



DATE: 10 November, 2008

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SUBJECT: BLA STN 125285/0

PRODUCT: Influenza recombinant HA trivalent

SPONSOR: Protein Sciences Corp

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**Summary:**

Significant deficiencies were noted upon review of 3.2.S (Drug Substance) and 3.2.P (Drug Product). These include: insufficient characterization and incomplete validation of the drug substance manufacturing process; insufficient justification of the minimum potency specification; incorrect determination of release potency specification; additional information is needed for complete evaluation of some release tests. We recommend that the product not be approved and a CR letter issued so that deficiencies can be addressed.

**Introduction:**

This BLA is for licensure of baculovirus-expressed insect cell-derived recombinant hemagglutinin (rHA), under the trade name FluBlok. The biochemical name is “purified recombinant hemagglutinin (derived from H1, H3 and B strains)”. This trivalent product is a sterile solution with no added preservatives for intramuscular immunization. Each 0.5ml dose contains 135 ug (45 ug of each strain) rHA and will be for active immunization of adults 18 yrs and older. We have reviewed modules that describe manufacture and specification of drug substance (monovalent bulk rHA for H1, H3 and B strains) and drug product (trivalent formulation).

**1. Manufacture sites and contract laboratories**

Protein Sciences Corp., Meriden, CT: At this site, rHA monovalent bulk concentrates (drug substance) are manufactured; release tests of drug substance are performed; stability tests of drug substance are performed. PSC is also responsible for determining DNA content and lot release of drug product.

Hospira, McPherson, Kansas: At this site, the drug product is: formulated (potency of drug substance is determined at Hospira prior to formulation); filled; excipient testing performed; vial/stopper/seal tests performed; release tests performed (with exception of DNA ----(b)(4)---, stability tests performed; packaged and labeled.

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## 2. Manufacture

### 2.1 General information

The purified recombinant influenza hemagglutinin (rHA) drug substances included in the FluBlok drug product are derived from strains representing influenza A subtypes H1N1 and H3N2 and influenza B. The rHA genes are to be cloned from the strains approved by FDA on an annual basis. Full-length HA genes from the selected viruses are cloned into the baculovirus *Auotgrapha californica* nuclear polyhedrosis virus (AcNPV). PSC has developed the expresSF+ cell line, which can be propagated in a serum-free medium, as the substrate for recombinant baculovirus infection and rHA production. This is a non-transformed, non-tumorigenic, continuous cell line derived from the fall army worm, *Spodoptera frugiperda*. PSC has utilized this system to manufacture rHA of 13 H1N1, H3N2, H5N1, and B influenza stains under several IND's, including multiple strains of each since 2004 under IND-11951.

The 2007/2008 vaccine formulation that has been investigated in support of the BLA contains rHA proteins of A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004. The anticipated properties of rHA's are full length, uncleaved, with molecular weights of approximately 65 kD. Reference sequences are obtained from either of two online databases, the Influenza Sequence Database or GenBank. Matching between database sequences and insert regions of rHA clones is determined in terms of amino acid sequence.

Based on PSC's experience with rHA substances of numerous influenza strains, the monovalent bulk proteins are ----(b)(4)--, and the purified rHA's migrate on SDS-PAGE -----(b)(4)----- with molecular weights of about 65 kDa. -----

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----- By electron microscopy, rosette-like micelle structures are observed. Purified rHA can agglutinate avian red blood cells, which indicates its ability to recognize sialic acid receptors as well as its higher order association into rosettes.

### 2.2 Raw materials

Raw materials are discussed in 3.2.S.2.3.9. PSC implements a raw materials and vendor management program in which raw materials containing the highest risk (final formulation components) are under the tightest control (most extensive testing). Each raw material has been assigned a PSC-part number (A through F) to allow for segregation upon receipt. Table 3.2.S.2.3.9-2 lists chemical name, grade, category and name of vendor and manufacturer for all raw materials. -----

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## 5. Analytical procedures

In-process tests that are performed during manufacture include:

The SRID assay (SOP QT0077) is used as a potency assay. The assay has been adequately validated at PSC and transferred to Hospira (validation report R-08-006). Despite validation, it was noted by both the sponsor and the reviewers that SRID values sometimes exceed the absolute amount of rHA in the product. This indicates that the SRID assay provides inaccurate quantitation of rHA. It should be kept in mind that the SRID assay provides the amount of antigen that is recognized by antibodies relative to the amount of reference antigen. The reference antigen used by Protein Sciences is the CBER reference antigen. This is a preparation of formalin-inactivated egg-grown virus that has SRID values assigned 'equivalent' to the absolute amount measured in the preparation as determined by SDS-PAGE/densitometry. Consequently, it is expected that the actual amount of HA that reacts in the SRID is LESS than the assigned value. Therefore, when the SRID assay is run to quantify rHA in a pure preparation, it is highly likely that the concentration assigned is not equivalent to the actual amount that reacts (the actual amount that reacts is likely to be less than that assigned). While this difference has not been an issue with egg-grown vaccine preparations (because the absolute amount of HA is not known), the discrepancy is easily noted for rHA in which total protein concentration is measured by BCA assay. -----



