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Subject: Serology Review Memo for BLA 125363/0 (MenHibrix)

Sponsor: GlaxoSmithKline (GSK)

Submissions Reviewed

STN 125363/0 Original BLA
STN 125363/0.5 (amendment received 3/15/2010)

BLA Summary/Background

On 12 August 2009, GSK submitted a Biologics License Application (BLA) for Meningococcal Groups C and Y and Haemophilus influenzae b Tetanus Toxoid Conjugate Vaccine. Clinical development of this vaccine, which was originally designated Hib-MenCY-TT, was conducted under US IND -(b)(4)-. The proprietary name is MenHibrix.

MenHibrix is a non infectious vaccine that contains *Neisseria meningitidis* serogroup C capsular polysaccharide (PSC), *Neisseria meningitidis* serogroup Y capsular polysaccharide (PSY), and *Haemophilus influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate, PRP), each covalently bound to tetanus toxoid.

MenHibrix is a lyophilized vaccine supplied in a -(b)(4)- single dose glass container. The vaccine is to be reconstituted prior to intramuscular injection with a liquid saline diluent supplied in --- (b)(4) --- containing -(b)(4)- of 0.9% Sodium Chloride diluent. The reconstituted product contains 2.5 µg of PRP-TT, 5 µg PSC-TT and 5 µg PSY-TT per 0.5 mL dose volume.

MenHibrix is not currently licensed in any country or region. Clinical data supporting MenHibrix are provided in this BLA. The clinical database supporting licensure of the candidate MenHibrix vaccine includes results from 13 completed clinical studies. A total of 7,521 subjects received primary vaccination with the Hib-MenCY-TT vaccine and a total of 7,023 subjects received a dose of the Hib-MenCY-TT vaccine during the fourth dose phase studies. The MenHibrix

vaccine was co-administered with routinely used childhood vaccines. A total of 28,968 doses of the MenHibrix vaccine have been administered during the completed clinical studies to evaluate the safety profile of the MenHibrix vaccine administered at 2, 4, 6 and 12 to 15 months of age in the target population of infants and toddlers from 6 weeks to 15 months of age for licensure. Clinical study reports for these 3 studies are provided in the BLA.

MenHibrix is indicated for active primary immunization for prevention of invasive disease caused by *Haemophilus influenzae* type b and meningococcal disease caused by *Neisseria meningitidis* serogroups C and Y in infants and toddlers. MenHibrix vaccine is proposed to be administered as a 4-dose series (0.5-mL dose) by intramuscular injection at 2, 4, 6, and 12 through 15 months of age. The first dose may be given as early as 6 weeks of age.

The important serology issue regarding this review is the reproducibility of the results obtained in the human serum bactericidal assay (hSBA). The (b)(4)- in hSBA in retested serum samples raises questions about the validation of the hSBA. These issues are discussed in the “hSBA Assay Development history” section (section 1.1) and the hSBA conclusion section of this report. A second hSBA problem was related to the study Hib-MenCY-TT -008 and -010 where the vaccinees in the *PedvaxHIB* (*H. influenzae* type b vaccine) immunized group (control group) manifested an increase in hSBA-MenY response after the 4th immunization schedule. An investigation has shown that antibodies developed against the PRP carrier (meningococcal outer membrane proteins) used in the *PedvaxHIB* vaccine were responsible for the MenY hSBA activity (section 1.4 of this review). A third issue is related to the “specificity” assessment of MenC and MenY (b)(4)-. As discussed in section 2 (Assay validation) of this review, the sponsor assessed “specificity” of (b)(4)- by -----(b)(4)-----
----- (b)(4)----- renders the “specificity” test inadequate for MenC and MenY (b)(4)-.

REVIEW

This review includes the following serological assays used in the BLA. All the assays listed here are under module 5.3.5.4 (Other Study Reports), which is a subsection of module 5 (Clinical study reports).

- 1) **Human Serum Bactericidal Assay (hSBA)**
- 2) **Anti-*Neisseria meningitidis* type C and Y (b)(4)-**
- 3) ***Haemophilus influenzae* PRP (b)(4)-**
- 4) **Pneumococcal (b)(4)-**

Conclusions for each assay can be found at the end of each assay review.

Table 1 Key immunogenicity objectives and associated clinical studies from which support is derived

Immunogenicity Objective	Marker	Supporting Clinical Study
Non-inferiority to Hib control (post-dose 3 and 4)	Anti-PRP	Post-dose 3: 005, 007, 009 Post-dose 4: 006, 010 (008 supportive)
Immune response to MenC and MenY (post-dose 3, pre-dose 4, and post-dose 4)	hSBA-MenC, hSBA-MenY	Post-dose 3: 009 Pre-dose 4: 010 (MenC only) Post-dose 4: 010
Non-inferiority of co-administration with diphtheria, tetanus, and poliovirus antigens	Anti-diphtheria, anti-tetanus, anti-polio type 1, 2 and 3	009 (005 supportive)
Non-inferiority of co-administration with PT, FHA, and PRN antigens	anti-PT, anti-FHA and anti-PRN	005, 009
Immune response to hepatitis B (Descriptive)	Anti-HBs	005, 009
Non-inferiority of co-administration with <i>Prevnam</i> (post-dose 3 and post-dose 4)	Anti-PS (<i>S. pneumoniae</i> serotypes)	Post-dose 3: 005 Post-dose 4: 006
Non-inferiority of co-administration with MMR and V	Anti-measles, anti-mumps, anti-rubella, anti-varicella	Pooled data 008 and 010
Post-dose 2 Hib, MenC, and MenY immuno	Anti-PRP, hSBA-MenC, hSBA-MenY	007
One year persistence post-dose 4	Anti-PRP, hSBA-MenC, hSBA-MenY	013

PRP : polyribosylribitol phosphate
hSBA: human serum bactericidal assay
HBs: hepatitis B
MenC: Meningococcal serogroup C
MenY : Meningococcal serogroup Y
PT: pertussis toxoid
FHA: filamentous hemagglutinin
PRN: pertactin
MMR: measles, mumps, and rubella
V: anti-varicella antibody

1.1) Human Serum Bactericidal Assay (hSBA) Development History

Document name “Human Serum bactericidal Assay (hSBA) development History”.

File name: assay-dev-history

The evaluation of the response against MenC and MenY portion of the MenHibrix is based on serum bactericidal assay (SBA) performed with -----(b)(4)----- as the complement source. The hSBA assay used by GSK is based on a -----(b)(4)----- . The -(b)(4)--MenC assay was the basis of the primary immunogenicity endpoint that supported the licensure of GSK Biologicals’ Menitorix™ (*Haemophilus influenzae* type b and *Neisseria meningitidis* serogroup C- tetanus toxoid conjugate vaccine) in the UK, Brazil, and other selected European countries. There are several variations of the -(b)(4)- that have been used by different laboratories around the world. These variations include the -----(b)(4)-----

----- . These differences are summarized in the table below.

