Protein Sciences
CORPORATION

A Universal Manufacturing Platform for Seasonal Flublok Production

Bio-Production Summit
San Diego
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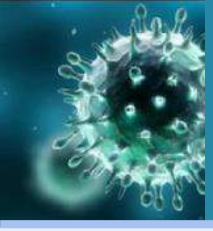


Outline

1. Protein Sciences Corporation Company Overview
2. Baculovirus Technology Platform (BEVS)
3. Flublok® – Recombinant Influenza Vaccine Production by BEVS
4. Continuous Flublok® Process Improvements
   - Resin Re-Use
   - Column Load
   - Yield Improvement
5. Conclusion

Protein Sciences Corporation Company Overview

- Founded in 1983 in USA (~125 employees)
- Manufacturing and developing
  - Vaccines
  - Biopharmaceuticals
- Flublok® seasonal influenza vaccine
  - Approved by USA Food Drug Administration (FDA)
  - Flublok ® is the first recombinant influenza vaccine
- 2009 BARDA contract was awarded for
  - To support licensure of Flublok ®
  - To continue development of Panblok (influenza pandemic vaccine)
- Headquarters in Meriden, CT
  - GMP facilities in CT and NY
  - 50L bioreactor in CT; 600L bioreactor in CT; 2,500L bioreactor in NY

Protein Sciences Corporation manufacturing and developing vaccines for 33 years

BEVS Platform for Vaccine and Protein Therapeutic Manufacturing : Workflow

1st step ("Plug and Play")

Engineer recombinant baculovirus with the gene of interest. Powerful promoter generates high yield of expressed protein

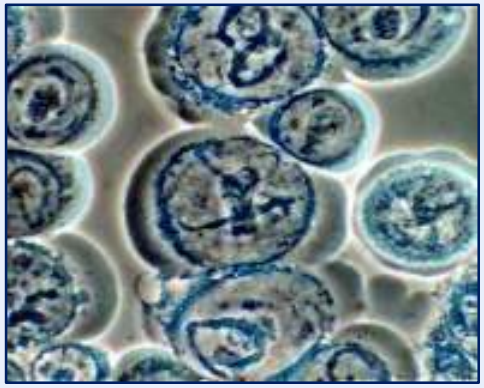
2nd step

Production of recombinant protein

3d step

Purify protein (purity > 90%)

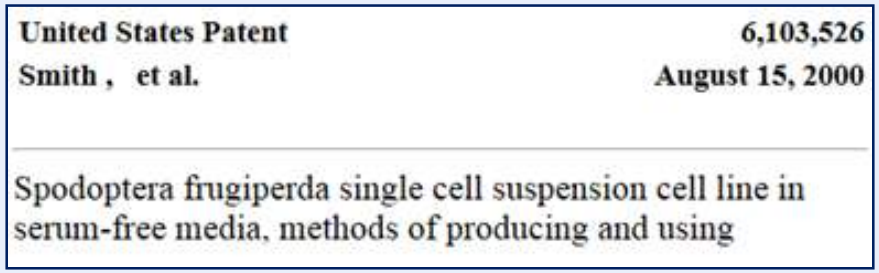
Formulate drug substance



- **Rapid production of biologically active proteins**
- **Scalable to large volumes with low cost media**
- **Linear scale-up from 10-50L scale to 459L, 1,800L and 18,000L scales**

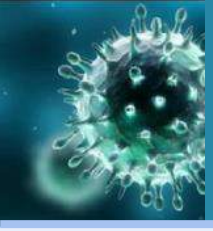
Advantages of *expres*SF+ Cells

- Qualified according to FDA guidance
  - Free of mycoplasma and spiroplasma
  - Free of retrovirus-like particles
  - Non-tumorigenicity in mice
- Easy to work
  - Suspension culture
  - Scalable for cGMP manufacturing up to 18,000L
  - Serum-free, protein-free, low cost growth medium
  - High density growth capability
  - Produces high titer AcNPV
  - Stable for > 50 passages
- Licensed by
  - Merck
  - Boehringer Ingelheim
  - uniQure
  - Japan Tobacco


United States Patent 6,103,526
Smith , et al. August 15, 2000

Spodoptera frugiperda single cell suspension cell line in serum-free media, methods of producing and using

- **Patented, unique insect cell line, approved for protein production in GMP environment**



Manufacturing Advantages of BEVS

- **Safe eukaryotic expression system**
- **Speed – change virus vector insert, not cell line**
  - Single Qualified Cell Line for **ALL** Products
  - Single Master Virus Bank for **ALL** Products
  - Multiple genes may be co-expressed
- **Expression**
  - High level protein expression
  - Ability to express large proteins
- **Processing**
  - Cleavage of signal peptides
  - Post-translational processing (i.e. - glycosylation)
  - Folding
- **Scale-up**

Flublok® - Next Generation Vaccine for Influenza

- **Recombinant Hemagglutinin (rHA) Antigen Vaccine**: **First Licensed Recombinant Influenza Vaccine**
  - Produced *in vitro* via BEVS technology platform
  - Cloned from FDA selected vaccine strains (wild type antigen)
  - Production requires: no eggs, no live influenza viruses, no bio-containment, no preservatives (thimerosal) or antibiotics, no latex
  - Approved for ages 18 and older
    - Pediatric trial was completed
- **Flublok rHA Antigens**
  - Highly purified protein
    - Membrane, glycosylated protein (monomer ~65kDa)
  - Correct 3-D structure
  - Biologically active (hemagglutination activity)
  - Induce protective immune responses
    - HA antibodies/surrogate marker of efficacy
    - Neutralizing antibodies

US FDA Approval January 16, 2013 (Trivalent)

US FDA Approval October 11, 2016 (Quadrivalent)

Seasonal Flu Vaccine is an Unique Business

- Influenza vaccines need to be updated every few years because of HA antigenic drift.
- WHO announces flu vaccine composition for Northern Hemisphere at the end of February.
- Development work
  - end of November - January (~60days)
- Vaccine released by FDA August-September.

