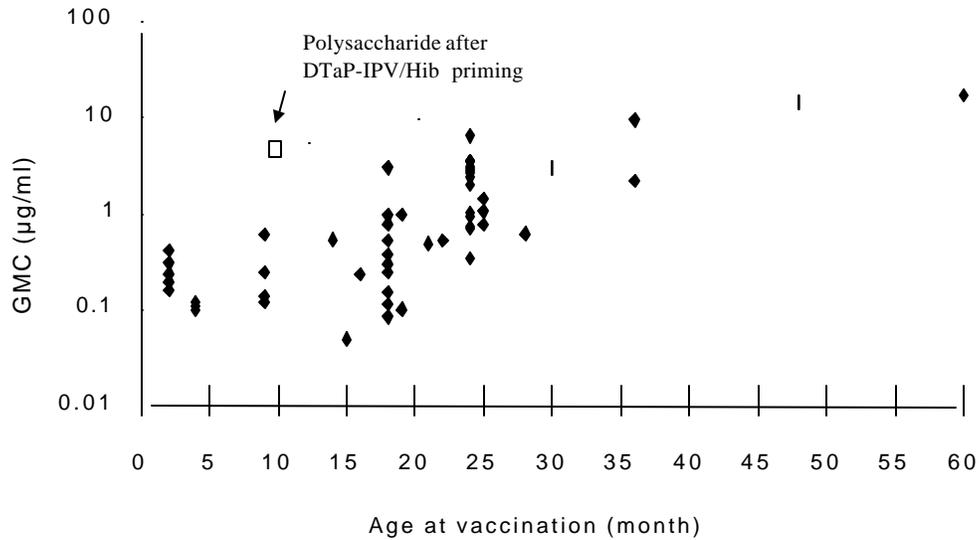
For the conjugate vaccines, factors other than circulating antibody levels play a crucial role in efficacy. The importance of immune memory in assuring protection against invasive disease has been graphically illustrated by experience from the United Kingdom. In the UK, children receive only primary immunization against Hib (2, 3, 4 months of age) and are not administered a booster dose. A decrease in antibody levels was observed with time post-vaccination in a cohort of children vaccinated using this schedule; the GMC for anti-PRP was $5.01 \mu\text{g/ml}$ one month after a primary course of PRP-T vaccine, decreasing to $0.83 \mu\text{g/ml}$ at one year of age (16, 19). The percentages of children with titers $> 1 \mu\text{g/ml}$ were, respectively, 90% and 48%. Despite the fact that many children had

titers below 1µg/ml, the observed efficacy up to 3 years of age remained > 94% (17). In fully vaccinated children, the estimated efficacy over 6 years of surveillance was 98.3% (20).

A widely used marker for the induction of immune memory following primary immunization is the response to a plain polysaccharide booster, which is intended to mimic natural exposure to wild type organisms.

Children initially primed with three doses of SmithKline Beecham's Hib conjugate vaccine as separate or mixed injections with DTaP-based combinations consistently mount an anamnestic response to a single dose of Hib plain polysaccharide booster with resultant high GMCs. In most studies, the booster was administered 6 months or more after primary immunization. In one study, a response to plain polysaccharide was evaluated following an early boost (given 4 months after completion of the primary series). Children primed with DTaP-IPV/Hib vaccine at 2, 4, 6 months of age were administered plain polysaccharide at 10 months of age (21). An increase in GMCs from 1.5 µg/ml prior to PRP administration to 4.8 µg/ml 10 days after PRP administration was observed. This latter value should be contrasted with the anti-PRP levels published for non-primed children receiving plain polysaccharide at the same age, i.e. less than 1 µg/ml, as shown in Figure 2. These results clearly show that the response to plain polysaccharide is anamnestic, and that an immune memory to PRP has effectively been induced. They furthermore demonstrated that immune memory is established early after primary vaccination.

