

North Carolina Biotechnology Center BPD, April 14th, 2011

Transfer, Late Stage Development, and Validation readiness of a fully disposable process for Monoclonal Antibody Manufacturing

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Talk Outline

- Monoclonal antibody (molecule, development history)
- Transfer to API facility
 - Fit-to-facility
 - Single-use
 - Challenges
- Drug substance manufacturing process validation
 - Optimisation and development of a single-use manufacturing system
 - Process characterisation and control strategy definition
 - Specific Challenges
- Outcomes
- Conclusions

Molecule

- Molecule
 - Humanised IgG1
 - GS-CHO cell line
 - Fed-batch process
 - Largely single-use, end-to-end
- Molecule history
 - Alliance partner (2007)
 - Acquired molecule from originating lab (Phase I material)
 - Transferred to GS-CHO (from NS0) cell line
 - Established Phase II – III process
 - Manufactured Drug Substance for Phase II, Phase III trials
 - Drug product manufacturing process also single-use
- Commercial Manufacturing & late stage process development
 - Lilly desire to include Lilly produced material in Phase III trials
 - Launch strategy (manufacturing site for clinical, commercial material)
 - Late-stage development and commercialisation to be done by Lilly

Drug Substance Production Process

- Largely single-use process
 - Affinity Capture resin cleaned and re-used
 - Using AKTA skids (Process, Pilot) with Affinity Capture column, membranes, filters
- Process defined by MacroGenics prior to transfer
 - Phase III appropriate, but additional process characterisation required to be validation ready
 - Control strategy for conformance lots based on process characterisation and pivotal history at Lilly
- Process history
 - Previous Phase III lots made by MacroGenics
 - Full scale demo lots at Lilly R&D as part of late stage process development

Drug Substance Production Process

Unit Operation 1 – Cell Culture
(Vial Thaw and Shake Flask Expansion, Wave Bioreactor Expansion, Production Bioreactor)

Shake Flasks, 20L Wave (seed expansion),
200L Wave (Production Bioreactor)

Unit Operation 2 – Clarification

Depth Filtration (MilliStak, Millipore); Intermediate
Hold (LevMix)

Unit Operation 3 – Affinity Capture Chromatography

20cm MAbSelect Resin column; AKTA Process, GE

Unit Operation 4 – Low pH Hold/Neutralisation

Standard disposables (bags, tubing, etc)

Unit Operation 5 – Anion Exchange Chromatography

AEX Q membrane, Sartorius Stedim; AKTA Pilot, GE

Unit Operation 6 – Nanofiltration

Virosart CPV filter, Sartorius (pumps, tubing, etc)

Unit Operation 7 – Ultrafiltration / Diafiltration / Formulation

Hollow fibre filter, Spectrum Labs; Uniflux 10, GE

Unit Operation 8 – Dispensing / Storage
(Drug Substance)

Final filter, Standard disposables (bags, tubing, etc)

35 day process (16 days in Production Bioreactor)

Lilly and Biologics

- Eli Lilly & Co.
 - Longstanding history with insulins
 - Marketed peptide therapeutics (Glucagon, Forteo, hGH)
 - Marketed biologic (Xigris)
 - Growing biologics business (ImClone - Erbitux, Lilly pipeline)
- Lilly, Kinsale, Ireland
 - 30yr history in small molecule commercial manufacturing (post-launch)
 - Building Biopharmaceutical capability (2011)
 - Launch/commercial facility
 - Lilly platform based (IE42 facility)
- Small molecule API parenteral facility adapted to run MAb process
 - Four lots (incl. 2 x Phase III) made (Q1 2009) within 14 months of facility selection decision
 - Additional Phase III campaign run in Q1 2010
 - Conformance lots in Q4 2010

Transfer Timelines

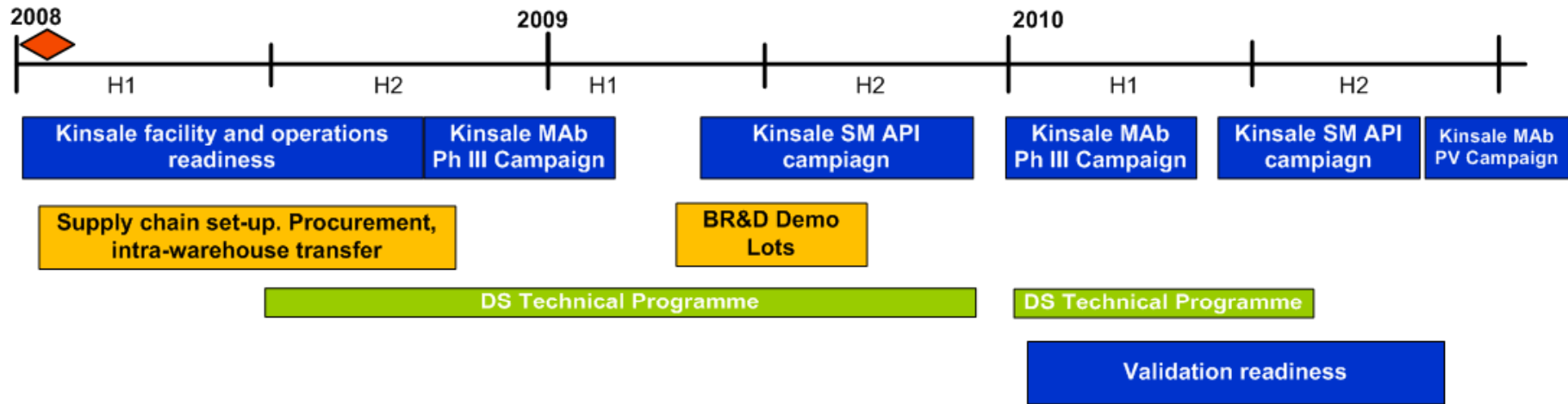


Figure 2. Overall timeline for process transfer, facility readiness and schedule of Phase III campaigns leading to process validation in Q4 2010. Red diamond indicates timing of facility selection decision. Activities at Lilly's Kinsale facility are shown in blue boxes, activities at Lilly Bioprocess R&D, Indianapolis, are shown in orange boxes. Drug substance technical programme (green boxes) was jointly executed between Kinsale and BR&D. **DS**, drug substance; **BR&D**, Bioprocess R&D; **CT**, clinical trial; **ENG**, Engineering batches; **PS**, primary stability; **PV**, process validation; **SM**, small molecule.

