



Our STN: BL 125285/0

Protein Sciences Corporation
Attention: Drs Manon M.J. Cox, MBA
1000 Research Parkway
Meriden, CT 06450-7159

Dear Drs Cox:

We have completed the review of your submissions dated through July 25, 2008, to your biologics license application (BLA) for Influenza Vaccine (FluBlok[®]), for the active immunization of adults 18 years of age and older against influenza disease, submitted under section 351 of the Public Health Service Act.

Our review finds that the information and data submitted are inadequate for final approval action at this time based on the deficiencies outlined below. The deficiencies in the CMC, Clinical/Statistical and Pharm-Tox sections are as follows:

CMC

1. We do not consider the validation of your manufacturing process to be complete for the following reasons:
 - a. The data provided are insufficient to assess consistency of the manufacturing process. Please provide tabulated results of critical process parameters and in-process tests of product yield for each manufacturing step for H1, H3 and B monovalent bulk lots prepared in 2007 and 2008.
 - b. We note from Section 3.2.S.2.5 "Process Validation" that the following manufacturing steps were not included in your process validation assessment:
--b(4)-----
------. Please provide a revised process validation protocol(s) and associated data for the manufacturing steps mentioned above. Please note that a Process Validation Protocol is a written plan pre-approved by the quality unit that specifies critical steps, controls, and measurements. The Process Validation Protocol states how validation will be conducted, identifying sampling, assays, specific acceptance criteria, production equipment, and operating ranges. Results obtained for each study described in the protocol should be evaluated in an associated process validation report.
 - c. Review of your process shows that --b(4)-----
----- and therefore we request that you demonstrate consistent manufacture as measured by yield and quality in three consecutive H1, H3 and B batches that

meet all specifications. The Process Validation study presented in the BLA does include 3 lots of B rHA that performed consistently in clinical trials. Please provide evidence that these lots were generated as consecutive batches, and submit in-process test results to demonstrate process consistency at each step in addition to yield and quality (--b(4)-----) of each monovalent bulk.

2. The manufacturing process is insufficiently characterized to ensure consistent product yield and potency. Your examination of batch records for H3/Wisconsin lots that failed to show lot-to-lot consistency in clinical responses did not identify a root cause, suggesting that critical process parameters for consistent manufacture are not yet in place. Conditions identified during process development that have a significant effect on step performance, product yield or product quality, need to be defined at manufacturing scale in order to establish operating parameters. Data to support limits should be provided for the critical parameters. Conditions that may impact the manufacturing process and for which we need additional supporting data to evaluate your process include:

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

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

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

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

-----.

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

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- f. –b(4)----- in downstream process steps: Please provide data to define parameters that support –b(4)----- performance.
–b(4)----- should be considered, with product yield and purity measured under different conditions. The data should be used to define operational parameters that support consistent product yield (-----b(4)-----) and purity.
- g. Re-use of columns: If columns will be used multiple times please provide lifetime studies to support reuse, cleaning, sanitization and storage. If small scale studies were performed to assess these items please provide the validation protocols and associated data. In addition, please explain how full scale studies were evaluated to confirm scaled down lifetime studies.
- h. Concerning the –b(4)-----: Please provide information on any preconditioning, cleaning, and sanitization or sterilization performed.
- i. –b(4)-----

3. The product specifications are inadequate as proposed.

- a. The potency of your product is determined by SRID with a target of 45 µg/dose of each HA until expiry. Your proposal of a minimum potency specification of –b(4)--- is unacceptable as it has not been justified nor demonstrated by clinical data. The minimum end-expiry potency specification must be supported by clinical data from studies conducted.
- b. Because the stability studies of 2007 and 2008 products show substantial loss of potency for all components (especially H1 and H3), you have proposed to –b(4)-- product in order to ensure sufficient product potency throughout the expiry dating period. After determining the minimum end-expiry potency specification, you should determine the –b(4)----- necessary to maintain this minimum potency value from release throughout your proposed dating period, also taking the precision of your stability estimate and your SRID assay variability into account to arrive at a minimum release specification.
- c. An upper release SRID potency specification should also be determined. The safety of the upper release potency specification that you determine must be supported by clinical data from studies conducted.

