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**To:** File: STN 125510/0

**Through:**  
William McCormick, OCBQ/ Director DBSQC

**Company:** Novartis Vaccine and Diagnostics, Inc.

**Product:** Fluad; STN: 125510/0

**Subject:** Review of Chemistry Related Procedures

**Recommendation:** Approval

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### Summary and Conclusion

This document constitutes the Review Memo for the analytical test methods and their validations for the following quality control lot-release tests:

**Monovalent Pooled Harvest (DS)**(b) (4)  
**Adjuvant (MF59C)**

Squalene Identity and Content

Polysorbate 80 Content / Sorbitan Trioleate Content

(b) (4)  


Appearance

(b) (4)  
**(b) (4) Filled Vaccine**

Squalene Identity and Content


CTAB

Extractable Volume

Total Protein (b) (4)

Appearance

pH

(b) (4)  


**Overall Conclusion of Review:** The proposed assay methods are adequately described and validated. Our recommendation is that they be approved for intended use as described.

**Background of Submission**

A new BLA was submitted for the Fluad vaccine, which is an adjuvanted vaccine for active immunization in persons 65 years of age and older against influenza disease caused by influenza virus subtypes A and type B contained in the vaccine. The antigens present in this vaccine are the same as that used to produce Agriflu (STN: 125297). The Drug Substance (Monovalent Pooled Harvest) from each of the three selected viral strains will be combined to produce the trivalent bulk product. The excipients present in the Monovalent Pooled Harvest (drug substance) and their concentrations are also the same as those in Agriflu. However, the vaccine contains a novel adjuvant MF 59C, which is an oil-in-water emulsion consisting of squalene, sorbitan trioleate and polysorbate 80.

## Review Narrative

### Drug Substance - Monovalent Pooled Harvest Tests

1.(b) (4) [REDACTED]

(b) (4) [REDACTED]

(b) (4) [REDACTED]


[REDACTED]

(b) (4) [REDACTED]


(b) (4) [REDACTED]



(b) (4)



(b) (4)



(b) (4)



## **Drug Product- Final Bulk and Filled vaccine**

### **16. Squalene Identity and Content by (b) (4)**

Reviewer: Ritu Agarwal


Information submitted and reviewed included:

- 125510/0 – 3.2.P.5. Control of Drug Product
- 125510/0 – 3.2.P.5.1 Specification
- 125510/0 – 3.2.P.5.4 Batch Analyses
- 125510/0 – 3.2.P.5.2 Analytical Procedures
  - SOP 306198: Squalene ID and Content by (b) (4)
- 125510/0 – 3.2.P.5.3 Validation of Analytical Procedures
  - ISU 07.014 VR3: Validation of the method used for the Determination of Squalene ID and Content
  - ISU 07.014 VR3: Validation Protocol for the method used for the Determination of Squalene ID and Content
  - Doc R/0069/02/14: Method Transfer report for the method used for the Determination of Squalene ID and Content

- 125510/0.16 – 1.2 Response to FDA information request dated 15 May 2015, Received on 17 July 2015
- 125510/0.23 – 1.2 Response to FDA information request dated 18 Sep 2015, Received on 29 Sep 2015

The MFC59C.1 adjuvant is added to diluted monovalent pooled harvest/s drug substance to a final squalene concentration of 9.75 g/L (b) (4). This corresponds to a specification of (b) (4) squalene in the FLUAD vaccine. Method


The identification and quantitation of squalene in final container product is performed by (b) (4) following the procedure described in SOP 306198. The method employs a (b) (4)




#### Method Validation

The method is used as a quantitative test for squalene in FLUAD final container samples (FLF 13.020). The following validation characteristics were evaluated: Specificity, Linearity and Range, Accuracy, Repeatability and Intermediate precision and Robustness.


(b) (4)




(b) (4)



(b) (4)



(b) (4)



(b) (4)

(b) (4)

(b) (4)

First Information request: The following IR was submitted to the sponsor on 15 May 2015. The response by Novartis received as Amendment 16 on 17 July 2015, is discussed below.

A. Regarding your Final Filled Drug Product, Squalene Identity and Content by (b) (4)

- i. Please submit SOP 306198 “Squalene Identity and Content by (b) (4) Please ensure that this includes the specific make of the (b) (4)

Review of response: As requested by CBER, the sponsor submitted the test method SOP, document 306198, which provide information on th (b) (4)

- B. In the Method validation report, Document 2933539-02 (ISU 07.014 VR 3 Rev. 7): Please provide details of preparation of final container FLUAD samples for Accuracy and Linearity studies to show that these validation characteristics were appropriately assessed in the drug product matrix.