[------(b)(4)-----]

The results of the -(b)(4)- performed at ------(b)(4)----- were used in the pre-licensure immunogenicity testing for meningococcal serogroup C conjugate vaccine that was approved in the UK. The -(b)(4)- developed by GSK has several differences compared to the --(b)(4)--. The sponsor conducted a bridging study with the ---(b)(4)--- based on a coefficient of correlation (r) and concordance correlation coefficient (CCC, Rc) calculation. Serum samples from study Hib-MenC-TT-012/013 were used for the bridging experiment and included samples that showed post-dose 3 responses in infants primed with either Hib-MenC-TT or MenC-CRM₁₉₇ monovalent vaccine (Meningitec®) as well as the pre-dose 4 and post-dose 4 in both groups, after administration of a booster dose of Hib-MenC-TT. The results of the rSBA-MenC bridging between the -(b)(4)- and GSK assays fulfilled both requirements for both the Hib-MenC-TT and the Licensed MenC Vaccine groups (LicMenC group) at all three serum sampling time points. For HibMenC, the slope was $r=0.8471$, accuracy was 0.8341, and CCC (Rc) was 0.7065. For LicMenC, the slope was $r=0.7852$, accuracy was 0.7931 and CCC (Rc) was 0.6227.

Strains Used in the SBA:

The strains used for development of hSBA were the same as those used in -(b)(4)-. Strain -(b)(4)- was used for the MenC hSBA and strain -(b)(4)- was used for the MenY hSBA. These strains were also used by -(b)(4)- and CDC.

Human Serum as Complement Source:

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

The analysis showed that conclusions were different when pools from Menomune™ or Hib-MenCY-TT conjugate vaccines were considered. The optimal conditions predicted with sera from recipients of Hib-MenCY-TT were selected: bacterial --- (b)(4) --- dilution of - (b)(4) - and - (b)(4) - of --- (- (b)(4) - final concentration). These conditions were further confirmed by evaluating the - (b)(4) - and - (b)(4) - versus - (b)(4) - samples which also included - (b)(4) - samples from Menomune™ study.

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

The results reported appear to indicate that a high number of Menomune immunized subjects who were negative in - (b)(4) - were positive in hSBA. The discordant results were observed mainly in the Menomune group in which a significant number of negative samples by means of - (b)(4) - were positive in hSBA. When the same analysis was performed using post vaccination samples from subjects who received Hib-MenCY-TT in study Hib-MenCY-TT-001 only, no specificity was calculated, since all subjects were positive by - (b)(4) -. However as shown in Table 6 the sensitivity and concordance between - (b)(4) - and hSBA are high. Correlation between (b)(4) and hSBA on samples from the pooled Hib-MenCY groups is shown in Figure 3.

[----- (b)(4) -----]

[----- (b)(4) -----]

To calculate the precision of hSBA, -(b)(4)- samples were run by -(b)(4)- technicians on -(b)(4)- days using the -(b)(4)- lot of b(4)'. The concordance between the -(b)(4)- operators ranged from 89% to 95%, with highest variation observed with samples in the low titer range.

The impact of lot-to-lot variability in b(4) was assessed by testing -(b)(4)- samples (Hib-MenCY-TT studies, conjugate vaccine recipients) with -(b)(4)- different b(4) lots using both -(b)(4)- and hSBA. The concordance and specificity of the hSBA results when compared to the -(b)(4)- results was calculated for each of the lots, which gave the same results, regardless of which lot of human complement was used as shown in Table 7.

[----- (b)(4) -----]

Based on these results b(4) was used in the clinical trials and hSBA was validated according to IHC guidelines. Document RDCIB-051 V1 describes the assay procedure and the documents BCHCPCV01 and BCHCPCV02 describe the full validation of the assays according to ICH guideline.

Development of hSBA-MenY Using an Experimental Design Approach:

The selection of optimal conditions for hSBA-MenY was also assessed using an experimental design as performed for MenC. The different conditions tested were as follows:

- Concentration of -----(b)(4)-----
- Dilution of the -----(b)(4)----- starting at absorbances between -(b)(4)-.
- -(b)(4)- dilutions of -----(b)(4)-----.

The experimental design for MenC was performed using -(b)(4)- different b(4) lots and by -(b)(4)- different technicians. However, the design did not allow for selection of a condition with higher desirability; therefore it was decided to continue using the initial procedure derived from the -(b)(4)- used for -----(b)(4)----- . Assessment of the assay's performance was done on the same subset of -(b)(4)- samples as used for the development of the hSBA-MenC. However, out of these (b)(4) samples only 150 results were analyzed (due to sample exhaustion). Limited concordance between -(b)(4)- and hSBA was observed for MenY, but similar to the results found for hSBA-MenC, the results from a second analysis performed on a single study (Hib-MenCY-TT-001, no Menomune group, i.e., no plain polysaccharide vaccine recipients), demonstrated improved hSBA and -(b)(4)- concordance. Specificity was not possible to calculate since all subjects had seroconverted with respect to -(b)(4)--MenY antibody titers -(b)(4)- post-vaccination as presented in Table 9. The correlation between both assays on samples from Hib-MenCY group only is presented Figure 5.

[-----(b)(4)-----]

[------(b)(4)-----]

Initial experiments to define the precision demonstrated a 36% CV when -(b)(4)- samples were tested by -(b)(4)- technicians at -(b)(4)- but using the -(b)(4)----- lot. The concordance between the -(b)(4)- operators ranged from 87% to 100%, with the highest variation being observed with samples in the lowest range of titers.

Variations in different complement lots were also evaluated. -(b)(4)- samples were tested with -(b)(4)- different b(4) lots in hSBA and results were compared to those obtained with -(b)(4)-. The concordance and specificity of the hSBA results when compared to -(b)(4)- results was calculated for each of the lots. The hSBA showed similar concordance, sensitivity, and specificity between the different lots of human complement. However, the concordance and specificity was lower than that obtained with hSBA-MenC and -(b)(4)--MenC (Table 10 below). The sponsor argues that unlike in -(b)(4)--MenC, the value of -(b)(4)--MenY in predicting protection against MenY disease has not been established. As a result despite a low level of concordance and specificity between -(b)(4)--MenY and hSBA-MenY, the assay is adapted for use in the evaluation of clinical samples.

[----- (b)(4) -----]

Assay Improvement:

GSK assessed three different factors for improvement of the hSBA assay. The first factor assessed was a ----- (b)(4) ----- factor. Reference sera do not exist for hSBA. To address the lack of standardization, a ----- (b)(4) ----- (adjustment) factor has been implemented by GSK. This ----- (b)(4) ----- factor is calculated based on the results of ----- (b)(4) ----- . The use of this ----- (b)(4) ----- factor is believed to be of importance in the absence of international reference sera and in the absence of a standard critical assay reagent (human complement for instance).

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

The second factor assessed was ----- (b)(4) ----- . Traditionally discontinuous titer calculation was used in SBA. Discontinuous calculation is the ----- (b)(4) ----- of a ----- (b)(4) ----- was reported as the titer. However, it has been shown that this method has a bias towards lower calculation of titers. The sponsor has implemented a ----- (b)(4) ----- that had been used in ----- (b)(4) ----- . The two methods are compared for the Hib-MenCY-TT-009 and -010 studies. Results have shown that the ----- (b)(4) ----- method has no significant impact on the % Seropositivity in either of the vaccinated groups whatever time-point is considered. Although titers released by ----- (b)(4) ----- are higher, this method is shown to increase the precision (repeatability) of the assay.

The third factor assessed was recovery for hSBA-MenC. According to the protocol the ----- (b)(4) ----- . Initial US phase II trials have shown that about ----- (b)(4) ----- of the plates were rejected due to too few CFUs for

the bacterial control with the active human complement. Rejection rate for hSBA-MenY remained at 10%. According to the protocol the -----(b)(4)-----

significantly reduced and CFU counts at the end of the assay were higher and less variable. As the new method significantly improved the robustness of the assay, the updated method was implemented.

