



MEMORANDUM

Date: October 10, 2012

To: Timothy Nelle, HFM-481
Chair, BLA Review Team

From: Rajesh K. Gupta, HFM-680
Deputy Director, Division of Biological Standards and Quality Control

Through: William McCormick, HFM-680
Director, Division of Biological Standards and Quality Control (DBSQC)

Subject: STN 125408 – Human Influenza Virus Types A (H1N1; H3N2) and B Hemagglutinin Vaccine, Purified, Inactivated (Madin Darby Canine Kidney, MDCK, Cells), Flucelvax, Review of Drug Substance and Drug Product Analytical Procedures

Cc: William McCormick, HFM-680
Brenda Baldwin, HFM-481
Timothy Fritz, HFM-481

On 31 October 2011, Novartis Vaccines and Diagnostics, Inc. (NVD) submitted a Biologics License Application (BLA) for Human Influenza Virus Types A (H1N1; H3N2) and B Hemagglutinin Vaccine, Purified, Inactivated (Madin Darby Canine Kidney, MDCK, Cells), Flucelvax. Division of Biological Standards and Quality Control (DBSQC) reviewed the analytical methods, associated validation protocols and reports and specifications on the Drug Substance (DS) and Drug Product (DP) in the original submission and amendments listed below. Additionally, DBSQC reviewed adequacy of tests performed to release DS and DP and tests for Mycoplasma and extraneous agents on the intermediates, production control cells and viral harvests (Reviewers from DBSQC: Rajesh K. Gupta, Lokesh Bhattacharyya, Alfred Del-Grosso, Manju Joshi, Muhammad Shahabuddin, James Kenney, Hyesuk Kong, Karen Campbell, Catherine Poole).

SUBMISSIONS REVIEWED

STN 125408/0, Sections 3.2.S.4.1, 3.2.S.4.2, 3.2.S.4.3, 3.2.S.4.5, 3.2.S.5, 3.2.P.5.1, 3.2.P.5.2, 3.2.P.5.3, 3.2.P.5.6, 3.2.P.6, Documents related to Methods in Section 3.2R

STN 125408/0.6 (amendment received 04/04/2012), Suitability of egg based single radial immunodiffusion (SRID) reagents

STN 125408/0.8 (amendment received 04/09/2012) Suitability of egg based single radial immunodiffusion (SRID) reagents, Additional method validation documents for SRID, lot release protocol template

STN 125408/0.12 (amendment received 06/20/2012) Response to Information Request on method validations

STN 125408/0.13 (amendment received 06/21/2012) Response to Discrepancy between results of SRID method performed at CBER and Novartis

STN 125408/0.21 (Sequence 0019, amendment received 08/06/2012) Clarifications on validation of Residual Infectious virus test

STN 125408/0.24 (amendment received 08/17/2012) Response to Information Request dated June 19, 2012 regarding waiving the Residual Infectious Virus test on -----(b)(4)- -----, Mycoplasma testing by indicator cell method on viral harvests and SRID testing on final container.

STN 125408/0.28 (amendment received 08/30/2012) Response to Integrity for samples shipped from Marburg to Holly Springs for the SRID

STN 125408/0.31 (amendment received 09/17/2012) Response to questions on Lot Release Protocol and updated version of LRP

STN 125408/0.34 (amendment received 09/21/2012) Response to questions on various test methods

STN 125408/0.38 (amendment received 10/10/2012) Method validation reports to support the residual infectious virus test

Methods Reviewed

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

- Single Radial Immunodiffusion (SRID)
- Residual Infectious Influenza Virus
- Total Protein --(b)(4)--
- --(b)(4)--:Total Protein --(b)(4)--
- Purity ----- (b)(4) -----
- Polysorbate 80
- CTAB (cetyltrimethyl- ammoniumbromide)
- Total DNA Content
- ----- (b)(4) -----
- Sterility Test

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

- Single Radial Immunodiffusion (SRID)
- Total Protein ----- (b)(4) -----
- Total DNA Content

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

Drug Product (Final Container)

- Sterility Test
- Endotoxin Content
- General Safety
- Extractable Volume
- Identity ----(b)(4)-----

Tests at early stages of Manufacture

- Test for Mycoplasma

RECOMMENDED ACTION

The data and documentation submitted to support the analytical methods used for testing of DS and DP of influenza virus vaccine, Flucelvax, were reviewed and a number of issues with regard to adequacy of methods used to release DP, method validations and use of appropriate reagents for the single radial immunodiffusion (SRID), potency test, were found. Agreement by NVD to include tests for endotoxin and residual infectious virus in -----(b)(4)----- and SRID potency test on the final container (DP), -----(b)(4)----- major deficiencies in methods for CTAB and Polysorbate 80 (discussed in a separate review memo by Dr. Lokesh Bhattacharyya) ensured acceptable safety, potency and purity of the product. With these agreements for additional tests on DP -----(b)(4)-----, I recommend approval of this application.