Review of response: In response, the overview tables of spiked squalene concentration and final reconstitution volumes of the drug product prepared for the linearity/accuracy studies were provided by the sponsor. The response is adequate.

Second Information request: After the review of response to the first IR, a new IR was submitted to the sponsor on 18 September 2015. The response by Novartis received as Amendment 23, on 29 September 2015, is discussed below.

- A. For the Final filled vaccine we have the following questions/comments regarding the Method validation report, Document 2933539-02 (ISU 07.014 VR 3 Rev. 7):
- Please provide the linear regression plots of analyte (squalene) concentration vs. response (peak area) for the squalene standard and your final container FLUAD samples used to obtain the validation data described in Tables 9 and 10 of your validation report. Please also submit data for linearity fit ( $R$  or  $R^2$ ), slope and distribution of residuals based on concentration vs. response.

Review of response: As requested by CBER, the sponsor provided the linear regression results for squalene amount vs peak area for final container samples and squalene standard. The regression coefficient of the linearity plot for standard and sample were (b) (4) in both the cases, and met the acceptance criteria of  $R$  to be (b) (4). The sponsor's data are adequate to demonstrate linearity in the drug product.

Conclusion: The method is described in sufficient details, and is suitably validated for lot release testing of the drug product.

## 17. CTAB Assay for the Drug Product

DBSQC Reviewer: Lokesh Bhattacharyya

Information submitted and reviewed includes:

- 125510/0 3.2.P.5 Control of Drug Product
- 3.2.P.5.1 Specification
- 3.2.P.5.2 Analytical Procedures
  - Analytical Procedures – Cetyl-trimethyl-ammonium bromide (CTAB) [NVD (b) (4)]
- 3.2.P.5.3 Validation of Analytical Procedures
  - 294160-01: Validation report of the CTAB determination in Flu vaccine, (b) (4) [NVD(b) (4)]
  - R/0401/09/13: Report for Analytical Method Transfer for the Determination of Cetyltrimethylammonium bromide (CTAB) Content in Agrippal Platform Products (b) (4) and Fluad) from the (b) (4) Site to the (b) (4) Site



- 293538-01: Qualification Report of the method for extractable volume determination in the phase of (b) (4) Filled Product with MF59 in syringe-vials for samples of FLUAD (b) (4) and tetravalent FLUAD (b) (4)
- 3.2.P.5.4 Batch Analyses
- 125510/0.5 1.11.1 Quality Information Amendment, received on 30 March 2015
- 3.2.S.4.2 Analytical Procedures
- SOP 295036 ver. 2: Standard Analytical Method for the Quantification of CTAB in Agrippal Platform Samples using (b) (4) [NVD (b) (4) ]
- 3.2.P.5.3 Validation of Analytical Procedures
- R/0689/12/10 Rev. 01: Validation of the standard analytical method for the quantification of CTAB in aqueous solutions using (b) (4) for Fluad Formulated Samples

The test is proposed to be performed at two locations: (b) (4). The proposed lot-release specification is  $\leq 24$  µg/mL.

#### Method

CTAB concentration is determined by the reaction of (b) (4) and CTAB in the sample. The resulting (b) (4)

(b) (4). The amount of CTAB present in a sample is determined by (b) (4)

(b) (4). The sponsor did not submit the SOP, however, the Analytical Procedure submitted contains the necessary details about the test method and the assay validity criteria.

#### Method Validation

The method was validated at the (b) (4) site (#R/0689/12/10) for the (b) (4), and is transferred to (b) (4) site. The validation characteristics and the results obtained at the (b) (4) site are summarized in Table 7 below.

(b) (4)

#### Assay Transfer

The method was transferred from the (b) (4) site to the (b) (4) site. The transfer report (R/0401/09/13) documents evaluation of linearity, LOQ, precision and accuracy (recovery) at the (b) (4) site, and comparability study at both sites. The results are summarized in Table 8.

