DEPARTMENT OF HEALTH & HUMAN SERVICES
FDA/CBER/OVRR/DBPAP

Memorandum

Date: March 1, 2015

From: Leslie Wagner, Chemist, LRSP/DBPAP
Clinical Serology and Bioassay Reviewer

To: BLA 125525/0
Quadracel - Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed Combined with Inactivated Poliovirus (DTaP-IPV) Vaccine

Through: Mike Schmitt, Lab Chief, LRSP/ DBPAP

Subject: Clinical Serology and Bioassay Review, and Approval Memorandum
Diphtheria & Tetanus Assay

Sponsor: Sanofi Pasteur Limited (SPL)

Table of Contents

General Information:

Sanofi Pasteur submitted a Biologics License Application (BLA) for "Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed Combined with Inactivated Poliomyelitis Vaccine" referred to as QUADRACEL. The clinical development of this vaccine in the United States was performed under BB-IND 14668, initially submitted 25 March 2011.

Sanofi Pasteur's currently approved 5-component acellular pertussis pediatric vaccine (DAPTACEL®) and Inactivated Polio Virus vaccine (IPOL®) are approved for use as a 5th dose booster in children 4 through 6 years of age and serve as a standard of care for this indication in the United States. Sanofi Pasteur plans to enhance this current offering by introducing QUADRACEL, the fully liquid DTaP-IPV combined vaccine as a 5th dose booster in children 4 to 6 years of age.

QUADRACEL (DTaP-IPV), manufactured by Sanofi Pasteur Limited, Toronto, ON, Canada, is a sterile suspension of Diphtheria and Tetanus toxoids and Acellular Pertussis vaccine adsorbed separately on aluminum phosphate combined with Inactivated Poliomyelitis vaccine types 1, 2, and 3 (DTaP-IPV), for intramuscular injection.

DTaP-IPV Vaccine is proposed for immunization against diphtheria caused by *Corynebacterium diphtheriae*, tetanus caused by *Clostridium tetanis*, pertussis (whooping cough) caused by *Bordetella pertussis*, and poliomyelitis caused by polioviruses types 1, 2 and 3 when given as a booster dose in children 4 years of age to < 7 years of age (prior to their 7th birthday).

This memo covers my review of the validation reports and additional data to support the use of the assays to quantitate antibodies against tetanus and diphtheria toxoids to generate pivotal data. In addition, review of the data generated in the Phase 3 studies and the repeat analysis of samples (biological validation) is provided.

Review Identifiers and Dates

Biologics License Application (BLA) Submission Tracking Number (STN): 125525/0

Submission received by CBER: March 24, 2014

Review completed: February 4, 2015

Material Reviewed:

The following general module sections of the BLA were reviewed:

- m1 Regional
- m2 Common Technical Document Summaries
- m5 Clinical Study Reports

A more detailed list of information in the BLA reviewed is provided below by amendment number:

Original submission - dated 23 March 2014

- m1.6 Meetings
- m2.5 Clinical Overview
- m2.7.1 Summary of Biopharmaceutical Studies and Associated Analytical Methods
- m2.7.3 Summary of Clinical Efficacy
- m5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies
- m5.3.5.1 Study Reports of Controlled Clinical Studies Pertinent to the Claimed Indication, Study M5I02

Amendment 0.2 - dated 27 June 2014

- m1.11.3 Efficacy information amendment
- m5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Related Master File, INDs and BLAs: BB-IND 14668

Executive Summary

This memo covers my review of the documents supporting the performance of the ELISA to quantitate antibody against the tetanus antigen and (b) (4) to quantitate antibody against diphtheria antigen. This memo also addresses my review of the serologic data for tetanus and diphtheria submitted in the Phase 3 study (M5I02) designed to provide the pivotal comparative data to support the efficacy of the tetanus and diphtheria antigens in QUADRACEL.

The serologic data in the study report for study M5I02 demonstrated noninferiority of the tetanus and diphtheria responses to QUADRACEL when compared to the control groups.