Further Validation of the Assays:

The firm performed additional validation of the hSBA-MenC and hSBA-MenY assays. Precision was reassessed for the hSBA-MenC and hSBA-MenY assays. Precision was initially evaluated in a Repeatability and Reproducibility method where -(b)(4)- technicians tested samples covering a range of titers. Following a subsequent CBER request submitted to the sponsor on June 30, 2008, GSK designed and performed a new R&R experimental plan. The sponsor designed new tests where a panel of -(b)(4)- samples was used to determine precision, covering the current analytical range of the assays, with additional samples in the lowest range of titers (titers -----(b)(4)-----

As stated above the sponsor has used -(b)(4)- sera to --(b)(4)-- results from different assays. However the sponsor had earlier reported -----(b)(4)----- data. By using the new variability formula both the ----(b)(4)--- and the old -----(b)(4)----- data were recalculated. The results indicated that when the assays were ----(b)(4)--- CV was found to be higher for MenC. For MenY, -----(b)(4)----- does not appear to affect variability. The variability appeared higher in the lower titers. When the variability was calculated at -----(b)(4)----- cut-off levels the variability was measured lower for MenC as the titers increased. Changes in the cut-off level did not impact MenY CV.

The initial SOP instructed that sera having titers ranging from --- (b)(4) -- be re-tested (designated as the -----(b)(4)-----). If the retested serum confirms the initial result, that titer is considered correct. If the retest yields different titers, a third test is performed. The result of the third test is considered correct. The technical cut-off of the assay is -(b)(4)-. Experience from testing the clinical samples has shown that there is high variability below titers of 1:16. As a result the sponsor has changed the --- (b)(4) ---. It is indicated that this change ensures the determination of sera -(b)(4)-, the clinical cut-off recommended by CBER. The firm also assessed the impact of the adjustment factor on assay precision. Evaluation of the adjustment factor to normalize results from different assays has shown that sera with titers -(b)(4)- manifest a high degree of variation. The adjustment factor was calculated based on the performance of two internal control sera containing high Ab levels against MenC and MenY PS. The -----(b)(4)----- factor was achieved by a formula where expected titers of the control sera were divided by the measured titers. During the IND phase, CBER raised a question about the high variability in sera with titers -(b)(4)- in assays using the adjustment factor. In response, GSK proposed two new methods for --(b)(4)--. The first method is called the -(b)(4)- method. In

this method, first tests were performed similar to the original adjustment factor method. Subsequently, geometric means of the adjustment factors calculated for each plate during that test session (there may be up to --(b)(4)-- during a test session) were calculated. The geometric means of the adjustment factors were then applied to each plate for final calculation of the sample titers. The second method is called -----(b)(4)----- by reagent ----(b)(4)----. This method involves the evaluation of new -----(b)(4)----- with -(b)(4)- samples. The results are compared with the reference lot. Subsequently a geometric mean of the adjustment factor is calculated from each plate used to document the bridging of the new reagent lot and is used as the common -----(b)(4)----- factor for each sample tested thereafter.

The sponsor analyzed data by using all three methods. The analysis was performed for both MenC and MenY. For each serogroup, samples for which hSBA repeat results were available (clinical samples subsequently used for bridging or development purposes) were identified. The results have confirmed that the original adjustment method yielded higher variability at -(b)(4)- titers. For MenY, current adjustment method yielded the best precision.

Following these attempts to ---(b)(4)--- assays the sponsor concluded that the current -----(b)(4)----- method yields the best precision for MenY hSBA and for MenC with titers -(b)(4)-. For samples in the -----(b)(4)----- the repeat test is applied to obtain the most accurate titers estimation. It is also stated that the number of samples from post-vaccination time points with a titer between 4 and 16 is very low for both C and Y. As a result, the probability of false positive values is therefore low.

The firm also assessed samples from studies Hib-MenCY-TT-009/010 falling in ---(b)(4)---. On October 8, 2008 CBER asked the sponsor to investigate in detail the effect of retesting of samples within the new -----(b)(4)----- on the precision for studies Hib-MenCY-TT-009/-010. The results have shown that of the 2689 results released (1017 -(b)(4)-, 852 -(b)(4)-, 820 -(b)(4)-), 8 samples were in the -----(b)(4)----- for MenC. Of these -(b)(4)- samples re-tested, only two samples needed a third assay to confirm their serological status (+ or -). As previously shown in the data submitted in October 2008, the precision of the assay is lower for the samples in the low titer range. It is worth noting that samples for which a third assay was needed exhibit a very high % CV as it is calculated using one value that differs from the 2 others considered as true with regards to the serological status.

When the samples for which only two assays are considered, the overall % CV of samples in the -(b)(4)- zone is -(b)(4)-.

For MenY, of the -(b)(4)- samples tested, 43 were in the -----(b)(4)-----. Of these 43 samples, only 3 needed a third assay to confirm their serological status (+ or -). Twenty-one samples belong to the pre-booster time point, all pre-booster samples with an initial titer < 8 were released as <8. The precision of the assay is lower for samples in the low titer range. It is worth noting that very high % CV are found with samples requiring three tests to determine their titer. The reason for this is that the calculated %CV uses one value that differs from the 2 others, these 2 being considered as true with regards to the serological status. For samples requiring only two assays, the overall %CV of samples in the ---(b)(4)--- is of 52.4%

GSK's overall conclusion was that the precision of the assay around the cut-off titer together with the ---(b)(4)-- rule were sufficiently precise with regard to the seropositivity status of the samples.

Sentinel samples:

In addition to including -----(b)(4)----- factors into each test and preparing QC charts to determine possible assay drift, GSK also included sentinel samples into the serum evaluation of MenCY-TT-009 and -010 clinical studies. The sentinel samples are sera from previous studies that had already been tested. Each week the same -(b)(4)- sentinel samples were included in the routine testing. The same samples were used throughout the testing in studies Hib-MenCY-TT-009 and -010 allowing comparisons of the titers from week to week as well as with the historical titers. Detailed discussion of the sentinel samples with respect to different clinical studies are provided below.

- Retest of Hib-MenCY-005

GSK instituted this QC testing after the observation that lower titers were being obtained with MenY hSBA with repeat testing. GSK indicates that nine samples from the study Hib-MenCY-005 were retested (Table 18). The actual number of samples on Table 18 is 10. It is not clear why these ten samples were retested. All ten sample titers were lower than previous measurements. Decrease in the measured titers was eight fold lower than the previous measurement (GMR of 0.15 current titer/reference titer). More importantly two samples that were positive in previous measurements yielded negative results (<1:4).

Table 18 Re-testing results of samples from study Hib-MenCY-TT-005.