Transfer to API facility

- Fit-to-facility
 - Not suitable fit for Bioprocess R&D GMP facility (Indianapolis) or IE42 facility (Kinsale)
 - Kinsale IE30 facility
 - Clean manufacturing facility
 - Manufactures Drug Substance for small molecule parenteral
 - ISO 7/8 areas
 - In-built equipment (e.g., gloved cabinets)
 - Open spaces available

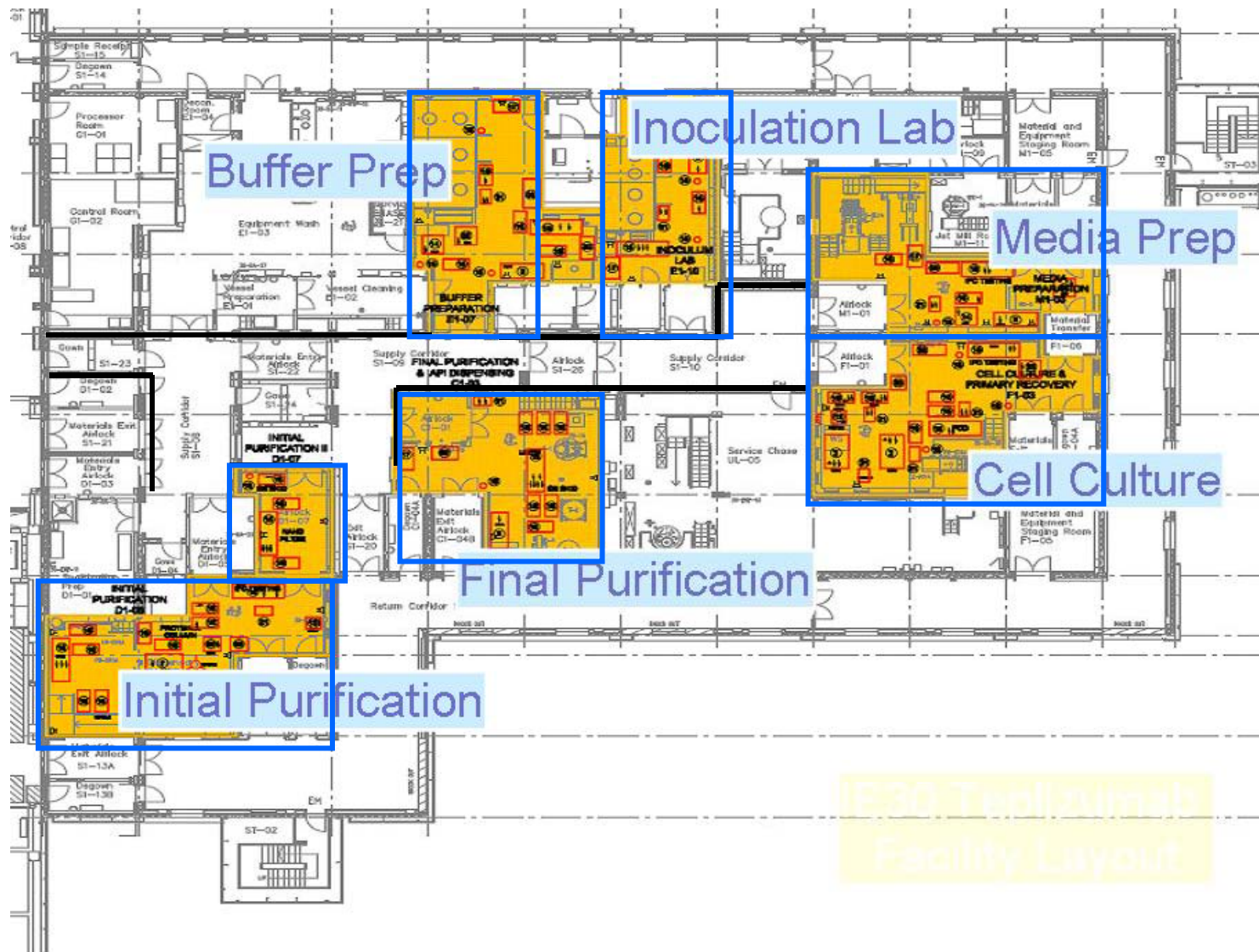
Transfer to API facility

- Considerations for Bioprocessing in a new site
 - Chosen facility lay-out
 - personnel and equipment flows
 - viral boundaries
 - Utilities (HVAC, power points, process gases)
 - Autoclave, parts washer
 - Warehousing space (10X more materials and consumables than small molecule process)
 - Cold storage (4°C, -20°C, -80°C)
 - Potential cross-contamination points with existing products
 - Data Historian/DCS access
 - Resources, capabilities (Tech Support, Quality, Operations, NPI)
 - Analytical support (in-house, out-sourced)
 - Phase appropriate capability on site in time?

Transfer to API facility

- Approach
 - Campaign-based use of IE30 facility (monoclonal antibody, small molecule parenteral)
 - Facility change over between campaigns
 - Materials procurement and release via Lilly US GMP facility
 - Analytical methods (Alliance partner, Lilly BR&D, out-sourced; Micro methods In-house)
- Established dedicated areas for:
 - Buffer prep
 - Media and feed prep
 - Inoculation and seed train expansion
 - Production Bioreactor and Primary Recovery
 - Initial (pre-viral) Purification: Affinity capture to AEX
 - Final Purification: Nanofiltrate to final formulation and fill
- Took over facility warehouse, incl. walk-in 4°C
- Installation of freezer area (-20°C, -80°C)

