Statistical Review and Evaluation Assay Only - Flucelvax

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville, MD 20852-1448
Statistical Review and Evaluation
BLA 125408/0 Assay Only

BLA/Supplement Number:	STN 125408/0
Product Name:	OPTAFLU®, Influenza vaccine (MDCK Cells)
Indication(s):	Prophylaxis against influenza disease caused by influenza virus subtypes A and B present in the vaccine
Applicant:	Novartis Behring
Date(s):	Submitted: October 31, 2011 Received: November 1, 2011 Action Due: August 31, 2012
Statistical Branch:	OBE/DB/Vaccine Evaluation Branch (VEB)
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Project Manager:	Timothy Fritz, Ph.D.; Brenda Baldwin, Ph.D.

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1. EXECUTIVE SUMMARY

The applicant (Novartis) submitted a biologics license application (BLA) (STN 125408/0 dated October 31, 2011) for their Madin Darby Canine Kidney (MDCK) cell-derived influenza vaccine (OPTAFLU®, also referred to as *cTIV*). This review focuses on the validations for the hemagglutination inhibition (HI) assays used in the clinical studies to support this application. There were two validation reports submitted under Module 5.3.1.4 "Reports of Bioanalytical and Analytical Methods for Human Subjects" on the HI test using egg-derived as well as cell culture-derived viral antigens.

Both validation reports evaluated the linearity, intra-assay and inter-assay precisions, and the robustness of the assay against varying working and stocking solutions. The specificity and accuracy were not evaluated. All the pre-specified acceptance criteria were met in the validations. However, these criteria might be limited to ensure the reliability of the targeted assessments. Specifically,

- The acceptance criteria for the assessment of linearity, which were based on the correlation coefficients (equivalent to R-square) between the dilutions and the corresponding titer results in the log scale, might not be adequate. This is because the R-square of the regression does not address the dilutional linearity, where the slope parameter of the regression is examined. Upon review of the fitted slopes in the same analysis, it appears that all 90% confidence intervals (CIs) of the slope estimates fell between ---(b)(4)--- (**Pages Determined to be Not Releasable: (b)(4).**), with the estimated slope ranging from ----(b)(4)---indicating a possible dilutional linearity bias (although this bias might be still acceptable for this type of assay). The bias appeared to be larger in HI assay using cell-derived antigens than in HI assay using egg-derived antigens.

- The acceptance criteria for the assessment of precisions (intra-assay and inter-assay), which were based on the deviations from the mean titers of ---------------(b)(4)----------- scale), although commonly used in the validation of flu HI assay, might be loose. However, the data showed no evidence of inadequate precision. The coefficients of variation (CV) in the experiments ranged from ---(b)(4)----% (see **Pages Determined to be Not Releasable: (b)(4).** through **Pages Determined to be Not Releasable: (b)(4).** in this review).

Finally, for the assessment of precision, it might be preferable to employ an experimental design that takes into account multiple repeats across multiple technicians over multiple days and/or runs to more efficiently and accurately characterize variance components (both intra-assay and inter-assay) from various factors.

Recommendations: The reviewer defers to the review committee on the acceptance of the HI assay and the immunogenicity results collected in the supporting clinical studies in this application.

The reviewer also suggests that the comparability of HI assay using cell-derived antigens with HI assay using egg-derived antigens be further investigated. ----------------(b)(4)---------------------- ---(b)(4)---

It is also recommended that the applicant be advised to use a multi-factor experimental design, where factors such as day and technician are considered in the same experiment, for future HI assay validations to more efficiently and accurately evaluate the intra- and inter-assay precision using a variance components approach.