Study	Samples	Time point	Reference titer	New titer	Ratio (New/ref)
HIB-MenCY-TT-005	(b)(4)	P3M5	(b)(4)	(b)(4)	0.11
HIB-MenCY-TT-005		P3M5			-/+
HIB-MenCY-TT-005		P3M5			0.92
HIB-MenCY-TT-005		P3M5			0.05
HIB-MenCY-TT-005		P3M5			0.33
HIB-MenCY-TT-005		P3M5			0.22
HIB-MenCY-TT-005		P3M5			0.52
HIB-MenCY-TT-005		P3M5			0.01
HIB-MenCY-TT-005		P3M5			-/+
HIB-MenCY-TT-005		P3M5			0.12
Geomean of ratios					0.15

An investigation into the possible causes of a --(b)(4)-- in MenY hSBA was conducted. GSK concentrated on the two main parameters that have changed since the initial hSBA assay. These parameters were the b(4) and the MenY -----(b)(4)----- . Re-evaluation of the bridging data showed that the lots of both of these components were properly bridged to previous lots. A

second set of samples (b)(4)- samples) from Hib-MenCY-TT-005 study were retested for MenY hSBA with the (b)(4)- that were used for the evaluation of the first set of 10 samples. Out of the (b)(4)- samples, 3 that measured positive during the initial testing measured negative in the re-test. Out of the remaining 9 samples, 7 manifested a decrease in hSBA titers (GMR of 0.77) while 2 samples manifested an increase in titers (GMR of 1.54 and 5.92 respectively). GSK also indicates that an additional (b)(4)- samples from the Hib-MenCY-007/-008 were retested. According to GSK, there was some degree of decrease in all 12 samples after retest (geomean ratio of 0.5), although none became negative. The data obtained after retesting of Hib-MenCY-007/-008 was not presented in the BLA. These data were requested from the firm in a teleconference dated February 28, 2010. These data have not been submitted for review.

GSK next sought to investigate the lots of (b)(4)- as a possible cause of the (b)(4)- in Hib-MenCY-TT-005 study serum hSBA titers. They compared the activity of (b)(4)- and the (b)(4)- lots that had been used in the original hSBA with the lots of (b)(4)- and the (b)(4)- that yielded (b)(4)- hSBA titers. The data were also evaluated with and without an adjustment factor. These data are shown in Table 20 below. The results showed that whatever the (b)(4) and (b)(4)- lots or calculation method used, the (b)(4)- of titer was consistent between the three conditions tested as well as concordance data when a cut-off of (b)(4)- is used. The firm concluded that the (b)(4)- of Hib-MenCY-TT-005 study sample titers is not due to a difference in activity of the (b)(4)- nor to a difference of sensitivity between (b)(4)-, (b)(4)-. They further concluded that the age of the samples could be a factor in the titers (b)(4)- because the samples were collected in 2005 (b)(4)- years prior to the retesting). They hypothesized that some components of the sera, such as an intrinsic inactivation of (b)(4)-, (b)(4)- might have been affected in a way that impacts the interaction between (b)(4) antibodies and strain MenY (b)(4)-. The sponsor also mentions that results from study Hib-MenCY-TT-008 were released in the period of time when a (b)(4)- in titers for study Hib-MenCY-TT-005 was observed. In addition, the sponsor also states that no (b)(4)- of titers or % SP for MenY was observed for study -008. However, it should be noted that although the sponsor did not present the data for study -008, they did indicate that on retest some (b)(4)- in titers was observed (geomean titers 0.5).

Table 20 Geomean ratio between historical data and data generated in re-test with different combination of (b)(4) lots

	(b)(4) (non adjusted)	(b)(4) (non-adjusted)	(b)(4) (adjusted)
Geomean	0.36	0.29	0.26
N samples + turning – (cut-off 1:8)	3/24	7/32	7/28

The sponsor indicates that no further investigation could be carried out because sera from study Hib-MenCY TT-005 were almost exhausted.

- Sentinel samples for Hib-MenCY-TT-009/010 testing.

Following the detection of a --(b)(4)-- in MenY hSBA titers of Hib-MenCY-TT-005 sera on retest, GSK introduced sentinel samples from study Hib-MenCY-TT-013 as part of routine testing. These sentinel samples were added in routine testing on a ----(b)(4)----- of the Hib-MenCY-TT-013 samples, -(b)(4)- were tested for hSBA-MenC and -(b)(4)- were tested for hSBA-MenY. The results from these samples were compared from week to week as well as to historical results. The data were evaluated according to the following:

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

The main outcome of these assessments is that sample titers as determined by hSBA-MenY dropped after the first week, but stayed constant during the remaining three weeks. However, sample titers from the hSBA-MenC changed minimally. The precision for hSBA-MenC using -(b)(4)- samples/4 repeats was calculated to be 77.14 %. Precision during validation experiments was 73.58%. For MenY, a precision of 43.11% was calculated from 15 samples/5 repeats. This observation is aligned with the validation experiments in which a precision of 45.20% was observed.

It is important to note that testing of the sentinel samples was performed weekly for four weeks. The major drift in samples from study -005 was observed after three years of storage. Therefore, although sentinel sample testing gives information on the repeatability of the results in the short term, they do not help explain the --(b)(4)-- in hSBA-MenY titers in study -005 retests.

Based on these results, GSK concluded that although no root cause was identified, the substantial -(b)(4)- in hSBA-MenY titers in the sera from study -005 study was not a generalized problem with MenY hSBA. GSK continued the phase III studies by adding sentinel tests to monitor the performance of the hSBA.

The history of hSBA development steps and methods used in the different clinical studies are summarized in Table 24 below.

Table 24 Table History of hSBA development steps and methods used in the different studies.

Studies	Testing date	Method		WS Lots		HC' Lots	
		hSBA C	hSBA Y	C	Y	C	Y
Hib-MenCY-TT-001 to Hib-MenCY-TT-004	May-2005 and before	(b)(4)					
Hib-MenCY-TT-005	Feb-Mar-2005	(b)(4)					
Hib-MenCY-TT-005	Dec-2005	(b)(4)					
Hib-MenCY-TT-005	Jan-Feb-2008	(b)(4)					
Hib-MenCY-TT-007	Jan-Feb-2006	(b)(4)					
Hib-MenCY-TT-007	June-Jul-Aug-2006	(b)(4)					
Hib-MenCY-TT-006	Aug-Sept-2006	(b)(4)					
Hib-MenCY-TT-008	Aug 2008 - Dec 2008	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
Hib-MenCY-TT-009	January-March 2009	(b)(4)	(b)(4)				
Hib-MenCY-TT-010	January-March 2009	(b)(4)	(b)(4)				
Hib-MenCY-TT-013	December 2008	(b)(4)	(b)(4)				

QC Charts:

QC charts assessing the performance of the two controls added to each assay were provided for studies Hib-MenCY-TT-005/-006/-007/-008/-009/-010/-013. The final value of controls is the geometric mean from (b)(4)- different routine tests. The range of acceptability is also calculated at the end of (b)(4)- runs. When a new control is introduced, its range is temporarily set based on limited runs. This is called “provisional range” (RP). The number after RP indicates the number of runs performed to determine the RP. After (b)(4)- test runs were completed the final ranges were calculated.

For MenC the controls did not yield a drift in titers. However, there were high numbers of “out of range” values until 19/08/2008, after which a recovery period for the (b)(4)- bacteria was introduced into the hSBA-MenC assay. Out of range values rendered plates invalid. The recovery method is when bacteria are allowed to recover ----- (b)(4) ----- with other reagents. Previously, bacteria were (b)(4)- with other test samples much earlier.

For MenY there was a drift in titers with control 1 for a short period (21/06/2006 – 04/07/2006). It is not known why Control 1 manifested a drift in titers during this period. Control 1 did not manifest a drift in titers in subsequent tests performed up to 09/08/2007.

No drift in either hSBA-MenC or hSBA-MenY is observed during the testing period of the two Phase III studies, Hib-MenCY-TT-009 and Hib-MenCY-TT-010. Although fluctuations in the assays have been observed during this period of time, the fluctuations have been minor and relatively low number of controls with values out of the range have been observed during that period of time.

