

Single Radial Immuno-Diffusion Memo, December 3, 2012-Q-Pan

• DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and
Drug Administration

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Chair, BLA Review Team

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Subject: STN 125419: BLA for Influenza A (H5N1) Virus Monovalent Vaccine, ID Biomedical Corporation of Quebec (dba GlaxoSmithKline Biologicals): Review of Single Radial Immuno-Diffusion (SRID) assay used in Potency and Identity Testing

On 22 February 2012, ID Biomedical Corporation of Quebec (dba GlaxoSmithKline Biologicals) submitted a Biologics License Application (BLA) for Influenza A (H5N1) Virus Monovalent Vaccine. This Memo covers the reviews of the analytical procedures and the associated validation reports for Single radial Immuno-diffusion assay (SRID) performed for testing of -----(b)(4)----- Drug Product (DP)

Submissions Reviewed

125419/0.0

- Section 2.3 Quality Overall Summary
- Section 2.3.S.: Control of -----(b)(4)-----:
- Section 2.3.P. Control of Drug Product-Antigen
- Section 3.2.S.4.2: Analytical Procedures
- HA Content by SRID
- Section 3.2.S.4.3: Validation of Analytical Procedures
- HA Content by SRID --- (b)(4)-----
- Section 3.2.P.5.2 Analytical Procedures
- HA Identity and content by SRID Antigen
- Section 3.2.P.5.3 Validation of Analytical Procedures
- HA content by SRID Antigen
- Section 3.2.R. Regional Information:
- HA Content Summarized SOP 9000018772 --- (b)(4)----
- HA Identity Summarized SOP 9000018770 Antigens
- HA content Translated SOP 9000018734 Antigens (titled: VR010 Radial Immunodiffusion for Low HA-concentration Influenza Vaccine- 9000018734-V08)

125419/0.4

- Section 1.11.1 Quality Information Amendment
- Request for Information from April 30, 2012- Method Validation Responses (questions 12-23)

125419/10

- Section 1.11.1 Quality Information Amendment

Review Summary-SRID Assay:

SRID test is used to determine Haemagglutinin (HA) content in the influenza vaccine using specific anti-HA antibodies and haemagglutinin reference standard. Test samples and reference antigen standard are treated with a zwitterionic detergent which allows the separation of antigens in an agarose gel containing a specific antiserum. A volume of the samples and reference dilutions are applied onto an agar plate containing a strain-specific antiserum. The plates are incubated in a moist chamber at room temperature to allow diffusion of the antigen. Reaction of the antigen with the antibody produces a zone of precipitation (which is in form of precipitin ring). After washing and drying the gel plates, precipitation zone, is visualized by Coomassie blue staining. The HA quantification is performed by comparing the ring diameters of samples with the diameters of known concentrations of the reference. The SRID can be used both as a qualitative test to identify as well as a quantitative test to determine concentration of haemagglutinin in the vaccine/sample.

GSK performed the validation of SRID method using: a) -----

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

----- Validation parameters evaluated include the specificity, linearity, linear range, accuracy, repeatability and intermediate precision. -----

----- (b)(4) -----
----- All the acceptance criteria were met during the validation.

Following the review of the methods/documents listed above, some documents clarification was needed. An information request (IR) was sent to the Sponsor on April 30, 2012. In this IR questions 12-14 were pertaining to SRID documents/method. Sponsor submitted an amendment (125419/0.4) on July 19, 2012. CBER IR question, GSK response ((italicized) and CBER comments to GSK responses submitted in amendment 0.4 are summarized below:

Question 12: In your Translated SOP 9000018734-V08 VR010 Radial Immunodiffusion for Low HA-Concentration Influenza Vaccine, you have stated that dilutions of ----(b)(4)----- will be prepared (from reference antigen at --(b)(4)-- starting concentration) to achieve concentrations of -----(b)(4)----- . We have following concerns:

a) The standard curve range for the SRID test for the seasonal influenza vaccine is ----- (----- (b)(4) -----), whereas the standard curve range for the current method is only ----- (b)(4) ----- . Please provide data on SRID ring diameter from multiple tests to demonstrate that the ring sizes at adjacent concentrations do not overlap.

