Production and Purification of Virus like particle (VLP) based Vaccine

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Outline

Motivation

- VLP vaccine candidates have become quite popular of late
- VLP-based processes are, however, currently quite diverse
- We undertook an effort to standardize the process
- We used hepatitis C VLP as a model
- This presentation will explain the approach taken and present the results obtained

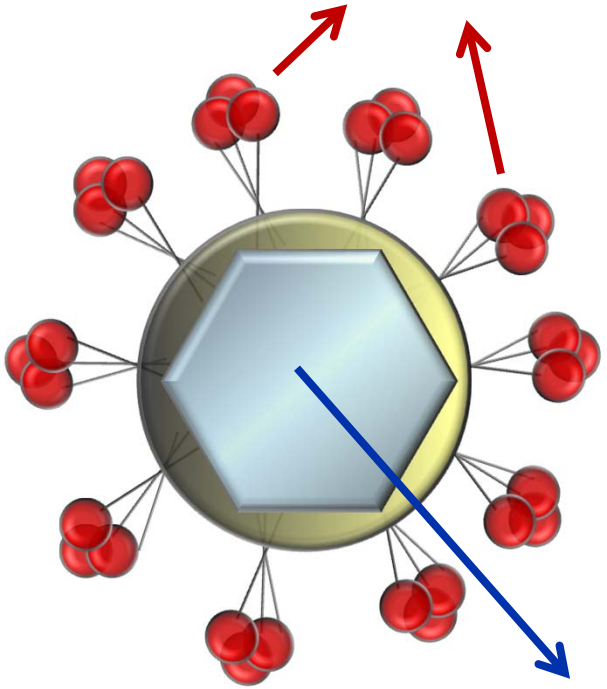
Why virus-like particles (VLPs)?

- Contain repetitive high-density displays of viral surface proteins that elicit strong T cell and B cell immune responses
- Non infectious because they do not contain genetic material, thus cannot replicate and are safer
- Their size (40-120 nm diameter) is optimal for uptake by dendritic cells
- Can be produced in a variety of cell culture systems
- Can self assemble *in vivo*
- Proven technology (Hepatitis B and Human Papilloma Virus vaccines)

Source: Roldão et al., *Expert Reviews in Vaccines* **9 (10)**, 1149-76 (2010)

VLPs for hepatitis C vaccine development

E1 and E2 glycoproteins from Hep C virus



Capsid and structure VLP
from retrovirus (murine leukemia virus)

Hepatitis C

- 170 million people infected
- Cirrhosis, liver cancer, death
- Current therapies only partially effective, costly and poorly tolerated
- No vaccine currently exists

