Vaccine Technology II

June 1-6, 2008 (Albufeira, Algarve, Portugal)

FluBlok, A High Dose Recombinant Hemagglutinin Influenza Vaccine

Manon Cox

June 2, 2008

Protein Sciences CORPORATION

A Vaccine Company for the 21st Century

"Making products where speed, cost and safety matters"

Agenda

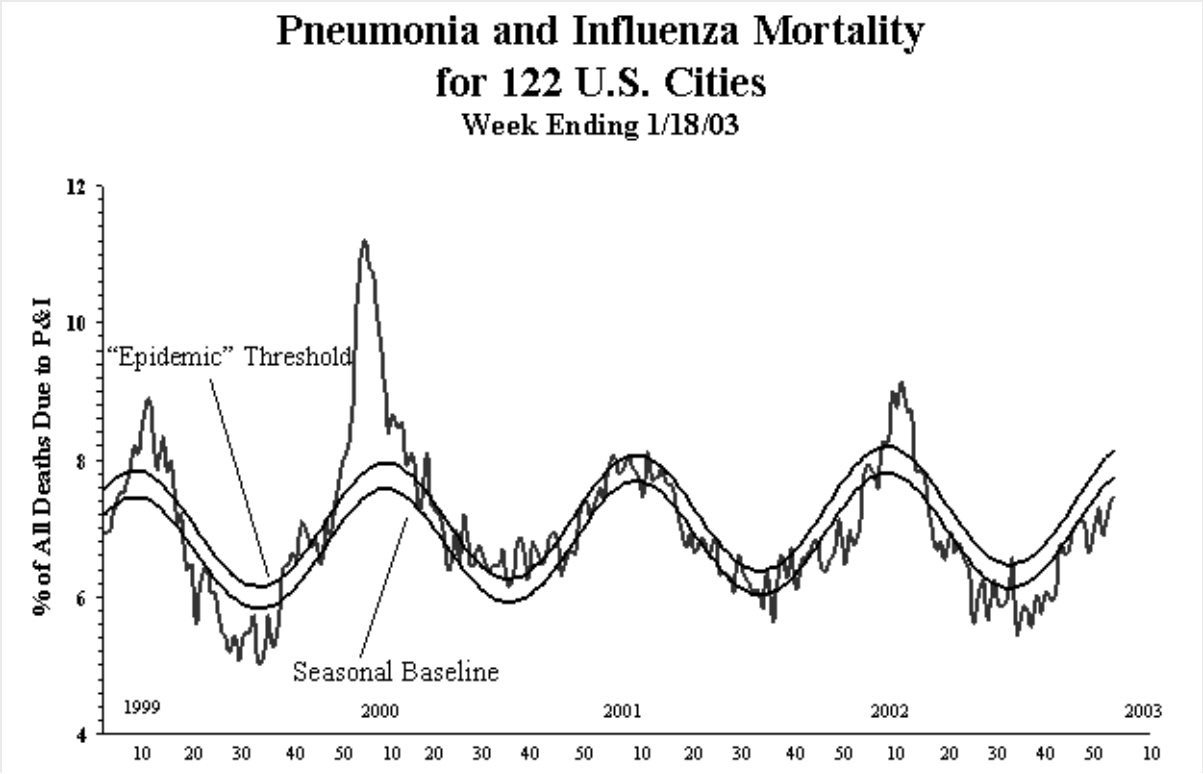
- **Influenza and the performance of TIV (2007-2008)**
- **Approaches to improve influenza vaccine**
- **FluBlok: rHA produced in insect cells**
- **Safety, immunogenicity and efficacy results from four studies performed in different populations**

Influenza (the "Flu")

- Contagious disease caused by the influenza virus
- Attacks the respiratory tract (nose, throat, lungs)
- "Flu-like Symptoms"
  - Fever, Headache, Tiredness, Dry Cough, Sore Throat, Nasal Congestion, Body Aches
- Average "Flu" Season - November to April
- Most people recover in one to two weeks
- Complications from influenza
  - Pneumonia, bronchitis, sinus and ear infections
- Individuals at high-risk for complications
  - 65 and older
  - Chronic heart or lung conditions
  - Very young children

Severity of Influenza

- 10-20% of the U.S. population gets influenza each year
- Influenza kills ~36,000 people per year in U.S.
  - 21,000 more than from HIV-related illnesses
- >200,000 hospitalized per year with the “Flu”



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The Influenza Viruses

- Orthomyxoviruses (Greek, myxa=mucus)
- Three types of influenza virus (A, B, and C)
  - A viruses
    - Divided into subtypes based on genetic and antigenic differences among surface proteins (HA & NA)
    - Current subtypes found in people are A(H1N1) and A(H3N2)
  - B viruses
    - No subtypes
  - C viruses
    - Cause mild respiratory illness
- Antigenic "Drift" of A and B viruses leads to epidemics every winter
- Antigenic "Shift" of A viruses leads to pandemics
  - 3x in the past 100 years

Licensed influenza vaccine

Characteristics

Trivalent vaccine: 2 A strains and 1 B strain

Protection correlates with hemagglutinin (HA) antibodies

Production process:

Chicken Embryo's

↓

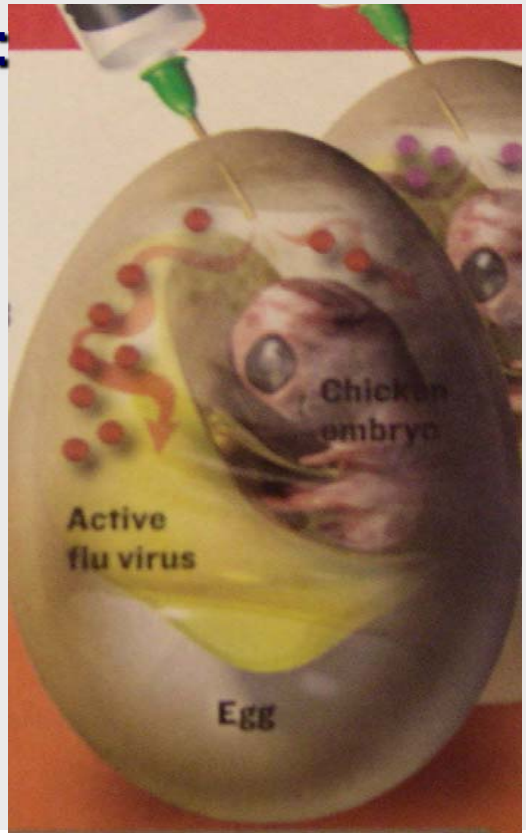
Isolation of Virus

↓

Kill Virus

↓

Isolate virus proteins



Long production cycle

One egg = one dose

Production affected by Avian influenza outbreaks

Adaptation required

Adverse reactions

Less effective in the elderly

Egg-based manufacturing is lengthy and inflexible

As yet only feasible in winter due to higher bioburden in summer months

Total duration of manufacturing process: 6 months

February — **July / August**

Egg supply organisation → WHO announces vaccine composition → Egg fertilization & incubation (11d) → Virus injection & incubation (3d) → Harvest of crude virus stocks → Inactivation, filtration, concentration → Formulation, filling, delivery

Regulatory controls

Review license amendments, ensure quality

Drawbacks

- Low flexibility
- Long planning cycles
- Egg contaminants
- Suboptimal antigen match

Faster and more flexible production methods are needed

Source: DataMonitor 2007

Performance of TIV (2007-2008)

Interim results for case-control study to estimate vaccine effectiveness for the prevention of medically attended, laboratory-confirmed influenza (MMWR, April 18, 2008)

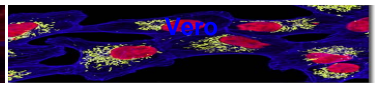
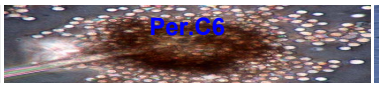
	Patients tested Positive for Influenza (N=191)[1]		Patients tested Negative for Influenza (N=425)		Adjusted VE % (95% CI)
	Vaccinated	Not Vaccinated	Vaccinated	Not Vaccinated	
All influenza					
All enrollees	36	155	165	260	44* (11, 65)
ACIP Recommended[2]	21	39	120	114	34 (-31, 67)
Healthy individuals 5-49[3]	15	116	45	146	54* (12, 76)

1.By RT-PCR
2.Children 6-59 months; adults ≥50 y and adults 5-49 with chronic medical conditions
3.Persons 5-49 years without chronic medical conditions
*Statistically significant

Approaches to Improve Vaccine Performance

- Increase the number of doses
- Increase hemagglutinin content
- Use of an adjuvant
- Vary type of preparation (whole virus, subvirion versus sub-unit)

Manufacturing alternatives: cell culture-based expression systems

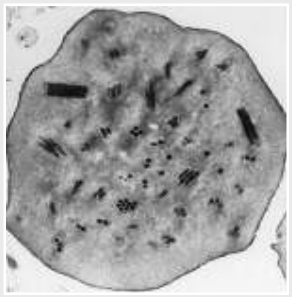
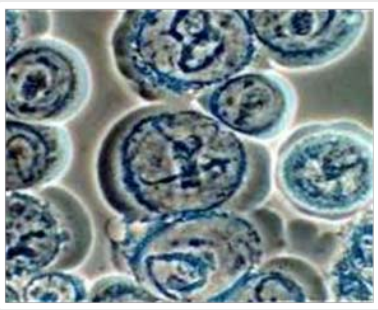
				
Origin	Madin Darby Canine Kidney	African green monkey kidney	Human embryonic retina	*Spodoptera frugiperda*
Relative yield	High	Low	Moderate	Very high
Tumorigenicity	High	Not demonstrated	Weak	None
Other uses	Veterinary vaccines	Other human vaccines, e.g. poliovirus vaccines, rabies vaccines	Other human vaccines, antibodies, gene therapy, therapeutic proteins	Established protein expression system
Key advantage	High viral yield	Proven track record, has been used in production of other human vaccines	High susceptibility to influenza virus	High yield, high scaleability, low costs
Key disadvantage	High tumorigenicity	Low virus yield	Unclear benefits, newcomer	Immunogenicity perceived as low
Companies involved	NovartisVaccines, Solvay, GlaxoSmithKline	Baxter	Crucell, Sanofi-Pasteur	Protein Sciences

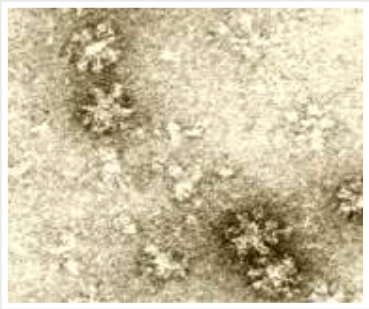
MDCK cells are furthest advanced and most popular approach

Source: DataMonitor 2007

FluBlok:
rHA produced in insect cells

Baculovirus Expression Vector System (BEVS)



- Engineer baculovirus with the gene of interest (e.g. Hemagglutinin)
- Baculoviruses highly specific to insect cells
- Powerful promoter generates high yield of protein of interest

