

Information Request, April 30, 2012-Q-Pan

**CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
OFFICE OF VACCINES RESEARCH AND REVIEW
DIVISION OF VACCINES AND RELATED PRODUCTS APPLICATIONS**

DATE:

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PAGES:

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TO:

GlaxoSmithKline Biologicals
Attention:Ms. Katalin G. Abraham
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FROM:

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Scientific Reviewer
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SUBJECT:

STN: BL 125419/0 – Request FOR Information

MESSAGE:

Dear Ms. Abraham:

Reference is made to your Biologics License Application dated February 22, 2012, for Influenza A (H5N1) Virus Monovalent Vaccine. We have the following request for information:

Regarding the Pediatric Plan:

1. Reference is made to your request for deferral of the Pediatric Research Equity Act (PREA) requirement to submit a pediatric assessment for children from birth through 17 years of age (refer to Module 1.9.2). Please note that a Pediatric Plan must be submitted for all deferred studies. A Pediatric Plan is a statement of intent that outlines the pediatric studies that you plan to conduct or are conducting (i.e., the pediatric studies that will comprise the pediatric assessment). The plan should address all pediatric subpopulations (from birth through 17 years of age), the development of an age-appropriate formulation (if necessary), and must contain a timeline for the completion of studies. We recommend that the timeline includes the dates that you will: (a) submit the protocols; (b) complete the studies; and (c) submit the study reports.

Regarding stability data:

2. Please submit updated stability data on the AS03-adjuvanted Quebec H5N1 influenza vaccine lots from the recent time points evaluated in the various stability plans.

Regarding validation information on clinical assays:

3. Please submit the full validation report for the microneutralization assay.
4. Please provide the original full validation report for the Haemagglutination Inhibition Test.
5. Please provide SOP PF-015 (*Determination of influenza antibodies in human sera with Haemagglutination Inhibition Test - Test with ----(b)(4)-----*), including method details and expression of the results.
6. Please provide the validation protocols QVALP-PF-015 and QVALP-PF-015-04 for the validation reports found in Module 5.3.5.4.3.
7. We have the following comments regarding the validation report CODE No. QVALR-PF-015, Version No. 01/E :*Determination of Influenza antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) - Test with ----(b)(4)-----*:
 - a. In Section 6.2: *Operator's validation results* (page 6), we noted that the HI results for the validation of operators are missing (Annex 2). Please provide this information.

- b. In Section 6.3: *Results for repeatability* (page 6), we noted that the HI results for precision and repeatability are missing (Annex 3). Please provide this information.
 - c. In Section 6.4: *Check of effective concentration of test* ---(b)(4)-----, we noted that although both a (b)(4) or a -----(b)(4)----- solution (batch no. ---(b)(4)--) were suitable for use in the HI test, you decided to use a -----(b)(4)----- because the results were closer to the declared titer of the tested control sera. For both the -----(b)(4)----- treatments, two test results from the (b)(4) control samples tested were observed to be “out of specification” (page 7). Please explain how you plan to address this finding in the context of assay validation.
 - d. In Section 6.5: *Comparison of the new (b)(4) method with manual testing*, you indicated that the clinical trial Flu-051 (Fluarix 2002/203) was tested manually and with the new (b)(4) equipment. A summary table was provided for seroconversion and seroprotection rates. While there was no significant difference between both methods, there was a tendency for variation in the seroconversion and seroprotection rates for the two test methods. In serum samples tested from subjects in the age group > 60 years, the (b)(4) method was less sensitive compared to the manual method for detection of seroprotection titers against H1N1 and B strains. For validation assessment, please submit the raw data with HI titers obtained for the serum samples using both methods for the tested samples. In addition, please clarify which HI test was used throughout the Q-Pan H5N1 Phase 3 trials (i.e., the (b)(4) method or manual testing).
8. We have the following comments regarding the validation report CODE No. QVALR-PF-015, Version No. 04/E: *Determination of Influenza H5N1 antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) - Test with horse blood cells*:
- a. Please provide SOP PF-015-06, <>Annex 9 (see Section 6: *Summary and Conclusion*, page 4).
 - b. Please provide the data for the ---(b)(4)----- treatment using horse blood cells.
 - c. Please specify the concentration of horse blood cells used in the HI test.
 - d. Regarding Section 4.3: *Robustness of horse blood cells*, we noted that data obtained with four serum samples showed identical HI titers when using fresh or stored horse blood cells (page 4). Please clarify whether the cells were stored ---(b)(4)----- the horse blood cells or in the form of whole blood.
 - e. Please provide the data that evaluate the effect of different antigen concentrations (Hemagglutination units) using horse blood cells.
 - f. In Section 5.1: *Control serum panel and intermediate precision*, the acceptance criteria for intermediate precision were fulfilled using a set of six serum samples (page 3). We noted that all four positive sera had high HI titers. Please indicate whether any of the serum samples tested had low HI positive titers (HI titer range: -(b)(4)-). This serum control panel should include samples with low to intermediate HI titers of ---(b)(4)----- to determine the precision, repeatability, and robustness of the assay. Please comment.

