Application Type	BLA
STN	125546/0
CBER Received Date	July 24, 2014
PDUFA Goal Date	March 24, 2015
Division / Office	OVRR
Committee Chair	Margaret Bash, M.D.
Clinical Reviewer(s)	Anuja Rastogi, M.D.
Project Manager	Edward Wolfgang, MSA; Kirk Prutzman, Ph.D.; Ramachandra S. Naik, Ph.D.
Priority Review	Fast Track
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
	Amelia Dale Horne, Dr.P.H. Chief, Vaccine Evaluation Branch, DB, OBE
	Estelle Russek-Cohen, Ph.D. Director, Division of Biostatistics, OBE
Applicant	Novartis Vaccines and Diagnostics, Inc.
Established Name	Meningococcal Group B Vaccine
(Proposed) Trade Name	Bexsero®
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc	Bexsero contains 50µg of each of the three purified recombinant protein antigens NadA, NHBA and fHbp, with 25µg of OMV measured as amount of total protein containing the PorA P1.4, and 1.5 mg of aluminum hydroxide per 0.5 ml dose.
Dosage Form(s) and Route(s) of Administration	0.5 mL suspension for intramuscular injection as a single dose pre-filled syringe.
Dosing Regimen	Two doses (0.5mL each) with an interval of at least 1 month between doses.
Indication(s) and Intended Population(s)	Active immunization against invasive disease caused by *Neisseria meningitidis* serogroup B strains of individuals from 10 years through 25 years of age.

Table of Contents

1. Executive Summary

BLA 125546/0 was submitted by Novartis Vaccines and Diagnostics, Inc. to seek U.S. licensure of the multicomponent Meningococcal group B vaccine Bexsero ®, indicated for active immunization against invasive disease caused by *Neisseria meningitidis* serogroup B strains of individuals from 10 years through 25 years of age. This review focuses on statistical aspects of serum bactericidal assays (hSBA) that were utilized to determine antibodies against serogroup B *Neisseria meningitidis* bacteria in the immunogenicity studies. The applicant set hSBA titre of (b)(4) [Novartis ----------(b)(4)----------] or 1:4 [----------(b)(4)----------] as the lower cut-off for protection. However, the validation of LLOQ appears to be inadequate for the --(b)(4)-- hSBA assay; LLOQ was not established for the --(b)(4)-- hSBA assay. Based on the review of the hSBA in the context of their intended use, CBER suggested the following LLOQs, depending on the laboratory that performed the assay and the indicator strain assessed: NVD -(b)(4)- lab – 1:8 for the NZ98/254 strain, and 1:16 for the H44/76, 5/99, and (b)(4)----- strains; ------(b)(4)------ lab – 1:8 for the 5/99 strain and 1:16 for the H44/76 and NZ98/254 strains. Secondly, the inter-laboratory hSBA comparison study indicates a moderate difference in the hSBA assay between the two laboratories for vaccine rMenB+OMV, the investigational vaccine of the current submission, based on Total Deviation Index ($TDI_{0.95}$), Coverage Probability ($CP_{1.0}$), Concordance Correlation Coefficient (CCC), and Bland-Altman plot. The data are not supportive for direct comparison between data generated in (b)(4) and (b)(4) or pooling of data from the two laboratories for integrated immunogenicity analysis. Nevertheless, among the studies in the current submission, there is only one study, V72P10, where the hSBA assay was performed in both laboratories. It was decided by the review team that study V72P10 is not the major study to support the immunogenicity objectives of the submission. Moreover, the immunogenicity data from different studies are not combined for the integrated analysis of immunogenicity data. Consequently, the overall impact of the inter-laboratory hSBA differences on the immunogenicity data analysis appears to be limited. This reviewer defers to the other members of the review committee for further considerations based on the totality of evidence submitted.

2. Clinical and Regulatory Background

The manual serum bactericidal assay using human complement (hSBA) was used for immunogenicity assessment in the pivotal clinical studies provided in this submission (Table 1). The hSBA was validated and performed in the Novartis -----(b)(4)------ at ------(b)(4)------ and the (b)(4) laboratory at ---(b)(4)---, respectively.

The -(b)(4)- hSBA assay was performed at ----------(b)(4)----------, Novartis Vaccines and Diagnostics in ------(b)(4)------- to assess the immune response against MenB strains in the clinical studies V72P10, V72_41, V72P4, V72P5, V72P13, and V72P16 (Table 1).

- The --(b)(4)-- hSBA assay was validated in 2008 against the first panel of indicator strains: H44/76, 5/99, and NZ98/254 serving as antigen-specific indicators for fHbp, NadA, and PorA P1.4 (OMV) vaccine antigens, respectively.

