Viral vectors - Overcoming process challenges to meet the clinical demands

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Questions

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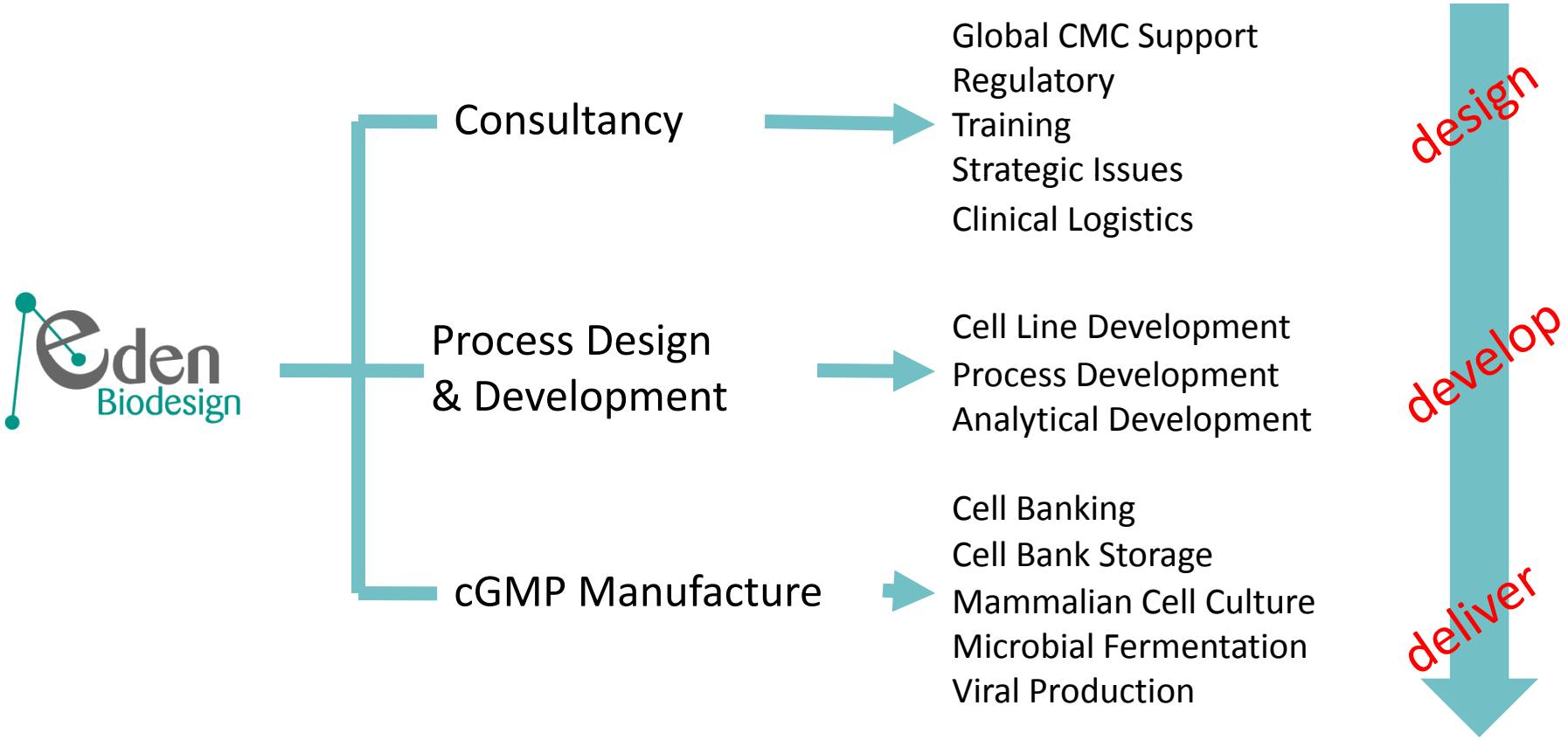
An unusual breadth and depth of services supported by considerable drug development experience and expertise



Eden Biodesign Maintains a Globally Integrated Biopharmaceutical Network

Research Triangle Park, NC
North American HQ

Liverpool, UK
Global HQ & cGMP Operations

San Diego, CA
Business Development Office

Clients on six of seven continents

● *Client Assignments* ★ *Eden Presence* ● *Strategic Partners*

Presentation Overview

- Challenges of viral production
- Historical processes
- Targets for success
- Upstream development requirements
- Case study
  - USP strategy
  - DSP & process sighting
- Scale up
- Conclusions

The Challenges…..

