MEMORANDUM **DEPARTMENT OF HEALTH AND HUMAN SERVICES**
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

Date: August 27, 2007

From: Drusilla Burns, Ph.D., Chief, LRSP, DBPAP, OVRR

Through: Milan Blake, Ph.D., Acting Director, DBPAP, OVRR

Subject: Review of BLA 125260, DTaP-IPV from GlaxoSmithKline

To: File

I have reviewed BLA 125260/0 which is a submission from GlaxoSmithKline (GSK) for a DTaP-IPV vaccine. In particular, I have focused on the following: 1) pertussis component manufacturing, 2) pertussis component testing results 3) stability of the pertussis component, and 4) pertussis component clinical serology. In addition, I have also reviewed 125260/0.3 which is an amendment to the application that contains the serological assay validation packages for the assays run at the ---------- to support Study 047 (Phase II study).

Summary of product and manufacturing:

This DTaP-IPV vaccine consists of a combination of GSK's Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) vaccine (Infanrix®, approved January 1997) and inactivated poliovirus vaccine (IPV). The DTaP and IPV components are the same as those found in GSK's Pediarix®, approved December 13, 2002).

The DTaP-IPV vaccine contains the following active ingredients per single dose of 0.5 ml: 25 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid, 25 µg of detoxified pertussis toxin (PT), 25 µg of formalin-treated filamentous hemagglutinin (FHA), 8 µg of formalin-treated pertactin (PRN), 40 units of D-antigen for poliovirus type 1 (Mahoney strain), 8 units of D-antigen for poliovirus type 2 (MEF-1 strain), and 32 units for D-antigen for poliovirus type 3 (Saukett strain).

DTaP-IPV is intended for active immunization against diphtheria tetanus, pertussis, and poliomyelitis, administered as the 5th dose of DTaP and as the 4th dose of IPV in children 4-6 years of age.

The manufacturing process of the pertussis antigens includes:

1. fermentation of *Bordetella pertussis*

2. extraction of PRN and PT/FHA components
3. purification of PT, FHA, and PRN antigens
4. detoxification of purified PT, FHA, and PRN antigens
5. adsorption of purified and detoxified PT, FHA, and PRN antigens onto aluminum adjuvant.

The pertusis antigens are manufactured by a process identical to that used to produce the pertussis antigens used in Infanrix and Pediatrix.

The adsorbed pertussis antigens are then formulated with pre-adsorbed diphtheria and tetanus concentrate and IPV concentrate. The final bulk is then filled into vials or syringes.

The three DTaP-IPV vaccine Phase III clinical consistency lots DC20A001, DC20A002, and DC20A003) were manufactured (formulated and filled) at pilot scale (-----formulation) in GSK Biological's clinical production facilities using pilot-scale equipment (----------). The bulk antigens were prepared at commercial scale in existing licensed facilities. The commercial demonstration lots---------- (syringes), ---------- (vials), ---------- (vials), ---------- (syringes) were formulated at commercial scale (----------).

Quality control test results for final bulk, and final container vaccine for each of these lots were satisfactory.

Vaccine Stability

Stability of unadsorbed pertussis antigen bulks

A storage period of ----------between diafiltration and adsorption has been validated for Infanrix. Because the pertussis antigens are identical at this stage for Infanrix and DTP-IPV, this storage period is reasonable.

Stability of adsorbed pertussis antigen bulks

GSK previously validated a ---------- shelf-life for the adsorbed pertussis antigen bulks for Infanrix. In these studies, three Infanrix lots were formulated with 2-PE-free, ----------old pertussis antigens and were followed on stability for ----------. All results were satisfactory. Because the pertussis antigen bulks are identical for Infanrix and DTP-IPV, this proposed shelf-life is appropriate.

Final formulated bulk stability

The first DTaP-IPV final bulk lots formulated at commercial scale (DTaP-IPV lots ---------- and ----------) were stored for up to---------- before filling into final containers. A maximal storage period of ---------- for final formulated bulk before filling is proposed. This storage period is reasonable.

