Application Type	BLA
STN	125525/0
CBER Received Date	March 24, 2014
PDUFA Goal Date	March 24, 2015
Division / Office	OVRR
Committee Chair	Matthew Steele, Ph.D.
Clinical Reviewer(s)	Anuja Rastogi, M.D.
Project Manager	Juan Lacayo, Ph.D.
Priority Review	
Reviewer Name(s)	Zhong Gao, Ph.D. Mathematical Statistician
Review Completion Date / Stamped Date	
Supervisory Concurrence	Tsai-Lien Lin, Ph.D. Team Leader, Viral and Bioassay Team
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Applicant	Sanofi Pasteur Limited
Established Name	Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine (DTaP-IPV)
(Proposed) Trade Name	Quadracel
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc	DTaP-IPV has been formulated to contain the following active ingredients per 0.5 mL dose: 15Lf Diphtheria Toxoid (D), 5 Lf Tetanus Toxoid (T), 20 µg Pertussis Toxoid (PT), 20 µg Filamentous Haemagglutinin (FHA), 3 µg Pertactin (PRN), 5 µg Fimbriae Types 2 and 3 (FIM), 40 D-antigen units Inactivated Poliovirus (IPV) Type 1 (Mahoney), 8 D-antigen units IPV Type 2 (MEF1), and 32 D-antigen units IPV Type 3 (Saukett).
Dosage Form(s) and Route(s) of Administration	Single-dose (0.5 mL) vial Intramuscular injection
Dosing Regimen	A single dose (0.5 mL) of Quadracel is to be administered as a fifth dose in the diphtheria, tetanus, pertussis vaccination (DTaP) series, and as a fourth or fifth dose in the inactivated poliovirus vaccine (IPV) series, in children who have received 4 doses of Pentacel®.
Indication(s) and Intended Population(s)	Active immunization against diphtheria, tetanus, pertussis, and poliomyelitis as a 5th dose booster in children 4 through 6 years of age

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1. EXECUTIVE SUMMARY

Sanofi Pasteur Inc. submitted an original BLA 125525/0 for Quadracel® - "Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed Combined with Inactivated Poliomyelitis Vaccine (DTaP-IPV)," indicated for active immunization against diphtheria, tetanus, pertussis, and poliomyelitis for use in children 4 through 6 years of age. This review focuses on statistical aspects of the serological assays used for the immunogenicity assessment in the pivotal study M5I02 that provides the core immunogenicity data to support the licensure of DTaP-IPV. In study M5I02, the criteria for demonstrating booster response are based on LLOQs (anti-Pertussis: PT, FHA, PRN, and FIM) or cut-offs (anti-Diphtheria toxoid, anti-Tetanus toxoid, and anti-Poliovirus types 1, 2, and 3). It is essential to thoroughly evaluate the assays for accuracy and precision in the area around the LLOQs and/or cut-offs due to their crucial role in defining serostatus. However, the validation of LLOQs appears to be absent or perhaps inadequate for the Diphtheria Toxin (b) (4) Assay, the Tetanus IgG ELISA, and the component Pertussis ELISAs. Secondly, the Tetanus IgG ELISA was not validated for its specificity. Assay specificity is essential for assessing unequivocally the immune response induced by individual components of a combination vaccine. The reviewer consulted with the product reviewers and other members of the review committee. It is understood that the assay validation studies in this submission were conducted years ago. For instance, the validation of the Diphtheria Toxin (b) (4) Assay was performed in 1997, and the Tetanus IgG ELISA was validated in 2001. These gaps will need to be addressed in the future to ensure that the assays are fully validated and up to the current standards. The reviewer defers to the review committee for further considerations of potential impact of these issues based on the totality of evidence submitted.

2. CLINICAL AND REGULATORY BACKGROUND

Sanofi Pasteur Inc. submitted an original BLA 125525/0 for Quadracel® - "Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed Combined with Inactivated Poliomyelitis Vaccine (DTaP-IPV)," indicated for active immunization against diphtheria, tetanus, pertussis, and poliomyelitis for use in children 4 through 6 years of age.