- A second validation was carried out in 2010 using three additional MenB test strains, i.e., low-expressing strain (b)(4) for fHbp, low expressing strain ------ (b)(4)-- for NadA, and -(b)(4)- as an NHBA-specific indicator strain.

The ---(b)(4)--- hSBA assay was performed at the ----------(b)(4)---------- in ---(b)(4)---. This procedure was validated using the three indicator strains: H44/76, 5/99, and NZ98/254. The assay using these three indicator strains was run for the clinical studies V72P10, V72P10E1, and V72_29 (Table 1).

An inter-laboratory comparative study was performed in the years 2006-2009 between ----------(b)(4)---------- to evaluate the comparability of the two serum bactericidal assays. In this study, sera of infants and adults from different clinical trials were evaluated against the indicator strains: H44/76, 5/99, and NZ98/254.

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

3.1 Review Strategy

This review focuses on the validation of two manual hSBA procedures, i.e., the -(b)(4)- procedure and the -(b)(4)- procedure, because both hSBA procedures were used to generate immunogenicity data in the pivotal clinical studies. Furthermore, the review examines comparability of the two hSBA procedures in determination of antibodies against serogroup B *Neisseria meningitidis* bacteria.

The ----------(b)(4)---------- has been --------(b)(4)---------- for the ----(b)(4)---- vaccine program. The assay was used to generate immunogenicity data on sera from a ----(b)(4)---- trial, V102_03, which is included in the submission. A validation plan of the --(b)(4)-- assay is provided in the submission. Nevertheless, the --(b)(4)-- assay has not been validated as yet. Therefore, the --(b)(4)-- assay is not reviewed under the current BLA submission. CBER will continue to work with Novartis on development and validation of ----(b)(4)---- under the IND for the ------(b)(4)------ vaccine.

Table 1: Overview of MenB Assays Used for Clinical Study Testing

Clinical Study	manual hSBA ---(b)(4)---	manual hSBA -----(b)(4)-----	(b)(4)-SBA ----(b)(4)----
V72P10	+	+	
V72_41	+		
V72_29		+	
V102_03			+
V72P5	+		
V72P4	+		
V72P13	+		
V72P16	+		

Source: Adapted from Table 1 in Section 5.3.1.4 Reports of Bioanalytical Methods

3.2 BLA/IND Documents that Serve as the Basis for the Statistical Review

- --(b)(4)-- hSBA validation report: Serum bactericidal assay (SBA) for determination of antibodies against serogroup B *Neisseria meningitidis* bacteria. --(b)(4)-- No. 249115. May 2008.
- --(b)(4)-- hSBA validation report: Serum bactericidal assay (SBA) for determination of antibodies against serogroup B *Neisseria meningitidis* bacteria. --(b)(4)-- No. 275751. September 2010.
- --(b)(4)-- hSBA validation report: Validation of the serogroup B serum bactericidal antibody assay. No. CD0168.
- Inter-laboratory comparison of the meningococcal capsular group B serum bactericidal antibody assay between Novartis Vaccines --(b)(4)-- and ----------(b)(4)----------. January 2012.

4. DISCUSSION OF INDIVIDUAL STUDIES

4.1 Validation of the hSBA Assay at ------(b)(4)-------

In 2008, the applicant conducted a validation study on the manual hSBA assay, at (b)(4) in -------(b)(4)------ , to evaluate the assay with respect to its use in routine testing of sera from clinical trials. The validation was carried out on the three *N. meningitides* serogroup B strains: H44/76, 5/99, and NZ98/254 as antigen-specific indicators of fHbp, NadA, and PorA P1.4 (OMV), respectively, using sera from clinical trials V72P5 and V72P3. The validation design, acceptance criteria, and results are summarized in Table 2.

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

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

Reviewer Comment:
Lower Limit of Quantification (LLOQ): The validation set hSBA (b)(4) as the lower cut-off for protection based on a theoretical rationale that the lower limit of the 2-sided 95% CI for a titer of(b)(4) is a titer of 4. Historically, Goldschneider et al. (1969) linked a titer of 4 in the SBA with human complement to protection against a meningococcal infection. However, the titers of the samples selected for the experiment for detection and

quantitation, accuracy, and precision assessment were not within an immediate range around the proposed LLOQs. Thus, the proposed LLOQs cannot be adequately evaluated for accuracy and precision profiles. During the review, the product reviewers recommended the LLOQs be set at 16 for the assays using H44/76 and 5/99 and at 8 for the assay using NZ9/254 based on the intermediate precision data. On September 5, 2014, CBER requested that the applicant use the LLOQs proposed by CBER for the immunogenicity analysis.