Insect cell / baculovirus VLP production platform

Recombinant baculovirus (BV) is used to infect insect cells

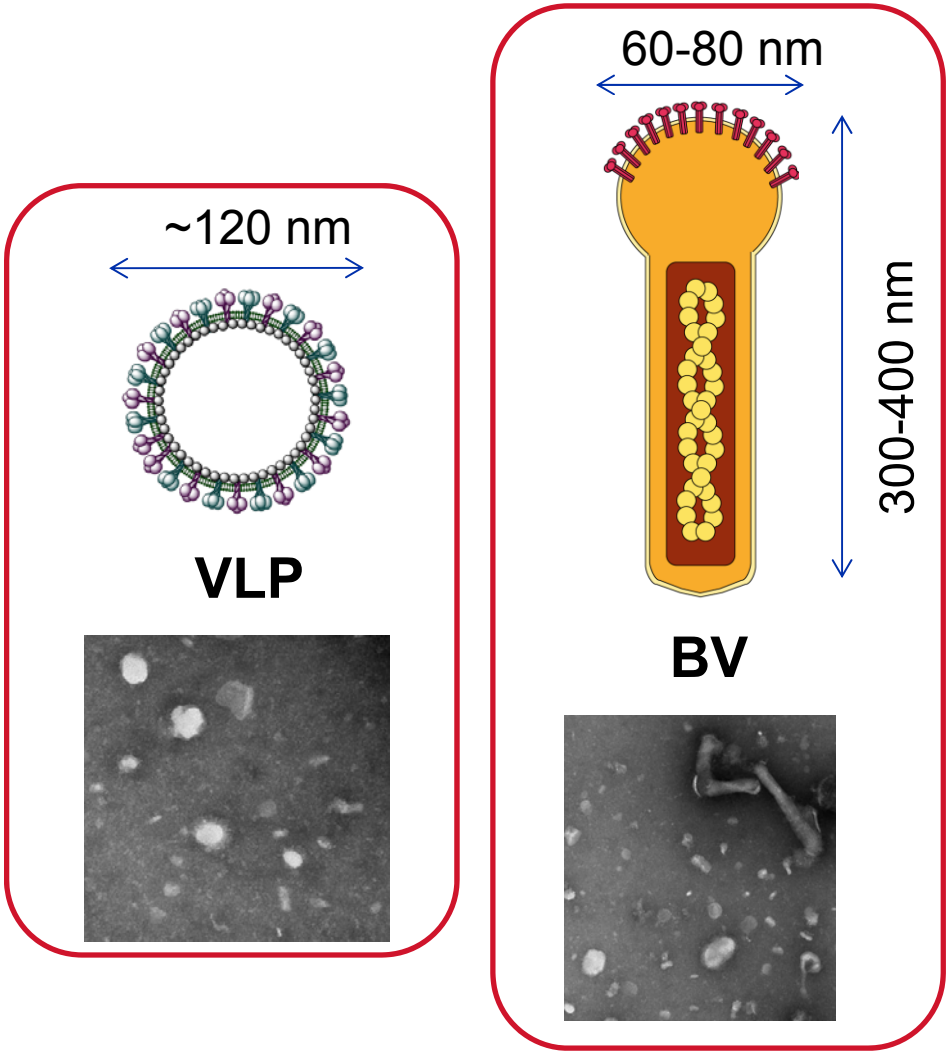
Key features

Transient production

High cell densities

Regulatory acceptance

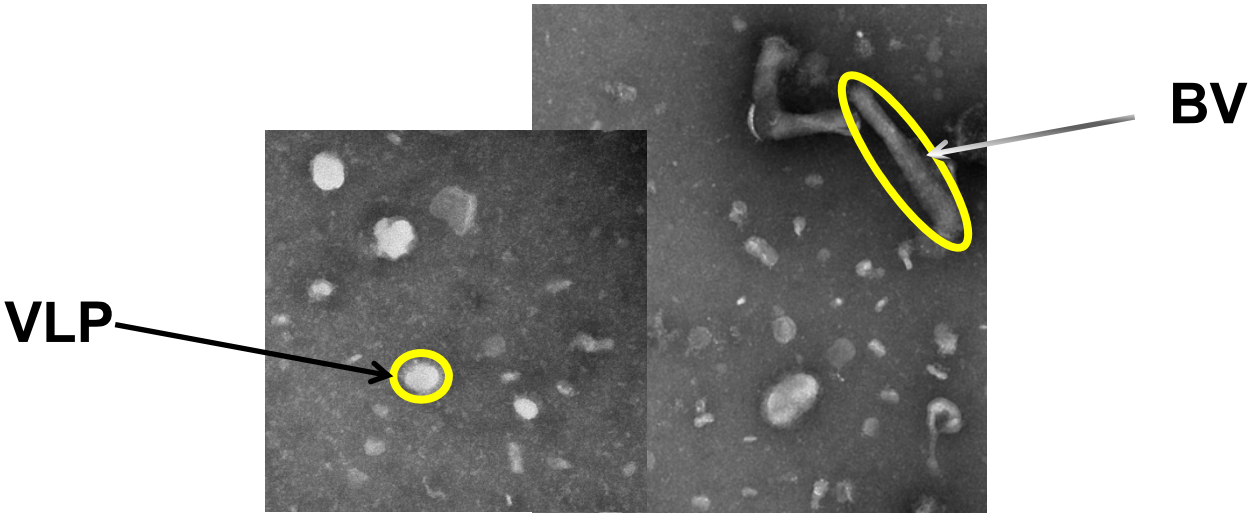
- Cervarix® (GSK)
- Flublok® (Protein Sciences)
- Several late-stage clinicals