Serological assays were used to assess immune responses for primary and observational objectives in the pivotal clinical trial M5I02 to support the licensure of Quadracel (DTaP-IPV). This review focuses on the following seven serological assays used for the immunogenicity evaluation.

- Diphtheria Toxin (b) (4) using (b) (4) and (b) (4) (b) (4).
- Tetanus Immunoglobulin G (IgG) Enzyme Linked Immunoassay (ELISA).
- Component Pertussis IgG ELISAs:
  - Pertussis Toxin (PT) IgG ELISA;
  - Filamentous Hemagglutinin (FHA) IgG ELISA;
  - Fimbriae types 2+3 (FIM 2+3) IgG ELISA;
  - Pertactin (PRN) IgG ELISA.
- Poliovirus (b) (4) using (b) (4) virus and (b) (4).

5. SOURCES OF DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

5.1 Review Strategy

The review focuses on the validation of the seven serological assays used for the immunogenicity assessment.

5.2 BLA/IND Documents That Serve as the Basis for the Statistical Review

- Summary of Serological Assays. Section 5.3.1.4 in the current submission (BLA 125525/0).
- Validation report for SOP #37S2, "(b) (4) for Diphtheria Antitoxin," Oct., 1997.
- Validation report for J000051, "ELISA Method for the Determination of tetanus antibodies in international units," C000149, Version 2. Oct., 2001.
- Validation report for SWI J003829, "ELISA Method for the Detection of Human Antibodies to Pertussis Toxin (PT) Antigen," C008666, Version 4.0. June, 2006.
- Validation report for SWI J003792, "ELISA Method for the Detection of Human Antibodies to Filamentous Haemagglutinin," C008396, Version 2.0. April, 2006.
- Validation report for SWI J003847, "ELISA Method for the Detection of Human Antibodies to Fimbrial Agglutinogens (2+3) Antigen," C008395, Version 2.0. April, 2006.
- Validation report for SWI J003848, "ELISA Method for the Detection of Human Antibodies to Pertactin (b) (4) Antigen," C008392, Version 2.0. April, 2006.
- Validation report for SWI J001656, "Poliovirus Determination by (b) (4) Testing," C007956, Version 1.0. Nov., 2005.

6. DISCUSSION OF INDIVIDUAL STUDIES

6.1 Validation of Diphtheria Toxin (b) (4) Assay

The diphtheria toxin (b) (4) is an (b) (4) functional assay that measures the level of diphtheria toxin neutralizing antibodies in human sera. (b) (4) . The validation was conducted to evaluate the assay in 1997. The validation results are summarized in Table 1. The applicant indicated that the diphtheria toxin (b) (4) is valid and suitable for its intended use to quantitate anti-diphtheria antibody levels in human sera.

(b) (4)

(b) (4)			
	(b) (4)		

(b) (4)

***Reviewer Comment**:*

(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.

(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.

(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.

6.2 Validation of Tetanus IgG ELISA

The tetanus IgG ELISA was used to evaluate anti-tetanus toxoid antibodies in human sera. The validation methods, acceptance criteria, and results are summarized in Table 2. The applicant indicated the validation study demonstrated acceptable performance of this test for the quantitation of anti-tetanus toxoid antibody levels in human serum samples.

(b) (4)

(b) (4)

***Reviewer Comment**:*

(1) <u>Range</u>: 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.

(2) <u>LOD</u>: 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) .