REVIEW SUMMARY

Inadequacies and deficiencies were found in testing methods proposed for the formulated trivalent bulk and final container, analytical method validations and suitability of SRID reagents used in the potency test. In the original submission, formulated trivalent bulk was not tested for -----(b)(4)----- and the final container was not tested for the potency test. There were major deficiencies in analytical method validations for residual infectious virus, residual CTAB and polysorbate 80 content.

Trivalent formulated bulk has not been claimed sterile. The sponsor cited the recent revised 21 CFR 610.12 sterility test requirement, which does not require the sterility test

for the bulk product. This caused a concern for developing a testing plan at CBER for lot release of the product after licensure as the sterility test is one of important tests performed at CBER. Secondly this was the first time that a formulated bulk has not been claimed sterile for a prophylactic vaccine product meant for parenteral use in a healthy population. After discussions with the CMC and facility reviewers and clarifications from the sponsor, it was found that the formulated bulk was tested for sterility as in-process test (b)(4), the bulk -----(b)(4)----- and the manufacturing step was validated for aseptic processing. Based on this information, it was decided to accept non-sterile trivalent formulated bulk and not to perform sterility test at CBER to release trivalent formulated bulk for lot release purposes. Sponsor did not propose a test for --(b)(4)--- at the trivalent formulated bulk stage. Since inactivated influenza vaccines are released at the trivalent formulated bulk to expedite availability of seasonal influenza vaccine and test for ---(b)(4)-- is an important test performed at CBER for lot release purposes, the sponsor was asked to include the test for ---(b)(4)-- at this stage to facilitate release of formulated trivalent bulk. The sponsor agreed to this request.

Sponsor did not propose a residual infectious virus test for the -----(b)(4)----- . After several discussions with the sponsor on its reluctance to perform this test at this stage due to lack of scientific merit of performing the test at ---(b)(4)----- stage versus the test performed at the -----(b)(4)-----, CBER recommended the sponsor to perform this test at the ----(b)(4)----- stage, which will be consistent with the other licensed inactivated influenza vaccines. CBER proposed that it will consider exemption from this test at a later stage. Finally, the Sponsor agreed to this proposal. There were major deficiencies in the validation of tests for residual infectious virus, residual CTAB and polysorbate 80. After a number of information requests and telephone discussions, the sponsor could not provide adequate data to demonstrate adequacy of residual infectious virus test in ---(b)(4)----. The sponsor was suggested to perform a verification study to demonstrate adequacy of residual virus test ----(b)(4)-----, which is a compendial method for egg based inactivated influenza vaccines. The sponsor provided these data and agreed to perform residual infectious virus test -----(b)(4)----- for release of the product in the US until the test --- (b)(4)---- is adequately validated. -----

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

Sponsor did not propose a SRID potency method on final container. This was a concern about adequate potency at the final container stage, particularly when the formulated trivalent bulk -----(b)(4)----- and due to the fact that influenza haemagglutinin (HA) is prone to polymerization and aggregation. CBER requested the sponsor to add this test on the final container to which sponsor agreed. The sponsor was performing the SRID method according to the -----(b)(4)----- which is slightly different than the method used by the CBER. Sponsor was advised to use the CBER based SRID method during the pre-BLA meeting to avoid any problems with the lot release, to which the sponsor had agreed. Initial comparative testing of monovalent bulks for SRID by the sponsor and the CBER generated discrepant results. The sponsor

had tested these monovalents by the (b)(4) method. During investigation, the sponsor re-tested the samples by the CBER method using CBER SRID reagents and obtained results which were comparable to those from CBER. Another complexity with this product was suitability of egg based SRID reagents for the product made in MDCK cells. Early in the review process, the sponsor was advised to demonstrate suitability of egg based reagents for their product and CBER recommended use of homologous cell based reagents, if these were available. After several discussions and testing at CBER, it was agreed to use homologous cell based SRID reagents for the H1N1 and B strains that are available from the National Institute of Biological Standards and Control (NIBSC), UK. CBER had participated in calibration of these reagents and had authorized the use of these reagents from NIBSC. For the H3N2 strain the sponsor demonstrated satisfactorily the suitability of egg based reagents.

The sponsor is not performing the test for non-cultivable Mycoplasma on the viral harvests by indicator cell method and provided an explanation on the problems, and challenges for this test. CBER recognizes these challenges and responded to the sponsor to implement a ---(b)(4)--- Mycoplasma test for viral harvests for the 2013-2014 influenza season. CBER offered to assist the NVD in the development of this test. NVD responded by acknowledging CBER's position and accepted to take the opportunity to further discuss this request with CBER. NVD further requested for an advice on the next steps to initiate these discussions. This requires a follow-up and development of a plan to set-up such a test on viral harvests to ensure absence of any Mycoplasma contamination of viral harvests with non-cultivable Mycoplasma.

