Statistical Review and Evaluation

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Subject STN: BL 125145/0
Pentacel (DTaP-IPV/Hib Combined)
Sanofi Pasteur

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I. BACKGROUND

This Biologics License Application (STN BL 125145/0) was submitted on July 26, 2005 by Sanofi Pasteur for PENTACEL™, the Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate) Reconstituted with Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed Combined with Poliovirus Vaccine Inactivated (HCPDT-IPV/PRP-T Vaccine. The clinical development of this vaccine in the United States has been investigated under BB-IND ----, initially submitted to CBER on July 21, 1999. The current statistical reviewer has been involved in this BLA submission since October 1, 2006.

PENTACEL Vaccine will be indicated for active immunization for prevention of disease due to H. influenzae type b, pertussis, diphtheria, tetanus and poliovirus types 1, 2, and 3 to be administered intramuscularly as a four-dose primary series at 2, 4, 6, and 15 to 18 months of age.

In a CR letter dated April 23, 2007, CBER requested the Applicant for additional information regarding pertussis serology assays used in the Pentacel pivotal studies including P3T06. In a telephone conference dated September 11, 2007, the Applicant proposed to submit the M5A10 post-dose 3 results in supporting the evaluation of the immune response of the Hib component of Pentacel.

This statistical review focuses on the Applicant's responses to the above issues and consists three individual reviews: the parallel line analysis (PLA, a pertussis ----- assay analysis method), a pivotal study P3T06, and M5A10 post-dose 3 immunogenicity results.

II. PARALLEL LINE ANALYSIS (PLA)

II.1 Background

Amendment BL 125145/0.48 which was submitted on October 02, 2007 contained the sample calculations for the parallel line analysis (PLA) using ----------. The applicant provides a complete description of the method used and sample calculations of the pertussis ----- as performed in Canada and the trending high control charts to demonstrate that the anti-PT ----- as performed in the US is stable over time.

In 1999 through 2001, the Canadian Pertussis ----- assay methods were updated following communication with CBER (October 1999 through April 2002, PLA 96-0660) to ensure consistency and quality of the assays by defining the Lower Limit of Quantitation (LLOQ), and reassessing stability and precision for each of the ------ over time. At that time, CBER also reviewed the parallel line data analysis program (used by ---------- ----------, which was ----- Calculation Software (---------- modeled according to the methods described by Manclark et al. CBER approved the assays for use as of December 2001.

In 2004, the Canadian Pertussis ------ were optimized and validated for use in ---------- ----------. To be consistent with the parallel line analysis (PLA) data calculation method as performed in ---------- implemented a PLA data calculation method using ----------. However, the parallel line analysis data reduction method utilized for sample calculation by ---------- was not modified. The only changes made were to include detailed criteria related to the reference curve and test sample acceptance.

II.2 Description of Procedures

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II. P3T06 STUDY

III.1 Introduction

This review focuses on the report entitled "Re-evaluation of Anti-Pertussis Toxin Responses for Subjects who Participated in Clinical Trial P3T06." The report summarizes the results from the re-assaying of P3T06 sera samples using a further-purified Pertussis Toxin (PT) ---------- ---------- ---------- in the ---------- assay. P3T06 is one of the pivotal studies in the Pentacel BLA submission.

Initially, sera collected from subjects participating in P3T06 were tested at ---------- ----------, using PT ----------. The purpose of the current study was to address the noninferiority of anti-PT GMCs and 4-fold rise rates post-Dose 3 and post-Dose 4 for Pentacel relative to DAPTACEL when tested with the ---------- assay using ----------. The analyses were performed based on re-testing of all available paired (pre-Dose1/post-Dose 3, or pre-Dose 1/post-Dose 4) sera (≥25 µL/sample), for subjects who participated in Study P3T06.

III.2 Study Design

Study P3T06 was designed to compare Pentacel vaccine to the US-licensed and concomitantly administered separate vaccines of DAPTACEL (DTaP), IPOL (IPV), and ActHIB (PRP-T). The study consisted of two stages, Stage I (age of 2-7 months) and Stage II (age of 12-17 months). Stage I had a randomized, controlled, multi-center design for evaluating the lot consistency of DAPTACEL in terms of immunogenicity and safety (double-blinded portion), when co-administered with other recommended vaccines. In an open-label manner, the study also compared the safety and immunogenicity of an infant series (Stage I, pooled Groups 1, 2, and 3) and 4th Dose (Stage II, Group1) DAPTACEL to the safety and immunogenicity of Pentacel. Subjects form Stage I DAPTACEL Groups 1, 2, and 3 (Lots 1, 2, and 3) were randomly allocated to participate in Stage II Groups 1, 2, and 3.

III.3 Re-Test Plan

In the original P3T06 study, statistical analyses were conducted on a total of 374 and 1167 subjects for Pentacel and DAPTACEL, who were included in the post-Dose 3 Stage I Per-Protocol (PP) Immunogenicity Population, and a total of 371 and 349 subjects for Pentacel and DAPTACEL Group 1 (received 4th dose of DAPTACEL and ActHIB at 15 months of age), who were included in the Stage II post-Dose 4 PP immunogenicity population.

