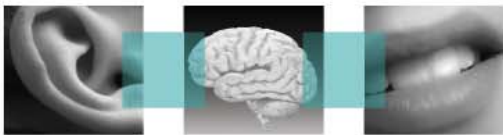


Accelerating Vaccine Development from Bench to Global Market

Risk and Opportunities



IBC Non-Antibody Protein Therapeutics Development and Manufacture
Successful Strategies for Bringing Protein Therapeutics to Market

March 4th 2009

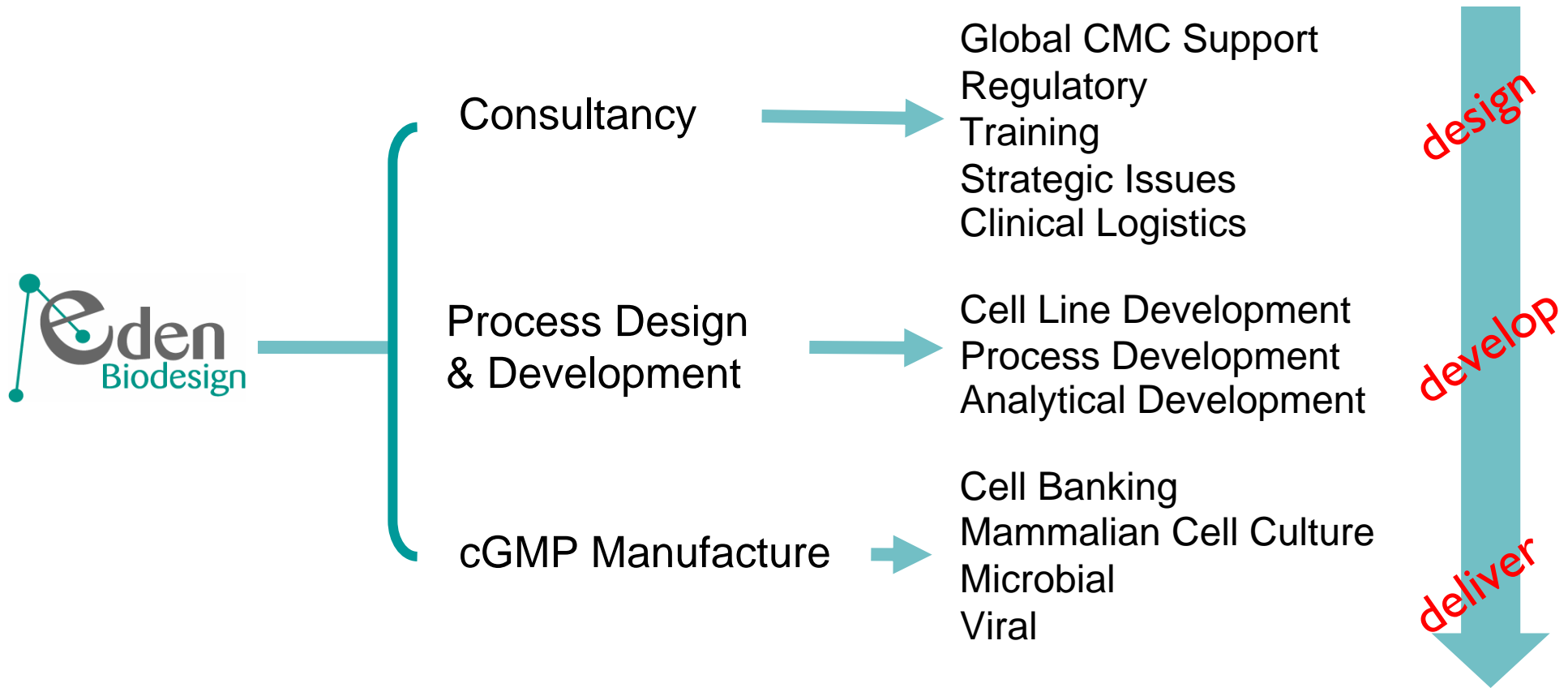
Presentation Outline

- ❑ Vaccine manufacturing technical regulatory challenges are greater than MAb's.
- ❑ New approaches to create valuable new vaccines suitable for global supply
 - ❑ Towards a platform approach similar to CHO/ProteinA for MAbs.
 - ❑ Leveraging new analytical methods
 - ❑ CMC packages suitable for global registration

Eden Biodesign



“Designing and developing valuable biopharmaceutical medicines by the application of good science from day one”



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
all five
continents

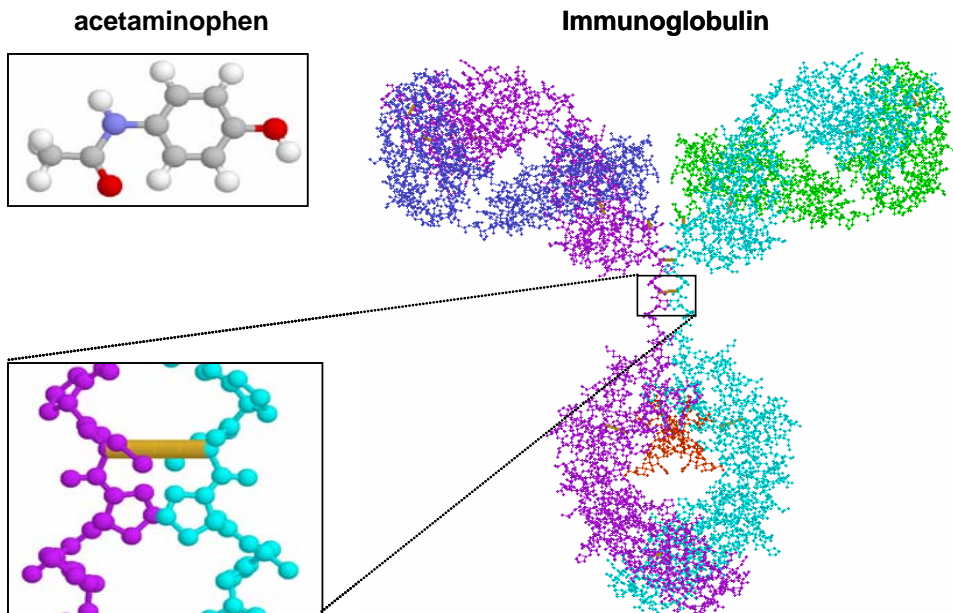
 **Client Assignments**

 **Eden Biodesign Presence**

 **Strategic Partners**

DESIGN • DEVELOP • DELIVER

mAbs are becoming 'small molecule-like'



- ❑ Commercial Trends
 - ❑ Mainstream product class
 - ❑ Biotech's mixed portfolio of small molecules and mAbs
- ❑ Manufacturing
 - ❑ Largely platform manufacturing design
 - ❑ Easily outsourced
 - ❑ Expanding international range of commercial facilities
 - ❑ Availability of experienced scientists and engineers
- ❑ Regulatory
 - ❑ Comparability protocols supporting flexibility scale-up options
 - ❑ Application of PAT, QbD initiatives.
 - ❑ CDER rather than CBER oversight

Vaccine Manufacturing Development Challenges



Product

Live
Attenuated
Inactivated
Purified Antigen
Conjugates
Recombinant
Whole cell Microbial
Viral
Virus Like Particles
Polysaccharides
DNA

Impact

Dedicated Facilities
No dominant production platform
Constraints on process change
Limited Transferability of skills and knowledge between products
High capital requirements and risk

Process

Cell Factories
Roller Bottles
Fermenters
Chromatography
Density gradient

Meeting the challenges



New approaches are required....

