Development of a Novel Recombinant Influenza Vaccine in Insect Cells

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New Cells for New Vaccines II

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New Cell for New Vaccines II

Topics:

- Baculovirus Expression Vector System (BEVS) technology
- Development and qualification of PSC's proprietary insect cell line

  Regulatory issues surrounding insect cells
- FluBIOk® Clinical study results

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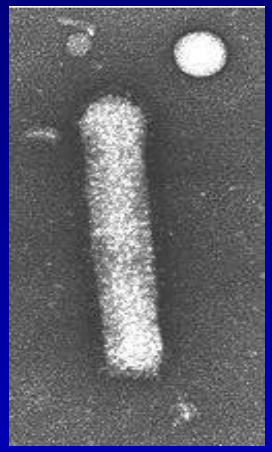
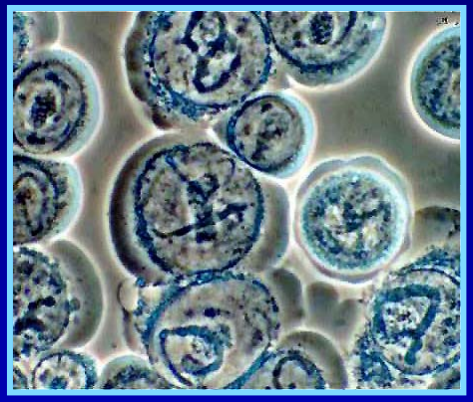
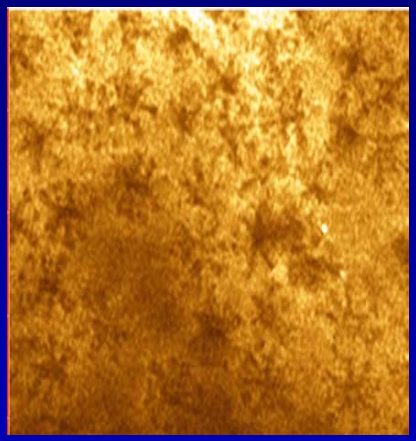
Technology

"Enabling products where speed, cost, and safety matter"

Highly Efficient Protein Expression System: 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 insect cells in a fermenter
- Infect cells with engineered virus
- Incubate infection for 48 - 72 hrs

- Highly purified protein
- rHA forms rosettes
- Formulate with PBS into vaccine

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Key Advantages of BEVS Technology

Authenticity of the antigen

- **Antigen in vaccine is an exact match to natural virus**

Speed

- Cloning to expression in weeks vs. months

Safety

- No live virus, no need for biocontainment
- >50,000 doses tested in humans with outstanding safety record

Versatility

- Cloned and expressed > 1,000 proteins

Reliable scale-up

- Current scale 500L; scale-up in progress

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Inherent Safety Associated with BEVS-based Production Technology

Baculovirus

- Daily exposure - typical serving of coleslaw contains 112 million polyhedra (each polyhedron contains multiple baculoviruses)[1]
- Limited Host Range (Lepidopteran Species of Insects)
- Do NOT Replicate in Mammalian Cells

Insect Cells

- Virtually No Known Adventitious Agents Can Replicate in both Insect Cells and Mammalian Cells
- Arboviruses are Rare Exceptions (West Nile Encephalitis)
- Derived from Non-biting Insects – Low Adverse Events

PSC has delivered > 50,000 doses in >5,000 subjects

[1]Heimpel et al (1973) Environmental Entomology, vol2 (1), pp. 72.

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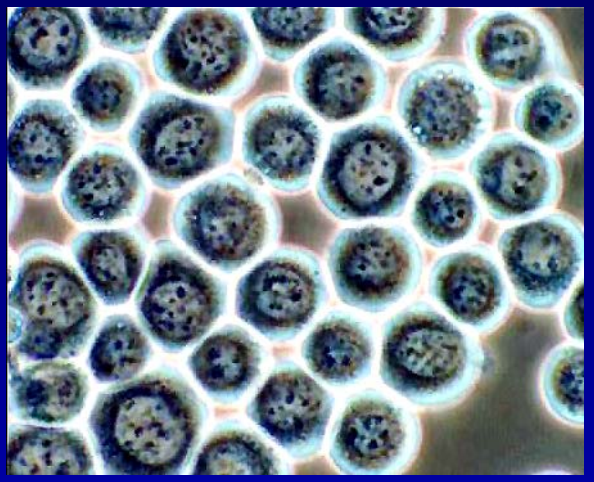
Proprietary Cell Line - Serum-free *expres*SF+®

