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Priority Review	No
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Review Completion Date / Stamped Date	
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Applicant	Novartis Vaccines and Diagnostics, Inc.
Established Name	Influenza Vaccine (IV)
(Proposed) Trade Name	FLUAD 65
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc	Contains haemagglutinin (HA) and neuraminidase (NA) antigens from the influenza virus subtypes A (H1N1 and H3N2) and B, and MF59C.1 adjuvant
Dosage Form(s) and Route(s) of Administration	Suspension for intramuscular injection supplied in 0.5 mL single-dose pre-filled syringes
Dosing Regimen	A single 0.5mL dose
Indication(s) and Intended Population(s)	Active immunization of persons 65 years of age and older against influenza disease

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1. EXECUTIVE SUMMARY

BLA 125510/0 was submitted by Novartis Vaccines and Diagnostics, Inc. (NVD) to seek licensure of the inactivated influenza vaccine FLUAD, indicated for active immunization of persons 65 years of age and older against influenza disease caused by influenza virus subtypes A (both H1N1 and H3N2) and B contained in the vaccine. This review focuses on the Hemagglutination Inhibition (HAI) assay and Single Radial Immunodiffusion (SRID) assay. The HAI assay, performed by (b) (4) for the Phase-3 pivotal trial V70_27, used the initial starting dilution (b) (4) instead of the conventional starting dilution (b) (4). The applicant conducted a study to compare the titers generated from the current HAI assay (b) (4) and from a newly developed HAI assay with starting dilution (b) (4). The neutralization condition of the new HAI assay (b) (4). (b) (4). The Deming regression analysis of the titers from the two assays showed that the 95% confidence interval of the slope was within (b) (4) for each of the antigens. Nevertheless, the review team considered that the serum dilution definition of the HAI assay (b) (4) was not consistent with how serum dilution is traditionally defined by CBER in this assay, and the comparability study may not be applicable in this situation. Thus, CBER requested the applicant to recalculate titers based on the (b) (4) initial serum dilution for the V70_27 pivotal trial. The applicant agreed to recalculate titers according to CBER's recommendation. Secondly, the applicant conducted a study to verify the applicability of the SRID assay, initially validated for Agriflu®, to the determination of HA content in samples of (b) (4). Filled Product of FLUAD. The re-verification results appear to be acceptable. Additionally, the SRID method was transferred from (b) (4). A study was conducted to assess equivalency of the method between the two sites based on dual site testing, and also evaluate the method for accuracy, precision, and linearity in (b) (4). The study results appear to be acceptable.

2. REGULATORY BACKGROUND

Novartis Vaccines and Diagnostics, Inc. (NVD) submitted an original BLA 125510/0 to seek licensure of the inactivated influenza vaccine FLUAD 65 indicated for active immunization of persons 65 years of age and older against influenza disease caused by influenza virus subtypes A (both H1N1 and H3N2) and B contained in the vaccine.

During the clinical development of the product, multiple Hemagglutination Inhibition (HAI) assays were performed by different laboratories for different clinical studies. (b) (4) performed its HAI assay for the immunogenicity analysis of the Phase 3 pivotal study V70_27 which evaluated safety, tolerability, immunogenicity, and lot-to-lot consistency of the product. The (b) (4), used its HAI assay to conduct the immunogenicity analyses for some Phase 2 studies and non-US Phase 3 studies.

Single Radial Immunodiffusion (SRID) assay was used to determine specific hemagglutinin (HA) content (b) (4) of different antigens. The method was validated for the determination of HA of Agriflu® final product. In 2011, the applicant conducted a study in (b) (4) to verify the applicability of the SRID assay to the determination of HA content in samples of (b) (4) Filled Product of FLUAD. The method

was transferred to the (b) (4), for manufacturing release and stability testing for multiple vaccine products including FLUAD. A transfer study was conducted to evaluate equivalence in the method between (b) (4).

3. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

3.1 Review Strategy

This review focuses on the Hemagglutination Inhibition (HAI) that was developed and performed by (b) (4), and used for the Phase 3 pivotal study V70_27. The (b) (4) HAI assay, used for the supportive studies, was validated in 1997 and had been reviewed in previous submissions. The review also focuses on the re-verification of the Single Radial Immunodiffusion (SRID) assay and the study on the transfer of the assay from (b) (4).

3.2 BLA/IND Documents that Serve as the Basis for the Statistical Review

- (1) Hemagglutination Inhibition (HAI) assay
 - Standard Operating Procedure: Hemagglutination Inhibition (HAI) Test for Titrating Influenza Virus A and B Specific Antibodies – Turkey RBCs. (b) (4). Document #: TSOP.119.057. Submitted to BLA 125510/0.2.
 - Information Amendment on CBER's Information Request originally received on 06 January 2015. Novartis Vaccines and Diagnostics, Inc. Submitted to BLA 125510/0.3.
 - Validation report for HI assay with initial starting dilution of (b) (4) "Validation of the Hemagglutination Inhibition (HAI) Test for Titrating Influenza A and B specific Antibodies (A/Brisbane/59/2007[H1N1], A/Brisbane/10/2007[H3N2], and B/Florida/4/2006). (b) (4), 2008. Submitted to BB-IND (b) (4).
 - Comparison of assay conditions of the hemagglutination inhibition (HAI) test for titrating influenza virus A and B. 1/8/2013. Document number: REPT.119.00092-FDX.
- (2) Single Radial Immunodiffusion (SRD) assay
 - Validation Report of the SRID method, applied to Flud product, phases of (b) (4) Filled Product and Packaged Product with MF59 and (b) (4), for the US market. Report Number: ISU 07.007 VR 15 Rev.2.
 - Interim report for the analytical method transfer of SRID testing for (b) (4) filled samples, from the (b) (4) site, Novartis Vaccines and Diagnostics. Document number: R/0400/09/13.

4. DISCUSSION OF INDIVIDUAL STUDIES

4.1 Hemagglutination Inhibition (HAI) Assay

(1) HAI assay procedure (TSOP.119.057) used for the Phase-3 pivotal study V70_27

The hemagglutination inhibition (HAI) assay (TSOP.119.057), developed and performed by (b) (4), was used for the immunogenicity analyses in the Phase 3 pivotal study V70_27. It was noted that the HAI assay used the initial starting dilution (b) (4) instead of the conventional starting dilution (b) (4) recommended by CBER. The deviation

may have an impact on the immunogenicity data from the Phase 3 pivotal trial V70_27, by doubling the titer values. On February 18, 2015, CBER sent the following IR to the applicant.

Regarding your response on February 12, 2015 (amendment 3) to our question about the HAI assay test and titer calculation by (b) (4), we have noted that the volume of virus (b) (4). Please correct accordingly the HAI titer of all subjects impacted by this error and provide the revised titers to the BLA no later than March 23, 2015.

In the applicant's response dated March 16, 2015, the applicant acknowledged that the immunogenicity evaluation of study V70_27, performed by (b) (4) in 2011, used a HAI method that considered the volume of virus added to the serum when calculating the final serum dilution and thus the titer definition. However, the applicant maintained that the reported titers were correct based on the following rationales.

- Starting in 2012, (b) (4) revised their HAI method such that HAI titers are determined by the (b) (4). This was to align with the industry standard described in WHO/BS/2012.2190. In addition, the new method was developed to maintain an assay that results in similar titers to TSOP.119.057. This led to a modification of the (b) (4) for the new method.
- As part of the procedural change, a comparison study was conducted to compare the titers generated by the new and existing methods, using a panel of pre- and post-vaccination serum samples. The study showed that the titers for all three strains resulting from the two methods were comparable, using regression analysis with acceptance criteria based on confidence intervals for the slope being within (b) (4). Also, the intercept and 95% CI were (b) (4) for H1N1, (b) (4) for H3N2, and (b) (4) for B, respectively.