In study M5I02, for both the anti-tetanus and anti-diphtheria responses, the lower limit of the 95% confidence intervals (CI) of the ratio of the geometric mean concentration (GMC) met the criterion in subjects who receive DTaP-IPV as a 5^{th} dose when compared to subjects who receive DAPTACEL + IPOL as a 5^{th} dose; similarly the difference (DTaP-IPV minus DAPTACEL + IPOL) in post-vaccination booster response rates for both anti-diphtheria and anti-tetanus between groups was non-inferior.

The validation reports and other additional data submitted in the BLA and cross referenced INDs were sufficient to support the adequate performance of the assays. No aberrant or unusual data were noted in the clinical study reports that would indicate performance issues with the assays.

Review

Serologic Assay for the Tetanus Antigen

Validation reports, SOPs and assay stability reports were cross referenced from IND 14668 in support of the data generated by these assays in the Phase 3 studies.

The following documents were submitted in support of the performance of the assays to quantitate responses to the tetanus antigen:

- Q_0277546, Method Instruction, "ELISA Method For The Determination Of Tetanus Antibodies In International Units
- Q_0249865, Validation Report for J000051, "ELISA Method for the Determination of Tetanus Antibodies in International Units"
- RED_00073544, Demonstration of the Long-Term Performance of the Anti-Tetanus IgG ELISA using Plots of Control Results
- RED_00069240, Biological Validation: M5I02 Tetanus ELISA, Diphtheria (b) (4), Polio (b) (4) (Types 1, 2, & 3) and Pertussis ELISAs (PRN, FHA, FIM, PT)
- Transfer Report for B000806 Transfer of SOP #A001837 "Determination of Diphtheria Antitoxin in International Units" from Clinical Serology Bldg. (b) (to Clinical Serology Bldg. (b) (4)
- Transfer of SWI J000051 "ELISA Method for the Determination of Tetanus Antibodies in International Units" from Bldg. (b) (to Bldg. (b) (4)
- Transfer Validation Report for SWI J000051 "ELISA Method for the Determination of Tetanus Antibodies in International Units" from Bldg. (b) (to Bldg. (b) (4)

The Tetanus IgG ELISA was used to quantitate the amount of anti-tetanus antibodies in human serum. This is the assay used by (b) (4) to evaluate the tetanus serological response to the vaccine.

The assay to measure anti-tetanus antibodies in human serum is based on standard direct ELISA methodology. Briefly (b) (4) .

The tetanus ELISA was originally validated in 2001 and was updated in 2003 (v. 02) to re-establish the Limit of Quantitation (LOQ) from (b) (4) . The sponsor submitted the control trending results from November 2001 to April 2014 which included the time period in which samples from Study M5I02 were tested. The sponsor has maintained the same reference standard ((b) (4)) for this assay since 2001, however different lots of (b) (4) have been used over the years. No trends were noted in the report or control charts.

The tetanus assay was originally validated in Building (b) (4) (Validation Report C000149) and included the following parameters: precision, accuracy, dilutability, limit of detection, and limit of quantitation. To ensure the validity of the transfer from Building (b) (to Building (b) (4) the current validation report assessed the accuracy and precision using SWI J000051 (same as Q_0277546) Transfer Report for C000586,"ELISA Method for the Determination of Tetanus Antibodies in international Units" from Bldg (b) (to Bldg (b) (4). The report states that upon review of the original Transfer Report (C000586) in 2004, the % difference between the results from Building (b) (and Building (b) (4) to assess accuracy were found to have been calculated incorrectly; originally (b) (4) of the titers were within (b) (4) of each other. Upon re-calculation, (b) (4) samples did not meet the (b) (4) criterion. An additional assessment was performed using Concordance Analysis and Control Equivalency applied to the original transfer data. Concordance Analysis is the most direct way to show comparability from two laboratories because it is a measure of both the precision and accuracy; additionally it provides a robust method for small sample sizes. According to the Concordance Analysis the upper 95% CI of the difference (most conservative approach) was (b) (4), meeting the criterion of less than (b) (4). An equivalency analysis of the high and low controls was performed to demonstrate assay stability. The analysis revealed that the (b) (4) means were equivalent between two laboratories. The results of the validation transfer report indicate that precise and equivalent diphtheria and tetanus antibody results can be determined in (b) (4) relative to (b) (4).