DETAILED REVIEW AND COMMENTS

A. -----(b)(4)-----

1. *Single Radial Immunodiffusion (SRID)*

SRID is used to determine the HA content in the inactivated influenza vaccines. CBER has years of experience for this method and have specific requirements for this test for the products marketed in the US. NVD had been following a method described in the (b)(4) and had agreed to follow the CBER's method. CBER provides reagents (reference antigen and antibodies) to the manufacturers of inactivated influenza vaccines. In some cases, when specific reagents are not available at CBER, CBER authorizes the use of SRID reagents from other World Health Organization (WHO)'s Essential Regulatory Laboratories (ERL), which include the NIBSC in UK, Therapeutic Goods Administration (TGA) in Australia and National Institute of Infectious Diseases (NIID) in Japan.

In this method, test sample and the reference antigen are treated with a Zwittergent 3-14 detergent which separates the HA antigen into trimers. Detergent treated antigens at various dilutions are applied in the wells of an agarose gel containing specific antibodies. Reaction of the antigen with the antibody at the optimal concentration

produces a ring of precipitation (i.e. precipitin ring); size of ring depends upon the concentration of antigens. By using a reference antigen of known concentration, a standard curve is generated from SRID ring sizes against concentration of HA antigen in the reference antigen. HA concentration of unknown sample is calculated from the standard curve using parallel line method.

------(b)(4)-----
------. Subsequently, additional work was done as per US-SRID method, using CBER reagents and following parallel line model to calculate the results.

Documents Reviewed:

- Document No.: 263311-01: Analytical Test Method Validation Report: Single radial immunodiffusion assay for the quantitative analysis of Influenza antigen in monovalent bulks and filling lots of FluCC A/Brisbane
- Document No.: 281129-01: Analytical Test Method Validation Report: Single Radial Immunodiffusion Assay for the Quantitative Analysis of Influenza Antigen in Monovalent Bulks of FluCC H3N2-Victoria
- Document No.: 279025-01: Analytical Test Method Validation Report: Single Radial Immunodiffusion Assay for the Quantitative Analysis of Influenza Antigen in Monovalent Bulks of FluCC B-Brisbane 2010
- Document No.: 281230: Validation Summary Report for the Determination of Hemagglutinin Content by SRD (USA), SOP 269284, for Testing FCC Monovalent Bulk Samples (validation done using a single monovalent bulk lot for A/Victoria/210/2009 X-187)
- Interim Qualification Report: Qualification Report for suitability of CBER egg-based influenza reference reagents for use with the flu cell culture generated material for testing of HA potency (submitted in an amendment 125408/0.6)
- Qualification Report for suitability of CBER egg-based influenza reference reagents for use with the flu cell culture generated material for testing of HA potency (submitted in an amendment 125408/0.8).
- Document No.: 288715: Summary Report for the Verification of the Method SOP 269284: Determination of Hemagglutinin Content by SRD (USA), for Testing FCC A/Brisbane (H1N1) Monovalent Bulk Samples
- Document No.: 288716: Summary Report for the Verification Protocol for the Determination of Hemagglutinin Content by SRD (USA) for Testing FCC B/Brisbane Monovalent Bulk Samples.
- Document No.: 291286: Summary Report for the Verification of the Method SOP 269284: Determination of Hemagglutinin Content by SRD (USA), for Testing FCC ----(b)(4)--- Samples
- Document No. 294861: Summary Report for the Verification of the SOP 269284: Determination of Hemagglutinin Content by SRD (USA), for Testing FCC B/Wisconsin/1/2010 Monovalent Bulk Samples (submitted in an amendment 125408/0.34)

- Document No. 294866 Qualification Report for CBER Reference Antigen A/Victoria/361/2011, IVR-165 (Lot 73) and CBER Antiserum A/Victoria/361/2011 (Lot H3-Ab-1211) for Use with SOP 269284, “Determination of Hemagglutinin Content by SRD (USA)”
- Document No. 294861 (Summary Report for Verification of SOP 269284: Determination of Hemagglutinin Content by SRD (USA) for Testing FCC B/Wisconsin/1/2010 Monovalent Bulk Samples.
- Document Number: 294865 (Summary Report for Qualification of B/Hubei-Wujiagang/158/2009 antigen (Lot B-Ag-1111) and antibody (Lot B-Ab-1207).

Review

During early validation (Document IDs 281129, 279025 and 263311) monovalent bulks from 3 different strains from previous influenza seasons were evaluated ----- (b)(4) ----- . Validation parameters evaluated during this study included precision (i.e. repeatability and intermediate precision), specificity and accuracy. Subsequently, parallel line method (CBER) was used to evaluate validation parameters with one monovalent lot from each of A/Victoria/210/2009 X-187 strain (Doc No. 281230), A/Brisbane-H1N1 monovalent bulks (Doc. No. 288715), B/Brisbane/60/2008 monovalent bulks (Doc No. 288716) and ----- (b)(4) ----- . Validations parameters evaluated were: specificity, linearity, repeatability, intermediate precision, accuracy, range and robustness. Results from these validation studies for the SRID met the acceptance criteria.

