STATISTICAL REVIEW AND EVALUATION

BLA Number:	125297.0, Amendments 16, 22
Product Name:	Influenza Virus Trivalent Subunit (A/A/B hemagglutinin and neuraminidase; embryonated hen's eggs) Vaccine, Inactivated (AGRIFLU®)
Applicant:	Novartis Vaccines and Diagnostics, Inc.
Date Submitted:	May 29, 2009, October 13, 2009
Statistical Reviewer:	Tsai-Lien Lin Mathematical Statistician, VEB/DB/OBE
Through:	A. Dale Horne Branch Chief, VEB/DB/OBE
To:	Anissa Cheung Ira Berkower Rajesh Gupta
CC:	Henry Hsu, Christopher Egelebo, chron

1. EXECUTIVE SUMMARY

In response to the questions regarding the validation of --b(4)----assay listed in the 4/27/2009 CR letter, the applicant recalculated the assay validation data, using the --b(4)------ model suggested by CBER. However, due to the nonlinearity displayed in many of the assay curves, the applicant proposed to drop the acceptance criteria for assay results which ensure the adequacy of the model fit, i.e., ------b(4)----------. The statistical reviewer considers a model fit criterion necessary to ensure the validity of the assay results.

Upon review of CBER comments delivered on 8/14/09 and -b(4)- documents provided by DPQ, the applicant has accepted CBER's recommendation and will have an additional -b(4)- validity criterion of -b(4)- included for their routine testing. Thus, all previous statistical concerns conveyed to the applicant have been acceptably addressed. There are no further statistical issues with the applicant's -b(4)- assay.

2. BACKGROUND

Amendment 16 contains Novartis' Complete Response to the deficiencies listed in the 4/27/2009 CR letter, including attachments to several of the responses. After reviewing the responses in Amendment 16, an Information Request was sent to the applicant on 8/14/2009 and DPQ's -b(4)- test method was shared with Novartis. Amendment 22 contains the Company's responses to these Information Requests. This statistical review will focus on the CMC comments regarding the validation of -b(4)- Assay.

3. STATISTICAL EVALUATION

3.1 AMENDMENT 16

CMC Comment 3(a) – Validation of -b(4)- Assay

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

Company Response to CMC Comment 3a:

Novartis recalculated the data of validation experiments using the -b(4)--- model (---b(4)-------------------------) and re-evaluated the validation parameters. The outcome of the validation analysis was reported in Technical Report 261657-01. However, not all of the original criteria set for validation and for acceptance of test results were met. Based on the FDA document "Guidance for the validation of bioanalytical method," Novartis proposed a modified set of validation acceptance criteria allowing larger RSD and deviation from theoretical value for the assessment of precision, accuracy, and LLOQ. The revised validation criteria were fully met by the recalculated data with the b(4) model.

Novartis also proposed to change their acceptance criteria for test results. There were 3 acceptance criteria for -b(4)-- in the original SOP: ---b(4)--- ----------------- -- The applicant proposed to drop the --b(4)-- criteria and only keep the -b(4)---------criterion. The rationale is that many assays have a curvilinear behavior when data are analyzed on a ------- b(4)----------------, while the original acceptance criteria are set for analysis on an

----------b(4)----scale, resulting in a wide failure of these criteria (about 41% of the standard curves and 62% of the sample curves fail at least one of the -b(4)---------- criteria).

Reviewer's Comments:

1. Whether the FDA guidance quoted by the Applicant is applicable to thisb(4) assay and whether the revised validation acceptance criteria are acceptable need to be reviewed by product reviewers.

2. It is surprising to see that such a large percentage of assays fail the original b(4) acceptance criteria. The reviewer verified that the applicant's ----b(4)---- potency calculation using the b(4) model was correct. The criterion causing the greatest problem is --b(4)----------. For a line with only b(4) data points, this criterion may be unnecessarily restrictive. There are also >10% of the curves failing theb(4) criterion. Examination of the plots of those dose-response curves which fail theb(4) criterion revealed that nonlinearity is the main problem. However, dropping b(4)criteria of ---b(4)---------- would leave no control over the adequacy of model fit. Furthermore, as the fit gets worse, it gets harder to detect any difference between --b(4)-------, since the applicant tests ---b(4)---------- (a significance test). Given the nonlinearity displayed in many of the applicant's assay curves, --b(4)------- is crucial for assay validity. Therefore, a criterion which provides assurance of the adequacy of model fit is necessary.