- Recent resurgence in live viral products
  - Gene therapy
  - Vaccines
- Typical Tox & Phase I = E+15 VP (E+13/14 IU)
- Typically research based processes

“Historical” Processes

- Adherent based systems
  - CPE as harvest indicator
  - Small scale
- CsCl purification
  - Process is an impurity
  - High Cap ex outlay
  - Scalable? Volume limited
- Inappropriate analytics
  - Activity indicating plaque assay – time consuming etc
  - Limited information on product quality

The Bioprocessing Targets…

- Suspension USP processes
  - Scalable
  - Appropriate for multiple backgrounds
- Platform DSP processes
  - Scalable
  - Appropriate impurity profiles
- Rapid development strategies
  - MOI/POI/POH studies
  - Process sighting
  - MVSS feasibility
- Rapid, platform & appropriate analytics
- Reduced C of G's

Upstream Development

- Generation of suspension cell lines
  - Serum weaning
  - Media development
  - Kinetics & aggregation state
- Cell banking investigation (Development based)
  - DMSO hold
  - Cell bank size
  - QC cell bank revival specifications
  - Process sighting

Case Study

Case Study

- Ad5 serotype containing transgene of clinical importance
- Suspension adapted cell line
- Rapid USP development strategy
  - Small scale and comparative
  - Appropriate analytics
- Platform DSP
  - Process sighting and scale up

Case Study - Appropriate Analytics

- Rapid development strategy
  - Rapid Titre (Hexon staining)

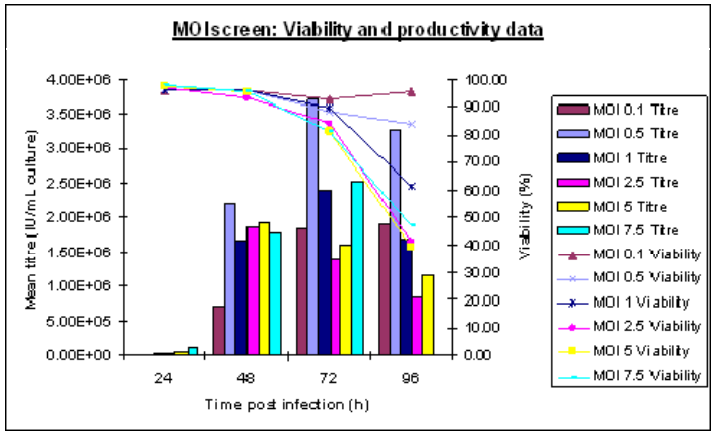
- Process sighting & exemplification (Pilot scale)
  - Rapid Titre
  - CIM ® QA HPLC (Whitfield RJ *et al.*, *J Chromatography A*. 2009)
  - HCDNA
  - HCP
  - Residual Benzonase
  - *Transgene (product specific)*
  - *Activity (product specific)*

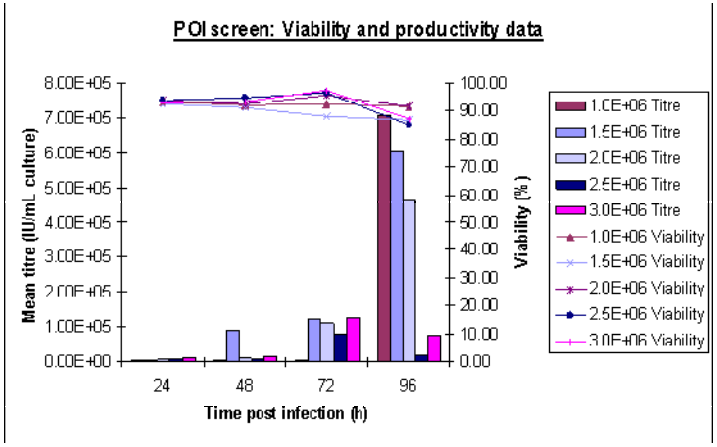
Case Study - Typical Analysis

Step	Sample	Volume (mL)	Analysis
Harvest	**Bioreactor sample**	**1955**	**RT**
Cell disruption	**Post lysis**	1000	AEX, DNA[1]
Clarification 1	**Clarified supernatant**	938	RT, SDS, AEX, DNA[1]
Nuclease reduction	**Post Benzonase**	**988**	**RT, SDS, AEX, DNA[1]**
UF/DF	**Permeate**	**1788**	**SDS, AEX, DNA[1]**
	Retentate	**230**	**RT, SDS, AEX, DNA[1]**
Clarification 2	**Post 0.65 µm filtration**	**192**	**SDS, AEX, DNA**
Anion exchange	**Peak 1 (also QS load)**	**14**	**RT, SDS, AEX, DNA[2]**
	Peak 2	**17.5**	**SDS, AEX**
Group separations	**Main peak eluate**	**27**	**RT, SDS, AEX, DNA[2]**

RT = Rapid titre
SDS = Reduced SDS-PAGE
AEX = Anion exchange HPLC
DNA[1] = Analysis via Picogreen assay
DNA[2] = Analysis via qPCR