Evolved from Sf9 Cells

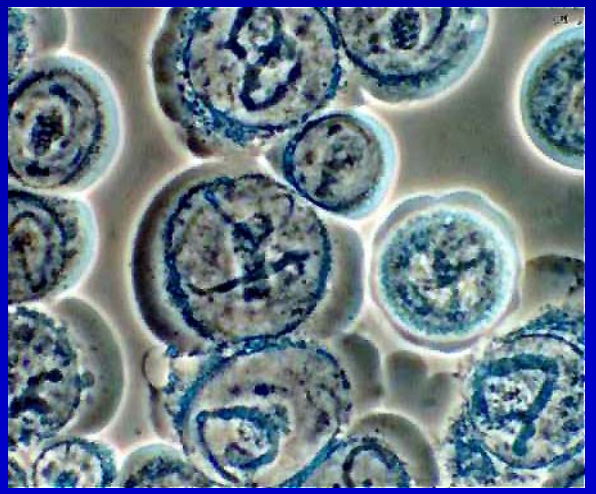
- Selective pressure in serum-free media with added insulin (0.4 mM)
- Unique phenotypic and genotypic properties
- Patented
- Qualified as per ICH Q5A and 1993 Points to Consider

Ideal for Manufacturing

- Serum-free – low cost media
- Stable for > 50 passages
- Infected with low MOI $\leq$ 1
- Produces high titer AcNPV
- cGMP at 500L scale
- Excellent safety record in clinical trials



Uninfected



Infected

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Evaluation of New Cell Substrates

Safety and Purity Considerations for Novel Cell Substrates

- Adventitious agents (infectivity)
- Nucleic acids
  - retrotransposons
- Host cell proteins
  - allergies
  - glycosylation impact
- Other possible contaminants induced by raw materials, e.g. FBS

Testing and Qualification of the *expres*SF+ MCB

*expres*SF+ Master Cell Bank

generated 1993; new bank in 2004

Identity Testing

- Karyotyping analysis
- Isoenzyme analysis
- Cell morphology/Growth Characteristics

(Growth, Infectivity and Protein Production)

Microbial Contaminants

- Sterility (21 CFR 610.12)
- Mycoplasma and Spiroplasma

(Direct and Indirect, Agar and Broth – 1993 PTC)

Tumorigenicity

- Tumorigenicity – 16 weeks, nude mice

Testing and Qualification of the *expres*SF+ MCB

Adventitious Virus Testing

- In Vitro - Vero, MRC-5, BHK-21, and Sf9 cells (28 days CPE/hemadsorption)
- In Vivo - Suckling mice, adult mice, and embryonated chicken eggs (1993 PTC, 21 CFR 630.35)
- Developing assays for arboviruses

Retrovirus Testing

- EM of $\geq$ 200 cells
- Co-cultivation/PERT[1]

[1] Sf9 cells are known to contain retrotransposons and thus are generally positive for RT.

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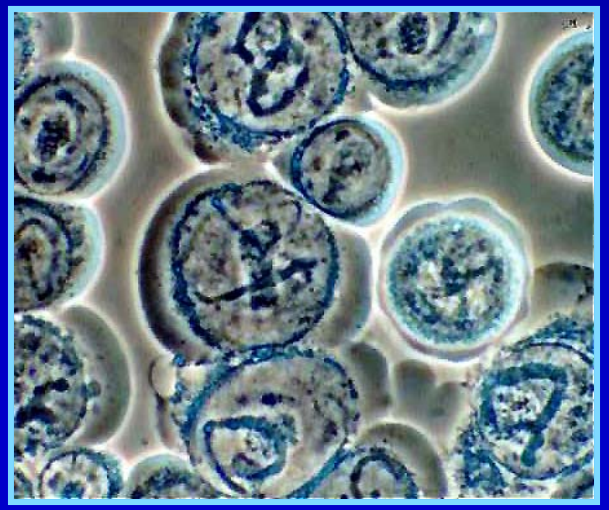
Testing and Qualification of the *expres*SF+ WCB

Microbial Contaminants

- Sterility (21 CFR 610.12)
- Mycoplasma and Spiroplasma
  - Direct and Indirect – 1993 PTC

Identity

- Cell morphology/Growth Characteristics
  - Growth
  - Infectivity
  - Protein Production



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Testing of End of Production (>50p) *expres*SF+ cells