- ❑ Adoption of innovative facility design permitting multi-technology vaccine production.
- ❑ More “platform like” approaches
- ❑ Evaluation and application of new analytical tools to create a data rich platform for process optimisation comparable to mAb development
- ❑ Knowledge management focusing on the quality of the end product: clinical vaccine and ‘good’ paper

Towards a Platform Approach

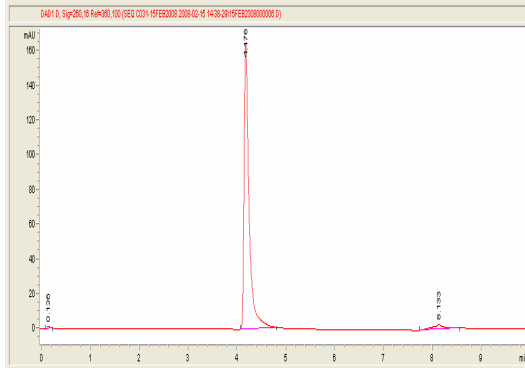
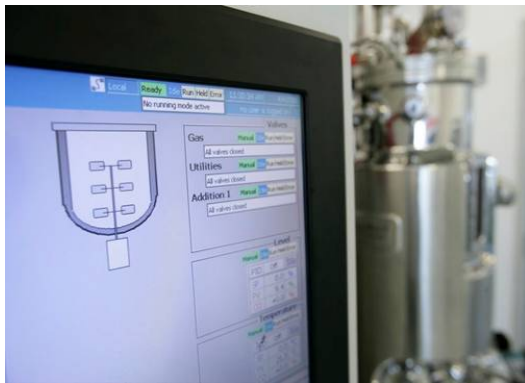
Pandemics, bioterrorism, emerging markets require a more “platform like” vaccine production approach that:

- Is robust
 - Fewer unit operations, ambient processing, hold steps, etc.
- Is easily transferred
 - Rapid global production
 - Must have a realistic supply chain
- Is reproducible
- Is scalable
- Is economically viable
- Negates classical stepwise development and reduces timelines
- Can be applied as part of tech. transfer using generic parameters

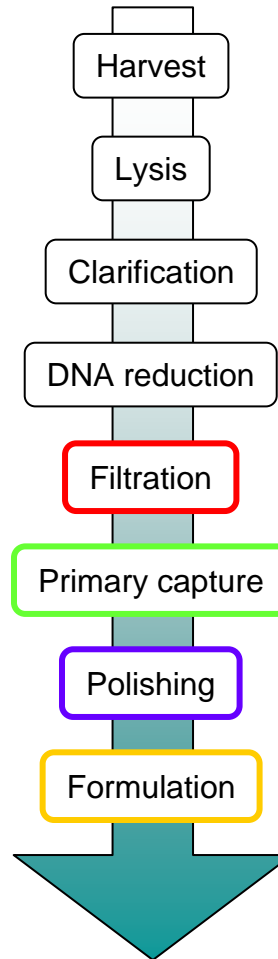
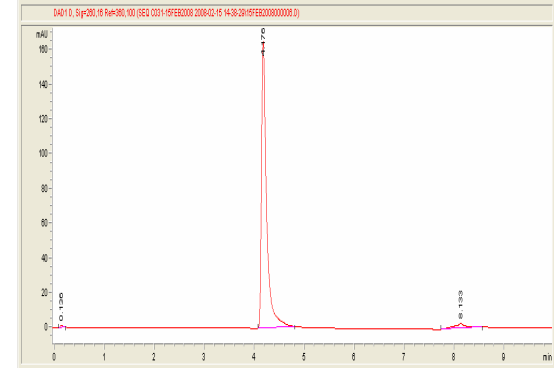
Towards standard platforms



VLPs
Pichia pastoris



Viral
HEK293

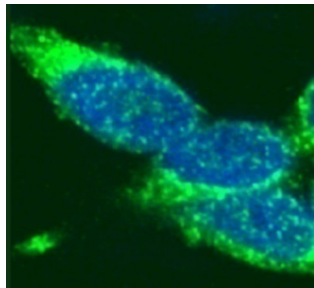


Purified Bulk Antigen

Disposables major role vaccine platform



- ❑ Disposable processing systems
 - ❑ Reduced contamination risk; rapid turnaround; less validation
- ❑ Stirred tank bioreactors where possible
 - ❑ Increased control of cell physiology during growth and production
 - ❑ Facilitate online (PAT) analytics (e.g. NIRS) for real time monitoring
- ❑ High recovery chromatographic methods for purification
 - ❑ Including disposable columns
- ❑ Established supply chain for global production