9. We have the following comments regarding the validation report CODE No. QVALR-PF-015, Version No. 05/E: *Determination of Influenza H5N1 antibodies in human sera with Haemagglutination Inhibition Test (H.I.T.) - revalidation for A/Indonesia/05/2005 x PR8-IBCD-.RG2*:
 - a. Please provide the data for ---(b)(4)----- treatment using horse blood cells.
 - b. Please specify the concentration of horse blood cells used in the HI test.
 - c. Please provide the data that evaluate the effect of different antigen concentrations (Hemagglutination units) using horse blood cells.
10. We have the following comments regarding the validation report PRO.MPCR.002: *Detection of Influenza A and B by PCR Roche and subtyping of Influenza A hemagglutinin*:
 - a. In the PCR amplification parameters (page 7), please confirm that the ----- (b)(4)----- times for the pandemic H1N1 2009 are only (b)(4) second.
 - b. Please provide PRO.MPCR 001 (*Basic use of the real-time PCR device ----- (b)(4)-----*).
 - c. Regarding your statement in Section 5.1: Check the validity of the control results, “uncertain PCR test: the test is complete. Output the *negative* release clarify the reason for not repeating any uncertain PCR tests.
 - d. In Table 5.2: Interpretation of the PCRs, a CP value of “(b)(4) and above” was reported to be “negative” (page 9). Please explain how you arrive to the meaning of this parameter. Also, please define CP and explain how it is different from the commonly used term “cycle threshold (Ct or CT)” used for RT-PCR data.
 - e. In Annex #3: Preparation of positive controls of Influenza A and B (page 15), please note the following:
 - i. It is not clear how the RT-PCR template was optimized just based on viral culture supernatant dilutions. Please provide the TCID₅₀ for the virus particles in the culture supernatant isolated at a cytopathic effect of 3+ following MDCK infection that were used to prepare controls for the RT-PCR assay.
 - ii. Please provide the reasoning for selecting a working dilution for each strain that gives a CP of about 23. Since this dilution may be different for Influenza A and Influenza B, please state the dilutions used for each strain. Please explain how this dilution correlates with RNA copies/ml for the template used in the RT-PCR assay. Other studies (e.g., see publication JCM 2009, 9; 2675-77) showed a detection of 50 fg of viral RNA (~5200 viral genome copies) in RT-PCR assays.
 - f. In Annex #6: Output of PCR influenza reports in -(b)(4)-, please clarify the use of two codes for the same outcome (e.g., MIAP and MIAPA for [Influenza A] PCR: positive and Influenza- B PCR: negative on page 22) and for HA subtyping on page 26.
 - g. Please indicate what (b)(4) stands for in the statement “When you have a positive influenza A, you must request the ---(b)(4)--- (page 24).
 - h. The improved RT-PCR assay detects pandemic H1N1-2009, H5 and Influenza B at a 10⁻⁶ dilution and H7, H1 and H3 at a 10⁻⁵ dilution. No false positive

results were detected. Since the result shown is up to 10⁻⁶ dilution for pandemic H1N1-2009, please indicate whether there were any dilutions below 10⁻⁶ tested for pandemic H1N1-2009 virus to identify the detection limit of pandemic H1N1-2009 using this RT-PCR assay.

11. We have the following comments regarding the validation report PRO.MICV.003:

Preparation of specimens in virology:

- a. Please provide the complete composition of the antibiotic solution (b)(4) and the rationale for using ---(b)(4)----- per 1 ml of specimen.
- b. Please clarify the use of the same code "CVH" for the group of viruses belonging to the HSV group and VZ, VZV, Varicella groups (page 7).

Regarding validation information on HA content by SRID:

12. In your Translated SOP 9000018734-V08 VR010: *Radial Immunodiffusion for Low HA-Concentration Influenza Vaccine* (Module 3.R: Regional Information), you stated that dilutions of ---(b)(4)----- will be prepared (from reference antigen at ---(b)(4)----- starting concentration) to achieve concentrations of (b)(4) and ---(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.
- b. Since the specification for final product is set as "Not less than (b)(4) µg HA/ml", similar dilutions of the final product in SRID assay would lead to -----(b)(4)----- mg HA/ml. Based on these concentrations and the 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.