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The HAI assay description is adequate. The assay that is described is 'standard': -----

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The HAI validation report (revision 28 Feb 2007) from Cincinnati Children's Hospital is also included in the BLA. PSC provided validation parameters that are used to define assay specificity, precision, repeatability, day-to-day variation, analyst-to-analyst variation, robustness (b)(4), titer range, and linearity. Each of these parameters is validated for one lot of BEVS-derived HA antigen for each virus type/subtype, using sera samples from either Flublok recipients or unvaccinated controls. While titers are comparable, the sensitivities of the HAI assays with BEVS-HA and egg-grown antigen were not compared.

## 6. Drug Substance and Drug Product Specifications

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6.2. Drug Product Specifications. Drug Product specifications are listed in 3.2.P.5 and shown in the Table 3.2.P.5.1 (included at the end of this report). Specifications for appearance, identity, endotoxin, and ----- (b)(4) ----- . Specifications for drug product that are distinct ----- (b)(4) -----:

Purity. (b)(4). While higher purity is the goal, (b)(4) is the lower limit of acceptable purity. Purity is calculated ----- (b)(4) ----- . This calculation needs to be included in the formulation batch records or worksheet (included in CR letter comment #11b).

Baculovirus DNA: The specification listed for total or baculovirus DNA is inappropriate – it should be corrected to show a specification of  $\leq 10$  ng/trivalent dose. Figure 3.2.S.3-6 suggests the sensitivity of this assay is approx ----- (b)(4) ----- . This is appropriate for this assay. 3.2.S.3-6 shows results for baculovirus DNA content -----

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----- . We have requested total DNA content and information regarding ----- (b)(4) ----- in CR letter comment #3g.

Total DNA: Assay description, validation and results have not been provided. This is requested and it is noted that the specification should be set at  $\leq 10$  ng/trivalent dose (not per strain, CR letter comment #10c).

Sterility testing is performed as per 21CFR 610.12. The proposed specification is “No growth observed,” as required by 21CFR 610.12. All batches used for clinical studies PSC01, PSC03, PSC04, and PSC06 have met this specification.

Potency specification is stated as ----- (b)(4) ----- for each HA component (H1, H3, and B)”. The minimum potency specification of ----- (b)(4) ----- that is proposed is unacceptable as it has not been justified nor demonstrated by clinical data. We have asked the firm to support the minimum end-expiry potency specification with clinical data (CR letter comment #3a).

FluBlok has shown significant immunogenicity across these previous studies, and the study results indicate that recombinant rHA vaccines may be significantly more immunogenic than the standard egg-grown inactivated vaccine when used at higher antigen concentrations. PSC has released drug product for clinical studies PSC03, PSC04, and PSC06 with a potency specification of 45  $\mu$ g ----- (b)(4) ----- as measured by SRID per dose. All formulation, release, stability and validation work to date has been performed using this specification. At the request of the FDA at the September 21, 2007, preBLA meeting, however, PSC tightened the specification for release of drug product to ----- (b)(4) ----- for each HA component (H1, H3, and B)”. It would be more accurate however to list the specification as the targeted dose of 45  $\mu$ g/dose, with the understanding that assay variability may result in commercial drug product at (b)(4) of this value. The sponsor needs to have a target dose (45 $\mu$ g/dose) and then formulate to meet that target dose at expiry (i.e. take into consideration stability). This comment is included in CR letter #3b. The SRID doses used in clinical trials (Table 3.2.P.5.6-2, included at the end of this report) shows the potency values for drug product batches released

for clinical trials to support this license application as well as for the first process validation run performed by Hospira.

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Total protein is NOT listed as a specification for the Drug Product. It is important to add this concentration, since there is a specification of “at least 45 µg rHA”. An upper limit of total protein should also be established based on clinical safety data. This comment is included in CR letter #3d.

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The fill volume specification is “not less than labeled volume”. The labeled volume is 0.5 mL per vial. FluBlok® is a sterile liquid, with no added preservatives, for intramuscular injection. FluBlok is supplied in single-dose vials containing one dose (0.5 mL). This specification is appropriate for this product.

## 7. Stability

**7.1 Drug Substance.** Stability of the Drug Substance is described in 3.2.S.7. For each of the strains in the 2007-08 FluBlok formulation (A/Solomon Islands/3/2006, A/Wisconsin/67/2005, B/Malaysia/2506/2004) multiple batches of rHA drug substance are being tested for stability under normal storage and stressed conditions. The study includes 3 batches of A/Solomon Islands, 3 batches of B/Malaysia, and 2 batches of A/Wisconsin (with a third A/Wisconsin batch being tested at limited timepoints). -----

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protein concentration. Potency by SRID has also only been monitored through 3 months. The data suggest that A/Solomon Islands rHA is more stable than other H1 strains, with little reduction in potency of H1 and B components at 3 months. In contrast, H3 (A/Wisconsin) shows substantial loss of potency at this time point. The accelerated stability aspect of this study was terminated after the 1-month time point, when H1 and H3 strain components had lost at least 25% of potency. -----(b)(4)-----