Identity

- Isozyme analysis
- Cell morphology/Growth Characteristics

Sterility

Adventitious Virus Testing

- In Vitro - Vero, MRC-5, BHK-21, and Sf9 cells (28 days CPE/hemadsorption)
- In Vivo - Suckling mice, adult mice, and embryonated chicken eggs (1993 PTC, 21 CFR 630.35)

Retrovirus Testing

- EM of $\geq$ 200 cells
- Co-cultivation/PERT

Tumorigenicity

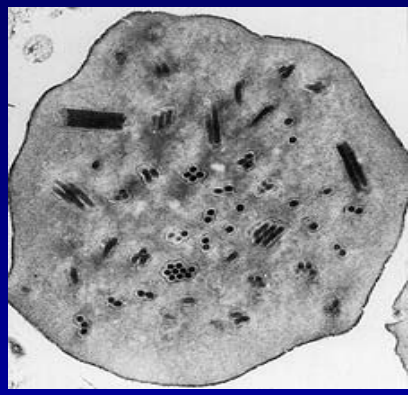
Technology
Cell Line: Serum-free *expres*SF+

Regulatory Advantages

- FDA finds acceptable for vaccine production
- Non-tumorigenic
- Serum-free medium
- Stable for > 50 passages
- cGMP at 500L scale
- Excellent safety record in clinical trials
- Most mammalian viruses cannot replicate in insect cells and vice versa

  (exception – Arboviruses)

Technology
PSC Virus Background

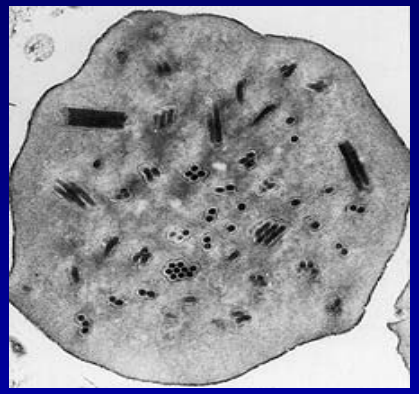


Autographa californica (Alfalfa Looper)

Nuclear Polyhedrosis Virus (AcNPV), E2 isolate

- AcNPV polyhedra were isolated from a single-field-collected alfalfa looper larva
- Propagated and plaque-purified/cloned
- Introduced restriction sites to facilitate generation and cloning of recombinant viruses
- SF+ cells were infected with parental, modified AcNPV
- Harvested supernatant was frozen and designated Master Virus Bank (MVB)
- DNA is isolated from MVB, linearized and recombined with transfer plasmid to form Working Virus Banks

Technology
MVB Qualification Testing



Identity

- Southern Blot
- Comparability to 1994 MVB

Microbial Contaminants

- Sterility (21CFR610.12)
- Mycoplasma/spiroplasma (Direct and Indirect, 1993 PTC)

Adventitious Agents

- In Vitro Assay (VERO, MRC-5, BHK21, S2; 28 days; CPE/hemadsorption)
- In Vivo Assay - Suckling mice, adult mice, and embryonated chicken eggs (1993 PTC, 21 CFR 630.35)

FluBIOk® - Next Generation Vaccine for Influenza

"Making products where speed, cost, and safety matter"

Trivalent recombinant hemagglutinin (rHA) vaccine

- Produced *in vitro* via insect cell culture technology
- Cloned from WHO/CDC recommended strains
- Easier to produce, no eggs, no live viruses, no bio-containment required, no preservatives
- Contains 3x45ug rHA

FluBIOk rHA Antigens

- Highly purified (95%)
- Correct 3-D structure
- Biologically active
  - Hemagglutination activity
- Induces protective immune responses
  - HAI antibodies
  - Neutralizing antibodies

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FluBlOk 2004 Phase II/III Field Study Summary of Results

Clinical dose (45ug/strain)

- Efficacy: 100% (even against drifted H3 strain)
- Effectiveness: 54% reduction ($p \leq 0.05$) in CDC-ILI vs. placebo

Efficacious and effective without neuraminidase

Highly immunogenic

- H3 component - high and long lasting titers
- Protective levels for all antigens for at least 6 months

FluBlOk protects against “drifted” strains

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FluBIOk 2006 Phase III Study PSC03: FluBIOk vs. FluZone Safety and Immunogenicity

- Randomized double-blinded Study
- Subjects: Age ≥ 65 yr (medically stable)
- Subjects randomized to received FluZone, or trivalent rHA containing 45 µg of each rHA
- Total enrollment: 869 subjects