Case Study - USP Approach

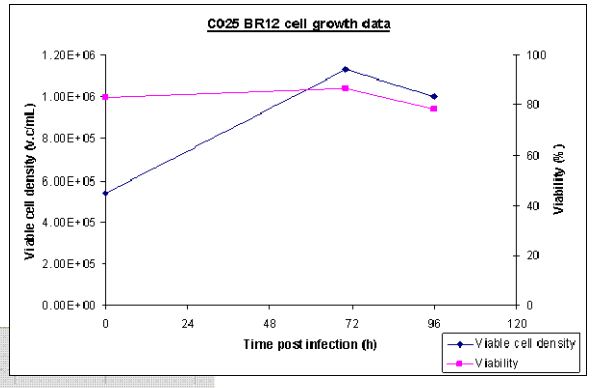


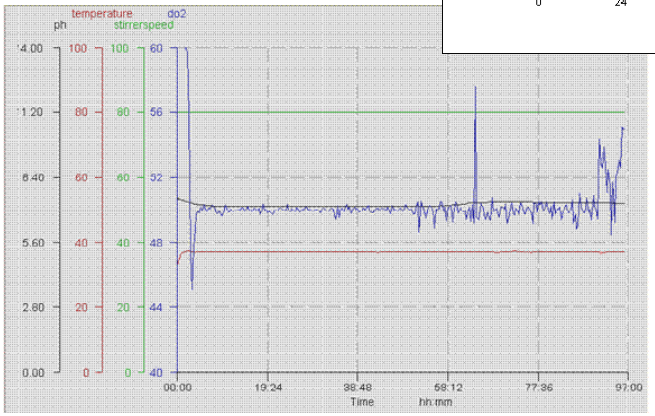


- Rapid development strategy
- 50mL shake flasks scale
- MOI, POI & POH
  - Primary indicator - process viability
  - Process titre determined
- Lower than expected MOI's
  - Smaller MVSS
- Process titre variance
  - 96 h duration confirmed

Case Study - Bioreactor

- 2.5 L stirred tank format
  - Process sighting
  - Baseline process controls
  - Developed critical controls
    - MOI = 0.5
    - POI = 5.0E+05 vc/mL
    - POH = 96 h





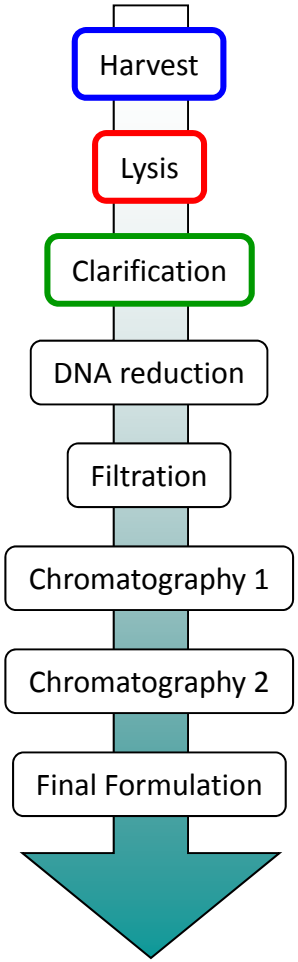
BioXpert

- Harvest titre = 4.5 E+10 IU (2L)

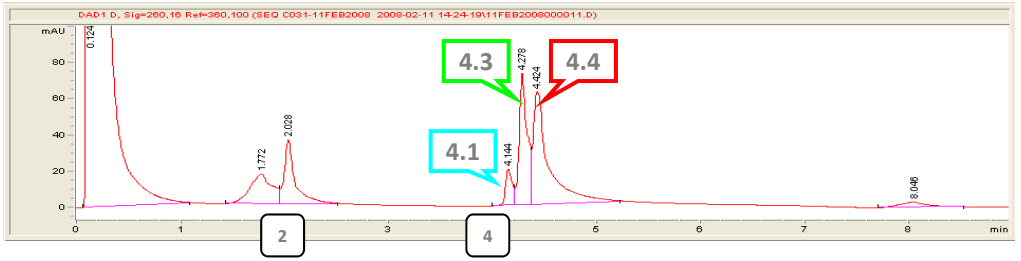
Case Study – DSP Approach

- Platform CIM® QA monolith process
  - Process sighting
  - Identify process changes if required
  - Decisions based on impurity profile & scalable unit activities