(b) (4)

The comparability study reported results from (b) (4) lot of the drug product (b) (4) with CTAB to make (b) (4) with final CTAB concentrations (b) (4) and tested at (b) (4) sites. The results show (b) (4) recoveries at the (b) (4) sites with respective CV values (b) (4). Analysis of the results by the (b) (4) show that the difference between the (b) (4) sites are within 95% confidence interval and the distributions of the recoveries at the (b) (4) sites are within the acceptance criteria for recovery (Tables ?? and ?? above) of (b) (4).

#### Information Request and Review

- b. Based on the review of the information provided by the sponsor, the following IR was submitted. The sponsor's response was received as Amendment 5 on 30 March 2015. The IR and review of the sponsor's response are discussed below.
- i. Please include an appropriate RSD of (b) (4) measurements at each dilution of standard, control and samples as a Test Validity Criterion in your SOP and submit for review.

Review of the Response: The sponsor proposed to update the SOP # 315799 to include an RSD of (b) (4) is applied to the (b) (4) values of (b) (4) measurements. This is consistent with the validation report and is acceptable.

- ii. In your validation report (Atlas No. 294160-01), you concluded method specificity based on the accuracy results only. We do not agree that you have demonstrated method specificity adequately because the accuracy is determined by (b) (4) method. Please provide data from the analysis of a representative CTAB-(b) (4) matrix for the (b) (4).

(b) (4) Vaccine to demonstrate no or negligible (b) (4) by the method, to demonstrate method specificity.

Review of the Response: In response, the sponsor updated the validation report to include results from the method development data from CTAB (b) (4) samples containing representative concentrations of components present in the sample matrix for (b) (4) Vaccine. The results show that the (b) (4) value from the components present in the sample matrix were less than half of the (b) (4) of the lowest standard. The (b) (4) are not negligible, as stated in the response, however, under the circumstances, the sponsor will be over-estimating the amount of CTAB in the (b) (4) Vaccine and still have to keep it within the specification limits. Thus, the response is acceptable.

- iii. You have demonstrated linearity of you assay by data from the standard only. We do not agree that you have demonstrated linearity of your assay adequately. Please provide linearity data using representative sample matrix of the (b) (4) Vaccine and demonstrate parallelism between the standard and the samples within a reasonable confidence interval.

Review of the Response: In response, the sponsor reanalyzed the data from the accuracy study presented in the Assay Transfer report (included in Table above) and showed that the slopes are (b) (4). These samples were obtained by (b) (4) CTAB to the (b) (4) Vaccine in the range (b) (4). In addition, the sponsor presented linearity results from the standard in the concentration range (b) (4) in response to IR question d (below). Our calculation shows that the ratio of the mean slope of the standard curves to that of the dilution curves of CTAB in the (b) (4) Vaccine is (b) (4). The results demonstrate parallelism between the standard and the samples over the assay range.

- iv. It is not clear what the lines mean in Figure 1 of your validation report (Atlas No. 294160-01). Clearly, they are not linear-regression fit lines. Please explain the significance of the lines in the figure.

Review of the Response: The sponsor acknowledges that the curves presented in Figure 1 of validation report 294160-01 are not what are typically presented for linearity analysis. The data are presented with appropriate analysis and the results of slope, y-intercept,  $r^2$  value shown in a table. In all cases, the  $r^2$  values met the acceptance criterion (b) (4). The slopes from the table were compared with the data provided in response to the IR b.iii above to assess parallelism. The response is satisfactory.

- v. You concluded that the Limit of Quantitation (LOQ) of the assay is (b) (4) based on the standard curve only. Please provide accuracy, precision and linearity data from your samples to support LOQ of your method.

Review of the Response: In response, the sponsor referred to the data provided in response to the IR b.iii above to recalculate the LOQ to be (b) (4) (equivalent to (b) (4) when a (b) (4) dilution is employed). However, since the LOQ was found to be (b) (4), based on the analysis of the standard, which is higher than (b) (4), LOQ value of (b) (4) is acceptable.

- vi. Please provide robustness data for your method by evaluating the effect of variations of shaking time and concentrations of the reagents around the parameters specified in your SOP.

Review of the Response: The sponsor included robustness data in the updated validation report (294160-01) submitted as part of Amendment 5. Robustness was evaluated by varying concentrations of the reagents (b) (4) around the respective nominal values. The results for (b) (4) of the (b) (4) variations failed to meet the acceptance criterion, (b) (4) difference from the results obtained by the method described in the SOP. The sponsor concluded that no variation from the concentrations reagents defined in the SOP is permissible. This is acceptable.