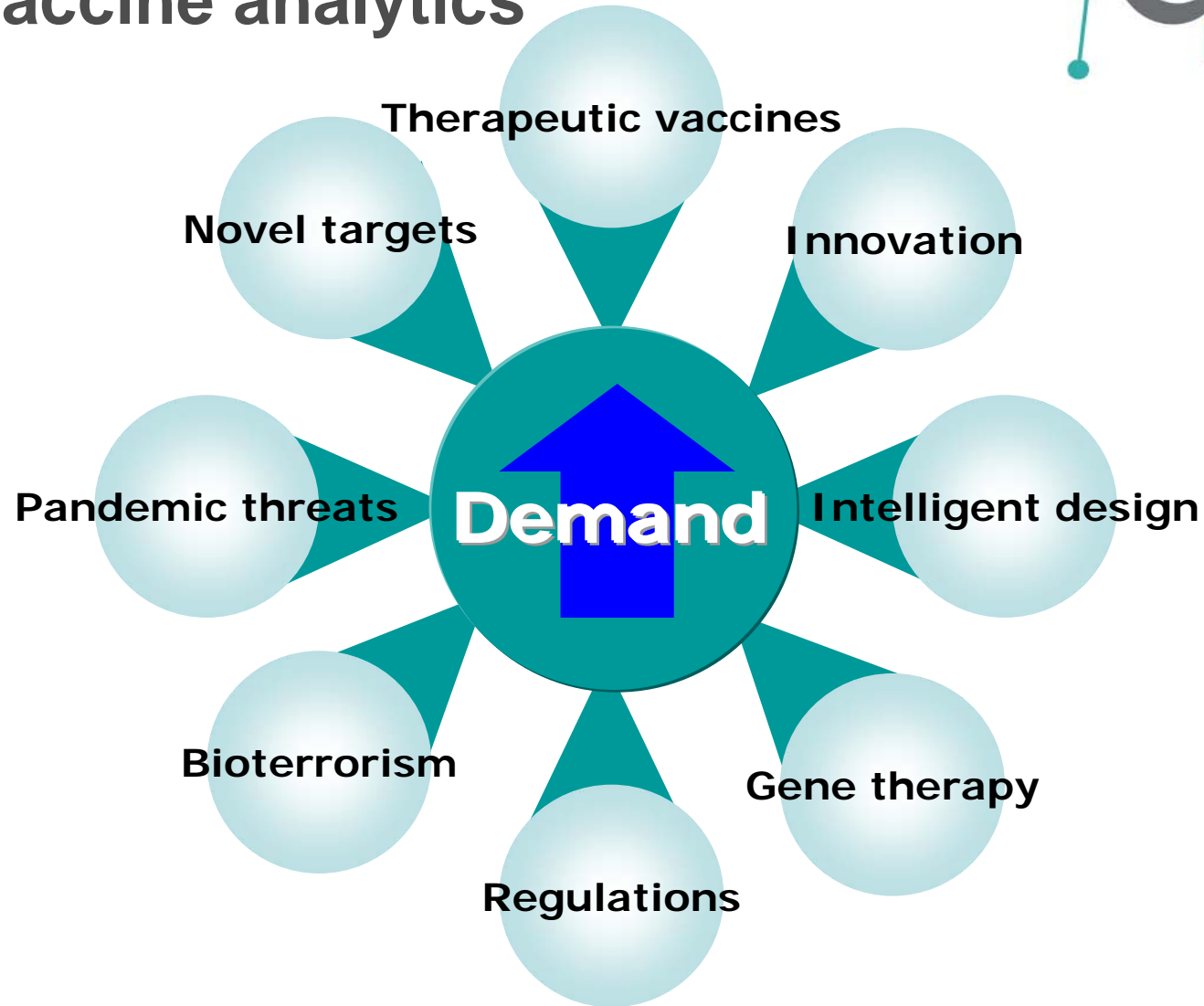


Courtesy Thermo Fisher

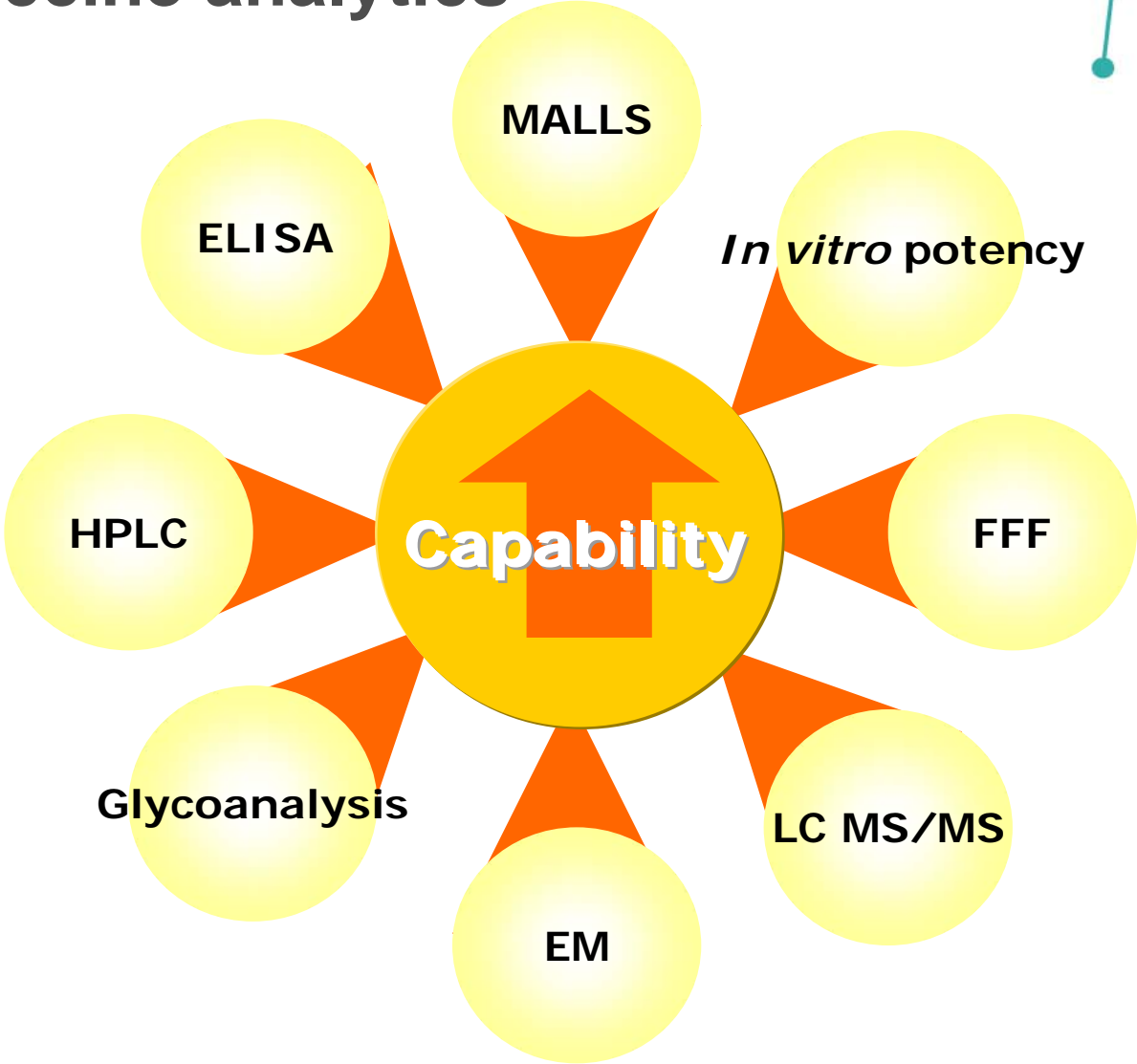


Courtesy GE Healthcare

Factors increasing demand for advanced vaccine analytics



Techniques increasing capability for advanced vaccine analytics



CASE STUDY 1: Process design and control

– Adenoviral vector



- Effective process development requires high quality analytics
- Currently available analytical techniques are frequently unreliable and/or slow:
 - Plaque assays (IU/mL)
 - Time consuming (> 3 days)
 - OD₂₆₀ (TP/mL)
 - Can only be applied on purified product
 - Assay variability
 - Anion Exchange-High Performance Liquid Chromatography

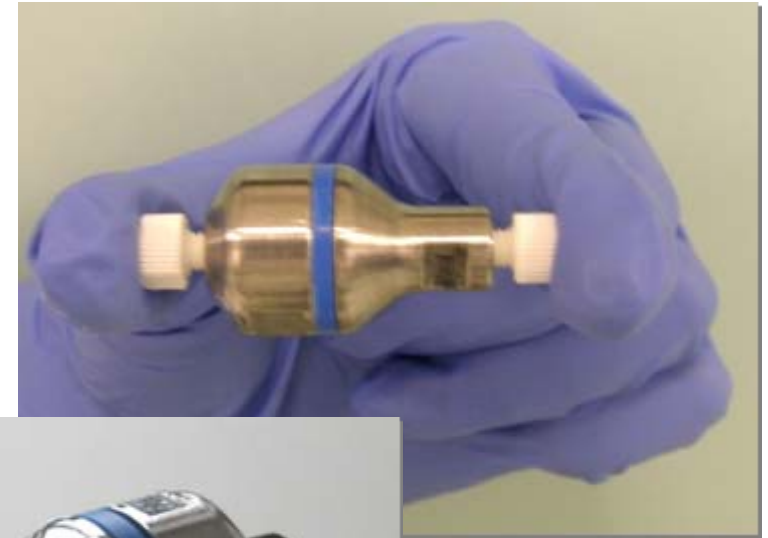
CASE STUDY 1: Process design and control