The absolute minimum quantity required to perform a single valid PT ------ assay was determined to be 25 µL. Table 1 (Table 4.1 in P3t06 Retest Report) presents the sample availability results. The total number of Stage I pre-Dose 1 and post-Dose 3 sera matching pairs with ≥25 µL/sample was 144 for Pentacel and 486 for DAPTACEL. For Stage II, the total number of pre-Dose 1 and post-Dose 4 sera matching pairs with ≥25 µL/sample was 113 for Pentacel (Group 4) and 128 for DAPTACEL (Group 1).

Table 1: Sample Availability at Stage I (Post-Dose 3) and II (Post-Dose 4)

Selection Criteria	Pentacel	DAPTACEL
Post-Dose 3		
Per-Protocol for Stage I	374	1167
All Pertussis serological results post-Dose 3	318	1015
PT serological result pre-Dose 1	219	712
≥25 µL of sera remaining Post-Dose 3 and Pre-Dose 1	144	486
Post-Dose 4		
Per-Protocol for Stage II	371	349
All Pertussis serological results post-Dose 4	366	345
PT serological result pre-Dose 1	207	222
≥25 µL of sera remaining Post-Dose 4 and Pre-Dose 1	113	128

Two major issues regarding re-test samples are discussed below:

III.3.1 Representative Sera Samples

Sample representativeness with the original US eBLA submission data was demonstrated if the lower and upper bounds of the 2-sided 95% CI of the GMC ratio [P3T06 PP population ------- assay) / re-test sample (------ assay)] for each pertussis antigen were >2/3 and <1.5 at pre-Dose 1, post-Dose 3, and post-Dose 4. Table 2 (for Pentacel results, Table 5.1 in the P3t06 Retest Report) and Table 3 (for DAPTACEL results, Table 5.2 in the P3t06 Retest Report) presents results of equivalence comparisons of the pre-Dose 1, post-Dose 3, and post-Dose 4 anti-PT antibody GMCs of the selected samples and those reported and submitted for the overall Pentacel and DAPTACEL PP Populations of Study P3T06.

Results demonstrated that the samples selected were representative of the originally tested PP Population, since both the lower and upper margins of the 95% CI of the GMC ratio for all pertussis antigens were >2/3 and <1.5.

III.3.2 Power Calculation

The sample sizes (as shown in Table 1) were based on subjects with sufficient sera sample pairs in the PP populations at Stages I and II, respectively. Power was based on evaluating non-inferiority by observing the upper bound of a 2-sided 95% CI of the difference in 4-fold rise rates being <10% and by observing the upper bound of a 2-sided 95% CI of the ratio of anti-PT GMCs being <1.5. Rate estimates were based on the original US eBLA submission data from Pentacel Study P3T06.

Table 4 (Table 4.2 in the P3t06 Retest Report) shows power estimates at Stages I and II. Within a primary hypothesis, the type I error rate is controlled to a level α by specifying that a vaccine regimen is non-inferior to its comparator if and only if the PT responses are found to be non-inferior for that regimen versus its comparator. There was no attempt to control the type I error rate across the primary hypotheses or across Stages I and II.

Table 2: Equivalence Comparison of GMCs at Pre-Dose 1, Post-Dose 3, and Post-Dose 4, Pentacel Subjects, PP Population vs. Re-test Sample

Pentacel Subjects		**Re-Test Sample**			**PP Population**			**PP Population/ Re-Test Sample**	
Bleed	**Antigen**	**M**	**Geometric Mean**	**95% CI**	**M**	**Geometric Mean**	**95% CI**	**Ratio**	**(95% CI)**
Pre-Dose 1	PT (EU/mL)	163	3.56	(3.21; 3.95)	270	3.80	(3.49; 4.14)	1.07	(0.93; 1.22)
	FHA (EU/mL)	163	4.49	(3.82; 5.27)	272	4.64	(4.10; 5.25)	1.03	(0.85; 1.26)
	FIM (EU/mL)	163	11.40	(10.34; 12.57)	269	11.75	(10.85; 12.72)	1.03	(0.91; 1.17)
	PRN (EU/mL)	163	3.00	(2.59; 3.48)	272	3.06	(2.72; 3.43)	1.02	(0.84; 1.23)
Post-Dose 3	PT (EU/mL)	144	89.79	(81.55; 98.86)	318	89.98	(84.19; 96.17)	1.00	(0.89; 1.13)
	FHA (EU/mL)	144	74.62	(66.59; 83.62)	318	73.68	(68.52; 79.23)	0.99	(0.87; 1.13)
	FIM (EU/mL)	144	270.11	(239.00; 305.27)	318	268.15	(247.21; 290.87)	0.99	(0.86; 1.15)
	PRN (EU/mL)	144	37.63	(32.43; 43.67)	318	36.05	(32.27; 40.27)	0.96	(0.79; 1.16)
Post-Dose 4	PT (EU/mL)	113	183.53	(159.01; 211.84)	366	174.03	(159.27; 190.17)	0.95	(0.79; 1.13)
	FHA (EU/mL)	113	112.61	(95.96; 132.15)	366	107.94	(99.42; 117.20)	0.96	(0.81; 1.14)
	FIM (EU/mL)	113	636.22	(539.70; 750.00)	367	553.39	(496.11; 617.27)	0.87	(0.70; 1.08)
	PRN (EU/mL)	113	89.70	(75.01; 107.27)	367	93.59	(83.98; 104.31)	1.04	(0.84; 1.30)