In the comparison study, (b) (4) compared the titers generated with TSOP.119.057 (HAI with starting dilution (b) (4) and TSOP.119.00510 (HAI with starting dilution (b) (4) and modified neutralization conditions) by testing a panel of (b) (4) samples from individuals who received the 2012-2013 Northern Hemisphere Influenza Virus Vaccine. The Deming regression analysis of the titers from the two assays showed that the 95% confidence interval of the slope was within (b) (4) for each of the three antigens (A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2), and B/Hubei-Wujiagang/158/2009). The study also reported that the mean difference in log2 transformed titer between the two methods was between -1 and 1. Although the study suggested that the two methods generated titers numerically close, it did not answer the basic question whether the modifications of the neutralization conditions were justifiable considering that the modifications were intended to make up for the 2-fold difference caused by the difference in dilution definition. After internal discussions, CBER sent the following request on April 1, 2015 to ask the applicant to recalculate titers based on the (b) (4) initial serum dilution for the V70_27 pivotal trial.

We do not agree that the reported HAI titers for pivotal trial V70_27 as determined using SOP.119.057 are correct, because the serum dilution definition under SOP.119.057 is not consistent with how serum dilution is traditionally defined by CBER in this assay. The comparability study (Document # REPT.119.00092-FDX) you provided is not applicable in this situation.

Because you are seeking approval under the accelerated approval pathway and possible licensure will be based solely on the immunogenicity data (and no efficacy data), the HAI results from the V70_27 pivotal trial will need to be re-calculated. We request that you re-calculate your titers based on the (b) (4) initial serum dilution and include revised datasets as well as an updated version of the clinical study report as previously requested.

On May 5, 2015, Novartis agreed to recalculate titers based on the (b) (4) initial serum dilution for the V70_27 pivotal trial.

(2) Validation of HAI assay procedure (TSOP.119.057)

The HAI assay procedure TSOP.119.057, with initial serum dilution of (b) (4), was validated by (b) (4) in 2008. The HAI assay was validated against A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Florida/4/2006. Table 1 summarizes the validation experiment design and acceptance criteria. The applicant concluded that the HAI assay met the acceptance criteria.

Table 1 Summary of the experiment design and acceptance criteria for the HAI assay procedure TSOP 119.057

(b) (4)

(b) (4)

Reviewer Comment:

Precision: The applicant used coefficient of variation of log2 GMT (standard deviation of log2 titers divided by mean of log2 titers) to measure precision in the validation. This is not an appropriate way of calculating %CV when data are analyzed on the log scale. Geometric coefficient of variation is the correct measure for precision of titer values. The Percent Geometric CV, calculated by the reviewer, ranges from (b) (4) in the current HAI^{(b) (4)} assay precision study, which was lower than (b) (4) GCV adopted by the applicant as the acceptance criterion in the their validation of HAI^{(b) (4)} assay at 2012. The assay precision appears to be satisfactory, although the calculation of %CV is not correct.

Linearity: The reviewer conducted additional analysis to assess linearity based on 95% confidence interval of the slope. The results showed that the 95% confidence interval of the slope was well within (b) (4) for each of the strains.

4.2 Single Radial Immunodiffusion (SRID) Assay

(1) Validation of SRID testing procedure

The SRID assay was validated for the determination of HA content in the samples of

(b) (4)

final product of Agriflu®. The applicant conducted the study to verify the applicability of the SRID assay to the determination of the HA content on the samples of

(b) (4)

Filled Product of FLUAD. The validation was performed by using

(b) (4)

Table 2 summarizes the validation parameters, testing methods, acceptance criteria, and results. The applicant concluded that the pre-defined acceptance criteria were met for the validation parameters.

(b) (4)

(b) (4)

Source: Reviewer's summary of Validation Report of the SRID method, applied to FLUAD product, phases of (b) (4), Filled Product, and Packaged Product with MF59 and (b) (4), for the US market. Report Number: ISU 07.007 VR 15 Rev.2.