1.2) MenY and MenC hSBA SOP

GSK provided SOPs for the hSBA as follows:

- “Measurement of bactericidal activity of human serum antibodies to *Neisseria meningitidis* serogroup C (without –b(4)-----) in presence of human complement” is about MenC hSBA.
- “Measurement of bactericidal activity of human serum antibodies to *Neisseria meningitidis* serogroup Y (without –b(4)----- in presence of human complement” is about MenY hSBA. Document number SOP 9000005592-V2. File name, sop-rd-cib-052-v2

These SOPs contain the procedures and materials used to perform the hSBA for MenY and MenC. The firm indicates that these SOPs were used in the R&D “ClinLabs Function Immunology” and “Bacterial development Unit” laboratories in Belgium and the -----(b)(4)-----.

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

(b)(4)-----

(b)(4)-----

(b)(4)-----

This assay determines the -----(b)(4)----- titer of human sera in the presence of human complement and it is performed in -----(b)(4)-----.

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

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

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

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

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

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

The exact dilution corresponding to ----(b)(4)--- is calculated by interpolation of the sample curve:

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

The calculated ---(b)(4)--- titers is next divided by the adjustment factor to achieve the final titers. Adjustment factor for each control is calculated with the following formula:

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

Each plate is evaluated for the assay criteria which were then used to determine the validity of the results.

The cut-off of the assay is defined as a titer of -(b)(4)-.

There were also conditions requiring repeat testing of the samples.

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

The (b)(4)- zone is defined as ---(b)(4)--- for both MenY and MenC. It should be noted that the (b)(4)- zone is defined as ---(b)(4)--- in the Men C SOP document RD-CIB-051 V1 (5.3.1.4.3) and the MenY SOP document RD-CIB-052 V1 (5.3.1.4.3). The (b)(4)- zone was changed in parallel to the change in the threshold as a result of CBER's recommendations (June 30, 2008). The initial threshold put forward by the sponsor was (b)(4)-. This value was subsequently changed to (b)(4)-. As a result, the (b)(4)- zone titers are changed to (b)(4)- in the Men C SOP document RD-CIB-051 V2 (5.3.1.4.3) and the MenY SOP document RD-CIB-052 V2 (5.3.1.4.3). When the samples that fall in the (b)(4)- zone are retested, if the positive or negative status of the sample is confirmed then the result obtained in the first assay is the final titers. If the second test does not confirm the first test, then a third test is performed to confirm the final titers.

1.3) hSBA Validation

1.3.1. Men C hSBA Validation

Validation of the MenC hSBA was provided in document BCHCPCV01 “Measurement of bacterial activity of human serum antibodies to *Neisseria meningitidis* serogroup C in presence of human complement”. This document contains validation test results for the MenC hSBA described in the SOP RD_CIB_052. The validation is based on ICH guidance documents Q2A and Q2B.

The firm assessed the following parameters during validation of this assay:

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

Evaluation of these validation parameters and the results were acceptable.

1.3.2) Men Y hSBA Validation

Document name “Measurement of bacterial activity of human serum antibodies to Neisseria meningitidis serogroup Y in presence of ---b(4)-----
Document number: BYHCPCV01. File name: val-byhcpcv01.

a result of this cross reaction, antibodies directed against MenY -(b)(4)----- lead to an -(b)(4)-hSBA titers in *PedvaxHIB* immunized subjects.

Table 1 Study Hib-MenCY-TT-008 and -010. Percentage of subjects with hSBA-MenY titers greater than or equal to (b)(4) and GMTs prior to and 42 days after fourth dose vaccination (ATP cohort for immunogenicity)

Study	Group	Timing	N	(b)(4)				GMT		
				n	%	95% CI		Value	95%CI	
						LL	UL		LL	UL
Hib-MenCY-TT-008	Hib-MenCY	Pre- dose 4	107	96	89.7	82.3	94.8	77.6	55.5	108.4
		Post- dose 4	122	119	97.5	93.0	99.5	1002.2	740.9	1355.7
	Hib	Pre- dose 4	38	0	0.0	0.0	9.3	2.0	2.0	2.0
		Post- dose 4	35	16	45.7	28.8	63.4	18.3	7.7	43.6
Hib-MenCY-TT-010	Hib-MenCY	Pre- dose 4	329	306	93.0	89.7	95.5	119.1	101.1	140.3
		Post- dose 4	342	338	98.8	97.0	99.7	1389.5	1205.0	1602.2
	Hib	Pre- dose 4	103	6	5.8	2.2	12.2	2.5	2.1	2.9
		Post- dose 4	120	87	72.5	63.6	80.3	48.6	31.9	74.0

Hib-MenCY = Hib-MenCY-TT + M-M-R II + Varivax (+ Prevnar) primed with Hib-MenCY-TT + Pediarix + Prevnar

Hib = *PedvaxHIB* + M-M-R II + Varivax (+ Prevnar) primed with *ActHIB* + Pediarix + Prevnar

GMT = geometric mean antibody titer calculated on all subjects

N = number of subjects with available results

n/% = number/percentage of subjects with titer within the specified range

Reviewer Conclusions on hSBA

The -(b)(4)- in MenY hSBA titers in sera from clinical study -005 samples on retest raises questions regarding the validity of the assay. Because the licensure of this product is based on serology assays only, the *Neisseria meningitidis* serotype Y (MenY) hSBA assay should be properly validated and should perform reliably and consistently. The validation report for the MenY hSBA, (BYHCPCV01) entitled “Measurement of bactericidal activity of human serum antibodies to *N. meningitidis* serogroup Y in presence of ---b(4)----- is provided in section 5.3.5.4.3. of the BLA. This validation report contains data supporting the repeatability of the assay. However, the data presented in section “6.1. Re-test of Hib-MenCY-TT-005” of “hSBA Assay Development History” document, raise questions regarding the validity of the MenY hSBA. Here, the retesting of ten samples from the Hib-MenCY-005 study in a MenY hSBA yielded a significant -b(4)---- in the titers of all samples. The measured titers were eight fold lower than the previous measurement (GMR of 0.15 current titer/reference titer). More importantly, two samples that were positive in the initial measurement yielded negative results (<1:4). Testing of a second set of samples (-(b)(4)- samples) from the Hib-MenCY-TT-005 study, using the -----b(4)----- that were used for the evaluation of the first set of nine samples, yielded a similar -(b)(4)- in the hSBA titers. This section also indicates that an additional -(b)(4)- samples from the Hib-MenCY-007/-008 clinical studies were also retested for MenY hSBA. Although these retest data were not provided, GSK states that there was some degree of -b(4)--- (GMR 0.5) in all -(b)(4)- samples after the retest. During a teleconference with the firm on February 9, 2010, CBER requested that GSK submit

the results of the (b)(4)- retested Hib-MenCY-007/-008 study sera. The firm has not submitted these data to date.