Accuracy: Accuracy measures the closeness of test results generated by the assay to the true or expected value of the analyte. The validation evaluated accuracy by calculating % deviation of each of the three determinations from the mean of the three determinations at each dilution level. Actually, this approach measures dispersion of three determinations of a sample rather than accuracy of the assay. The reviewer conducted an accuracy analysis by calculating % deviation of GMT of three determinations of a diluted sample from the expected titer (Table 3). Here the expected titers of diluted samples were calculated from titers of the neat samples and dilution factors. For strain 5-99, % deviation ranges from -6.4% to -27.3%; for strain 44-76, % deviation ranges from 2.1% to 28.0%; for strain NZ98-254, % deviation ranges from 2.7% to -26.7%. The result shows that % deviation tends to increase with dilution factor. Overall, the % deviation from expected value is lower than 50% for all strains at all dilution levels.

***Table 3** Accuracy analysis results of the hSBA assay for three strains*

Strain	Sample	GMT	Expected Titre	% Dev
5-99	V72P3-05S-04006	7025.9	7025.9	
5-99	V72P3-05S-04006 1:2	3287.4	3513.0	-6.4
5-99	V72P3-05S-04006 1:4	1312.0	1405.2	-6.6
5-99	V72P3-05S-04006 1:10	549.1	702.6	-21.8
5-99	V72P3-05S-04006 1:50	102.2	140.5	-27.3
5-99	V72P3-05S-04006 1:250	21.7	28.1	-22.9
44-76	V72P3-05S-01004	390.5	390.5	
44-76	V72P3-05S-01004 1:2	210.2	195.3	7.7
44-76	V72P3-05S-01004 1:4	99.6	97.6	2.1
44-76	V72P3-05S-01004 1:10	40.3	39.1	3.2
44-76	V72P3-05S-01004 1:20	21.6	19.5	10.8
44-76	V72P3-05S-01004 1:50	10.0	7.8	28.0
NZ98-254	V72P5-08S-01030	385.3	385.3	
NZ98-254	V72P5-08S-01030 1:2	184.2	192.7	-4.4
NZ98-254	V72P5-08S-01030 1:4	98.9	96.3	2.7
NZ98-254	V72P5-08S-01030 1:8	42.0	48.2	-12.8
NZ98-254	V72P5-08S-01030 1:16	17.7	24.1	-26.7
NZ98-254	V72P5-08S-01030 1:32	9.7	12.0	-19.8

***Source:** Reviewer's analysis based on data contained in the --(b)(4)-- hSBA validation report (--(b)(4)-- No. 249115).*

Linearity: The applicant used the mean of the three determinations for each sample as the reference value for the linearity assessment. Such analysis actually evaluated precision of the three determinations at different dilutional levels rather than dilutional linearity. Dilutional linearity is typically evaluated based on the linear relationship between observed titers and expected titers, derived from titer of a neat sample and dilution factors. Secondly, the acceptance criterion for linearity did not address slope of regression line, which is important for linearity assessment.

In 2010, the applicant conducted a second validation study on the manual hSBA assay at ---(b)(4)--- in response to CBER's concern that the strains 44-76 and 5/99 express relatively high levels of antigen and therefore may not necessarily be considered representative. The study was intended to validate the hSBA assay using supplemental strains ----------(b)(4)---------- that express lower levels of NadA -------(b)(4)-------- and fHBP (-(b)(4)-) antigens. The study also evaluated strain -(b)(4)- for the immunogenicity assessment of the NHBA vaccine antigen.

The manual hSBA assay had limited use in testing against the supplemental strains in the studies for adolescents and adults 10-25 years of age that were submitted to support licensure (Table 4). The manual hSBA (--(b)(4)--) was only used to test --(b)(4)-- (NHBA) in study V72P10. High background hSBA sero-positivity rates against -(b)(4)- were observed among non-immunized adolescents in study V72P10. Subsequently, the applicant identified and characterized a supplemental test strain, --(b)(4)--, with the intention of extending and supporting observations made for the primary clinical NHBA indicator strain. Because the applicant decided to ----------(b)(4)----------, validation of the ----(b)(4)---- strain will be performed in the validation of -(b)(4)- in the context of the ----(b)(4)---- IND -(b)(4)- program.

Table 4 Indicator strains tested in the manual hSBA to support rMenB+OMV NZ immunogenicity in studies in adolescents and adults 10-25 years of age

Studies	**H44-76 (fHbp)**	**5-99 (NadA)**	**NZ98-254 (PorA P1.4)**	**(b)(4)-- (NHBA)**
V72P10	--- --(b)(4)--	--- --(b)(4)--	--- --(b)(4)--	--- --(b)(4)--
V72P10E1	--- --(b)(4)--	--- --(b)(4)--	--- --(b)(4)--	--
V72_41	--- --(b)(4)--	--- --(b)(4)--	--- --(b)(4)--	--
V72_29	--- --(b)(4)--	--- --(b)(4)--	--- --(b)(4)--	--

Note: (1) The supplemental test strains (b)(4) (fHbp), --(b)(4)-- (NadA), and --------(b)(4)-------- (NHBA) were tested by ----------(b)(4)----------;
(2) All indicator strains were tested by -(b)(4)- in study V102-03.

