QbD Based Process Development Strategies for Antibodies

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Director of Process Development

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ABOUT COOK PHARMICA

- **CONTRACT DEVELOPMENT AND MANUFACTURING ORGANIZATION**
- **WHOLLY-OWNED SUBSIDIARY OF COOK MEDICAL**
- **LEGACY OF LIFE SCIENCES INNOVATION SINCE 1963**
- **900,000 FT^2 (83,600 M^2) FACILITY IN BLOOMINGTON, INDIANA - USA**

CLINICAL AND COMMERCIAL

DEVELOPMENT

- PROCESS DEVELOPMENT
- ANALYTICAL DEVELOPMENT
- FORMULATION DEVELOPMENT

DRUG SUBSTANCE

- CELL CULTURE MANFACTURING
- CAPACITY TO 250 L, 600 L, (2) 2,500 L

DRUG PRODUCT

- BARRIER ISOLATOR TECHNOLOGY
- VIAL FILLING
- LYOPHILIZATION
- SYRINGE FILLING
- SAFETY DEVICE AND AUTOINJECTOR ASSEMBLY
- BLISTER THERMOFORMING
- KITTING AND CARTONING

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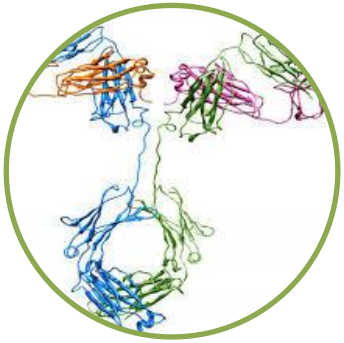
GROWTH IN DIVERSITY OF PROJECTS AND CLIENTS

Client Partners

- Early Stage and Evolving Biotech
- Large Pharmaceutical Companies
- Biosimilar Ventures and Established Subsidiaries
- Sub-contractor for US Government (BARDA)



Molecule/ Product Classes

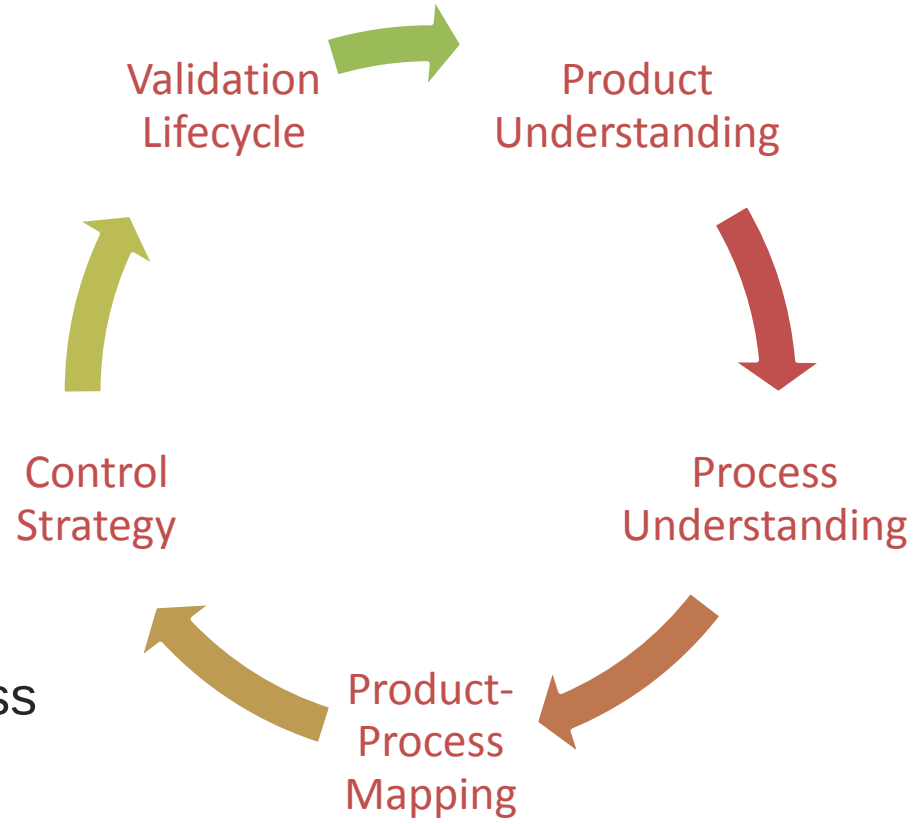
- Mabs and Fusion Proteins (Innovator, Biosimilar, Biobetter)
- Recombinant Therapeutics Proteins (Enzymes, Interferons)
- Peptides, Oligonucleotides, and Proteoliposomes
- Vaccines (drug product only)

Lifecycle Phase

- Preclinical Development
- Phase I/ II/ III Development, Reg./ Tox. and cGMP Manufacture
- PV and Continued verification
- Commercial Manufacturing

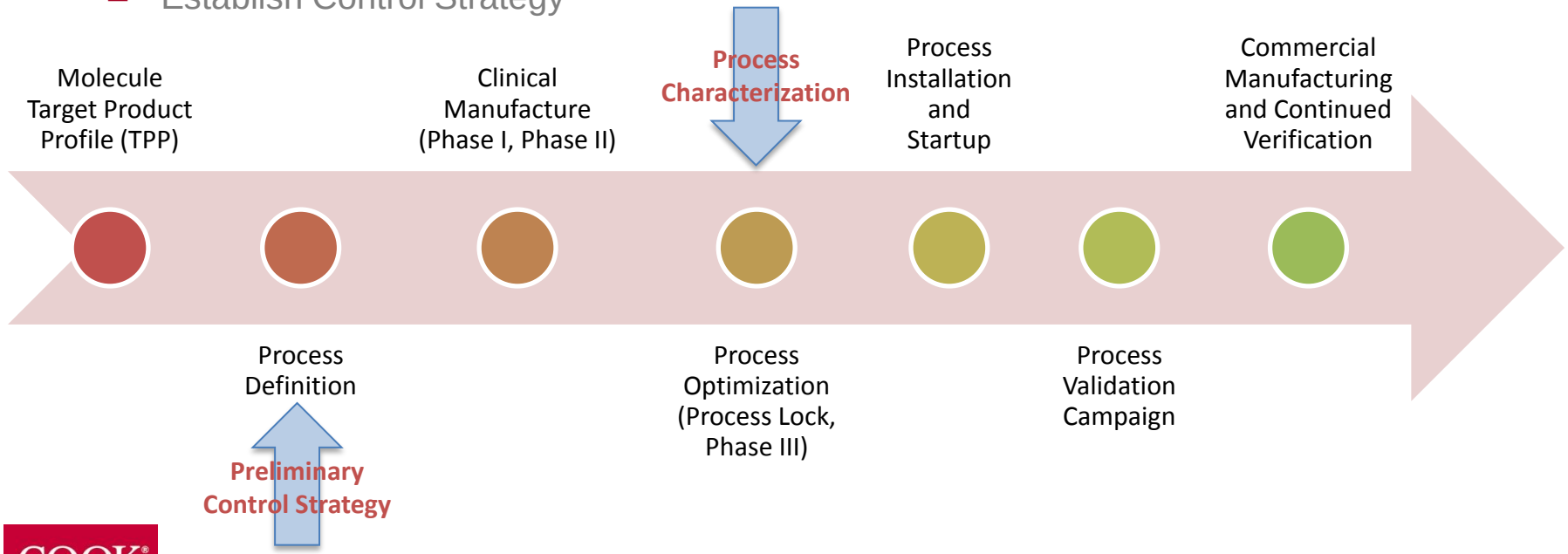
PRESENTATION OUTLINE

- mAb - Molecule to Market Lifecycle
- Product Understanding
  - Criticality Assessments
  - Preliminary Analytical Control Strategy
- Process Understanding
  - Risk Management
  - Process Characterization
- Product-Process Mapping
  - Design Space
  - CPP-CQA Linkages
- Control Strategy – PV Readiness with well-defined/ well-characterized process



MOLECULE TO MARKET

- Process Definition (Phase I, Phase II)
  - In-Depth Product Characterization but little or no process characterization
  - Preliminary Control Strategy and Process Definition for meeting TPP
  - Initial scale-up for GMP Manufacture
- Process Optimization (Phase III)
  - In-depth process characterization
  - DOE Driven Studies and establish Design Space
  - Final Scale-up and process lock
  - Establish Control Strategy



PRODUCT UNDERSTANDING: IN-DEPTH CHARACTERIZATION

Prior to early development, in-depth molecule characterization supports criticality risk assessment of product quality attributes and insight into stability

Functional Analysis

- Antigen Binding
- Cell Based Binding

Structural Analysis

- N-Glycan Analysis/ Glycosylation
- N-Terminal Sequencing
- C-Terminal Lysine
- Post translational modification(PTM) analytics
- Peptide Mapping (LC-MS)

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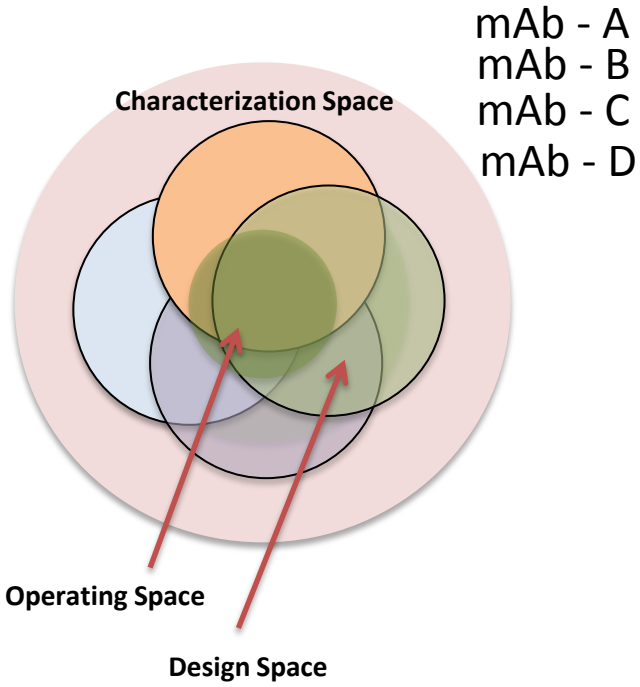
PRELIMINARY CONTROL STRATEGY

Based on a criticality risk assessment, a testing plan based on criticality of product attributes is established (One or more analytical methods associated with each CQA)

