STATISTICAL REVIEW AND EVALUATION

BLA

BLA/Supplement Number:	125419/0
Product Name:	Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted
Indication(s):	For active immunization for the prevention of disease in persons 18 years of age and older at increased risk of exposure to the influenza A virus H5N1 subtype contained in the vaccine.
Applicant:	ID Biomedical Corporation of Quebec (dba GlaxoSmithKline Biologicals)
Date Submitted:	February 22, 2012
Review Priority:	Standard
Statistical Branch:	OBE/OB/VEB
Primary Statistical Reviewer:	**Tielin Qin** Mathematical statistician
Concurring Reviewer (1):	**Tsai-Lien Lin,** Team Leader Viral and Bioassay Team
Concurring Reviewer (2):	**A. Dale Horne,** Branch Chief Vaccine Evaluation Branch
Medical Office/Division:	OVRR/DVRPA
Review Committee Chair:	Carmen Collazo-Custodio
Project Manager:	Kirk Prutzman, Jeremy Wally

1.   EXECUTIVE SUMMARY

BLA 125419/0 is for licensure of the influenza (H5N1) virus monovalent vaccine which will be used for active immunization for the prevention of disease in person 18 years of age and older at increased risk of exposure to the influenza A virus H5N1.

Several findings were identified in the validation reports for the HAI assay. First, although CBER sent an information request on April 26, 2012 for the original full validation report, all the reports for the HAI assay submitted to this BLA are partial validation reports. These partial validations appear to be conducted when significant changes occurred. Some assay parameters, such as linearity and accuracy, were not evaluated in these partial validations. Second, for most validation parameters, the criteria used appear to be loose; whether the assay parameters evaluated and the criteria used provide adequate control for the HAI assay should be determined by the product reviewers. Regarding acceptability of this particular HAI assay for this specific submission, I defer to the product reviewers to evaluate further based on their relevant past experience.

2.   INTRODUCTION

BLA 125419/0 is an original application for licensure of the influenza (H5N1) virus monovalent vaccine, which will be used for active immunization for the prevention of disease in person 18 years of age and older at increased risk of exposure to the influenza A virus H5N1. This statistical assay review evaluates the validation reports for the HAI assay, the neutralization assay, and the SRID assay that were submitted to this BLA.

3.   STATISTICAL EVALUATION

3.1   Validation Report CODE No. QVALR-PF-015, Version No. 01/E: *Determination of Influenza antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) - Test with ---b(4)-----------*

The aim of this validation report is to validate the HAI assay using the –b(4)----- method for the quantification of influenza antibodies in human sera. The validation parameters evaluated include precision (repeatability and intermediate precision), validation of operators, robustness, effective concentration of neuraminidase, and comparison of the --b(4)------- and manual method.

1. Validation of serum panel and Intermediate Precision

At least b(4) sera as control sera were determined on at least at ---b(4)------------ days in duplicate with the three actual influenza virus strains by different lab technicians. The acceptance criteria are that the geometric means of the daily duplicates shall not vary by more than +/- b(4) titer steps compared with the geometric mean of all determinations, and the difference between the daily results for one control serum shall not be more than b(4) titer steps.

2. Validation of operators

   Each of b(4) operators tested b(4) validated control sera in duplicate with the three actual influenza virus strains. The acceptance criterion is that not more than b(4) of the geometric means of all duplications shall vary by more than b(4) titer steps compared with the validated control sera titers.

3. Precision-Repeatability

   Three validated control sera were tested b(4) times at the same day with the actual strains in duplicate. The acceptance criteria are the b(4) geometric means of one test ---b(4)-----------------------------shall not vary by more than b(4) titer steps from each other and they shall not vary by more than b(4) titer steps from the validated titer.

4. Effective concentration of test ----b(4)------------------------

   -b(4)--- control sera were inactivated with a –b(4)---, respectively.The acceptance criterion is that not more than b(4) of the geometric means of all duplications shall vary by more than b(4) titer steps compared with the validated control sera titers. The –b(4)---------- solution which gave the best correspondence with the validated titers should be used.

5. Comparison of the new –b(4)----------- method with manual testing

   Sera of clinical trial Flu-051 (Fluarix 2002/203) were tested in parallel with –b(4)--- and with the manual test method. The results were evaluated statistically by significance test. There should be no significant difference between the final results received with the different test methods. The results of test for significant differences between the determined parameters are shown in Tables 1 and 2. Therefore, the applicant concluded that there are no significant differences between the two methods.

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b(4)

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b(4)

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6. Robustness tests

The influence of various method variations (such as: -----b(4)-- --- ---------------------- was investigated. The mean value of duplicates varying by more than b(4) titer steps from the declared value for each standard serum indicates an influence of the method variation.

