STATISTICAL REVIEW AND EVALUATION

BLA Number:	125363.0
Product Name:	MenHibrix (Meningococcal Groups C and Y and Haemophilus b Tetanus Toxoid Conjugate Vaccine)
Applicant:	GlaxoSmithKline Biologicals SA
Date Submitted:	August 12, 2009
Statistical Reviewer:	Tsai-Lien Lin Lead Mathematical Statistician Viral and Bioassay Team, VEB/DB/OBE
Through:	A. Dale Horne Branch Chief, VEB/DB/OBE
To:	Joseph Temenak David Staten
CC:	Meghan Ferris Barbara Krasnicka Mustafa Akkoyunlu Iryna Zubkova Milan Blake Estelle Russek-Cohen Henry Hsu DB chron

1. EXECUTIVE SUMMARY

GSK submitted BLA 125363 to seek licensure of a combination vaccine Hib-MenCY-TT intended for the vaccination of US infants at 2, 4, and 6 months of age, with a fourth dose to be administered at 12 to 15 months of age. The primary meningococcal immunogenicity endpoints in the clinical studies have been defined in terms of assays measuring serum bactericidal activity with human compliment used as the exogenous complement source (i.e., hSBA assays). This statistical review covers the quality control data of hSBA assays, and the validation of ELISA assay for measuring the antibody to the PRP polysaccharide of *Haemophilus influenza* type b. In addition, at the product reviewer Dr. Zubkova's request, Section 5.2.2. of the validation report of -----(b)(4)----- assay is reviewed.

hSBA Assay:

A reduction of hSBA MenY titers of (b)(4) re-tested Hib-MenCY-TT-005 samples was observed and was further confirmed by re-testing (b)(4) additional Hib-MenCY-TT-005 samples and (b)(4) Hib-MenCY-TT-007/-008 samples. The 4 weeks re-test results of those Hib-MenCY-TT-013 samples used as sentinel samples in the routine testing of Hib-MenCY-TT-009/-010 samples also showed a –(b)(4)--- of MenY titers from the initial reference values, except for week 1. However, the week to week comparisons showed no apparent drift. A large amount of missing and <LLOQ values in the dataset could make the results difficult to interpret.

Overall, it cannot be confirmed based on the applicant's analyses that no assay drift has occurred prior to or during the testing of studies -009 and 010 samples. The sentinel sample dataset contains more samples than stated and with missing codes unexplained. It is also not clear what the "concordance" values presented by the applicant are. The applicant did not provide the requested information regarding this dataset during the BLA review. As a result, the statistical reviewer cannot verify the applicant's results or perform additional statistical analyses to evaluate the assay drift issue.

Hib PRP ELISA Assay:

The applicant evaluated the validation parameters of precision (repeatability and intermediate precision), accuracy, linearity, specificity, and robustness, etc, and discussed the parallelism test. There is no evidence of unsatisfactory assay performance. However, the design and analysis methods used by the applicant to assess precision and parallelism are not statistically sound. For future assay development, statistical approaches suggested in the draft -b(4)- guidances on Biological Assay Validation and Design and Development of Biological Assays are recommended.

------(b)(4)------- Assay:

There is an error in the logistic model used to fit the calibration curve. This could be just a typo. Since the applicant used a commercially available software to do the curve fitting, the error is not likely to produce any impact on the assay results generated.

CONCLUSIONS

- A ---(b)(4)--- of hSBA MenY titers was observed consistently for re-tested samples from studies Hib-MenCY-TT-005 and -007/-008. No conclusion on assay drift can be drawn based on the results from the sentinel samples in the testing of studies 009 and 010 samples. Clarification of the data set and the analysis results presented in the submission is needed for further evaluation.

- There is no evidence of unsatisfactory performance of the Hib PRP ELISA assay. However, the design and analysis methods used by the applicant to assess precision and parallelism are not statistically sound. Better approaches are recommended for future validations.

- There is an error in the logistic model used for fitting the ----(b)(4)------- assay calibration curve. This may have been a typo and is not likely to impact the assay results.

2.   BACKGROUND

GSK submitted BLA 125363 to seek licensure of a combination *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroups C and Y – tentanus toxoid conjugate vaccine (Hib-MenCY-TT) intended for the vaccination of US infants at 2, 4, and 6 months of age, with a fourth dose to be administered at 12 to 15 months of age. Based on discussions with CBER, the primary meningococcal immunogenicity objectives have been defined in terms of assays measuring serum bactericidal activity with human compliment used as the exogenous complement source (i.e., hSBA assays).

GSK's hSBA-MenC and –MenY assays were developed based on the existing assays measuring bactericidal activity with (b)(4) compliment (b)(4) assays). The validation of hSBA assays has been reviewed in IND (b)(4).