Critical Quality Attribute	Analytical Methods	Analytical Control Strategy			
		In-Process	Release	Stability	Char.
➢ IgG	Protein A - HPLC	x	x		
➢ Protein Concentration	UV-A280	x	x	x	
➢ Protein ID	Peptide mapping (LC-MS)		x		x
➢ Functional Analysis	ELISA Binding ; Cell-Based Assays				x
➢ Product-related Impurities (DNA, Protein A, HCP)	ELISA based Methods	**x**	x		
➢ Adventitious Agents (Bioburden, Endotoxin)	Plate Count Methods, LAL	x	x		
➢ Charge Variants (Deamidation, Sialylation, etc.)	IEX-HPLC, cIEF/ iCE	x	x	x	x
➢ Size Variants (aggregation, dimers, fragmentation)	SEC-HPLC , SDS-PAGE, CE-SDS	x	x	x	x
➢ Process Impurities (Triton X-100, residual solvents, etc.)	RP-HPLC	x	x		
	GC	x	x		
➢ Glycan Profile	FL-HPLC, MALDI MS				

PROCESS DEFINITION – A TECHNOLOGY PLATFORM APPROACH

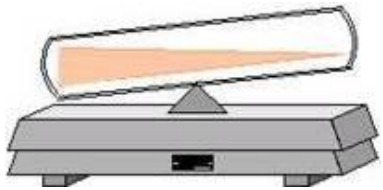
- A key strategy for success and quick turnaround is an established development platform
  - Standardized processes with established design space
  - Well-characterized standard equipment, resins etc. with established scale-up criteria and engineering design space
- An established technology platform allows for significantly reduced parameter screening to develop a process for a class of molecules that meets product TPP and provides assurance of quality
  - No loss of flexibility with appropriate technology platform



Concept in harmony with QbD – A Systematic Approach to Leveraging Prior Knowledge…

Cell Culture Platform

- Standard cell expansion processes (e.g. WAVE® bags)
- Standard Off-the-shelf media options with baseline feed strategy
- 2L and 20L platform with established design charts for scale-up
- Standardized processes: Feed Strategy, Inoculation Density, Temperature shift, pH/ pCO2 control, Gassing Strategy etc.
- Minimal experimentation to establish high titer process and meet critical product attributes and target product profile



WAVE Bags

Expansion

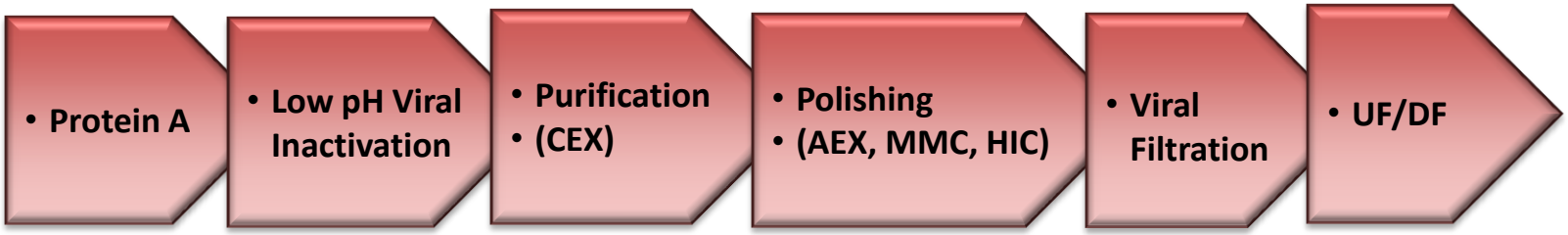
2L Platform

Scale-up

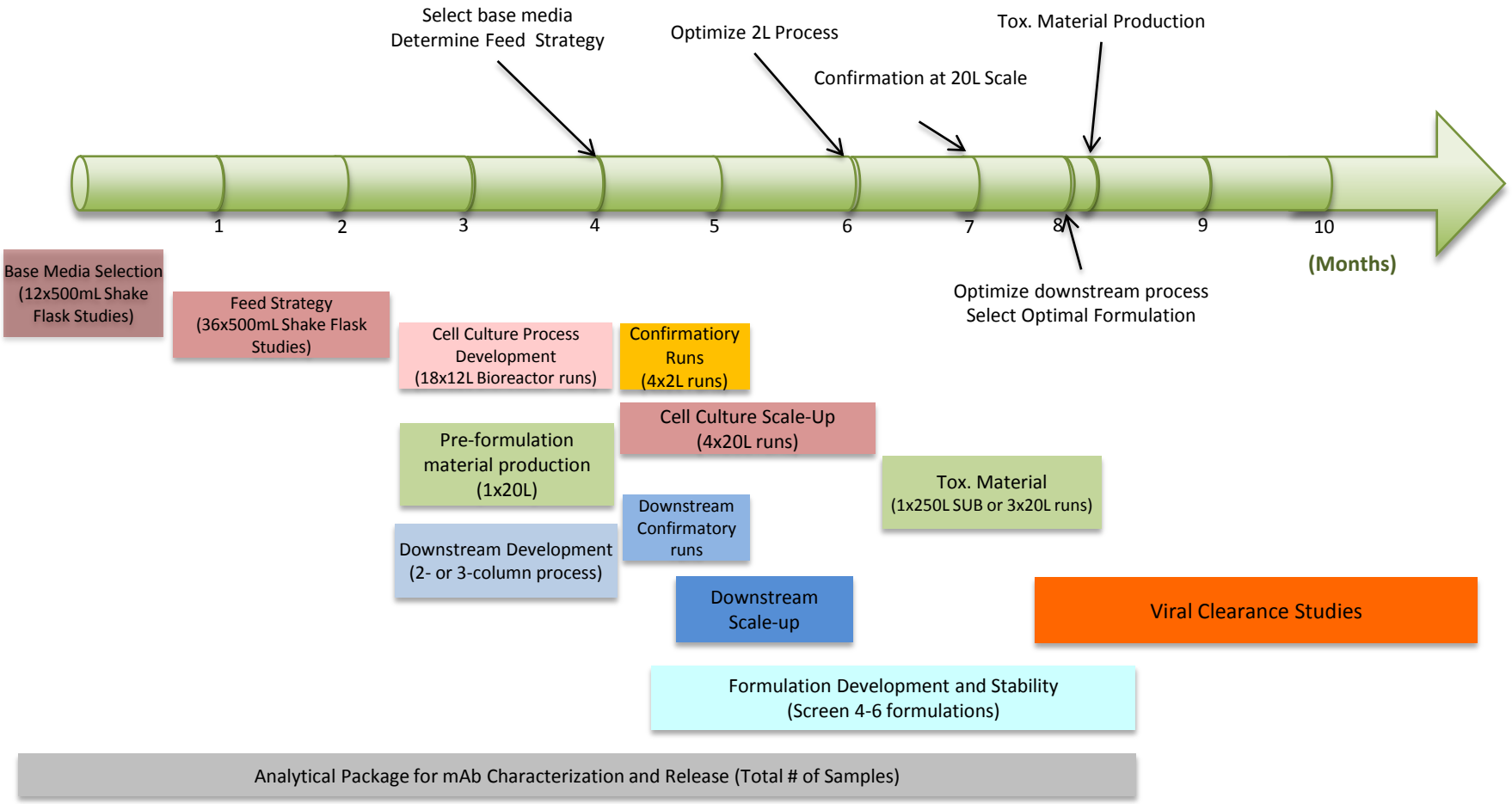
20L Platform

Downstream Platform

- Standard 2 or 3 column process
- Well-characterized resin library and established vendors
- Established column packing and operation and scale-up criteria
- Established viral filtration technologies
- Minimal screening experiments to establish baseline process to meet in-process controls and product attributes



TYPICAL DEVELOPMENT PROGRAM FOR EARLY PHASE MOLECULES



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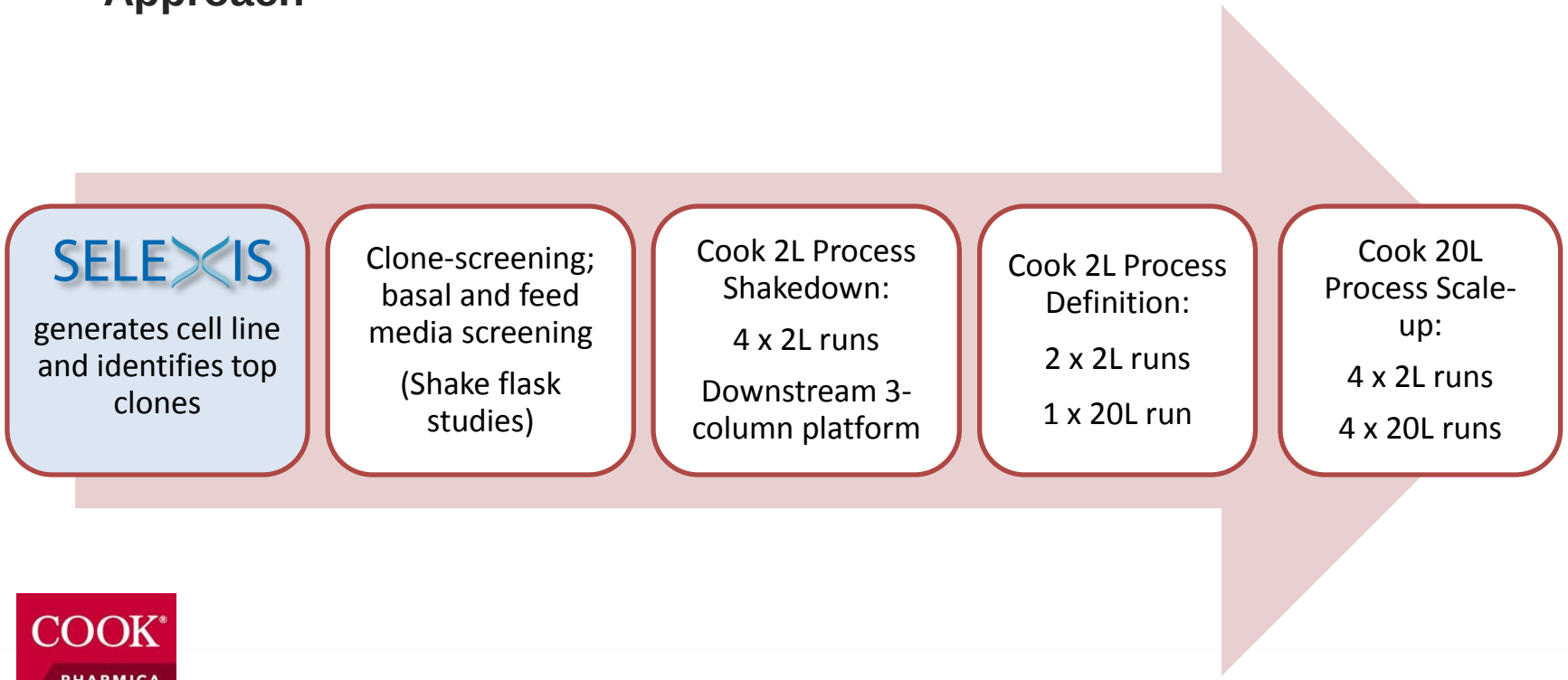
CASE STUDY – RAPID DEVELOPMENT OF A BIOSIMILAR