Reviewer's Comments:

General comments:

1. *This validation report was completed on Feb. 09, 2002.*

2. *The validation report is a partial validation. The aim of this validation was to validate the HAI assay when the assay was changed from manual testing to the –b(4)-- method. Intermediate precision, validation of operators, repeatability, robustness, effective concentration of –b(4)------------, and comparison of the –b(4)-- and manual methods were evaluated. However, linearity, accuracy, specificity, range, LOD, and LOQ were not evaluated. Although the reviewer requested the full validation report, the applicant has not provided it.*

3. *The acceptance criterion the applicant used in this validation is that the geometric means of the duplicates should not vary by more than +/- b(4) titer steps compared with the geometric mean of all determinations. This type of criterion does not have adequate statistical rigor to assure that the HAI assay has adequate performance. An assay with large variability and/or poor accuracy may still meet the criterion due to the non-quantitative nature of the acceptance criterion. Since a reportable HAI titer is the geometric mean of the duplicates, the titer values are already considered as continuous data. Therefore, it is preferred to assess precision by estimating the %RSD or %CV which provides much more information on the assay performance. Whether this criterion provides adequate control for the HAI assay should be determined by the product reviewers. Please see the comments and recommendations below for specific parameters.*

Below are the comments for specific validation parameters:

4. *Precision: The applicant evaluated intermediate precision and repeatability separately. To accurately assess precision, a multifactor design of experiment is preferred, which takes all important sources of variability (e.g., analyst and day) into consideration simultaneously and permits the estimation of variance components due to each source of variability.*

5. *Robustness tests: The applicant evaluated the effect of each robustness factor on method variability separately. It is preferred that an experiment design, which can investigate the effects of selected method variations simultaneously, be employed.*

6. *Comparison of the new –b(4)--- method with the manual testing: The strategy that the applicant used in this study is not optimal. The conclusion that there are no significant differences in mean titers, serocoversion rates, and seroprotection rates between both methods for testing sera in clinical study Flu-051 (Fluarix 2002/203) does not guarantee that these two methods will generate similar results for each individual pair of sera. Comparison of the new –b(4)-- method with the manual testing should be evaluated by using proper statistical methods for assessing agreement between two methods (e.g,. Deming's regression, Concordance Correlation Coefficient, etc.) with appropriate, pre-specified acceptance criteria.*

3.2 Validation Report CODE No. QVALR-PF-015, Version No. 02/E: *Determination of Influenza antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) - Test with –b(4)--------------*
(This validation report was submitted on July 18, 2012 as the responses to our IR letter on April 26, 2012).

Reasons for the update:
- Some changes in the software program for -----(b)(4)---------.
- Revalidation of operators and control sera.
- Change of test ---b(4)------------- batch.

1. Validation of serum panel and Intermediate Precision

   At least b(4) sera as control sera were determined on at least –b(4)------ days in duplicate with the three actual influenza virus strains by different lab technicians. The acceptance criteria are that the geometric means of the daily duplicates shall not vary by more than +/- b(4) titer steps compared with the geometric mean of all determinations, and the difference between the daily results for one control serum shall not be more than b(4) titer steps.

2. Validation of operators

   Each of b(4) operators tested b(4)validated control sera in duplicate with the three actual influenza virus strains. The acceptance criterion is that not more than b(4) of the geometric

means of all duplications shall vary by more than b(4) titer steps compared with the validated control sera titers.

3. Precision-Repeatability

Three validated control sera were tested b(4) times on the same day with the actual strains in duplicate. The acceptance criteria are the b(4) geometric means of one test (--b(4)--------
---------------------) shall not vary by more than b(4) titer steps from each other, and they shall not vary by more than b(4) titer steps from the validated titer.

4. LOD, LOQ, and range

For the assay design and according to SOP PF-015, the applicant claimed that the ---b(4)--
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--b(4)----------------
--b(4)---
-------------.
---b(4)---

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5. Effective concentration of test –b(4)-----------

-b(4)---- control sera were inactivated with both a –b(4)--
---------- The acceptance criterion is that not more than b(4) of the geometric means of all duplications shall vary by more than b(4) titer steps compared with the validated control sera titers. The –b(4)--------------------- which gave the best correspondence with the validated titers should be used. The applicant concluded that –b(4)----------------------------
-- are suitable for use to test HAI. Therefore, the applicant decided to use a –b(4)--------------.

