Statistical Review and Evaluation - Agriflu, July 10, 2008

BLA Number:	125297
Product Name:	Influenza Virus Trivalent Subunit (A/A/B hemagglutinin and neuraminidase; embryonated hen's eggs) Vaccine, Inactivated (Agrippal®)
Applicant:	Novartis Vaccines and Diagnostics, Inc.
Date Submitted:	July 10, 2008
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1. Executive Summary

Novartis uses ---b(4)---------- assay to determine the HA content in the influenza vaccine. The potency determinations for all non-clinical, clinical, and stability batches have been calculated using the -b(4)--------- assay method in Siena. However, for commercial US distribution, --b(4)---------- will be released according to b(4) determinations calculated from the -b(4)---------- assay method and using CBER reagents.

Based on the documents submitted in the original BLA and Amendment 2, Novartis' b(4) assay calculations are not correct and the validation of the ---b(4) ---------- assay is also not acceptable.

The -b(4)- assay method performed for all non-clinical, clinical, and stability batches is not calculated correctly. Insufficient details regarding the --b(4)----- assay method are provided. However, it appears that Novartis also did not perform the --b(4)-- assay method correctly. The validation report of --b(4)----assay using CBER reagents and (incorrectly calculated) --b(4)------- method showed other problems. The precision should be calculated over the reportable values, not the means of several runs of several ---b(4)--. The linearity acceptance criteria also did not include the assessment on ----b(4)---.

2. Background

----------b(4)---------- assay is used by Novartis to determine the Haemagglutinin content in the influenza vaccine. The potency determinations for all non-clinical, clinical, and stability batches have been calculated using the -b(4)--assay method in Siena. However, in order to release batches for commercial US distribution, --b(4)---------- will be released according to --b(4)-determinations calculated from the ---b(4)---------- assay method and using CBER reagents.

Sections 3.2.S.4.2 and 3.2.S.4.3 of the original submission contain information on the b(4) assay, including the description of -b(4)- method and --b(4)---------- assay method, the validation protocols, the qualification report, validation reports of -b(4)------- method completed previously, and some raw data of the validation experiments. On November 7, 2008, Novartis submitted in Amendment 2 a new b(4) method validation report, using ----------b(4)---------and reagents. In the original submission, this assay is performed at two sites, Siena (Italy) and --b(4)--- The --b(4)---------- assay methods are calculated differently at two sites. In the Amendment 2, only the assay performed in Siena and its validation report (b(4) 07.007 VR 5 Rev.1) are presented. This statistical review is based on the information available in the original submission and the Amendment 2, but will focus on the assay performed in Siena.

3. Statistical Evaluation

3.1 Assay Calculation

- -----b(4)--------- ---------- -----b(4)---------- ----b(4)---------- where, ----b(4)---------- ----b(4)---------- ----b(4)---------- ----b(4)---------- ----b(4)----------
- ----b(4)---------- ---------- ----b(4)---------- b(4) ---b(4)---------- ------b(4)----------

Reviewer's Comments:

1. Novartis' "modified" --b(4)----- assay calculation is based on the ------b(4)--- lines for the standard and test sample estimated separately with separate intercepts. However, for a -b(4)----- assay model to be statistically valid the two intercepts must

not be significantly different and a common intercept should be assumed in the calculation (see ----b(4)----- ----------). The potency calculation assuming unequal intercepts will be close to the correct value if the two intercepts are very close to each other. Although the assay analysis acceptance criteria require "----------b(4)----------," such a criterion is not adequate to ensure the validity of the -b(4)- method given the data range. Thisb(4)criterion may lack sufficient statistical justification and the -b(4)----- method may not be appropriate in some cases.

2. The --b(4)------ assay method (3.2.S.4.2 Analytical Procedures [b(4)-] pages 4-5, Amendment 2) is not clearly described. From Novartis' formula for calculating the haemagglutinin concentration in the sample---b(4)--- ----------. Furthermore, (b(4)) in the formula is not a mathematically correct estimate of b(4) potency. The -b(4)---------- assay should be performed on the ----b(4)----------. CBER's recommended --b(4)-------- assay method for influenza vaccine is to be performed on the ----------b(4)------- scale. In addition, --b(4)--------- should be verified.
3. It is also not clear how the -------b(4)-----analysis is performed in Novartis' --b(4)-- assay with respect to the estimation of the common-b(4)-. If the common -b(4)- is calculated by ---b(4)---------- (as done in -b(4)--), it is only an approximation. The correct method should be --b(4)----------.

3.2 Assay Validation for b(4) USA method

The validation parameters evaluated include precision (repeatability and intermediate precision), accuracy, linearity, specificity, quantification limit, range, and robustness.

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

Reviewer's Comments:

1. The evaluation of precision is performed on the means of b(4) runs of --b(4)----- not the reportable values. The %CV calculated over theb(4) means will be smaller than the %CV for the reportable values.
2. The acceptance criteria for linearity are not adequate for assessing -b(4)----- linearity. Note that --b(4)---- linearity also requires the ---b(4)---------- between

the observed versus the expected values to be within an acceptable range around b(4).

4. Conclusions

Overall, based on the documents submitted in the original BLA and Amendment 2, Novartis'-- b(4)- assay calculations are not correct and the validation of the --b(4)---------- assay is also not acceptable.

The --b(4)----- assay method performed for all non-clinical, clinical, and stability batches is not calculated correctly. Insufficient details regarding the --b(4)-------- assay method are provided. However, it appears that Novartis also did not perform the --b(4)------- assay method correctly. The validation report of -b(4)- assay using CBER reagents and (incorrectly calculated) --b(4)---------- method showed other problems. The precision should be calculated over the reportable values, not the means of several runs of several ----b(4)----. The linearity acceptance criteria also did not include the assessment on ----b(4)---.