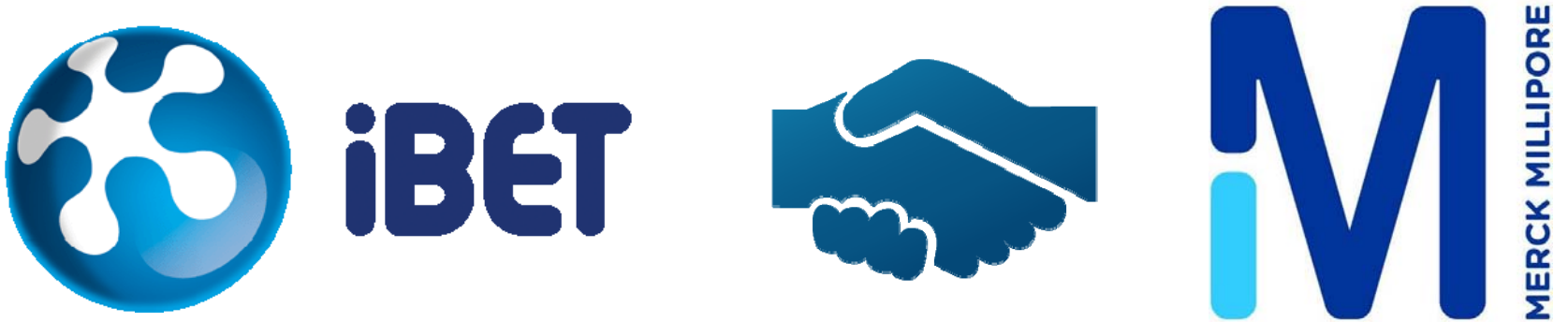
Source: Mena et al., *Expert Reviews in Vaccines* **10 (1)**, 1063-81 (2011)

Challenges in VLP vaccine production

- Low production yields
- Stability of enveloped VLPs
- Difficulties in baculovirus (BV) removal lowers recovery
- No established platform processes for purification