Summary
The data support adequate performance parameters for the use of the tetanus assay to generate data for clinical studies with threshold, fold rise and geometric mean endpoints. No issues with the validation reports were noted during review of this assay for IND 14668.

The statistical assay reviewer (Z. Gao) noted some potential deficiencies with the tetanus assay, these are indicated in italics, however 'defers to review committee for further considerations of potential impact of these issues based on the totality of evidence submitted'. In my opinion, these deficiencies do not constitute grounds for rejection of the suitability of the assay for the following reasons:

1. *Range: The range is the interval between the upper and lower concentration/amount of analyte in the sample for which it has demonstrated that the assay has a suitable level of accuracy, precision, and linearity. The validation lacks specification of assay range.*

   Response: While the range was not explicitly stated it can be inferred based on information determined from studies measuring the limits of quantitation (upper and lower), linearity, precision, and accuracy. It can be estimated from the available data as (b) (4). In addition during routine use, samples whose results are greater than the reference serum (b) (4) are further (b) (4) within the working range of the assay.

Additionally, the sponsor has provided data assessing the precision at the lower limit of quantitation (b) (4), accuracy and precision of a high titered control sample at the upper limit of quantitation (b) (4), and accuracy and precision of (b) (4).

2. *LOD: Limit of detection (LOD) is the lowest concentration of an analyte that the assay procedure can reliably differentiate from background noise. The method for determining LOD focused on the lowest result that can be calculated by the software* (b) (4). *However, the method did not provide evidence on whether the proposed LOD can differentiate from background noise. In fact, section 11.4 Dilutability of the validation report reported that background was* (b) (4) *for Tetanus. The background noise appears to be substantially higher than the proposed LOD* (b) (4).

   Response: The Lower Limit of Quantitation (LLOQ) was initially established (in 1998) at (b) (4) with an estimated LOD of (b) (4). The LLOQ was revalidated in 2001and was revised to (b) (4). The original LOD was extrapolated (not empirically determined) due to difficulty in obtaining very low titer/negative sera. For the purpose of the clinical studies conducted for this BLA, determination of the LOD is not a required attribute that needs to be demonstrated based on the clinical endpoint of (b) (4).

3. *Linearity: The dilutability experiment covers only a small range of low concentrations. It is essential to evaluate linearity/internal accuracy across the entire assay range. Additionally, the dilutability experiment tested only* (b) (4) *samples. However, the starting dilution* (b) (4) *and* (b) (4) *are recommended to be used for the clinical sample testing in the validation report. Therefore, the linearity assessment presented in the validation report appears to be inadequate.*

   Response: According to the SOP, clinical samples are routinely tested at (b) (4); however the procedure allows for some flexibility depending upon concentration but requires supervisory approval. The procedure states that a sample may be tested at dilutions as (b) (4). Ideally the validation should have included a demonstration of proportional dilution of a sample covering all potential dilutions. However I offer the following considerations to support the dilutional linearity of this method: (1) the potential for interference would most likely occur with low titered sera because the serum matrix would constitute the majority of the sample when analyzed at a low dilution. The sponsor submitted evidence that (b) (low-titered samples analyzed both undiluted and diluted (b) (4), appear to have good recovery and there was no statistically significant differences between diluted and undiluted samples; (2) for studies of the LLOQ, the sponsor tested (b) (4) low-titered sera (b) (4). Acceptable precision ((b) (4)) was obtained for all samples at the (b) (4) dilution, whereas only one sample had acceptable

precision at the (b) (4) dilution. This data supports acceptable precision can be achieved with low titered sera (less than (b) (4)) run at an appropriately lower starting dilution ((b) (4)). In addition, the reverse cumulative distribution curves generated using the clinical data indicate that the shapes of the curves were sufficiently close between comparator groups even though the curves were not overlapping. This indicates that whatever bias may be present, due to dilutional non-linearity, is consistent across the range of the assay and unlikely to have affected the data or the clinical outcome.