Drug Substance Production Process in Lilly

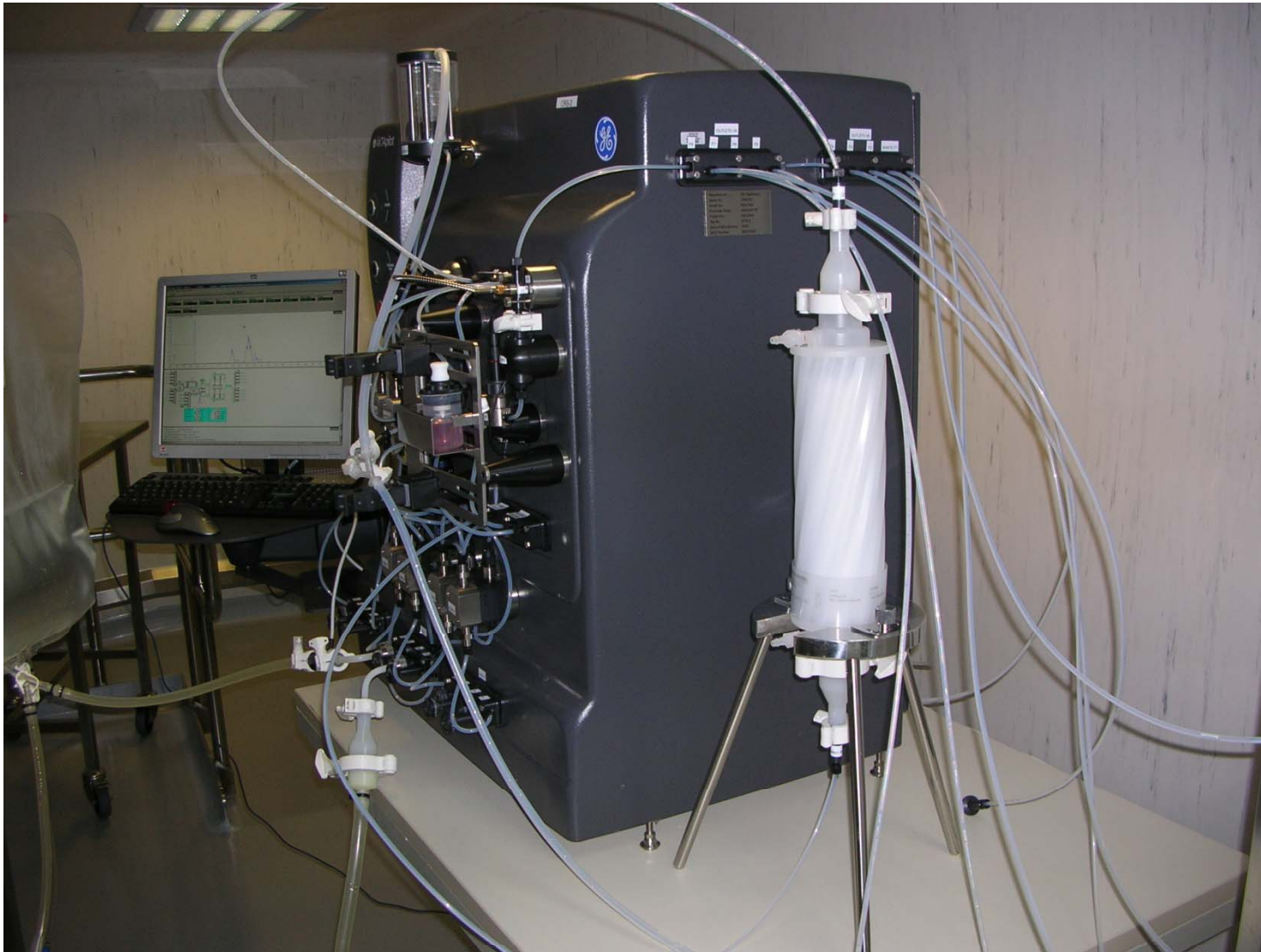


Drug Substance Production Process in Lilly Kinsale: Facility Adaptation



Adaptation of an ISO 8 classified area in a facility used for small molecule parenteral API manufacturing. [Top Panel] Open space is shown, containing glycol loops. [Right panel] 200L disposable bioreactor systems in place in same area. A process gas (air, O₂, CO₂) manifold has been added to the rear wall.

Drug Substance Production Process in Lilly Kinsale: Facility Adaptation



AEX chromatography via membrane AEX and skid

Single-use: Challenges

- General
 - Procurement
 - ca.10X increase in materials and consumables vs small molecule process
 - Supplier Quality systems
 - Capacity to support
 - New considerations (e.g., ADC, L+E, adventitious agents)
 - Warehousing (space, cold storage, logistics)
 - For first campaign procured and released through US GMP facility
 - Inter-warehouse transfer
 - Check and release at Kinsale site

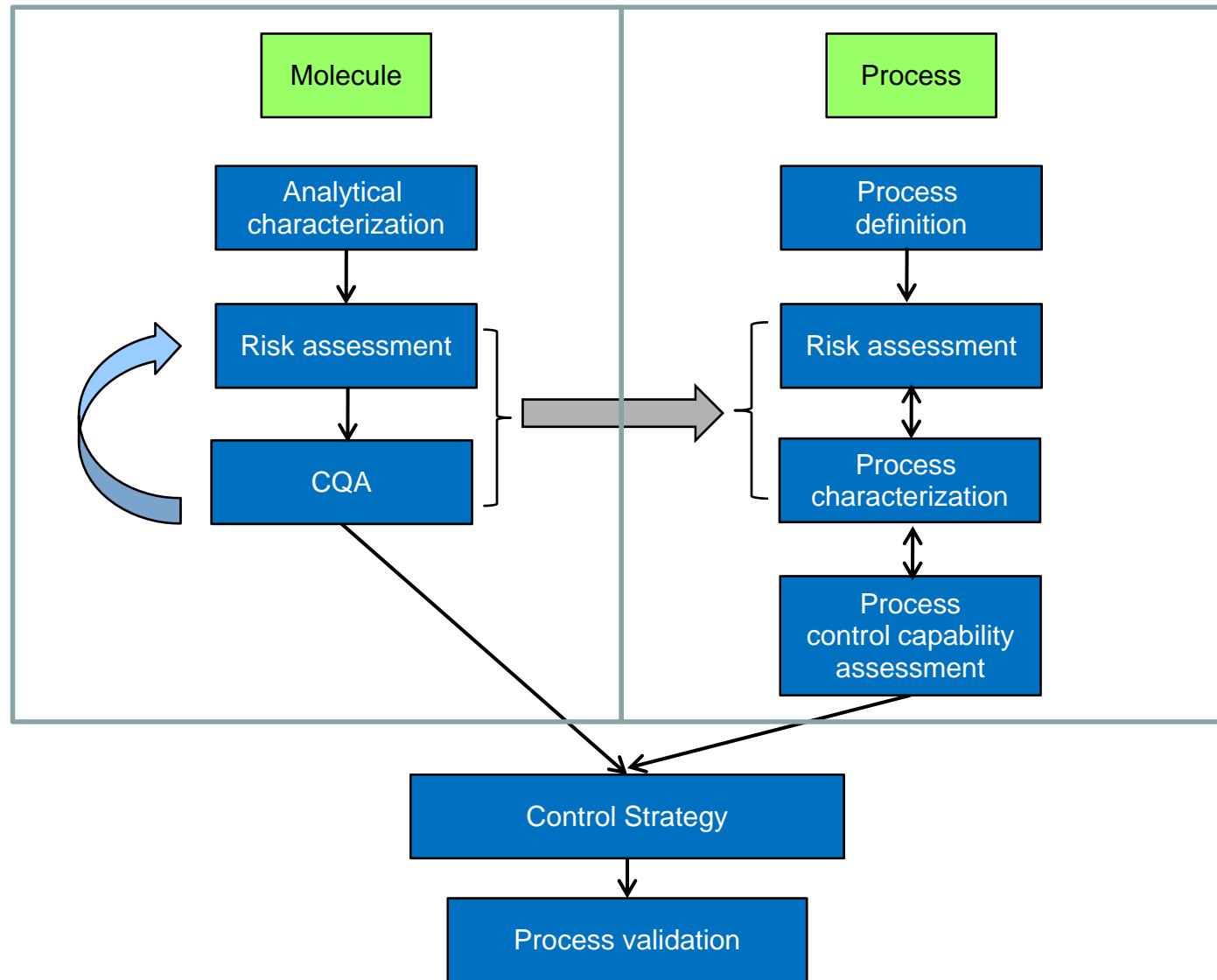
Single-use: Challenges

- Seed train expansion
 - No issues
- 200L Production Bioreactor (Wave System)
 - Poor experience with “in bag” probes
 - At-line process gas measurements: CO₂, O₂, metabolites (NOVA)
 - Modified (20L Wave) temperature probe (under disposable bioreactor)
 - Tubing sets
 - Recommend procuring and stocking generic tubing sets (e.g., lengths of pre-sterilised C-flex Size 16)
 - Off-the-shelf availability of these is poor from suppliers (client-specific manufacturing)
 - Numerous options for custom design, but time consuming and Supplier Quality considerations