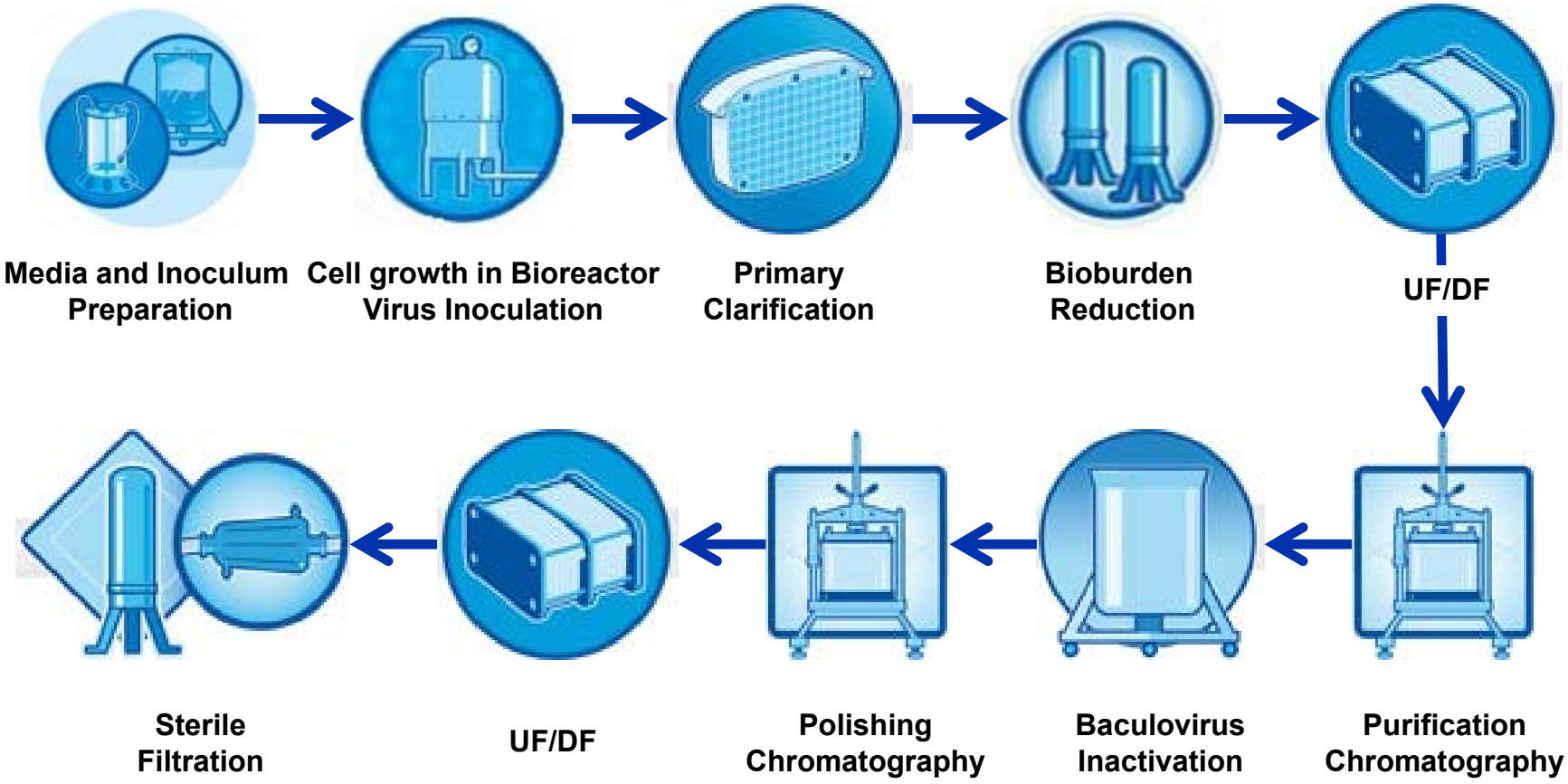
Work carried out in collaboration with iBET