We recognize the limitations of the validation, including the studies on dilutional linearity; however I feel the assay is suitable for the purpose of this study. Response rates for the tetanus assay are typically on the order of (b) (4) times the clinically relevant cut-off value (b) (4) . In this study at least 97% of subjects achieved a post-booster level (b) (4) times the cut-off ((b) (4)), which is indicative of long term protection. The tetanus assay has been used for licensing all the firms' tetanus containing vaccines and disease has been well controlled using vaccine licensed using this assay.

4. *Precision: Precision was not adequately assessed in the area around the serostatus cut-off ((b) (4)) in this validation.*

Response: Adequate demonstration of precision was performed by the sponsor in the original validation; this was confirmed during transfer studies of the assay when it was moved from building (b)(to building (b) (4) . In the original validation (b) (4) samples tested were at or below the clinically relevant endpoint (b) (4) . No trend of %CV was noted with respect to concentration. All but one of the low titered samples had %CV values below (b) (4) which is consistent with the capabilities of this type of assay.

5. *Specificity: This validation did not evaluate the potential impact of the antibodies to the antigen of other organisms on the measurement of tetanus antibody level.*

Response: Specificity was not specifically addressed in the validation. This was partially inferred from studies where they (b) (4) in my opinion the internal accuracy was very good. I have not requested this specifically for re-validation as there may be an issue in finding truly negative serum.

Serologic Assay for the Diphtheria Antigen

Validation reports, SOPs and assay stability reports were cross referenced from IND 14668 in support of the data generated by these assays in the Phase 3 studies.

The following documents were submitted in support of the performance of the assays to quantitate responses to the diphtheria antigen:

- Q_0277558, Method Instruction, "Determination of Diphtheria Antitoxin in International Units"
- Q_0293450, Validation Report for SOP #37S2, "(b) (4) for Diphtheria Antitoxin"
- RED_00073616, Control Performance of Diphtheria (b) (4) from January 1999 through March 2014
- RED_00073544, Demonstration of the Long-Term Performance of the Anti-Tetanus IgG ELISA using Plots of Control Results
- RED_00069240, Biological Validation: M5I02 Tetanus ELISA, Diphtheria (b) (4), Polio (b) (4) (Types 1, 2, & 3) and Pertussis ELISAs (PRN, FHA, FIM, PT)
- Transfer Protocol B000806 "Transfer of SOP #A001837 (37S31) Determination of Diphtheria Antitoxin in International Units from Clinical Serology Bldg. (b) (to Clinical Serology Bldg. (b) (4)"
- Transfer Report for B000806 Transfer of SOP #A001837 "Determination of Diphtheria Antitoxin in International Units" from Clinical Serology Bldg. (b) (to Clinical Serology Bldg. (b) (4)

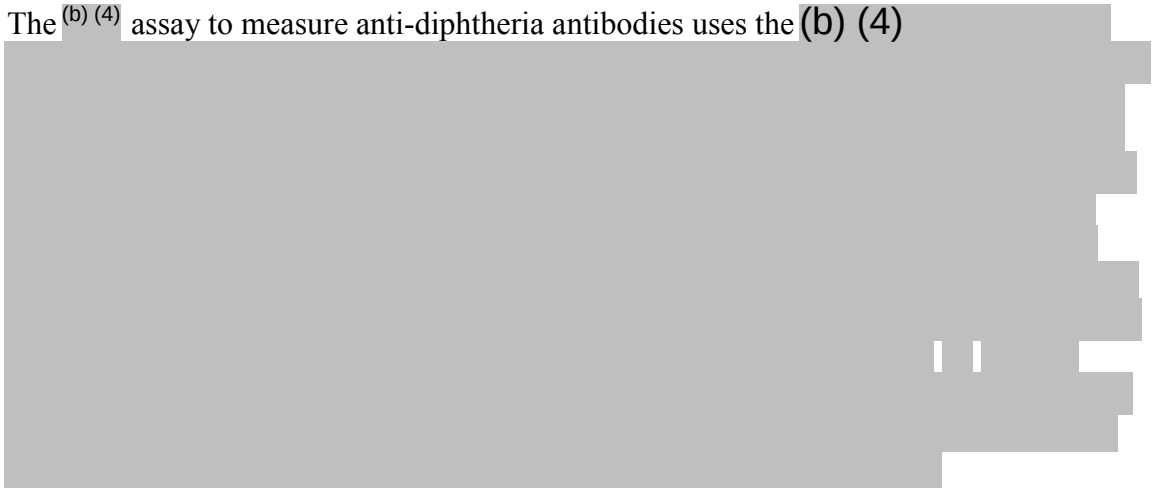
The protocol states that a (b) (4) was used to quantitate the amount of Diphtheria Toxin neutralizing antibodies in human sera. This is the y assay used by (b) (4) to evaluate the diphtheria serological response to the vaccine.