GSK Response: *“Please note that for the seasonal influenza vaccine, the standard is set at (b)(4) µg HA/ therefore leading to standard dilutions from (b)(4) µg HA/ml to (b)(4) µg HA/ml. For the Quebec H5N1 drug product, the standard is set at --- (b)(4) ---/ ml therefore leading to standard dilutions from ---- (b)(4) ----- HA/ml. The Both the seasonal and the lower HA concentration methods require generating diffusion ring of similar diameters (between -- (b)(4) ----- for the standard at 100% dilution). As the HA content in the trivalent seasonal drug product before dilution is 2-fold greater than the Quebec H5N1 drug product, the required dilution factor to obtain a similar ring diameter for the seasonal drug product is also 2-fold greater than required for the Quebec H5N1 drug product.*

Figure 1 and Figure 2 present some agar plate pictures from different analyses of Quebec H5N1 drug product. These gels and the corresponding diffusion ring diameter show that there is no overlap observed between adjacent concentrations.”

CBER Comment: GSK explains that that for both seasonal influenza vaccine and lower HA concentration methods, assay requirement is to generate diffusion ring of similar diameters (between 8 and 10 mm for the standard at 100% dilution). Based on this Quebec H5N1 drug product, the standard is set at 20 µg HA/ ml (i.e. which is 100%) leading to standard dilutions from ---- (b)(4) -----

----- HA/ml. The representative data provided in the response shows that the diffusion ring diameter variation do allows proper differentiation between closely related dilutions. **Sponsors response is acceptable.**

b) Specification for final product is set as “Not less than(b)(4) HA/ml”, similar dilutions of the final product in SRID assay would lead to ----(b)(4)----- mg HA/ml. Based on these concentrations and concentrations of reference antigen used in the test, there is not enough overlap between the reference and the sample curves to demonstrate meaningful parallelism between these 2 curves. Please explain.

GSK Response: "In the SRID assay, there is a full overlap between the two curves as the linear regressions are generated by the diffusion ring diameters (in log scale of the y-axis) as a function of the dilution factor (in log scale of the x-axis) and the identical four serial dilution factors (100%, 85%, 70% and 50%) are applied on both the reference standard at (b)(4) mg HA/ and the sample to be analyzed. As seen in -----

(b)(4)

----- The linear regression analyses shows that the assay is suitable to determine the HA concentration ((b)(4) HA/ml in this example) and appropriately determine if the sample meet the release or end of shelf-life specifications. In fact, the lowest HA concentration of the working range determined by the method validation is ----(b)(4)----.

CBER Comment: -----

(b)(4)

----- Based on the explanation provided sponsors response is acceptable.

Question 13: The linearity of the SRID has been performed by diluting reference and sample to (b)(4) concentrations each and measuring the ring diameter. Correlation coefficient was calculated by plotting log concentration vs. log diameter. Linearity of the method should be demonstrated by diluting samples in a matrix at different concentration and testing the diluted samples in a standard SRID assay using appropriate reference antigen. The test should be performed as per requirement for testing either monovalent or ---(b)(4)- as appropriate. The reportable results from such tests should be used to perform linear regression analysis. Please comment.

GSK Response: “In response to CBER’s request, GSK is providing the following information. The validation study for this test was designed and carried out according to the ICH guidance on Validation of Analytical Procedures Q2 (R1). The validation design was built to demonstrate that the method is specific, linear, accurate and precise. As per ICH guidance: “A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of the synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.” During the validation of the method and as described in Module 3.2.S.4.3 HA Content by SRID Monovalent --- (b)(4)-- Module 3.2.P.5.3 HA Content by SRID – Antigen, the Company opted for demonstrating the linearity of the assay on the matrices representative of the ----(b)(4)----- drug product. Indeed, for both SRID validations (i.e. ----(b)(4)----- and drug product), the linearity was demonstrated using a matrix of known HA concentration, as determined with a validated SRID method, and then diluted with PBS to (b)(4) different HA concentrations. In both validations, the matrix is composed of seasonal strains as a worst case representative of the -----(b)(4)----- drug product. The (b)(4) matrix concentrations were analyzed, using the method parameters to be validated for both the ----(b)(4)----- drug product, with the appropriate reference antigen standard at the same (b)(4) concentrations in triplicate-----
----- (b)(4)-----