– Adenoviral vector



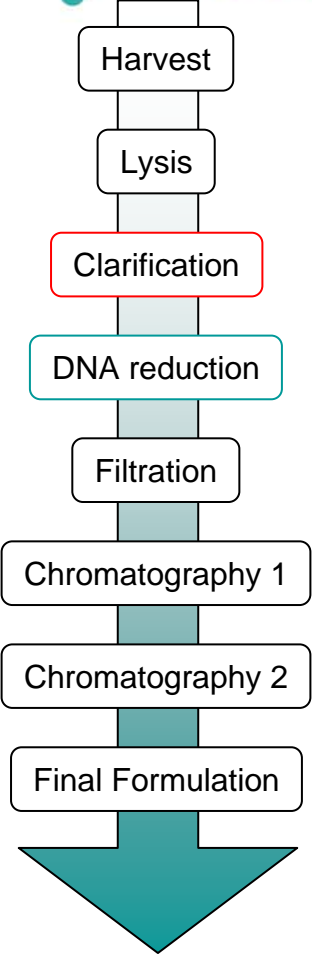
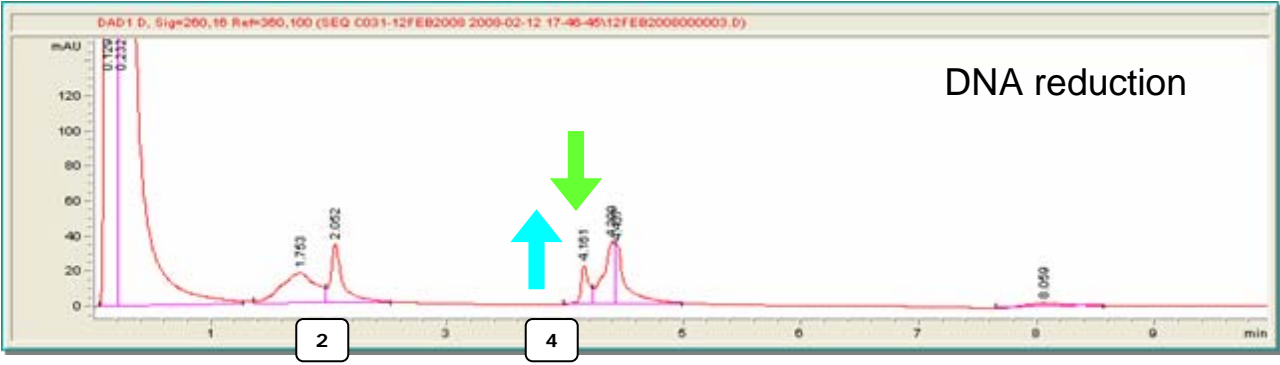
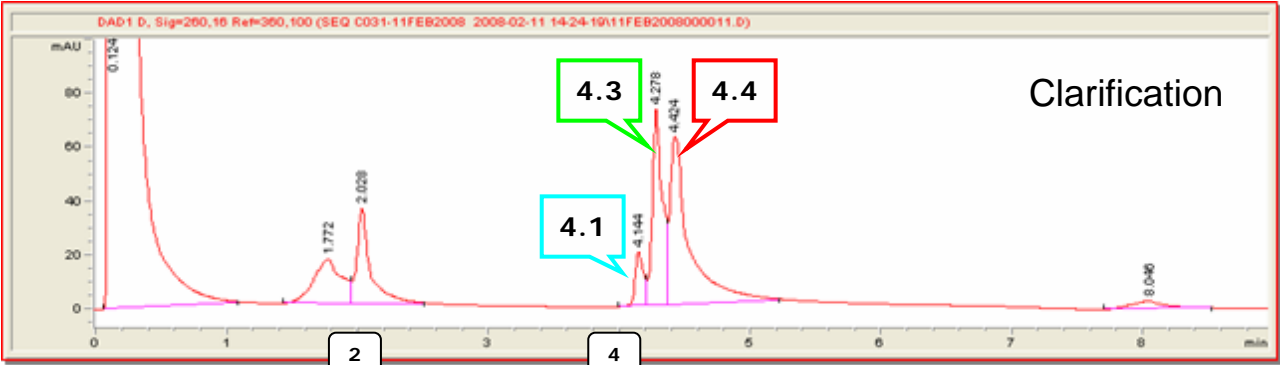
CIM[®] QA Biomonolith HPLC column

- ❑ Column size = 76 μ L
- ❑ Max pressure = 150 bar
- ❑ Column temperature = 22°C
- ❑ Injection volume = 25 μ L
- ❑ Flow rate = 1 mL/min

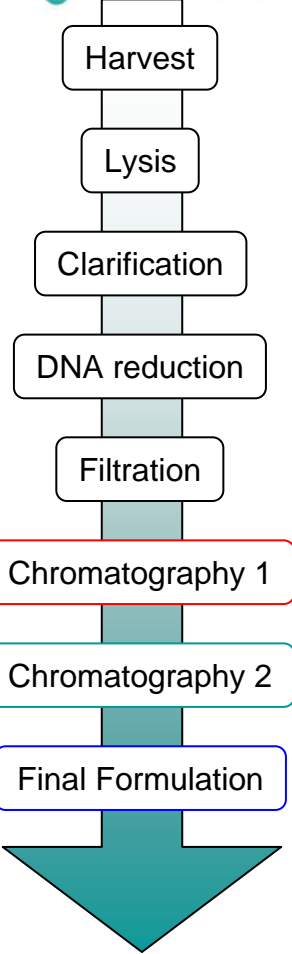
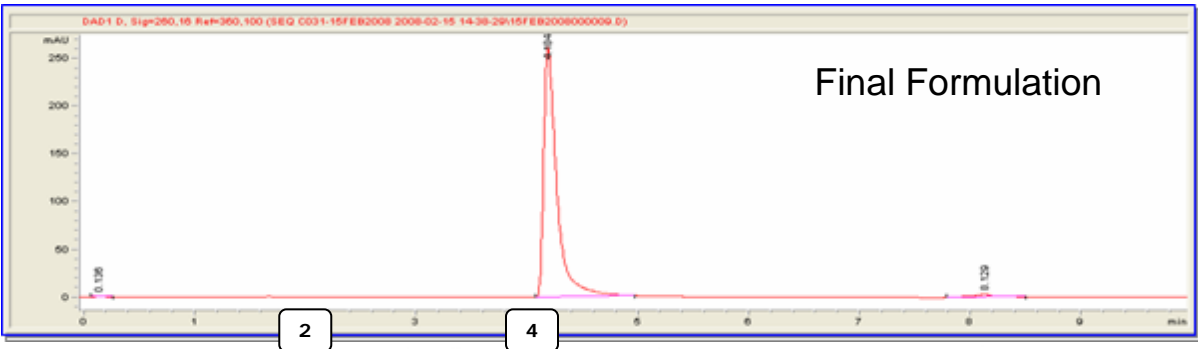
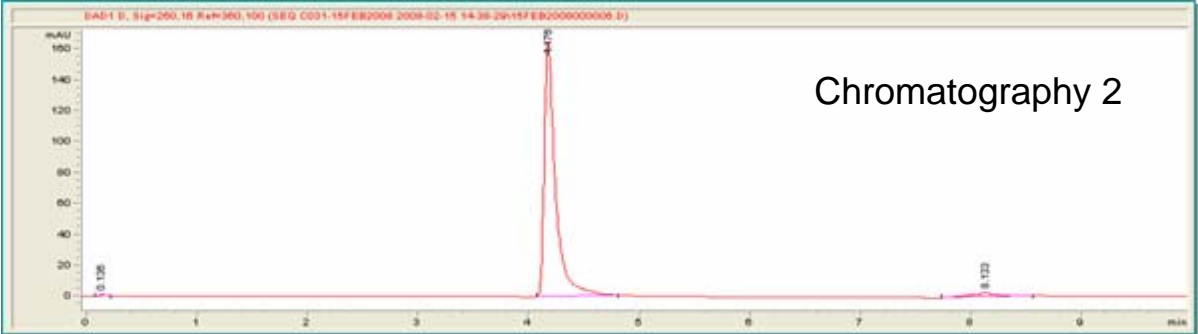
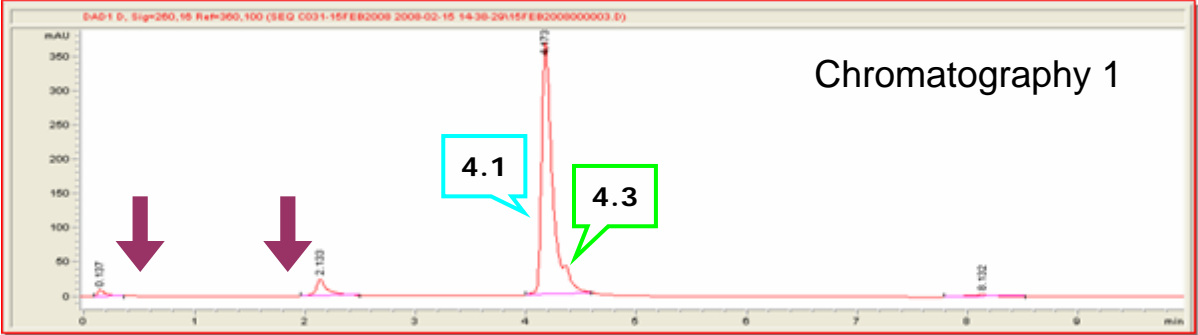