6. Comparison of the changed method with previous method

--b(4)-------------------------- serum pairs from the clinical trial FLU-052 were tested with the changed –b(4)--- method, and compared with the results determined with the previous –b(4)--- method. The results were evaluated statistically by a significance test. There is no significant difference between the final results obtained with the different test methods. Therefore, the applicant claimed that there are no significant differences between the previous –b(4)----------- method and the changed method (with adapted software).

$$\Big[\qquad\qquad\qquad\Big]$$

b(4)

Reviewer's Comments:

1. *This is a partial validation report. The validation was conducted when a new -b(4)-----------was used. Therefore, only precision, LOD, LOQ, and range were evaluated in this report.*

2. *The acceptance criterion: Please see the comments for Validation report QVALR-PF-015, Version No. 01/E.*

3. *Precision and comparison of the new –b(4)----- with the old –b(4)------: Please see the comments for Validation report QVALR-PF-015, Version No. 01/E.*

4. *LOD, LOQ, and range: Lower quantification limit is usually defined as the lowest level that demonstrates satisfactory precision and accuracy, not the first pre-dilution..*

3.3 Validation Report CODE No. QVALR-PF-015, Version No. 04/E: *Determination of Influenza antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) - Test with horse blood cells*

Reason for the update:
- Test for H5N1 antibodies in human sera with horse blood cells instead of –b(4)----- blood cells.

1. Validation of serum panel and intermediate Precision

At least b(4)sera were tested on b(4) different days in duplicate. The geometric means of the daily duplicates shall not vary by more than +/- b(4) titer steps compared with the geometric mean of all determinations.

2. Repeatability

Three validated control sera are tested b(4) times (including –b(4)------) on the same day against the H5N1 strain in duplicate. The b(4) geometric means of one test row (--b(4)--------------------) shall not vary by more than b(4) titer steps from each other, and they shall also not vary by more than b(4) titer steps from the validated titer.

3. Robustness of horse blood cells, influence of storage time of blood on the test

-b(4)----- validated control sera were tested in duplicate:
- ---b(4)--------------------------
- ---b(4)----------------------------------

The geometric means of each duplicate shall not vary by more than b(4) titer steps compared with the validated control serum titers. The applicant concluded that the storage of the horse blood cells over –b(4)------- has no influence on the test and the analysis of titers.

Reviewer's Comments:

1. *This is a partial validation report. The validation was conducted when the assay condition was changed from using –b(4)---- blood cells to using horse blood cells. Therefore, only precision and robustness were evaluated in this report.*

2. *The acceptance criterion: Please see the comments for Validation report QVALR-PF-015, Version No. 01/E.*

3.4   Validation Report CODE No. QVALR-PF-015, Version No. 05/E: *Determination of Influenza antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) – Revalidation for A/Indonesia/05/2005 x PR8-IBCDC-RG2*

Reason for the update:
- Test for H5N1 antibodies in human sera for strain A/Indonesia/05/2005

1. Repeatability

Three validated control sera are tested –b(4)--- (including-b(4)---------) on the same day in duplicate. The b(4) geometric means of one test row (--b(4)-----------------) should not vary by more than b(4) titer steps from each other, and they should not vary by more than b(4) titer steps from the validated titer as well. The acceptance criteria for repeatability were fulfilled.

2. Intermediate precision on control serum panel

--b(4)----- sera were tested on b(4) different days in duplicate. The b(4) geometric means of the daily duplicates should not vary by more than +/- b(4) titer steps compared with the geometric mean of all determinations.

3. Overtime reproducibility

All the results of the control sera were recorded, and were presented on a graph as a function of the date of testing. All the results were within the predefined titer range.

4. Robustness of horse blood cells, influence of storage time of blood on the test

--b(4)---- validated control sera were tested in duplicate:
- -----b(4)------------------.
- -----b(4)---------------------------.

The geometric means of each duplicate shall not vary by more than –b(4)- titer steps compared with the validated control serum titers. The applicant claimed that the storage of the horse blood cells over –b(4)------------ has no influence on the test and the analysis of titers.

5. Specificity

The assay specificity was checked with the HAI results obtained on the 398 pre-vaccination samples of GSK trial H5N1-007. All the pre-vaccination samples (100.00%) display negative HAI results. The applicant concluded that the assay specificity is higher than 99%, and can be considered as excellent for an immunoassay.

Reviewer's Comments:

1. *This is a partial validation report. The validation was conducted when the virus strain was changed. Therefore, only precision, overtime reproducibility, specificity, and robustness were evaluated in this report.*

2. *The acceptance criterion: Please see the comments for Validation report QVALR-PF-015, Version No. 01/E.*

3.5   Validation Report CODE No. PQ-Report #2006093: *Validation of serum neutralization assay*
(This validation report was submitted on July 18, 2012 as the responses to our IR letter on April 26, 2012).

1. Validation of serum panel and intermediate Precision

Control sera were validated with influenza virus strain A/Vietnam/1194/2004 –b(4)------. At least b(4)sera were determined on at leastb(4)different days in triplicate by different technicians. The titers of the daily triplicates should not vary by more than +/- b(4) titer steps compared to the geometric mean of all determinations. The difference between the daily results for each control serum should not be more than +/- b(4) titer steps.