- **Background**
  - Collaboration with SELEXIS for a biosimilar trastuzumab (model molecule), using a client-owned SELEXIS cell line for demonstrating proof-of-concept.
- **Objective**
  - Create scalable process via a technology platform approach for commercial-ready titers with minimal development
- **Approach**



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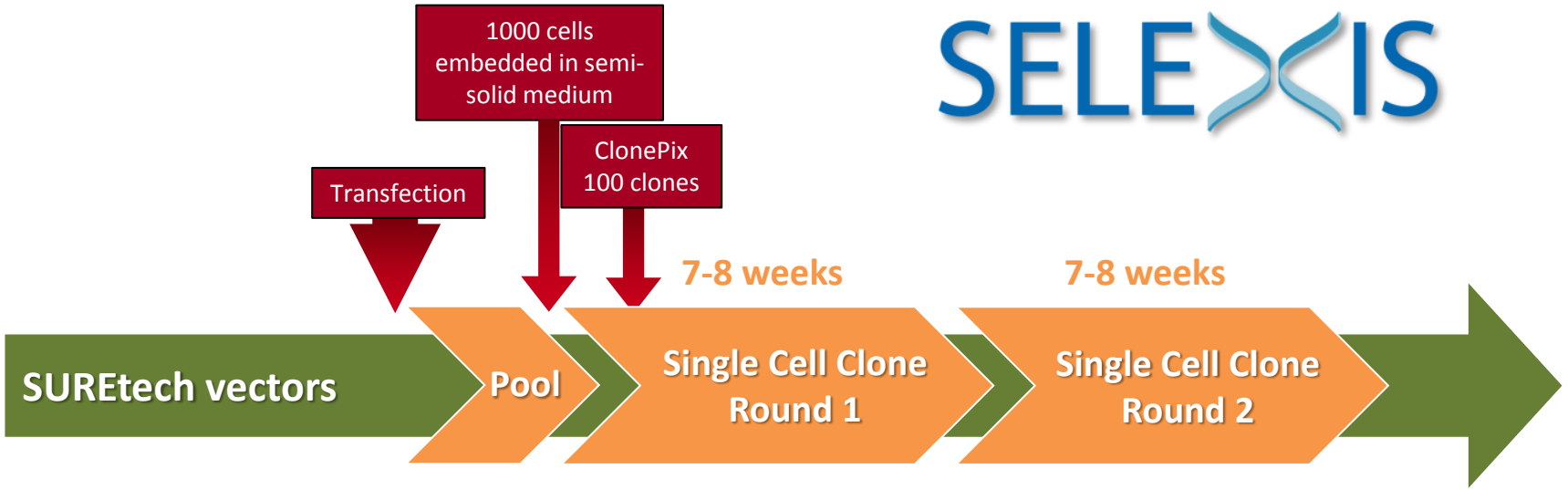
MAMMALIAN CELL LINE DEVELOPMENT PLATFORM



Generation of high performance and stable cell lines using the SURE*technology* Platform™ for cGMP manufacturing

- SGE high-productivity expression vector
- Single-cell cloning in chemically-defined media
- Suspension growth in chemically-defined basal media (commercial media)
- Optimized feed strategy (commercial feed)
- Robust growth to high cell densities

Candidate Clones

- Productivity assay
- Functional assay
- Manufacturing
- Preclinic/tox

Clonal Cell Lines

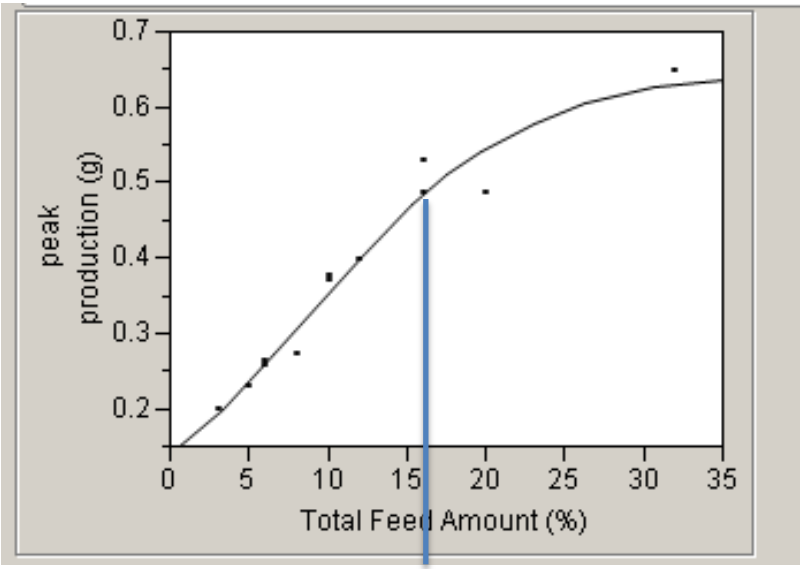
- Fed batch process
- cGMP manufacturing
- Clinical trial supply
- Market supply
- Research cell bank

INITIAL SHAKE FLASK STUDIES: FEED STRATEGY SCREENING WITH FULL FACTORIAL DOE

Variables			
Feed Concentration	1%	2%	NA
Feed Frequency	Daily	Bi-daily	NA
Feed Timing	D3-7	D3-12/13	D3-18

Concentration	Frequency	Timing
1	Bi-daily	D3-7
1	Bi-daily	D3-12/13
2	Daily	D3-7
1	Bi-daily	D3-18
2	Daily	D3-12/13
1	Daily	D3-7
2	Bi-daily	D3-7
1	Daily	D3-18
2	Bi-daily	D3-18
2	Daily	D3-18
1	Daily	D3-12/13
2	Bi-daily	D3-12/13

→ 12 flask DoE full factorial



16%

Non-linear relation between total feed amount and production → 16-20% best range for good titer with reduction in wasted feed (cost)
JMP analysis indicates daily feeds D3-18 best

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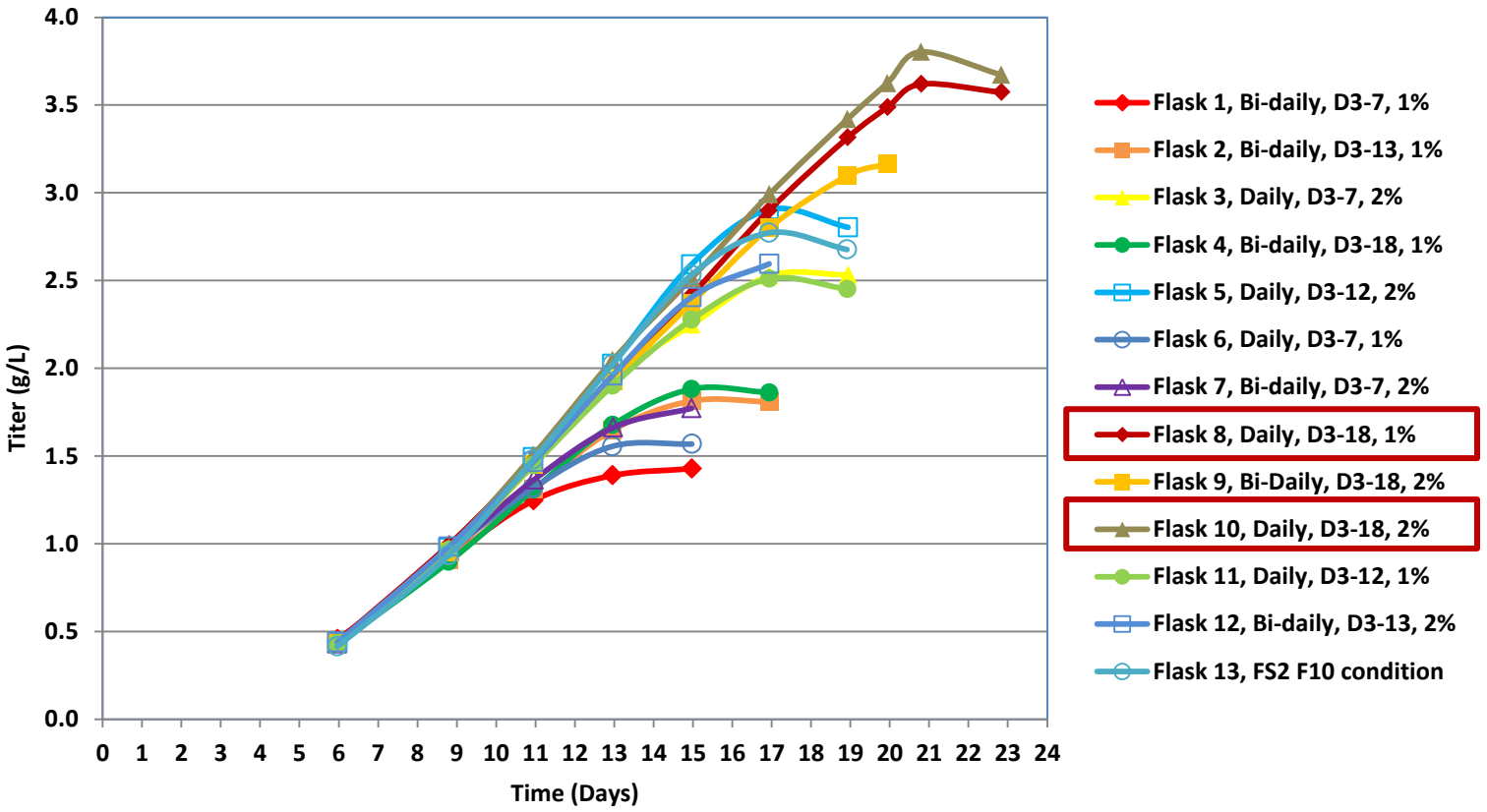
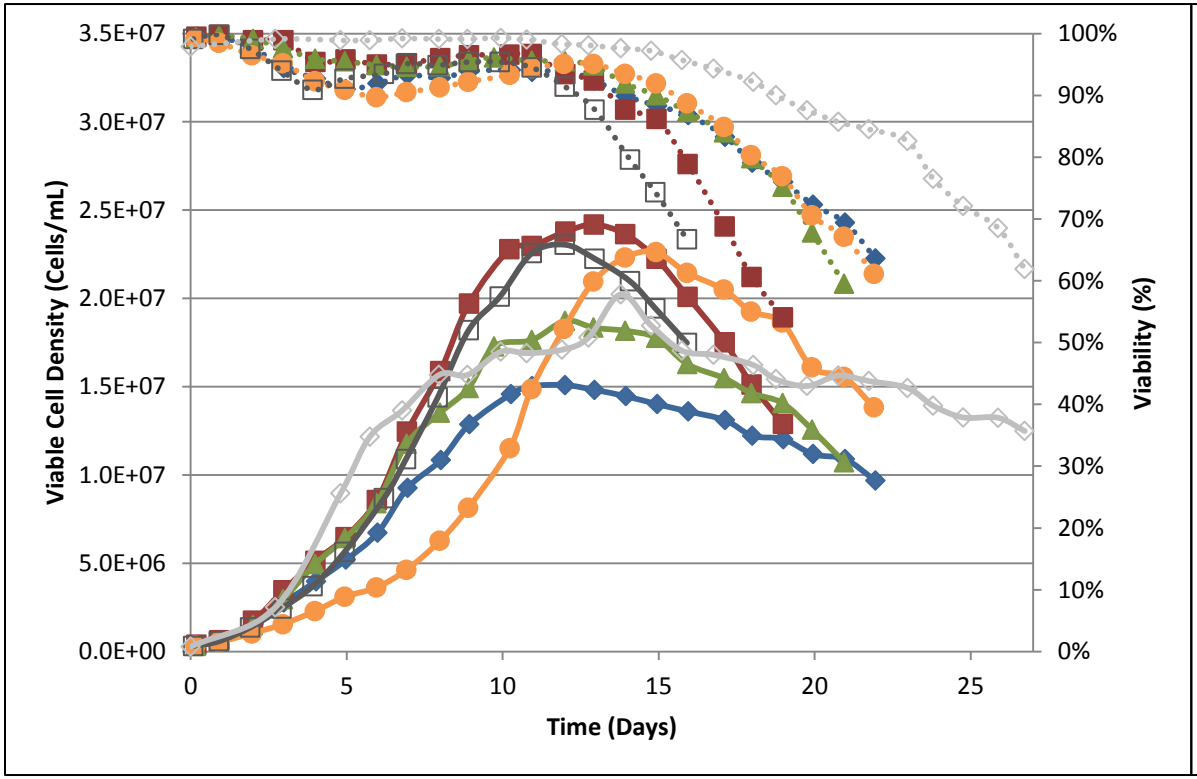
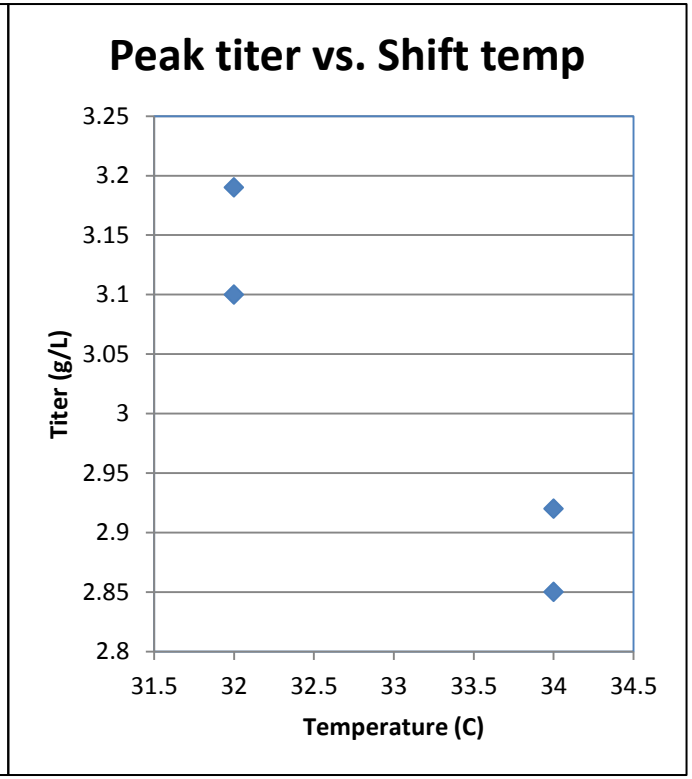
RESULTS – TITER DRIVEN OPTIMIZATION OF FEED STRATEGY