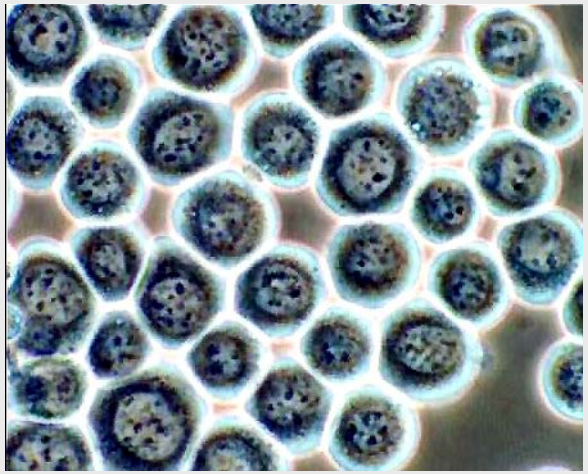
- Culture expression of insect cells in a fermenter
- Infect cells with engineered virus
- Incubate infection for ~48 - 72 hours

- Protein forms rosettes
- Purify protein to > 90% into final product
- Formulate with PBS into vaccine

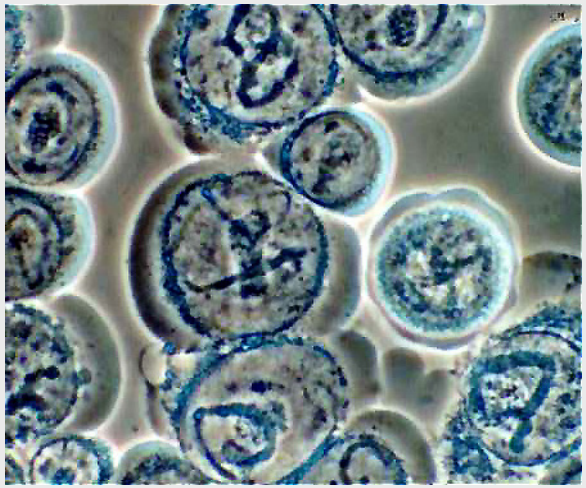
FluBlok® Approval → Validation

Technology Improvements
SF+ Serum-free Cells

- **Evolved from Sf9 Cells**
  - *Selective pressure* in serum-free media with added insulin (0.4 **m**M)
  - unique phenotypic and genotypic properties
- **Ideal for Manufacturing**
  - serum-free
  - stable for > 50 passages
  - infected with low MOI < 1
  - produces high titer AcNPV
  - cGMP at 500L scale
  - excellent safety record in Phase I and Phase II clinical trials
  - patent
  - available for commercial and non-commercial use