- vii. In Table 2.3 of your Analytical Method Transfer Report (Doc. Ref. No. R/00401/09/13 Rev. 01), you included (b) (4) concentrations, one as (b) (4). It seems that you have applied a (b) (4) and you need to use this (b) (4) to match your results with the expected concentrations. But use of such (b) (4) is not indicated in the description of Analytical Procedure that you have submitted. Please explain your (b) (4), why you need to use it (background information) and why the (b) (4) is not indicated in the description of the Analytical Procedure, and provide details of data showing how you came up with the (b) (4).

Review of the Response: The sponsor explained that there is no (b) (4) to be applied to this method when performed for routine sample analysis. The term (b) (4) was obtained by (b) (4). Thus, it represents the calculated amount of CTAB spiked into the drug product for analysis. The response clarifies the confusion.

- viii. You have only evaluated reproducibility between the (b) (4) sites but no comparability data in your Analytical Method Transfer Report. Please provide comparability data for sufficiently large number of lots from both sites to permit adequate statistical evaluation of comparability together with statistical evaluation of the data. We recommend that you submit data from at least (b) (4) lots, the same lots being analyzed at both sites.

Review of the Response: The sponsor indicated that the method has been validated fully at the (b) (4) and linearity, LOQ, precision, and accuracy were evaluated independently at the (b) (4) site. In view of the independent validation at the (b) (4) site, this is acceptable.

**18. Test for the Extractable Volume—Final Container Vaccine**

DBSQC Reviewer: Lokesh Bhattacharyya

Information submitted and reviewed includes:

- Analytical Procedures – Extractable Volume [NVD (b) (4)]
- R/0461/10/13: Report for the Analytical Method Transfer of Extractable Volumes test for Adjuvanted Agravit Platform Product in Pre-filled Syringes, from the (b) (4) Site to the (b) (4) Site

Extractable volume is determined by (b) (4)

The proposed specification is (b) (4) 0.50 mL.

The method is qualified by the assessment of repeatability and intermediate precision. It is not clear from the qualification report (293538-01) at which site the qualification was done. However, in response our IR, the sponsor informed that the method is qualified to be performed at the (b) (4) site and will be performed at that site only. The results obtained by (b) (4), each performing (b) (4) tests of different lots of the Flud Vaccine (b) (4) product show CV (b) (4). All results are above (b) (4), meeting the proposed specification, (b) (4).

**Information Request and Review**

- c. Based on the review of the information provided by the sponsor, the following IR was submitted. The sponsor's response was received as Amendment 5 on 30 March 2015. The IR and review of the sponsor's response are discussed below.
  - i. In your analytical procedure, you indicated that the weight is divided by (b) (4) while in the qualification report (Report No. 293538-01) you used (b) (4). Please revise the SOP to replace (b) (4) because you have qualified your method using (b) (4).
  - ii. Please provide the data on the determination of (b) (4) that you used in method qualification, analytical transfer, and will continue to use in lot release testing at (b) (4) sites.

**Review of the Response:** In response to IR I and ii above, the sponsor explained that the Extractable Volume will be determined at the (b) (4) site only using the (b) (4) value approved for Fluvirin vaccine product, which has been licensed for the US market. This test will only be performed using (b) (4) in (b) (4) following the BLA approval.

- iii. In section 3.1 of your qualification report (Report No. 293538-01) you indicated that operator 2 calculated the (b) (4) manually. Please explain how and provide a comparison of the (b) (4) data and the data obtained by a (b) (4).

Review of the Response: The sponsor provided the method of manual calculation of (b) (4). This is acceptable.

- iv. In your Analytical Method Transfer Report (Doc. Ref. No. R/0003/13 Rev. 01), you did not provide any comparability data between the two sites. Please provide comparability data between (b) (4) sites.

Review of the Response: The sponsor responded that this test will be performed at the (b) (4) site only. Thus, independent qualification of the method at (b) (4) sites should be acceptable for method transfer. This reviewer agrees particularly because this is a (b) (4) method.

- v. For the precision data you presented in the Analytical Method Transfer Report, the syringes were filled (b) (4), not by using the actual manufacturing process. Thus, the data is not representative. Please provide precision data from (b) (4) sites using syringes that are filled by actual manufacturing process.