Daily Feed of 1% and 2% are top performers for titer

2L PROCESS SHAKEDOWN – UNIVARIATE STUDY WITH TEMPERATURE SHIFT



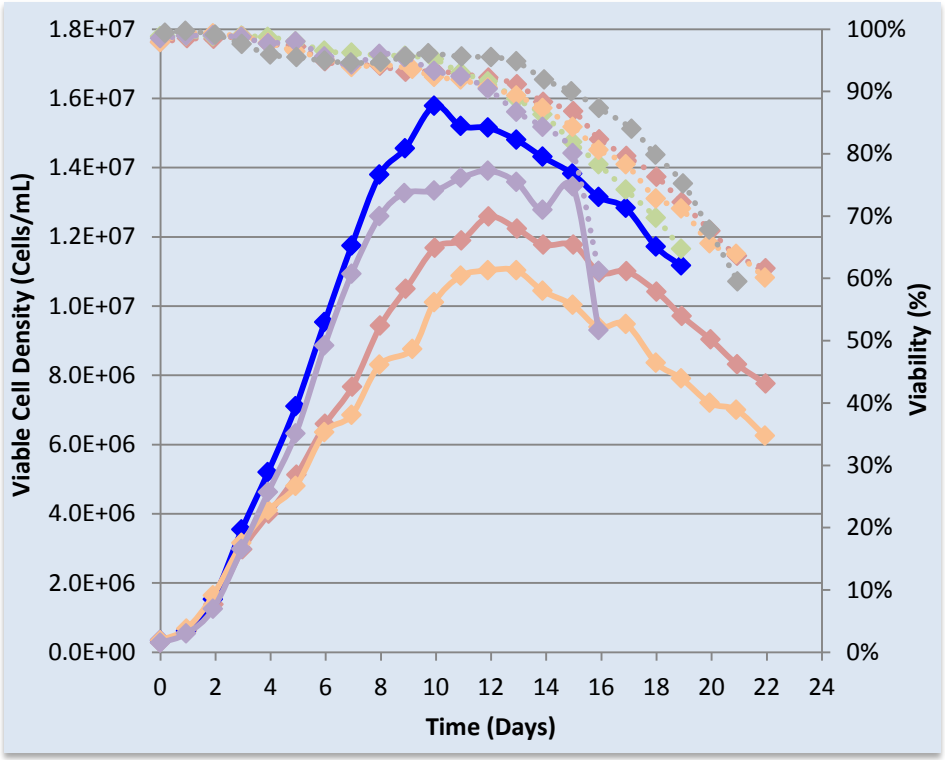


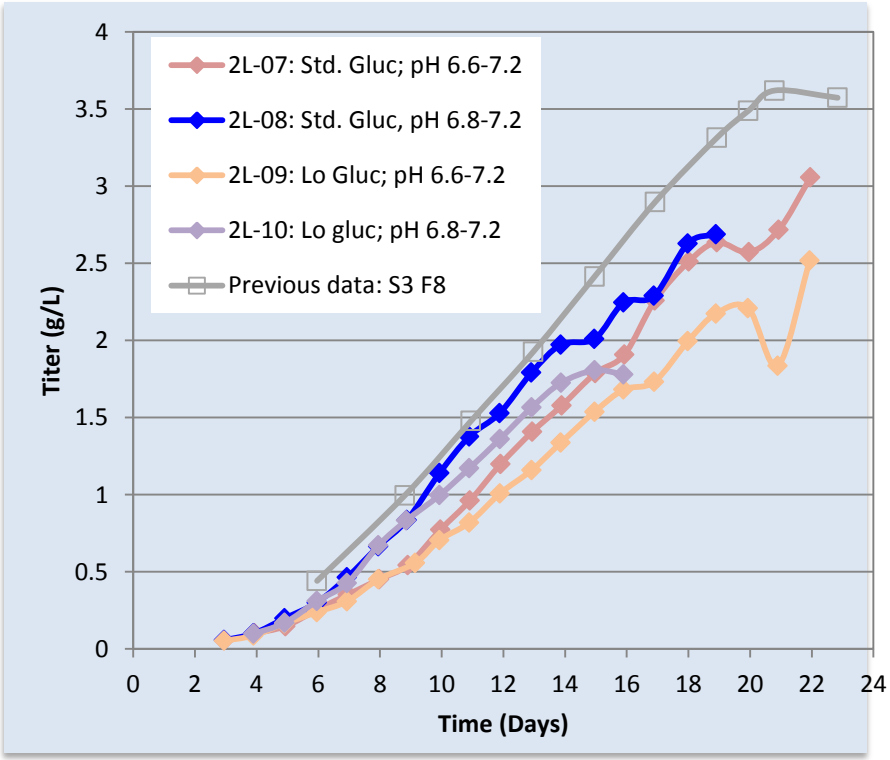
A temperature shift of 32C seems more favorable for titer.

2L PROCESS DEVELOPMENT: DEFINE PH AND GLUCOSE LEVELS

Factors Tested:

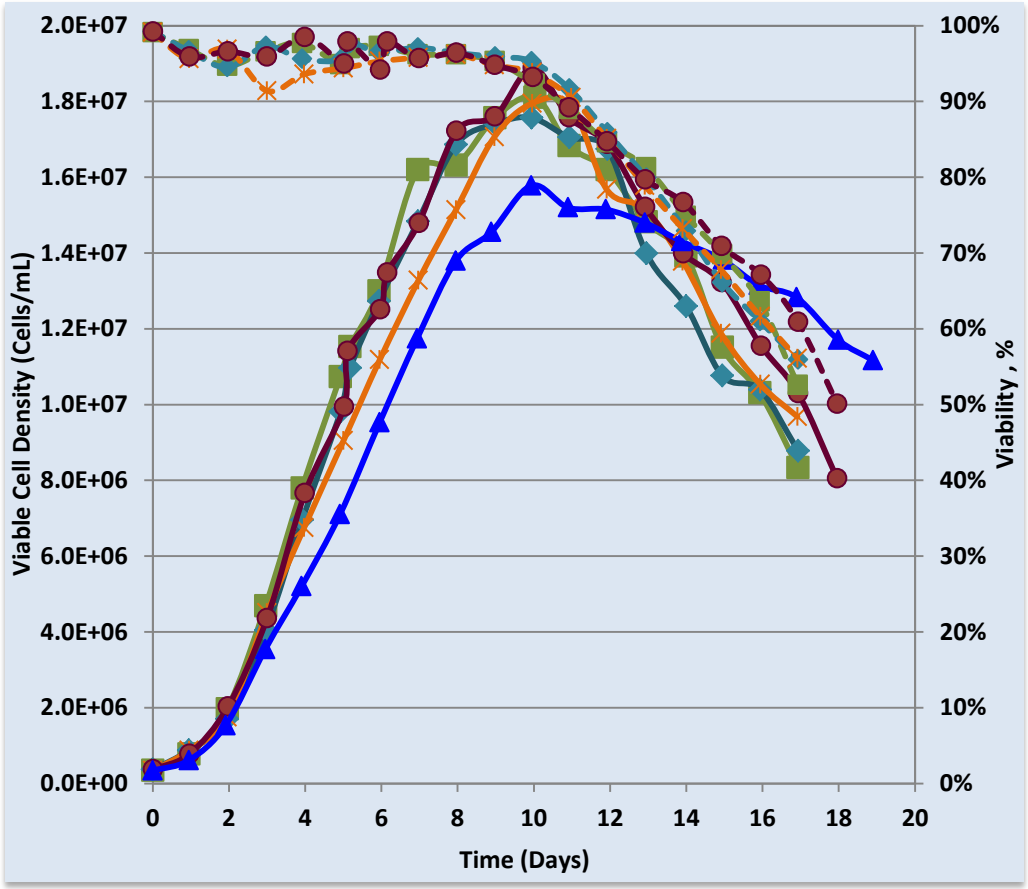
- Low Glucose vs Std. Glucose Levels
- pH range 6.6 – 7.2 vs 6.8 – 7.2