Suitability of Egg based SRID Reagents for the Influenza Vaccine made in MDCK cells

NVD was advised at an early stage of review of the BLA to submit data and a report on suitability of egg based reagents for their product made in MDCK cells. The sponsor submitted a qualification report summarizing the suitability of CBER’s egg based SRID reagents for determination of HA content of their monovalent bulk samples by SRID method using strains from 2011-12 influenza season (Amendments 125408/0.6 and 125408/0.8). Egg-based reference reagents, when used with the NVD’s influenza vaccine made in MDCK cells met the pre-defined criteria for non-parallelism. Calculated HA contents were underestimated against HA content determined from ----- (b)(4) ----- (Recovery 33 to 64%). NVD’s explanation for underestimation was loss of SRID activity in these samples, which were produced several months back. Underestimation of HA content may result in having more HA content in the vaccine, but the upper limit of HA content is controlled by the ----- (b)(4) ----- content in the product. Therefore, there is no safety related risk due to high HA content. This report supported use of egg-based reagents for use in HA potency determination by SRID for the

influenza vaccine made in MDCK cells. However, this needs to be verified for each new strain.

Discrepancy in SRID Results obtained at NVD and CBER

SRID results from the tests performed at CBER for monovalent bulk lots of 3 strains were more than 20% lower for 8 of 15 lots tested than the results provided by sponsor. Sponsor clarified in an amendment 125408/0.13 that discrepancy in SRID results between NVD and CBER was due to different reagents and methods used to perform the SRID test at NVD (b)(4) method and NIBSC reagents). During an investigation, samples were re-tested by the sponsor using the CBER reagents and the method. The re-test generated results that were within 20% of those obtained at CBER.

Use of Heterologous Egg based Reagents for testing of 2012-2013 vaccine

NVD communicated to CBER that they have used cell based reagents for the H1N1 strain, A/Brisbane/10/2010 cell derived (obtained from NIBSC), homologous egg based reagent for H3N2 strain, A/Victoria/361/2011 IVR-165 (obtained from CBER) and heterologous B strain egg based reagents, B/Hubei-Wujiagang/158/2009 BX-39 (obtained from CBER).

CBER responded with an information request (IR) sent via email by Dr. Timothy Fritz on 09/07/12 requesting NVD to provide the following information.

Q.3 Please provide a qualification report to show that A/Victoria/361 egg-based reagents are suitable for the SRID testing of MDCK cell grown vaccine.

Q.4. Because a cell-based reference antigen for B/Wisconsin is available from NIBSC, CBER recommends using this reference for SRID testing. Please comment.

In response to CBER's information request NVD submitted an amendment, 125408/0.34 with the qualification reports demonstrating suitability of CBER's egg-based reagents for H3N2 and B strains for their product made in MDCK cells. Use of homologous H3N2 egg-based reagents was acceptable. However, CBER did not recommend the use of heterologous reagents for B strain based on CBER's experience with discrepant results when heterologous reagents were used during the H1N1 pandemic. Heterologous reagents for the H1N1 strain were re-calibrated by CBER. However, sponsor refused to test this year's vaccine with the homologous B strain reagents, available from NIBSC. This caused a concern, particularly when the sponsor has been using the homologous B strain reagents for releasing vaccine in Europe. Further, NVD argued that they did not know if they could use reagents from NIBSC which seemed strange when they were already using NIBSC reagents for the H1N1 strain. To move forward with this issue, CBER decided to test all lots with the homologous reagents B strain

reagents for lot release at CBER and communicated to the sponsor in a tele-con on September 17, 2012. Subsequently, CBER tested 3 launch lots of the product using homologous B strain reagents and these lots met the specifications for SRID. This was communicated to the sponsor in a tele-con on October 5, 2012 and results were communicated later. At this tele-con, sponsor told that they don't plan to release any more lots of influenza vaccine this year. CBER recommended that in case NVD decides to market any more lots of the product this year, these should be tested with the homologous B strain reagents from NIBSC.

Integrity of Samples shipped from Marburg to Holly Springs for the SRID

In an information request on August 15, 2012, the following request was made to NVD about integrity of samples shipped from Marburg to Holly Springs for the SRID.

“Regarding samples that are shipped from Marburg to Holly Springs for the SRID test, please describe how you maintain the sample integrity during the sample shipping and storage. Please provide data to demonstrate that there is no significant difference in the potency of the monovalent bulk during shipping. We suggest that you compare the SRID results using the same sample tested at both the Marburg and Holly Springs locations.”