Source: Adapted from Table 4.1-1 in the Summary of Clinical Pharmacology Studies (Section 2.7.2).

4.2 Validation of the hSBA Assay at -----(b)(4)------

The manual hSBA assay was also performed at the ----------(b)(4)---------- in ------(b)(4)------ to deal with the issue of capacity limitation during the testing of the Phase 2b/3 clinical sera. As shown in Table 4, sera from V72P10 and the extension study, V72P10E1, and study V72_29 were tested at (b)(4). In 2009, the validation of the manual hSBA assay, at (b)(4), was carried out on the three *N. meningitides* serogroup B strains: 44/76-SL, 5/99, and NZ98/254 as antigen-specific indicators of fHbp, NadA, and PorA P1.4 (OMV), respectively. The validation design, acceptance criteria, and results for specificity, accuracy, precision, linearity, and LLOQ are summarized in Table 5.

--(b)(4)--

--(b)(4)--

***Reviewer Comment**:*
Lower Limit of Quantification (LLOQ): The ---(b)(4)--- assay validation study did not provide evidence that the lower cut-off of titer 4 is capable of differentiating a lower titer positive sample from a negative sample. Further, LLOQ was not established or validated for the ---(b)(4)--- assay. The product reviewers proposed an LLOQ of 8 for the assay against 5/99 and 16 for the assays against H44/76 and NZ98/254, with the limit of detection of 4 for all assays, based on the precision, bias, and linearity data as well as consideration of absence of negative samples. On September 5, 2014, CBER requested that the applicant use the LLOQs proposed by CBER for the immunogenicity analysis.

Acceptance Criteria for Precision and Linearity: The validation acceptance criteria appear to be inconsistent for some parameters between the validation of the ---(b)(4)--- assay and that of the --(b)(4)-- assay, including but not limited to precision and linearity. For precision, the ---(b)(4)--- assay validation was based on percentage of samples with SBA titer being within ± 1 SBA titer of the median of replicates, while the --(b)(4)-- assay validation was based on %CV. For linearity, the acceptance criterion used in the ---(b)(4)--- assay validation was percentage of diluted sample results being within ± 1 SBA titer of the undiluted sample result. In contrast, the --(b)(4)-- assay validation based the acceptance criterion on the correlation coefficient, and also reported slope of regression line. The inconsistencies in validation acceptance criteria between the two assays may impact the comparability of validation of the two assays. In addition, the

validation criteria used at the ---(b)(4)--- lab assumed that the hSBA titers are semi-quantitative, which is not correct. The interpolated titers are continuous data.

4.3 Manual hSBA Inter-Laboratory Comparison Study

An inter-laboratory comparative analysis was performed in the years 2006-2009 between ----------*(b)(4)*---------- to evaluate the comparability of the two serum bactericidal assays. The serum samples of infants (study V72P9) and adults (study C60P1 and V72P5) were tested against three different MenB strains 44/76-SL, NZ98/254, and 5/99. The participants (infants or adults) of these trials received the following different vaccine formulations: MeNZB^{TM} (a serogroup B outer membrane vesicle (OMV) vaccine prepared from strain NZ 98/254), recombinant serogroup B vaccine rMenB (NadA, fHbp, and NHBA), and 4CMenB/Bexsero (rMenB+OMV).

(1) Agreement Statistics for Continuous Data

In the hSBA bridging study, sera of infants (V72P9) and adults (C60P1 and V72P5) were evaluated at the ----------*(b)(4)*---------- in the years 2006-2009. A pair of titer measurements was generated by the (b)(4) and (b)(4) lab, respectively, for each sample. The agreement statistics for continuous data were evaluated using the post-vaccination serum samples. The applicant used the total deviation index ($TDI_{0.95}$) for agreement assessment. $TDI_{0.95}$ is the boundary value below which 95% of the absolute difference from target values lies (Lin, L et al., Journal of the American Statistical Association 2002; 97(457): 257-270). The applicant expected the $TDI_{0.95}$ to be around or below 1.0 based on their SBA experience. Total deviation index ($TDI_{0.95}$), along with coverage probability ($CP_{1.0}$), was calculated for vaccine rMenB and rMenB+OMV and the three strains, to assess comparability between the ----------(b)(4)---------- hSBA assays (Table 6). Here, $CP_{1.0}$ denotes the coverage probability of the absolute difference within 1.0 from target values in the log2 titer scale (1 titer step). However, the applicant did not provide $TDI_{0.95}$ and $CP_{1.0}$ for vaccine MeNZB. The reviewer provided $TDI_{0.95}$ and $CP_{1.0}$ for MeNZB with the three strains in Table 6 to assess the inter-laboratory hSBA comparability for MeNZB.