Figure 2: GMC 10 days after one dose of PRP in DTaP-IPV/Hib primary vaccinated children compared to published data on GMC 1 month after one dose of PRP in unprimed children, as a function of age



4 and 5. Comparison of isotypes and subclasses and demonstration of functional capacity of the anti-PRP antibodies elicited by SmithKline Beecham’s DTaP-based Hib conjugate combination vaccines

It is important to ascertain that the biological activity of the antibodies elicited by a DTaP-based Hib conjugate combination vaccine is not different from that of monovalent Hib conjugate vaccines.

The functional capacities of antibodies induced by SmithKline Beecham’s DTaP-based Hib conjugate combinations were assessed using several different assays to confirm that these parameters were not negatively affected.

Antibody avidity, isotype and subclass

Antibody avidity is an important marker of successful priming, as memory responses are characterized by the production of high avidity antibodies, which are predominantly IgG1.

Despite the differences in the levels of anti-PRP antibodies found after vaccination, antibody avidity was not different in children administered Hib conjugate vaccines either separately or as a mix with DTaP-based combination vaccines. The results from one representative clinical trial are presented in Table 3.

In addition, the primary response to vaccination with the DTaP-based Hib conjugate combinations was found to be predominantly IgG, with higher levels of IgG1 relative to IgG2. This is not different from what has been described for licensed monovalent Hib conjugate vaccines.

Table 3. Avidity and geometric mean concentrations (GMC) of anti-PRP antibodies in subjects primed with DTaP-HBV and PRP-T as mixed or separate injections and after a plain PRP booster

Priming Vaccines	Timing	N	Avidity		GMC	
			nM ¹	(95% CI)	mg/ml	(95% CI)
DTaP-HBV + PRP-T mixed	Post-primary	24	2.42	(2.01–2.91)	1.72	(1.12–2.64)
	Pre-booster	7	2.73	(2.38–3.13)	0.40	(0.18–0.85)
	Post-booster	20	2.75	(2.31–3.29)	17.3	(10.2–29.4)
DTaP-HBV + PRP-T separate	Post-primary	18	2.54	(2.10–3.08)	7.07	(3.78–13.2)
	Pre-booster	24	2.67	(2.23–3.19)	0.89	(0.57–1.40)
	Post-booster	23	2.85	(2.37–3.43)	34.5	(17.4–68.2)

N = number of subjects

Table taken from reference 18.

Biological activity in an opsonophagocytosis assay

The opsonophagocytosis assay measures the ability of anti-PRP to kill Hib organisms in the presence of complement and polymorphonuclear cells; this capacity thus represents an important defense mechanism against infection by Hib.

When corrected for GMC, geometric mean opsonic activity did not differ. The unconjugated PRP booster elicited antibodies with the same geometric mean ratio after priming with either mixed or separate administration of Hib conjugate vaccine.

Table 4. Opsonic activity determined in post-booster sera from subjects given primary courses of DTaP-based combinations with PRP-T as separate and mixed injections, then boosted with separate or mixed injections of PRP-T or unconjugated PRP

Priming Vaccines	Booster Vaccines	N	Opsonic Activity		Ig (mg/ml)		Opsonic/Ig ratio	
			GMT	(95% CI)	GMC	(95% CI)	GMR	(95% CI)
DTaP + PRP-T separate	DTaP + PRP-T separate	34	462	(267–801)	84.2	(53.5–132.5)	5.49	(3.86–7.80)
DTaP/PRP-T mixed	DTaP/PRP-T mixed	64	216	(58–295)	36.9	(27.8–49.2)	5.85	(4.86–7.04)
DTaP-HBV + PRP-T sep.	DTaP-HBV + PRP sep.	14	412	(166–1023)	58.2	(28.0–121.3)	7.07	(4.4–11.3)
DTaP-HBV/PRP-T mixed	DTaP-HBV/PRP mixed	17	97	(41–225)	20.3	(11.8–35.0)	4.76	(2.8–8.1)

N = number of subjects

Table adapted from reference 18

Animal passive protection assay

Sera obtained post-primary vaccination with SmithKline Beecham's DTaP-based Hib conjugate combination vaccines were evaluated for their ability to prevent bacteremia in the infant rat model. No differences have been found in the protective capacity with respect to the monovalent Hib conjugate vaccines.