Table 3: Equivalence Comparison of GMCs at Pre-Dose 1, Post-Dose 3 and Post-Dose 4, DAPTACEL Subjects, PP Population vs. Re-test Sample

DAPTACEL Subjects		Re-Test Sample			PP Population			PP Population/ Re-Test Sample	
Bleed	Antigen	M	Geometric Mean	95% CI	M	Geometric Mean	95% CI	Ratio	(95% CI)
Pre-Dose 1	PT (EU/mL)	514	4.05	(3.79; 4.32)	795	3.98	(3.77; 4.20)	0.98	(0.90; 1.07)
	FHA (EU/mL)	514	5.15	(4.69; 5.65)	806	4.87	(4.52; 5.25)	0.95	(0.84; 1.07)
	FIM (EU/mL)	513	12.19	(11.47; 12.95)	797	12.20	(11.61; 12.82)	1.00	(0.93; 1.08)
	PRN (EU/mL)	514	3.18	(2.93; 3.44)	799	3.15	(2.94; 3.37)	0.99	(0.89; 1.10)
Post-Dose 3	PT (EU/mL)	486	61.09	(57.47; 64.93)	1016	63.82	(61.22; 66.53)	1.04	(0.97; 1.12)
	FHA (EU/mL)	486	29.83	(27.91; 31.89)	1016	29.22	(27.91; 30.60)	0.98	(0.90; 1.06)
	FIM (EU/mL)	486	260.87	(240.75; 282.67)	1015	267.18	(253.15; 282.00)	1.02	(0.93; 1.13)
	PRN (EU/mL)	486	41.78	(38.21; 45.69)	1016	43.25	(40.68; 45.99)	1.04	(0.93; 1.15)
Post-Dose 4	PT (EU/mL)	128	182.35	(156.32; 212.71)	346	168.48	(153.52; 184.89)	0.92	(0.77; 1.10)
	FHA (EU/mL)	128	62.68	(54.94; 71.51)	345	64.02	(58.81; 69.69)	1.02	(0.87; 1.20)
	FIM (EU/mL)	128	618.90	(515.01; 743.74)	347	513.54	(457.72; 576.17)	0.83	(0.67; 1.03)
	PRN (EU/mL)	128	182.67	(154.53; 215.94)	347	186.07	(168.16; 205.88)	1.02	(0.84; 1.24)

Table 4: Power Estimates at Stages I (Post-Dose 3) and II (Post-Dose 4)

Antigen	Endpoint	Estimated 4-fold Rise Rate or GMC Standard Deviation	Non-inferiority Definition	Sample Size Per Group (Pentacel / DAPTACEL)	% Power
Post-Dose 3					
Pertussis PT	≥4-fold rise	94%	$<\delta = 10\%$	144/486	95.6
Pertussis PT	GMC	0.60	$<\delta = 1.5$	144/486	~100.0
Stage I Combined					**95.6**
Post-Dose 4					
Pertussis PT	≥4-fold rise	97%	$<\delta = 10\%$	113/128	95.1
Pertussis PT	GMC	0.86	$<\delta = 1.5$	113/128	95.3
Stage II Combined					**90.6**

III.4 Statistical Methods

III.4.1 Objectives, Hypotheses, and Endpoints

Stage I Objective: The primary objective of Stage I was to compare the 4-fold rates and the GMCs elicited by the PT pertussis antigen in Pentacel with those of DAPTACEL when these vaccines are co-administered with other recommended vaccines, after the infant series.

Stage I Hypothesis: The anti-PT antibody response elicited by the infant series of Pentacel concurrently administered with a pneumococcal conjugate (Pnc7) and hepatitis B (HepB) vaccines would be non-inferior to responses (pooled responses from three vaccine lots) elicited by DAPTACEL concurrently administered with Haemophilus influenzae type b (Hib), inactivated poliovirus (IPV), Pnc7, and HepB vaccines, as assessed by the difference of the 4-fold rise rates [post-Dose 3 (--- assay) / pre-Dose 1 (--- assay) ≥4] and the ratio of their GMCs.

Stage I Endpoints: Non-inferiority for the PT pertussis antigen was demonstrated if:

- The upper bound of the 2-sided 95% Confidence Interval (CI) of the difference in 4-fold rise rates (Groups 1-3 Combined - Group 4) was <10%.
- The upper bound of the 2-sided 95% CI of the ratio of the GMCs (Groups 1-3 Combined GMC / Group 4 GMC) was <1.5.