-----The methods adequacy (for both the ----(b)(4)----- drug product) for their intended uses has been demonstrated by the specificity results showing that excipients and potentially interfering compounds present in the matrix do not interfere in the HA quantification along with the accuracy and precision results that met their method validation requirements. The same validation approach has been previously presented for the seasonal influenza vaccine FluLaval (STN: BL 125163-210, Approved December 14, 2011) as the SRID method and method validation used for the seasonal ----(b)(4)----
---- is the same as that used for the Quebec H5N1 --- (b)(4)-----.

Examples of a sample and standard linearity calculations presented in Module 3.2.P.5.3 HA Content by SRID – Antigen for the drug product method (9000018734) validation are shown below in Figure 4 and Figure 5. -----
(b)(4)-----
----- demonstrating the linearity of the method.”

CBER Comment: GSK's response explains the approach for demonstrating the linearity of the assay using matrices representative of the ----(b)(4)----- drug product. Samples were diluted to (b)(4) concentrations in parallel with the appropriate reference antigen standard at the same (b)(4) concentrations in triplicate---(b)(4)-----

-----In addition sponsor has stated that the same validation approach has been previously presented for the seasonal influenza vaccine FluLaval (STN: BL 125163-210, Approved December 14, 2011) as the SRID method and method validation used for the seasonal ---(b)(4)----- is the same as that used for the Quebec H5N1 --- (b)(4)----. **Based on the explanation provided and on the information that similar validation approach has been approved for seasonal influenza vaccine FluLaval, sponsor's response is acceptable.**

Question 14: Accuracy studies have been performed by testing samples of vaccine diluted to specific concentrations and using these target concentrations as the theoretical values. The Accuracy is calculated as:

$$\text{Accuracy} = \frac{\text{Average experimental value} \times 100}{\text{Theoretical value}} = \text{-----}$$

Please clarify how were the original concentrations of these samples were determined.

GSK Response: *In response to CBER's request, GSK is providing the following information. The concentration of the matrices used for the ----(b)(4)----- drug product SRID assay, prior to dilution and preparation, was determined with a validated method used for the seasonal trivalent drug product.*

The matrix original HA concentration, which was determined by the validated method following the standard testing procedure for the seasonal trivalent drug product, was diluted to obtain three different theoretical concentrations.

CBER Comment: GSK response is acceptable.

Subsequently additional information request was sent to GSK (via email from Jeremy Wally on September, 25, 2012) to clarify questions regarding information provided on the SRID assay. CBER IR question, GSK response and CBER comment to GSK responses submitted in an amendment (125419/0.10) on September 25, 2012 are summarized below:

Question1: For VR010 Radial Immunodiffusion for Low HA-concentration Influenza Vaccine- 9000018734-VO8, the SOP states that -----
----- (b)(4)----- . Please clarify following in your SOP:

- Please clarify what is meant by “at least three tests” and describe any instances when more than 3 tests are performed.
- Please provide the specifications for potency value and standard deviation (SD) for any cases when more than 3 tests are used to calculate results.
- Please describe how the out of specification results are handled and additional testing performed if the calculated potency value from the initial 3 tests is out of specification.
- Please specify the potency specifications (along with SD) for ---(b)(4)-- final container vaccine based on number of tests performed.

GSK Response: *“Please note that analytical methods, as is the case for the SRID method 9000018734, are not market specific. Consequently, the procedure described in the SOP is general where only the analytical result interpretation will reflect any market specific requirements, if applicable.*

Please refer to GSK’s response to Question #4 from the July 30, 2012 CBER information request from C. Collazo (CBER) to K. Abraham (GSK) contained within this submission as m1.11.1 Quality Information Amendment – Response to Request for Information from July 30, 2012 – CMC (Question 4) for the requested information.”