#	Season	Changes			
		A (H1N1)	A (H3N2)	B	Q-Valent B
1	2017-2018				
2	2016-2017		●	●	●
3	2015-2016		●	●	
4	2014-2015				
5	2013-2014		●	●	●
6	2012-2013		●	●	
7	2011-2012				
8	2010-2011	●	●		
9	2009-2010			●	
10	2008-2009	●	●	●	
11	2007-2008	●			
12	2006-2007		●	●	
13	2005-2006		●		
14	2004-2005		●	●	
15	2003-2004				
16	2002-2003			●	
17	2001-2002			●	
18	2000-2001	●	●	●	
19	1999-2000			●	

B Yam
B Vic
B unknown
NA

- Annual reformulation of seasonal flu vaccine
- Flu vaccine manufactures are under high pressure by timeline
- 1st batches of drug substances produced at risk

Adaptation of Process to Seasonal Changes

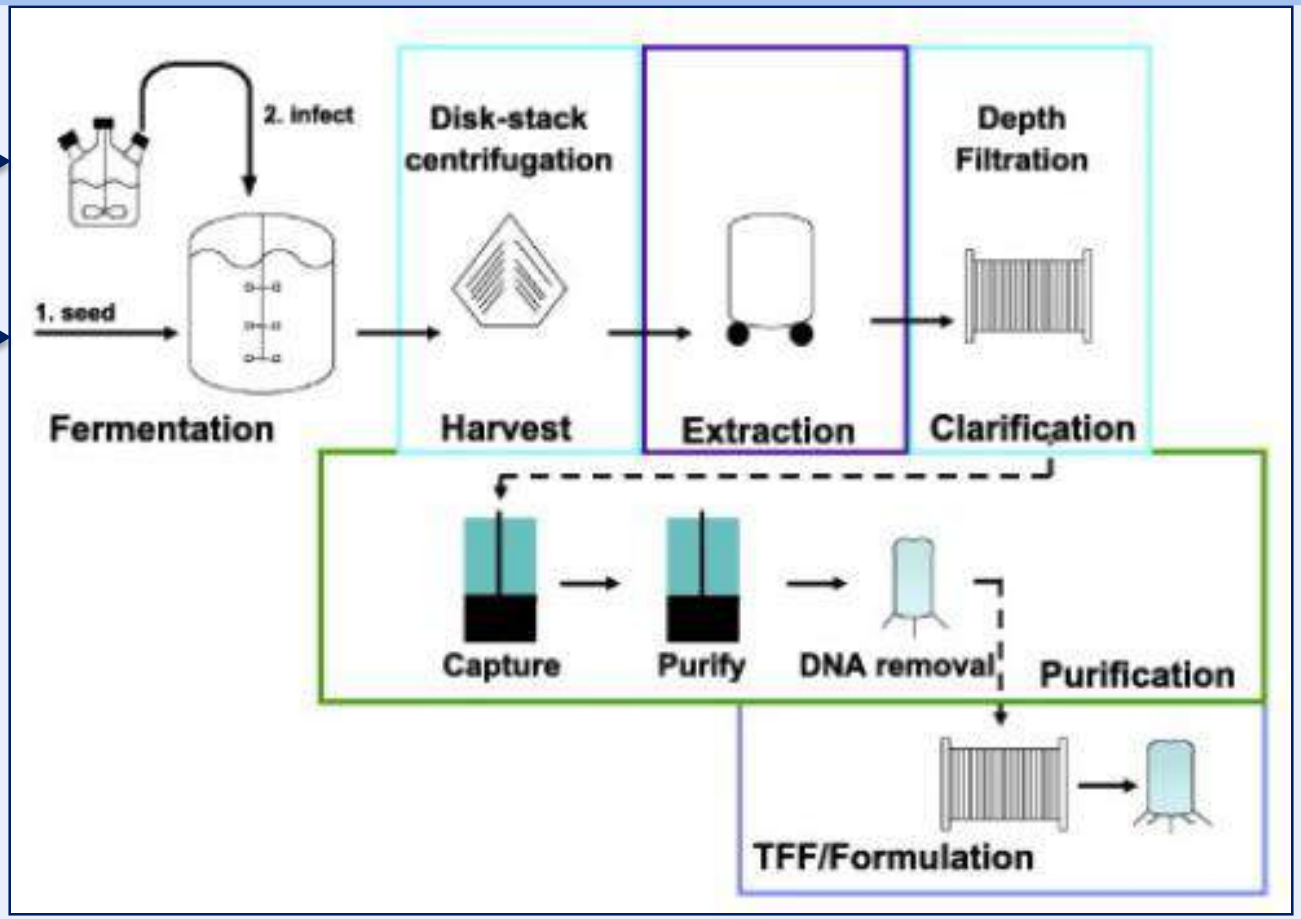
FDA Selected Vaccine Strains by Season			
Season	**H1N1 strain**	**H3N2 strain**	**B strain (+Q-Valent B)**
2017-18	A/Michigan/45/2015 (?)	A/Hong Kong/4801/2014 (?)	B/Brisbane/60/2008 (?) B/Phuket/3073/2013 (?)
2016-17	A/California/7/2009	A/Hong Kong/4801/2014	B/Brisbane/60/2008
2015-16	A/California/7/2009	A/Switzerland/9715293/ 2013	B/Phuket/3073/2013
2014-15	A/California/7/2009	A/Texas/50/2012	B/Massachusetts/2/2012
2013-14	A/California/7/2009	A/Texas/50/2012	B/Massachusetts/2/2012
2012-13	A/California/7/2009	A/Victoria/361/2011	B/Wisconsin/1/2010
2011-12	A/California/7/2009	A/Perth/16/2009	B/Brisbane/60/2008
2010-11	A/California/7/2009	A/Perth/16/2009	B/Brisbane/60/2008
2009-10	A/Brisbane/59/2007	A/Brisbane/10/2007	B/Brisbane/60/2008
2008-09	A/Brisbane/59/2007	A/Brisbane/10/2007	B/Florida/04/2006
2007-08	A/Solomon Islands/03/2006	A/Wisconsin/67/2005	B/Malaysia/2506/2004
2006-07	A/New Caledonia/20/99	A/Wisconsin/67/2005	B/Malaysia/2506/2004
2005-06	A/New Caledonia/20/99	A/California/7/2004	B/Shanghai/361/2002