- d. While –b(4)----- at the time of monovalent bulk production, this –b(4)-- may be –b(4)- at the time of product fill. Taking this into consideration as well as purity of –b(4)- the maximum amount of total protein in a dose of vaccine is not defined. Please establish an upper limit specification for total protein based on clinical safety data.
 - e. The SRID assay measures the relative amount of antigenic rHA in the drug substance. The data submitted indicate instances where this value is in excess of the amount of rHA that is physically possible (as determined by total protein and purity), indicating that while the potency assay provides the means to determine the relative amount of rHA that is antigenic, it does not accurately quantify this amount. As a result, you formulated the product based on the concentration determined by SRID, with the criterion that -----(b)(4)-----). We accept this change in formulation as a temporary remedy, and expect you to establish a potency assay that accurately quantitates the amount of antigenically intact rHA in your product within an acceptable time frame. Please submit your plans to develop reagents or methods to meet this need.
 - f. Drug Product specifications do not currently include –b(4)----- or total protein content. Please provide relevant data to support inclusion or exclusion of these specifications.
 - g. DNA content of drug product is not stated in the BLA. Please provide the DNA concentration of all Drug Product lots manufactured in 2007 and 2008. ---(b)(4)-- ----- is inadequately characterized. As discussed during the teleconference meeting between CBER and you on December 6, 2007, please provide information -----(b)(4)----- . Please note if the total DNA is in excess of 10 ng in each product dose (not per strain as indicated in your table of drug product specifications), we may request an additional process step for DNA digestion.
4. Regarding your completed and proposed stability studies:
- a. Please address the presence of particles in some monovalent bulk batches during the stability studies, in the context of the acceptance criterion of "—b(4)-----" and explain how this parameter is monitored.
 - b. The photostability study of FluBlok revealed that extensive exposure to light reduces product potency. This is prevented when the product is stored as instructed in its secondary packaging material. A statement to store unpackaged vials of product in the dark should be included on carton/container labels and in the package insert.
 - c. We note that you proposed a post-approval stability protocol and commitment (in 3.2.P.8.2) to monitor the stability of at least three batches of 2008/09 FluBlok formulation. We recommend that this stability study be performed with drug product formulated according to comment 2b, above.

d. The stability data for monovalent bulks show –b(4)----- less than –b(4)---
----- Since this –b(4)----- has been an important parameter in
evaluating potency, please justify your statement that monovalent bulks are stable
for at least –b(4)----- . We note that you proposed a post-approval
stability commitment (in 3.2.S.7) to analyze three batches of monovalent bulk
drug substance from each strain of the 2008/09 vaccine formulation. We
recommend proceeding with this study at this time, examining the first three bulk
substance batches of each strain.

5. Additional information is needed to complete our review of Adventitious Agent Testing:

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

b. Regarding---b(4)-----: To allow us to complete
our evaluation of the results submitted on July 1, 2008:

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

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

c. Regarding the –b(4)----- assays (Section 3.2.A.2.2 Viral):

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

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

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

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

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

7. Since yield and purity of the product is strain dependent, please provide an overall manufacturing optimization plan for new strains. Please include process parameters for each process step.

8. With regard to inadequately described in-process tests:

- a. Assessment of contamination is performed by --b(4)------. Please describe the sensitivity of this method for each step that lists this as a critical parameter, providing volume and number of fields observed in each case.
- b. Please provide volume of sample and resultant sensitivity of bioburden tests and justify your alert limits and action limits in this context.
- c. Please provide data that support removal of the --b(4)----- included in your --b(4)-----, or specify residual amounts in the drug substance. Given that --b(4)----- is easily contaminated with --b(4)-----, please provide a Certificate of Analysis or data to show that it is free of contaminants.

9. Data in the baculovirus clearance report are not sufficient to determine whether baculovirus removal is achieved to an acceptable level during monovalent bulk purification. --b(4)-- -----

If sufficient baculovirus clearance by your process is not evident upon examination of this further information, we will request further study with changes in methodology such

–b(4)–-----

10. Additional information is needed to assess the following assay methods or validations:

- a. We have the following concerns regarding the validation of your purity assay described in report QVRQT005:
 - i. Specificity of the assay relies on a –b(4)–-----
----- The total amount of impurity determined by this assay does not include contaminating proteins that –b(4)–-----