2. BACKGROUND

Under Module 5.3.1.4 "Reports of Bioanalytical and Analytical Methods for Human Subjects," the applicant submitted two validation reports on the hemagglutination inhibition (HI) assay for the seasonal influenza strains of types H3N2 (A/Wisconsin), H1N1 (A/Solomon), and B (B/Malaysia) using virus material passaged on eggs (Doc. No 251875) as well as on Madin Darby Canine Kidney (MDCK) cells (Doc. No. 253630). This assay (using egg-derived antigens) was used to measure immune responses in six Phase II and Phase III studies to support vaccine immunogenicity in this application. Both HI assays using the egg-derived antigens and HI assays using the cell-derived antigens had been originally used to evaluate the immunogenicity of several clinical studies. However, mainly due to resource constraints, only the US study V58P5 has been re-analyzed after the "pipetting issue" using HI egg-derived antigens and HI cell-derived antigens, while all other supporting studies have been retested only with HI egg-derived antigens, since the HI egg-derived assay was historically used for licensure of influenza vaccines.

The cell-derived HI assay provided slightly higher HI titers than the egg-derived HI assay for cTIV vaccines. The applicant stated that the cell-derived antigens would be the most appropriate for testing immune responses to cTIV, as it was produced in the same way (i.e., seed strain passaged in eggs followed by virus propagation in MDCK cells) as cTIV and therefore a more sensitive indicator for HI antibody in cTIV vaccines. ------------------(b)(4)--

3. ASSAY VALIDATION

3.1 Descriptions

Table 1 below describes the time, materials and instruments used in the validations for HI assays using both egg-derived antigens and cell-derived antigens. The sample pool was from over (b)(4) sera from the subjects who received one dose of a trivalent, non-adjuvanted influenza vaccine used for the northern hemisphere during the 2007/2008 flu season.

Table 1 : Materials and Instruments Used

	Egg-derived	Cell-derived
Validation Time	May and June 2008	June 2008
Vaccine Strain Subtypes	A/Wisconsin, A/Solomon Island, and B/Malaysia; North hemisphere 2007/2008	A/Wisconsin, A/Solomon Island, and B/Malaysia; North hemisphere 2007/2008

	Egg-derived	**Cell-derived**
Sample Pools	Sample 1: High titer (>1:40) Sample 2: Low titer (≤1:40) Sample 3: Negative	Sample 1: High titer (>1:40) Sample 2: Low titer (≤1:40) Sample 3: Negative
Positive Controls	Sheep controls from NIBSC	Sheep controls from NIBSC
Negative Controls	Serum from a non-vaccinated individual who was repeatedly tested to give no HI titer against the respective test strain	Serum from a non-vaccinated individual who was repeatedly tested to give no HI titer against the respective test strain

Deviations from the Analytical Test Method Validation Protocol

Specificity was not validated for HI assay using egg-derived antigens because it was validated in 2004 (Doc. No. 226105) per the applicant.

3.2 Specificity

---(b)(4)--

3.3 Linearity

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3.6 Robustness

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[(b)(4)]
3.7 Working Range

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4. SUMMARY AND CONCLUSIONS

This statistical review was performed to evaluate the validation for the HI assays using the egg-derived antigens as well as the cell-derived antigens.

Both validation reports evaluated the linearity, intra-assay and inter-assay precisions, and the robustness of the assay against varying working and stocking solutions. The specificity and accuracy were not evaluated. All the pre-specified acceptance criteria were met in the validations. However, these criteria might be limited to ensure the reliability of the targeted assessments. Specifically,

- The acceptance criteria for the assessment of linearity, which were based on the correlation coefficients (equivalent to R-square) between the dilutions and the corresponding titer results in the log scale, might not be adequate. This is because the R-square does not address the dilutional linearity, where the slope parameter of the regression is examined. Upon review of the fitted slopes in the same analysis, it appears that all 90% confidence intervals (CIs) of the slope estimates fell between ---(b)(4)--- (**Pages Determined to be Not Releasable: (b)(4).**), indicating a possible dilutional linearity bias (although this bias might still be acceptable for this type of assay). The bias appeared to be larger in HI assay using cell-derived antigens than in HI assay using egg-derived antigens.

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