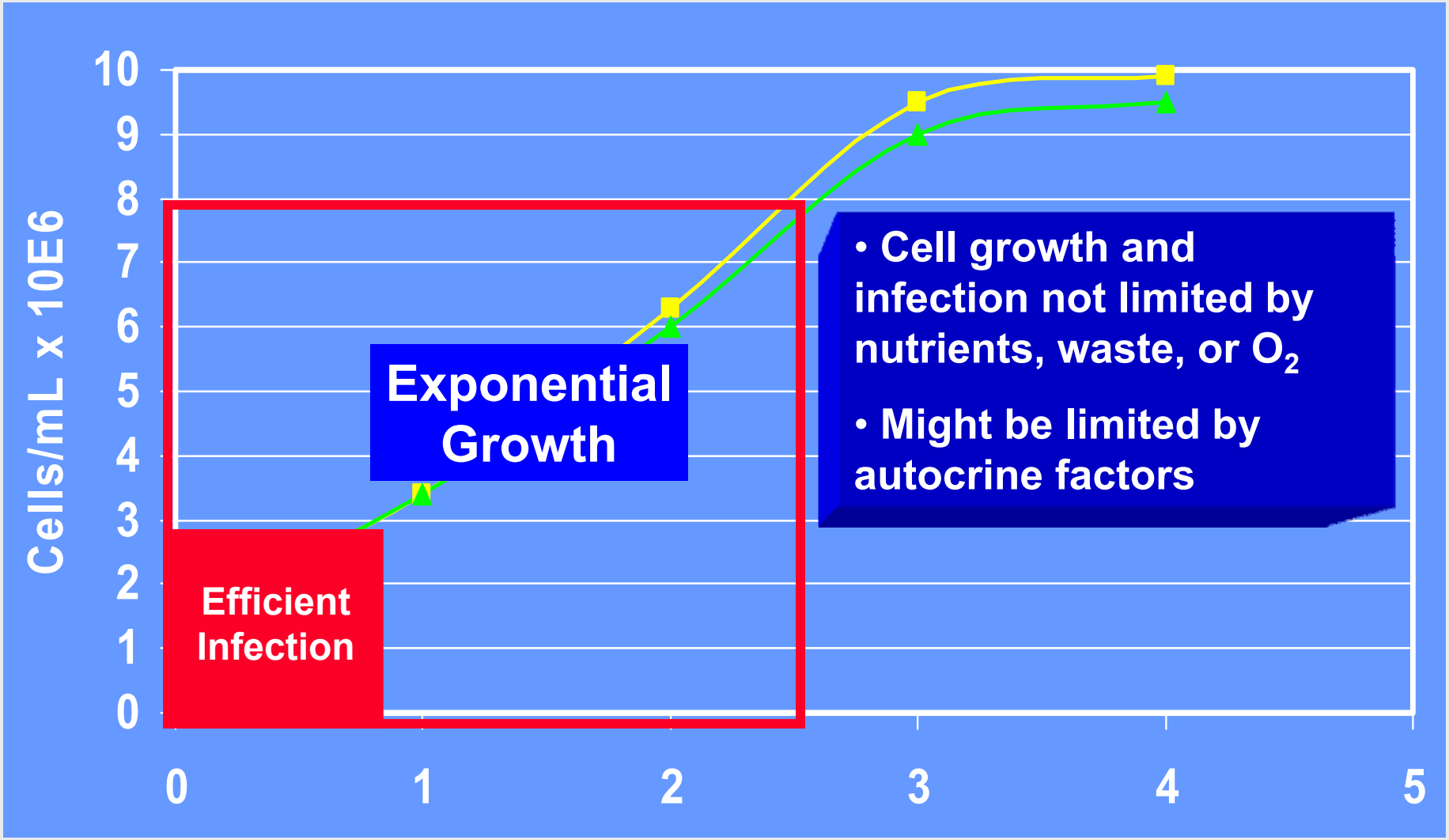


Uninfected SF+

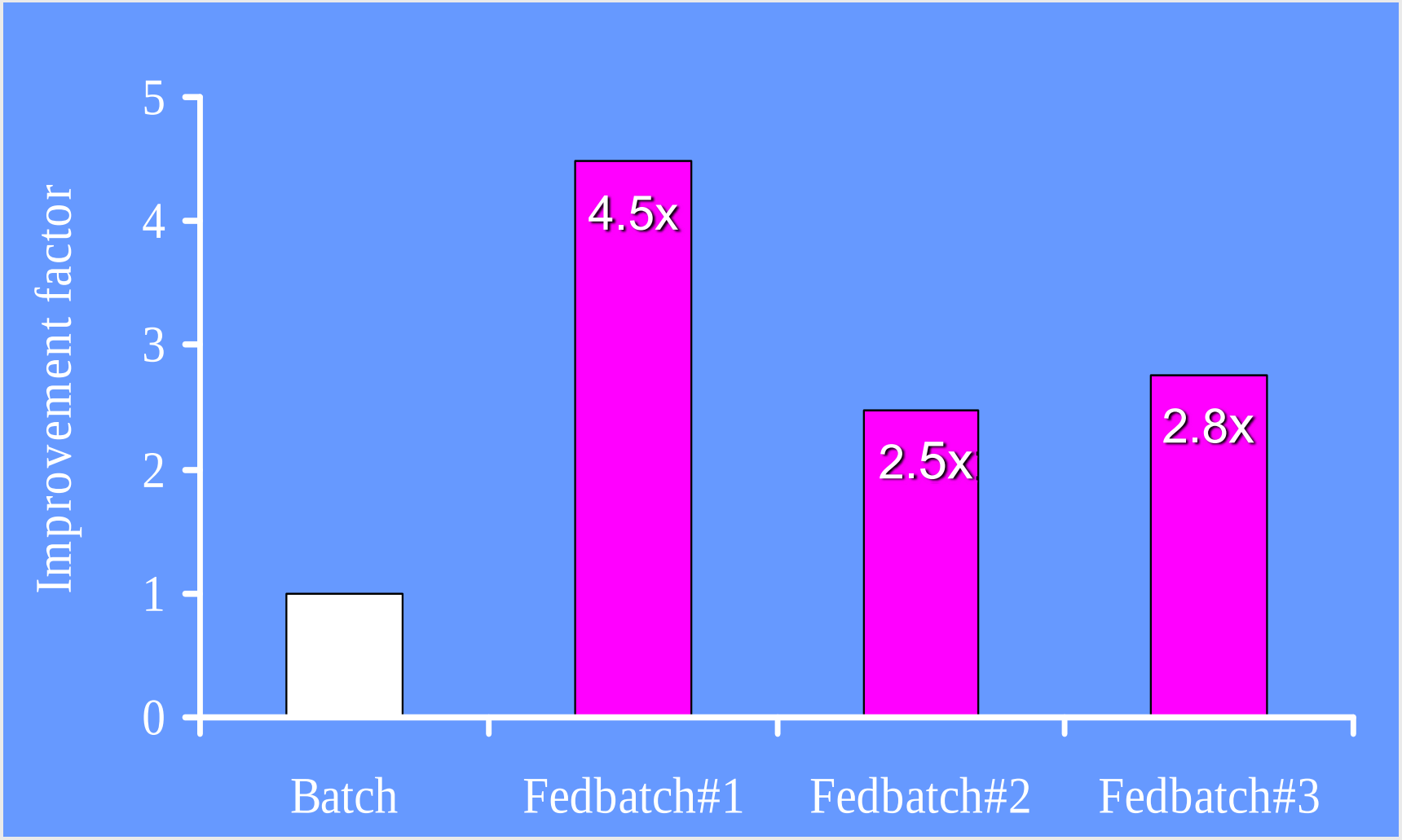


Infected SF+

SF+ Cell Growth and Infection

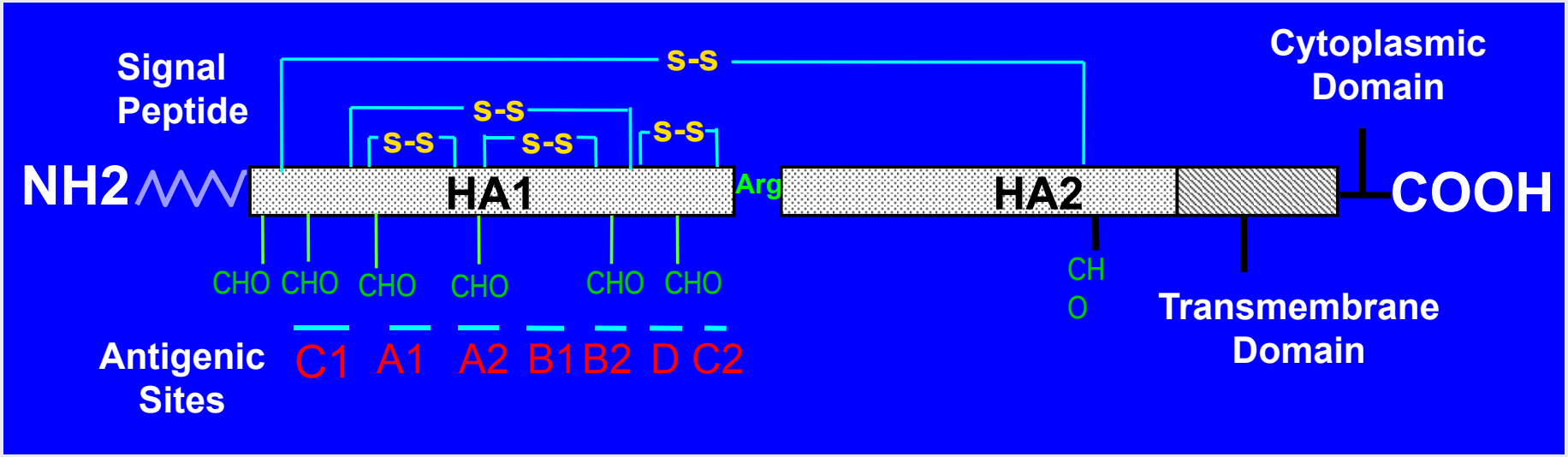


HA yields: Feed's Importance

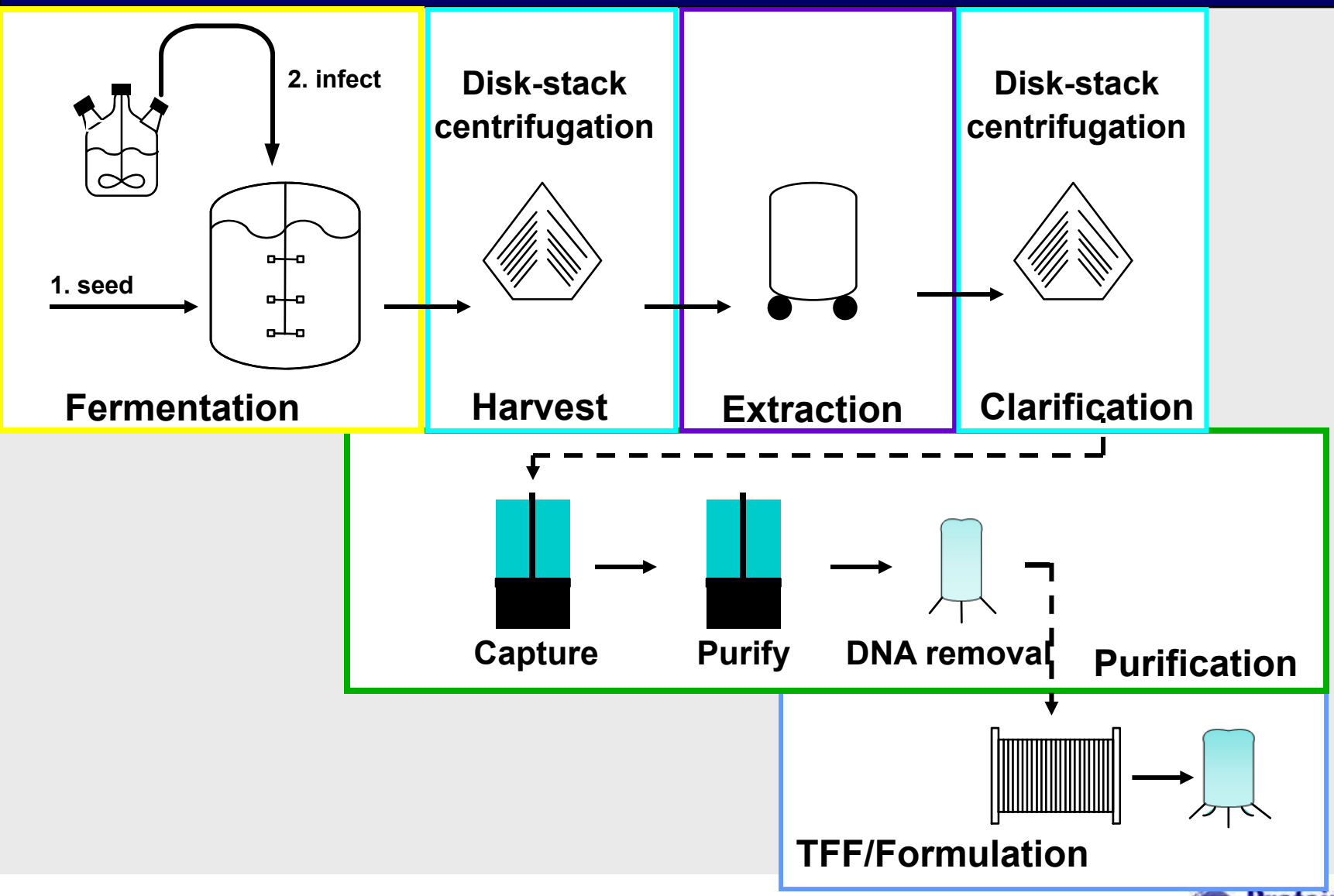


Hemagglutinin properties

- Trimeric integral membrane protein
- Cleavage of HA with host protease into HA1 and HA2 needed for fusion activity
- HA1 and HA2 linked by disulfide bonds
- Contains four antigenic sites (A, B, C, and D)
- Contains many glycosylation sites
- Hydrophobic transmembrane domain