The same (b)(4) scale FluBlok batch ---(b)(4)-- was also the subject of a photostability study. -----

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----- “Primary packaging” means 2 mL glass vials without labels (nude vials), and “secondary packaging” means 2 mL vials in cartons representative of packaging to be used for FluBlok. Negative controls were 2 mL glass vials wrapped in foil. Test parameters are appearance, --- (b)(4) ---, total protein by BCA, and potency by SRID. The only one of these parameters for which there were differences between primary or secondary packaging and the negative control was potency. A/Solomon Islands rHA in nude vials had a dramatic loss of potency (from 34 ug/dose to <7.5 ug/dose). A/Wisconsin and B/Malaysia rHA components in nude vials had potency reduced by greater than 50% under this light condition. However, none of the 3 had an appreciable loss of potency when contained in secondary packaging. PSC cites ICH Guidance Q1B as saying an acceptable change observed in the “marketing pack” or secondary package does not necessitate a package redesign or product reformulation. We are not concerned about photo-instability of product in bare vials under these rather extreme light conditions when it appears that normal secondary packaging negates the problem. We do however recommend inclusion of instruction to store the product in the dark (CR letter comment #4b).

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**Table 3.2.S.2.6-4 Drug Substance Batches Used to Formulate Trivalent Drug Product Batches in FluBlok Development**

Year of Manufacture (Study Number)	Drug Product Composition and Batch Number	Drug Substance Batches by Subtype and Strain		
		H1	H3	B
2003 (DMID 03-119)	Composition	A/New Caledonia/20/99	A/Panama/2007/99	B/Hong Kong/330/2001
	0316P-A	(b)(4)	(b)(4)	(b)(4)
	0316P-B	(b)(4)	(b)(4)	(b)(4)
	0316P	(b)(4)	(b)(4)	(b)(4)
2004 (PSC01)	Composition	A/New Caledonia/20/99	A/Wyoming/3/03	B/Jiangsu/10/03
	50-04011A	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----
	50-04011B	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----
2006 (PSC02 & PSC03)	Composition	A/New Caledonia/20/99	A/Wisconsin/67/2005	B/Ohio/01/2005
	50-06019 (PSC03)	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----
	50-06020 (PSC02)	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----
2007 (PSC04 & PSC06)	Composition	A/Solomon Islands/03/2006	A/Wisconsin/67/2005	B/Malaysia/2506/2005
	50-07010 (Lot A)	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----
	50-07011 (Lot B)	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----
	50-07014 (Lot C)	----- (b)(4) -----	----- (b)(4) -----	----- (b)(4) -----

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**Table 3.2.P.5.1-1 FluBlok Drug Product Specifications**

Test	Method (Reference)	Acceptance Criteria
Appearance	Visual inspection ----(b)(4)----	Colorless, clear liquid essentially free of visible particles
Identity	----(b)(4)--- -----	----(b)(4)--- ----- -----
Bacterial Endotoxin	----(b)(4)--- -----	----(b)(4)----
Sterility	Membrane Filtration (21 CFR 610.12)	No growth observed
Potency	SRID (PR-1468)	----(b)(4)--- -----
Purity	Weighted Average of Drug Substance Purities	----(b)(4)----
DNA Content	----(b)(4)--- -----	----(b)(4)----
	----(b)(4)--- -----	
----(b)(4)----	----(b)(4)----	----(b)(4)----
----(b)(4)----	----(b)(4)--- -----	----(b)(4)----
General Safety	21 CFR 610.11	All animals survive and weigh no less than at time of injection
Fill Volume	----(b)(4)----	Not less than labeled volume



**Table 3.2.P.5.6-2. Potency Data on Batches of Drug Product at Release.** Potency was measured by SRID. Target formulation was 45µg/dose and actual result per batch is shown below. Potency (as measured by SRID) specification was 45µg/dose --(b)(4)--- for PSC03, PSC04, and PSC06 batches.

Purpose	Year of Manufacture	Batch Number	H3 Potency (µg/dose)	B Potency (µg/dose)	H1 Potency (µg/dose)
PSC01 Trial	2004	50-04011A	45	45	36 <sup>1</sup>
PSC03 Trial	2006	50-06019	48	42	44
PSC04 & PSC06 Trial	2007	50-07010 (Lot A)	44	50	41
	2007	50-07011 (Lot B)	50	48	46
	2007	50-07014 (Lot C)	42	44	44
Process Validation/Stability	2007	CM7-515	44	48	45

<sup>1</sup> After formulation of Batch 50-0411A, it was determined that the concentration of the H1 component in the high-dose formulation was 35 µg, rather than the target dose of 45 µg. This deviation was reported on October 12, 2004 (Memorandum from Manon Cox, COO to Director DVRPA) in an amendment to BB-IND 11951 (Serial 2 dated 10/12/04).