Final container stability

24-month stability data are available for the three clinical consistency lots used in the Phase III study. These lots differ from commercial lots in that they were formulated at pilot scale. The commercial demonstration lots have been placed on stability and will be followed for 36 months, the proposed dating period. GSK previously submitted a comparability protocol for demonstrating that the commercial-scale lots were comparable to those lots used in the pivotal clinical study. CBER agreed that the proposal for bridging the commercial and clinical manufacturing facilities is satisfactory. One of the components of that comparability protocol is that "Results for the commercial scale lots will be compared to results obtained for the three clinical consistency lots. To be considered acceptable/validated, results for the commercial scale lots must be within the historical range obtained with the clinic-scale product". However this criterion was not strictly met.

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Additional stability data for the commercial lots may shed light on whether these discrepancies are only due to variability of the test.

COMMENT:

- Please provide any updated stability data that you might have for the commercial demonstration lots ----------.

When the stability data for the phase III clinical lots were reviewed (Section 3.2.P.8.3), the following were noted.

1. Potency for pertussis antigens at the 24-month time point was somewhat lower than that observed at earlier time points. Additional stability data might add insight into whether this represents a real downward trend or whether this represents variability of the test.

COMMENT:

- Please provide any updated stability data that you might have for the phase III clinical consistency lots DC20A001, DC20A002, and DC20A003.

2. Results of ---------- were found satisfactory except for that for unbound PT of lot DC20A001B at the 24-month time point ------. GSK found no reason to invalidate the test result but when a retest was performed, the result was below the detection limit ------- and in line with that observed at previous stability time points. In-vivo potency for PT was in line with that observed for other lots. Therefore, I have relatively little concern about this test result.

Pertussis Serology

Pertussis ELISAs

Study DTaP-IPV-048

ELISA validation reports for pertussis assays conducted by GSK were submitted in the application. Certain additional information is needed to confirm that the assays are performing in a manner such that the data produced are meaningful and support GSK's conclusions.

COMMENTS:

- Please submit data which support the precision and accuracy of the assay for samples at or near the lower limit of quantitiation and at or near the cut-off values of $\geq$5 EU/ml.

- Please submit data which support the precision of the assays over their entire working ranges.

- For each critical reagent used in the pertussis ELISAs, please submit a summary of the data generated to qualify the batch(es) used in the critical assays presented in this submission.

- Please provide data that demonstrate that the pertussis assay ELISA assays behaved in a stable manner and that critical assay parameters did not change from the time that the assays were validated (ELISA validation reports are dated 1998) until the time that critical assays presented in this submission were conducted.

Study DTaP-IPV-047

Pertussis immunogencity data generated in a phase II study (DTaP-IPV-047) were submitted in this application. GSK considers the immunogenicity data generated in this trial to be supportive. The pertussis ELISA assays used to evaluate the clinical samples from this trial were assayed in the ----------. GSK submitted pertussis assay validation reports for the ------ ---------- in amendment 0.3. I found the validation report to be missing critical information such that the validity and soundness of the data generated using these assays cannot be evaluated. Without this information, I do not consider the pertussis immunogenicity data from this study to be supportive.

COMMENT:

1. The validation reports received for the pertussis ELISA conducted in the ---------- are missing critical information that would justify the use of these assays. Without this information, pertussis immunogenicity data from Study DTaP-IPV-047 will not be considered supportive. The following information is needed to complete the validation study report for each pertussis antigen:

- A detailed description of the methods and software used to calculate ELISA units/ml in each test sample and representative calculations
- A detailed description of each of the critical reagents
- The specifications for each critical reagent
- For each critical reagent, a summary of the data generated to qualify the batch(es) used in the critical assays presented in this submission
- For each coating antigen, the source, a summary of the purification process (if available) and any testing to ensure purity (i.e. absence of other vaccine antigens)
- Details describing how the -------- pertussis reference serum was calibrated against FDA control serum lot #3
- Data demonstrating the assay has acceptable precision and accuracy over the entire working range
- Data supporting the precision and accuracy of the assay for samples at or near the lower limit of quantitation and at or near the assigned cut-off value of 5 EU/ml
- Data demonstrating specificity of the assay

2. Dilutional linearity studies for each of the pertussis antigen ELISAs shows considerable bias as the sample is diluted. Such a large bias might affect interpretation of the data generated by these assays. Please comment.