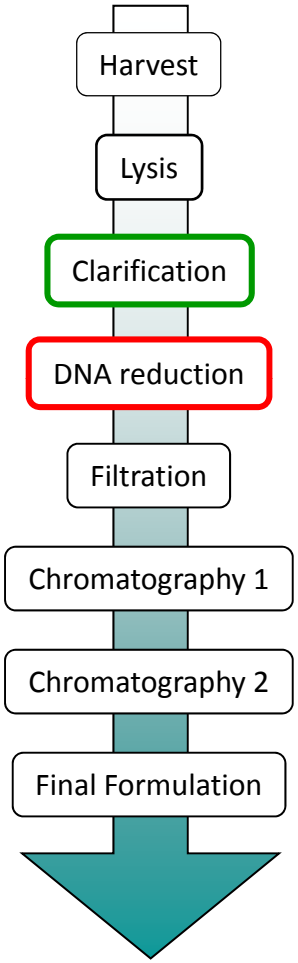
Case Study - DSP

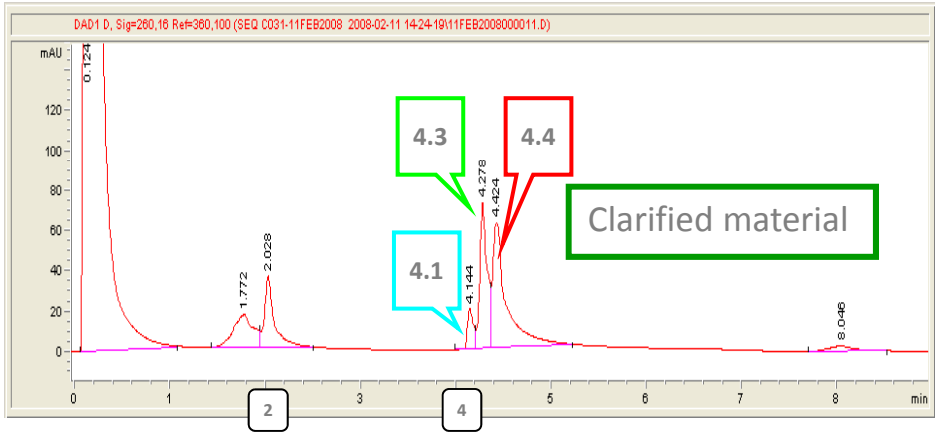


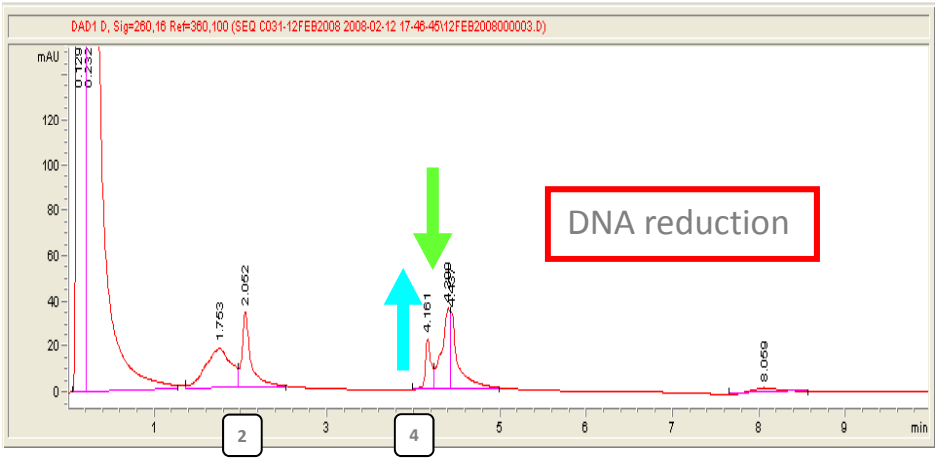
- Harvest = Batch centrifugation
  - TFF under development
  - Natural -80C hold point if required
- Lysis = Lysis buffer (50% volume reduction)
- Clarification = Batch centrifugation