Table 6 The total deviation index ($TDI_{0.95}$) and coverage probability (CP_1) for the combinations of vaccines and strains: ----------*(b)(4)*---------- (95% confidence bound is included)

	rMenB [a] $TDI_{0.95}$	rMenB [a] CP_1	rMenB+OMV [a] $TDI_{0.95}$	rMenB+OMV [a] CP_1	MeNZB [b] $TDI_{0.95}$	MeNZB [b] CP_1
44/76-SL	2.10 (3.02)	0.61 (0.42)	1.82 (2.43)	0.69 (0.53)	3.58 (4.43)	0.25 (0.12)
5-99	0.91 (1.25)	0.96 (0.81)	1.82 (2.34)	0.70 (0.56)	1.65 (2.27)	0.73 (0.54)
NZ98-254	0.91 (1.25)	0.95 (0.81)	1.80 (2.31)	0.70 (0.57)	4.93 (5.98)	0.11 (0.04)

Source: a. Summarized from Figure 2-7 in the inter-laboratory comparison study report.
b. Reviewer's analysis based on the data provided in the inter-laboratory comparison study report.

Reviewer Comment:
For vaccine rMenB+OMV, the investigational vaccine of the current submission, $TDI_{0.95}$ was 1.82, 1.82, and 1.80 in the log2 titer scale, respectively, for strains 44/76-SL, 5-99, and NZ98-254. Additionally, $CP_{1.0}$ was 69%, 70%, and 70% for strains 44/76-SL, 5-99, and NZ98-254, respectively. The applicant expected the $TDI_{0.95}$ to be around or below 1.0 based on their SBA experience. The results indicate a greater-than-expected inter-laboratory difference in the hSBA assay for the post-vaccination sera between ----------(b)(4)----------.

For vaccine rMenB, the inter-lab titer difference varied among the strains. $TDI_{0.95}$ was 0.91 for strain 5-99 and NZ98-254, while $TDI_{0.95}$ was 2.10 for strain 44/76-SL. For MeNZB, there appears to be substantial inter-lab difference for strain 44/76-SL and NZ98-254. $TDI_{0.95}$ was 3.58 and 4.93, respectively, for strain 44/76-SL and NZ98-254. Additionally, $CP_{1.0}$ was only 25% and 11% for strain 44/76-SL and NZ98-254, respectively.

Assay agreement was further evaluated with the concordance correlation coefficient (CCC) (Lin, L et al., Journal of the American Statistical Association 2002; 97(457): 257-270). The CCC can be expressed as the product of the accuracy and precision coefficients. For the three strains and two vaccines, CCC was generally greater than or close to 0.85 except CCC for 44/76-SL and rMenB+OMV (0.763). Further, the results showed that accuracy was greater than 0.90 for the combinations of three strains and two vaccines rMenB and rMenB+OMV, while the estimate of precision ranged from 0.77 to 0.99. The results suggested that precision/variability instead of accuracy (systematic difference between the two labs) may play a more substantial role in the observed inter-lab difference.

The applicant also used Bland-Altman plots to evaluate the inter-laboratory hSBA comparability for rMenB and rMenB+OMV (Figure 1). The applicant did not provide Bland-Altman plots for vaccine MeNZB. To assess the inter-laboratory hSBA comparability for MeNZB, the reviewer provided the Bland-Altman plots for MeNZB with strains 44/76-SL, 5-99, and NZ98-254 in Figure 2.