In an amendment 125408/0.28, sponsor responded with the following response

In order to ensure sample integrity, Novartis ships samples utilizing ---(b)(4)--- to maintain appropriate storage conditions. Analytical Quality Control will work with validation and sample management to carry out a shipping study according to a pre-approved protocol employing same courier, flight path, and containment/monitoring ---(b)(4)----- container, etc) . As part of this shipping validation study, Analytical Quality Control will test samples per the SRID method to ensure sample integrity during shipping process. This data will be provided to the agency once completed.

This response is acceptable based on monitoring and tracking of temperature during shipment, which will be further supported by the studies committed by the sponsor. These studies should be completed before the next influenza season. Lack of this study is not a concern for the product to be released in 2012-13 season as CBER is testing all lots for SRID potency.

Conclusion: SRID method is suitable for intended purpose using CBER authorized reagents. Suitability of egg based reagents to be used in determining the HA potency by SRID must be evaluated for each new strain.

2. *Residual Infectious Influenza Virus*

The absence of residual infectious influenza virus is tested according to the -----
----(b)(4)----- inoculated into cell
cultures which are -----(b)(4)-----

Documents Reviewed

- Document #105243-05
- Document#231855
- Document #403031

Review

The original submission had high level summaries of development and validation of the test method and a complete evaluation of the method was not possible. Based on the review of the available information CBER made an information request to the sponsor on May 4, 2012 to provide the following documents and information.

Comment 11a

CBER's Comments (Communicated May 4, 2012)

In Document No. 231299, Section 5.1, data presented indicate that all 3 strains of the control virus preparation were detected at ----(b)(4)----. In section 4.3, the document states that "lower dilutions may still be completely or partially positive, since a tailing effect is to be expected." Please explain the tailing effect leading to detection of ----(b)(4)----- in 100% of experiments for all 3 strains.

NVD's Response (Amendment 125408.12)

NVD provided a theoretical explanation which did not provide a satisfactory response to the comments. NVD's response is given below.

The tailing effect of positive responses at (b)(4) end dilutions is displayed in the (hypothetical) graph shown in Figure 1.11.1-1 provided in amendment 0.12, which is based on the statistical conditions of a Poisson distribution.

As shown in the graph, at lower dilutions and (b)(4) values below (b)(4), the percentage of positive reactions will be reduced gradually, following a sigmoidal dose/effect curve. The course of those curves varies for different viruses and strains and their cell substrates and also depends on specific test variables, such as incubation times until reading of the test results and the degree of cytopathic

Our test for absence of residual infectious virus uses a sample volume of -----

(b)(4)

The theoretical explanation you have provided in response to Question 11a shows that -----

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

NVD's Response (Amendment 125408.19, Sequence 0021)

(b)(4)

This explanation was not satisfactory to CBER.

Comment 11b

CBER's Comments (Communicated May 4, 2012)

In Document No. 231299, Section 6, you mentioned that the detection limit of the ---(b)(4)--- method was compared with that of the ----(b)(4)----- method. Please indicate if the amount of the virus inoculum in the two methods was the same for the two methods for a meaningful comparison.

NVD's Response (Amendment 125408.12)

The amount of the virus inoculum in the two methods was different. As defined by the ---(b)(4)-----, the test in ---(b)(4)----- applied a total sample volume of -----(b)(4)-----, each). The ---(b)(4)----- method uses a total sample volume of -----(b)(4)-----, each). Therefore, due to -----(b)(4)-----, the ---(b)(4)----- test is more sensitive than the test used for embryonated egg vaccines.

NVD stated that amount of the virus inoculum in two methods was different. The intent of this CBER query was to ensure that NVD performed a proper comparability study between the new ---(b)(4)----- method and previously used ---(b)(4)---- method using equal amount of virus made in MDCK cells to demonstrate that ---(b)(4)-- method is at least as sensitive as the (b)(4) method.(or more sensitive as they claim). In their response the sponsor did not address CBER's concern. Further comments were communicated to NVD as given below.

CBER's Comments on NVD's Response (Communicated June 19, 2012)

CBER agrees that testing (b)(4) times more sample using the ---(b)(4)----- method may provide (b)(4) times greater assurance of absence of residual infectious virus than the (b)(4) method performed in ----(b)(4)----- . However, this assumption is based on similar levels of sensitivity of detecting live virus using -----(b)(4)----- . Please provide data on the comparative sensitivity of ----(b)(4)----- using virus preparations made in MDCK cells. This information should be submitted within the next 2 weeks. CBER is willing to discuss this issue further by tele-con, if necessary.

NVD's Response (Amendment 125408.19, Sequence 0021)

The results of all measurements passed the pre-determined acceptance criteria. Thus it has been demonstrated that the execution of the test according to SOP 105243 consistently produces correct and reproducible results. The method is valid for -----(b)(4)----- . In accordance with the defined requirements the detection limit of this method is -----(b)(4)----- . The tested examples of this validation show that a value of -----(b)(4)----- can be achieved, as one would expected with high probability (compare Appendix 1). Due to the limitation of the statistical probability, however, the technically reproducible detection limit of ---(b)(4)----- is maintained.