Downstream Process



FluBlok: Potential Benefits
(contains 3x45µg recombinant hemagglutinin)

- Influenza rHA antigens are produced in insect cells – protein based vaccine with low endotoxin content
- rHA protein is highly purified and does not contain egg protein or other contaminants from eggs
- Selection or adaptation of influenza virus strains that produce at high levels in eggs is not required =>the best genetic match
- Cloning, expression and manufacture of FluBlok within 2 months
- FluBlok does not require large amounts of embryonated chicken eggs
- Manufacturing of FluBlok does not require biocontainment facilities
- Manufacture of rHA does not include formalin inactivation or organic extraction procedures

Clinical Studies to Support BLA under accelerated approval mechanism

BLA filing to support licensure April 2008

- PSC01: Efficacy Study in Healthy Adults (451 subjects; 1:1:1)
  - Placebo controlled and two doses of FluBlok
- PSC03: Efficacy Study in Adults older than 65y (869 subjects; 1:1)
  - Active controlled study (FluZone®)
- PSC04: Field Efficacy Study in Healthy Adults (4650 subjects; 1:1)

  (Interim Day 28 Safety and Immunogenicity available)
  - Placebo controlled
- PSC06: Non-inferiority Immunogenicity/Efficacy Study in 600 Healthy Adults (50-64y)

  (Interim Day 28 Safety and Immunogenicity available)
  - Active controlled study (FluZone®)

Clinical studies of rHA vaccines conducted under BB-IND 11951

Study Age Range	**Strain, Dose of rHA_0**	**N^1 at 135µg Dose (3 x 45µg)**	**Control**
PSC01 18-49 yr (2004-2005 influenza season)	45µg A/New Caledonia/20/1999 (H1N1) 45µg A/Wyoming/3/03 (H3N2) 45µg B/Jiangsu/10/03 (Also 15µg H1 & B, 45µg H3) (N=151)	**153**	Saline (N=154)
PSC03 ≥65 yr (2006-2007 influenza season)	45µg A/New Caledonia/20/1999 (H1N1) 45µg A/Wisconsin/67/2005 (H3N2) 45µg B/Ohio/1/2005	**436**	Fluzone (N=433)
PSC04 18-49 yr (2007-2008 influenza season)	45µg A/Solomon Islands/3/2006 (H1N1) 45µg A/Wisconsin/67/2005 (H3N2) 45µg B/Malaysia/2506/2004	**2344**	Saline (N=2304)
PSC06 50-64 yr (2007-2008 influenza season)	45µg A/Solomon Islands/3/2006 (H1N1) 45µg A/Wisconsin/67/2005 (H3N2) 45µg B/Malaysia/2506/2004	**300**	Fluzone (N=302)
Total Safety Database ≥18 yr		**3233**	3193

Solicited Adverse Events in Adults During the First 7 Days After Administration of FluBlok, Placebo, or Comparator Influenza Vaccine

	Study PSC01		Study PSC04		Study PSC06		Study PSC03	
	Adults age 18-49 yrs		Adults age 18-49 yrs		Adults age 50-64 yrs		Adults age ≥65 yrs	
	FluBlok*	Placebo	FluBlok	Placebo	FluBlok	Fluzone	FluBlok	Fluzone
Number of Subjects	153	154	2344	2304	300	302	436	433
Local Adverse Events								
Pain	61%	17%	37%	8%	51%	55%	22%	23%
Redness	5%	2%	4%	2%	8%	8%	10%	12%
Swelling	10%	3%	3%	2%	8%	10%	11%	13%
Bruising	7%	4%	3%	3%	5%	5%	3%	5%
Systemic Adverse Events								
Headache	42%	41%	15%	15%	20%	21%	11%	9%
Fatigue	16%	18%	15%	14%	13%	21%	9%	10%
Muscle Pain	20%	12%	10%	7%	13%	14%	7%	9%
Fever‡	0%	2%	<1%	<1%	<1%	0	<1%	0%
Joint pain	5%	5%	4%	4%	5%	6%	5%	6%
Nausea	8%	6%	6%	5%	4%	5%	4%	3%
Chills	3%	2%	3%	3%	4%	5%	4%	4%
Sweating	3%	5%	NA	NA	NA	NA	3%	2%

NOTE: Subjects are only counted once based on the most severe response reported by subjects on the memory aid. Results >1% reported to nearest whole percent; results >0 but <1 reported as <1%.

* Data restricted to 135µg formulation.

† NA=data not available (not collected during the study).

‡ Fever defined as ≥99.8°F (37.7°C). In PSC03, fever was defined as >100.4°F.

PSC01 - FluBlok Phase II/III Field Study Summary of Results

Efficacy	▪ **In PSC01, commercial dose level (135µg total rHA) provided:** ➢ **100% (95% CI: 29.7, 100) efficacy against culture confirmed CDC-ILI** ➢ **87.3% (95% CI: 5.5, 99.7) efficacy against culture confirmed respiratory illness (CDC-ILI not required)** ➢ **54.4% reduction in CDC-ILI (regardless of culture results)** ▪ Lower dose level (75µg total rHA: 15µg H1 15µg B, 45µg H3) not selected for further development also demonstrated 71% "protective efficacy" and 30% reduction in CDC-ILI vs. placebo ▪ Significant dose response effect confirmed for H1 and B
Highly Immunogenic	▪ Protective levels for all antigens for at least 6 months ▪ H3 component – high and sustained immunogenicity and long lasting titers
Protection Against Drifted Strains	▪ Excellent protection against viruses that had changed (drifted strains included in efficacy estimates provided above)

PSC01 - Results Published In JAMA

PRELIMINARY COMMUNICATION

Safety and Immunogenicity of a Baculovirus-Expressed Hemagglutinin Influenza Vaccine
A Randomized Controlled Trial

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Context A high priority in vaccine research is the development of influenza vaccines that do not use embryonated eggs as the substrate for vaccine production.