The diphtheria (b) (4) assay was validated in 1997. The sponsor submitted the control trending results January 1999 to March 2014, covering the time period samples for Study M5I02 were tested. Different lots of critical reagents (reference, toxin, and control) have been used in this assay over time; minor trends were observed but were due to lot changes of reagents and all results were within acceptable performance ranges. The Minimum Detectable Antitoxin (MDA) is defined as the lowest concentration of the reference standard that neutralizes a standard diphtheria toxin challenge dose; overall the reference has performed within the range of (b) (4) from January 1999 to March 2014.

The Sanofi serologic assay for antibodies to diphtheria is based on an assay developed in the 1970's. This assay has been used for decades to assess the responses to diphtheria toxoid in combination vaccines. In general, responses to diphtheria are robust and well above the conventionally accepted sero-protective level of (b) (4) and the disease has been well controlled. Based on the general use of the assay and the reliability of the reagents, the assay appears to be robust and reliable. However, the current methods and validations at Sanofi are not consistent with current practices. We have no reason to

believe that the data generated by the assay to date are unreliable, in fact the standard and control data submitted to date indicate consistent performance. Sanofi was made aware of the lack of information related to the assay and be given the opportunity to address the weaknesses in the assay documentation over time through IND 14468.

The (b) (4) assay to measure anti-diphtheria antibodies uses the (b) (4)



The assay was originally validated in (b) (4) (Validation Report V04-433A, Validation Report for SOP #37S2, (b) (4) for Diphtheria Antitoxin") and included the following parameters: precision, limit of detection, selectivity, and ruggedness. The assay was subsequently transferred between laboratories at (b) (4) from Building (b) (to Building (b) (4). To verify the validity of the transferred assay prior to evaluation of clinical samples, precision, accuracy, specificity, and dilutability were tested, and the results showed that diphtheria (b) (4) results obtained in Building (b) (4) are equivalent to results produced in Building (b) ((Transfer Report, C000552, Transfer Report for B000806, "Transfer of SOP #A001837, "Determination of Diphtheria Antitoxin in International Units" from Clinical Serology Bldg (b) (to Clinical Serology Bldg (b) (4)"). The transfer validation study was primarily composed of samples ((b) (4)) with concentrations with a range of (b) (4) ; the primary study endpoint for anti-diphtheria titers is based on demonstration of a 2- or 4-fold rise in titers to vaccination (depending upon pre-vaccination levels). A Reverse Cumulative Distribution plot of the anti-diphtheria responses show that greater than (b) (4) of subjects had titers less than (b) (4) (b) (4) pre-vaccination and were at least (b) (4) post-vaccination.

The statistical assay reviewer (Z. Gao) noted some potential deficiencies with the diphtheria assay, these are indicated in italics, however 'defers to review committee for further considerations of potential impact of these issues based on the totality of evidence submitted'. In my opinion, these deficiencies do not constitute grounds for rejection of the suitability of the assay for the following reasons:

1. *Selectivity/Specificity: The validation showed that the presence of antibodies to the antigen of other organisms did not inflate diphtheria toxin neutralization antibody titers when the sera samples were known to have low diphtheria toxin neutralization antibody titers. However, the validation lacks the*

selectivity/specificity assessment of positive samples at different titer levels. Since the mechanisms of potential interference between diphtheria toxin antibodies and the antibodies of the other organisms, if any, are unclear, it is essential to ensure that diphtheria toxin neutralization antibody titers can be accurately measured for positive samples with different titer levels in the presence of antibodies to the antigen of other organisms.