CASE STUDY 1: Process design and control

– Adenoviral vector



CASE STUDY 1: Process design and control – Adenoviral vector



CASE STUDY 1: Process design and control

– Adenoviral vector



Conclusions

- CIM® QA HPLC allows rapid analysis of adenovirus particles
 - Can be used to analyse crude lysate and purified preparations
 - Potential for application in absolute quantification
- Gradient method has been developed to separate adenovirus particles from host-cell proteins and particle fragments
- Method and column performance is consistent and robust
- Invaluable in the development and exemplification of a adenovirus purification platform
- Work accepted for publication
 - Whitfield RJ et al., J Chromatography A. 2009

CASE STUDY 2: Impurity characterisation by MS



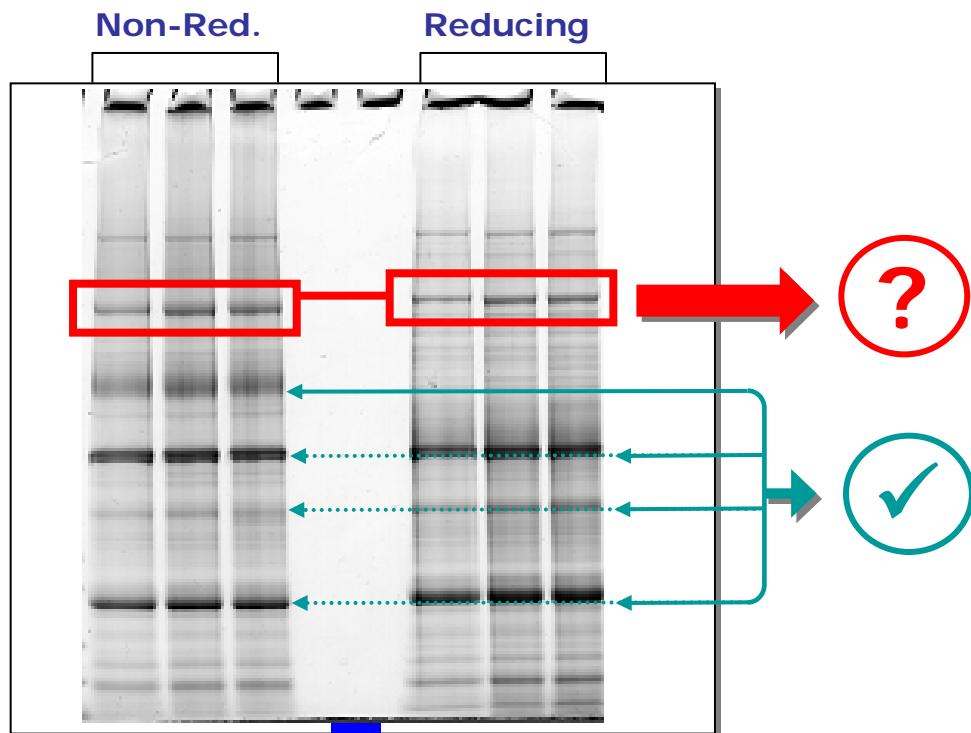
Introduction

- One release test for this product was SDS PAGE.
- A previously unencountered protein band (122.6 kDa) was detected
- Identifying unknown proteins is difficult by traditional techniques.
- Proteomics techniques were applied to identify this contaminant.
- Robotic gel excision and in-gel tryptic digestion performed, followed by MALDI-ToF MS.
- The identity of this protein, and proteins contained within other bands, was confirmed.

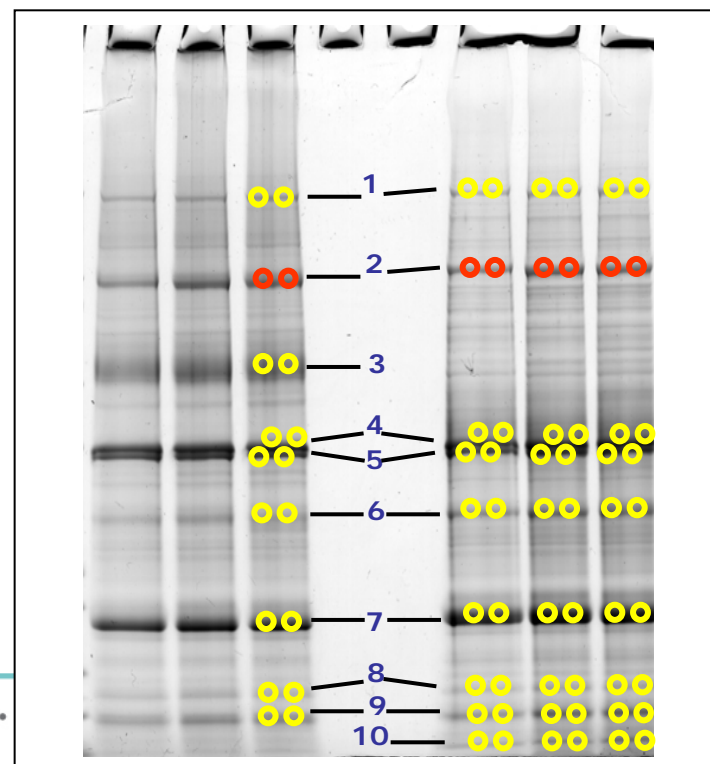
CASE STUDY 2: Impurity characterisation by MS