Reviewer Comment:

Linearity: Additional regression analysis for the measured and theoretical concentrations by the reviewer showed that the slope and 95% confidence interval for measured and theoretical concentrations were (b) (4) for strain B/Brisbane, (b) (4) for strain H3N2 (X-187), and (b) (4) for strain H1N1 (X-181). The 95% confidence intervals were within the range (b) (4), used in the acceptance criteria.

(2) Transfer of SRID from (b) (4)

The SRID method was originally validated in (b) (4). The method was transferred to the (b) (4) site. A study was conducted to assess whether the method when performed in (b) (4), was equivalent to when performed in (b) (4) using reagents, equipment and analysts from the relative sites. The study assessed the method for accuracy, precision, and linearity in (b) (4). The local verification results showed that accuracy, linearity, and intermediate precision met the pre-defined acceptance criteria. The study also performed dual site testing to determine equivalence by the Two One Sided t-Test (TOST). The initial testing results showed that, for strains IVR-165,

BX-39, and B/Brisbane, the one-sided lower and upper 95% confidence limits were within the acceptance limits of (b) (4); however, for strain X-181, the one-sided lower and upper 95% confidence limits were (b) (4) which were not fully contained within the acceptance limits. (b) (4)

(b) (4) results showed that the one-sided lower and upper 95% confidence limits were within the acceptance limits. Table 3 summarizes the study parameters, test methods, acceptance criteria, and the results of the transfer study.

(b) (4)

(b) (4)

- **Source:** the reviewer's summary of the transfer study report "Interim report for the analytical method transfer of SRID testing for (b) (4) and filled samples, from the (b) (4) site to the (b) (4) site, Novartis Vaccines and Diagnostics. Document number: R/0400/09/13."

Reviewer Comment: *Additional analysis by the reviewer showed that the relative bias [(measured potency/target potency – 1) × 100%] at individual concentration level ranged from (b) (4). Overall, linearity of the assay appears to be acceptable.*

The second testing for X-181 was performed using sample neat to avoid re-introduction of additional inaccuracy in the process of dilution. The TOST analysis was performed on the original data instead of converted percentage recovery data. The one-sided lower and upper 95% confidence limits for the difference between two sites were (b) (4) HA/mL to (b) (4) HA/mL. The applicant converted the limits to (b) (4) using the overall mean of the data from both sites, which were within the (b) (4) acceptance criterion. On the original scale, the one-sided lower and upper 95% confidence limits (b) (4) HA/mL to (b) (4) HA/mL were within the (b) (4) margin of the mean of the data from the (b) (4) site (b) (4) HA/mL, i.e. (b) (4).

5. CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

The HAI assay, developed and performed by (b) (4), was used for the immunogenicity analyses in the Phase 3 pivotal study V70_27. The HAI assay used the initial starting dilution (b) (4) instead of the conventional starting dilution (b) (4) recommended by CBER. The applicant conducted a study to compare the titers generated from the current HAI assay (b) (4) and from a newly developed HAI assay with starting dilution (b) (4). The neutralization condition of the new HAI assay (b) (4). The Deming regression analysis of the titers from the two assays showed that the 95% confidence interval of the slope was within (b) (4) for each of the antigens. Nevertheless, the review team considered that the serum dilution definition of the HAI assay (b) (4) is not consistent with how serum dilution is traditionally defined by CBER for this assay, and therefore the comparability study may not be applicable in this situation. Thus, CBER requested the applicant to recalculate titers based on the (b) (4) initial serum dilution for the V70_27 pivotal trial.

5.2 Conclusions and Recommendations

The applicant agreed to recalculate titers according to CBER's recommendation. Then the applicant conducted a study to verify the applicability of the SRID assay, initially validated for Agriflu®, to the determination of the HA content on the samples of (b) (4) Filled Product of FLUAD. The re-verification results appear to be acceptable. Additionally, the SRID method was transferred from (b) (4) . A study was conducted to assess equivalency of the method between the two sites based on dual site testing, and also evaluate the test for accuracy, precision, and linearity in (b) (4) The study results appear to be acceptable.