In order to investigate the (b)(4) and the (b)(4)- lots as possible causes of the (b)(4)- in serum hSBA titers, the firm next performed a set of MenY hSBA assays with lots of (b)(4) and (b)(4)- used at the time of initial data release (lot ---b(4)---) and compared these results with the data generated with new (b)(4)- and (b)(4)- lots. Regardless of the (b)(4) and (b)(4)- lots used in the hSBA, the results of these tests also confirmed the (b)(4)- in hSBA titers with Hib-MenCY-TT-005 study sera. Based on these results, the firm excluded the (b)(4) and the bacterial (b)(4)- as a possible cause for the reduction in the MenY hSBA observed in MenCY-TT-005 study sera. The firm then suggested that (b)(4)- of the MenCY-TT-005 clinical study samples could be a possible cause of the drift in titers since these study samples were first collected and tested in 2005, while the retests were performed in 2008. Possible changes in some of the serum components such as ---b(4)----- was presented as a hypothesis for the ---b(4)--- of the hSBA titers. However, the sponsor did not provide any evidence showing that: 1) there was a change in these molecules over time and 2) these changes effected hSBA. In the February 9, 2010 teleconference, we asked GSK whether they had further investigated these two possibilities and generated data that could support their hypothesis. We also asked whether they had a plan to evaluate possible changes in the (b)(4)- used in the hSBA because changes in the antigenic components of the ---b(4)--- during storage or after passage could be affecting the sensitivity of bacteria to hSBA. GSK indicated in response that a proper and full characterization was not conducted on the strain (b)(4)- used in the MenY hSBA when it was first obtained from CDC. Nevertheless, the firm noted that a recent characterization of the strain showed that the strain was (b)(4)-. However, since the strain was not characterized initially and during the time of storage, it was not possible to assess any change in the strain. To date, CBER has not received further information on the characterization of the MenY strain (b)(4)- or on the possible reasons for the drift in the MenY hSBA.

Because the licensure of this product is based on serology assays only, it is crucial that the sponsor provide scientific evidence showing that the MenY hSBA assay is working reliably and consistently. The (b)(4)- in titer, in particular for study MenCY-TT-005 suggest that the MenY hSBA assay performance is not reliable and not consistent. The communication of the following specific comments to the sponsor is recommended:

- a. In the “assay development history” document (Section 5.3.5.4.3. GSK hSBA Assay Development History) the sponsor indicated that the (b)(4)- separate retests of samples from the Hib-MenCY-005 clinical study in a MenY hSBA yielded a significant (b)(4)- in the majority of tested samples. The sponsor also indicated that an additional (b)(4)- samples from the Hib-MenCY-007/-008 clinical study were also retested for MenY hSBA and some degree of decrease (GMR 0.5) was observed in all (b)(4)- samples after retest. The sponsor needs to submit the results of the (b)(4) retested Hib-MenCY-007/-008 clinical study sera.
- b. In order to explain the possible causes of the (b)(4)- in serum hSBA titer in the Hib-MenCY-TT-005 study, the sponsor suggested that the effect may be due to

adverse effects of age on essential serum components of the assay. The sponsor needs to provide complete data in support of their hypothesis that the proposed changes in serum components affect the hSBA titers as observed in the Hib-MenCY-TT-005 study.

- c. On a teleconference dated February 9, 2010, the sponsor and CBER discussed the possibility that a change in ---(b)(4)--- due to storage might have altered sensitivity in the hSBA. The sponsor needs to provide detailed information on the characterization of the MenY strain -(b)(4)-.

2) Anti-*Neisseria meningitidis* type C and Y -(b)(4)-

2.1) Anti-*Neisseria meningitidis* type C and Y -(b)(4)- SOP

Document name “Measurement of total -(b)(4)- antibody to Anti-*Neisseria meningitidis* type Y polysaccharide (by -----(b)(4)-----)”.

(b)(4)

Calculation of -(b)(4)- concentration:

[illegible]

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

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

Critical assay Criteria:

In order to be able to assess the assay validity Low Positive and High Positive control titers as well as the LOQ (Limit of Quantitation) needed to be determined.

LOQ equals to the concentration of the standard corresponding to the lowest % OD for which calculations are valid (here (b)(4)-) multiplied by the lowest test sample dilution (here (b)(4)-). LOQ needed to be below the assay cut-off level of (b)(4)- for the assay to be valid. Once the validation of assay was verified then the validity of results needed to be evaluated. Absorbances above the limit of linearity of the ----(b)(4)---- were not considered in calculating the results (in general (b)(4)-). Both points of duplicate samples were used for the calculation of LOQ and the intra-duplicate CV% was (b)(4)-.

Reasons for repeat testing:

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

2.2) Validation of *Neisseria meningitidis* type C and Y polysaccharide (b)(4)-

Validation for MenC (b)(4)- was provided in documents ----- (b)(4) -----
“Measurement of Total (b)(4)- antibody to *Neisseria meningitidis* type C polysaccharide (by ----- (b)(4) -----, human serology)” and validation for MenY (b)(4)- was provided in document --- (b)(4) --- “Measurement of Total (b)(4)- antibody to *Neisseria meningitidis* type Y polysaccharide (by ----- (b)(4) -----, Human serology)”.

The firm originally validated the type C and type Y ----- (b)(4) ----- format where samples were ---- (b)(4) ----. Subsequently they switched to the ----- (b)(4) ----- to increase the assay productivity. The validation results presented in this document contain key performance characteristics that have been re-evaluated for the ---- (b)(4) ---- format.

The validation results evaluated in this document contain the following key performance characteristics of the ----- (b)(4) -----.

Limit of detection (LOD):

LOD is determined as ---(b)(4)--- for MenC and MenY.

Limit of Quantification (LOQ):

LOQ is determined as --(b)(4)-- for MenC and --(b)(4)-- for MenY.

Cut-off level:

Cut-off level is an arbitrary number assigned based on the LOQ and LOD values. It is set as -(b)(4)- for MenC -(b)(4)-.

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

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

Analytical range:

The lower limit of Analytical range is the cut-off limit of -(b)(4)-. Upper limit is not defined because samples can be diluted until a value can be calculated.

Precision:

Repeatability and Intermediate precision (inter laboratory variation) is calculated for MenC and MenY. Both the MenC and MenY average coefficient of variation for intra-assay replicates and triplicates was -(b)(4)-. The intermediate precision assessment showed that MenC coefficient of variation of titers was -(b)(4)-and MenY titers were -(b)(4)-.

Accuracy:

Accuracy is the closeness of agreement between the value which is accepted either as a conventional true value or as an accepted reference value and the value obtained by applying the test procedure. To assess accuracy -(b)(4)- CDC sera with assigned values were tested at GSK. The correlation between CDC values and the values obtained with the -(b)(4)- procedure used at GSK Bio showed a correlation coefficient “r” of -(b)(4)-.

The sponsor also performed a bridging study with ------(b)(4)-----

----- that had been previously validated. A regression analysis (correlation coefficient, slope and intercept of the regression line) of a series of assays performed under -----(b)(4)-----, using samples within a range of -----(b)(4)----- MenC -(b)(4)- antibody level showed that correlation coefficient $R = 1.00$; intercept = -0.02 and slope = 0.99.

Further bridging was done by assessing the concordance between the two assays at -----(b)(4)----. A series of assays were performed under -----(b)(4)----- using samples within a low titers range of -----(b)(4)---- MenC -(b)(4)- (assayed at the dilution -(b)(4)-). The results showed that concordance between the -----(b)(4)----- was 94%.

Accuracy assessment for MenY -(b)(4)- was done by testing -(b)(4)- CDC sera with assigned values at GSK. The correlation between CDC values and the values obtained with the -(b)(4)- procedure used at GSK Bio showed a correlation coefficient r of 0.96.

Linearity:

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

Specificity:

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

Interference:

Interference tests showed that ------(b)(4)----- do not affect the measured MenC or MenY -(b)(4)- the antibody levels.