STAGE 1: SDS PAGE



STAGE 2: GEL PLUG EXCISION

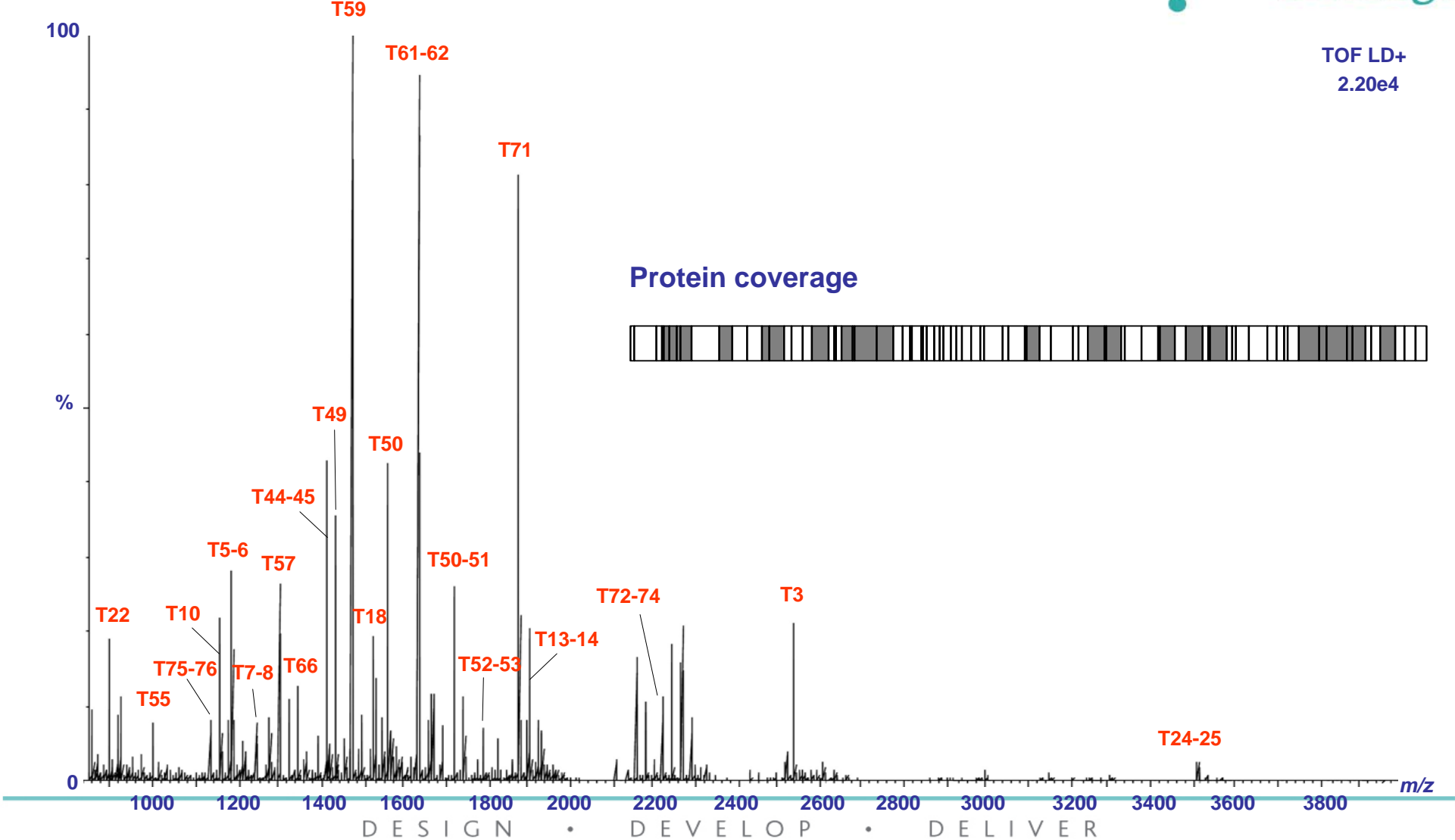


CASE STUDY 2: Impurity characterisation by MS

STAGE 3: MALDI-ToF OF TRYPTIC DIGESTS



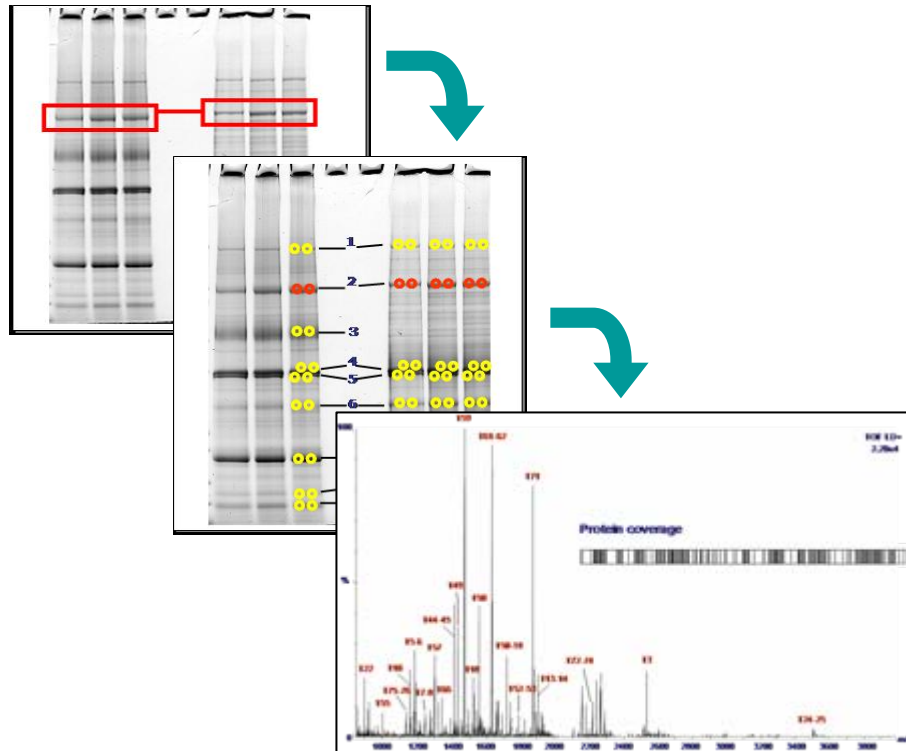
TOF LD+
2.20e4



CASE STUDY 2: Impurity characterisation by MS



STAGE 4: DATABASE SEARCHING



Mascot: Sequence Query

Your name: Email:

Search title:

Database: MSDB

Taxonomy: All entries

Enzyme: Trypsin Allow up to: 1 missed cleavages

Fixed modifications: Carbamidomethyl (C), Carbamyl (K), Carbamyl (N-term), Carboxymethyl (C), Deamidation (NQ)

Variable modifications: Biotin (K), Biotin (N-term), Carbamidomethyl (C), Carbamyl (K), Carbamyl (N-term)

protein mass: kDa ICAT:

peptide tol. ±: 50 ppm MS/MS tol. ±: 0.8 Da

peptide charge: 1+ Monoisotopic: Average:

Query: 1085.640, 1114.610, 1134.599, 1150.559, 1163.626, 1311.675,

Instrument: Default

Overview: Report top: 5 hits

Start Search ... Reset Form

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CASE STUDY 2: Impurity characterisation by MS

STAGE 4: DATABASE SEARCHING



Mascot: Sequence Query

Your name: Email:

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Query: 1085.640, 1114.610, 1134.599, 1150.559, 1163.626, 1311.675

Instrument: Default

Overview Report top 5 hits

Start Search ... Reset Form

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Mascot Search Results

User:
 Email:
 Search title:
 Database: MSDB 20030707 (1206286 sequences; 383022796 residues)
 Timestamp: 17 Jul 2003 at 16:59:44 GMT
 Top Score:

Probability Based Mowse Score

Score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event. Protein scores greater than 73 are significant ($p < 0.05$).