 - ii. Linearity of the purity assay is shown for –b(4)–-----
-----, and therefore the limit of quantitation (LOQ) is –b(4)–-----.
The limit of detection is somewhat lower (–b(4)–-----). Since an amount below the LOQ would not be included as an impurity and the maximum amount of sample loaded is –b(4)–-----, impurities that constitute b(4)– of the sample are not included. This seems reasonable unless there are a large number of impurities present at these low levels. In order to justify the purity specification, please estimate the number of impurities present at these low levels based on –b(4)–----- below LOQ in analysis of 2007 and 2008 monovalent bulk preparations.
 - iii. Please validate the accuracy of the assay for contaminants that are either smaller or larger than—b(4)–-----.
 - vi. The accuracy of your assay for purity is not well defined. Please provide a direct measure of rHA purity (for example, quantification of –b(4)–----- using –b(4)–----- as the standard in –b(4)–-----) in comparison to purity as measured in the current assay, or present results from your Host Cell Protein assay to support the accuracy of your purity assay.
- b. The Host Cell Protein (HCP) assay results are not consistent with specifications for purity. In order to assign % HCP values that do not conflict with the specification for purity –b(4)– please revise your protocol to b(4) a suitable amount of HCP standard –b(4)– so that acceptance criterion for this test can be set at –b(4)–
- c. The SOP and validation report for quantitation of total DNA (–b(4)–-----) have not been submitted. Please submit these items for our review.
- d. The description of the endotoxin test is incomplete. Please provide the source and qualification of the standard used in the –b(4)–----- assay.
- e. The calculated maximum amount of –b(4)–-----) is stated as being close to –b(4) of the acceptable amount of this excipient (Table 2.3.S-18, in

location have yet to be resolved. You must satisfactorily resolve these issues prior to approval of the application.

CLINICAL/STATISTICAL

Regarding study PSC04 entitled, “Evaluation of the Immunogenicity, Safety, Reactogenicity, Efficacy, Effectiveness and Lot Consistency of FluBlok Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine In Healthy Adults Aged 18 to 49”:

13. A total of 59 subjects that were randomly selected to be included in the HI antibody immunogenicity subset remain unaccounted for in your analyses.
 - a. Your statistical analysis plans for study PSC04 outlined a total of 450 subjects to be included in the immunogenicity subset and you reported that a total of 480 subjects in study PSC04 were vaccinated and randomized to the FluBlok immunogenicity subset. Day 28 HI antibody immune response results in the datasets were available, however, for only 393 of these subjects. Your analysis only included 391 subjects. Please account for the two missing subjects.
 - b. Your BLA amendment on June 28, 2008 (STN 125285/0.5), in which you responded to our written concern (sent by fax on June 12, 2008) about missing immune response data among some subjects in this study, identified the disposition of 30 subjects (lost to follow up or withdrew consent) but indicated that you have not yet determined the disposition of 59 subjects. You further stated that study PSC04 is an ongoing study and that subjects may not have been included in the analysis because “the analysis was performed at a specific point in time prior to the actual completion of the study”. The disposition of those 59 subjects should be provided, including the two missing subjects as noted above. The HI antibody immune responses from those 59 subjects should be included in the statistical analyses, if data are available. If the immune responses are not available, sensitivity analyses should be performed using appropriate methods dependent upon the type(s) of the response variable(s).
14. The study report and electronic datasets for Study PSC04 did not contain an evaluation of the secondary immune response endpoint among subjects randomized to receive placebo. The pre-specified secondary endpoint for the HI antibody immune responses to the B strain were achieved in study PSC04, but the integrity of these results would be strengthened by comparison to a placebo group to ensure that administration of FluBlok alone was responsible for the HI antibody immune response results to the B strain. Although the other studies submitted to the BLA used a control group for the HI antibody immune response endpoints, after completing our review we noted that the other studies failed to meet one of the two endpoints for the B strain. For example, according to the results from study PSC03, the non-inferiority hypothesis was not met with respect to differences in seroconversion rates between FluBlok and Fluzone for the B antigen. In addition, according to the results from study PSC06, there is no evidence that the seroconversion rate is $\geq 40\%$ for B/Malaysia, that is, the null hypothesis cannot be rejected at the level of $\alpha = 0.025$. The pre-specified secondary immunogenicity endpoints

on a subset of subjects who received placebo in study PSC04 should be obtained from stored sera if available.

15. According to results of statistical analyses performed on PSC04 datasets, three investigated lots did not meet the pre-defined criteria for the lot-to-lot consistency. In particular, lot-to-lot consistency was not demonstrated for the A/Wisconsin (H3N2) strain, in which the confidence intervals on the pairwise GMT ratios were almost all completely outside the equivalence margins of 0.67 and 1.5. It is desirable to demonstrate lot-to-lot consistency before pooling the immune response results in the secondary immune response analysis that would support accelerated approval of FluBlok. Please provide additional information about potential causes of the observed differences and their impact on estimates of efficacy.
16. According to the data from study PSC04, geometric mean titers at Day 28 for H3N2 strains were 396.88, 178.8, and 241.2 for lots A, B, and C, respectively. We note that there are considerable differences between geometric mean titers for H3N2 strains and that similar differences were observed for studies PSC03 and PSC06. The GMTs for H3N2 for these studies were 338.35 and 105.1, respectively. Please provide more information on potential causes of this disparity of these results.
17. GMTs were almost comparable for H1 and B strains for all lots (Interim Clinical Study Report, page 60). However, when applying to the PSC04 study data an analysis of covariance with adjustment for the covariates Lot and HI Assay, the results indicated that:
 - a. The estimations of GMTs per lot, GMT ratios, and 95% CI were different from estimations obtained without adjustment.
 - b. GMTs were not comparable in some instances for H1 and B (e.g., Lot A vs. Lot B).