Robustness:

To determine whether there is any position (or "end-of-run") effect due to delays in the addition of reagents, -(b)(4)- were positioned on the ------(b)(4)----- . No major position effect on the measured MenC or MenY -(b)(4)- antibody level was observed.

Recommended Serum Dilutions:

Based on the titration experiments recommended initial serum dilutions ranged from -----(b)(4)---
----- for MenC and ------(b)(4)-----
----- for MenY.

Reviewer conclusions on MenC and MenY -(b)(4)-

The specificity assay for the validation of MenC and MenY -(b)(4)- only investigates the
------(b)(4)-----

-(b)(4)- is an important aspect of specificity assay. The sponsor needs to perform -(b)(4)- experiments with---(b)(4)--- polysaccharide also. The remaining information presented in the SOP and the validation documents are acceptable.

3) Haemophilus influenzae PRP ELISA

3.1) PRP ELISA SOP

Document “SOP RD-CIB-004-E V1-0” b(4) ELISA) SOP to conduct the ELISA assay used to measure serum antibodies against PRP antigen in Hib immunized subjects.

ELISA procedure:

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

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

|
Calculation of titers:

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

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

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

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

3.2) PRP ELISA Validation

Document “val-pppcv01-PRP ELISA validation” contains the validation assays PRP ELISA.

Assay validity criteria presented in this document are in line with the criteria put forward in the FDA and International Committee on Harmonization (ICH) document “Q2(R1): Validation of Analytical Procedures: Text and Methodology”. The criteria in this document are Specificity, Linearity, Range, Accuracy, Precision, Detection Limit, Quantitation Limit and Robustness. The validity tests have shown that this assay could reliably measure anti-PRP antibodies ≥ 0.100 $\mu\text{g/ml}$. The assay cut-off level is $-(b)(4)-\mu\text{g/ml}$, which also means that sera $-(b)(4)-\mu\text{g/ml}$ are seropositive for anti-PRP.

Reviewer Conclusion on PRP ELISA

The “ELISA SOP” presented here describes the protocol, the reagents used to perform the ELISA and the calculation method used to determine anti-PRP ----- $(b)(4)$ ----- . The “validation report” evaluates the specifications and limitations of the assay and the calculation method used to determine the ----- $(b)(4)$ ----- . Specifically, parameters such as LOD, LOQ, cut-off level, as well as the “-- $(b)(4)$ -- around the cut-off level” are defined and the serum Ab titer calculation with a four parameter curve fitting method is described. The performance of the calculation method is presented with step by step example where the roles of high positive and low positive controls as well as the reference standard are demonstrated in the interpolation of data and the calculation of titers. Finally, the performance of the assay in consistently obtaining reliable and reproducible results is investigated as recommended in the International Conference on Harmonization ----- $(b)(4)$ ----- documents. For this purpose, Precision, Accuracy, Linearity, Parallelism, Specificity, Duplicate testing, Robustness, Assay drift, Analytical Run length, as well the recommended Serum Dilutions are determined. The results of these investigations showed that ELISA used to measure serum ----- $(b)(4)$ ----- antibodies performs reliably and reproducibly.

One (1) Page Determined to be Non-Releasable: (b)(4)

The acceptance for the results is dependent on the validity of the assay.

There were conditions when the assay had to be repeated. A critical criterion for repeat testing of samples is whether the measured concentrations fall within the -----(b)(4)----- around the -----(b)(4)----- for this assay is defined as -----(b)(4)----- . When a sample that is in the --(b)(4)-- is retested if the “positive” or “negative” status of the sample is confirmed, the result found in the first assay is the final titers. If no confirmation is obtained, then the result of a third assay decides which of the previous 2 results is confirmed. The confirmed result is considered as the final titer.

4.2) Pneumococcal -(b)(4)- validation reports

The two versions of the documents -----(b)(4)----- “Measurement of Total -(b)(4)- antibody to *Streptococcus pneumoniae* polysaccharides in human serum (by -----(b)(4)-----): Performance characteristics and validation” are reviewed.

The sponsor has submitted -(b)(4)- validation reports supporting the SOP RD-CIB-035v02. The second report ----(b)(4)---- is an updated version of ----(b)(4)---- and it was submitted in response to CBER’s May 23, 2007 dated questions (document “Strep Efficacy-Oct07”) regarding the extrapolation of results for the validation studies obtained with the “----- (b)(4) -----” to the “----(b)(4)----”. Please note that May 23, 2007 CBER letter and the sponsor’s October 2007 response to CBER’s letter are related to -(b)(4)- study report of GSK’s Havrix application. In addition, the sponsor also submitted additional justification for using -(b)(4)- as a threshold in their ELISA in response to a CBER’s question asked during the review of Hiberix (April, 23, 2009). The sponsor reported that the ----(b)(4)---- threshold levels decrease to --(b)(4)-- as a result of -----(b)(4)----- and the non-vaccine serotype -(b)(4)-. In the letter to the sponsor dated April 23, 2009, CBER requested a justification for the change in the threshold levels. In their response letter (June 1, 2009), the sponsor provided bridging data between their --(b)(4)-- and the WHO non---(b)(4)---. In their response the sponsor indicated that their decision to switch to ----(b)(4)---- was in line with the year 2000 WHO recommendations. GSK state that they followed the WHO recommendation to compare the --- (b)(4) --- with a well characterized non----(b)(4)---- as a reference. The sponsor refers to the 2003 WHO meeting where two laboratories were chosen as reference laboratory for the conventional non----(b)(4)---. These two laboratories are the -----(b)(4)-----

----- . The response letter of GSK contains the summary of the comparison data derived from a bridging study between GSK ----(b)(4)---- and the WHO reference -(b)(4)- run at the -(b)(4)- reference laboratory. The sponsor then calculated the Correlation and Concordance values to assess the bridging.

The initial comparison was made by using -(b)(4)- pediatric sera from individuals immunized with GSK’s 11 valent pneumococcal vaccine. These data were published by the sponsor in 2006 (Henckaerts, 2006). The results of this study showed that --- (b)(4) --- threshold concentration was -(b)(4)- and it was equivalent to 0.35 µg/ml concentration of the WHO non----- (b)(4) -----.

A second study containing -(b)(4)- sera from toddler pre- and post-booster conjugate immunization studies at the age of 15 to 18 months was also performed by GSK and the WHO reference laboratory. Combination of the two studies (-(b)(4)- samples) once again suggested that the ---(b)(4)-- threshold was -(b)(4)-.

A third study involved the analysis of 118 pediatric sera obtained one month after a 3-dose Prevnar primary immunization. Once again the samples were tested by -(b)(4)- (the reference laboratory) and GSK. Here also there was a high degree of correlation and a high concordance between both assays for all seven serotypes. Concordance agreement was 96% and the kappa value was 0.78. Concordance correlation coefficient (CCC) for the seven serotypes ranged from 0.8492 for 6B to 0.9551 for b(4)

Based on these results the sponsor suggested that the use of -(b)(4)- as the threshold in ---(b)(4)-- ---- is appropriate in the studies where Prevnar is co-administered with an investigational vaccine as in the case of MenHibrix, the subject of this BLA.

The validation tests were done using SOP “Measurement of Total -(b)(4)- Antibody to *Streptococcus pneumoniae* polysaccharides in human serum (by -----(b)(4)-----
-----.