3. Please provide data that demonstrate that the pertussis assay ELISA assays behaved in a stable manner and that critical assay parameters did not change from the time that the assays were validated until the time that critical assays presented in this submission were conducted.

Clinical Serology Results

The pivotal phase III study submitted to support the requested indication for the vaccine was DTaP-IPV-048. This was an open (double-blind for consistency lots), randomized, multicenter clinical trial of the safety, immunogenicity, and consistency of three manufacturing lots of GSK's DTaP-IPV candidate vaccine compared to that of separate injections of GSK's DTaP vaccine (Infanrix) and Aventis Pasteur's IPV vaccine (IPOL) administered as a booster dose in healthy children 4 to 6 years of age. In regards to pertussis immunogencity, the study objectives were:

Primary objectives:

- To demonstrate the lot-to-lot consistency of three manufacturing lots of DTaP-IPV vaccine in terms of pertussis toxoid (PT), filamentous hemaglutinin (FHA), and pertactin (PRN) geometric mean concentrations (GMCs) in a subset of subjects one month after vaccination

  Criteria for lot consistency:
  For each pair of lots and for each antigen, the lower and upper limits of the 95% CI on the GMC ratio were within the pre-defined limits [0.67, 1.5].

- To demonstrate the non-inferiority of DTaP-IPV vaccine compared to Infanrix + IPOL administered separately in terms of booster responses

  Criteria for non-inferiority of DTaP-IPV vaccine (1 month after vaccination)
  For each antigen, the upper limit of the two-sided standardized asymptotic 95% CI for the difference between the Infanrix + IPOL group and (minus) the DTaP-IPV group in the percentage of subjects with a booster response was less than or equal to the pre-defined clinical limit of 10%

  For pertussis antigens, a booster response is defined as
  - Initially seronegative subjects (pre-booster antibody concentration below cut-off of <5 EU/ml) with an increase of at least four times the cut-off one month after vaccination (post-booster antibody concentration $\geq$20 EU/ml
  - Initially seronpositive subjects with pre-booster antibody concentration $\geq$5 EU/ml and <20 EU/ml with an increase of at least four time the pre-booster antibody concentration one month after vaccination
  - Initially seropositive subjects with pre-booster antibody concentration $\geq$20 EU/ml with an increase of at least two time the pre-booster antibody concentration one month after vaccination

Secondary Objectives

- To evaluate the lot-to-lot consistency of three manufacturing lots of DTaP-IPV vaccine in terms of pertussis booster responses one month after vaccination
- To evaluate DTaP-IPV vaccine compared to Infanrix+IPOL administered separately in terms of pertussis GMCs one month after vaccination

GSK also performed a secondary analysis in which they examined what they call “seropositivity status” which they define as

- Anti-PT $\geq$ 5 EU/ml
- Anti-FHA $\geq$ 5 EU/ml
- Anti-PRN $\geq$ 5 EU/ml

Results for lot consistency analysis:

Primary endpoint:

Table 26 Ratios of post-vaccination antibody GMCs/GMTs (adjusted for baseline concentration) between DTaP-IPV lots one month after vaccination (ATP Cohort for immunogenicity)