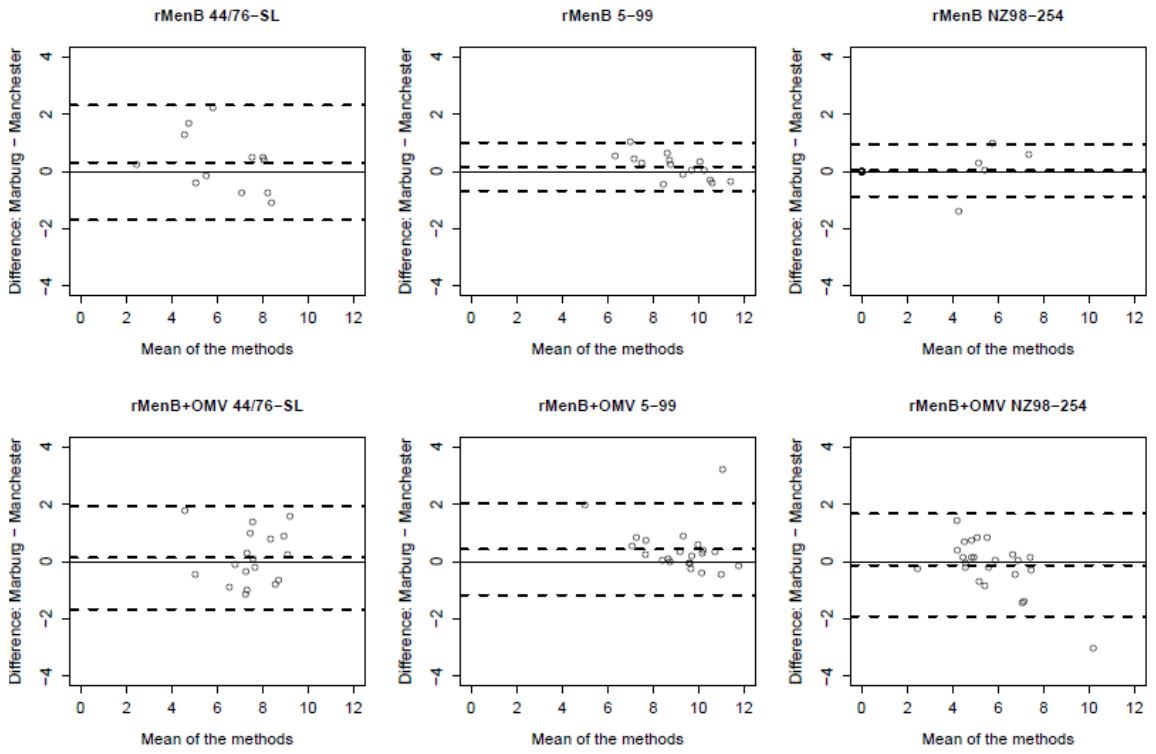

Figure 1 Bland-Altman plot for vaccine rMenB and rMenB+OMV and strains 44/76-SL, 5-99, and NZ98-254. Solid line – line of equality (difference between two assays is equal to zero); dotted lines – mean difference and 95% limits of agreement. Here, 95% limits of agreement are mean difference ± 1.96 × standard deviation of differences (Bland and Altman, Lancet 1995; 346: 1085-1087).
Source: The Bland-Altman plots in Figures 2-7 in the inter-laboratory comparison study report, reproduced by the reviewer based on the data provided in the report.

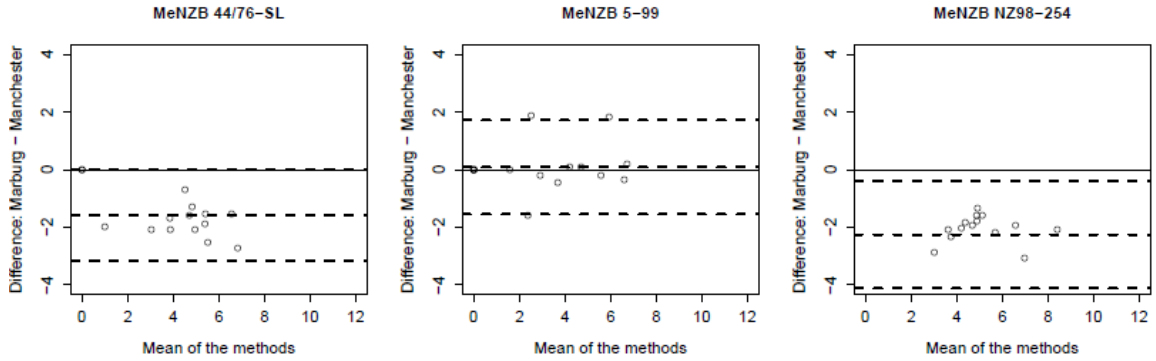

Figure 2 *Bland-Altman plot for vaccine MeNZB and strains 44/76-SL, 5-99, and NZ98-254. Solid line – line of equality (difference between two assays is equal to zero); dotted lines – mean difference and 95% limit of agreement.*
Source: *Reviewer's analysis based on the data provided in the inter-lab comparison study report.*

Reviewer Comment*:*
The Bland-Altman plots in Figure 1 show that the mean difference was not far away from the line of equality for vaccines rMenB and rMenB+OMV with the three strains.