(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 undiluted and diluted* (b) (4) *samples. However, the starting dilution* (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.*

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

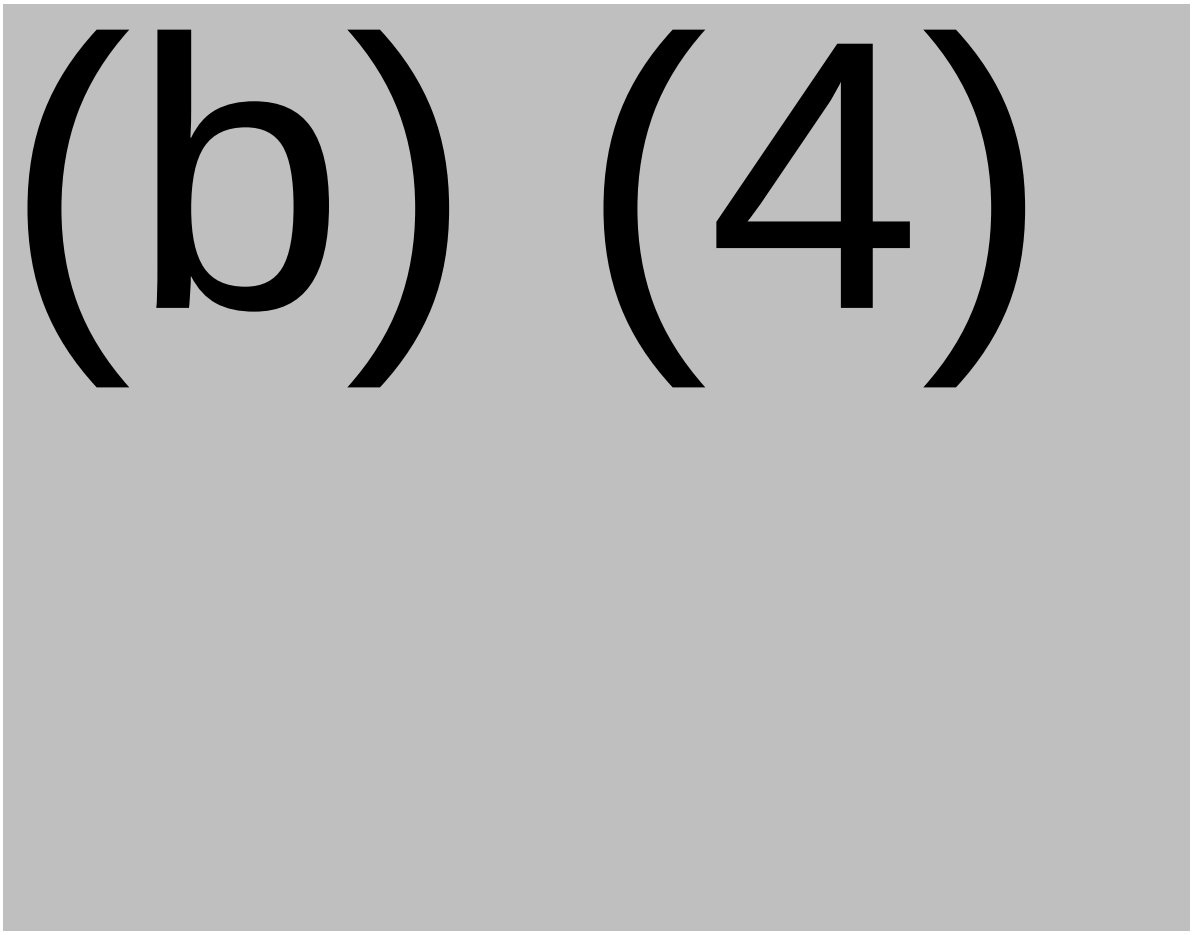
(5) *Specificity: This validation did not evaluate the potential impact of the antibodies to the antigen of other organisms on the measurement of tetanus antibody level.*

6.3 Validation of Component Pertussis IgG ELISA

The component pertussis IgG ELISAs (anti-PT, anti-FHA, anti-FIM 2/3, and anti-PRN) were used to evaluate the immune response to pertussis antibodies in human sera. Table 3 summarizes the validation methods and acceptance criteria for the component pertussis IgG ELISAs. The applicant concluded that the component pertussis IgG ELISAs were acceptable for the quantitation of anti-PT, anti-FHA, anti-FIM 2/3, and anti-PRN antibodies in human sera.

Table 3: Component Pertussis IgG ELISAs Validation Methods and Acceptance Criteria

(b) (4)



5.3.1.4).