Objective To determine the dose-related safety, immunogenicity, and protective efficacy of an experimental trivalent influenza virus hemagglutinin (rHA0) vaccine produced in insect cells using recombinant baculoviruses.

Design, Setting, and Participants Randomized, double-blind, placebo-controlled clinical trial at 3 US academic medical centers during the 2004-2005 influenza season among 460 healthy adults without high-risk indications for influenza vaccine.

Interventions Participants were randomly assigned to receive a single injection of saline placebo (n = 154); 75 µg of an rHA0 vaccine containing 15 µg of hemagglutinin from influenza A/New Caledonia/20/99(H1N1) and influenza B/Jiangsu/10/03 virus and 45 µg of hemagglutinin from influenza A/Wyoming/3/03(H3N2) virus (n = 153); or 135 µg of rHA0 containing 45 µg of hemagglutinin each from all 3 components (n = 153). Serum samples were taken before and 30 days following immunization.

Main Outcome Measures Primary safety end points were the rates and severity of solicited and unsolicited adverse events. Primary immunogenicity end points were the rates of 4-fold or greater increases in serum hemagglutinin inhibition antibody to each of the 3 vaccine strains before and 28 days after inoculation. The prespecified primary efficacy end point was culture-documented influenza illness, defined as development of influenza-like illness associated with influenza virus on a nasopharyngeal swab.

Results Rates of local and systemic adverse effects were low, and the rates of systemic adverse effects were not different in either vaccine group than in the placebo group. Hemagglutinin inhibition antibody responses to the H1 component were seen in 3% of placebo, 51% of 75-µg vaccine, and 67% of 135-µg vaccine recipients, while responses to B were seen in 4% of placebo, 65% of 75-µg vaccine, and 92% of 135-µg vaccine recipients. Responses to the H3 component occurred in 11% of placebo, 81% of 75-µg vaccine, and 77% of 135-µg vaccine recipients. Influenza infections in the study population were due to influenza B and A(H3N2), and influenza A infections were A/California/7/2004–like viruses, an antigenically drifted strain. Seven cases of culture-confirmed CDC-defined influenza-like illness occurred in 153 placebo recipients (4.6%) compared with 2 cases (1.3%) in 150 recipients of 75 µg of vaccine, and 0 cases in recipients of 135 µg of vaccine.

Conclusions In this study, a trivalent rHA0 vaccine was safe and immunogenic in a healthy adult population. Preliminary evidence of protection against a drifted influenza A(H3N2) virus was obtained, but the sample size was small. Inclusion of a neuraminidase component did not appear to be required for protection.

Trial Registration clinicaltrials.gov Identifier: NCT00328107

JAMA. 2007;297:1577-1582 www.jama.com

All currently licensed influenza vaccines in the United States are produced in embryonated hen's eggs. There are several well-recognized disadvantages to the use of eggs as the substrate for influenza vaccine. Eggs require specialized manufacturing facilities and could be difficult to scale up rapidly in response to an emerging need such as a pandemic. It is usually necessary to adapt candidate vaccine viruses for high-yield growth in eggs, a process that can be time consuming, is not always successful, and can select receptor variants that may have suboptimal immunogenicity.[1] In addition, agricultural diseases that affect chicken flocks, and that might be an important issue in a pandemic due to an avian influenza virus strain, could easily disrupt the supply of eggs for vaccine manufacturing. Therefore, development of alternative substrates for influenza vaccine production[2] has been identified as a high-priority objective.

One potential alternative method for production of influenza vaccine is expression of the influenza virus hemagglutinin (HA) using recombinant DNA techniques. In this study, we evaluated an experimental influenza vaccine consisting of recombinant HA expressed in insect cells by a

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PSC03: Difference in Proportions of Subjects with Seroconversion or Significant Increase in HI Titers at Day 28

STRAINS	ALL SUBJECTS		SUBJECTS ≥75	
	FluBlok N=431	Fluzone N=430	FluBlok N=163	Fluzone N=159
	Number Of Subjects (%) [95% CI]			
A/New Caledonia				
Seroconversion[1] or significant increase[2] [2-sided 95% CI]	187 (43) [38.7, 48.2]	140 (33) [**28.1***, 37.2]	64 (39) [31.8, 46.8]	48 (30) [**23.1**, **37.3**]
Difference in proportions between TIV and FluBlok	-10.8		-9.1	
2-sided 95% CI	-17.3, -4.3		-19.4, 1.3	
P-Value[3]	0.001		0.087	
Meets CBER criterion for non-inferiority? ‡	**YES**		**YES**	
A/Wisconsin				
Seroconversion[1] or significant increase[2] [2-sided 95% CI]	335 (78) [73.5, 81.6]	248 (58) [52.8, 62.4]	129 (79) [72.9, 85.4]	86 (54) [46.3, 61.8]
Difference in proportions between TIV and FluBlok	-20.1		-25.1	
2-sided 95% CI	-26.2, -13.9		-35.0, -15.1	
P-Value	<0.001		<0.001	
Meets CBER criterion for non-inferiority? ‡	**YES**		**YES**	

CBER Criterion for non-inferiority: lower bound of the two-sided 95% CI for the % of subjects achieving seroconversion should meet or exceed 30%."