In the Quality Information Amendment – Response to Request for Information from July 30, 2012 – CMC (Question 4), GSK included: “In addition, further to discussions with CBER’s lot release group (Dr. Gupta and Dr. Joshi) on August 16, 2012, and in order to allow CBER to release the Quebec H5N1 final bulk according to their historical lot release requirements for influenza vaccine, the potency estimated by the SRID assay for the(b)(4) ----will be calculated with 3 valid gels only. Re-testing with 3 gels will only be performed if there are not 3 valid gels from the initial sequence. If there are 3 valid gels and the mean obtained is below the minimum release acceptance criteria (MRAC) for the --- (b)(4)--- it will be considered out-of specification and investigated accordingly. Hence, the HA content specification for the Quebec H5N1 ----- (b)(4) at release will be not less than ----- (b)(4)---- and n= 3 gels). Therefore, the specification for the (b)(4) presented in the original BLA (m3.2.P.5.1 Specifications Antigens --(b)(4)--- (STN 125419, sequence 0000, submitted February 22, 2012)) is being revised to restrict the potency assessment from 3 valid SRID gels only, i.e., N = 3.

As stated in m3.2.P.2.2 Drug Product - Shelf-life Evaluation Antigens, in order to calculate a single mean value globally for all markets (as the Eur. Ph. requires taking all valid gels into account), the SRID assay for the final container potency will be run with a minimum of 3 gels (typically --- (b)(4)--- are prepared to reduce repeated testing). The mean result will therefore be calculated including all the gels performed that meet the method and restricted standard deviation (b)(4) acceptance criteria. Re-testing with a minimum of 3 gels will only be performed if there are less than 3 valid gels from the initial testing sequence. If there are at least 3 valid gels from a sequence and the mean result obtained is below the minimum release acceptance criteria (MRAC) for the final container, it will be considered out-of-specification and investigated accordingly. The

Company would like to highlight that CBER will perform lot release on the final bulk and thus the testing voluntarily implemented on the final container is an additional control to monitor the extent of HA loss.

CBER Response: As regards to testing strategy for --(b)(4)-- i.e. specification at release- -----(b)(4)-----n= 3 gels is acceptable. However, GSK's response for testing potency of final container stated that "potency will be run with a minimum of 3 gels (typically (b)(4) gels are prepared to reduce repeated testing). The mean result will therefore be calculated including all the gels performed". In the view of this reviewer, proper statistical evaluation of this testing strategy needed to be assessed. DBSQC requested and deferred to the Statistical reviewers on the submission to evaluate the suitability and related statistical implication of the testing strategy proposed by GSK. Statistical Reviewers concerns and comments regarding the minimum release acceptance criterion (MRAC) for final container and --(b)(4)- were communicated to sponsor as IR on November 09, 2012 (email from Kirk Prutzman). On November 27, 2012, GSK provided a slide presentation in which they discussed their proposal for the SRID specifications for the --(b)(4)----- final container.

A conference call was held on November 28, 2012. GSK presented the slides which were provided to CBER on November 27, 2012, and went over the history of the submission, including the advice given during the IND phase of the candidate vaccine. GSK's proposed criteria for HA potency specifications for ---(b)(4)----- and Final Container Release and End of Shelf Life are shown in Table below

Table

	Mean Result HA/ml) (µg	Assay Variability	Assay Replicates
----- (b)(4) -----	(b)(4)	----- (b)(4) -----	(b)(4)
Final Container Release and End of Shelf Life	(b)(4)	----- (b)(4) -----	Min 3-gel

During the discussion GSK responded that they routinely run (b)(4) for SRID testing of the final container and all gels that meet the assay validity criteria are used for the calculation of HA content. They use a minimum of 3 valid gels to calculate the mean. "Valid" gels are gels that meet the acceptance criteria for the SRID assay and these acceptance criteria are the same as those applied for the seasonal vaccine. The (b)(4) are performed to avoid the need for repetition of the assay, especially important during a pandemic situation. Variation greater than -----(b)(4)---- in the gels performed leads to rejection of assay results.