Response: The assay is based on the specificity of the antigen-antibody reaction. Previously, specificity was evaluated twice, once during the original validation and again when the firm transferred the method to another building. Specificity data revealed the lack of a diphtheria antibody response in the presence of polio, and RSV antibodies and diphtheria toxin. This indicated the assay only measures what was intended, diphtheria antibodies. While the firm did not demonstrate specificity with samples containing anti-diphtheria antibodies, demonstration that the test system does not react with irrelevant antibodies in the matrix suggests it is specific for the intended purpose. All sera contain IgG specific for other antigens (including pathogens that the individual has been exposed to), yet they showed adequate accuracy and precision, implying specificity.

2. *Linearity: The linearity of this assay was not evaluated in this validation. This assay determines neutralizing antibody units for the test samples relative to the reference serum. It is essential to assess whether the assay is able to obtain test results which are directly proportional to the concentration (amount) of analyte in the test sample.*

   Response: The sponsor addresses linearity in a report (Transfer Report for B000806 Transfer of SOP #A001837 "Determination of Diphtheria Antitoxin in International Units" from Clinical Serology Bldg. (b)(to Clinical Serology Bldg. (b) (4)), where they refer to linearity as dilutability. (b) (4) samples with titers (b) (4) (b) (4) ; these samples were run in the assay and the titers observed in the diluted samples were all within one dilution of the expected titers. This demonstrates that the assay detects a proportional drop in concentration by dilution over a range of titers. This covers the titers that were observed in the clinical study reported in this BLA.

3. *Precision: The precision evaluation was not conducted on samples with titers covering the assay range. Especially, precision was not assessed in the area around the cut-off point for serostatus.*

   Response: Even though precision is not specifically known at cut-off value ((b) (4) (b) (4)), precision was determined at a higher level, which is more in line with values seen for the booster responses (b) (4) subjects had at least (b) (4)). We recognize the limitations of the validation; however I feel the validation is suitable for the purpose of this study. The assay has been used for licensing all their diphtheria containing vaccines, response rates are typically on the order of (b) (4)

(b) (4) the cut-off value (b) (4), and disease has been well controlled using vaccine licensed using this assay.

Clinical Study Data for the Tetanus & Diphtheria Antigens

Pivotal Study M5I02, Safety and Immunogenicity of DTaP-IPV (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed Combined with Inactivated Poliovirus Vaccine) Compared to DAPTACEL (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) + IPOL (Poliovirus Vaccine Inactivated) as the 5th Dose in Children 4 to 6 Years of Age.

- Subjects in Group 1 received 3 vaccines concomitantly: a dose of DTaP-IPV vaccine, a dose of MMR vaccine, and a dose of V vaccine.
- Subjects in Group 2 received 4 vaccines concomitantly: a dose of DAPTACEL vaccine, a dose of IPOL vaccine (DAPTACEL + IPOL), a dose of MMR vaccine, and a dose of V vaccine.
- Subjects in Group 3 received up to 3 vaccines concomitantly: a dose of DTaP-IPV with or without a dose of MMR and V vaccine(s).
- Subjects in Group 4 received up to 4 vaccines concomitantly: a dose of DAPTACEL vaccine, a dose of IPOL vaccine, with or without a dose of MMR vaccine, and a dose of V vaccine.