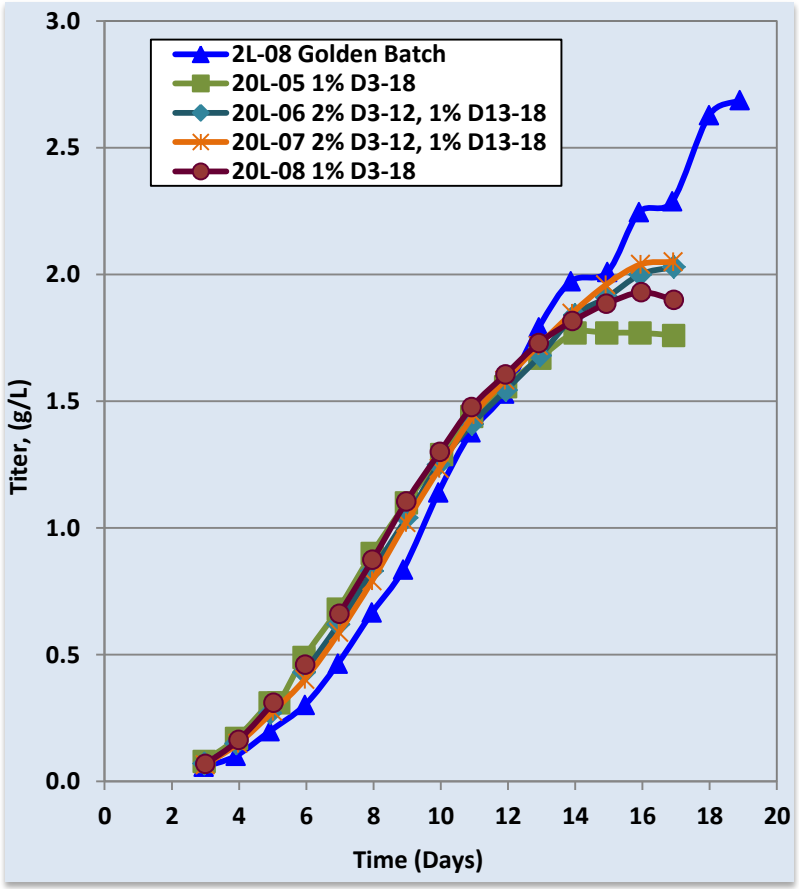




Results: Maintaining pH above 6.8 early benefits cell growth, but shortens culture longevity; Standard glucose levels gives better titer

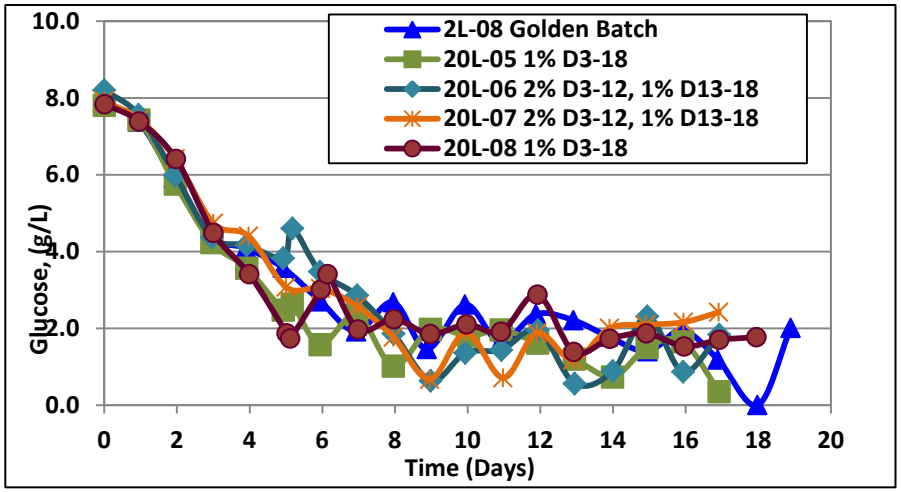
PROCESS CONFIRMATION AND SCALE-UP

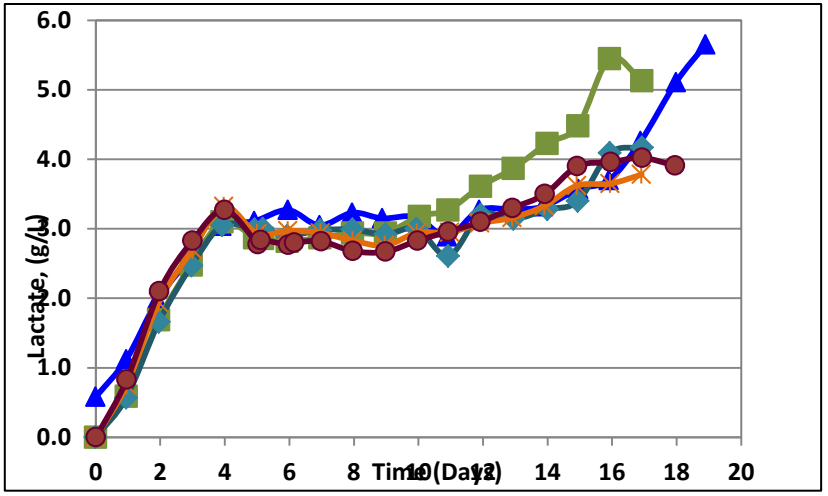


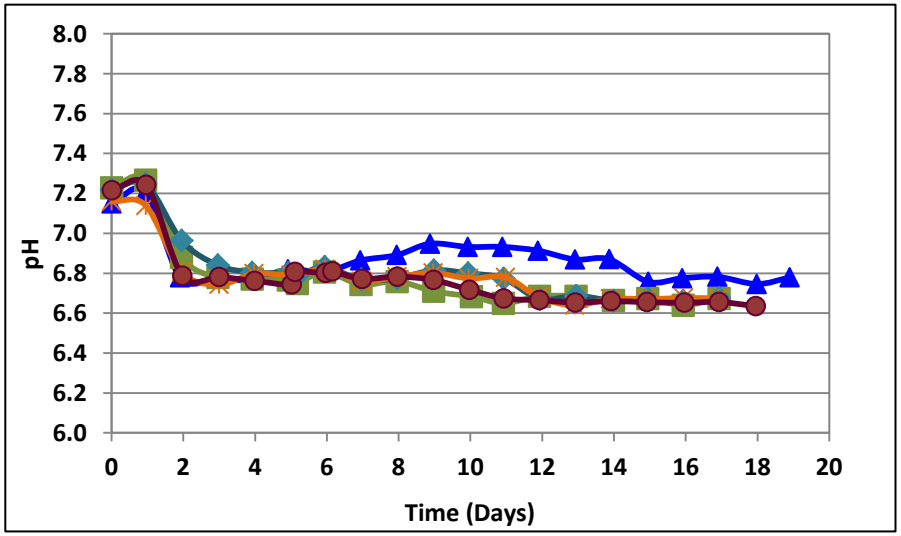


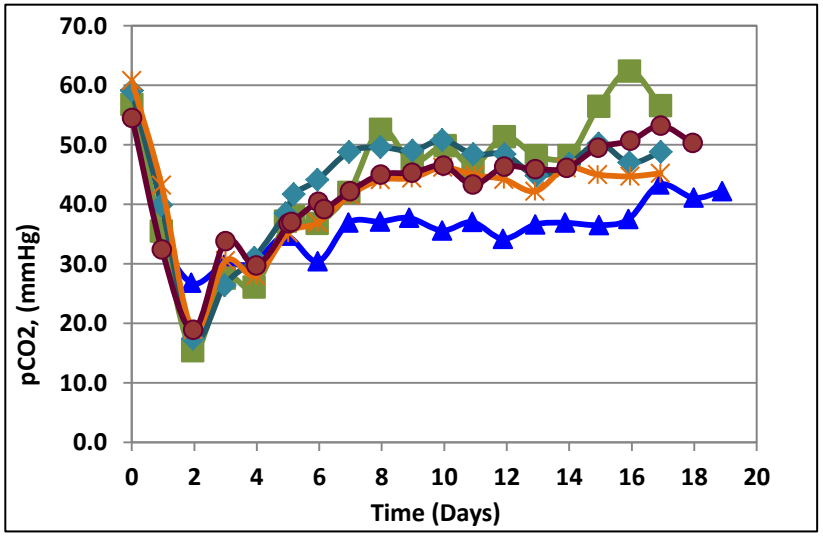
- 2L-08 baseline condition – “Golden Batch”
- Scale up to 20L