Disposables: Challenges

- Primary Recovery and Protein Purification
 - Largely issue free
 - Small parts pre-use cleaning
 - Industry practice: small parts washed ± autoclaving
 - Access to parts washer and autoclave in multi-product facility?
 - Off-the-shelf sterile parts or Pyrogen-free
 - WFI system (e.g., hoses)
 - Beware of small print!!
 - e.g., 0.6m² 0.45/0.2um membrane sterile, 1.2m² not sterile; Virosart, Q membranes
- Custom single-use designs
 - Opportunity to incorporate small parts, tubing assemblies, in to a single solution

Late stage process development and validation



Approach to Process Validation

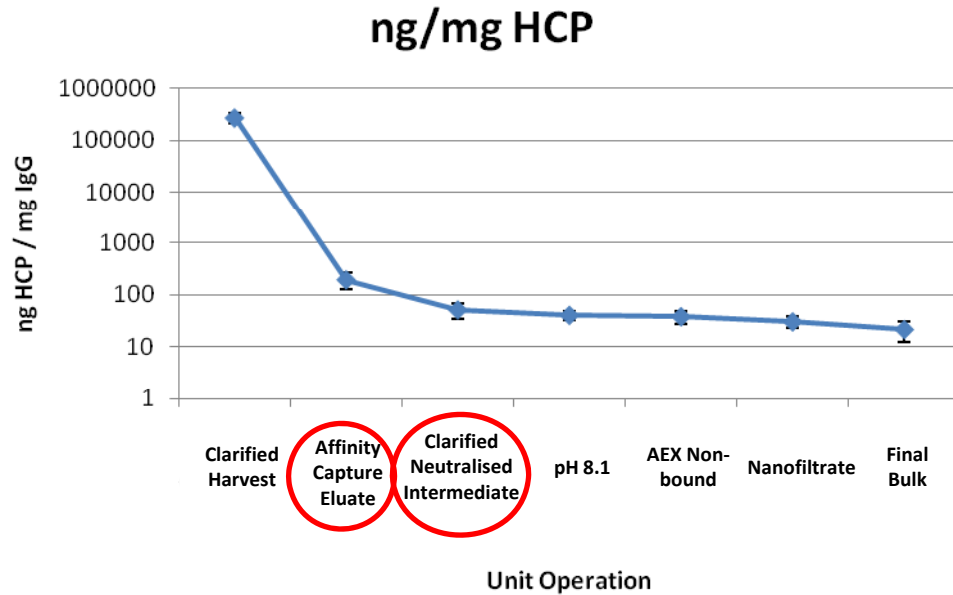
- Broad categories of validation controls:
 - Microbiological controls (including process controls)
 - Analytical testing
 - In process
 - Batch release
 - Process Characterisation
 - Parametric controls
 - Process Support Qualification studies
 - Mixing studies, cleaning validation, etc
 - L+E assessments
 - Supplier Quality systems (GMP)
 - Raw materials
 - Consumables

Control parameters for validation

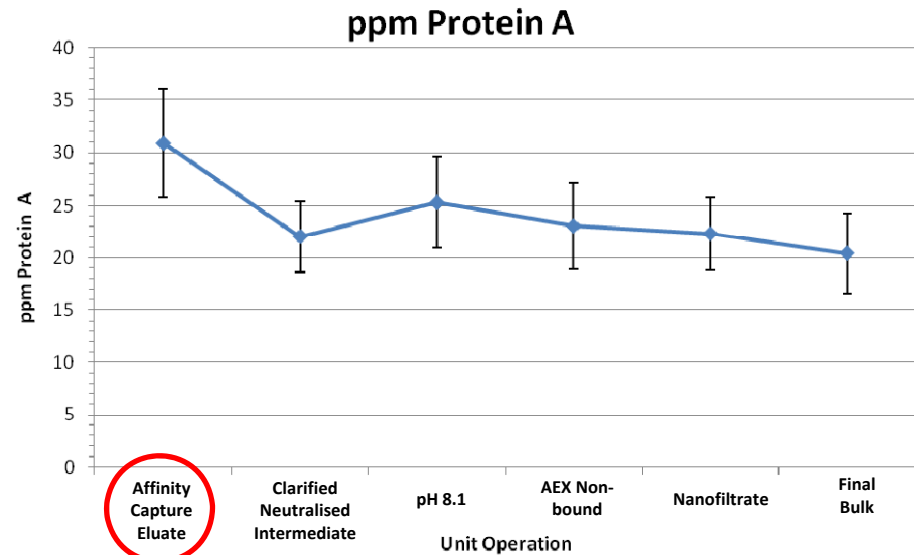
- Process Microbiological control
 - Extensive sampling in 2 x Phase III campaigns
 - Evaluation + Risk Assessment (and Reduction) = Final Micro control strategy
 - Approach taken does not differ considerably from fixed (stainless steel) platforms

- Analytical control
 - Batch release specifications (derived from molecule CQAs, stability indicated issues, Drug Product considerations)
 - In process testing:
 - Characterisation of **clearance and control** of process and product related impurities
 - Confirm function of unit operations (e.g., clear Host Cell Protein at Affinity capture chromatography)
 - Additional/ongoing process characterisation testing (contingency for likely scenarios, e.g., glycoform testing at batch release)

Control parameters for validation



Averaged in-process data for residual GS-CHO Host Cell Protein (HCP). Error bars indicate 1 standard deviation. Data averaged across three to 10 lots per unit operation.



Averaged in-process data for residual Protein A. Data averaged across three to 10 lots per unit operation. Error bars indicate 1 standard deviation.