iBET: Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

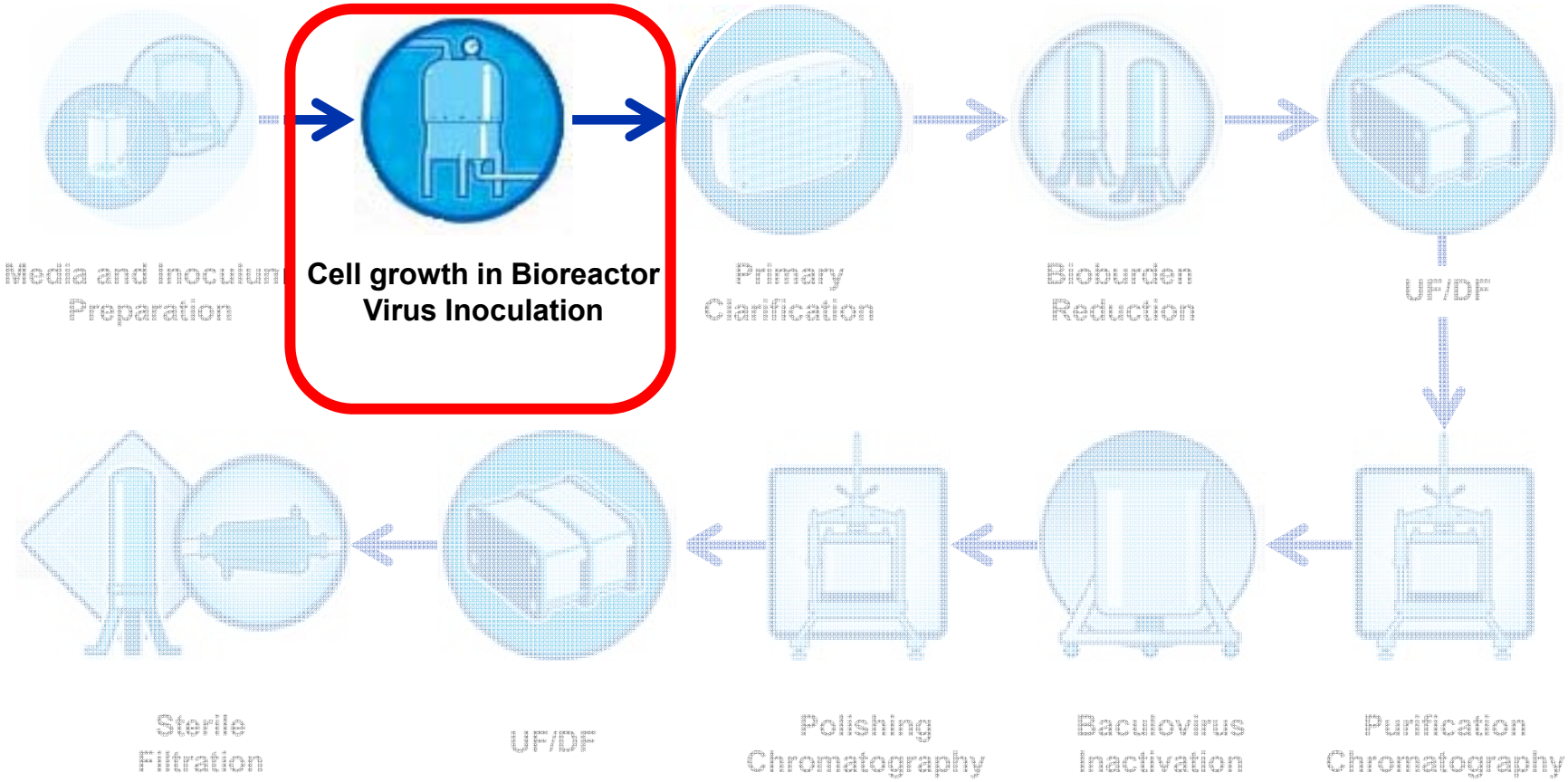
Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform



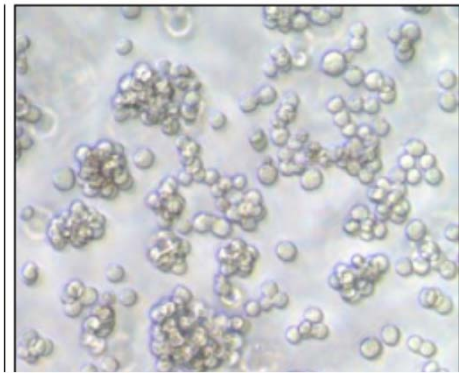
Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform



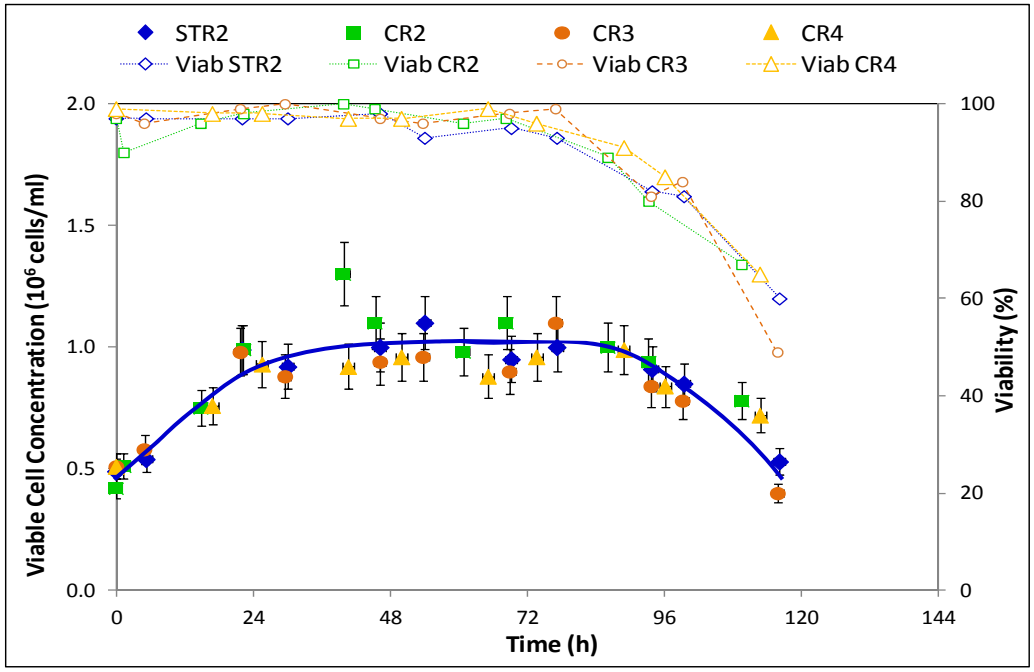
Insect cell culture

- Cell culture was carried out in stirred tank glass bioreactor and disposable bioreactor (Mobius® 3L bioreactor)
- Sf9 insect cells and Sf900II cell culture media were used in the process
- Mobius® 3L bioreactor was first operated at same conditions previously used for stirred tank glass bioreactors
  - Cell aggregation
  - Formation of foam
  - Longer lag phase
  - Lower viable cell concentration