PROCESS SCALE-UP: METABOLITE PROFILES









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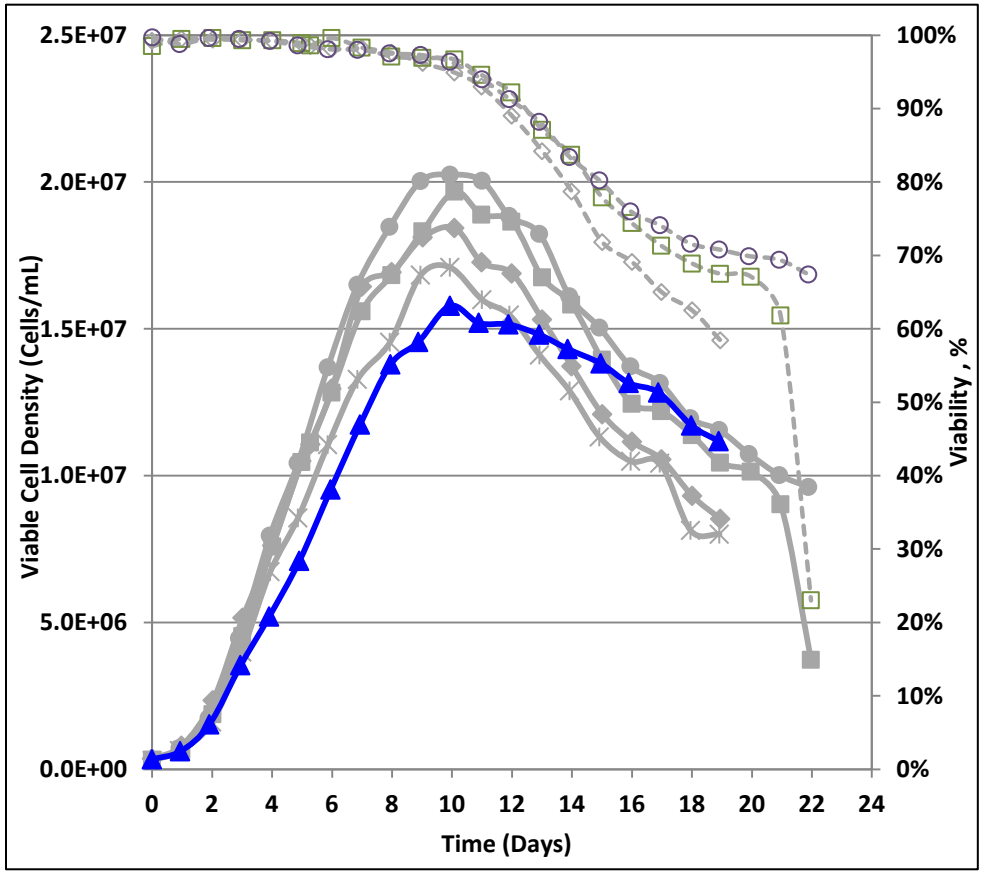
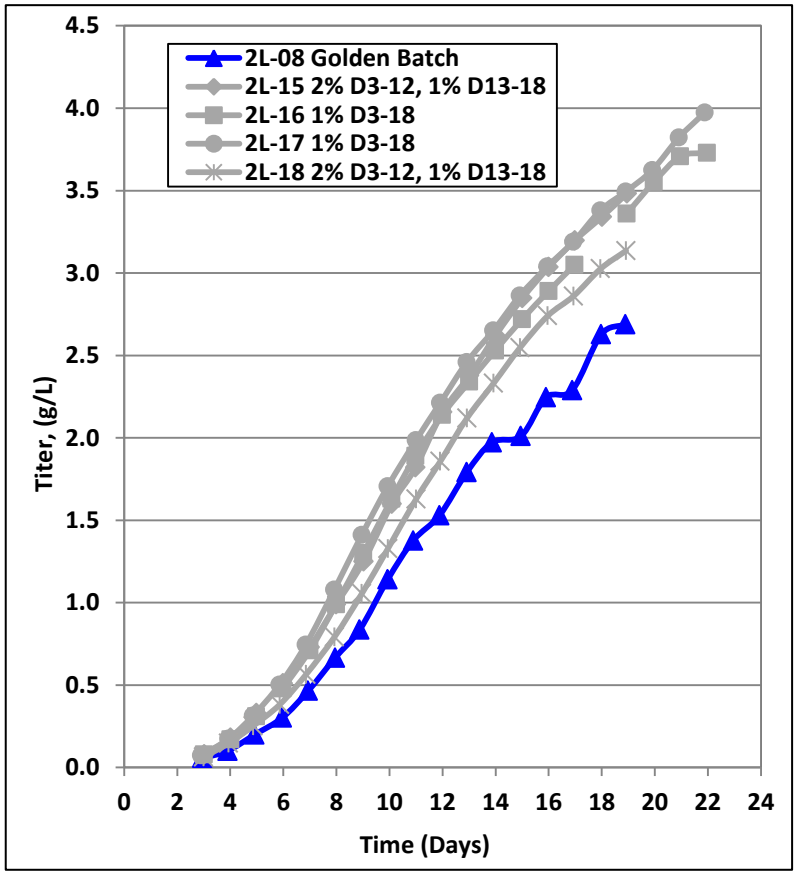
HIGH TITER PROCESS DEVELOPMENT – WORK IN PROGRESS

- Optimized seed train in 2L
- Diluted vs concentrated feeds
- Additional work required for optimization of 20L process





Standard 3-column process

Results from a pilot scale downstream process for a 20L scale bioreactor run:

Process Step	Pool Conc. (g/L)	Pool Vol. (L)	Total g	Step Yield (%)	SEC-HPLC Monomer (%)	HCP by ELISA (ng/mg)
Clarified Harvest	1.59	18.91	30.61	100	NA	NA
MabSelect Sure/Viral Inactivation Cycle 1	4.29	3.4	14.57	93.8	96.4	1075.4
MabSelect Sure/Viral Inactivation Cycle 2	4.22	3.25	13.72	94.4	96.6	NA
Combined Viral Inactivation Pool*	4.26	5.67	24.15	NA	95.1	962.8
POROS XS Pool**	6.09	3.4	20.73	87.5	99.1	3.9
POROS Q Pool	1.02	17.76	18.12	99.7	99.9	< 1
Viral Filtration Pool	0.96	19	18.24	100.7	99.8	< 1
UFDF Pool	21.89	0.82	18.06	99.0	99.1	< 0.05

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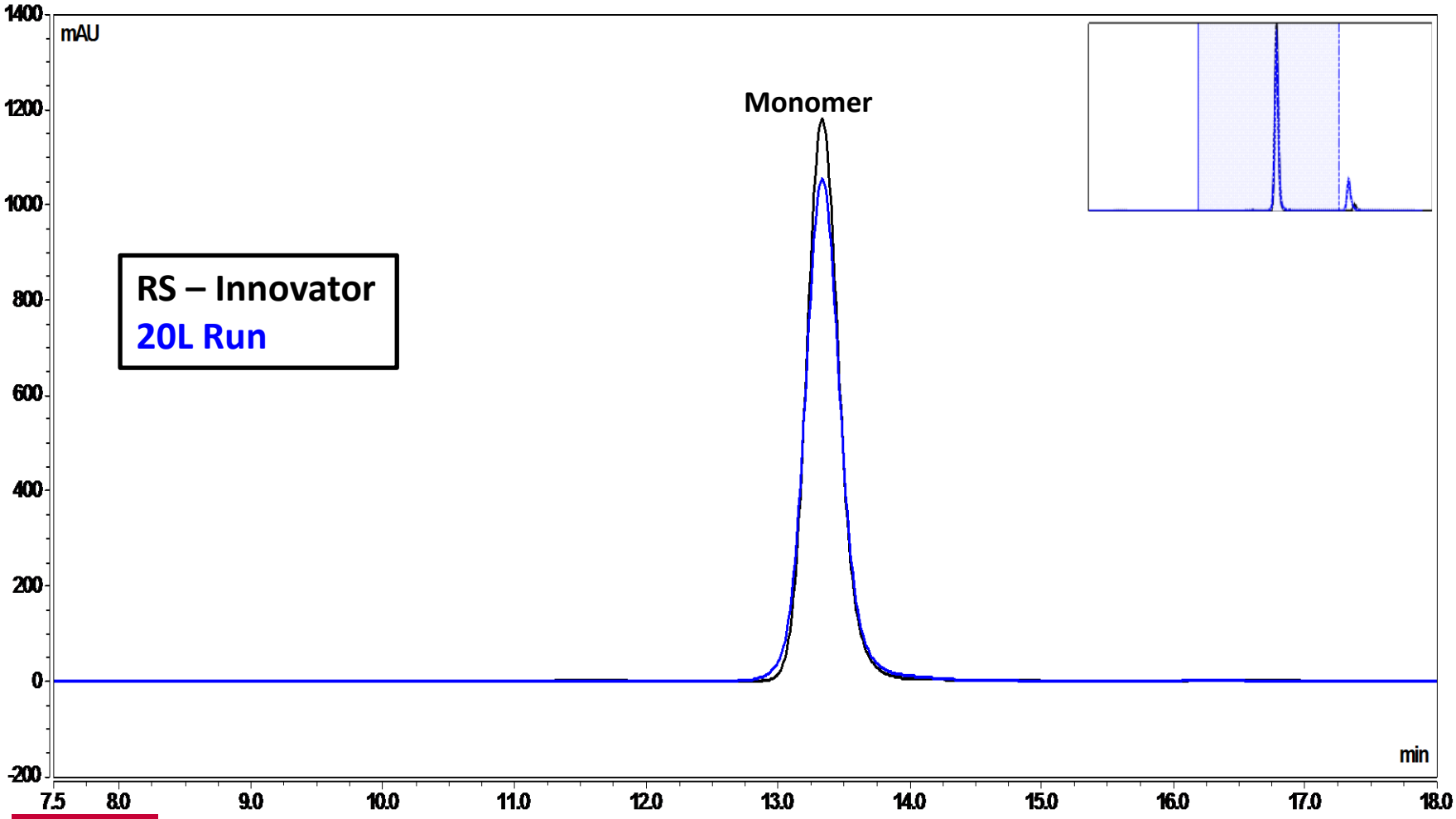
RESULTS: SIZE VARIANTS BY SEC-HPLC