Case Study - DSP

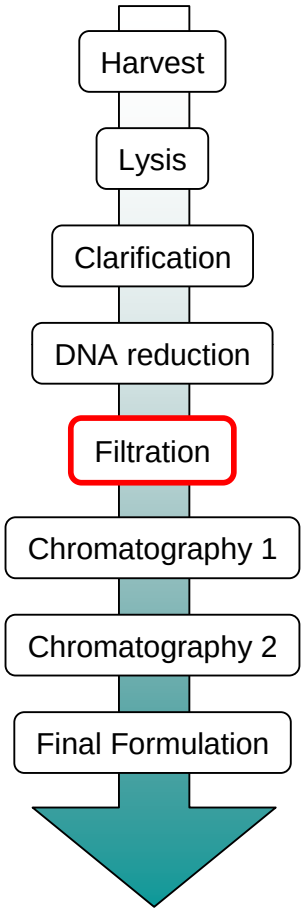




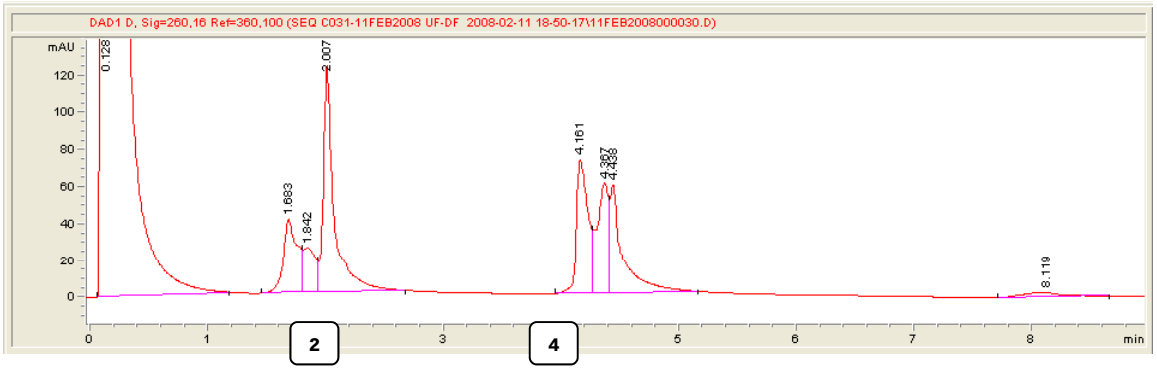


- HCDNA = Below levels of detection

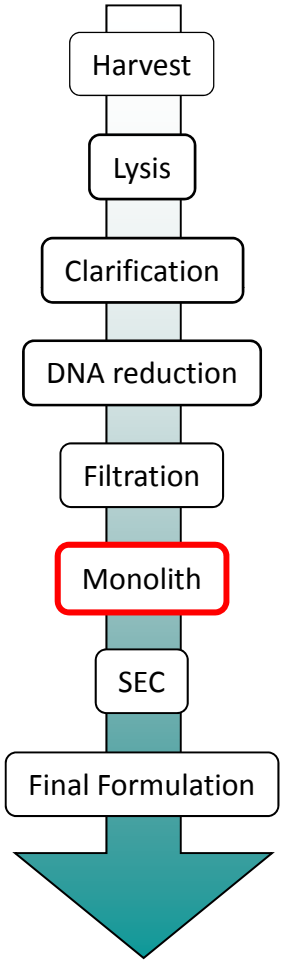
Case Study - DSP

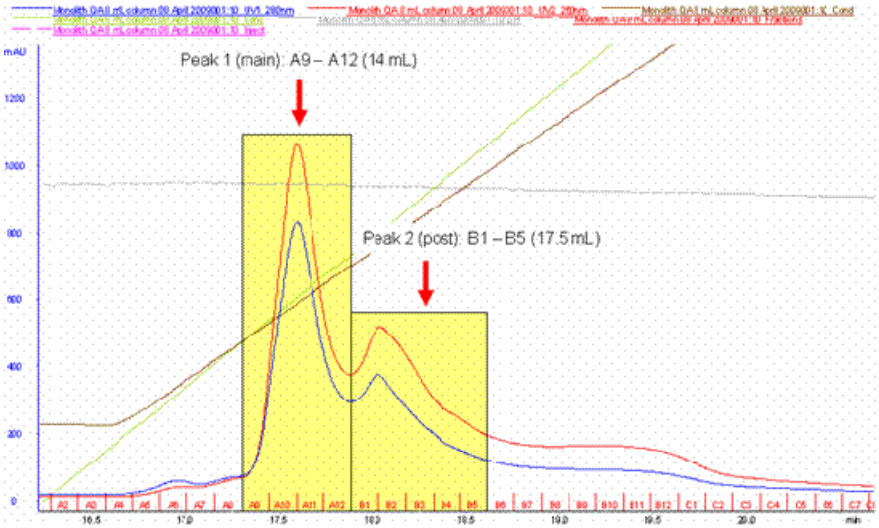


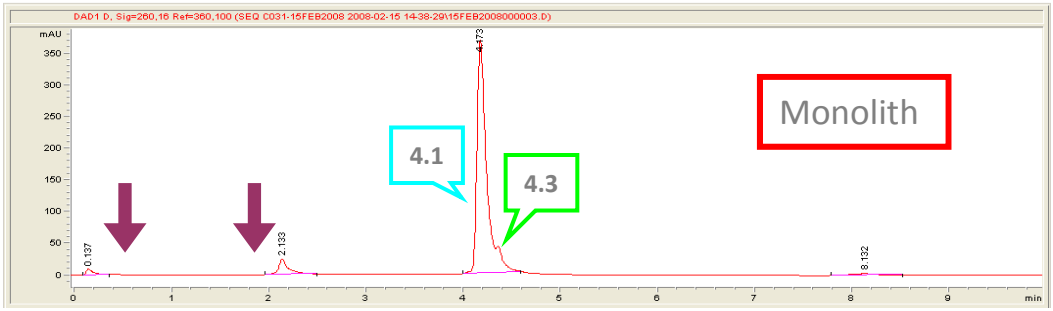
- UF/DF exchange into appropriate AEX buffer
- Reduce residual benzonase & 5X vol reduction