○ (Analytical in process) Process validation acceptance criteria applied

Equipment validation

- Adopted “standard” approach to single-use equipment validation
 - URS / FRS, IOQ
 - Unicorn methods validated and GMP controlled
- For single-use consumables
 - QRM approach to risk ranking
 - Mix of audits/site visits, Quality agreements, questionnaires, depending on resulting risk rating
- Issues observed with single-use consumables:
 - Tubing assemblies not properly assembled (loose fittings/connections)
 - Bag integrity issues
 - Impellers disconnecting from mixing bags

Control parameters for validation

- Process Support Validation studies
 - E.g., Mixing studies, cleaning validation, hold times
 - Approach taken does not differ considerably from fixed (stainless steel) systems
 - E.g., grouping/family approaches, component impact assessments
 - Timing: ideally have this information incorporated prior to PV campaign
 - Avoid “surprises” (e.g., under-mixing) immediately prior to PV
 - Generate data in last (pre-PV) Ph III campaign
 - Confirm (if required) during PV batches
 - Leachables + Extractables

Challenges with single-use systems: control parameters

Issues addressed during late stage development	
Issue	Resolution **
IgG reduction issue at Primary Recovery	* Integrated bag (hold container), filter, sampling and aeration system: - Link between %D.O. and IgG reduction
Control of IgG polymer formation at pH adjustment of DSP intermediates	* Bulk recirculation system, with in-line addition of titrant.
Manual mixing steps (pre-sampling, pH adjustment of intermediates)	Widespread use of mixing/rocker platforms (Wave)

* Design of custom disposable solution

** Control capability assessment showed robust, well-controlled, system post-change.

DSP, Downstream Processing

Challenges with single-use systems: control parameters

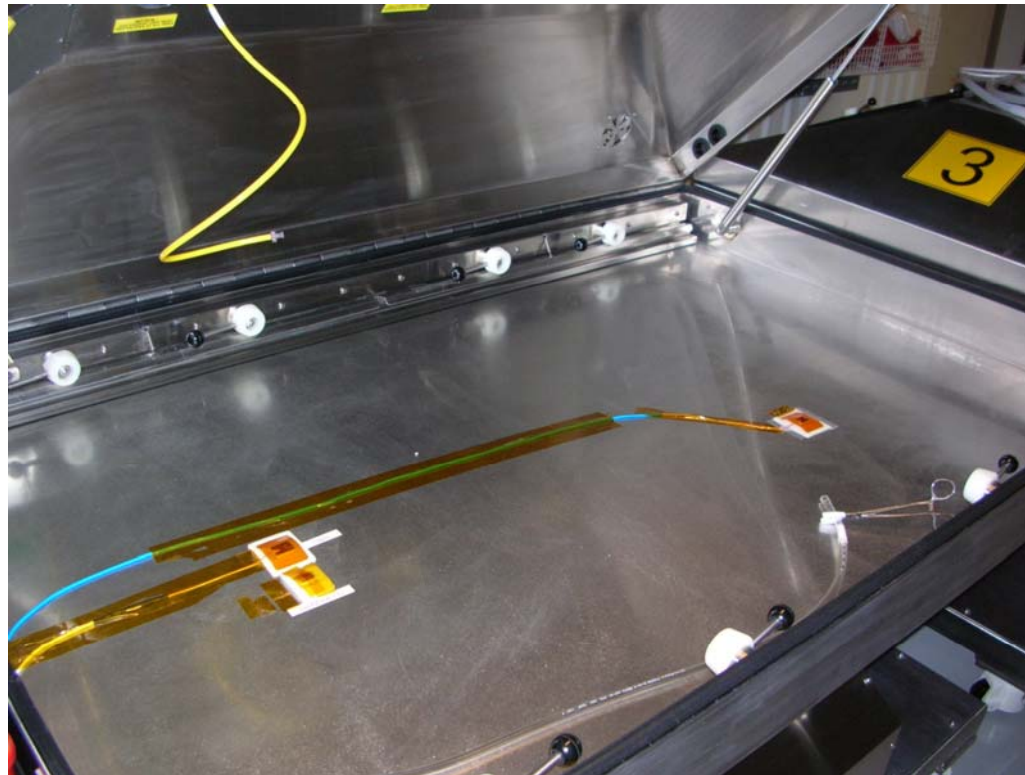
- Parametric control:
 - Following CQA identification, classified and established ranges for control parameters
 - Similar approach to that taken currently by Lilly for platform biologics
 - Issues of concern with disposable system for conformance campaign
 - Temperature control in Production Bioreactors (Wave System)
 - Gas (D.O., PCO₂) control in Production Bioreactors (Wave System)
 - Production Bioreactor inoculation seeding density
 - Temperature control in DSP intermediates (viral clearance parameters)
 - Control capability and measurement uncertainty for probes (e.g., temperature in DSP)
- Aware that better *de novo* solutions existed for some issues encountered
 - Adaption of these not always appropriate given commercial phase (post-CT)

Challenges with single-use systems: Bioreactor temperature

- Temperature control in Production Bioreactors (Wave System)
 - Temperature is CPP (significant impact on >1 product CQA)
 - FMEA identified detectability concern regarding temperature probes
 - Any calibration drift would only be seen at next calibration (pre- and post-campaign)

Challenges with disposable systems: Bioreactor temperature

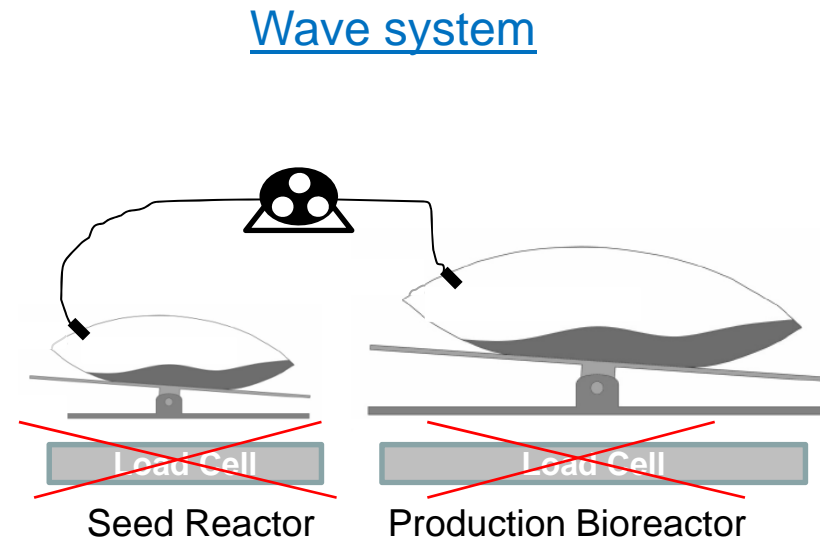
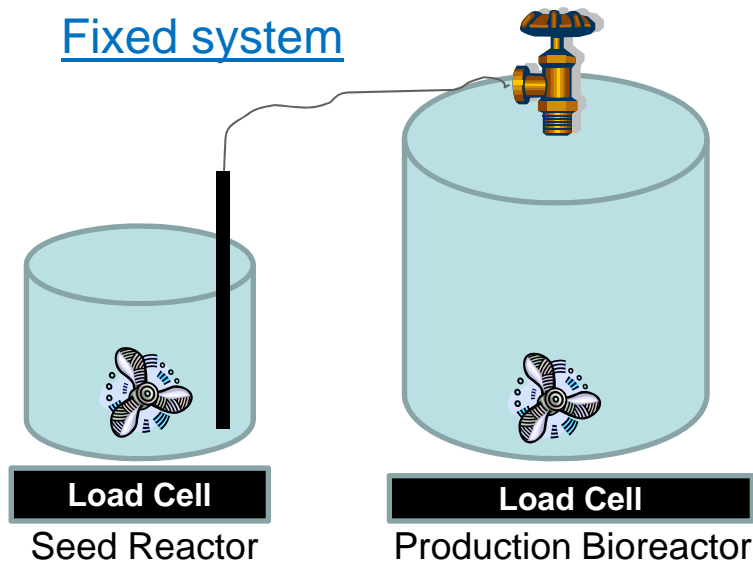
- Single thermocouple on unit base plate (poor experience with Wave in-bag probes; using Sartorius Cultibags in Wave System)
- Installed second probe and “referee” probe (qualified system)
- “Customised” probes: from 20L Wave systems