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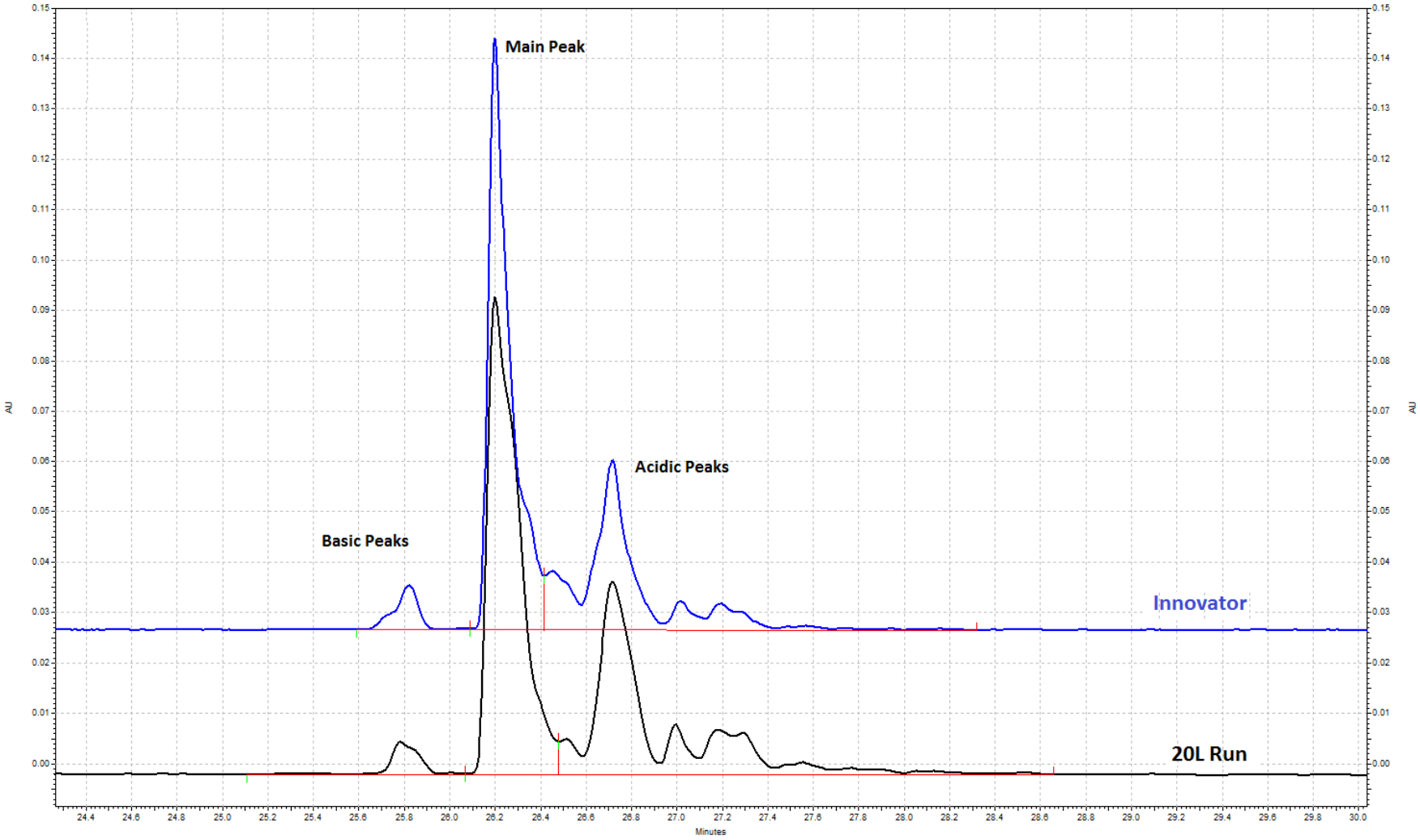
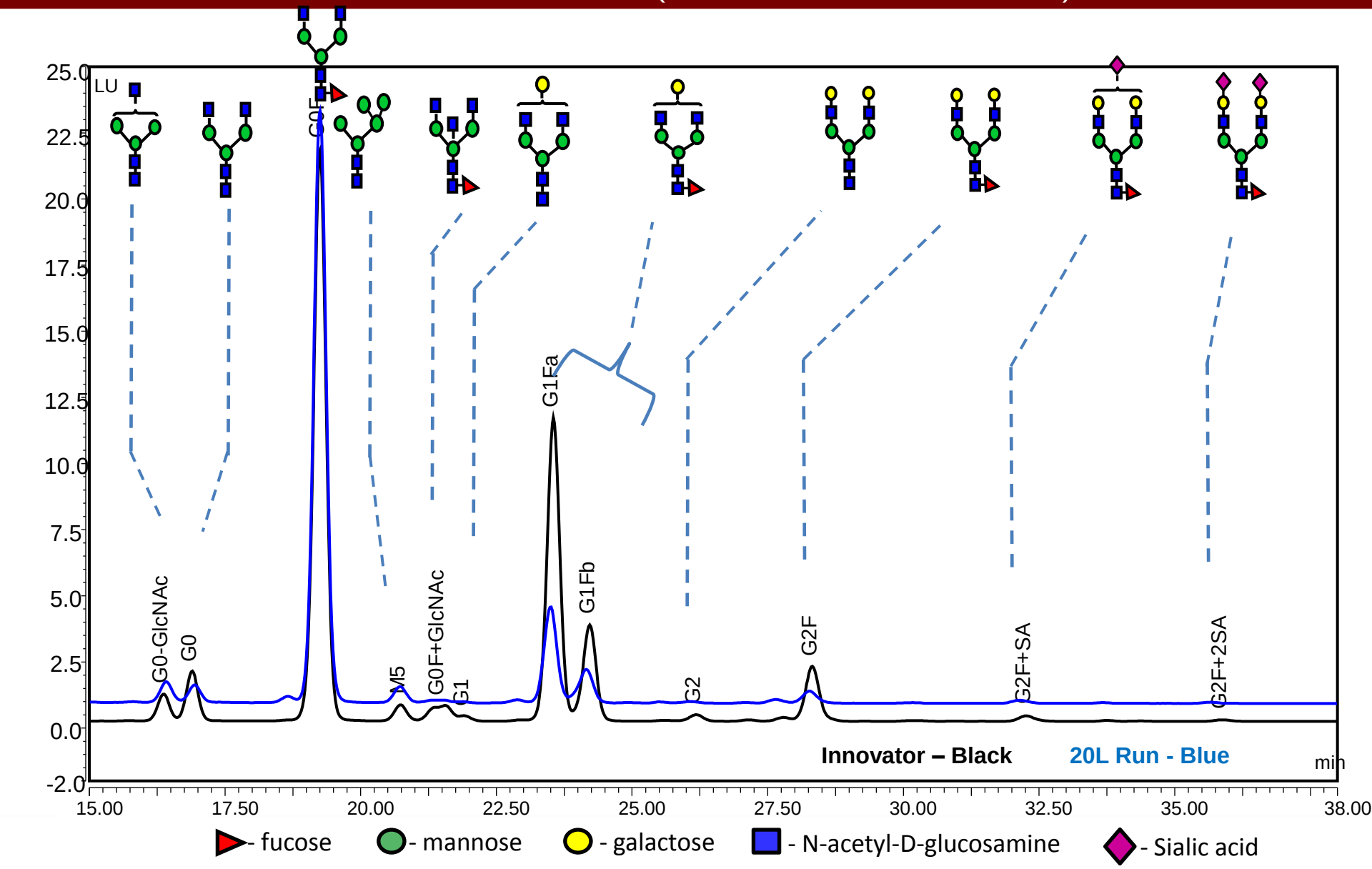
RESULTS: CHARGE VARIANTS BY CIEF



RESULTS: GLYCOSYLATION ANALYSIS (N-GLYCANS WITH FL-HPLC)



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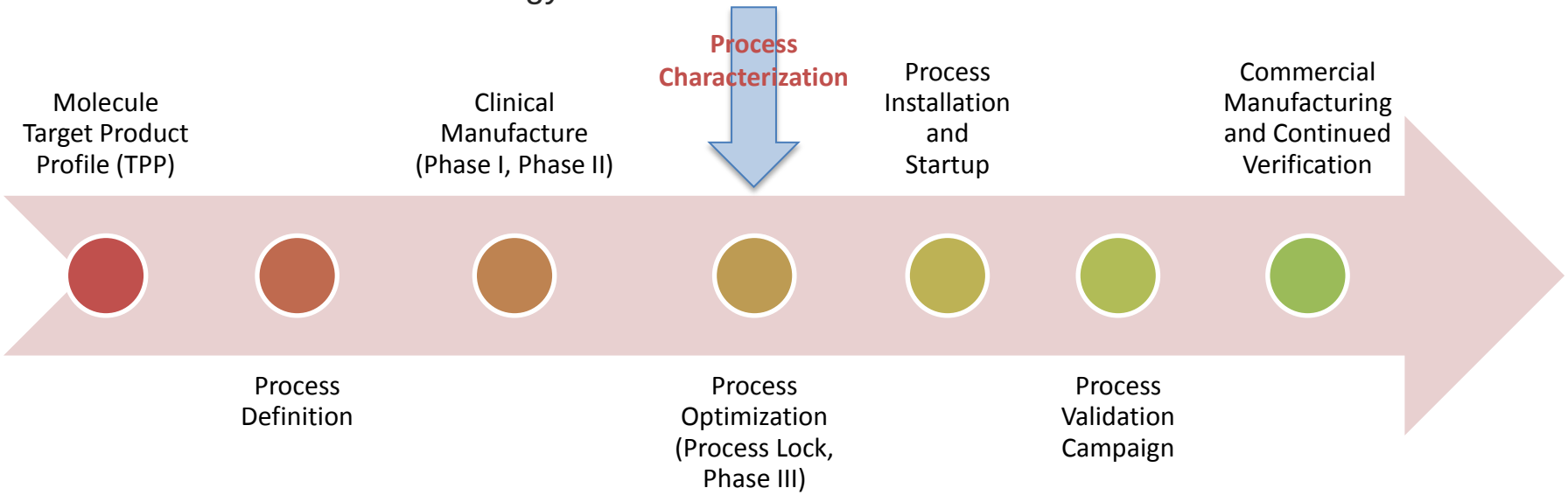
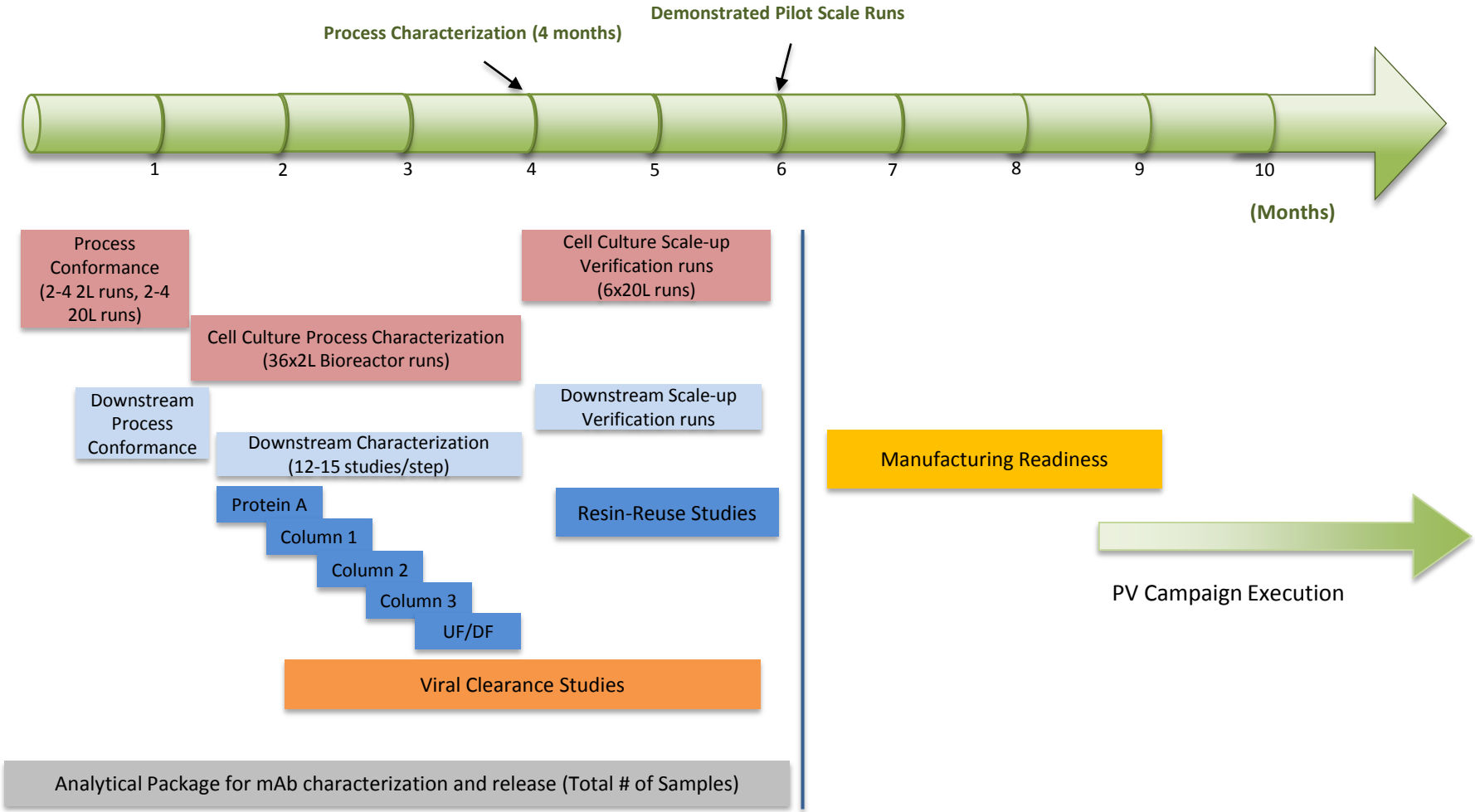