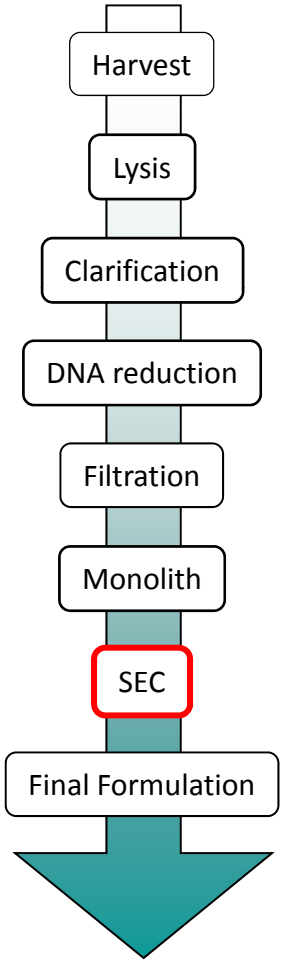
CIM® QA Monolith column chromatography

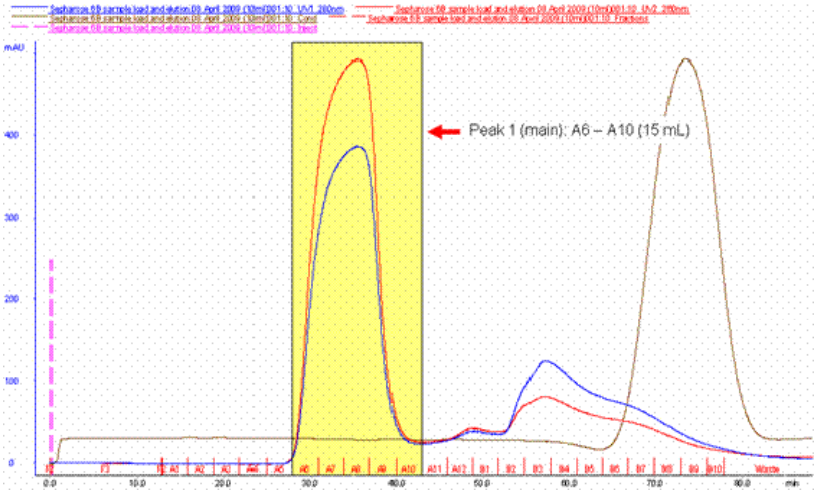


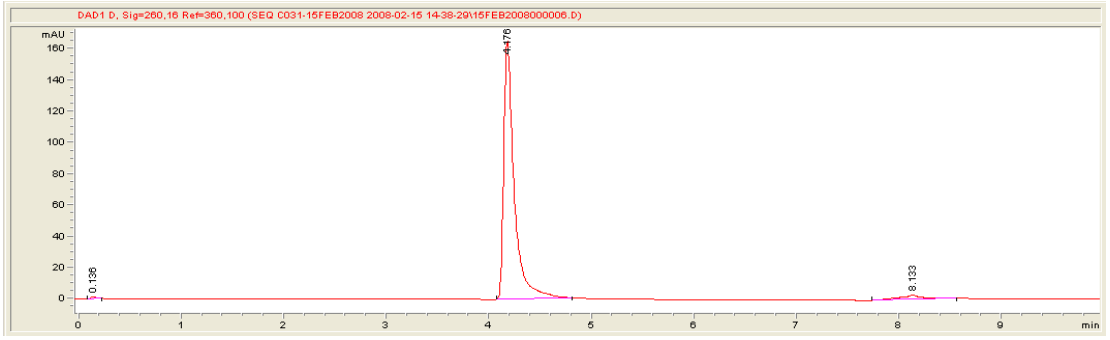




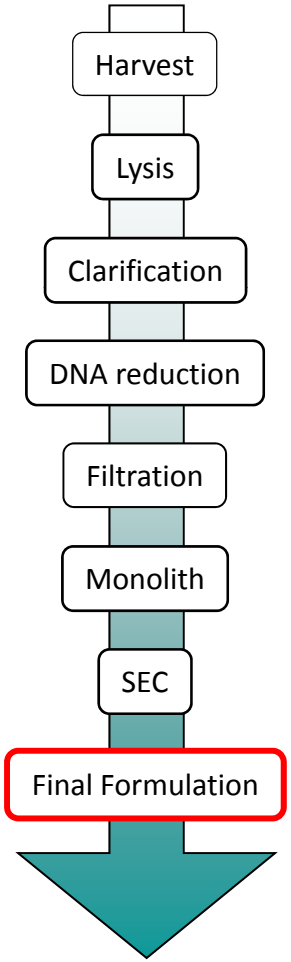
Size Exclusion Chromatography



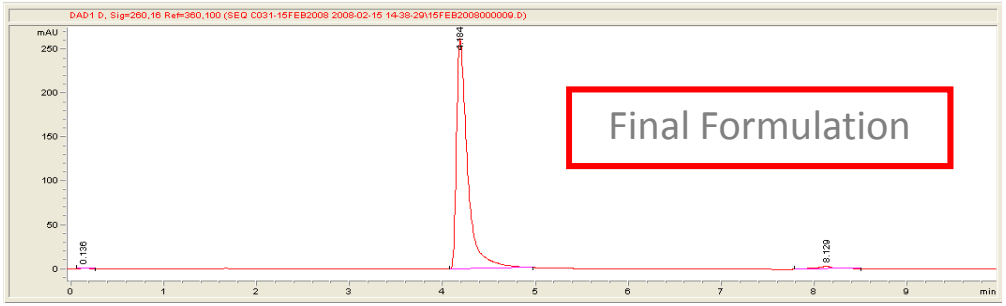




Final Formulation

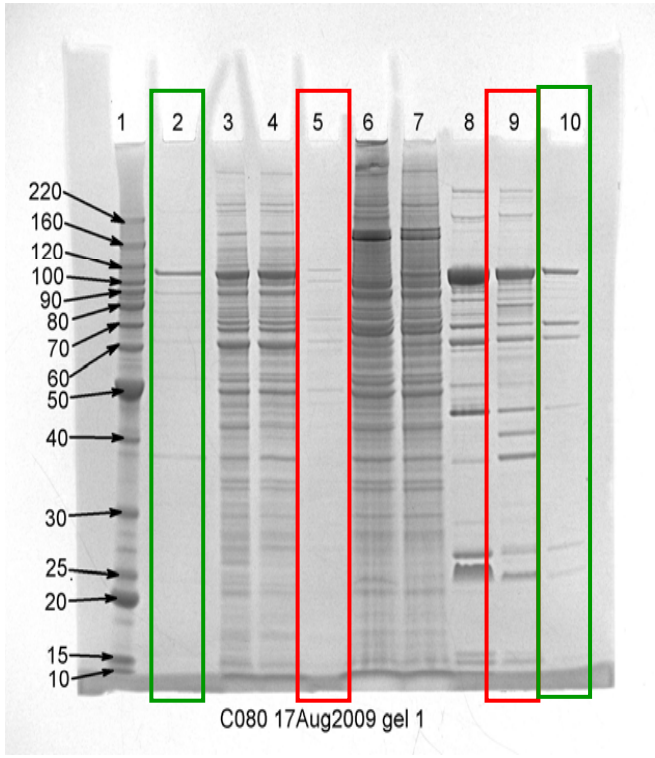


- UF/DF into final buffer formulation
  - Client supplied formulation
  - Appropriate concentration (Concentration/reduction)
  - Appropriate for route of administration



Case Study – Product Integrity

Lane	Sample	Vol. loaded (µL)
1	Benchmark protein ladder	5
2	Adeno-X-Lac viral stock	15
3	Post Clarification	5
4	Post Benzonase	5