Primary Objectives (Immunogenicity)

- To compare the pertussis (pertussis toxoid [PT], filamentous haemagglutinin [FHA], pertactin [PRN], and fimbriae types 2 and 3 [FIM]) booster responses and geometric mean concentrations (GMCs) (as measured by enzyme-linked immunosorbent assay [ELISA]) following DTaP-IPV vaccination (Group 1) to those elicited following DAPTACEL + IPOL vaccination (Group 2) when administered as a 5th dose
- To compare the diphtheria and tetanus booster responses and GMCs (as measured by neutralizing assay and ELISA, respectively) following DTaP-IPV vaccination (Group 1) with those elicited following DAPTACEL + IPOL vaccinations (Group 2) when administered as a 5th dose
- To compare the IPV booster responses and geometric mean titers (GMTs) (as measured by neutralizing assay) following DTaP-IPV vaccination (Group 1) with those elicited following DAPTACEL + IPOL vaccinations (Group 2) when administered as either a 4th or 5th dose.

Primary Immunogenicity Endpoints (Diphtheria & Tetanus)
For diphtheria and tetanus antibodies, the percentage of subjects demonstrating the booster response and the GMCs were to be assessed. The criterion for demonstrating a booster response was as follows:

- Subjects whose pre-vaccination antibody concentrations were < 0.1 IU/mL demonstrated the booster response if they had a post-vaccination level ≥ 0.4 IU/mL

- Subjects whose pre-vaccination antibody concentrations were ≥ 0.1 IU/mL but < 2.0 IU/mL demonstrated the booster response if they had a 4-fold rise (i.e., post-/pre-vaccination ≥ 4)
- Subjects whose pre-vaccination antibody concentrations were ≥ 2.0 IU/mL, demonstrated the booster response if they had a 2-fold response (i.e., post-/pre-vaccination ≥ 2)

Observational Immunogenicity Endpoints (Diphtheria & Tetanus)
The following serological endpoints were measured on Day 0 prior to vaccination and 28 days after the vaccination in Group 1 and Group 2:

- Geometric means of fold-rises (GMFR) for anti-diphtheria and anti-tetanus
- Seroprotection rates for anti-diphtheria and anti-tetanus antibodies
    - Anti-diphtheria antibody concentrations ≥ 0.1 IU/mL and ≥ 1.0 IU/mL
    - Anti-tetanus antibody concentrations ≥ 0.1 IU/mL and ≥ 1.0 IU/mL
    - RCDCs were generated for diphtheria and tetanus, antibody concentrations for pre- (Day 0) and post-vaccination (Day 28)

The results for response rates are presented in the table below (from Table 5.4 of the clinical study report). Number of subjects in each group ranged from 248 to 256. Subjects receiving DTaP-IPV had slightly lower response rates against tetanus and diphtheria antigens than those receiving DAPTACEL + IPOL.

Table 5.4: Non-inferiority comparison of post-vaccination anti-tetanus and anti-diphtheria booster response rates between groups – PP Analysis set

	DTaP-IPV (N=263)		DAPTACEL + IPOL (N=253)		Non-Inferiority Comparison		
	n/M	%	n/M	%	Difference in Rates (%) (DTaP-IPV) – (DAPTACEL+IPOL)	(95% CI)	Non-Inferiority Achieved
Anti-tetanus (ELISA - IU/mL)	213/253	84.2	209/248	84.3	-0.1	(-6.5; 6.3)	Yes
Anti-diphtheria (TNA - IU/mL)	249/256	97.3	247/249	99.2	-1.9	(-4.8; 0.6)	Yes

Reference: Section 9, Table 9.14
n: number of subjects with booster response
M: number of subjects with available data
Note: Non-inferiority is supported by the data if the lower limit of the 2-sided 95% CI is > -10%.

The results for GMCs are presented below (from Tables 5.5 and 5.6 of the clinical study report). Values are International Units/ml. Number of subjects in each group ranged from 254 to 262. Pre-vaccination GMCs were comparable between groups. Post vaccination GMCs in subjects receiving DTaP-IPV were non-inferior against all tetanus and diphtheria antigens than in those receiving DAPTACEL + IPOL.