13. The linearity of the SRID was performed by -----
----- (b)(4) -----

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

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

Average experimental value x 100

Accuracy= % Recovery = -----
Theoretical value

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

Regarding other validation reports:

15. We have the following comments regarding the report *Formaldehyde Content by* ---(b)(4)----- (Module 3.2.S.4.3):

- a. It is not clear what --- (b)(4) --- was used in the validation of this method. Please confirm whether H5N1 was used as the --- (b)(4) ---. If a different --- (b)(4) --- was used in the validation studies, please indicate the --- (b)(4) --- used and explain why that --- (b)(4) --- should be considered representative of H5N1.
- b. You stated on page 6 of the validation report for the formaldehyde content assay, "... --- (b)(4) --- solution is added" to prepare --- (b)(4) ---. Please describe the final concentration of thimerosal in --- (b)(4) --- and --- (b)(4) --- and explain how these solutions are representative of the --- (b)(4) ---.
- c. Pages 7 and 8 of the validation report show that the linearity of the assay was demonstrated only with the --- (b)(4) ---, which were prepared in (b)(4). Linearity should also be studied with the --- (b)(4) --- matrix and the regression line shown to be parallel to that of the standard curve. Please provide linearity results in the --- (b)(4) --- matrix and a comparison of linear-regression fit of results with that obtained with a standard curve generated in (b)(4).
- d. Page 8 of the validation report shows that the Y-intercepts (k_0) are about as much or greater than the absorbance at --- (b)(4) ---. Given the large value of the intercepts, it is difficult to agree that the range of the assay is --- (b)(4) ---. Please recalculate the assay range taking the high value of Y-intercept (residuals) into account and submit the results.
- e. The summary of the test method SOP included in the submission shows that the standard solutions are prepared at --- (b)(4) --- concentrations. This range is not consistent with the range determined by the validation studies, --- (b)(4) ---. Please explain this discrepancy or revise the SOP to make it consistent with the assay range and submit the revised version.

16. Regarding the validation report *Protein Content by* --- (b)(4) --- ((Module 3.2.S.4.3), we note on page 5 of the report that you stated, "The theoretical concentrations of the samples were --- (b)(4) ---." Page 7 of the report mentions an additional theoretical concentration, --- (b)(4) ---. Please describe how these theoretical concentrations were determined.

17. We have the following comments regarding the validation report *Sodium Deoxycholate by* (b)(4) (Module 3.2.S.4.3):

- a. -----

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

----- However, both Figures 1 and 2 show plots of Intensity vs. Minutes. They appear to be chromatograms and do

- not have m/z axes, as stated in the text. Please provide the actual ---(b)(4)---- results (----(b)(4)-----), which are described in the text.
- b. The linearity of this assay was demonstrated only with standards. Please provide linearity results also in the worst-case scenario matrix used in the validation and a comparison of linear-regression fit of the results in product matrix with that obtained with standards
 - c. You indicated on page 4, "The analyses of linearity were performed using a linear regression with a weighting of 1/x." Please provide details of the analysis. Also, please explain why the "weighing" method is appropriate.
 - d. Intermediate precision was studied by (b)(4) analysts only. This should also be done over -(b)(4)- days and using more than (b)(4) column. Please provide comparative results performed over at least (b)(4) days and also using at least (b)(4) columns.
18. Regarding the report *Protein Content by* ---(b)(4)----- (Module 3.2.P.5.3), you stated that the linearity of the method was studied using ---(b)(4)---- standards." Please indicate the material(s) used as the standard for this assay. Also, please describe how the concentrations were determined.
19. We have the following comments regarding the report ----- (b)(4)----- (Module 3.2.P.5.3):
- a. A quadrivalent influenza drug product was used to represent the drug product during method validation. Please describe what drug product was used and why a quadrivalent product was used instead of the actual drug product. Please explain why the quadrivalent influenza drug product is representative of the Influenza A drug product.
 - b. The linearity of the assay was demonstrated only with the standards, which were prepared in water. Please provide linearity results in product matrix and the comparison of linear-regression fit of the results in product matrix with that obtained with standards.
 - c. Please describe the conditions, including temperature and exposure to light, under which the samples were held during the *Post-preparative Stability of Sample* study.
20. Table 5 (page 14) of the report *Thimerosal Content by* ----(b)(4)----- (Module 3.2.P.5.3) provided *Accuracy and Repeatability* results using ----- --(b)(4)-. It is not clear how the accuracy percents of thimerosal were calculated. Also, the table did not show the results from the --(b)(4)- matrix. Please provide the results from the --(b)(4)-matrix and explain the calculation of accuracy percents.
21. We have the following comments regarding the report *Tocopherol and Squalene Identity and Content by* --(b)(4)- (Module 3.2.P.5.3):
- a. According to the SOP for this assay (Doc # 9000010419 – Version 01), the range of concentrations of the standard solutions is -----(b)(4)----- for both tocopherol and squalene for the determination of tocopherol and squalene content in AS03A. However, the validation studies indicate that the target concentrations of the analytes were ---(b)(4)--- each and the validation has been done in the range of ---(b)(4)----- of each, which is below the range of the concentrations of the standards in the SOP. Please explain why we should accept the validation of this assay.