Because no reference sera exist for hSBA, to address the lack of standardization, a ---(b)(4)------ factor and quality control charts have been implemented to monitor the assay stability and to identify any possible assay drift.

This statistical review covers the QC results of hSBA assays, and the validation of ELISA assay for measuring the antibody to the PRP polysaccharide of *Haemophilus influenza* type b. In addition, at the product reviewer Dr. Zubkova's request, Section 5.2.2. of the validation report of ----(b)(4)------ assay will be reviewed.

3.   STATISTICAL EVALUATION

3.1   hSBA ASSAY - SENTINEL SAMPLES

3.1.1   Re-test of Hib-MenCY-TT-005 and -007/-008 samples

A -(b)(4) of hSBA MenY titers of (b)(4) re-tested samples was observed, while no drift was predicted based on the QC chart. This --(b)(4)--- of sensitivity was further confirmed by re-testing (b)(4) additional samples from study Hib-MenCY-TT-005, and(b)(4) samples from studies Hib-MenCY-TT-007/-008 tested in parallel. Table 1 presents the results of these re-tests.

**Table 1   Re-testing Results of Samples from Studies Hib-MenCY-TT-005
and -007/-008**

Study	Number of samples re-tested	Ratio of GMT (re-test / ref)	Number of positive samples lost after re-testing
Hib-MenCY-TT-005	(b)(4)	0.15	2 / 9
Hib-MenCY-TT-005	(b)(4)	0.77	3/10
Hib-MenCY-TT -007/-008	(b)(4)	0.50	0 / ?

Two samples out of the (b)(4) re-tested samples from study -005 were negative initially. Among the 10 samples positive initially, the GMT ratio for the 7 samples positive after re-testing was 0.50. No detailed information was provided on the re-testing results of samples from study -007/-008. The applicant stated that no positive samples were lost after re-testing. However, it is not known how many out of the (b)(4) re-tested -007/-008 samples were positive initially.

The applicant investigated whether a change in (b)(4) lot or the ---(b)(4)---- used for the re-testing could have caused the reduced sensitivity, using a panel of samples from study -005. The result suggests that the reduction of titer is not due to a difference in (b)(4) lot or bacterial ----(b)(4)---. The applicant concluded that the --(b)(4)----- sensitivity for the retested -005 samples was an artifact that might be linked to the age of the samples.

Reviewer's Comments: A consistent pattern of -(b)(4)- sensitivity is observed for all (b)(4) re-testings conducted for study -005 and studies -007/-008 samples. The potential impact of this problem is not only on the study results of studies -005, -007, and -008, but also on the hSBA MenY titer results obtained after the initial testing of studies -005, -007, and -008 samples.

3.1.2   Sentinel samples from Hib-MenCY-TT-013 for -009/010 testing

Samples from study Hib-MenCY-TT-013 were added in routine testing of -009 and -010 samples as sentinel samples on a weekly basis. The re-test results for 4 weeks from these sentinel samples were compared from week to week, as well as to reference initial results. The applicant reported the following results (Table 2):

Table 2   Results of Sentinel Samples from Studies Hib-MenCY-TT-013

Comparisons with reference titers

	GMR (wk1/ref)	GMR (wk2/ref)	GMR (wk3/ref)	GMR (wk$_{b(4)}$ref)
MenC	1.00	0.89	0.81	0.78
MenY	0.96	0.63	0.67	0.68

	r (wk1/ref)	r (wk2/ref)	r (wk3/ref)	r (wk $_{b(4)}$/ref)
MenC	0.94	0.97	0.96	0.96
MenY	0.64	0.78	0.78	0.83

	Concordnce (wk1/ref)	Concordance (wk2/ref)	Concordance (wk3/ref)	Concordance (wk $_{b(4)}$/ref)
MenC	81.25%	91.93%	96.15%	83.87%
MenY	96.43%	96.77%	97.30%	91.43%

Comparisons from week to week

	GMR (wk2/wk1)	GMR (wk3/wk1)	GMR (wk3/wk2)	GMR ($_{b(4)}$/wk1)	GMR ($_{b(4)}$/wk2)	GMR ($_{b(4)}$/wk3)
MenC	0.94	0.87	0.92	0.79	0.83	0.84
MenY	0.99	1.08	1.01	1.09	0.94	0.95

	GMR (wk2/wk1)	GMR (wk3/wk1)	GMR (wk3/wk2)	GMR ($_{b(4)}$/wk1)	GMR ($_{b(4)}$/wk2)	GMR ($_{b(4)}$/wk3)
MenC	0.97	0.98	0.98	0.98	0.98	0.98
MenY	0.94	0.94	0.92	0.95	0.96	0.94