Review of the Response: The sponsor explained that the objective of the study is to demonstrate adequate method performance at the (b) (4) site. Thus, using syringes that are filled by actual manufacturing process should not be necessary. The (b) (4) filled syringes are representative of the products obtained by the actual manufacturing process. This is acceptable, particularly given that this is a (b) (4) method.

#### **19. Test for Total Protein in (b) (4) (Drug Product) by (b) (4)**

DSBQC Reviewer: Tao Pan

Information submitted and reviewed included:

- 3.2.P. Influenza Virus Vaccine-Injection-Novartis Vaccines and Diagnostics (b) (4)
  - 3.2.P.5. Control of Drug Product
  - 3.2.P.5.1. Specifications
  - 3.2.P.5.2. Analytical Procedures
  - Analytical Procedures
  - Analytical Procedure Total Proteins (b) (4) (SOP 306039)
  - 3.2.P.5.3. Validation of Analytical Procedures
  - Validation of Analytical Procedure Intro
  - Validation of Analytical Procedures Total Protein
  - Attachment 28 (b) (4) Report Eng (ISU07.013VR3Rev.5)
  - Attachment 30 (b) (4) Protocol Eng (ISU07.13VP3Rev.0)
  - Attachment 31 LVP Transfer Report (R/0394/09/13)

The Fluad vaccine drug product is presented as a 0.5 mL single dose sterile suspension for injection, containing three viral strain surface antigens and the adjuvant MF59C.1. The total protein content of the final drug product is measured to calculate the content of proteins other than hemagglutinins that is determined by (b) (4)

SRID result from the total protein amount. The specification for lot release is (b) (4) dose.

Method:

The total protein content is determined by the (b) (4) method which is based on the (b) (4). The resulting (b) (4). The amount of protein present in a sample is determined by (b) (4). The method was developed and validated at the sponsor's (b) (4) site and transferred to the (b) (4) site. Information was provided by the sponsor (SOP 306039), including descriptions on the preparations of the standards and samples, assay method execution, assay result evaluation, reportable result generation, and assay validity criteria, it is adequate and clear.

Validation:

This method was initially validated as a quantitative assay for drug product (validation protocol: ISU07.13VP3Rev.0; validation report: ISU07.013VR3Rev.5) at (b) (4) site, the assay characteristics validated include: precision (repeatability and intermediate precision), accuracy, specificity, linearity, and range. The assay was transferred (transfer report: R/0394/09/13) and currently used in the sponsor's (b) (4) site for the lot release of drug product.

(b) (4)

(b) (4)

(b) (4)



1 page determined to be not releasable: (b)(4)

(b) (4)

### Conclusion

Based on the assay validation report, and the assay transfer report, it can be concluded that the total protein content by (b) (4) method has been validated for its intended use: the determination of total protein content in drug product, it has been successfully transferred to the (b) (4) facility, and is approvable for the release testing of drug product at the current site.

### **Appearance and pH**

DBSQC Reviewer: Kouassi Ayikoe

Documents submitted and reviewed

- 3.2.P.1 Description and Composition of Drug Product
- 3.2.P.5.1 Specifications ((b) (4) Vaccines and Final Filled Vaccines).
- 3.2.P.5.2 Analytical Procedures-Drug Product
  - SOP 278840 “Analytical Procedures – pH”
- 3.2.P.5.3 Validation of Analytical Procedures (Drug Product)

### **20.Appearance**

For Final Filled Vaccine, the appearance and visible particles testing are accomplished by visual inspection against both (b) (4). The validations were performed at (b) (4) followed by method transfer from (b) (4). After inspecting a minimum of (b) (4) samples per lot from each site, no deviation from conformity was observed; therefore, the products meet the specification.

### **21. pH**

Method - As a (b) (4) method the pH of the sample is measured after the calibration of the instrument with at least (b) (4). The calibration tolerance must be within (b) (4) of the certified value. As depicted in the SOP CQS

(b) (4)

For (b) (4) Vaccine, sample pH, a (b) (4) method, depicted in (b) (4) is measured after calibration of the instrument with suitable reference solutions. The procedure is described in the SOP # 278840 and the specification is 6.9 7.7 unit pH. The validation of the pH for the (b) (4) Vaccine is same as that established for the (b) (4)

The pH determination and validation for Final Filled Vaccine are carried on the same way as in Drug Products.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

4 pages determined to be not releasable: (b)(4)