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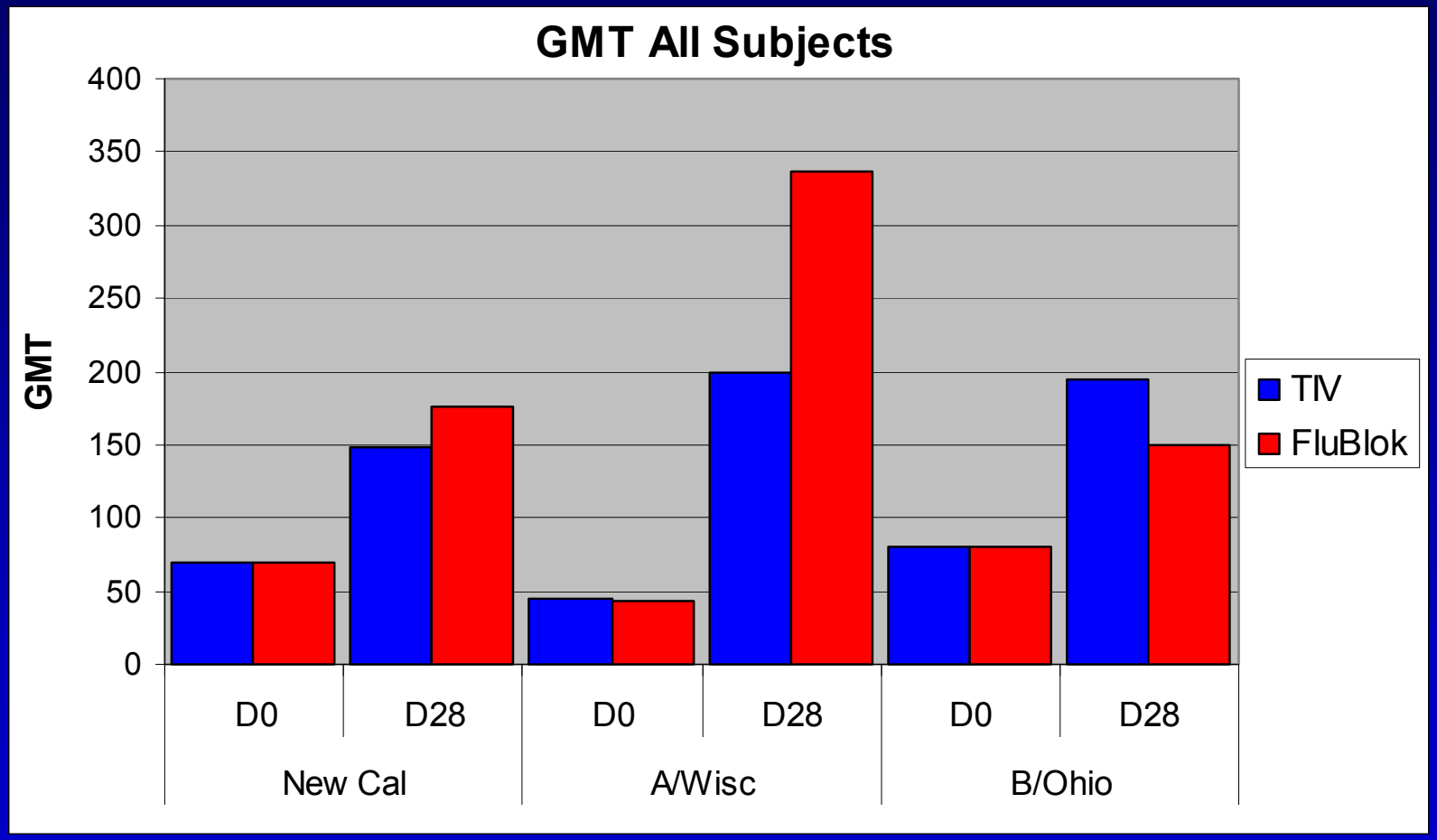
PSC03 GMT Results
FluBlOk (135µg) vs. TIV (45µg)



TIV/FluBlOk GMT ratio D28 not to exceed 1.5 →
Endpoint met for all three antigens