	Concord (wk2/wk1)	Concord (wk3/wk1)	Concord (wk3/wk2)	Concord ($_{b(4)}$/wk1)	Concord ($_{b(4)}$/wk2)	Concord ($_{b(4)}$/wk3)
MenC	86.67%	86.36%	92.31%	92.59%	86.67%	86.36%
MenY	100%	100%	100%	93.33%	94.29%	93.33%

The GMRs at each week compared to the reference were calculated based on all samples with valid titers at that week, and the GMRs week to week were calculated based on samples for which a valid result was available at both weeks. The r-values and concordance were also calculated. The applicant concluded that comparing to the reference results, the GMRs showed no significance for MenC and a low reduction of titer for MenY was observed except for week 1. The r value and concordance results also showed a high level of agreement assessing that no drift occurs during period of testing.

Reviewer's Comments: The applicant stated that there were (b)(4) sentinel samples for hSBA MenY. However, in the raw data table provided by the applicant, there are (b)(4) samples. Many test results are missing or below LLOQ. The missing values can have different codes: e.g., "/", "IR", "TC", or "TD." CBER requested the applicant to provide

explanations for those missing data codes and the values assigned to <LLOQ results in the analysis, as well as to clarify the number of samples included in the analysis. CBER has not received the information requested in March, 2010. As a result, the analysis cannot be verified by the statistical reviewer. Due to the large amount of missing <LLOQ values, how they are handled can potentially impact the results of GMR. In addition, the applicant did not provide any explanation on the r and concordance values presented. The reviewer verified that r-values are the correlation coefficients between log-transformed titers at different times, ignoring all <LLOQ and missing values. However, it is not clear what the "concordance" values are. They do not appear to be concordance correlation coefficients.

The comparisons from week to week examine the assay drift that occurred during the period of week 1 to week b(4) and cannot assess whether drift has occurred prior to week 1. Due to the large amount of missing values in the data set, there appears to be some inconsistency between the comparisons with reference titers and the comparisons from week to week. For example, MenY titer appears to drop from week 1 to week 2 based on the comparisons with reference titers (GMR drops from 0.96 to 0.63); while the GMR of week 2 to week 1 is 0.99 based on the week to week comparisons, suggesting no change from week 1 to week 2. How missing values should be treated in the analysis needs to be further evaluated. Furthermore, the correlation coefficient is not an appropriate statistical tool for assessing assay drift. The titers can reduce significantly over time, yet still have high correlation between different time points.

3.1.3 Conclusions

A reduction of hSBA MenY titers was observed consistently for re-tested samples from studies Hib-MenCY-TT-005 and -007/-008. In addition, the applicant's analyses cannot confirm that no assay drift has occurred prior to or during the testing of studies -009 and --010 samples. The sentinel sample dataset contains more samples than stated and with missing codes unexplained. It is also not clear what the "concordance" values presented by the applicant are. The applicant did not provide the requested information regarding this dataset during the BLA review. As a result, the statistical reviewer cannot verify the applicant's results or perform additional statistical analyses to evaluate the assay drift issue.

3.2   HIB PRP ELISA ASSAY

The PRP ELISA assay validation report, dated 1/6/1999, was reviewed. This assay validation report has been previously submitted under Hiberix and Havrix, which were approved in 2009.

3.2.1   PRP ELISA assay analysis method

The anti-PRP antibody titers are calculated by interpolation of the standard curve which is fitted by a 4-parameter logistic model:

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

d = upper asymptote
a = lower asymptote
c = dilution associated with the point of symmetry of the sigmoid and is located at the
    midpoint of the sigmoid curve.
b = related to the slope of the curve.

The upper and lower asymptotes are not estimated by curve fitting. In the case of full sigmoidal curve, the highest and lowest OD values are assigned as the upper and lower asymptotes. In the case of partial curve, the a or d values obtained from the Low Positive Control or High Positive Control will be used. The ODs are transformed to %OD for standardization purpose. The titer for each dilution of a test sample is calculated by interpolation of the standard curve and adjusted for dilution factor. The titer for a test sample is then calculated by taking the average of the titers for those dilutions with %OD falling within the 10%-70% range.

3.2.2   PRP ELISA assay validation

The validation parameters evaluated include precision (repeatability and intermediate precision), accuracy, linearity, specificity, and robustness, etc. The report also discussed parallelism test.

Precision: To assess repeatability, samples representing a range of PRP (b)(4) antibody levels were assayed in duplicate or triplicate. The %CVs for (b)(4)-assay replicates were averaged. The average %CVs are well below 10%. To assess intermediate precision, samples representing a range of PRP titers were assayed in (b)(4)- on ----(b)(4)---------- by --(b)(4)-- technicians. The %CVs were calculated for each sample and averaged across samples. When (b)(4) technicians were involved, the average %CV is 16%.