- Alarm limits and response procedures set (GMP)

Challenges with disposable systems: Bioreactor inoculation

- Production Bioreactor inoculation seeding density:
 - Inoculation seeding density a CPP (impacts two CQAs)
 - Only partial transfer from Seed Reactor required to reach target inoculation cell density in Production Bioreactor
 - Concern over robustness given nature of inoculum transfer (cell settling [towards transfer line] during inoculation)



- Fixed gradient tray holder (fabricated locally)
- Split transfer, with intermediate mixing
- Defined cell counting windows pre- and post-inoculation

Challenges with disposable systems: Bioreactor inoculation

- Undertook process variability (and reduction) assessment
 - Identify additional factors contributing to process variability
- Approach implemented prior to second Phase III campaign
 - Data analysis (seed density post-inoculation) and operator feedback show more robust and streamlined process.

Challenges with disposable systems: DSP temperature

- Temperature control in DSP intermediates (viral clearance parameters)
 - Temperature control for Viral Inactivation step (CPP; 15 – 25°C)
 - Original system has no in-line temperature probe
 - Processing area temperature control by facility temp (controlled, alarmed)
 - Temperature confirmed at VI step (identical approach taken for Viral Clearance validation study)
 - Using bench-top probe
 - Measurement uncertainty (MU) assessment of temperature probe showed MU @ 2σ of 2.4°C (thermocouple)
 - Prompted probe change to RTD probe to improve accuracy and robustness

Challenges with disposable systems: DSP temperature

- All probes may not be equivalent:
 - Change in pH probe (precision issues) for Low pH VI
 - New probe (calibrated) was reading ca.0.4 pH units higher than old probe in same intermediates and buffers
 - Both probes are amperometric

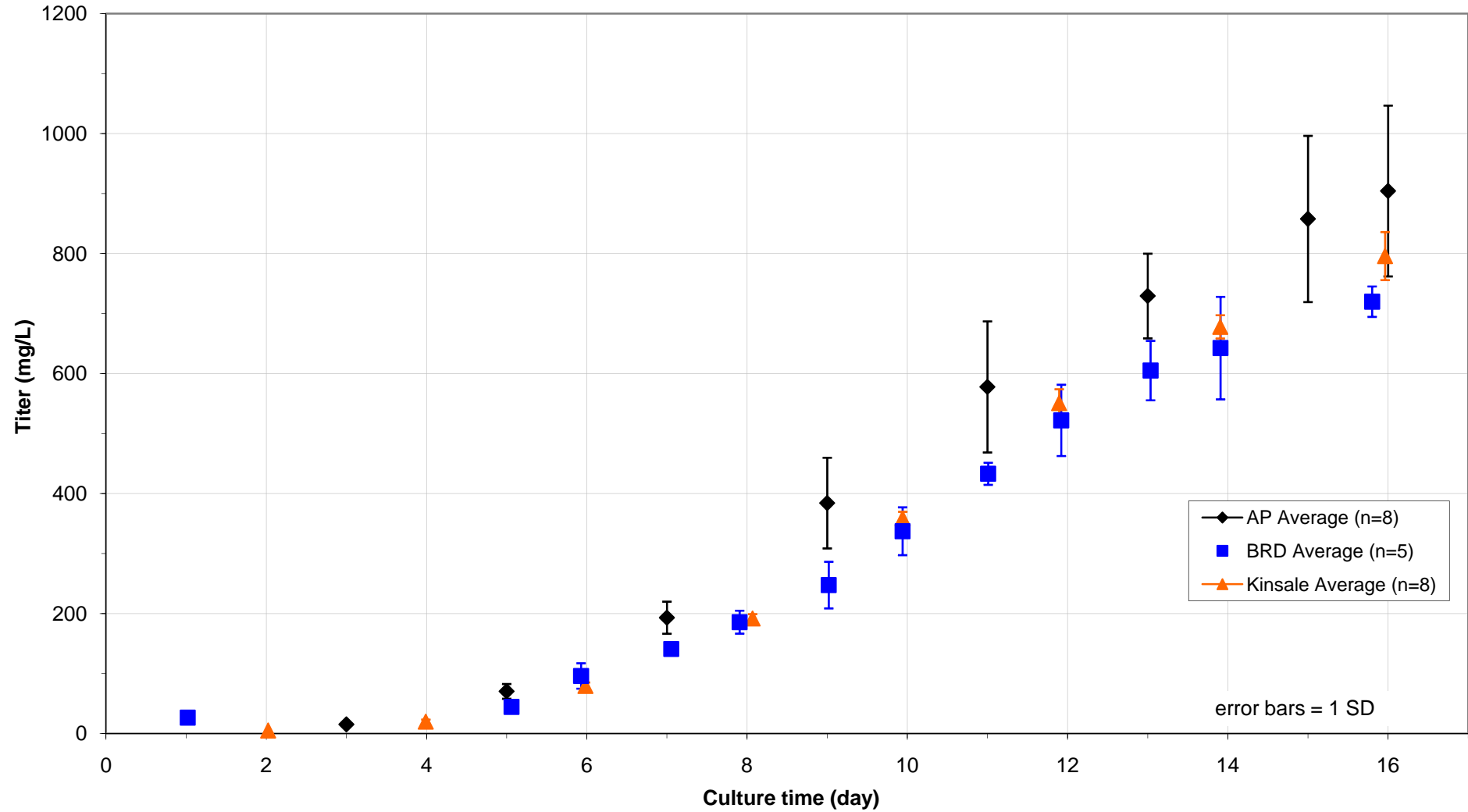
Outcomes

- Both Process and Product assessed as comparable between original site (Alliance partner), development site and manufacturing site
 - documented assessment