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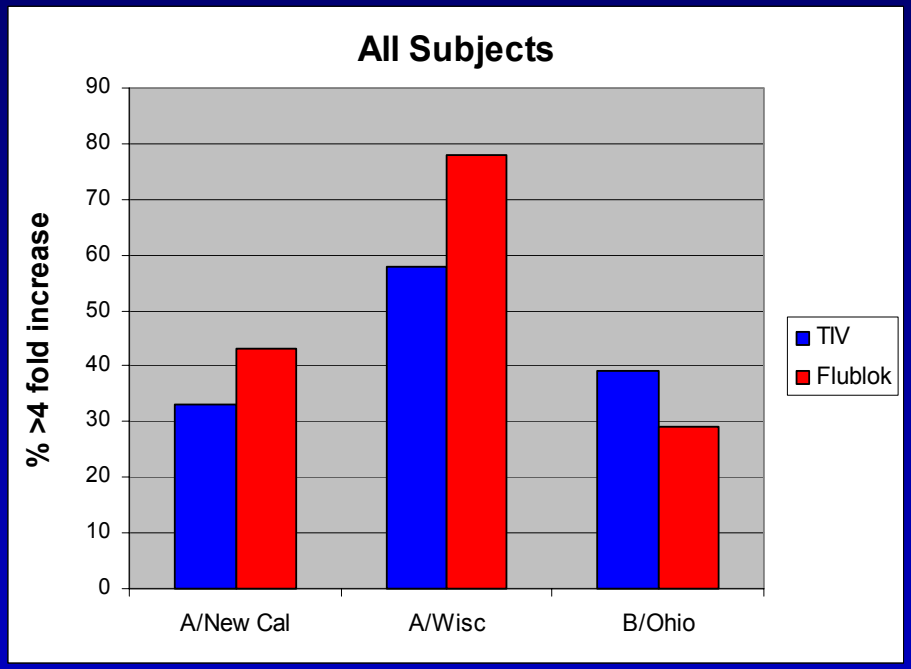
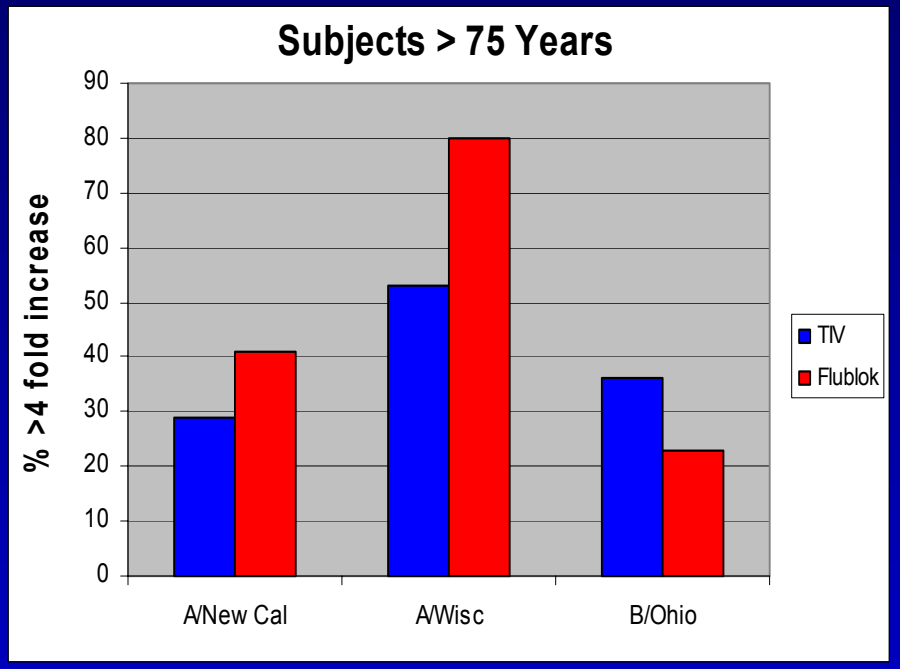
PSC03 Serology Results
FluBlOk (135µg) vs. TIV (45µg)





Difference in sero-conversion observed in population as a whole versus 279 subjects aged 75 or older

Note: TIV contained B/Malaysia and FluBlOk contained B/Ohio antigen

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FluBlOk® - Next Generation Vaccine for Influenza
"Making products where speed, cost, and safety matter"

The BEVS technology provides:

- Speed, Cost and Safety
- Rapid response to emerging strains
- No need to handle live viruses
- Authentic antigen (no changes due to adaptation of the virus to egg or cell culture)

Next steps for PSC

- Preparing our launch facility
- Two clinical studies in progress for 2007-2008 to support accelerated and traditional approval
- BLA filing starting Q4 2007 (Accelerated Approval)
- FluBlOk product approval expected in 2008
- Development of prophylactic pandemic vaccine

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