5	UF/DF Permeate	5
6	UF/DF Filtered Retentate	5
7	LFT + PLW	5
8	AEX Peak 1	5
9	AEX Peak 2	5
10	GS Pool	5



Reducing SDS-PAGE

Case Study – Process Sighting

- Harvest titre = 4.5 E+10 IU (2L)
  - Assay interference with spent media
- Final product 4.56E+11 IU (2L)
- Final product 8.02E+12 VP (2L)
- VP:IU ratio ~17.5:1 (Typically 20:1)
- Good impurity profile
  - Residual HCDNA, HCP, Benzonase – not detectable

Scale up

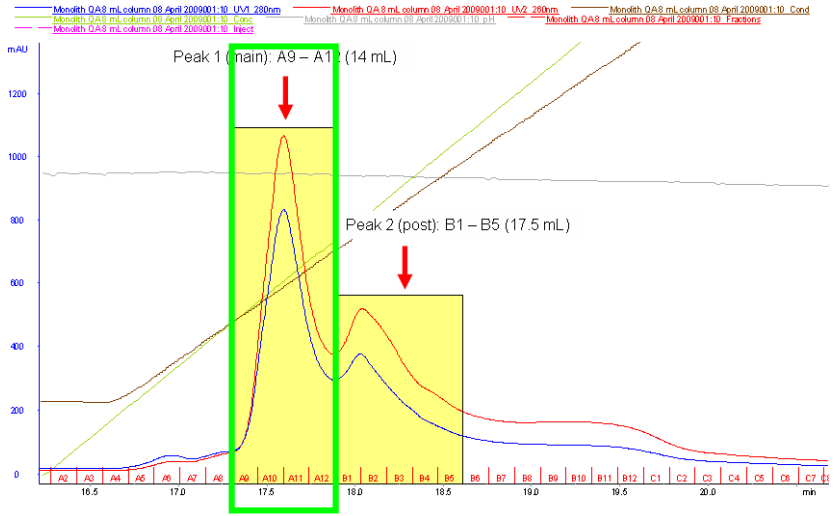
- Appropriate for variant cell backgrounds
  - Developed process controls applied
  - Culture kinetic remain similar to 2L scale bioreactor
- DSP appropriate for scale up
  - Differences in AEX profile at scale
  - Impurity profile