In an analogous test method in ----(b)(4)-----, which is defined by ---(b)(4)----- for egg-derived vaccine preparations, the detection limits were also evaluated for 3 test virus strains (see Analytical Test Method Validation Report Document #403031):

Strain A/New Caledonia (H1N1): Detection limit -----(b)(4)-----

Strain A/Panama (H3N2): Detection limit -----(b)(4)-----

Strain B/Guangdong (B): Detection limit -----(b)(4)-----

As far as a direct comparison of different test systems admits, one may conclude that the detection limit of the method using -----(b)(4)----- validated here is either equal or 10-fold lower than the conventional test in -----(b)(4)-----.

- Document# 403031 [Attachment-9: Analytical Validation Report - Absence of replication competent influenza virus

If necessary Novartis can further discuss this topic with CBER by arranging a telephone call.

In their response NVD mentioned that they did not perform a direct comparative study of the two methods using same virus made in MDCK cells.

Comments 11c, 11d and 11e (Request for documents Communicated May 4, 2012)

Test method SOP 105243 for testing residual infectious virus by ---(b)(4)-----
EDMS document No. 231855, describing study to determine detection limit of
---(b)(4)----- method in -----(b)(4)----- preparations containing HA.

Analytical Test Method Validation Report 403031 for evaluation of detection
limit in ---(b)(4)----- for the 3 test virus strains.

NVD's Response (Amendment 125408.12)

The sponsor provided the requested documents.

Follow-up on Comments 11a and 11b

To resolve issues from questions 11a and 11b, to which NVD had provided conflicting and confusing information and no further documents to support reliability of the residual infectious virus test in ---(b)(4)----, a telecom was arranged on August 24, 2012. In the telecom with regard to (11a) CBER indicated that some of the information provided by Novartis appeared contradictory. NVD clarified that they had tested -----(b)(4)----- not ---(b)(4)---- and that this was why 100% of the experiments were positive for all 3 strains. CBER requested that Novartis provide the validation protocol and testing reports and to indicate which lots of virus were tested.

With regard to (11b) CBER indicated that NVD provided a theoretical explanation of why the -----(b)(4)----- method should be more sensitive than the -----(b)(4)----- method for detecting residual infectious virus. CBER clarified that NVD had not provided actual data showing the comparability between the 2 methods. CBER suggested 2 possible options for demonstrating comparability:

Option 1

If Novartis is unable to demonstrate that the test for residual infectious virus performed in ---(b)(4)----- is not at least as sensitive as the test performed in -----(b)(4)-----, Novartis needs to test first 3 -----(b)(4)----- lots from each annual influenza season for residual infectious virus in both the -----(b)(4)----- and ---(b)(4)-----
All 3 lots for all seasonal strains must pass the test in both substrates. Subsequent lots during the season can be tested by the ---(b)(4)----- method only.

Option 2

Alternatively, if Novartis can provide validation data demonstrating that the sensitivity of the residual infectious virus test performed in ---(b)(4)--- is at least as great as the test performed in ----(b)(4)----, then Novartis may release -----(b)(4)----- by the test performed in ---(b)(4)---- only. We suggested that a possible experiment to validate the sensitivity of the assay (which should be demonstrated for at least 3 strains representing H1N1, H3N2 and B types) was as follows:

----(b)(4)----- (at least 3 lots for each strain) spiked with serial dilutions of the same strain of MDCK cell grown influenza virus of known concentration ---(b)(4)----- should show at least similar or higher sensitivity in detecting spiked virus by the ---(b)(4)--- test as compared to the ----(b)(4)----- test. As per theoretical explanation provided during the call that the sensitivity of --(b)(4)----- is significantly higher than the -----(b)(4)-----, they can perform the test at one spike level of ---(b)(4)---- and show that such spike cannot be detected by (b)(4), but readily detected by ---(b)(4)----.

Novartis indicated that they would provide testing data as soon as possible.

CBER's Information Request (Email from Dr. Timothy Fritz, dated September 7, 2012 and the subsequent tele-con on September 13, 2012)

The information provided by NVD via e-mail on September 5, 2012 for CBER comment 11a did not include the residual infectious virus validation documents with virus lots and titers as requested in the August 24, 2012 tele-con between CBER and Novartis. Please provide this information.

In the information provided by NVD via e-mail on September 5, 2012 for CBER comment 11b, NVD did not discuss the 2 options agreed upon by CBER and Novartis in an August 24, 2012 tele-con.

NVD's Response (Amendment 125408.34)

In this amendment, NVD did not provide the validation protocol and report as agreed upon during earlier tele-con on August 24, 2012 in response to comment 11a. It seemed that sponsor did not have this data. To move forward on this issue, it was agreed upon between CMC and DBSQC reviewers that the safety of the product will be ensured if the NVD agrees to one of the options in 11b and CBER will be satisfied with the response. During tele-con on September 14, 2012, this was communicated to the sponsor.