***Reviewer Comment**:*

(1) <u>*Specificity/Selectivity*</u>*:*

The ELISA assay for the detection of antibodies to Pertussis Toxin (PT) antigen did not meet the pre-defined specificity/selectivity acceptance criteria for the PT antigen, in both initial validation and re-validation. In the re-validation, (b) (4) *. Subsequently, the applicant claimed sufficient specificity of the assay based on its ED50 analysis, where ED50 refers to effective dose at which point a 50% competition is observed.*

The ELISA for the detection of antibodies to Fimbrial Agglutinogens (2+3) antigen (FIM) did not meet the pre-defined specificity/selectivity acceptance criteria because (b) (4) *PT antigen. Similarly, the applicant used the ED50 analysis as the basis for claiming sufficient specificity of the assay.*

The PT and FIM ELISA specificity issues represent two scenarios, i.e., (b) (4) *being adequate. The reviewer considers that ED50*

analysis may be helpful in evaluating potential impact of interference of (b) (4) *in scenario (b). However, it remains a question whether the ED50 analysis can adequately evaluate maximal binding specificity of* (b) (4) *in scenario (a). The reviewer defers to the product reviewers on whether specificity is adequate based on the ED50.*

(2) <u>LLOQ</u>:

LLOQ was used to define the criteria for demonstrating booster response based on anti-Pertussis antibodies. For the component Pertussis IgG ELISAs, LLOQ was established based on Limit of Blank (LOB) and Limit of Detection (LOD). The previously established LLOQ values (b) (4) *adjusted to a higher value* (b) (4) *based on precision profiles. In the recovery experiments for accuracy assessment, samples* (b) (4) *reference generated expected values ranging from* (b) (4) *, while samples* (b) (4) *of reference generated expected values less than* (b) (4) *. Therefore, the accuracy assessment did not adequately cover the area around the LLOQs.*

(3) <u>Precision</u>:

The summary of serological assays and the original validation reports presented different acceptance criteria for the precision analysis. The original validation reports defined the acceptance criteria as the upper 95% CI for the overall %CV being (b) (4) *in contrast, the summary of serological assays defined the criteria as overall %CV being* (b) (4) *The upper 95% CI limit for inter-assay %CV was higher than* (b) (4) *. Therefore, the difference in the criteria may cause inconsistent results. Secondly, the precision results were reported to the hundredth decimal place or as whole number. For the repeatability assessment of the Pertactin* (b) (4) *ELISA, it is noted the degree of freedom (DF) is drastically different between the whole number analysis and the hundredth place data analysis (Table 6 in the validation report for Pertactin* (b) (4) *.*

(4) <u>Linearity</u>:

The linearity of an assay is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The applicant's dilutability experiment evaluated only (b) (4) *samples. The reviewer used the recovery experiment data for accuracy assessment to conduct a dilutional linearity analysis (Figure 1). The results showed no substantial deviation from linearity.*

(b) (4)

(b) (4)

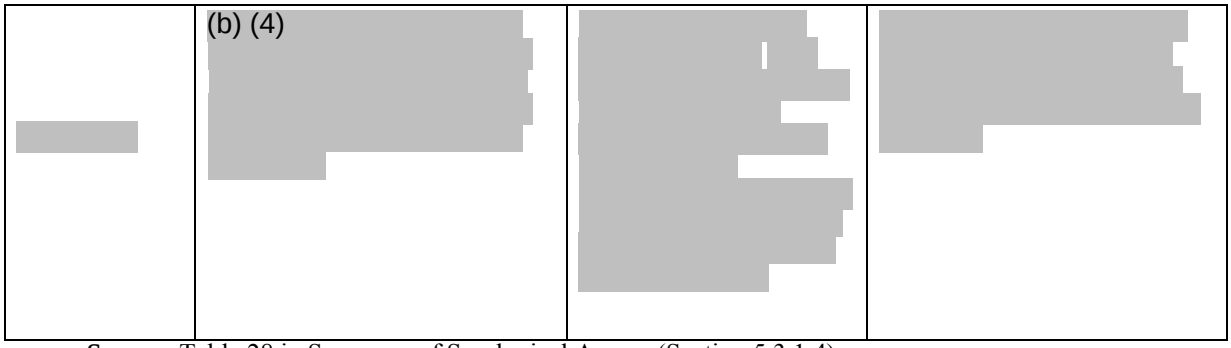
(b) (4)