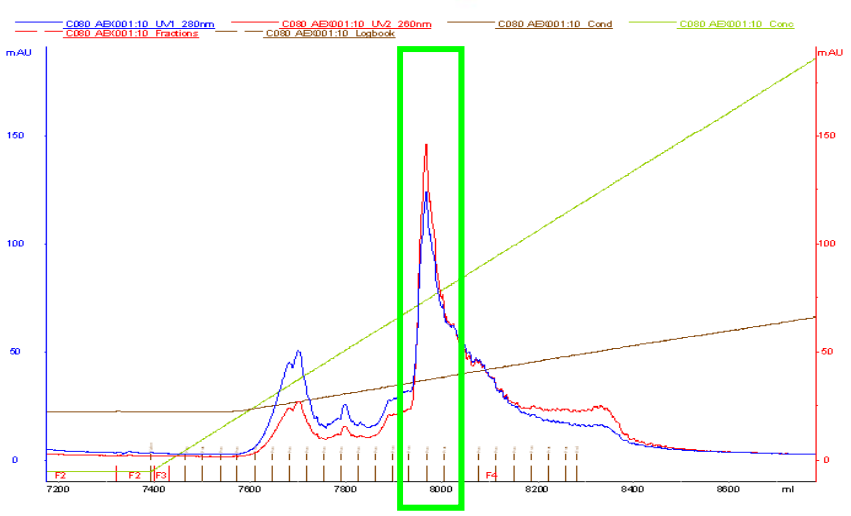
2.0 – 20L Scale up

2L (STR & DSP

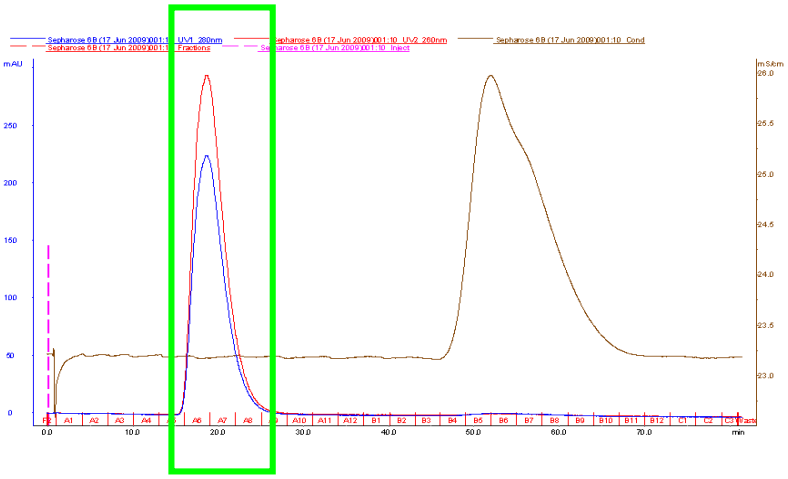
20L (STR & DSP

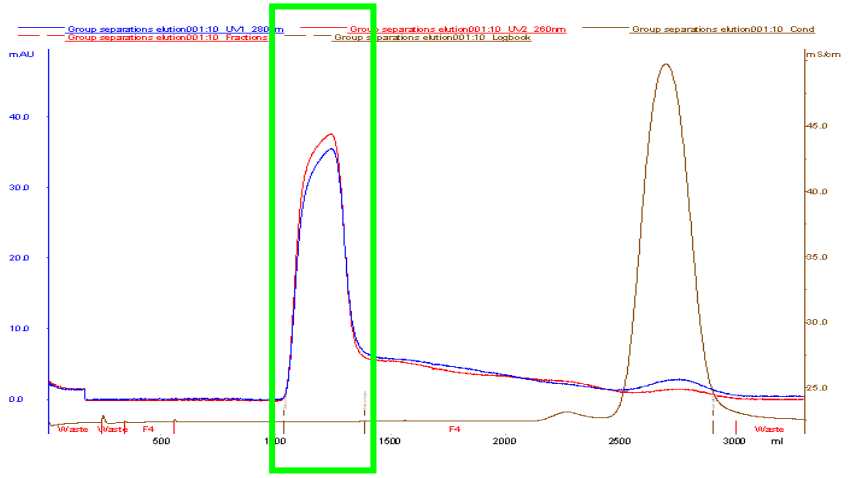
CIM ® QA Monolith





SEC (Group sep)





Conclusions

- Suspension based process supports:
  - Rapid, comparative development strategy
  - Scaled, STR based batch production
- DSP process tolerates challenge from various USP backgrounds (Ad5 serotype) and scale up issues
  - Robust analytics demonstrate identical impurity profiles as 2 & 20L scale
- Platform analytics support both rapid development activities and clinical batches
- Delivery of ~5.0E+15 VP (~2.4E+14 IU) achievable
  - Reproducible impurity profile

Acknowledgments

- Eden Biodesign:
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  - Miloš Barut
  - Aleš Štrancar

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