MONOCLONAL ANTIBODY CHARACTERIZATION APPROACH



EXAMPLE PROCESS CHARACTERIZATION DOE FOR A CEX STEP FOR TRASTUZUMAB

- Cation Exchange Chromatography
  - POROS XS® Resin in Bio-Rad small-scale column (1.2 – 1.3 mL CV)
- Method
  - Equilibration: 10 mL of 50 mM Sodium Acetate
  - Load: pH and conductivity adjusted material
  - Equilibration Wash: 10 mL of 50 mM Sodium Acetate
  - Salt Strip: 10 mL of 50 mM Sodium Acetate, 500 mM NaCl

Column #	Pattern	pH	Conductivity	Load Rate
1	−−−	4.5	3	40
2	000	5	4.5	60
3	+++	5.5	6	80
4	+−+	5.5	3	80
5	+−−	5.5	3	40
6	−++	4.5	6	80
7	−−+	4.5	3	80
8	−+−	4.5	6	40
9	++−	5.5	6	40

Full Factorial Screening Design:

- Factors: pH, Conductivity, Load Rate
- Response: Yield, CHO-HCP Level

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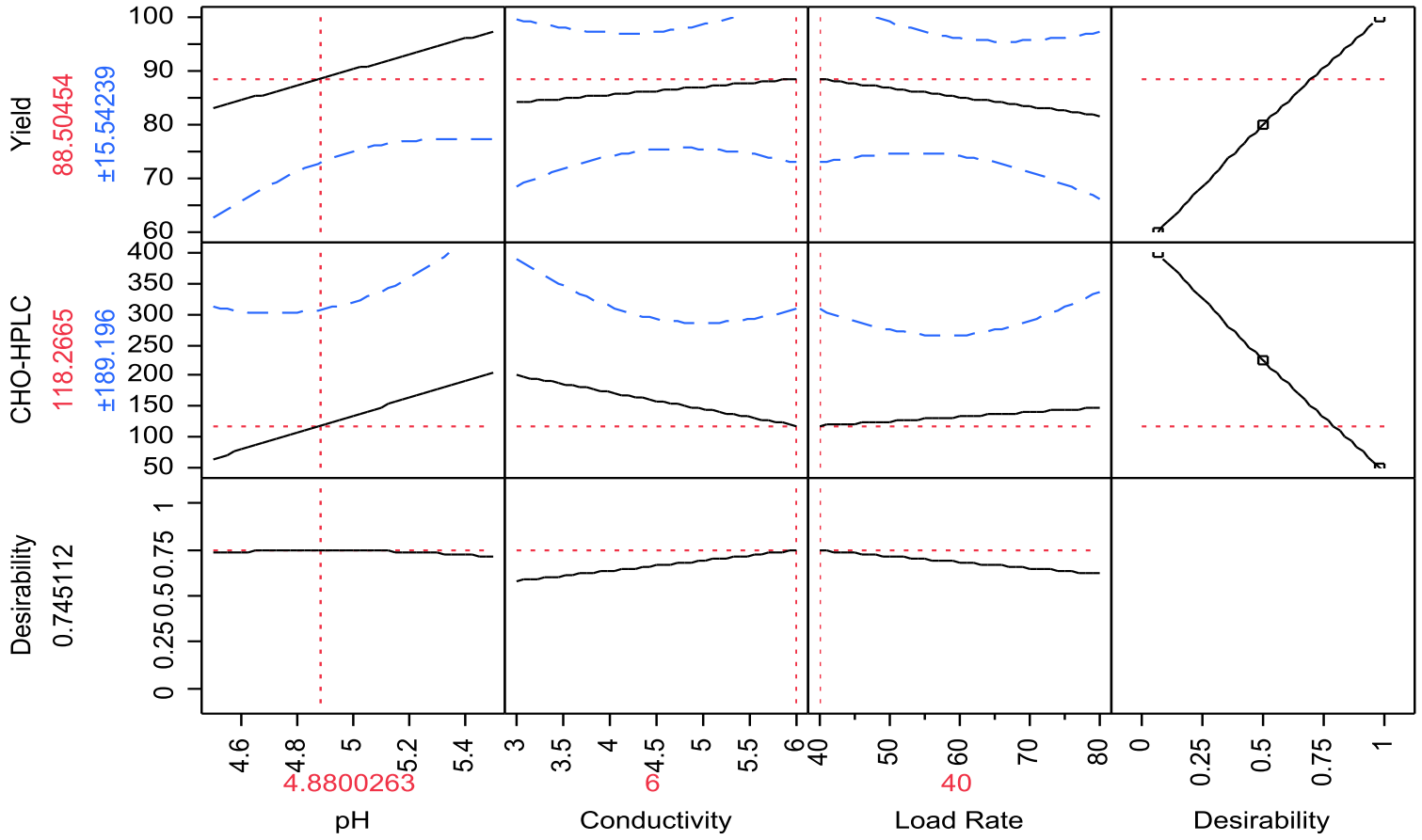
STATISTICAL ANALYSIS: PREDICTION PROFILER – MAXIMIZING DESIRABILITY



Target Operating Range: pH operating Space based on maximum desirability. Acceptable range defined depending on desired HCP clearance

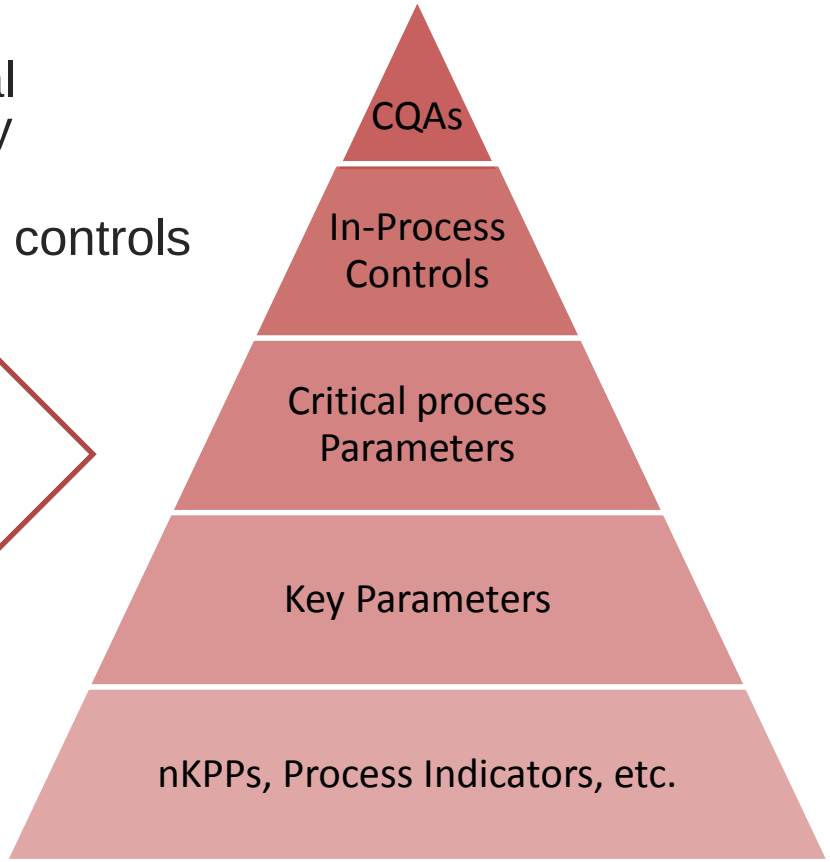
(Additional Optimization DOE studies required)

DEFINE CONTROL STRATEGY - MANUFACTURING READINESS FOR PV

- Upon completion of characterization studies, a **Control Strategy** is defined suitable for process validation
  - Define Acceptable Ranges for critical process parameters (CPPs) and Key Process Parameters (KPPs)
  - Define specifications and in-process controls and limits for all CQAs

Process Characterization → **Design Space** → **Control Strategy**

Risk Assessment of facility, equipment, process etc. feeds into control strategy



SUMMARY

- A flexible technology platform allows for rapid development for early phase clinical manufacturing
  - Leverage prior knowledge and experience
  - Reduced parameter screening
  - Well-characterized equipment, resin library and methods
- Phase-appropriate QbD as a critical enabler for successful validation and robust process – *molecule to market*
  - Process characterization as part of late stage development

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