- b. The validation report shows a representative chromatogram from a sample and indicates that this was evaluated against the chromatogram of “other ingredients of the formulation.” However, you did not include a chromatogram of the “other ingredients of the formulation” sample in the validation report. This result is important to ensure that no peak coelutes with either tocopherol or squalene because the peak purity results were not included in the submission as part of the specificity of the assay. Please provide either the chromatogram of the “other ingredients of the formulation” sample or results of the peak purity analysis for tocopherol and squalene.
 - c. It is not clear whether the results presented in Table 2 (page 5) of the report were obtained from solutions prepared in ---(b)(4)--- matrix (in which, according to the SOP, the standards were prepared) or in the product matrix. Please provide linearity results from solutions prepared in both ---(b)(4)--- (for standard solutions) and in product matrix and the comparison of linear-regression fit of the results between the two matrices.
 - d. Table 2 of the report (*Linearity* study) did not show the value of the Correlation Coefficient (R). Please provide these results.
 - e. A placebo formulation was used in the *Accuracy* study (page 6). Please describe the placebo formulation in detail.
 - f. You stated on page 6 of the report (*Accuracy* section), “The % recoveries were computed using a ---(b)(4)--- level - linear through ---(b)(4)--- model.” However, the results shown in Table 2 and the text on page 5 of the report (under *Linearity*) indicate that the intercept of linear-regression fit of the standard line “was different from 0.” Please explain this discrepancy.
 - g. The *Repeatability* study was done at around --(b)(4)-- concentration, which is below the range otherwise studied in the validation ----(b)(4)----- and also below the specified range of the standard in the SOP ----(b)(4)----- Please explain how the submitted *Repeatability* results are relevant to this assay.
 - h. Intermediate precision was studied over a (b)(4) validation scheme only. This should also be done by (b)(4) analysts and using more than (b)(4) column. Please provide comparative results obtained by at least (b)(4) analysts and also using at least (b)(4) columns.
22. The accuracy shown in the report ----- (b)(4) ----- (Module 3.2.P.5.3, Table 4, page 5) was studied using ----- (b)(4) ----- standards at ----(b)(4)-----, which is below the ----(b)(4)--- of the adjuvant (around -(b)(4)-) shown in Table 1 (page 3). Please provide additional results to demonstrate the *Accuracy* of the measurement around the measured --- (b)(4) ---- of the adjuvant using standards of ----(b)(4)-----.
23. We have the following comments regarding the report *Polysorbate Identity and Content* by ----(b)(4)----- (Module 3.2.P.5.3):
 - a. The linearity of this assay was demonstrated only with standards (page 4). Please provide linearity results also in product matrix and a comparison of linear-regression fit of results with that obtained with the standards.
 - b. Table 3 and Table 8 have the title “Corrected Optical Densities.” The report did not provide any description on how these “Corrected Optical Density” values were calculated and their significance in the validation study. Please explain.

- c. Intermediate precision was studied by (b)(4) analyst only over (b)(4) days. This should also be done by (b)(4) analysts. Please provide comparative results obtained by at least (b)(4) analysts.
- d. You stated in the *Conclusion* section (page 10) that the validation was done in the range of (b)(4) µg polysorbate/tube. However, it is difficult to agree with this conclusion because: (i) the linearity was studied in the range --(b)(4)----- µg/tube of the standard only (Table 1) and a linearity study was not done with the product matrix, (ii) the Repeatability was studied at -(b)(4)-- µg/tube, and (iii) the Accuracy studied in the sample matrix with ----(b)(4)----- (assuming per tube) (Table 7), which (Repeatability and Accuracy) do not cover the concluded range of the assay. Furthermore, there is no correlation among these three sets of results. Please explain why ----(b)(4)---- is the appropriate range of this assay.