2. Validation of operators

Each operator tested at least b(4)validated control sera in triplicate. The acceptance criterion is that not more thanb(4)of the geometric means of all triplications should vary by more thanb(4) titer steps compared with the validated control sera titers.

3. Precision-Repeatability

b(4) validated control sera were tested b(4) times on the same day in triplicate. The acceptance criterion is that the b(4) titers of the replicates of one serum should not vary by more than b(4) titer steps from the validated titer.

4. Robustness

The influence of various method variations (such as: ---b(4)---
--) was investigated. The mean value of triplicates varying by more than b(4) titer steps from the declared value for each standard serum indicates an influence of the method variation. The applicant concluded that no influence on the test results can be observed by the method variation factors.

5. Linearity

--b(4)--- according to the SOP, and the dilution factors were –b(4)--------------------. Each dilution was considered as a sample and tested accordingly. The neutralization titer of a serum should not vary by more than +/- b(4) titer steps compared to the validated titer. The applicant concluded that the deviation from linearity is within the accepted range of difference.

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

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

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

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

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8. –b(4)-----------------------------

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

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

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

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

---b(4)---

Reviewer's Comments:

General comments for the neutralization assay validation:
1. The acceptance criterion: Please see the comments for Validation report QVALR-PF-015, Version No. 01/E.

2. Accuracy, range, and specificity were not evaluated in the validation study.

Below are comments for the specific validation parameters:

3. *Precision: The applicant evaluated the intermediate precision and repeatability separately. It is preferred to use a multifactor design of experiment which takes all important sources of variability (e.g., analyst and day) into consideration simultaneously, and permits the estimation of variance components due to each source of variability.*

3.6   Validation of Analytical Procedures HA content by SRID

Specificity, accuracy, repeatability, intermediate precision, linearity, and range were evaluated.

Linearity

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

Intermediate Precision

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

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

Range

The range was established based on the linearity, accuracy, and precision determination for each type of sample. The applicant claimed that all of the acceptance criteria for linearity, accuracy, and precision were met for the working range of ----b(4)----------------
----------μgHA/mL.

Reviewer's Comments:

1. *To assess precision, it is recommended to use a multifactor design of experiment which takes all important sources of variability (e.g., analyst and day) into consideration simultaneously and permits the estimation of variance components due to each source of variability.*

2. *Intermediate precision was evaluated only at one concentration (b(4)µgHA/mL).*

3. *Linearity was evaluated at b(4) concentrations (--b(4)---------------------------------), and accuracy and repeatability were evaluated at concentrations of –b(4)----------µgHA/mL. However, intermediate precision was evaluated only at concentration b(4)µgHA/mL. Therefore, the intermediate precision results may not be adequate to support the assay working range of ---b(4)-------------µgHA/mL.*

3.7   PHARMACEUTICAL DEVELOPMENT DRUG PRODUCT-SHELF-LIFE EVALUATION

Decay Rate Assessment-Drug Final Container Thimerosal Content-Inverted Position

A statistical analysis was conducted to assess the comparability of the final container thimerosal content slopes for inverted vials, and the result has shown a p-value of 0.12. The applicant explained that, in the case of 3 lots, a 0.25 significance level is recommended by the ICH Q1E guidelines. As b(4) lots were available, the applicant argued that the usual b(4)significance level can be used. Therefore, the applicant concluded that the null hypothesis of equal slope cannot be rejected, and a common slope estimate was used for a linear regression on the stability data for the final container thimerosal content stored in the inverted position.

Reviewer's Comment:
I consider the applicant's justification acceptable.

Quality information amendment- Response to Request for information Received on April 30, 2012 (Question 2)

Drug Substance Batches ----b(4)----------------------------

  ---b(4)---
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1 page determined to be not releasable: b(4)

4. SUMMARY AND CONCLUSIONS

ID Biomedical Corporation of Quebec (dba GlaxoSmithKline Biologicals) submitted several validation reports for the HAI assay, the neutralization assay, and the SRID assay in this submission.

Several findings were identified in the validation reports for the HAI assay. First, although CBER sent an information request on April 26, 2012 for the original full validation report, all the reports for the HAI assay submitted to this BLA are partial validation reports. These partial validations appear to be conducted when significant changes occurred. Some assay parameters, such as linearity and accuracy, were not evaluated in these partial validations. Second, for most validation parameters, the criteria used appear to be loose; whether the assay parameters evaluated and the criteria used provide adequate control for the HAI assay should be determined by the product reviewers. Regarding acceptability of this particular HAI assay for this specific submission, I defer to the product reviewers to evaluate further based on their relevant past experience.

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    cc:
    Review Team

Estella Russek-Cohen
John Scott
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