Reviewer's Comments: Taking average of %CVs is not a statistically appropriate approach for estimating precision. To evaluate precision, instead of utilizing the convenient test results from available samples to obtain average %CVs, a well designed experiment should be conducted and the variance component approach is recommended to estimate the day-to-day, analysis-to-analysis variability, and to further calculate the (b)(4)-assay and (b)(4)-assay precision. While there is no particular evidence that the precision of the PRP ELISA assay is not satisfactory, the applicant is advised to perform future validations using Design of Experiment approach and proper statistical methodology.

Accuracy: The assay accuracy was evaluated by comparing the titers measured by (b)(4) to the titers measured by (b)(4)-- procedure which is considered the golden standard of PRP antibody testing. The regression analysis of log-transformed (b)(4) titers (y) versus (b)(4) titers (x) in –(b)(4)----- laboratory showed that the agreement between the two assay methods is good, with r^2=0.95, slope=1.022, and intercept=0.075.

In addition, two studies were conducted to compare (b)(4) results for clinical study samples with in-house (b)(4) results. Study 1 consisted mainly of pre and post-primary samples, while Study 2 consisted of pre and post-booster samples. The regression analyses also showed good agreement (r^2=0.95 and 0.98, slope=1.037 and 1.040, and intercept=0.020 and 0.063 for Studies 1 and 2 respectively). Comparable distribution of titers between the two methods is observed for both studies.

Linearity: (b)(4) sera from PRP vaccinees were -----------(b)(4)------------------------------- --. The resulting PRP antibody levels corrected for predilution factor were very close for all predilutions, suggesting satisfactory dilutional linearity.

Parallelism: Parallelism between the standard and test sample curves is a validity requirement for titer calculation. The applicant assessed parallelism by calculating the interdilutional %CV (i.e., %CV of the measured titers from those dilutions that give %ODs within the linear range of 10%-70%, or %CV of the titer values used to calculate the mean titer for a sample). Parallelism is accepted if %CV is (b)(4). The justifications for this unusually loose %CV criterion are: no parallelism test is used for (b)(4) which served as the reference for the development of ELISA PRP assay; and there was no association between %CV and ratio of titers between (b)(4) and ELISA assays.

Reviewer's Comments: Using %CV to assess parallelism is a rather unusual and crude way for assessing parallelism. The fact that no parallelism test is required for (b)(4) assay does not imply that a parallelism criterion is of no practical use for ELISA assay and thus set at an overly loose level. The applicant stated that sample and standard curves are usually highly parallel in this assay with %CV<20%. For future assay development, a parallelism test in line with the methods suggested in the new USP draft guidance on Design and Development of Biological Assays is recommended.

3.2.3 CONCLUSIONS

There is no evidence of unsatisfactory assay performance. However, the design and analysis methods used by the applicant to assess precision and parallelism are not statistically sound. For future assay development, statistical approaches suggested in the draft USP guidances on Biological Assay Validation and Design and Development of Biological Assays are recommended.

3.3   -------(b)(4)----- ASSAY – Dose response curve

Dr. Zubkova requested Section 5.2.2 be reviewed by a statistician. Section 5.2 of the a-HBs assay validation report describes the calibration curve of the assay. A 4-parameter logistic model is fitted to the dose response curves. The logistic function given in 5.2.2 is as follows:

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

Where,

        y = OD on a linear scale
        x = dose on a logarithmic scale
        A = lower asymptote
        B = upper asymptote
        C = dose at the inflection point of the sigmoidal curve
        D = curvature parameter related to the slope of the curve

Reviewer's Comments: There is an error in the logistic model given in the report. The correct model is:

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

This could be just a typo. The applicant used a commercially available software to do the curve fitting. A correct function should have been used in the program. Therefore, it is not likely to produce any impact on the assay results generated.

4. CONCLUSIONS

- A --(b)(4)--- of hSBA MenY titers was observed consistently for re-tested samples from studies Hib-MenCY-TT-005 and -007/-008. No conclusion on assay drift can be drawn based on the results from the sentinel samples in the testing of studies 009 and 010 samples. Clarification of the data set and the analysis results presented in the submission is needed for further evaluation.

- There is no evidence of unsatisfactory performance of the Hib PRP ELISA assay. However, the design and analysis methods used by the applicant to assess precision and parallelism are not statistically sound. Better approaches are recommended for future validations.

- There is an error in the logistic model used for fitting the ------(b)(4)--------- assay calibration curve. This may have been a typo and is not likely to impact the assay results.