Concise Protein Summary Report

[Switch to full Protein Summary Report](#)

To create a bookmark for this report, right click this link: [Concise Summary Report \(.data/20030717/FTTtGGet.dat\)](#)

Re-Search All Search Unmatched

- [VJCH2](#) Mass: 204809 Total score: **121** Peptides matched: 15
 vitellogenin II precursor [validated] - chicken

[CAA31942](#) Mass: 204679 Total score: **120** Peptides matched: 15
 GGVITIIG NID: - Gallus gallus

[Q9CU59](#) Mass: 24156 Total score: 60 Peptides matched: 5
 Adult male thymus cDNA, RIKEN full-length enriched library, clone:5830450E06, full length

PROTEIN ID



VJCH2	Mass: 204809	Total score: 121	Peptides matched: 15
CAA31942	Mass: 204679	Total score: 120	Peptides matched: 15
GGVITIIG NID: - Gallus gallus			
Q9CU59	Mass: 24156	Total score: 60	Peptides matched: 5
Adult male thymus cDNA, RIKEN full-length enriched library, clone:5830450E06, full length			



CASE STUDY 2: Impurity characterisation by MS



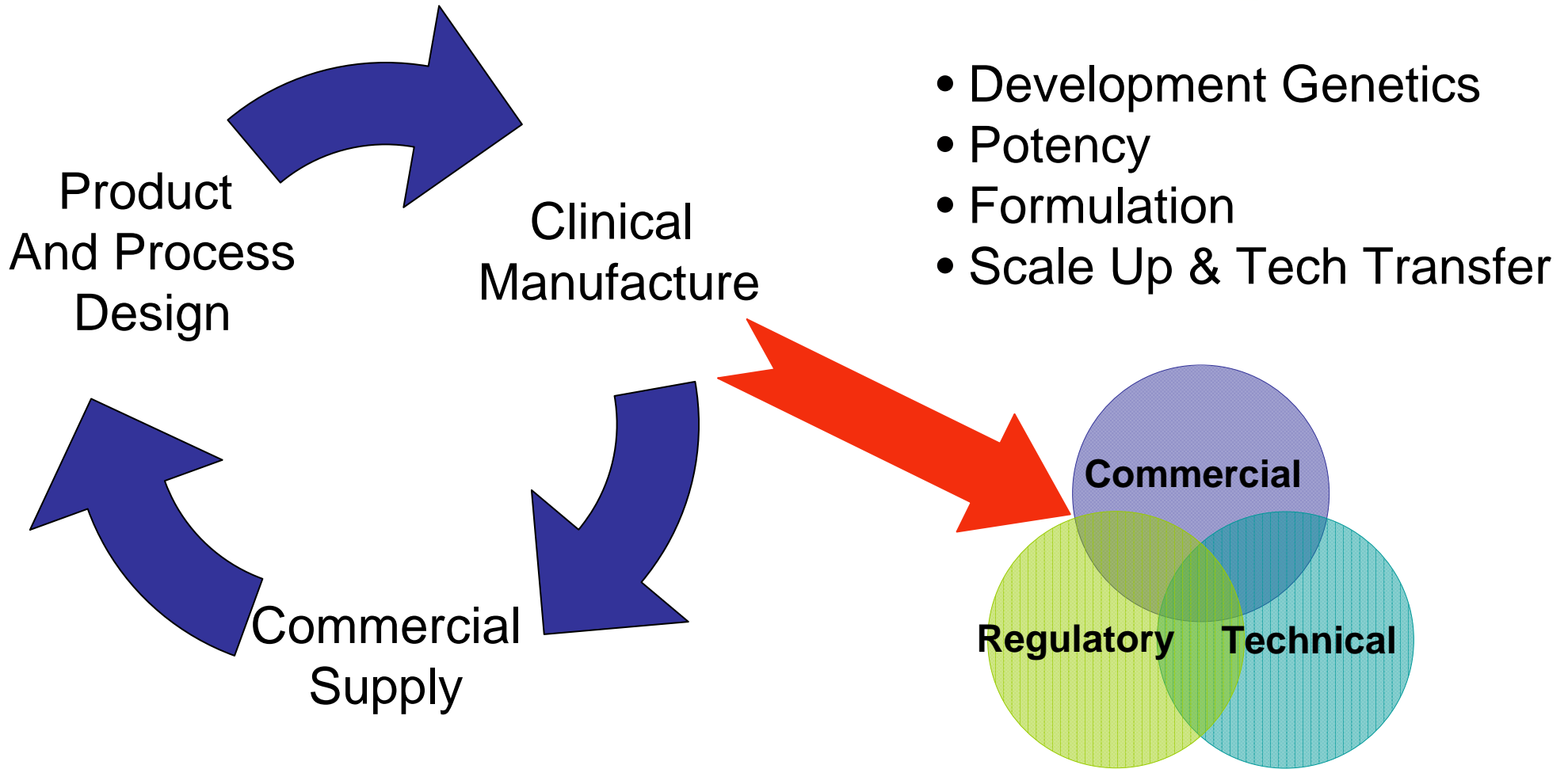
RESULT: Contaminant successfully identified

- Contaminant = harmless protein, in-process artefact
- Process slightly modified, protein band disappeared
- Subsequent process changes could be easily assessed

Presentation Outline

- ❑ Vaccine manufacturing technical regulatory challenges are greater than MAb's.
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 - ❑ Leveraging new analytical methods
 - ❑ CMC packages suitable for global registration

Risk Reduction During Development



Regulatory Intelligence



From: Donna.2.Boyce@gsk.com
[mailto:Donna.2.Boyce@gsk.com]
Sent: Monday, March 31, 2008 11:58 AM
To: Henchal, Laraine
Subject: welcome back

Hi,
Hope you had a nice holiday..do you have any updates regarding the potency spec for me?
best regards,
Donna

From: Henchal, Laraine
Sent: Monday, March 31, 2008 1:37 PM
To: 'Donna.2.Boyce@gsk.com'
Subject: Potency release spec

Yes, looks like we will have release potency between ----- (including those values; so ----- at release). End-expiry OK at ---, but with a 24 month dating period for now. With the drop-off on the numbers at -- months, we would like to see further stability data -----, which can be done with supplement. 24 months may even be enough for you -----... So the release criteria will need to be reflected in an amended lot release protocol...I know that Joe Quander had some format questions for you earlier.

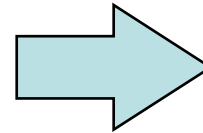
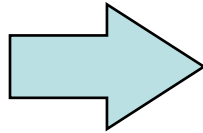
Laraine S. Henchal, MAS
Microbiologist Reviewer, Team Leader
Viral Vaccines Branch
Division of Vaccines and
Related Products Applications

End of Production Cells (EOP)

The genetic stability has been analysed for one full-scale fermentation for each type of end of production cells (culture purity, species identity, host strain identity, restriction endonuclease mapping, plasmid retention and DNA sequencing).

EPAR, Rev 8, 2008 Gardasil

Basics Ignored: *Development Genetics*



Vector construction, host strain source, raw materials, storage and stabilityfully documented!