- **At least one vaccine antigen typically changes with each season**

Universal HA Antigens Process in Insect Cells

Recombinant Baculovirus Scale-up (P4, P5)

***expres*SF+ Cells Seed Train**

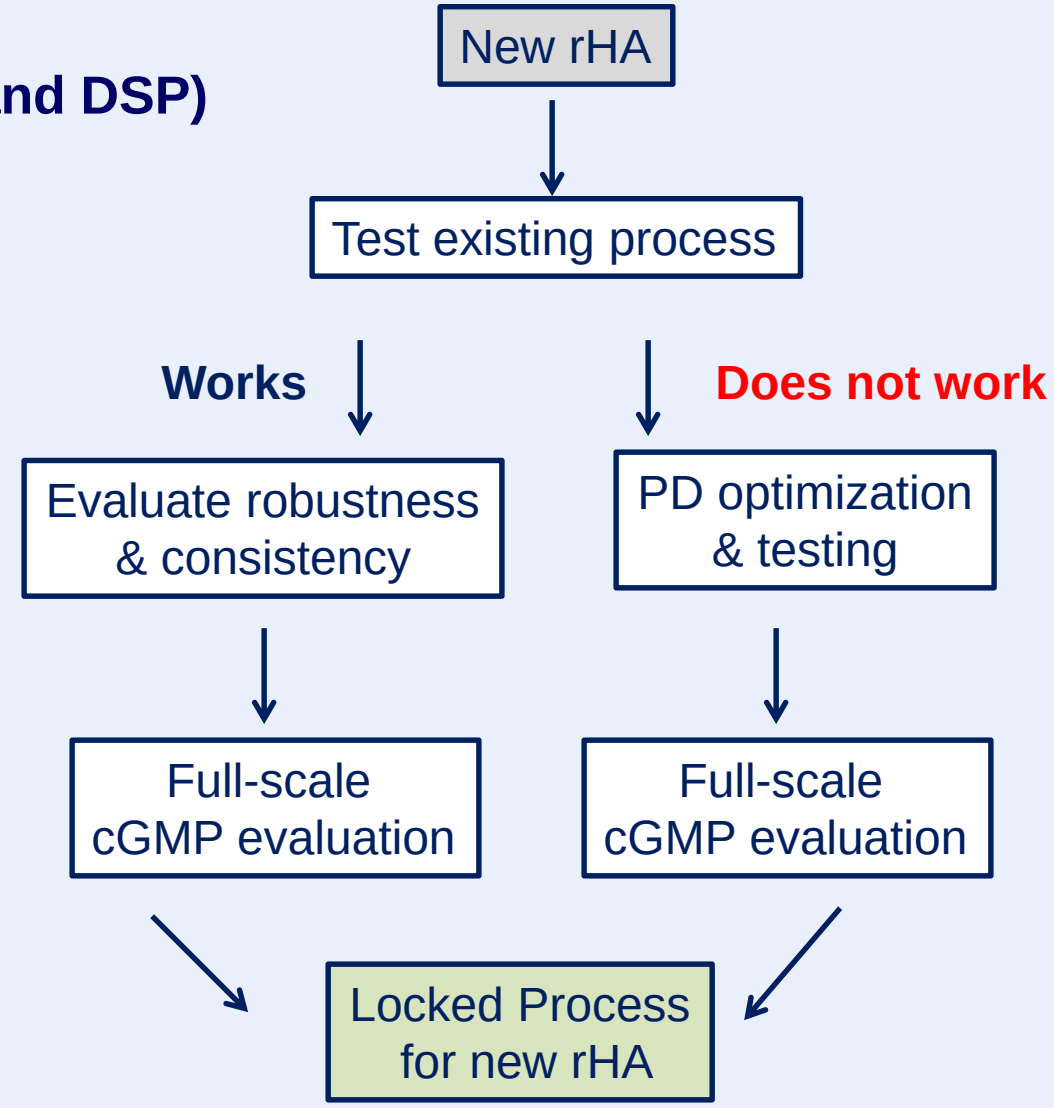


- Scale down model at 1L, 4L, 10L and 50L scales
- Technology transfer to 459L, 1,800L and 18,000L scales
- Less than one week for production of one HA lot
- rHA protein is highly purified recombinant protein

Process Development
Road Map to a New Seasonal Process

SOP driven process (USP and DSP)

- Defines scale down models for Process Development.
- Provides criteria for evaluation of results (yield, purity and biochemical characterization).
- Provides data summary sheets for documentation.
- Provides guidelines and instructions for evaluating new antigens.