These assay performances are related to the -----(b)(4)-----
------. The validation is based on the ICH (International Conference on Harmonization) guidance documents Q2A [ICH, 1995] and Q2B [ICH, 1996].

Limit of detection (LOD):

The LOD is calculated as ---(b)(4)---.

Limit of Quantitation (LOQ):

The LOQ was measured as ---(b)(4)---.

Cut-off level:

The cut-off level is determined based on the LOD and LOQ values as well as the value recommended at the WHO meeting on pneumococcal antibody measurements, March 10, --(b)(4)---
-----, 1999 was --(b)(4)-- (Characteristics of a suitable -(b)(4)- for estimation of pneumococcal polysaccharide antibodies. -(b)(4)-).

The cut-off level is set as -(b)(4)-.

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

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

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

The lower limit of analytical range is defined by the assay cut-off (b)(4)-. The upper limit is not defined because samples can be (b)(4)-until a value can be calculated.

Precision:

The “Repeatability” and “Intermediate precision (inter laboratory variation)” aspects of Precision are calculated. Overall the precision of the (b)(4)- was estimated to be (b)(4)-.

Accuracy:

The US FDA (b)(4)- was used to determine “Accuracy”. The average of the (b)(4)- values was calculated for each serotype, and then compared with the assigned value by calculating the ratio. In order to bridge (b)(4)- samples were tested by using the (b)(4)- format or with (b)(4)- for (b)(4)-. A regression analysis where acceptance criterion of benchmarking is R^2 (b)(4)- was performed. To determine the concordance at (b)(4)- between the (b)(4)- and the (b)(4)-, a series of samples (at least (b)(4)- per serotype) were tested by both methods. Selected samples had low antibody concentration (b)(4)-. The percentage of concordance is calculated as being the number of samples with double positivity or double negativity status at assay cut-off (by mean of (b)(4)-) divided by the total number of samples used, multiplied by (b)(4)-.

Linearity:

(b)(4)- tests were used to determine Linearity. In (b)(4)- experiments the expected PS (b)(4)- levels in the samples that were mixed at a 50/50 ratio were compared with the measured concentrations. The percentage recovery was calculated as (b)(4)-

In dilution experiments (b)(4)- samples exhibiting anti-PS (b)(4)- concentration within the overlapping concentration range covered by two consecutive (b)(4)- dilutions used in the (b)(4)- (were used to further document the linearity of the tests. These (b)(4)- samples are distributed as following: (b)(4)- samples tested for (b)(4)- samples for (b)(4)- samples for (b)(4)- samples for (b)(4)- samples for (b)(4)-. For each sample, the ratio of the concentration generated by the (b)(4)- was calculated, and then averaged across all samples. A ratio close to 1 indicated equivalent results when different dilutions are used, demonstrating the linearity of the assay.

Specificity:

The specificity data presented in this document are from the (b)(4)-. It is indicated that the equipment and the (b)(4)- times are the same as the ones used for the (b)(4)-. Specificity was assessed by (b)(4)-

Robustness:

----- (b)(4) ----- times at different steps of the SOP are introduced and results were compared with those obtained by using SOP. To determine whether there is any position (or "end-of-run") effect due to delays in the addition of reagents, (b)(4)- sera were positioned on the ----- (b)(4) ----- of the run. The antibody concentrations were calculated for each sample, at each (b)(4)- position.

---- (b)(4) --- effect:

Serum samples representing a range of antibody concentrations were aliquoted into ---- (b)(4) ----

----- . The ratios of results obtained in both tests were close to 1 for all PS tested.

Assay stability:

The values for Low positive and the High positive controls in the ---- (b)(4) ----- obtained between March 2003 to September 2003 were plotted. The limits of ---- (b)(4) ----- represent the average antibody concentration plus (b)(4)- standard deviations. There was no major over this time period.

Recommended ----- (b)(4) -----:

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

References:

Henckaerts I, Goldblatt D, Ashton L, et al. Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with or without 22F inhibition at low antibody concentrations in paediatric sera. Clin Vaccine Immunol. 2006; 13(3):356-60.

Reviewer Conclusion on pneumococcal -(b)(4)-

The SOP and the validation assays as well as the sponsor's response to CBER's questions support the use of their (b)(4)- to measure anti-pneumococcal (b)(4)- in trials where MenHibrix and Prevnar were used concomitantly. The "-(b)(4)- SOP" presented here describes the protocol, the reagents used to perform the (b)(4)- and the calculation method used to determine pneumococcal polysaccharides present in 7-valent pneumococcal vaccine, Prevnar. The "validation report" evaluates the specifications and limitations of the assay. Specifically, parameters such as LOD, LOQ, cut-off level, as well as the "----- (b)(4) -----" are defined. Finally, the performance of the assay in consistently obtaining reliable and reproducible results is investigated as recommended in the ICH Q2A and Q2B documents. For

this purpose, Precision, Accuracy, Linearity, Parallelism, Specificity, Duplicate testing, Robustness, Assay drift, Analytical Run length, as well the recommended ----(b)(4)---- are determined. The results of these investigations showed that -(b)(4)- used to measure -(b)(4)- antibodies against pneumococcal polysaccharides performs reliably and reproducibly.

COMPLETE RESPONSE LETTER ITEMS

GSK should resolve the following issues prior to approval of the BLA:

1. You use the Human serum bactericidal assay (hSBA) as a serological correlate of protection following the immunization with MenHibrix. Briefly, this assay is based on -----(b)(4)-----
----- The titer which ---(b)(4)--- of the bacteria is then calculated. Because the licensure of this product is based on serology assays only, you must provide scientific evidence showing that the *Neisseria meningitidis* serotype Y (MenY) hSBA assay is validated and is working reliably and consistently. The -(b)(4)- in titer, in particular for clinical study MenCY-TT-005 suggest that the assay performance is neither reliable nor consistent. Please provide scientific evidence showing that the *Neisseria meningitidis* serotype Y (MenY) hSBA assay is properly validated for its intended use and is working reliably and consistently. You should provide the following evidence in support of the reliability and consistency of this assay:
 - a. In the “assay development history” document (Section 5.3.5.4.3. GSK hSBA Assay Development History) you indicated that the -(b)(4)- separate retests of samples from the Hib-MenCY-005 clinical study in a MenY hSBA yielded a significant -(b)(4)- in the majority of tested samples. You also indicate that an additional -(b)(4)- samples from the Hib-MenCY-007/-008 clinical study were also retested for MenY hSBA and some degree of -(b)(4)- (GMR 0.5) was observed in all -(b)(4)- samples after retest. Please submit the results of the -(b)(4)- retested Hib-MenCY-007/-008 clinical study sera.
 - b. In order to explain the possible causes of the -(b)(4)- in serum hSBA titer in the Hib-MenCY-TT-005 study, you suggested the effect may be due to adverse effects of age on essential serum components of the assay. Please provide complete data in support of your hypothesis that the proposed changes in serum components affect the hSBA titers as observed in the Hib-MenCY-TT-005 study.
 - c. On a teleconference dated February 9, 2010, you discussed with CBER the possibility that a change in ----(b)(4)---- due to storage might have altered sensitivity in the hSBA. Please provide detailed information on the characterization of the MenY strain -(b)(4)-.
2. In the validation documents for MenC and MenY -(b)(4)- you have determined “specificity” of the assay by -----(b)(4)-----
----- is an important aspect of “specificity” determination. Please provide additional data showing the results of -----(b)(4)-----.