Stage II Objective: The primary objective of Stage II was to compare the 4-fold rise rates and the GMCs elicited in toddlers after the 4th dose of the PT pertussis antigen in Pentacel (Group 4) with those elicited by DAPTACEL co-administered with ActHIB (Group 1).

Stage II Hypothesis: The anti-PT antibody response elicited in toddlers by the 4th dose of Pentacel (Group 4) would be non-inferior to those elicited by DAPTACEL concurrently administered with ActHIB (Group 12), as assessed by the difference of the 4-fold rise rates [post-Dose 4 (--- assay) / pre-Dose 1 (--- assay) ≥4] and the ratio of their GMCs.

Stage II Endpoints: Non-inferiority for the PT pertussis antigen was demonstrated if:

- The upper bound of the 2-sided 95% Confidence Interval (CI) of the difference in 4-fold rise rates (Group 1 - Group 4) was <10%.
- The upper bound of the 2-sided 95% CI of the ratio of the GMCs (Group 1 GMC / Group 4 GMC) was <1.5.

III.4.2 Statistical Analysis

Measurements:

1. PT Pertussis 4-fold rise rates

   The following algorithm was used to calculate fold-rise:

   - If the numerator was <LLOQ and the denominator was <LLOQ, then fold-rise was calculated as 0.5LLOQ/0.5LLOQ,

- If the numerator was <LLOQ and the denominator was ≥LLOQ, then fold-rise was calculated as 0.5LLOQ/(Raw Value of Denominator),
- If the numerator was ≥LLOQ and the denominator was <LLOQ, then fold-rise was calculated as (Raw Value of Numerator)/LLOQ,
- If the numerator was ≥LLOQ and the denominator was ≥LLOQ, then fold-rise was calculated as (Raw Value of Numerator)/(Raw Value of the Denominator)

where the denominator was the pre-Dose 1 value and the numerator was the post-Dose 3 or post-Dose 4 values (all values as reported by ---- using the ---------- ----------, depending on which stage of the study was being analyzed.

2. PT Pertussis GMCs

   At the subject level, if a serology value was <LLOQ, then for the analysis of GMC, the value was imputed as 0.5LLOQ prior to the log-transformation.

Population Analyzed

- Per-Protocol Immunogenicity Population:

  The PP population included all subjects who satisfied the following:
  - Met all inclusion and exclusion criteria,
  - Received all 3 (for Stage I comparisons) or 4 doses (for Stage II comparisons) of study vaccines as per randomization within window intervals,
  - Post-Dose 3 (for Stage I comparisons) and post-Dose 4 (for Stage II comparisons) blood sampling performed in the specified windows.
  - Had a valid serology test result post-Dose 3 (for Stage I comparisons) or post-Dose 4 (for Stage II comparisons) for at least 1 DAPTACEL or Pentacel antigen.

- Populations Used in the Re-test Analyses

  All immunogenicity analyses were conducted in an available sample from the PP populations of Study P3T06 Stage I and Stage II, respectively. At each stage, all subjects in the re-test sample satisfied the following:
  - Part of the PP population.
  - Serological results for each pertussis antigen, post-Dose 3 (Stage I) or post-Dose 4 (Stage II).
  - PT serological result, pre-Dose 1.
  - ≥25 µL of sera remaining pre-Dose 1, and post-Dose 3 (Stage I) or post-Dose 4 (Stage II).

III.4.3 Results of Non-Inferiority of Anti-PT Immunogenicity

Stage I Primary Objective

4-fold Rise Rates

Table 5: Non-Inferiority Comparison of 4-Fold Rise Rates, Post-Dose 3, Pentacel vs. DAPTACEL

Anti-PT (EU/mL)		Pentacel			DAPTACEL			Non-Inferiority Comparison DAPTACEL - Pentacel		Non-Inferiority Yes/No
Population \ Criteria	**Bleed**	**n/M**	**%**	**95% CI**	**n/M**	**%**	**95% CI**	**%**	**(95% CI)**	
Representative Sample ≥4-fold rise (Re-tested results)	Post-Dose 3	137/143	95.8	(91.1; 98.4)	420/481	87.3	(84.0; 90.2)	-8.49	(-12.92; -4.05)	Yes
PP Population ≥4-fold rise (Original results)	Post-Dose 3	205/219	93.6	(89.5; 96.5)	613/712	86.1	(83.3; 88.6)	-7.51	(-11.63; -3.39)	Yes

As shown in the above Table 5 (Table 5.3 in the P3t06 Retest Report), Pentacel anti-PT 4-fold rise rates, based on the re-assay serology results for the representative sample, were non-inferior to those of the separately administered US licensed standard of care vaccines DAPTACEL, IPOL, and ActHIB after the 3rd dose (infant series). Four-fold rise rates for the PT antigen were higher in the Pentacel group than in the pooled DAPTACEL group (95.8% versus 87.3%, respectively) and very similar to the previous rates observed in the PP Population of study P3T06, tested at ---------- (93.6% versus 86.1%, respectively).