Scale Down Model for New Strains of Flublok®

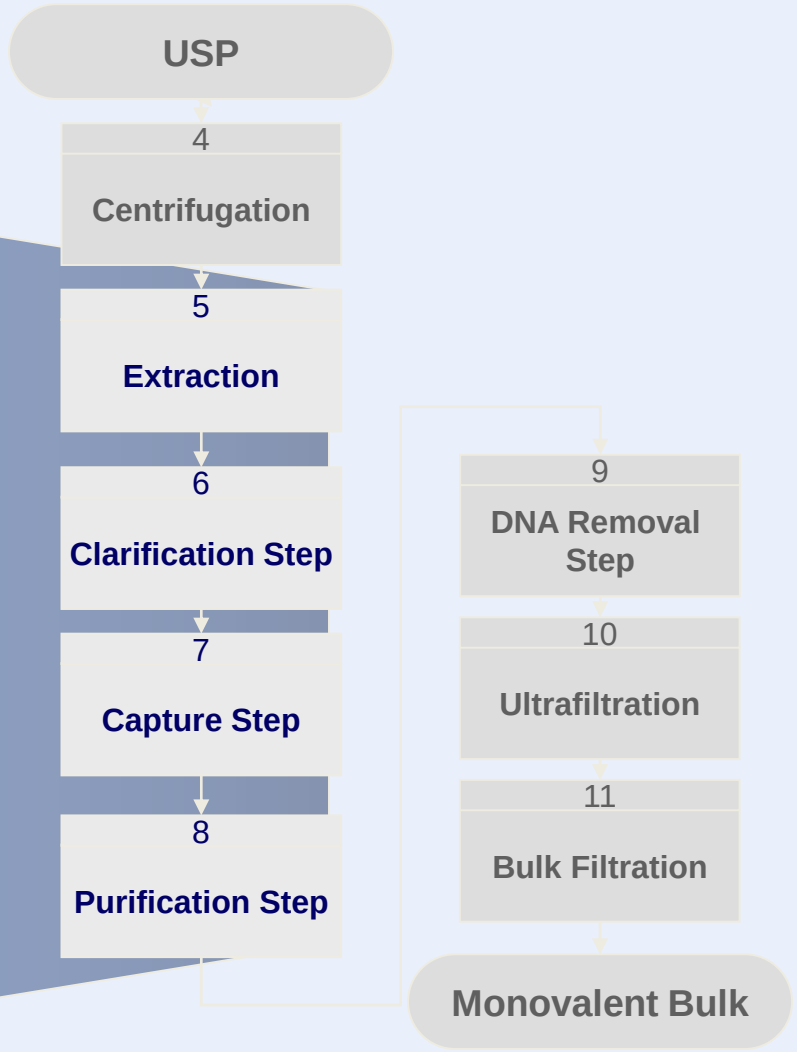
SOP defines rHA specific process changes and requirements:

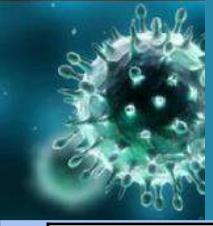
Step 5: Identify pH limits and detergent concentration.
Confirm rHA stability for process step duration at defined pH limits.

Step 6: Identify required conditioning for step 7 (pH/conductivity adjustments).
Confirm adequate clarification.

Step 7: Identify pH and/or conductivity requirements and ranges, elution strategy.
Confirm yield and purity of Eluate.

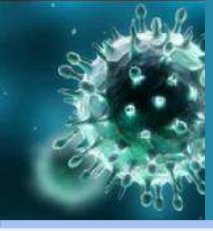
Step 8: Identify/confirm operating pH/conductivity range, load conditioning.
Confirm yield and purity of Eluate.





Development of a Universal Process

Step	Type/Subtype Variability? (B, H1, H3)	Seasonal Strain Variability?	Variable Parameters
Fermentation	no	no	universal step
Harvest	yes	infrequent	viability
Extraction	yes	often	pH; [detergent]; [salt]
Depth Filtration or Clarification	yes	infrequent	pH; [detergent]; [salt]
IEX Chromatography (Capture)	yes	often	pH; [salt]; [detergent]; resin type
HIC Chromatography (Purification)	yes	some	pH; [salt]; [detergent]
Q Filtration or DNA removal	no	no	universal step
Ultrafiltration	no	no	universal step



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Flublok® Constant Process Improvement: *Resin Reuse Study*