However, the inter-lab difference appears to change over the assay mean for some vaccine/strain combinations, such as rMenB with 44/76−SL, rMenB with 5−99, and rMenB+OMV with 5−99. In these cases, mean difference may be inadequate for the evaluation of assay agreement. For further assessment, the reviewer performed Deming regression to examine the linear relationship between titers measured by the ---(b)(4)--- and ------(b)(4)------ assays (Figure 3). It is noted that, for vaccine MeNZB, the mean difference was away from the line of equality for strain 44/76-SL and NZ98-254, respectively (Figure 2). The Deming regression analysis also showed that the regression line deviated from the perfect agreement line for the two strains (Figure 3). The results suggest that there exist substantial inter-lab differences in hSBA for MeNZB and strains 44/76-SL and NZ98-254. Additionally, the inter-lab difference in hSBA varied substantially among vaccines and strains.

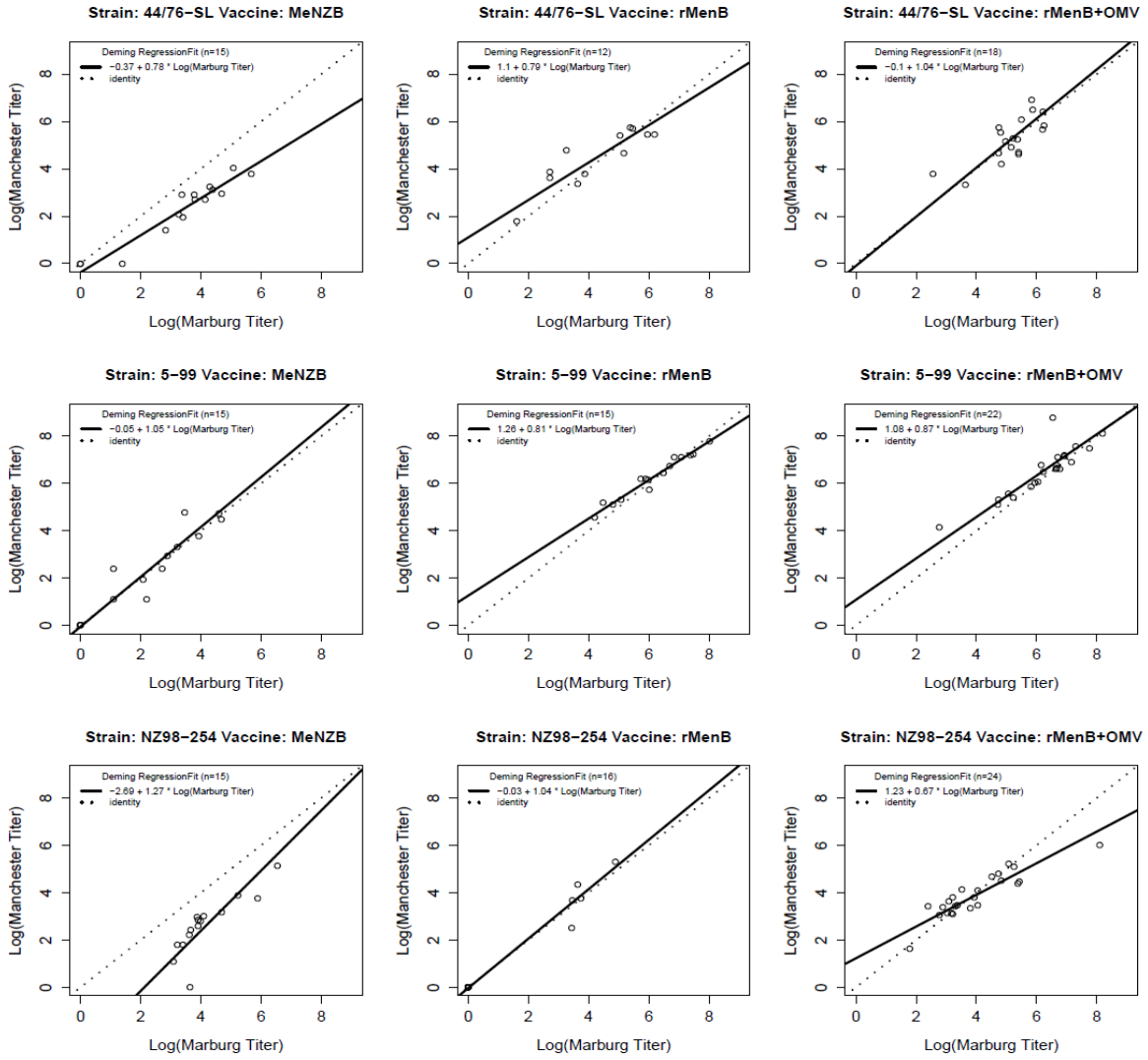

Figure 3 *Scatter plot with Deming regression for the inter-lab comparison for the vaccine and strain combinations*
Source: *Reviewer's analysis based on the data provided in the inter-lab comparison study report.*

(2) Agreement Statistics for Categorical Data

The applicant evaluated overall agreement, positive agreement, and negative agreement between the ----------*(b)(4)*---------- hSBA using both pre- and post-vaccination immunogenicity data (Table 7) and combining all 3 vaccines. In this analysis, the continuous data were dichotomized to below (<) and above (≥) the cut-off titer of 4. The applicant concluded that the overall agreement and positive agreement were at least 90%, although the negative agreement was 81% and 85% for strain 44/76-SL and NZ98-254. The reviewer's analysis indicates that the Kappa coefficient of agreement is 0.85 for all three strains, 0.80 for strain 44/76-SL, 0.92 for strain 5-99, and 0.82 for strain NZ98-254.