GMCs

Table 6: Non-Inferiority Comparison of Anti-PT GMCs, Post-Dose 3, Pentacel vs. DAPTACEL

Anti-PT (EU/mL)		Pentacel			DAPTACEL			Non-Inferiority Comparison DAPTACEL / Pentacel		Non-Inferiority Yes/No
Population \ Criteria	**Bleed**	**M**	**Geometric Mean**	**95% CI**	**M**	**Geometric Mean**	**95% CI**	**Ratio**	**(95% CI)**	
Representative Sample Concentration (Re-tested results)	Post-Dose 3	143	102.62	(93.91; 112.15)	485	61.88	(58.29; 65.70)	0.60	(0.53; 0.68)	Yes
PP Population Concentration2(Original results)	Post-Dose 3	318	89.98	(84.19; 96.17)	1016	63.82	(61.22; 66.53)	0.71	(0.65; 0.77)	Yes

As shown in the above Table 6(Table 5.4 in the P3T06 Retest Report), anti-PT GMCs were higher in subjects, who received Pentacel (Group 4, GMC=102.62 EU/ml) than in those who received DAPTACEL, IPOL, and ActHIB in Groups 1-3 (GMC=61.88 EU/ml). Consequently, the noninferiority comparisons based on the ratio of the GMCs met the non-inferiority criteria. The applicant claims "the upper bound of the 95% CI of the ratio did not include 1, demonstrating the **statistical superiority** of the anti-PT responses elicited by 3 doses of Pentacel. The same was true for the 95% CI of the GMC ratio of the original P3T06 results, where Pentacel was also shown to be **statistically superior** to DAPTACEL at the end of the infant series."

Reviewer's comments: *Given that the statistical superiority was not considered in the primary hypothesis, it is inappropriate to make such conclusion*

.

Stage II Primary Objective

4-fold Rise Rates

Table 7: Non-Inferiority Comparison of 4-Fold Rise Rates, Post-Dose 4, Pentacel vs. DAPTACEL

Anti-PT (EU/mL)		**Pentacel**			**DAPTACEL**			**Non-Inferiority Comparison DAPTACEL - Pentacel**		**Non-Inferiority Yes/No**
Population \ Criteria	**Bleed**	**n/M**	**%**	**95% CI**	**n/M**	**%**	**95% CI**	**%**	**(95% CI)**	
Representative Sample ≥4-fold rise (Re-tested results)	Post-Dose 4	106/113	93.8	(87.7; 97.5)	116/127	91.3	(85.0; 95.6)	-2.47	(-9.08; 4.14)	Yes
PP Population ≥4-fold rise (Original results)	Post-Dose 4	225/231	97.4	(94.4; 99.0)	231/238	97.1	(94.0; 98.8)	-0.34	(-3.31; 2.63)	Yes

Table 7 (Table 5.5 in the P3T06 Retest Report) presents Pentacel and DAPTACEL and ActHIB anti-PT 4-fold rise rates with 95% CI, based on the re-assay serology results for the representative sample. Pentacel anti-PT 4-fold rise rates were non-inferior to those of DAPTACEL and ActHIB in toddlers (after the 4th dose). Four-fold rise rates for the PT antigen were very similar between the two groups, (i.e., 93.8% for the Pentacel group, and 91.3% for the DAPTACEL group). The same was true for the PP Population, tested ---------- (97.4% versus 97.1%, respectively).

GMCs

Table 8 (Table 5.6 in the P3T06 Retest Report) presents the GMCs with a 95% CI after a 4th dose of Pentacel in Group 4, and 3 doses DAPTACEL, IPOL, and ActHIB followed by a 4th dose of DAPTACEL and ActHIB in Group1. The anti-PT GMCs were similar in subjects from the representative set, who received Pentacel (Group 4, GMC=107.89 EU/ml) and those who received DAPTACEL and ActHIB (GMC=100.29 EU/ml). The anti-PT GMC responses elicited by 4 doses of Pentacel were non-inferior to the GMC responses elicited by 4 doses of DAPTACEL.

Table 8: Non-Inferiority Comparison of Anti-PT GMCs, Post-Dose 4, Pentacel vs. DAPTACEL

Anti-PT (EU/mL)		**Pentacel**			**DAPTACEL**			**Non-Inferiority Comparison DAPTACEL / Pentacel**		**Non-Inferiority Yes/No**
Population \ Criteria	**Bleed**	**M**	**Geometric Mean**	**95% CI**	**M**	**Geometric Mean**	**95% CI**	**Ratio**	**(95% CI)**	
Representative Sample Concentration (Re-tested results)	Post-Dose 4	113	107.89	(93.68; 124.26)	128	100.29	(86.02; 116.94)	0.93	(0.75; 1.15)	Yes
PP Population Concentration (Original results)	Post-Dose 4	366	174.03	(159.27; 190.17)	346	168.48	(153.52; 184.89)	0.97	(0.85; 1.10)	Yes

III.4.4 Conclusion

Re-test sera samples from subjects immunized with Pentacel or DAPTACEL were re-analyzed with the --- assay using the ----------. The anti-PT findings of this re-test study confirmed the conclusions based on the existing P3T06 data regarding the non-inferiority of the anti-PT antibody responses elicited by Pentacel.