Lot A	N	Adjusted GMC/GMT	Lot B	N	Adjusted GMC/GMT	GMC/GMT ratio			Lot-to-lot consistency criterion met (Yes/No)
						Lot A/ Lot B	95% CI		
							LL	UL	
Anti-D									
DTaP-IPV Lot 1	280	17.460	DTaP-IPV Lot 2	282	17.996	0.970	0.871	1.080	Yes
DTaP-IPV Lot 1	280	17.460	DTaP-IPV Lot 3	282	18.161	0.961	0.863	1.070	Yes
DTaP-IPV Lot 2	282	17.996	DTaP-IPV Lot 3	282	18.161	0.991	0.890	1.103	Yes
Anti-T									
DTaP-IPV Lot 1	279	9.796	DTaP-IPV Lot 2	283	10.050	0.975	0.866	1.097	Yes
DTaP-IPV Lot 1	279	9.796	DTaP-IPV Lot 3	282	11.160	0.878	0.780	0.988	Yes
DTaP-IPV Lot 2	283	10.050	DTaP-IPV Lot 3	282	11.160	0.901	0.800	1.014	Yes
Anti-PT									
DTaP-IPV Lot 1	272	67.9	DTaP-IPV Lot 2	273	72.4	0.938	0.828	1.063	Yes
DTaP-IPV Lot 1	272	67.9	DTaP-IPV Lot 3	277	70.5	0.963	0.850	1.091	Yes
DTaP-IPV Lot 2	273	72.4	DTaP-IPV Lot 3	277	70.5	1.026	0.906	1.162	Yes
Anti-FHA									
DTaP-IPV Lot 1	281	814.7	DTaP-IPV Lot 2	280	932.2	0.874	0.783	0.976	Yes
DTaP-IPV Lot 1	281	814.7	DTaP-IPV Lot 3	283	860.3	0.947	0.849	1.057	Yes
DTaP-IPV Lot 2	280	932.2	DTaP-IPV Lot 3	283	860.3	1.084	0.971	1.209	Yes
Anti-PRN									
DTaP-IPV Lot 1	280	606.8	DTaP-IPV Lot 2	281	608.0	0.998	0.867	1.148	Yes
DTaP-IPV Lot 1	280	606.8	DTaP-IPV Lot 3	284	581.8	1.043	0.907	1.200	Yes
DTaP-IPV Lot 2	281	608.0	DTaP-IPV Lot 3	284	581.8	1.045	0.909	1.202	Yes
Anti-poliovirus type 1									
DTaP-IPV Lot 1	270	2113.5	DTaP-IPV Lot 2	266	2126.8	0.994	0.836	1.181	Yes
DTaP-IPV Lot 1	270	2113.5	DTaP-IPV Lot 3	273	2142.3	0.987	0.831	1.172	Yes
DTaP-IPV Lot 2	266	2126.8	DTaP-IPV Lot 3	273	2142.3	0.993	0.836	1.180	Yes
Anti-poliovirus type 2									
DTaP-IPV Lot 1	274	2361.8	DTaP-IPV Lot 2	268	2112.9	1.118	0.951	1.314	Yes
DTaP-IPV Lot 1	274	2361.8	DTaP-IPV Lot 3	265	2346.7	1.006	0.856	1.183	Yes
DTaP-IPV Lot 2	268	2112.9	DTaP-IPV Lot 3	265	2346.7	0.900	0.765	1.060	Yes
Anti-poliovirus type 3									
DTaP-IPV Lot 1	269	3754.6	DTaP-IPV Lot 2	255	3376.7	1.112	0.941	1.314	Yes
DTaP-IPV Lot 1	269	3754.6	DTaP-IPV Lot 3	263	3631.4	1.034	0.876	1.220	Yes
DTaP-IPV Lot 2	255	3376.7	DTaP-IPV Lot 3	263	3631.4	0.930	0.787	1.099	Yes

Data source: Appendix Table IIIA

Adjusted GMC (GMT) = geometric mean antibody concentration (titer) adjusted for baseline concentration (titer)

N = Number of subjects with both pre- and post-vaccination results available

95% CI = 95% confidence interval for the adjusted GMC (GMT) ratio (ANCOVA model: adjustment for baseline concentration (titer) - pooled variance with more than 2 groups); LL = lower limit, UL = upper limit

criteria for claiming lot-to-lot consistency - 95% CI for the point estimate of the between-lot GMC(GMT) ratio completely within the range (0.67, 1.5)

Secondary endpoint:

Table 23 Percentage of subjects with booster responses for Anti-PT, Anti-FHA, Anti-PRN antibodies one month post-vaccination (ATP Cohort for immunogenicity)