Therefore, combination of DTaP-based vaccines and Hib conjugates does not interfere with the quality of the antibodies produced by the primary vaccination course or as a result of a booster challenge with unconjugated PRP.

Post-marketing field effectiveness of SmithKline Beecham's DTaP-based Hib conjugate combination vaccines in Germany

SmithKline Beecham's DTaP-based Hib conjugate combination vaccines (DTaP/Hib and DTaP-IPV/Hib) have been in widespread use in Germany since 1996. They have become the preferred choice for Hib vaccination in this country. Their field effectiveness against invasive Hib disease is being evaluated in an ongoing post-marketing surveillance program carried out under the auspices of the "Erhebungseinheit Für Seltene Pädiatrische Erkrankungen in Deutschland" (ESPED). Surveillance has shown that invasive Hib disease in Germany is associated with absent or delayed vaccination. The extensive use of DTaP-based Hib conjugate combination vaccines has not resulted in an increase in invasive Hib disease. The overall effectiveness of these combinations has been shown to be 97.4% (22).

Conclusions

In conclusion, lower anti-PRP responses as assessed by % of subjects with anti-PRP levels >1 ug/ml, are attained following vaccination with SmithKline Beecham's DTaP-based Hib conjugate combination vaccines.

In accordance with the "Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Disease" (April 1997):

"If antibody levels induced by the combination vaccines are lower than those induced by the component vaccines, a "protective" antibody level might still be attained. In such case, the manufacturer should provide data or information to support the premise that the lower response will not affect the protective efficacy of the product."

Evidence has been presented that supports the efficacy of our DTaP-based Hib conjugate combination vaccines based on the following:

- Adequate responses following the primary series, with attainment of seroprotection levels of antibodies ($\geq 0.15 \mu\text{g/ml}$) in a high proportion of children.
- Achievement of antibody titers in the range of licensed conjugated vaccines, previously shown to be highly protective.
- Early induction of immune memory with IgG dominated anamnestic responses that further indicate successful priming.
- Functional capacity and antibody characteristics comparable to those induced by separate vaccination.
- Demonstration of field effectiveness

It is concluded that the protection afforded by Smith Kline Beecham's DTaP-based Hib conjugate combination vaccines is not different from that elicited by other licensed conjugate Hib vaccines.

References:

1. Frasch CE. *Haemophilus influenzae* type b conjugate and combination vaccines. Clin Immunother. 1995; 4:376-386.
2. Schmitt HJ, Zepp F, Muschenborn S et al. Immunogenicity and reactogenicity of a *Haemophilus influenzae* type b tetanus conjugate vaccine when administered separately or mixed with concomitant diphtheria tetanus toxoid and acellular pertussis vaccine for primary and for booster immunizations. Eur J Pediatr 1998 157: 208-214.
3. Zepp F, Schmitt HJ, Kaufhold A, et al. Evidence for induction of polysaccharide specific B-cell-memory in the first year of life: plain *Haemophilus influenzae* type b-PRP (Hib) boosters children primed with a tetanus-conjugate Hib-DTPa-HBV combined vaccine. Eur J Pediatr 1997; 156:18-24.
4. Halperin SA, King J, Law B, Mills E, Willems P. Safety and immunogenicity of *Haemophilus influenzae*-tetanus toxoid conjugate vaccine given separately or in combination with a three-component acellular pertussis vaccine for the first four doses. Clin Infect Dis 1999; 28: 995-1001.
5. Schmitt HJ, Knuf M, Uwamwezi MC, Ortiz E, Sanger R. Immunogenicity and reactogenicity of a separate and combined DTPa-HBV-IPV+/Hib vaccine. Proceedings of the 17th Annual Meeting ESPID Crete 1999
6. Greenberg DP, Wong VK, Partridge S, Chang SJ, Howe BJ, Ward JI. Immunogenicity of a booster dose of Hib conjugate vaccine in children with impaired immune responses following primary vaccination with DTaP-HepB-PRP-T vaccine. Proceedings of the 36th IcaAC, New Orleans 1996.
7. Dagan R, Igbaria K, Piglansky L, et al. Safety and immunogenicity of a combined pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus and *Haemophilus influenzae* type b-tetanus conjugate vaccine in infants, compared with a whole cell pentavalent vaccine. Pediatr Infect Dis J 1997; 16:1113-1121.
8. Pichichero ME, Passador S. Administration of combined diphtheria and tetanus toxoids and pertussis vaccine, hepatitis B vaccine, and *Haemophilus influenzae* type b (Hib) vaccine to infants and response to a booster dose of Hib conjugate vaccine. Clin Infect Dis 1997; 25:1378-1384.
9. Aristigui J, Dal-Ré R, Garrote E, González A, Arrate JP, Pérez A. Assessment of the immunogenicity and reactogenicity of a quadrivalent diphtheria, tetanus, acellular pertussis and hepatitis B (DTPa-HBV) vaccine administered in a single injection with