6.4 Validation of Poliovirus (b) (4)

The poliovirus (b) (4) is an (b) (4) functional assay that measures the antibody response to poliovirus types 1, 2, and 3 by measuring the level of neutralizing antibodies to poliovirus in human sera. A validation study was performed to evaluate performance of the assay. The validation methods, acceptance criteria, and results are summarized in Table 4. The applicant concluded that this assay is valid and acceptable for the intended use to quantitate anti-poliovirus antibody levels in human sera.

(b) (4)

(b) (4)			
	(b) (4)		



Source: Table 28 in Summary of Serological Assays (Section 5.3.1.4).

10. CONCLUSIONS

10.1 Statistical Issues and Collective Evidence

The pivotal study M5I02 provides the core immunogenicity data to support the licensure of DTaP-IPV. In study M5I02, the criteria for demonstrating booster response are based on LLOQs (anti-Pertussis: PT, FHA, PRN, and FIM) or cut-offs (anti-Diphtheria toxoid, anti-Tetanus toxoid, and anti-Poliovirus types 1, 2, and 3). Ideally, the assays should be thoroughly evaluated for accuracy and precision in the area around the LLOQs and/or cut-offs due to their crucial role in defining serostatus. However, validation of LLOQ and/or cut-off may not be adequate for some assays.

- For the Diphtheria Toxin (b) (4) Assay, accuracy and precision were not assessed in the area around the cut-off of (b) (4) . The LOD was established based on the lowest dilution of serum used in the test, and LLOQ was not determined and validated.
- For the Tetanus IgG ELISA, the cut-off of (b) (4) was not adequately validated for accuracy and precision.
- LLOQ was used to define the criteria for demonstrating booster response based on anti-Pertussis antibodies. For the component Pertussis IgG ELISAs, LLOQ was established based on Limit of Blank (LOB) and Limit of Detection (LOD). The previously established LLOQ values (PT (b) (4) , FHA (b) (4) , FIM (b) (4) , Pertactin (b) (4)) were adjusted to a (b) (4) value (PT (b) (4) , FHA (b) (4) , FIM (b) (4) , Pertactin (b) (4)) based on precision profiles. In the recovery experiments for accuracy assessment, (b) (4) of reference generated expected values ranging from (b) (4) , while (b) (4) of reference generated expected values less than (b) (4) . Therefore, the accuracy assessment did not adequately cover the area around the LLOQs.

Assay specificity is essential for assessing unequivocally the immune response induced by individual components of a combination vaccine.

- The Tetanus IgG ELISA was not evaluated for its specificity.
- The Fimbrial Agglutinogens (FIM) ELISA did not meet the pre-defined specificity/selectivity acceptance criteria. (b) (4) samples showed a greater than (b) (4) competition for the (b) (4) PT antigen. Instead, the applicant used the ED50 analysis as the basis for claiming specificity of the assay.

10.2 Conclusions and Recommendations

The assay validation studies in this submission were conducted years ago. For instance, the validation of the Diphtheria Toxin (b) (4) Assay was performed in 1997, and the Tetanus IgG ELISA was validated in 2001. The validation of assay performance at LLOQs and/or cut-offs, used as the criteria for demonstrating booster response, may not be adequate for the Diphtheria Toxin (b) (4) Assay, the Tetanus IgG ELISA, and the component Pertussis ELISAs. Secondly, the Tetanus IgG ELISA was not validated for its specificity. The reviewer consulted with the product reviewers and other members of the review committee. These gaps will need to be addressed in the future to ensure that the assays are fully validated and up to the current standards. The reviewer defers to the review committee for further considerations of potential impact of these issues based on the totality of evidence submitted.