PSC03 -continued

CBER Criterion for a non-inferiority: the upper bound of the two-sided 95% CI on the ratio of the GMT's does not exceed 1.5. This criterion is met for FluBlok for all antigens

Timepoint	Vaccine Group	*A/New Caledonia (H1)*		*A/Wisconsin (H3)*	
		GMT	**95% CI**	**GMT**	**95% CI**
Day 0	Fluzone (N=430)	70.2	(62.8, 78.6)	44.7	(39.2, 51.0)
	FluBlok (N=431)	69.0	(62.1, 76.6)	42.7	(37.6, 48.4)
Day 28	Fluzone (N=430)	148.1	(134.2, 163. 4)	199.2	(176.8, 224.4)
	FluBlok (N=431)	176.8	(159.4, 196.0)	338.5	(299.7, 382.5)
	Ratio GMT TIV/FluBlok	0.84	(0.81, 0.86)	0.59	(0.57, 0.60)
	Meets CBER Criterion?	**YES**		**YES**	

CBER Guidance to Support Accelerated Approval in Placebo Controlled Trial

- The lower bound of the two-sided 95% CI for the percent of the subjects achieving seroconversion for HI antibody should meet or exceed 40%
- The lower bound of the two-sided 95% CI for the percent of the subjects achieving an HI Antibody titer ≥40 should meet or exceed 70%

Strains	Titer ≥40	95% CI	Seroconverted*	95% CI
H1N1 A/Solomon Island/3/2006	98%	97.2, 99.7	78%	74.2, 82.3
H3N2 A/Wisconsin/67/2005	96%	94.6, 98.3	81%	76.6, 84.5
B B/Malaysia/2506/2004	96%	93.9, 97.9	53%	48.3, 58.1

* ≥4-fold increase and minimum titer 40

PSC06 - Field Study - Serology Results
FluBlok (135µg)

- Total enrolled 602 subject (50-64 y) at 6 centers
- Average age: 56 years; 63% female
- HAI Titers were determined
- Data used to support accelerated approval in the US

CBER Guidance to Support Accelerated Approval Applied to PSC06

	No. of Subjects (%)	
	FluBlok (N=299)	Fluzone (N=302)
A/Solomon Islands/03/06		
Seroconversion[1] or Significant Increase[2]		
Yes	216 (72)	200 (66)
2-sided 95% CI	[66.8, 77.2]	[60.6, 71.5]
Meets CBER criterion?[3]	**YES**	**YES**
Difference in Proportions (TIV vs FluBlok)	-6.0	
2-sided 95% CI	[-13.4, 1.4]	
P-value	0.113	
Meets CBER criterion?[4]	**YES**	
B/Malaysia/2506/04		
Seroconversion[1] or Significant Increase[2]		
Yes	122 (41)	124 (41)
2-sided 95% CI	[35.2, 46.6]	[35.5, 46.8]
Meets CBER criterion?[3]	**NO**	**NO**
Difference in Proportions (TIV vs FluBlok)	0.3	
2-sided 95% CI	[-7.7, 8.2]	
P-value	1.000	
Meets CBER criterion?[4]	**YES**	
A/Wisconsin/67/05		
Seroconversion[1] or Significant Increase[2]		
Yes	183 (61)	132 (44)
2-sided 95% CI	[55.4, 66.8]	[38.0, 49.5]
Meets CBER criterion?[3]	**YES**	**NO**
Difference in Proportions (TIV vs FluBlok)	-17.5	
2-sided 95% CI	[-25.4, -9.5]	
P-value	<0.001	
Meets CBER criterion?[4]	**YES**	

1 4-fold or greater increase from pre-vaccination to Day 28 with a minimum Day 28 titer of 1:40.

2 Pre-vaccination titer below limit of detection and Day 28 titer ≥1:40.

3 Lower bound of 2-sided 95% CI should meet or exceed 40% (for persons <age 65 years)

4 Upper bound of 2-sided 95% CI on the difference between seroconversion rates should not exceed 10%CI based on Clopper-Pearson exact method; P-value based on Fisher's Exact Test

CBER Guidance to Support Accelerated Approval Applied to PSC06

			A/Solomon Islands/03/06		*A/Wisconsin/67/05*		*B/Malaysia/2506/04*	
Visit	**Dose Group**	**N**	**GMT**	**95% CI**	**GMT**	**95% CI**	**GMT**	**95% CI**
Day 0	FluBlok	299	28.71	(25.59, 32.21)	18.57	(16.37, 21.06)	48.49	(43.38, 54.19)
	Fluzone	302	27.77	(25.07, 30.76)	18.20	(16.07, 20.62)	49.18	(43.77, 55.25)
Day 28	FluBlok	299	181.34	(159.61, 206.02)	105.41	(91.01, 122.09)	110.93	(100.07, 122.97)
	Fluzone	302	139.74	(124.64, 156.66)	60.88	(53.58, 69.18)	116.03	(104.16, 129.25)
Ratio of GMT[1] at Day 28			0.77	(0.75, 0.79)	0.58	(0.53, 0.62)	1.05	(1.01, 1.09)
Meets CBER criterion?			**YES**		**YES**		**YES**	

[1] Ratio of GMT=(GMT TIV/GMT FluBlok)

Ratio should be lower than 1.5

FluBlok Manufacturing

- Launch of FluBlok in house
  - 2008 - 600-L (1-2 million doses)
  - 2009 – 1,800L (5-10 million doses)
  - 2010+
    - **2 x 5000L in new facility in Meriden (up to 60 million doses); or**
    - **LSM outsource**

Outlook – 2008 and beyond

- FluBlok to market
  - BLA Filing April, 2008
  - Approval anticipated in time for 2008/09 flu season
- PSC04 and PSC06
  - Safety and antibody titers submitted
  - Field data – Q3/Q4
- Tentative FDA approval date –October 2008