Basics Ignored: *Regulatory Approval*



Proven quality of the biotechnological product

- ✓ a validated and reproducible PROCESS
- ✓ a characterised and consistent PRODUCT



pre-determined acceptance criteria

Regulatory Intelligence



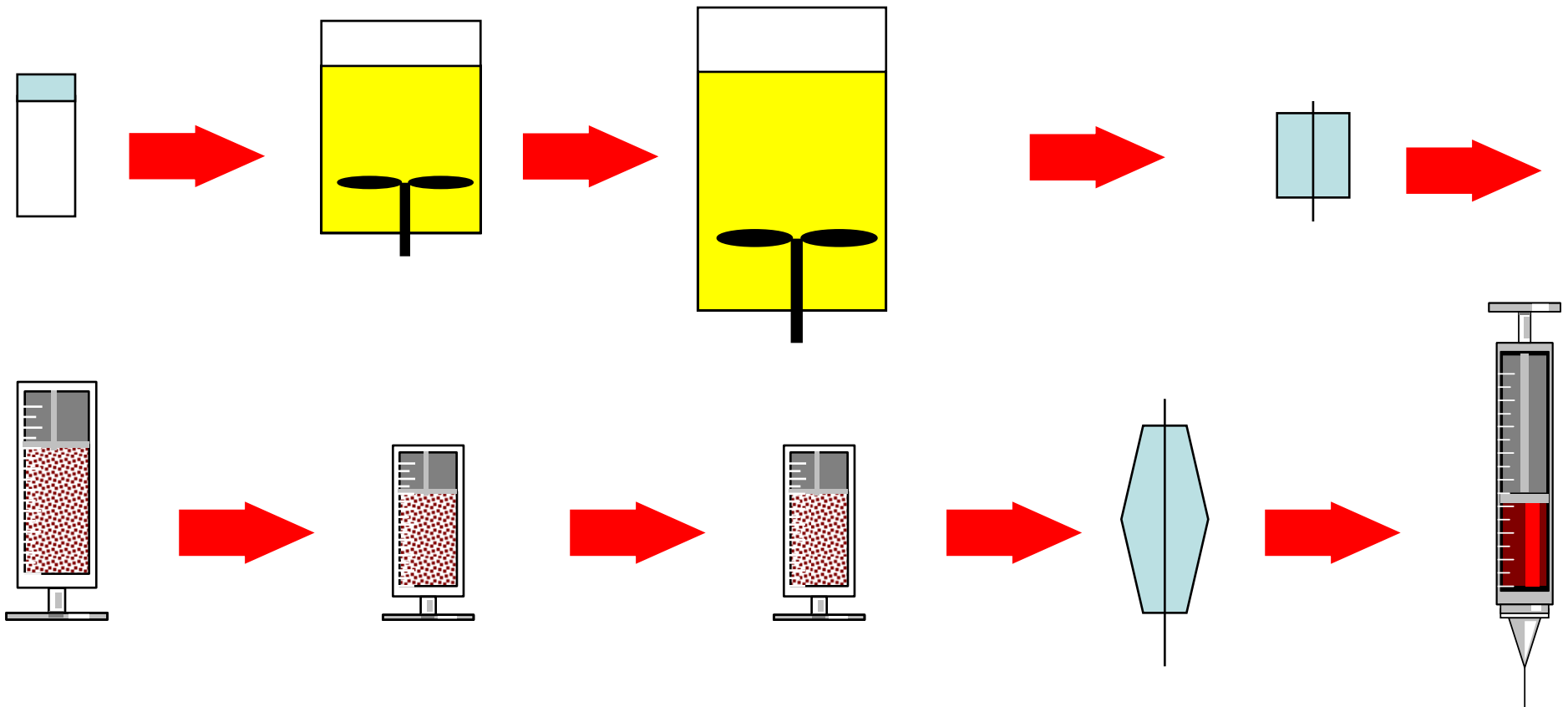
Brorson 98-0012

6/10/98

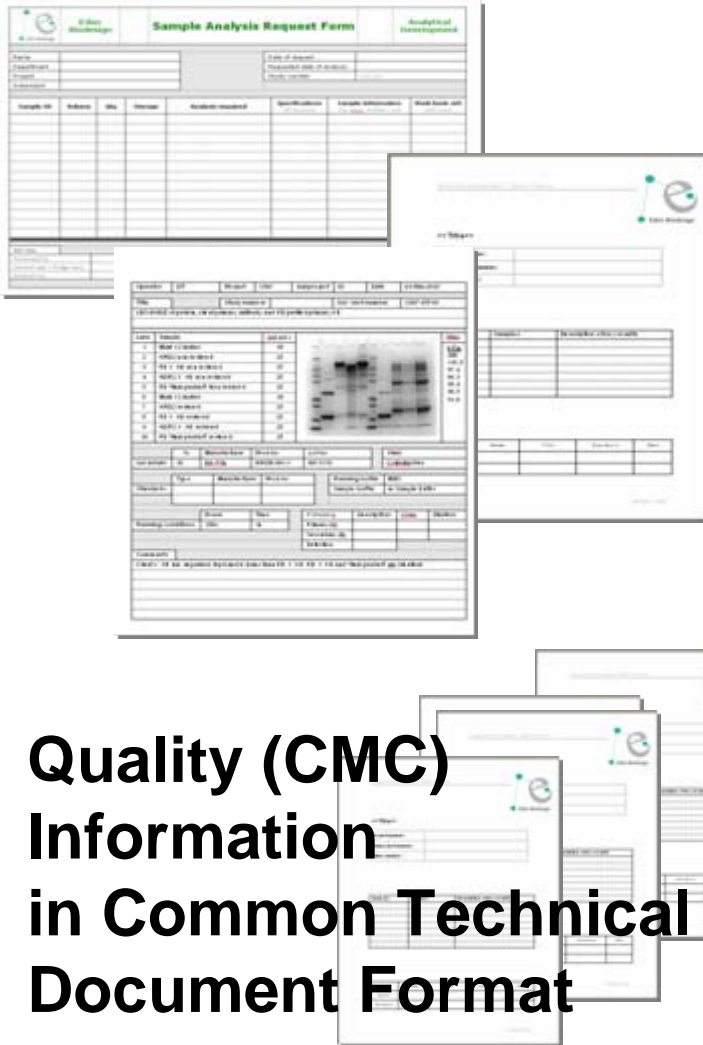
- assay was validated for linearity (60-130%), accuracy, repeatability, intermediate precision, range and specificity.
- SDS-PAGE gels are run reduced and non-reduced on 8-18% linear gradient gels. The gels were validated for repeatability, intermediate precision and LOD (0.02%)

**23 PAGES
DETERMINED NOT
TO BE RELEASABLE**

Basics Ignored: *Focus on antigen not finished product*



Knowledge Management



- Quality of Manufacturing throughout product life cycle
- ❑ Reference Standards
 - ❑ Qualified Assays
 - ❑ Structure conceptual process design

In conclusion



Vaccine manufacturing development still faces challenges that have been largely overcome for mAbs and other recombinant products

Opportunities:

- ❑ Multi-technology facilities based on design, containment, disposables and new regulatory flexibility
- ❑ Utilisation of new analytical methods to provide data to challenge the process = product orthodoxy
- ❑ Development and acceptance of more “platform like” production schemes for novel vaccine types
- ❑ Global vaccine development enhanced through focused knowledge management using technical summary reports as building blocks of value