				Booster Response			
						95% CI	
Antibody	Group	Pre-vaccination status	N	n	%	LL	UL
Anti-PT	Group 1	S-	198	181	91.4	-	-
		S+ (<20 EL.U/mL)	62	59	95.2	-	-
		S+ (≥20 EL.U/mL)	12	11	91.7	-	-
		Total	272	251	92.3	88.4	95.2
	Group 2	S-	186	170	91.4	-	-
		S+ (<20 EL.U/mL)	72	67	93.1	-	-
		S+ (≥20 EL.U/mL)	15	13	86.7	-	-
		Total	273	250	91.6	87.6	94.6
	Group 3	S-	182	169	92.9	-	-
		S+ (<20 EL.U/mL)	83	81	97.6	-	-
		S+ (≥20 EL.U/mL)	12	7	58.3	-	-
		Total	277	257	92.8	89.1	95.5
Anti-FHA	Group 1	S-	7	7	100	-	-
		S+ (<20 EL.U/mL)	62	62	100	-	-
		S+ (≥20 EL.U/mL)	212	196	92.5	-	-
		Total	281	265	94.3	90.9	96.7
	Group 2	S-	2	2	100	-	-
		S+ (<20 EL.U/mL)	66	66	100	-	-
		S+ (>=20 EL.U/mL)	212	204	96.2	-	-
		Total	280	272	97.1	94.4	98.8
	Group 3	S-	5	5	100	-	-
		S+ (<20 EL.U/mL)	58	58	100	-	-
		S+ (>=20 EL.U/mL)	220	205	93.2	-	-
		Total	283	268	94.7	91.4	97.0
Anti-PRN	Group 1	S-	22	22	100	-	-
		S+ (<20 EL.U/mL)	71	69	97.2	-	-
		S+ ≥20 EL.U/mL)	187	179	95.7	-	-
		Total	280	270	96.4	93.5	98.3
	Group 2	S-	27	27	100	-	-
		S+ (<20 EL.U/mL)	87	87	100	-	-
		S+ (≥20 EL.U/mL)	167	163	97.6	-	-
		Total	281	277	98.6	96.4	99.6
	Group 3	S-	25	24	96.0	-	-
		S+ (<20 EL.U/mL)	71	71	100	-	-
		S+ (≥20 EL.U/mL)	188	184	97.9	-	-
		Total	284	279	98.2	95.9	99.4

Data source: Appendix table IIIA
Group 1: DTaP-IPV lot 1 + *M-M-R*$_V$
Group 2: DTaP-IPV lot 2 + *M-M-R*$_V$
Group 3: DTaP-IPV lot 3 + *M-M-R*$_V$
S+ = subjects with titers ≥5 EL.U/mL
S- = subjects with titers <5 EL.U/mL
N = number of subjects with available results at PRE and POST time point
n/% = number/percentage of subjects with a booster response
95% CI = exact 95% confidence interval; LL = Lower Limit; UL = Upper Limit
Total = subjects either seropositive or seronegative at pre-vaccination
Booster response defined as :
For initially seronegative subjects, antibody concentration ≥ 20 EL.U/mL one month post-vaccination

Additional analysis (seropositivity)

Table 22 Percentage of subjects with Anti-PT, Anti-FHA, and Anti-PRN antibody concentrations of at least 5 EL.U/mL, and geometric mean antibody concentrations before and one month after vaccination according to treatment lots (ATP Cohort for immunogenicity)