Regarding facilities information:

- 24. A component of the oil-in-water emulsion, α-tocopherol, is known to be sensitive to --(b)(4)--. A minor breach in container closure integrity could lead to reduction of α-tocopherol content in the emulsion, thus decreasing the immunostimulatory properties of the AS03 adjuvant. Please provide the validation studies performed to assure container closure integrity of the AS03 adjuvant including positive and negative controls. The studies need to assess the suitability of the method used, and to determine the limit of detection and sensitivity of the method (minimum leak detected), so as to assure that gas (air) leaks could be detected.
- 25. You have listed the use of several autoclaves and ---(b)(4)-- for the sterilization of the equipment used in the manufacture of the AS03 adjuvant. The validation reports you provided do not describe the location of the biological indicators and thermocouples used, and why they are considered worst case. Please provide this information.
- 26. Please provide a list of the shared contact equipment used in the manufacture of AS03 adjuvant and other U.S. and/or non-U.S. licensed products. For each piece of equipment, please list the products manufactured.
- 27. . Please provide a list of contact and non-contact equipment used in the manufacture of AS03 adjuvant that are not used in the manufacture of other U.S. licensed products.
- 28. Please provide a list of the shared contact equipment used in the manufacture of H5N1 (Drug Substance, Drug Product) and other U.S. and/or non-U.S. licensed products. For each piece of equipment, please list the products manufactured.
- 29. Please provide a list of contact and non-contact equipment used in the manufacture of H5N1 (Drug Substance, Drug Product) that are not used in the manufacture of other U.S. licensed products.
- 30. Please describe the use of the (b)(4) and (b)(4) tanks during the manufacture of AS03 adjuvant. You have provided media simulations to support the use of the (b)(4)-- connectors ((b)(4) tanks). Please clarify if you have performed aseptic media simulations to support the connectors for the (b)(4) tanks.

31. In the Validation Protocol 20080319, you stated, “a description of the different validation steps is given in aseptic process simulation validation procedure.” Please provide the steps validated in this study.
32. In the Validation Protocol 20070009 for the --(b)(4)----- inspection machine, you stated that the “challenge kit is prepared using the --(b)(4)---- adjuvant product; the challenge kit includes (b)(4) defective vials ((b)(4) vials with glass debris and (b)(4) vials with black particulates (pieces of stopper) and (b)(4) vials without particulate defects.” Please specify the size and number of contaminants in the challenge kit to determine the sensitivity of detection. In addition, you reported that the acceptance criterion is “Not less than (b)(4) of global defective vials are rejected,” yet the data presented show the efficiency of manual inspection is $\geq 99.25\%$ for glass and black particles. Please explain.
33. You have provided the first page (page 1 of 2) and its translation for the media simulation performed on January 13, 2011 (No. V11FB15Y01). In the French Version, two items in Environmental Monitoring are flagged with *, however in the English translation, the two items are checked as C (C= conform). Please explain the discrepancy and provide the second page and its translation.
34. You have stated in the Validation Protocol 20080397 that the sterilization temperature for the validation is worst case – b)(4) less than routine sterilization (page 2 of 9); however, you stated on page 3 of 9 that the (b)(4) station in Bldg. (b)(4) does not technically permit the application of the worst case temperature. Please clarify.

Regarding the Pharmacovigilance Plan:

35. Reference is made to previous reports on narcolepsy following administration of another AS03-adjuvanted vaccine, *Pandemrix* (D-Pan H1N1), and to recent publications from Finland regarding increased incidence of childhood narcolepsy after administration of AS03 adjuvanted H1N1 vaccine. Because of these reports of narcolepsy with other AS03-adjuvanted influenza vaccines, we suggest that you consider including narcolepsy on the adverse events list for close monitoring (Module 1.16 Pharmacovigilance Plan, section 3.1.1.2), though elevated risk of narcolepsy has not been observed in adults who received this or any other AS03-adjuvanted vaccine.
36. In the Pharmacovigilance Plan (m1.16), you proposed to conduct post-authorization active safety surveillance using a Pandemic Cohort (section 3.1.3.1.1.). The objectives were stated. Please clarify the study design and population, outcomes of interest, ascertainment of outcome, ascertainment of exposure, statistical analysis, and sample size and power calculation, in order to achieve those objectives.