Table 7 Summary table with categorical agreement statistics

Strain	Overall agreement (%)	Positive agreement (%)	Negative agreement (%)
44/76-SL	90	95	81
5-99	93	93	92
NZ98-254	91	98	85

Source: Table 7 in the inter-lab comparison study report

5. CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

(1) LLOQ

The applicant set hSBA ----------*(b)(4)*---------- as the lower cut-off for protection. Historically, Goldschneider et al. (1969) linked a titer of 4 in the SBA with human complement to protection against meningococcal infection. However, validation of LLOQ appears to be inadequate for the hSBA assay conducted in (b)(4), (b)(4); LLOQ was not established or validated for the hSBA assay used in ----------*(b)(4)*----------. On September 5, 2014, CBER proposed new LLOQs and requested that the applicant use these LLOQs for the immunogenicity analyses.

Information Request dated 5 September 2014 (Item 1):
We have reviewed the validation reports for the hSBA conducted at -(b)(4)- and ---*(b)(4)*--- in the context of their intended use. Please be advised that due to the limitations of data submitted to support the lower limit of quantitation (LLOQ) for either dilutional linearity or precision, we consider the hSBA assays to be validated for a LLOQ of either 8 or 16, depending on the laboratory that performed the assay and/or the indicator strain assessed:
a. NVD ---(b)(4)--- lab: 8 for the NZ98/254 strain, and 16 for the H44/76, 5/99, and ---(b0(4)---- strains;
b. --------*(b)(4)*-------- lab: 8 for the 5/99 strain and 16 for the H44/76 and NZ98/254 strains.

(2) Inter-laboratory comparability of the hSBA assay

The hSBA comparison study indicates a moderate inter-laboratory difference in the hSBA assay for rMenB+OMV, the investigational vaccine of the current submission. $TDI_{0.95}$ was 1.82, 1.82, and 1.80 in the log2 titer scale, respectively, for strain 44/76-SL, 5-99, and NZ98-254. Additionally, $CP_{1.0}$ was 69%, 70%, and 70% for strain 44/76-SL, 5-99, and NZ98-254, respectively. Therefore, the data are not supportive for direct comparison between data generated in (b)(4) and (b)(4) or pooling of data from the two laboratories. Nevertheless, among the studies in the current submission, there is only one study, V72P10, where the hSBA assay was performed in both laboratories (Table 1). Moreover, the immunogenicity data from different studies are not combined for the integrated analysis of immunogenicity data. Therefore, the overall impact of the inter-laboratory hSBA differences on the immunogenicity data analysis appears to be limited.

5.2 Conclusions and Recommendations

The applicant set hSBA titer of ----------*(b)(4)*---------- as the lower cut-off for protection. However, the validation of LLOQs appears to be inadequate for both hSBA assays conducted in the two laboratories. Based on the review of the hSBA in the context of their intended use, CBER proposed new LLOQs, depending on the laboratory that performed the assay and the indicator strain assessed: NVD --*(b)(4)*-- lab – 1:8 for the NZ98/254 strain, and 1:16 for the H44/76, 5/99, and --*(b)(4)*-- strains; -------*(b)(4)*------- lab – 1:8 for the 5/99 strain and 1:16 for the H44/76 and NZ98/254 strains. Secondly, the hSBA comparison study indicates a moderate inter-laboratory difference in the hSBA for vaccine rMenB+OMV, the investigational vaccine of the current submission, based on $TDI_{0.95}$, $CP_{1.0}$, and Bland-Altman plots. The data are not supportive for direct comparison between data generated in ----*(b)(4)*---- or pooling of data from the two laboratories. Nevertheless, among the studies in the current submission, there is only one study, V72P10, where the hSBA assay was performed in both laboratories. It was decided by the review team that study V72P10 is not the major study to support the immunogenicity objectives of the submission. Moreover, the immunogenicity data from different studies are not combined for the integrated analysis of immunogenicity data. Consequently, the overall impact of the inter-laboratory hSBA differences on the immunogenicity data analysis is limited. This reviewer defers to the other members of the review committee for further considerations based on the totality of evidence submitted.