Please perform an analysis of covariance and draw clinical/statistical conclusions related to the assays and lots and provide relevant explanation.

18. You did not provide a tabular summary and source electronic datasets for the general medical history data collected on each subject in study PSC04. Please provide this information.
19. Subjects from Site 13 who were improperly randomized were not included as protocol deviations in your disposition of subjects for study PSC04. Please include these subjects as protocol deviations and re-submit a tabular summary and amended electronic datasets for the End of Study Record.

Regarding study PSC06 entitled, “Evaluation of the Safety and Reactogenicity of FluBlok, Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine, and Comparison of the Immunogenicity, Efficacy and Effectiveness of FluBlok to a Licensed Egg-Grown Influenza Vaccine in Adults Aged 50 to 64”:

20. The main purpose of study PSC 06 was to evaluate the safety and immunogenicity of FluBlok vaccine. Please note that your primary endpoints and hypotheses stated in the last version of the protocol and SAP were not consistent. Please clarify whether this was a consequence of modification of the primary hypotheses approximately 10 months ago. It appears that you did not adjust primary endpoints to the primary hypotheses. Your definitions of the primary endpoints (e.g., “*proportion of subjects in each vaccine group achieving a Day 28 post vaccination serum titer HAI antibody titer of 40 or greater for each vaccine antigen*”) were stated for both groups. However, your primary hypotheses were defined only for the FluBlok study group as stated in the primary hypotheses in the Statistical Analysis Plan, pages 9 and 10, and were to be tested only for the FluBlok group. Testing hypotheses constitutes an essential part of assessment of FluBlok vaccine. Please clearly present the primary hypotheses results, for FluBlok only, in one table.

Regarding study PSC03 entitled, “Comparison of the Immunogenicity, Safety and Reactogenicity of FluBlok, Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine, To a Licensed Egg-Grown Influenza Vaccine In Ambulatory Elderly Adults”:

21. Numerous differences were identified between your reported numbers of subjects experiencing various unsolicited adverse events and our review of the source electronic datasets. The differences found for unsolicited severe adverse events were of concern.
- a. It appears that your final study report may have counted subjects only once with adverse events in various categories even if that subject experienced multiple different events (by MedDRA Preferred Term or PT) within that category. For example, the total number of serious adverse events in your electronic source dataset was 42 and 45 reports for Fluzone and FluBlok, respectively, in contrast to your study report of 34 and 36 reports for Fluzone and FluBlok, respectively.
 - b. Other differences could not be accounted for by simply reporting the number of subjects with any adverse event per event category. For example, under severe adverse events regardless of causality, you reported severe adverse events in nine subjects randomized to Fluzone and six subjects randomized to FluBlok. Our review of your electronic datasets found severe adverse events in 24 subjects randomized to Fluzone with 30 different severe adverse events (by PT), and 26 subjects randomized to FluBlok with 29 different severe adverse events (by PT) in the electronic datasets.

Please address these differences in the safety assessments.

22. The evaluation of adverse events may have been affected by unblinding of some study subjects. You reported that the study staff at Site 5 had inappropriate access to the randomization code, and, therefore, subjects were unblinded at this site that enrolled a total of 126 subjects. We acknowledge that you have performed post-hoc analyses and report that this protocol violation did not impact the overall study results. The BLA submission, however, did not contain clarifications as to the nature of the inappropriate access to the randomization code and whether the staff involved evaluated safety parameters. The number of subjects “unblinded” by this event, the treatment assignment

of those subjects, and the role of the study staff in the evaluation of adverse events, should be provided.

The following are additional clinical/statistical deficiencies:

23. When you presented results related to non-inferiority hypotheses, you included P-values of superiority tests for the differences, e.g., seroconversion proportions (e.g., Clinical Study Report PSC03, page 56). Please note that such presentation is misleading when used in the tables in which you intend to report non-inferiority assessments. Please provide the tables for the results related to non-inferiority assessments only.
24. Please note that labels for the x-axis for figures of reverse cumulative distribution curves of the response variable for each study throughout the submission were not correct (e.g., clinical study PSC04, page 57, 58; Figures 1A, 1B, 1C). Each reverse cumulative function figure used the x-axis on the log 10 scale. However, you have used log (titers) as labels for the x-axis in these figures, but you were plotting reverse cumulative functions versus titers (not log titers) using the log 10 scale. Please resubmit the reverse cumulative distribution curves in the appropriate format.