IV. M5A10 POST-DOSE 3 IMMUNOGENICITY

IV.1 Introduction

M5A10 is designed to compare the immunogenicity and safety of 3 doses of DAPTACEL, ActHIB, and IPOL and a 4th dose of DAPTACEL and ActHIB (US-licensed schedule) with either: 4 doses of Pentacel; a 4th dose of DAPTACEL and ActHIB administered after 3 doses of Pentacel; or 4 Doses of HCPDTIPV and ActHIB in infants (infant series) and toddlers (4th dose). This statistical review focuses on the report that presents only anti-PRP results for the Infant Series (Stage I).

IV.2 Trial Design

M5A10 is a phase 3, randomized, controlled, multi-center, open-label study designed to demonstrate that the anti-diphtheria, anti-tetanus, anti-pertussis, and anti-PRP immune responses elicited by different formulation of the 5-component acellular pertussis vaccines are non-inferior to those elicited by 4 doses of DAPTACEL and ActHIB.

Approximately 2,160 children were enrolled in this 2-Stage study. The total duration of the trial was approximately 19 months. Children were randomly assigned to 1 of 4 following groups:

Group 1: DAPTACEL, IPOL, and ActHIB at 2, 4, and 6 months of age (Stage I) and DAPTACEL and ActHIB at 15 months of age (Stage II).

Group 2: Pentacel at 2, 4, and 6 months of age (Stage I) and DAPTACEL and ActHIB at 15 months of age (Stage II).

Group 3: HCPDT-IPV and ActHIB at 2, 4, and 6 months of age (Stage I) and at 15 months of age (Stage II).

Group 4: Pentacel at 2, 4, and 6 months of age (Stage I) and at 15 months of age (Stage II).

For Stage I of the study, all children received a pneumococcal vaccine (Prevnar) at 2, 4, and 6 months of age and a hepatitis B vaccine at 2 and 6 months of age. For Stage II of the study, all children received Prevnar, M-M-R II (measles, mumps, and rubella), and Varivax (varicella) or ProQuad (measles, mumps, rubella, and varicella) vaccines at 12 months of age.

Table 9 (Table 2.1 in M5A10 Hib Immunogenicity Report) presents the main Stage I activities.

Table 9: Trial Activities

Visit Number (V)	**V01**	*Phone calls*	**V02**	*Phone calls*	**V03**	*Phone calls*	**V04**
Visit Intervals [days]		*V01 + 3, V01 + 8, V01 + 30*	**V01 + [45-75]**	*V02 + 3, V02 + 8, V02 + 30*	**V02 + [45-75]**	*V03 + 3, V03 + 8*	**V03 + [30-48]**
Days in Age Window	**42 to 89**		**87 to 164**		**147 to 239**		**210 to 287**
Informed Consent Signed	√						
Inclusion & Exclusion Criteria	√						
Blood Sampling (BS)	BS1 (1.0 mL)						BS2 (5 mL)
Randomization[1]	√						
Vaccines - Group 1							
DAPTACEL, ActHIB, IPOL	√		√		√		
Vaccines – Groups 2&4[2]							
Pentacel	√		√		√		
Vaccines – Group 3							
DTaP-IPV, ActHIB	√		√		√		
Vaccines all groups							
Recombivax HB® / Engerix B®	√				√		
Prevnar®	√		√		√		
Termination Record							√

[1] A randomization number was not assigned until a minimum of 1.0 mL of blood was obtained at Visit 1.

[2] Subjects in Groups 2 and 4 received the same vaccines during Stage I. In Stage II, Group 2 subjects were to receive DAPTACEL and ActHIB, and Group 4 subjects – Pentacel.

IV.3 Statistical Methods

Only the immunogenicity objectives, hypotheses, and endpoints that pertain to the anti-PRP comparisons between the Pentacel (Group 2 & 4) and the DAPTACEL, IPOL, and ActHIB (Group 1) cohorts are included in this review.

Immunogenicity objectives, hypotheses, and Hib endpoints

The immunogenicity objectives, hypotheses, and Hib endpoints are summarized in Table 10.

Table 10: Immunogenicity objectives, hypotheses, and Hib endpoints.