   As to the test for --b(4)--, in this --b(4)---- case, an equivalence test may be preferred since it tests the right alternative hypothesis --b(4)----------. In practice, however, because CBER does not have an acceptable equivalence range for -b(4)- comparison defined yet, setting the equivalence range after data are collected is not necessarily a better solution. For future assays, if an acceptable equivalence range for the ratio between -b(4)------ can be defined, an equivalence approach for testing --b(4)----- would be a preferred method.

3. The problem of high percentage of b(4) failures due to nonlinearity shown in the applicant's assay curves when analyzed on the -b(4)------ scale is not seen or much less with the data from other manufactures, according to CBER DPQ laboratory. This high failure rate suggests that Novartis' assays exhibit a much -b(4)- degree of curvature than other manufactures' -b(4)- assay data. It is recommended that reasons for this curvilinear behavior and ways to obtain a -b(4)--dose-response curve should be investigated for future assays.

4. Because of the nonlinearity issue when dose-response curves are analyzed on the -b(4)-- scale, the reviewer investigated whether a -b(4)---------- model for ----------b(4)--- data would be a better model for Novartis' data. It was found that though linearity improved with the ----------b(4)--- scale, the validity underlying the ----------b(4)------- model (i.e.,---------b(4)----------) is not satisfied for a large percentage of assays, an even more serious problem. It appears that though the --b(4)------ model has a linearity issue sometimes, considering the assay design (same dilutions, similar

potency and shape of dose-response curves between the standard and test samples), the estimation of-b(4)---- potency is still reliable enough, provided the --b(4)---- validity is satisfied. Thus, the --b(4)------ model may still be the model of choice.

CMC Statistical Comment 3b:

(b) We notice that in the technical study 257249, you treat your modified --b(4)-------- method as the gold standard and select the --b(4)------- model that is the best match to the --b(4)-- method. CBER does not consider your modified --b(4)------- method as the gold standard. The chosen --b(4)------ model should be a theoretically valid model (as explained in 3a above) that gives satisfactory performance with respect to linearity and ----b(4)---------- for the standard and test samples. Please provide an EXCEL or SAS file for the raw data ------b(4)---- in your technical study 257249.

Company Response to Comment 3b:

Novartis included SAS transport files for the raw assay data in the Technical Report 261657 provided as Attachment 3.2.S.4.3 [b(4)]-11.

Reviewer's Comments:

The SAS data files submitted are the data for validation experiments. It is not clear whether the assay dataset used to evaluate various assay analysis models, as described in the Technical Study 257249, is a subset of the validation dataset. Nevertheless, for the purpose of evaluating the assay analysis models and various acceptance criteria, the submitted data also serve the purpose.

3.2 AMENDMENT 22

CMC Comment 1 – b(4) Method –*b(4)* as validity criterion

Your proposal to dropb(4) as the validity criterion for b(4) method is not acceptable. We have not heard of any problem from other manufacturers about linearity and do not expect more than 10% assays fail theb(4) criterion. A validity criterion which provides assurance of the fit of the analysis model is considered necessary. If you want, DPQ can share ourb(4) test method and MS Excel calculation worksheet with you.

Company Response to CMC Comment 1:

The applicant will have an additional b(4) validity criterion of b(4) included for routine testing.

Reviewer's Comments: *The applicant's response is acceptable.*

4. CONCLUSIONS

In response to the questions regarding the validation of b(4) assay listed in the 4/27/2009 CR letter, the applicant recalculated the assay validation data, using the --b(4)-- model suggested by CBER. However, due to the ---b(4)---------- displayed in many of the assay curves, the applicant proposed to drop the acceptance criteria for assay results which ensure the adequacy of the model fit, i.e., ---b(4)----------. The statistical reviewer considers a model fit criterion necessary to ensure the validity of the assay results.

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