PHARM TOX

Regarding the developmental toxicity study no. 2146-001:

25. Fifty (50) animals were allocated to the littering subgroup, 25 animals/group. In the vaccine treated group 23 of 25 animals delivered a litter (Table 16), 2 were not pregnant (Table 17). The total number of animals evaluated for clinical signs in the saline group was 24 and the total number of animals in the vaccine treated group was 21. Please explain and provide information on the 4 animals in the vaccine treated group and the 1 animal in the saline group that were apparently not evaluated for clinical signs.
26. Data in Table 5 and C-5 indicate that the average length of time of F0 females in diestrus, proestrus, estrus and metestrus was comparable among saline and vaccine treated groups. However cyclicity data indicate a difference in the number of animals “not cycling” (defined as at least ten days without estrus) in the vaccine group (7 of 50 animals (14%)) compared to the control group (2 of 50 animals (4%)). You state that this group difference is not statistically different. We find this difference of 4 % versus 14 % remarkable especially when considering the test species, i.e., --b(4)----- rats. Please comment and provide historical control data for this parameter from studies conducted in the testing facility using this test species.
27. Total preimplantation loss was higher (58) in the vaccine treated group compared to the saline control (32) (Table 12). In the saline treated group 8 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 25.0%. In the vaccine treated group 11 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 38.1%. In this group there were an additional 2 animals with a % preimplantation loss above 7.7% (Tables C-11 and C012). Please provide the historical control data from the testing facility with regard to this parameter.

28. Data in Table 1 of Appendix D show that on study day 1 (day of 1st injection) the maternal antibody titer to A/New Caledonia/20/1999 (H1N1) was about 9 x above that observed in the control group. Please explain. Furthermore, data in Table 1 Appendix D show that antibody titers to A/Wisconsin/67/2005 (H3N2) in fetal blood on GD 20 were approximately 5 x higher (GMT:338.2) compared to the titer in maternal blood on GD 20 (GMT: 62.3) and approximately 2 fold higher in pups on lactation day 2 (GMT:114.9). Please explain this finding as pups were not immunized with the test article.

We acknowledge that you have included a Pharmacovigilance plan in your BLA; however, we reserve our comments on this proposal for later, when this application is found suitable for approval. Depending on subsequent CBER evaluation and final labeling, CBER may request additions to the Pharmacovigilance plan.

Review and comment on the proposed final labeling will be completed when the application is otherwise acceptable. Extensive revision of the proposed final labeling may be required based on any additional information relating to the safety and effectiveness of this drug product.

The Pediatric Research Equity Act (PREA) requires that all NDAs, BLAs, or supplemental applications for a new active ingredient, new indication, new dosage form, new dosing regimen, or new route of administration contain a pediatric assessment unless a waiver or deferral has been obtained. You have submitted a proposal to defer pediatric studies for pediatric age groups 6 months through 17 years of age, as well as, a request for a partial waiver for infants from birth to < 6 months of age. This proposal will be formally reviewed by FDA's Pediatric Review Committee prior to final action on the application.

You may request a meeting or teleconference with us to discuss the steps necessary for approval. For PDUFA products please submit your meeting request as described in the FDA Guidance for Industry: Formal Meetings With Sponsors and Applicants for PDUFA Products February, 2000 (<http://www.fda.gov/cber/gdlns/mtpdufa.pdf>). For details, please also follow the instructions described in CBER's SOPP 8101.1: Scheduling and Conduct of Regulatory Review Meetings with Sponsors and Applicants (<http://www.fda.gov/cber/regsopp/81011.htm>).

Within 10 days after the date of this letter, you should take one of the following actions: (1) amend the application; (2) notify us of your intent to file an amendment; or (3) withdraw the application).

We stopped the review clock with the issuance of this letter. We will reset and start the review clock when we receive your complete response. Please also note that any amendment should respond to all deficiencies listed and that a partial reply will not be considered for review nor

will the review clock be reactivated until all deficiencies have been addressed. If you have any questions, please contact the Regulatory Project Manager, Captain Katherine Matrakas, at (301) 827-3070.

Sincerely yours,

Wellington Sun, M.D.
Director
Division of Vaccines and
Related Products Applications
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Research and Review
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