				≥5 EL.U/mL				GMC		
						95% CI			95% CI	
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL
Anti-PT	Group 1	PRE	272	74	27.2	22.0	32.9	3.8	3.5	4.2
		PI(M1)	285	285	100	98.7	100	66.9	61.1	73.3
	Group 2	PRE	273	87	31.9	26.4	37.8	4.1	3.7	4.5
		PI(M1)	280	279	99.6	98.0	100	74.1	66.6	82.4
	Group 3	PRE	278	95	34.2	28.6	40.1	4.1	3.7	4.5
		PI(M1)	284	283	99.6	98.1	100	71.4	64.7	78.6
Anti-FHA	Group 1	PRE	281	274	97.5	94.9	99.0	49.4	42.5	57.4
		PI(M1)	285	285	100	98.7	100	809.1	740.5	884.2
	Group 2	PRE	283	281	99.3	97.5	99.9	48.0	41.4	55.6
		PI(M1)	280	280	100	98.7	100	918.8	840.0	1005.0
	Group 3	PRE	283	278	98.2	95.9	99.4	53.9	46.0	63.1
		PI(M1)	285	285	100	98.7	100	869.2	795.2	950.2
Anti-PRN	Group 1	PRE	280	258	92.1	88.3	95.0	28.0	24.5	32.1
		PI(M1)	285	285	100	98.7	100	617.4	549.2	694.0
	Group 2	PRE	283	256	90.5	86.4	93.6	25.0	21.7	28.7
		PI(M1)	281	281	100	98.7	100	584.7	519.7	657.9
	Group 3	PRE	284	259	91.2	87.3	94.2	28.0	24.3	32.3
		PI(M1)	285	285	100	98.7	100	594.2	526.6	670.4

Data source: Appendix table IIIA
Group 1: DTaP-IPV lot 1 + *M-M-R*II
Group 2: DTaP-IPV lot 2 + *M-M-R*II
Group 3: DTaP-IPV lot 3 + *M-M-R*II
GMC = geometric mean antibody concentration
N = number of subjects with available results
n/% = number/percentage of subjects with concentration within the specified range
95% CI = 95% confidence interval; LL = Lower Limit; UL = Upper Limit
PRE =pre-vaccination blood sample at Day 0
PI(M1) =post-vaccination blood sample at month 1

Results for non-inferiority of DTaP-IPV compared to Infanrix + IPOL

Primary endpoint:

Table 36 Difference between groups in percentage of subjects in the pooled DTaP-IPV and DTaP plus IPV treatment groups with a booster response to DTaP antigens one month after vaccination (ATP Cohort for immunogenicity)

Antibody	Pooled DTaP-IPV			*Infanrix + IPOL*			Difference between groups (*Infanrix + IPOL* minus pooled DTaP-IPV) (%)	95% CI		Non-inferiority criterion met (Yes/No)
	N	n	%	N	n	%		LL	UL	
Anti-D	844	840	99.5	260	260	100	0.47	-0.98	1.21	Yes
Anti-T	844	816	96.7	261	245	93.9	-2.81	-6.55	-0.09	Yes
Anti-PT	822	758	92.2	256	237	92.6	0.36	-3.83	3.71	Yes
Anti-FHA	844	805	95.4	261	251	96.2	0.79	-2.50	3.21	Yes
Anti-PRN	845	826	97.8	261	253	96.9	-0.82	-3.79	1.14	Yes

Data source: Appendix table IIIA

Pooled DTaP-IPV = DTaP-IPV lots 1, 2, and 3 (pooled) + MMR_V

Infanrix + IPOL = *Infanrix + IPOL* + MMR_V

N = Total number of subjects with available results at PRE and POST timepoint.

n/% = number/ percentage of subjects with a booster response at post-vaccination.

95% CI, LL/UL = Standardized asymptotic 95% confidence interval around difference, Lower/Upper limit.

Criteria for claiming non-inferiority – upper limit of the 95% CI for the point estimate of the difference between groups in percentage of subjects with a booster response is 10% or less

Secondary Endpoint

Table 31 Adjusted ratios of Anti-PT, Anti-FHA, Anti-PRN GMCs one month after vaccination (ATP Cohort for immunogenicity)

					Adjusted GMC ratio (*Infanrix* + *IPOL* / Pooled DTaP-IPV)		
	Pooled DTaP-IPV		*Infanrix* + *IPOL*			95% CI	
Antibody	N	Adjusted GMC	N	Adjusted GMC	Value	LL	UL
Anti-PT	822	70.8	256	78.0	1.103	0.994	1.224
Anti-FHA	844	871.1	261	927.5	1.065	0.972	1.166
Anti-PRN	845	600.6	261	587.7	0.979	0.871	1.100