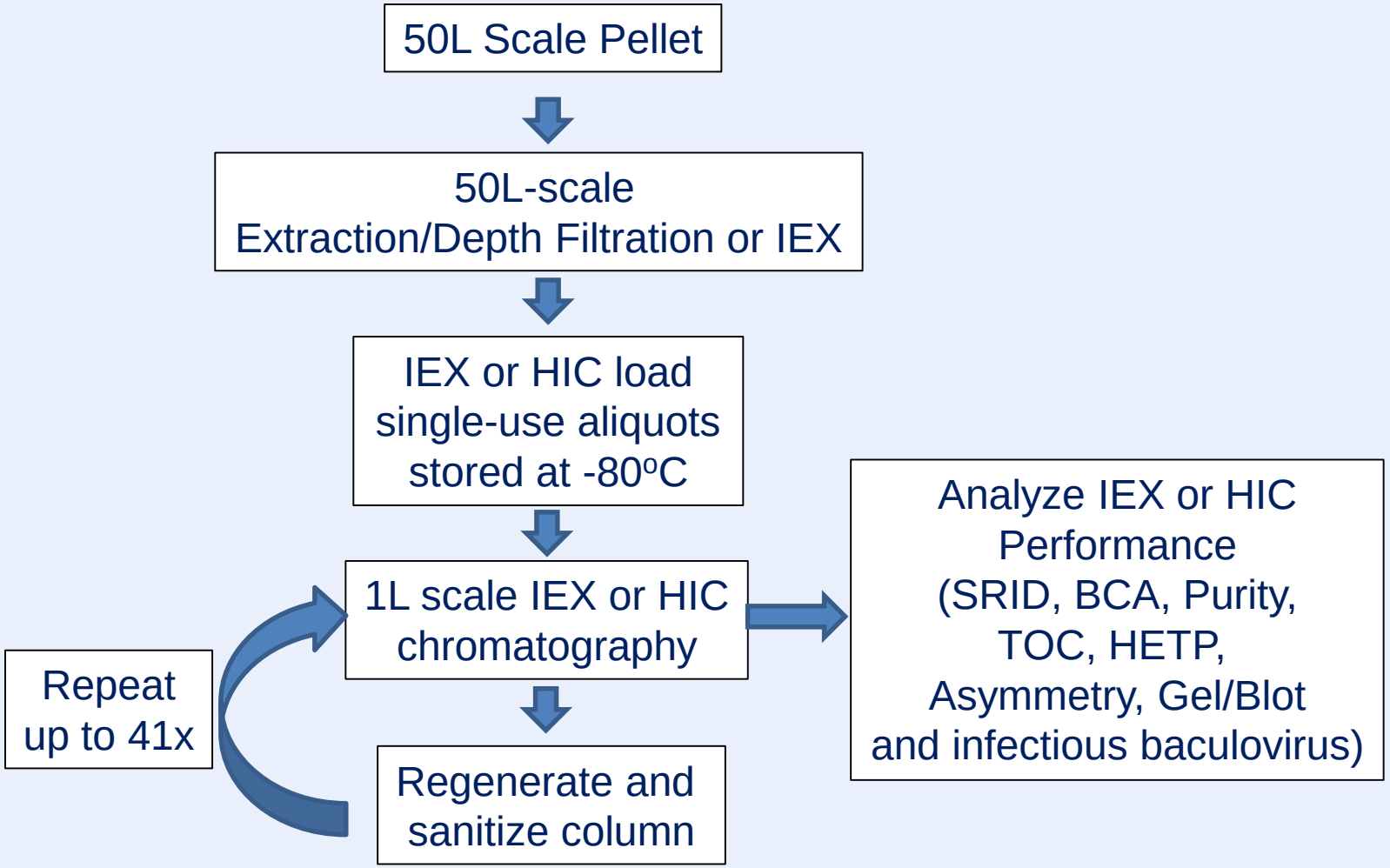
- Current limits of resin reuse were established using historical data from commercial scale purifications available at the time the limits were implemented, and do not necessarily represent the maximum resin re-use limits.
- Determine the maximum number of column resin reuse cycles for IEX and HIC for Flublok.

The Endpoint:

- When the column becomes unusable (clogged, dried out, etc.)
- Negative impact on rHA purification
- Upon completion of the 16-41th consecutive, successful purification
  - Tested by chromatogram comparison, SRID, BCA, Purity, rBV, TOC, HETP, Asymmetry and Gel/Blot

The B, H3 and H1 strains were selected to test

Resin Reuse Study:
Experimental Design



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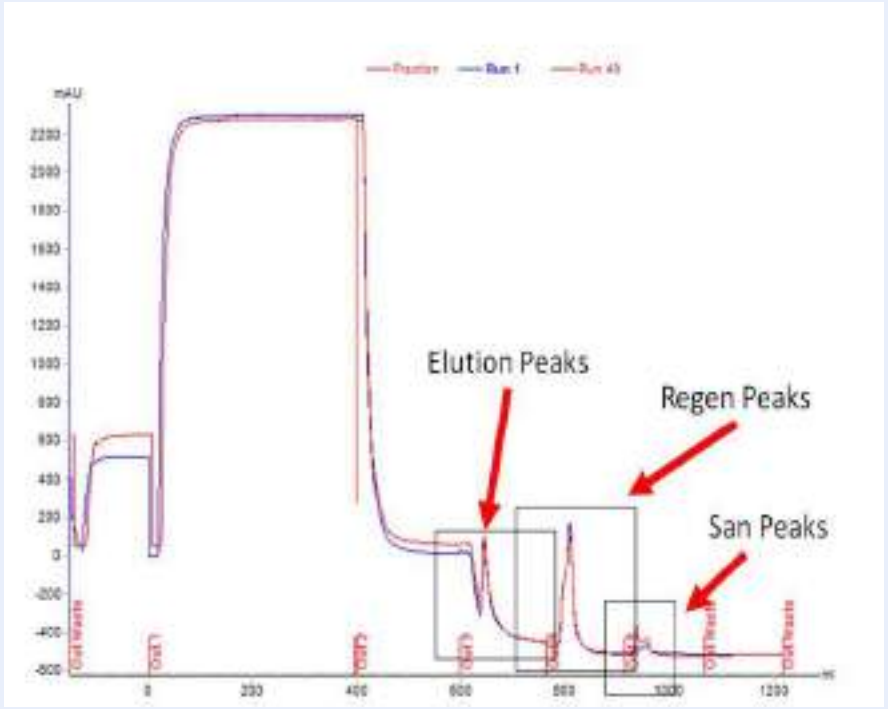
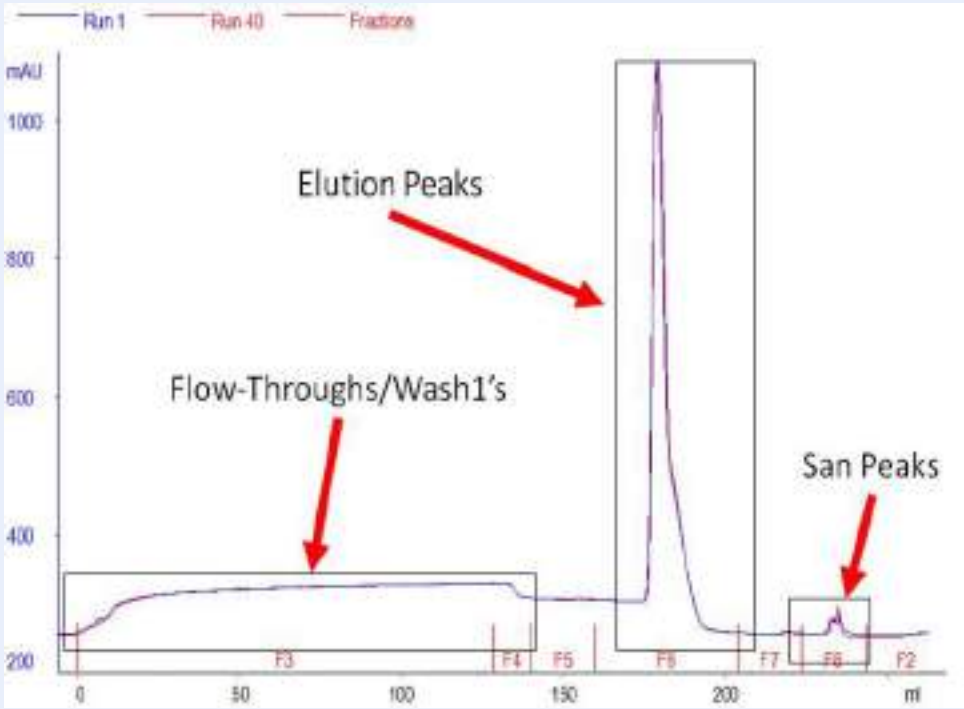
Resin Reuse Study:

IEX and HIC Chromatograms Overlay First and Last Run

IEX Chromatography



HIC Chromatography



- The overlays of IEX and HIC chromatograms between the first and last runs of the column reuse study are highly comparable

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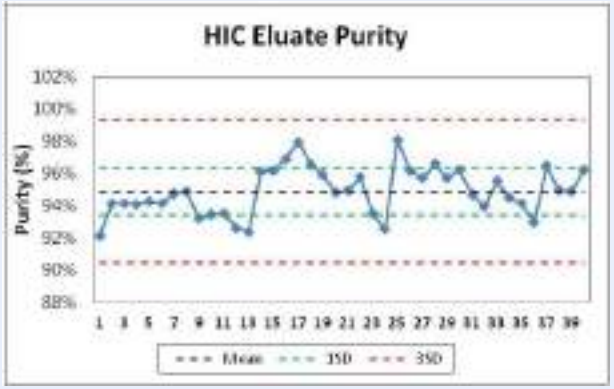
Resin Reuse Study:
IEX and HIC Selected Testing Results



- The step yields and purity are consistent throughout the 40 runs and support extending the column resin use up to 40 cycles

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Resin Use Study: Conclusion

Influenza Strain	Current Column Use Limit	
	IEX	HIC
H1	Increased from 7 to **15**	Increased from 7 to **15**
H3	Increased from 9 to **15**	Increased from 7 to **15**
2 x B	Increased from 4 to **40**	Increased from 4 to **40**

- Packed-column usage limits were re-evaluated by PD at small scale in 2015
- The resin reuse numbers were approved by FDA
- Resin reuse limits are under evaluation at commercial scale

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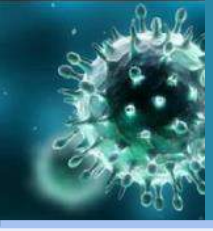
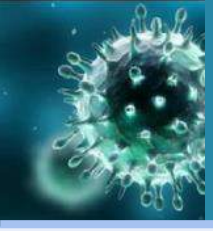


Flublok® Constant Process Improvement:
Column Load Study

- Load limits based on successful manufacturing runs; do not reflect actual maximum capacities
- Load criteria should be based on the real capacity of the resin.
  - To avoid deviations
  - Develop Fed-Batch process
- The Goal:
  - Find the maximum column capacities for IEX and HIC, by intentionally overloading them
- What is “Maximum Capacity?”
  - How much protein can be loaded before losing not more than 15% of rHA in the FT