This method is suitable for intended purpose.

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

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

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

5. **Purity** ----- (b)(4) -----

This method is described in SOP 222239, which is a standard -----
----- (b)(4) ----- technique. Validation documents (235802-02
and 265940-01) describe validation of the method to assess the purity of
--- (b)(4) ----- and final product. Specifications have been described for
detection of haemagglutinin and --- (b)(4) ----- . Information in the validation
report does not support identification of --- (b)(4) ----- by this method and the
method has not been validated for this purpose. During a discussion with the
CMC reviewer, it was agreed that the DVP CMC reviewer will complete review
of this method. It was also agreed that this method may not be necessary to ensure
safety, purity and potency of the product and therefore, it was decided not to
request data from this test in the lot release protocol.

6. **Polysorbate 80**

7. **CTAB (cetrltrimethyl- ammoniumbromide)**

These 2 methods have been reviewed by Dr. Lokesh Bhattacharyya, Lab Chief for
Laboratory of Analytical Development and Blood Related products. Dr.
Bhattacharyya will write the review ----- (b)(4) -----.

8. **Total DNA Content**

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

These 2 methods have been reviewed by the CMC reviewer and will be covered
in the review memo from the CMC reviewer.

10. **Sterility Test**

The methodology and verification of sterility test have been reviewed by Dr.
James Kenney, the Team Leader for the Laboratory of Microbiology, In-vivo
Testing and Standards. The review has been submitted in a memo by Karen
Campbell..

B. -----(b)(4)-----

1. *Single Radial Immunodiffusion (SRID)*

All aspects of this method have been reviewed in the -----(b)(4)----- Section above. The method is suitable for testing -----(b)(4)-----.

2. *Total Protein (----- (b)(4) -----)*

Total Protein is determined by a -----

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

----- specification for total protein in the ----- (b)(4) -----

Documents Reviewed

- Section 3.2.P.5.2.2.1 – Method Description
- Validation report Attachment 3.2.R.3-258579

Accuracy was evaluated by ----- (b)(4) -----
----- Repeatability was
determined as ----- (b)(4) ----- . Intermediate
precision was evaluated as ----- (b)(4) ----- .

CBER's Comments (communicated May 4, 2012)

Please submit SOP 103347 or a full procedural description of the Determination of Total Protein ----- (b)(4) ----- . This should include a specific description of the protein standard used for control of accuracy as well as the working range of the procedure.

NVD's Response (amendment 125408.12)

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

The working range of the method is defined as ----(b)(4)----- and is documented in the Analytical Validation Report, Document# 258579 attached to this amendment.

- Document# 258579 [Attachment-16: Analytical Test Method Validation Report -----(b)(4)-----]

An English translation of the requested SOP 103347-07 is also appended:

- Document# 103347-07 [Attachment- 17: SOP Total -----(b)(4)-----
-----]

CBER's Review of NVD's Response

The response is acceptable.

CBER's Comments (communicated May 4, 2012)

Section 3.2.P.5.3.2.1 Validation of Analytical Procedures Total Protein Determination -----(b)(4)----- and linked Validation Report Doc. No 25879-02:

- 9a. Validation characteristics do not include the defined range of this procedure. Please specify the procedural range as based on acceptable accuracy, linearity and precision.
- 9b. Precision is not addressed in the submitted report. It is stated in Section 4.3.1 that "Repeatability has already been calculated with different products listed in AVPP 254267 and does not need to be determined within this validation". An identical statement is made regarding Intermediate Precision in Section 4.3.2. Please submit the reports in which precision of this method as applied to the Optaflu ---(b)(4)----- has been evaluated.

NVD's Response (amendment 125408.12)

9a)

Spike experiments with ----(b)(4)----- prove specificity, accuracy, linearity and robustness in a procedural range of ----(b)(4)----- . Please refer to the following

Attachment:

- Document# 258579 [Attachment-16: Analytical Test Method Validation Report -----(b)(4)----- Method]

9b)

Precision data (repeatability and intermediate precision) are available from the validation study conducted in 2003, Analytical Validation Report Document# 403073 performed with Flu Cell Culture material. Further precision data are available from the validation study conducted in 2005 with ---(b)(4)--- material and documented in Analytical Validation report, Document# 233411. An overview of all validation activities is given in Analytical Validation Plan/Protocol, Document# 254267, Table 2-1.

The referenced documents are appended to this amendment:

- Document# 403073 [Attachment-18: Analytical Test Method Validation Report -----(b)(4)----- Method]
- Document# 233411 [Attachment-19: Analytical Test Method Validation Report -----(b)(4)----- method]
- Document# 254267 [Attachment-20: Analytical Test Method Validation Plan/ Protocol Determination of -----(b)(4)-----
-----]

CBER's Review of NVD's Response

Provided documents are acceptable and the method is suitable for intended purpose.