	Objective	Hypotheses	Endpoints
Primary	To compare the immune responses to PRP elicited by 3 doses of Pentacel to those elicited by 3 doses of DAPTACEL, IPOL, and ActHIB (US standard of care) as measured by seroprotection rates.	A non-inferiority test was used to compare the anti-PRP immune responses elicited by 3 doses of Pentacel (Groups 2&4 combined) to those elicited by 3 doses of DAPTACEL, IPOL, and ActHIB (Group 1). Non-inferiority was demonstrated if the upper bound of the 2-sided 95% Confidence Interval (CI) of the difference in seroprotection rates for ≥0.15 µg/mL or ≥1.0 µg/mL (Group 1 rate - [Groups 2&4] rate) was <10%.	Anti-PRP antibody (Ab) concentrations ≥0.15 µg/mL or ≥1.0 µg/mL post-Dose 3 were assessed 30 to 48 days after the 3rd dose of the primary series (V04) in Group 1 and Groups 2&4 combined.
Secondary	To compare the anti-PRP immune responses elicited by 3 doses of Pentacel to those elicited by 3 doses of DAPTACEL, IPOL, and ActHIB as measured by geometric mean concentrations (GMCs).	Non-inferiority testing was used to compare the anti-PRP responses elicited by 3 doses of Pentacel (Groups 2&4) to those elicited by 3 doses of DAPTACEL, IPOL, and ActHIB (Group 1). Non-inferiority was demonstrated if the upper bound of the 2-sided **90%** CI of the ratio of GMCs (Group 1 mean / [Groups 2&4] mean) was <1.5.	Anti-PRP Ab concentrations (seroprotection rates and GMCs) were assessed 30 to 48 days after the 3rd dose of the primary series (V04) in Groups 1 and 2&4 combined.
Observational	To present for Group 1 (DAPTACEL, IPOL and ActHIB) and Groups 2&4 (Pentacel) the anti-PRP seroprotection rates and GMCs with their corresponding 95% CI and reverse cumulative distribution curves (RCDC) post-Dose 3.	No hypotheses were tested for the observational endpoints. Descriptive summaries of the anti-PRP seroprotection rates, GMCs, and RCDCs were presented by treatment group.	Anti-PRP Ab concentrations (seroprotection rates and GMCs) were assessed 30 to 48 days after the 3rd dose of the primary series (V04) in Groups 1 and 2&4 combined.

Populations Analyzed

Two analysis populations are relevant to the immunogenicity analyses:

- Intent-to-Treat (ITT) immunogenicity population
- Per-Protocol (PP) immunogenicity population

For all immunogenicity analyses, the PP population was designated as the primary population and ITT population was also analyzed.

The intent-to-treat (ITT) population for immunogenicity for post-Dose 3 consisted of subjects who had 3 doses of study vaccine (regardless of treatment assignment errors), the post-Dose 3 blood draw, and a valid serology test result for at least 1 antigen.

The per-protocol (PP) population was a subset of the intent-to-treat (ITT) population for immunogenicity. The PP population consists of subjects who had to satisfy the following criteria:

- Meet all inclusion and exclusion criteria at study entry
- Receive the correct dose of all 3 doses of Pentacel; DTaP-IPV and ActHIB; or DAPTACEL, ActHIB, and IPOL according to the randomization schedule
- Receive Pentacel; DTaP-IPV and ActHIB; or DAPTACEL, ActHIB, and IPOL within the following time windows:
    - Dose 1: 42 to 89 days of age
    - Dose 2: Visit 1 + ≥45 days
    - Dose 3: Visit 2 + ≥45 days and ≤239 days of age
- Have blood sample 2 (post-Dose 3) drawn within the Visit 3 + (30-48 days) window and a valid serology test result for at least 1 antigen.

The PP summaries present the subjects grouped as they were randomized.

IV.4 Results

As the comparisons in this report will focus on those between subjects in Group 1 and Groups 2&4, the results presented are limited to these study groups.

IV.4.1 Disposition of Analyzed Population

Stage I of this trial was conducted at 38 US study centers, which provided a nationwide sampling of the subject population representative of the ethnic composition of the United States.

In Stage I, a total of 1084 subjects in Groups 2&4 combined and 543 subjects in Group 1 were randomized between 10 November 2005 and 21 September 2006. As the vaccination schedule was the same for Groups 2&4, subjects included in these groups were combined for all Stage I statistical analyses.

Of all randomized subjects, 916 and 455 subjects in Groups 2&4 and Group 1, respectively, satisfied the criteria for, and were included in the ITT Immunogenicity Population. Of these, 82 Group 2&4 subjects and 32 Group 1 subjects were protocol violators, leaving a total of **834** Group 2&4 subjects and **423** Group 1 subjects who satisfied the criteria for the PP Immunogenicity Population.

The mean age of subjects in both Groups 2&4 and Group 1 was 2.1 months. There were slightly more males than females in the study; however, the distributions were similar in Groups 2&4 (51.9% males and 48.1% females) and Group 1 (55.1% males and 44.9% females). The majority of subjects in these groups were Caucasian (74.6% in Groups 2&4 and 72.3% in Group 1), followed by Hispanic (12.6% in Groups 2&4 and 13.2% in Group 1) and Black (5.0% in Groups 2&4 and 6.1% in Group 1).

IV.4.2 Primary Objective: Seroprotection Rates for anti-PRP

Table 11 (Tables 4 and 5 in Appendix 2, M5A10 Hib Immunogenicity Report) presents the seroprotection rates after the 3rd dose of Pentacel in Groups 2&4 and

DAPTACEL, IPOL, and ActHIB in Group 1 for the PP immunogenicity population. The anti-PRP seroprotection rates elicited by Pentacel at the ≥0.15 µg/mL and ≥1.0 µg/mL antibody levels were non-inferior to those elicited by DAPTACEL, IPOL, and ActHIB.