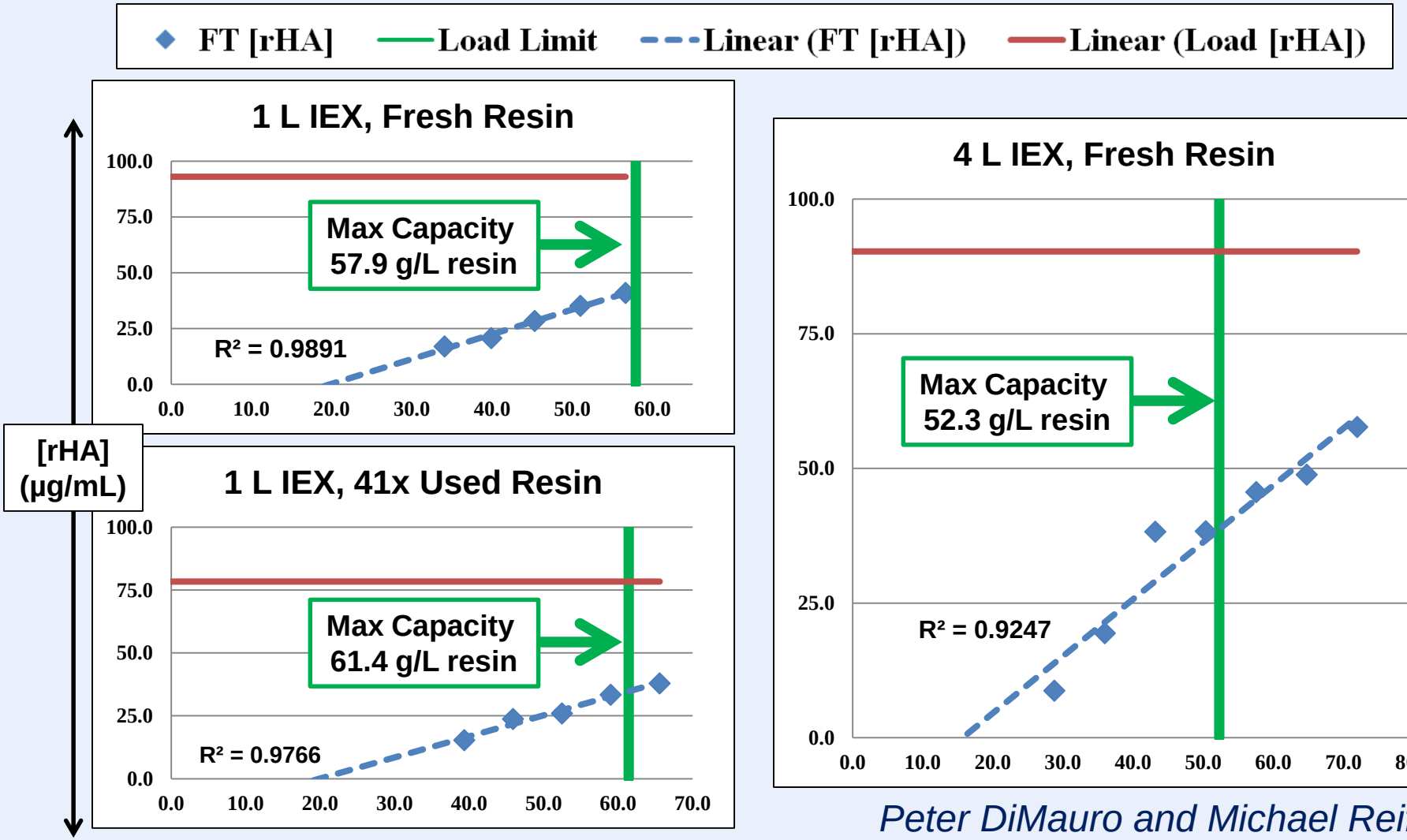
Column Load Study: *Experimental Design*

- Target load that might overload the column
  - IEX: 5x criteria
  - HIC: 2x criteria

- Purify with load at different scales and resin uses. Sample FT stream every 10% of Load
  - Scout at 1L scale, with fresh resin
  - Verify results at 4L scale, with fresh resin
  - Verify again at 1L scale, with used resin

- Identify where the 15% rHA loss point is

IEX Max Capacity Calculations:
FT Stream [rHA] vs Total Protein Loaded



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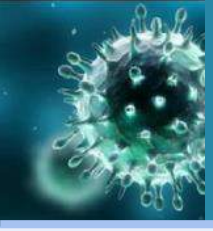
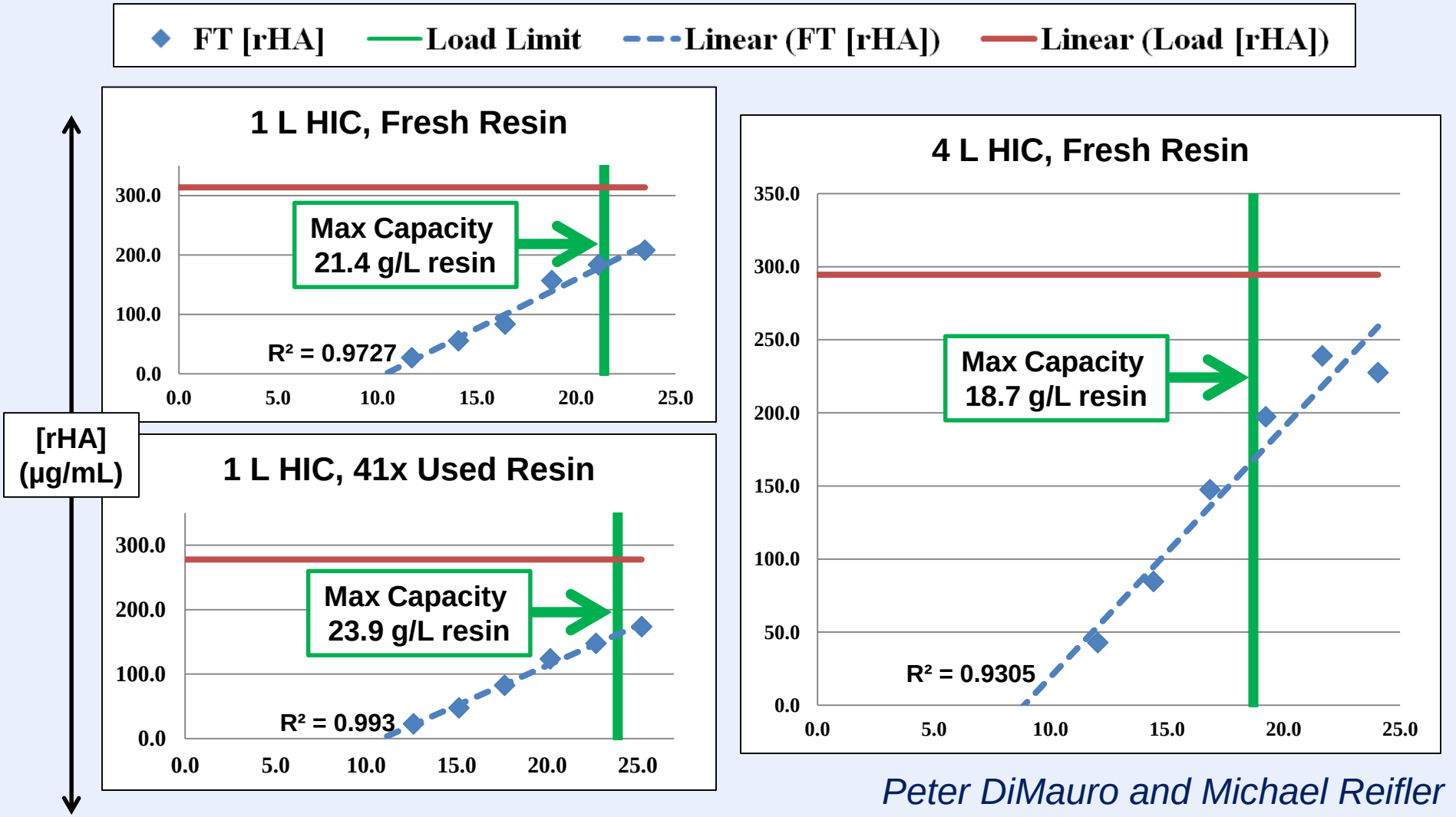
HIC Max Capacity Calculations:
FT Stream [rHA] vs Total Protein Loaded