- Haemophilus influenzae* type b conjugate vaccine, to infants at 2, 4 and 6 months of age. Vaccine 1998; 16:1976-1981.
10. Eskola J, Käyhty H, Takala A, et al. A randomized, protective field trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* type b disease. New Engl J Med. 1990; 323:1381-1387.
 11. Peltola H, Eskola J, Käyhty H, Taakla AK, Mäkela H. Clinical comparison of the *Haemophilus influenzae* type b polysaccharide-diphtheria toxoid and the oligosaccharide-CRM197 protein vaccines in infancy. Arch Pediatr Adolesc Med 1994; 148:620-625.
 12. Black SR, Shinefield HR, Lampert D, et al. Safety and immunogenicity of oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine in infancy. Pediatr Infect Dis J. 1991; 10:92-96.
 13. Black SB, Shinefield HR, Fireman B, et al. Efficacy in infancy of oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine in a United States population of 61 080 children. Pediatr Infect Dis J 1991; 10:97-104.
 14. Santosham M, Wolff M, Reid R, et al. The efficacy in Navajo infants of a conjugate vaccine consisting of *Haemophilus influenzae* type b polysaccharide and Neisseria meningitidis outer-membrane protein complex. New Engl J Med. 1991; 324:1767-1772.
 15. Ward J, Brenneman G, Letson GW, Heyward WL and the Alaska *H. influenzae* Vaccine Study Group. Limited efficacy of a *Haemophilus influenzae* type b conjugate vaccine in Alaska native infants. New Engl J Med 1990; 323:1393-1401.
 16. Booy R, Taylor SA, Dobson SRM, et al. Immunogenicity and safety of PRP-T conjugate vaccine given according to the British accelerated immunisation schedule. Arch Dis Child 1992; 67:475-478.
 17. Booy R, Heath PT, Slack MPE, Begg N, Moxon ER. Vaccine failures after primary immunisation with *Haemophilus influenzae* type-b conjugate vaccine without booster. Lancet 1997; 349:1197-1202.
 18. Eskola J, Ward J, Dagan R, Goldblatt D, Zepp F, Siegrist CA. Combined vaccination of *Haemophilus influenzae* type b conjugate and diphtheria-tetanus-pertussis containing acellular pertussis. Lancet 1999; 354:2063-2068.

19. Booy R, Hodgson S, Griffiths H, Chapel HM, Moxon ER. Antibody persistence after accelerated immunisation against *Haemophilus influenzae* type b. *BMJ* 1993; 306:971-972.
20. Moxon ER, Heath PT, Booy R, Azzopardi HJ, Slack MPE, Ramsay ME. The impact of Hib conjugate vaccines in preventing invasive *H. influenzae* diseases in the UK. *Vaccine* 1999; 17(suppl 3):S11-S13.
21. Dagan R, Amir J, Ashkenazi S, Ortiz E, Kaufhold A. Early anamnestic response to plain polyribosylribitol phosphate (PRP) challenge as evidence of induction of immune memory after combined DTPa-IPV/Hib primary vaccination. Proceedings of the 39th ICAAC, San Francisco, 1999.
22. Schmitt HJ, Siedler A, Niessing W, Weil J. DTPa Hib combination vaccines: population-based estimation of effectiveness by capture-recapture technique. Proceedings of the 39th ICAAC, San Francisco, 1999.