- Irish Medicines Board audit Jun 2009
 - IMP licence granted

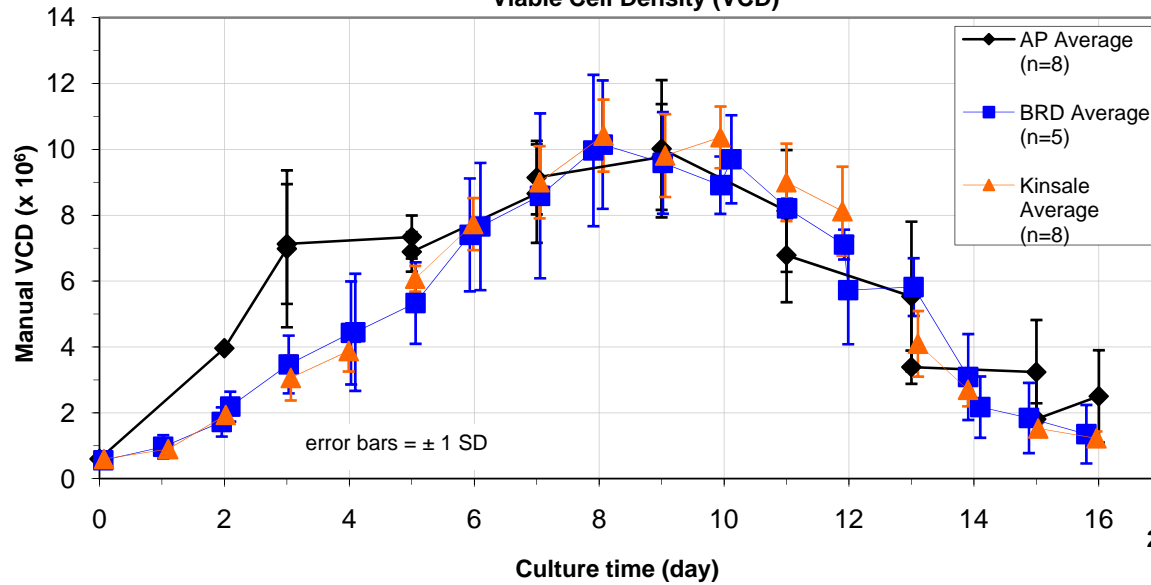
Outcomes: Production Bioreactor Titres

200L Wave Bioreactor
Titer (mg IgG/L)