CBER noted that the Minimum Release Acceptance Criteria are different for the --- -----(b)(4)----- the Final Container (FC) (Reference to slide 12 from GSK presentation). GSK responded that the overage is built in to sustain the shelf life. They are tightening the assay variability (there is more precision incorporated in the release

model), and whereas the (b)(4) is limited to only 3 gels being run, the FC calculations include all valid assay replicates ((b)(4) are run routinely and the test results will include all valid gels, with a minimum of 3 gels required). One value is reported globally for FC release and stability.

CBER explained that the intent of historical specifications applied to seasonal influenza virus vaccines is that (b)(4) mg HA/ml is the mean result and (b)(4)mg HA/ml is the lower confidence. GSK responded that if these criteria are applied, one clinical lot (lot AFLPA109A) would be "OOS" at release (Reference to slide 17 from GSK presentation titled "Overview of Manufactured Lots and Impact of 95 % LCB at (b)(4)-mg HA/ml"). This lot was used in clinical study Q-PAN H5N1-002 and the clinical data was used to support the BLA submission. Furthermore, the shelf-life of the vaccine would be significantly reduced (predicted 12 months). Overall, the impact of applying a 95 % LCB at (b)(4) µg HA/mL could lead to a --(b)(4)-- increase in overage, resulting in a decrease in the HHS stockpile, a decrease in manufacturing capacity, and an increase in manufacturing cost (Reference to slide 19 from GSK presentation titled "Impacts of 95 % LCB at (b)(4) mg HA/ml").

Based on the GSK's presentation and subsequent discussions CBER agreed that there are some differences with GSK's proposal as compared with what CBER typically accepts for seasonal influenza vaccines. At this point CBER agrees with GSK's proposal noting that this is thought of as being a unique situation for the Q-Pan H5N1 pandemic vaccine (which is dose sparing and contains an adjuvant) as compared to seasonal influenza vaccines and that the proposed release criteria may be considered without impact to CBER's long history for handling unadjuvanted inactivated seasonal influenza vaccines lot release.

Question2: For HA Content Summarized SOP 9000018772, the SOP states, "The HA content determination, based on the parallel line bioassay method, is performed with the validated spreadsheet. For the whole virion monovalent intermediate and monovalent drug substance, (b)(4) replicate gels are tested, and the geometric mean of the (b)(4) results is reported as the HA content." No specification is described regarding the standard deviation for the calculated results. Please include these specifications in the SOP and submit the revised SOP.

GSK Response: "In response to CBER's request, GSK is providing the complete SOP as well as the following explanation. An English summary of the SRID method SOP 9000018772 was provided (m3.2.R HA Content Summarized SOP 9000018772 Monovalent (b)(4)) in the original BLA (STN 125419, sequence 0000, submitted on February 22, 2012). The SOP currently contains the standard deviation criterion to be applied for the -----(b)(4)--- HA content individual gel results. As stated in the English translated SRID method 9000018772 SOP, provided herein as m3.2.R Single Radial Immunodiffusion 9000018772 VE007, the analysis is valid if the standard deviation is (b)(4)-. Please note that the SRID method SOP 9000018772 is also used and approved for the seasonal influenza vaccine FluLaval (as most recently provided to CBER for STN BL 125163, sequence 0079 on November 11, 2012).

CBER Response: GSK response is acceptable.

Conclusion:

The data submitted to support the SRID assay used for testing of -----(b)(4)----- Drug Product for Influenza A (H5N1) Virus Monovalent Vaccine was reviewed. Following initial review of the submission questions were communicated to the sponsor as information requests. During review it was noticed that there are some differences in GSK's proposed strategy and release criteria as compared with what CBER typically accepts for seasonal influenza vaccines. Following discussions with sponsor CBER agreed with GSK's proposal noting that this is thought of as being a unique situation for the Q-Pan H5N1 pandemic vaccine (which is dose sparing and contains an adjuvant) as compared to seasonal influenza vaccines and that the proposed release criteria may be considered without impact to CBER's long history for handling unadjuvanted inactivated seasonal influenza vaccines lot release. Based on the review of original submission, information provided in amendments (listed above) and the CBER's negotiations with GSK on setting specifications following the November 28, 2012 teleconference, I recommend approval of this application.