Data source: Appendix table IIIA

Pooled DTaP-IPV = DTaP-IPV lots 1, 2, and 3 (pooled) + *M-M-R*II

Infanrix + *IPOL*: *Infanrix* + *IPOL* + *M-M-R*II

Adjusted GMC = geometric mean antibody concentration adjusted for baseline concentration

N = Number of subjects with both pre- and post-vaccination results available

95% CI = 95% confidence interval for the adjusted GMC ratio (ANCOVA model: adjustment for baseline concentration - pooled variance); LL = lower limit, UL = upper limit

Additional analysis:

Table 30 **Percentage of subjects with Anti-PT, Anti-FHA, and Anti-PRN antibody concentrations of at least 5 EL.U/mL, and geometric mean antibody concentrations before and one month after vaccination (ATP Cohort for immunogenicity)**

				≥5 EL.U/mL				GMC		
						95% CI			95% CI	
Antibody	Group	Timing	N	n	%	LL	UL	value	LL	UL
Anti-PT	Pooled DTaP-IPV	PRE	823	256	31.1	28.0	34.4	4.0	3.8	4.2
		PI(M1)	849	847	99.8	99.2	100	70.7	66.8	74.8
	Infanrix + *IPOL*	PRE	257	89	34.6	28.8	40.8	4.2	3.8	4.7
		PI(M1)	261	261	100	98.6	100	80.4	72.4	89.1
Anti-FHA	Pooled DTaP-IPV	PRE	847	833	98.3	97.2	99.1	50.4	46.2	55.0
		PI(M1)	850	850	100	99.6	100	864.2	821.0	909.8
	Infanrix + *IPOL*	PRE	262	258	98.5	96.1	99.6	53.2	45.2	62.5
		PI(M1)	261	261	100	98.6	100	939.7	858.3	1028.8
Anti-PRN	Pooled DTaP-IPV	PRE	847	773	91.3	89.2	93.1	27.0	24.9	29.2
		PI(M1)	851	851	100	99.6	100	598.7	559.2	640.9
	Infanrix + *IPOL*	PRE	262	236	90.1	85.8	93.4	27.4	23.5	32.0
		PI(M1)	261	261	100	98.6	100	593.8	525.7	670.7

Data source: Appendix table IIIA

Pooled DTaP-IPV = DTaP-IPV lots 1, 2, and 3 (pooled) + *M-M-Rᵢ*

Infanrix + *IPOL*: *Infanrix* + *IPOL* + *M-M-Rᵢ*

GMC = geometric mean antibody concentration

N = number of subjects with available results

n/% = number/percentage of subjects with concentration within the specified range

95% CI = 95% confidence interval; LL = Lower Limit; UL = Upper Limit

PRE =pre-vaccination blood sample at Day 0

PI(M1) =post-vaccination blood sample at Month 1

GSK met their primary and secondary objectives as far as immunogenicity of the pertussis components is concerned, however the additional analysis in which they look at rates of seropositivity is uninformative because their cut-off value for seropositivity is near, or only slightly above, the lower limit of quantitation (LLOQ) for the pertussis ELISA's. Thus, a very high proportion of subjects had titers above the cut-off level even before the vaccination that occurred during the trial (see Table 22 on p. 8 and Table 30 on p. 11 of this review). Furthermore, because the cut-off values are in the lower, more variable range of the assay, false positives may occur due solely to assay variability. Thus this analysis has an unacceptably low sensitivity and should not be considered.

COMMENT:

We note that you conducted additional analyses in which you determined seropositivity status (ELISA values for pertussis antigens ≥ 5 EU/ml). We note that the cut-off values that you used are near, or only slightly above the LLOQs for the assays. These cut-off values are in the lower, more variable range of the assays such that false positives may occur solely due to assay variability. We also note that a large proportion of titers obtained pre-vaccination were at or above these levels. Thus, seropositivity is an insensitive method for evaluating differences between DTaP-IPV lots or between separate versus combined vaccines. Therefore, CBER considers seropositivity data to be uninformative and will not be considered as supportive. Please comment.