Table 11: Non-Inferiority Comparison of PRP Seroprotection Rates, Post-Dose 3 Groups 2&4 vs. Group 1 (PP and ITT Immunogenicity Populations)

		Groups 2&4		Group 1		Non-Inferiority Comparison Group 1 – Groups 2&4		
Population	**Criteria**	**n/M**	**%**	**n/M**	**%**	**%**	**95% CI**	**Non-Inferiority**
PP	≥ 0.15 µg/mL	775/826	93.8	380/421	90.3	-3.56	-6.84, -0.29	Yes
	≥ 1.0 µg/mL	620/826	75.1	315.421	74.8	-0.24	-5.33, 4.85	Yes
ITT	≥ 0.15 µg/mL	847/908	93.3	410/453	90.5	-2.77	-5.93, 0.38	Yes
	≥ 1.0 µg/mL	681/908	75.0	336/453	74.2	-0.83	-5.74, 4.09	Yes

Reviewer's comments: SAS and StatXact programs had been used for checking the figures in the above table. Based on the data provided by the applicant, all figures were matched.

IV.4.3 Secondary Objective: GMC for anti-PRP Immune Response

Table 14 (Tables 8 and 9 in Appendix 2, M5A10 Hib Immunogenicity Report) shows that the GMCs for the anti-PRP antigens elicited by Pentacel were non-inferior to the GMCs elicited by DAPTACEL, IPOL, and ActHIB. The same comparison using the upper bound of the 95% CI as the limit for non-inferiority was also performed, reaching the same conclusion of non-inferiority.

Table 12: Summary of Seroprotection Rates, Post-Dose 3 Groups 2&4 and Group 1 (PP and ITT Immunogenicity Populations)

			Groups 2&4			Group 1		
Population	**Bleed**	**Criteria**	**n/M or M**	**% or Geometric Mean**	**95% CI**	**n/M or M**	**% or Geometric Mean**	**95% CI**
PP	Post -Dose 3	≥ 0.15 µg/mL	775/826	93.8	92.0, 95.4	380/421	90.3	87.0, 92.9
		≥ 1.0 µg/mL	620/826	75.1	72.0, 78.0	315/421	74.8	70.4, 78.9
		GMC	826	2.52	2.25, 2.81	421	2.38	2.01, 2.81
ITT	Post -Dose 3	≥ 0.15 µg/mL	847/908	93.3	91.5, 94.8	410/453	90.5	87.4, 93.0
		≥ 1.0 µg/mL	681/908	75.0	72.1, 77.8	336/453	74.2	69.9, 78.1
		GMC	908	2.51	2.25, 2.79	453	2.37	2.02, 2.79

Reviewer's comments: SAS and StatXact programs had been used for checking the figures in the above table. Based on the data provided by the applicant, all figures were matched.

IV.4.4 Results of Additional Analyses

Reverse cumulative distribution curves (RCDCs) were generated for post-Dose 3 responses to PRP. The curves for Groups 2&4 and Group 1 show the same antibody concentration distribution in both the PP and ITT populations.

Post-Dose 3 anti-PRP immune responses were also analyzed by race/ethnicity.

IV.4.5 Conclusion

Study M5A10 shows that Pentacel provides anti-PRP (Hib) antibody responses that are non-inferior to those obtained with the separate administration of DAPTACEL, IPOL, and ActHIB (the US licensed component vaccines and standard of care since 2003).

V. COMMENTS TO THE REVIEW COMMITTEE

The following issues had been discussed in the internal meeting held on November 2, 2007. The committee members from the laboratory decided to accept this proposed parallel line analysis (PLA) using ----------

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VI. COMMENTS TO THE APPLICANT

In the report of "Re-evaluation of Anti-Pertussis Toxin Responses for Subjects who Participated in Clinical Trial P3T06" (page 19 of 253), you claim that "the upper bound of the 95% CI of the ratio did not include 1, demonstrating the **statistical superiority** of the anti-PT responses elicited by 3 doses of Pentacel. The same was true for the 95% CI of the GMC ratio of the original P3T06 results, where Pentacel was also shown to be **statistically superior** to DAPTACEL at the end of the infant series."

Please acknowledge that it is inappropriate to draw such conclusions given that statistical superiority was not considered in the primary hypothesis.

VII. REVIEWER'S RECOMMENDATION

Based on data presented in Study M5A10, Pentacel provides anti-PRP (Hib) antibody responses that are non-inferior to those obtained with the separate administration of DAPTACEL, IPOL, and ActHIB (the US licensed component vaccines and standard of care since 2003).

Results of the re-test sera samples of the existing P3T06 data show that the anti-PT antibody responses elicited by Pentacel are non-inferior to that elicited by DAPTACEL.

A minor concern regarding the claimed statements in Study P3T06 is addressed above in the Comments to the Applicant.

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