Table 5.5: Summary of anti-tetanus and anti-diphtheria GMCs pre- and post-vaccination - PP Analysis Set

		DTaP-IPV (N=263)			DAPTACEL+IPOL (N=253)		
		M	Geometric Mean	(95% CI)	M	Geometric Mean	(95% CI)
Anti-tetanus (ELISA - IU/mL)	Pre-vaccination	254	0.644	(0.548; 0.758)	248	0.608	(0.518; 0.715)
	Post-vaccination	262	6.42	(5.74; 7.18)	253	5.48	(4.88; 6.15)
Anti-diphtheria (TNA - IU/mL)	Pre-vaccination	257	0.510	(0.437; 0.596)	249	0.426	(0.362; 0.501)
	Post-vaccination	262	18.6	(16.6; 20.8)	253	15.5	(13.7; 17.6)

Reference: Section 9, Table 9.44

M: number of subjects with available data

Table 5.6: Non-inferiority comparison of post-vaccination anti-tetanus and anti-diphtheria GMCs between groups - PP Analysis Set

	DTaP-IPV (N=263)		DAPTACEL + IPOL (N=253)		Non-Inferiority Comparison		
	M	Geometric Mean	M	Geometric Mean	GMC Ratio (DTaP-IPV) / (DAPTACEL+IPOL)	(95% CI)	Non-Inferiority Achieved
Anti-tetanus (ELISA - IU/mL)	262	6.4	253	5.5	1.17	(0.998; 1.38)	Yes
Anti-diphtheria (TNA - IU/mL)	262	18.6	253	15.5	1.20	(1.01; 1.42)	Yes

Reference: Section 9, Table 9.16

M: number of subjects with available data

Note: Non-inferiority is supported by the data if the lower limit of the 2-sided 95% CI is > 2/3.

The reverse cumulative distribution curves for the antibodies to tetanus and diphtheria antigens showed that the curves for the subjects who received DTaP-IPV were the same shape as the curves for the subjects who received DAPTACEL + IPOL.
Review of all data, including the line listings, show no aberrant results.

To confirm that the assays used to generate the data used in the study were performing adequately and that no data were inappropriately excluded from the analysis, the following information requests were sent to the sponsor on 12 May 2014.

1. Please provide data to support the stability of the performance of the immunoassays used to assess responses to diphtheria, tetanus and pertussis from the time of validation to the analysis of samples in in study M5I02.

2. If you retested samples in your immunologic assays and replaced specific data points in study M5I02, please provide a summary of retesting either as part of the Clinical Study Report or separately. In this summary, we request you include a listing of the values replaced during data cleaning, reasons for sample retesting, and an assessment of the impact of the retesting and replacement of values.

In response to question 1, the sponsor submitted the following

- RED_00073616, *Control Performance of Diphtheria* (b) (4) *from January 1999 through March 2014*
- RED_00073544, *Demonstration of the Long-Term Performance of the Anti-Tetanus IgG ELISA using Plots of Control Results*

Plots of the reference and internal positive control were presented from validation through testing of samples for M5I02. The graphs included information when lot changes occurred for key reagents. No trends were noted.

In response to question 2, Sanofi submitted RED_00069240, Biological Validation: M5I02 Tetanus ELISA, Diphtheria (b) (4), Polio (b) (4) (Types 1, 2 & 3) and Pertussis ELISAs (PRN, FHA, FIM, PT), Version 2, 19 Jun 2014. The report indicates that no samples were repeated in the diphtheria assay; only two samples were re-tested for anti-tetanus antibody response based on the statistical analysis.

Recommendation

The immunoassays used to measure the antibody response to the diphtheria and tetanus components of Quadracel are adequate for the purposes for which they were used in this application. Demonstration of acceptable performance of the assays is essential in order to approve this Biologics License Application (BLA) because immunogenicity data provide the primary evidence supporting comparability of the new combination vaccine to the currently licensed products packaged separately.

On March 24, 2014 Sanofi Pasteur submitted an original BLA with Clinical Efficacy Data to support a label claim. I have reviewed all documents relating to immunoassay performance of the diphtheria and tetanus assays for SPL supplement STN 125525/0; the clinical data, assay validation reports and data supporting assay performance since validation indicate the assays were performing as expected. Serologic data in support of study M5I02 appear to have been generated in assays adequate for that use. I recommend approval of the application.