3. Total DNA Content

This method has been reviewed by the CMC reviewer and will be covered in the review memo from the CMC reviewer.

4. (b)(4)

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

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

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

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

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

5. --- (b)(4) ---

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

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

-----.

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

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

--(b)(4)---

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

Conclusion

The method is suitable for intended purpose.

6. Sterility Test

Trivalent formulated bulk has not been claimed sterile. The sponsor cited the recent revised 21 CFR 610.12 sterility test requirement, which does not require the sterility test for the bulk product. This caused a concern for developing a testing plan at CBER for lot release of the product after licensure as the sterility test is one of important tests performed at CBER. Secondly this was the first time that a formulated bulk has not been claimed sterile for a prophylactic vaccine product meant for parenteral use in a healthy population. After discussions with the CMC and facility reviewers and clarifications from the sponsor, it was found that the formulated bulk was tested for sterility as in-process test ----(b)(4)----- and the manufacturing step was validated for aseptic processing. Based on this information, it was decided to accept non-sterile trivalent formulated bulk and not to perform sterility test at CBER to release trivalent formulated bulk for lot release purposes.

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

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

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

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

C. Drug Product (Final Container)

- 1. Sterility Test**
- 2. Endotoxin Content**
- 3. General Safety**

The methodology and verification of sterility test, endotoxin content test and general safety test have been reviewed by Dr. James Kenney, the Team Leader for the Laboratory of Microbiology, In-vivo Testing and Standards. The review has been submitted in a memo by Karen Campbell.

4. Appearance and Extractable Volume Tests

In the Appearance test, clarity and opalescence of the vaccine is compared visually with water and opalescent reference suspensions. The test is as described in the (b)(4).

Documents Reviewed

- Section 3.2.P.5.2.3.4 – Method Description – Appearance
- Section 3.2.P.5.3.3.4 – Validation Statement

Extractable volume of single dose containers may be tested directly by extracted content volume using a ---(b)(4)----- . Extractable content of ---(b)(4)--- and prefilled syringes are tested by -----
----- (b)(4) -----
-----.

Documents Reviewed

- Section 3.2.P.5.2.3.5 – Method Description - Extractable Volume
- Section 3.2.P.5.3.3.5 Validation Statement

As standardized, (b)(4), simple physical test methods, validation is not required.

Conclusions

These methods are suitable for intended purpose

5. *Single Radial Immunodiffusion (SRID)*

Sponsor did not propose a SRID potency method on final container. This was a concern about adequate potency at the final container stage, particularly when the formulated trivalent bulk -----(b)(4)----- and due to the fact that influenza haemagglutinin (HA) is prone to polymerization and aggregation. CBER requested the sponsor to add this test on the final container to which sponsor agreed.

All aspects of this method have been reviewed in the ---(b)(4)----- Section above. The method is suitable for testing final container.

6. *Identity by Hemagglutinin Antigen Assay* ---(b)(4)-----

[illegible]

For validation of ---(b)(4)----- test, specificity and robustness parameters were evaluated.

Documents Reviewed

- Document No.: 288433-01: Analytical Test Method Validation Report: Identity FCC US in final container

Review

Specificity and robustness (i.e. -----(b)(4)-----

----- were evaluated during validation of
Hemagglutinin antigen assay by -----

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

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

Conclusion

The method is suitable for intended use.

D. Tests at early stages of Manufacture

1. Test for Mycoplasma

The methodology and validation of test for Mycoplasma have been reviewed by Dr. James Kenney, the Team Leader for the Laboratory of Microbiology, In-vivo Testing and Standards. The review has been submitted in a memo by Karen Campbell.

The sponsor is not performing the test for non-cultivable Mycoplasma on the viral harvests by indicator cell method and provided an explanation on the problems, and challenges for this test (amendment 125408.12). CBER recognizes these challenges and responded to the sponsor with the following comment.

“Regarding your June 19, 2012 (Sequence 12) response to Comment 10b of our May 4, 2012 Information Request: 10b. We understand Novartis’ concerns regarding using the indicator cell method for Mycoplasma testing of viral harvests. Your control cell Mycoplasma testing provides some assurance for viral harvests. We recommend that Novartis develop and implement a --- (b)(4) ---- Mycoplasma test for viral harvests for the 2013-2014 influenza season. CBER is willing to assist Novartis in the development of this test.”

NVD responded in an amendment 125408.24 by acknowledging CBER’s position and accepted to take the opportunity to further discuss this request with CBER. NVD further requested for an advice on the next steps to initiate these discussions.

This requires a follow-up and development of a plan to set-up such a test on viral harvests to ensure absence of any Mycoplasma contamination of viral harvests with non-cultivable Mycoplasma.