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Column Load Study: Conclusion

Actual max capacities for B-strain rHA exceed the present criteria.

Process Step	Current IEX Upper Load Criteria	Maximum Capacity		
		1L, Fresh Resin	4L, Fresh Resin	1L, 41x Used Resin
IEX	1x	3.5x	3.2x	3.7x
HIC	1x	1.7x	1.4x	1.9x

All other criteria met, based on resin reuse study:

- SRID step yield
- Purity, rBV
- TOC before/after runs
- HETP/Asymmetry

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Flublok® Constant Process Improvement: *Yield Improvement*

DSP Process Optimization

- **Target step – IEX (rHA was detected in post peak, low IEX step yield)**
  - Perform Proof of Concept Studies at 1L Scale
    - IEX elution conditions were modified
      - **Concentration of salt**
      - **Concentration of detergent**
      - **Volume of collected IEX eluate**
  - Perform 4L and10L Runs under a Protocol
  - Perform Stability Study and Biochemical Characterization
  - Tech Transfer to MFG (459L and 1,800L scales)
- **Applied for the following rHA proteins**
  - B/Brisbane, H3 A/Switzerland and B/Phuket

Yield Improvement:
rHA Production under Optimized Process

Process		Optimized Process			Non-Optimized Process
Step	Batch #	10L Scale	459L Scale	1,800L Scale	1,800L Scale
Yield Improvement		**3.9x**	**3.2x**	**3.4x**	**1x**

Process changes do not impact rHA quality and purity:

- Reducing and non-reducing protein profiles by SDS-PAGE analysis
- The HA0 content is ≥95% in all batches
- All batches are properly folded based on HA assay results
- All batches are properly folded and display resistance to trypsin digestion
- rHA particle size by DLS
- rHA protein has the expected thermal stability and the retention time is consistent with rHA rosettes

The surfactants concentration in all batches meets batch requirements

Rick Chubet, Peter DiMauro and Michael Reifler

Flublok® Continuous Process Improvement:
Yield Improvement by Fed-Batch

Block	Step Number	Process Step
USP	1	Cell Culture
	2	Virus Stock
	3	Protein Production
DSP	4	Centrifugation
	5	Extraction
	6	Depth Filtration
	7	IEX Chromatography
	8	HIC Chromatography
	9	Q Membrane Filtration
	10	Ultrafiltration
	11	Bulk Filtration

High Cell Density
And
Feed Addition

Downstream PD Fed-Batch Development

- **Units Operation was confirmed**
- **Fed-Batch is working**
- **Two fold yield improvement was achieved for H1, H3 and B strains**
- **Process changes do not impact rHA quality and purity**

Harvest
Double Bio-Mass

Extraction
Increase Amount of Extraction Buffer

Depth Filtration
Increase Size

IEX Step
No Changes

HIC Step
Increase Size

Q Filtration Step
No Changes

Ultrafiltration and 0.2 µm Filtration Steps
Increase Size

Peter DiMauro, Paul Morgigno, Dan Gelperin and Michael Reifler

Conclusion

- BEVS using SF+ cells is an established platform for the production of recombinant vaccine antigens and proteins.
- Flublok®, recombinant influenza vaccine can be manufactured within two months
  - Influenza vaccines need to be updated every year because of HA antigenic drift
  - The commercial Flublok® process needs to be optimized for the new antigens
- Continuous process and yield improvements are part of the Flublok® life cycle management

Acknowledgements

Flublok®, recombinant influenza vaccine, became a reality thanks to support of contract HHSO100200900106C and perseverance of the Protein Sciences Corp. Team

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Indresh Srivastava and Manon Cox

#FlublokProtected!#ShowMeTheVial