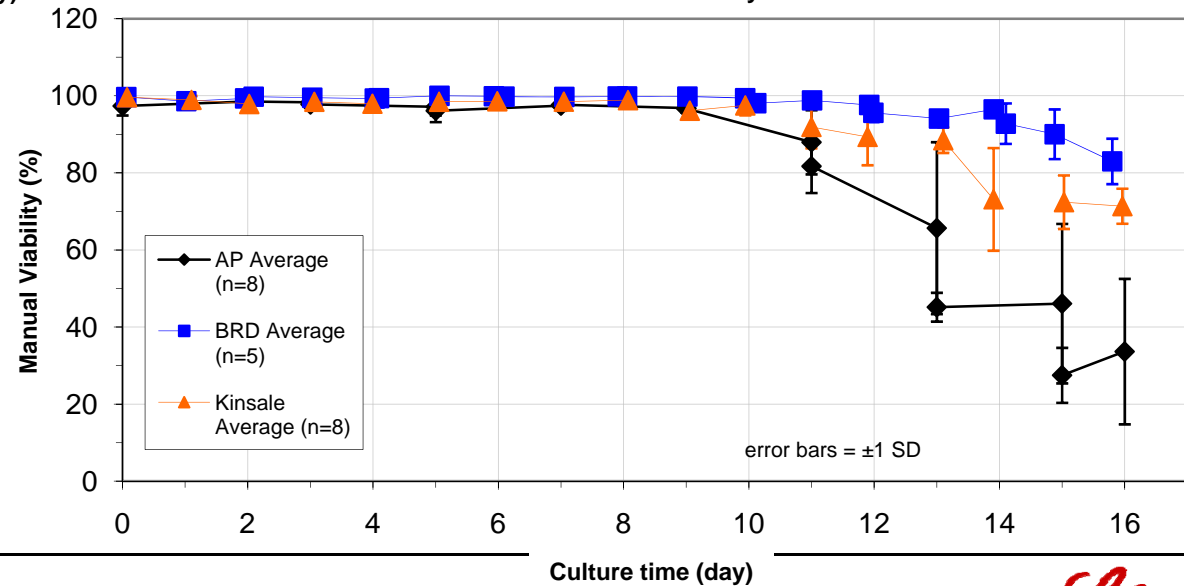


Outcomes: Production Bioreactor

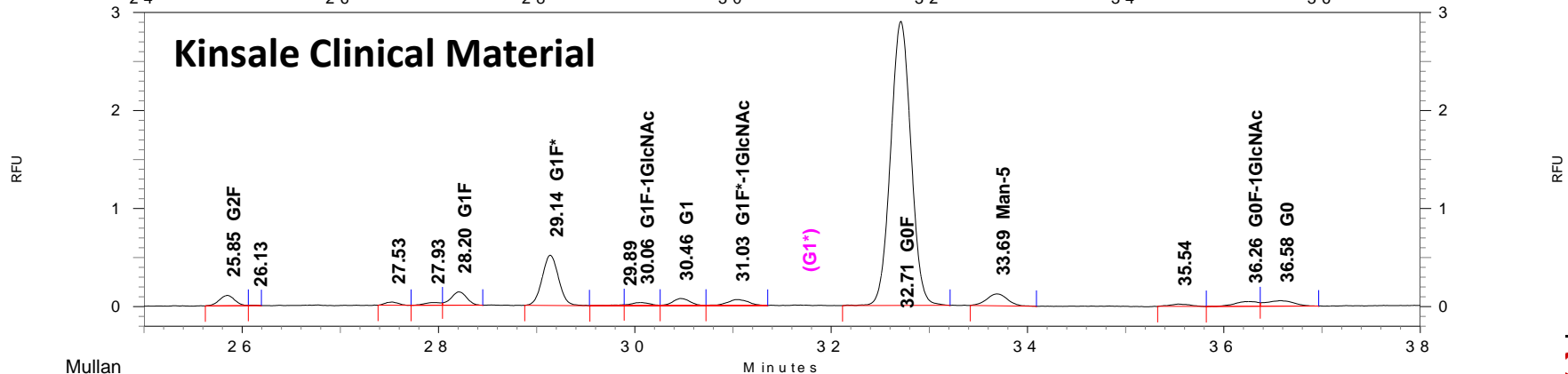
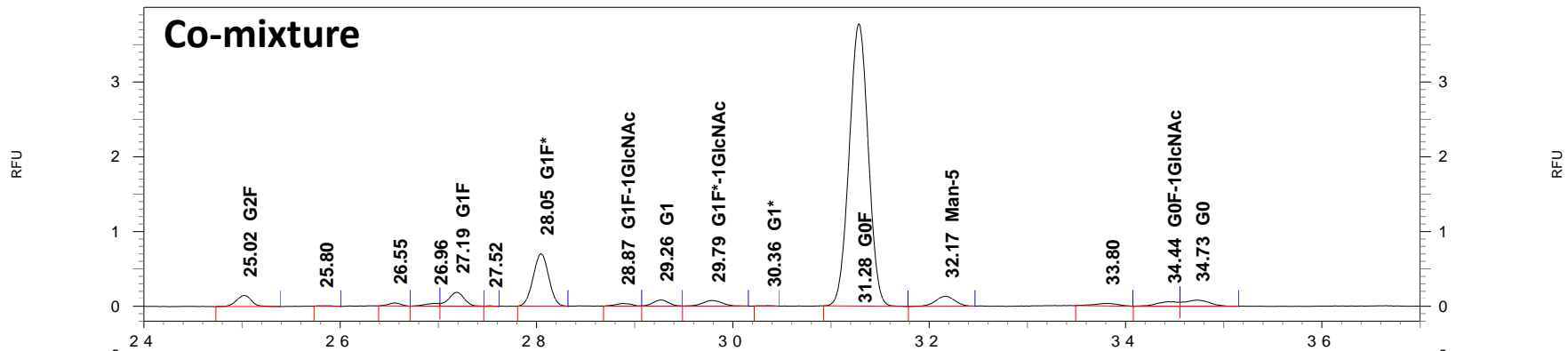
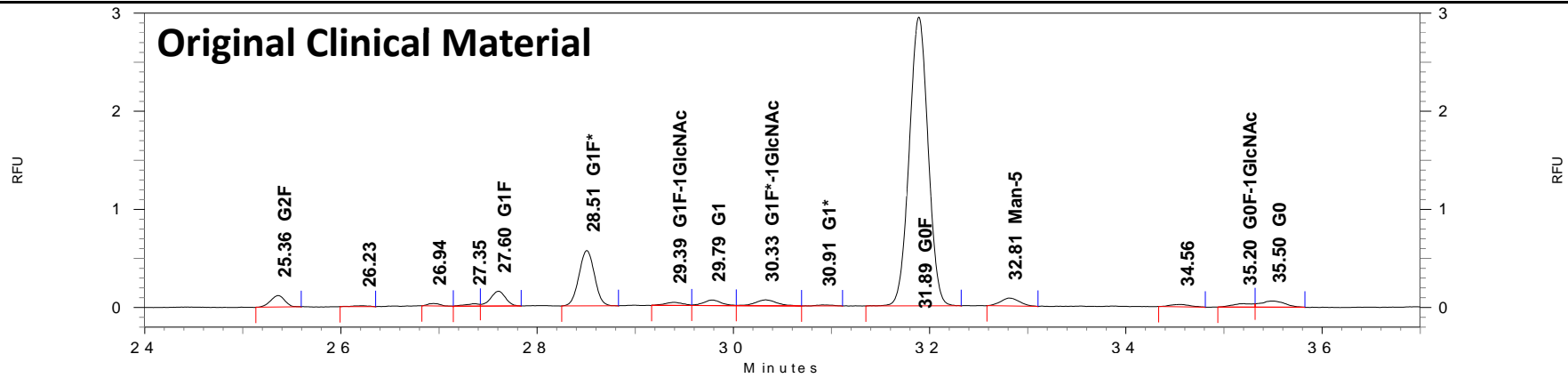
200L Wave Bioreactor Culture Performance
Viable Cell Density (VCD)



200L Wave Bioreactor Culture Performance
% Viability



Outcomes: Product Quality – Oligosaccharide profile (CE-LIF)



Readiness to validate

- Project discontinued in Oct 2010
 - Data review from Phase III trial: did not meet clinical end-point
- At time of project cessation:
 - Controlled, characterised, well understood process
 - Critical controls identified, control capability assessed (basis for SPC), GMP system fully developed
 - Previous two GMP campaigns (2009, 2010; total of seven lots) very comparable (process indicators, product quality)
- Series of formal gate reviews prior to “approval to validate”
 - Development and Manufacturing
 - Technical peer group (other product teams in Lilly)
 - Senior Management
- Documented process comparability
 - Between previous sites of manufacture and Lilly Kinsale site

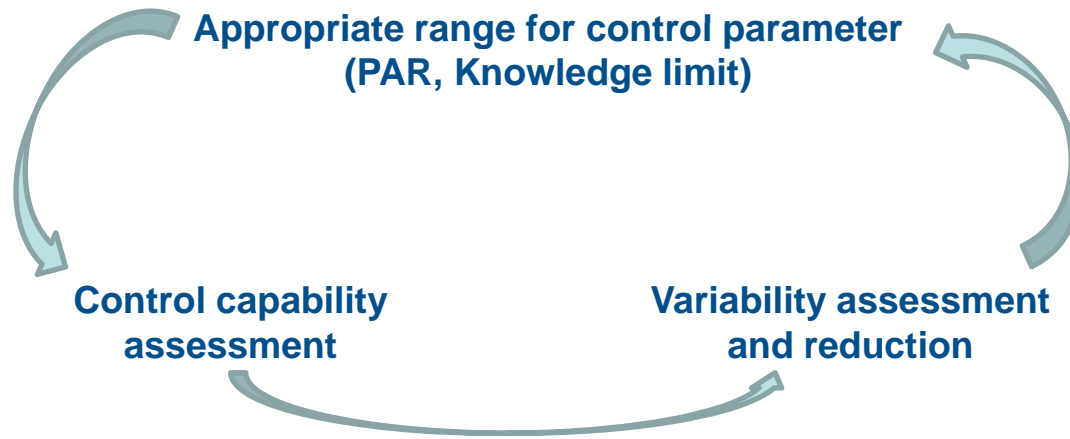


Concluding Points

- Several challenges arising from use of disposables
 - Procurement, Warehousing, Cold Chain
 - Off-the-shelf availability of “standard” items
 - Pump tubing sets, tubing extenders, small parts (e.g., pyrogen-free)
- Approach provides strategic flexibility
 - Support non-platform processes
 - In-licensed late-stage molecules, alliances

Concluding Points

- Disposable platforms are adaptable for process development
 - Customisation and appropriate qualification and assessment of systems (e.g., temperature control)
 - Importance of appropriate scaled models
- Disposable platforms are amenable to process validation
 - Challenge in late stages of commercialisation cycle to keep process “locked”
 - Defining the right operating space (PAR + control capability) for your system (e.g., D.O. control)
 - Timing is everything (e.g., mixing parameters, measurement uncertainty): understanding these in time to adjust process accordingly
 - There are solutions to challenges without recourse to process changes



Acknowledgements

Lilly Kinsale

Large Molecule Manufacturing Science & Technology

IE30 Operations

Quality Control Laboratory

Quality Operations

Procurement and Warehousing

Lilly R&D

R&D Mammalian Cell Culture

R&D Purification

Analytical R&D

Procurement and Warehousing

CMC

Regulatory Affairs

MacroGenics, Inc