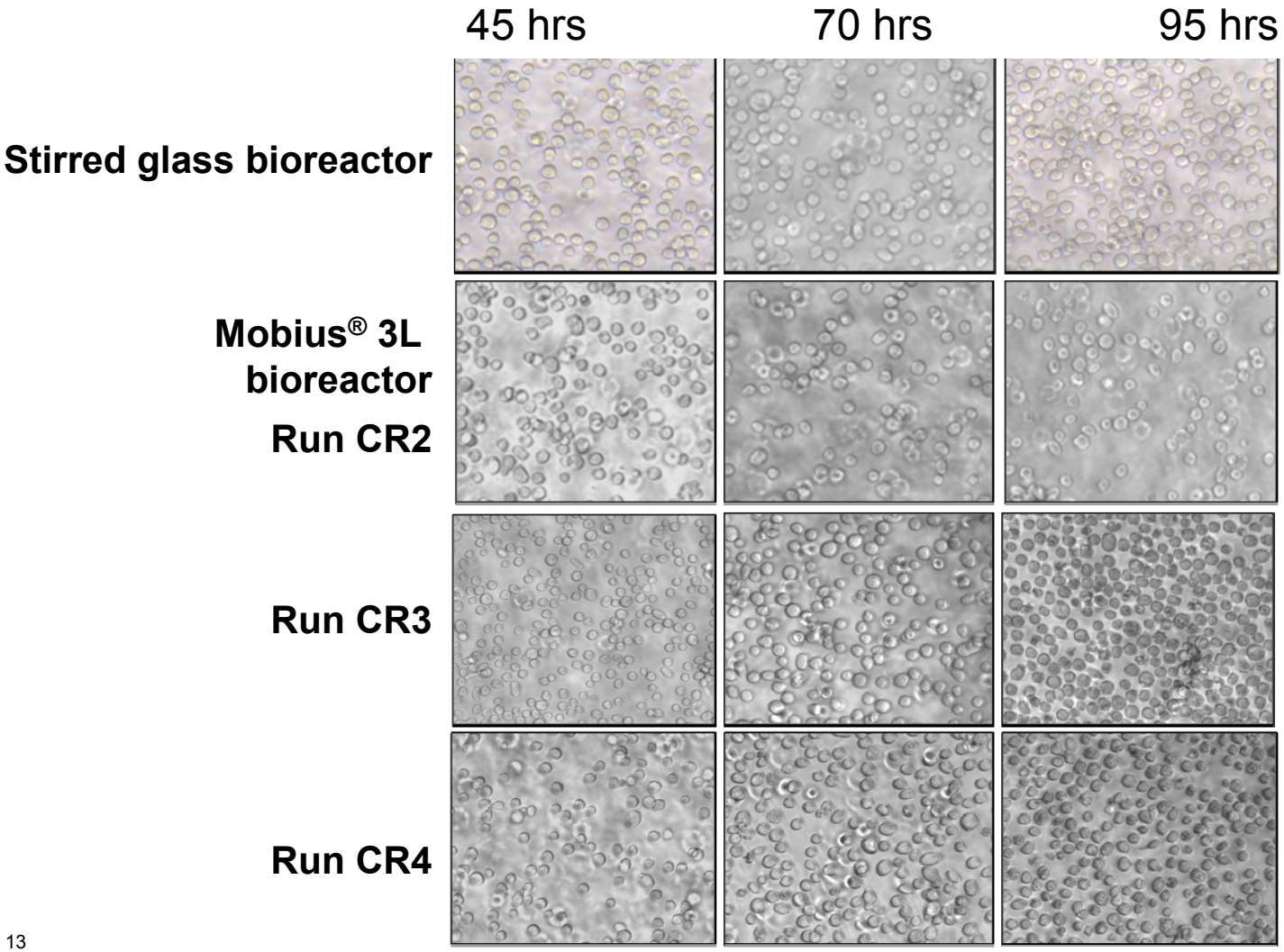


Insect cell culture conditions improved based on experience with Mobius® bioreactor

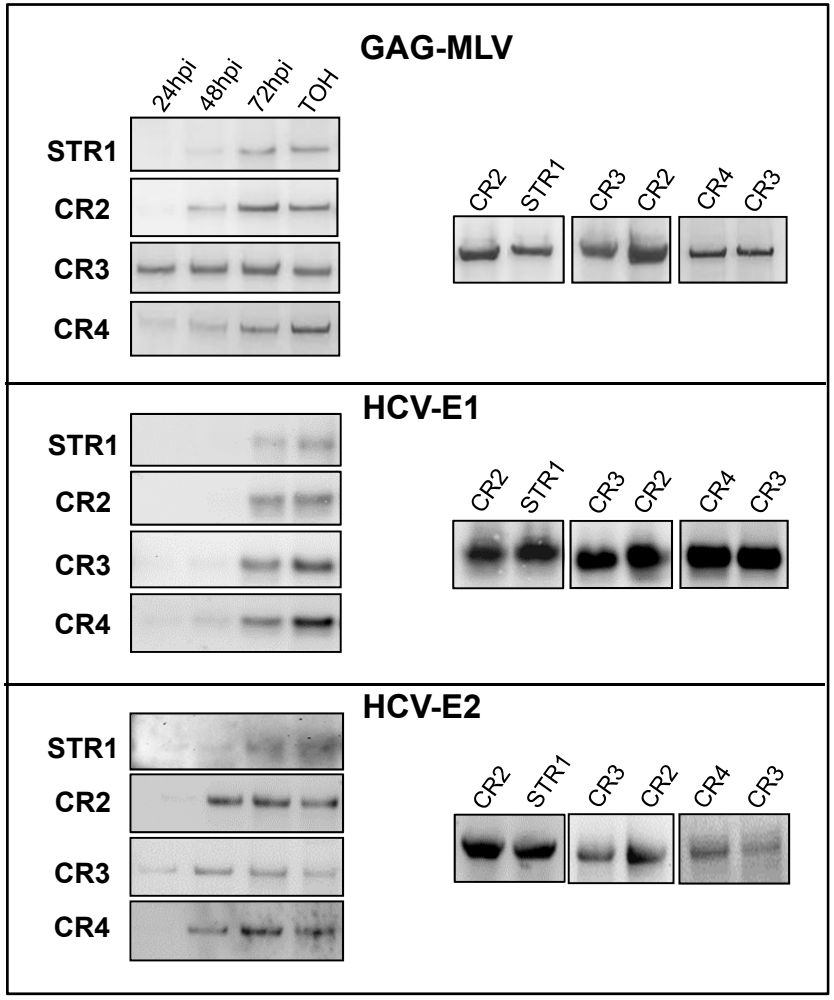
- Increased agitation rate
- Increased cell density of inoculation
- Replaced micro sparger with an open-pipe sparger

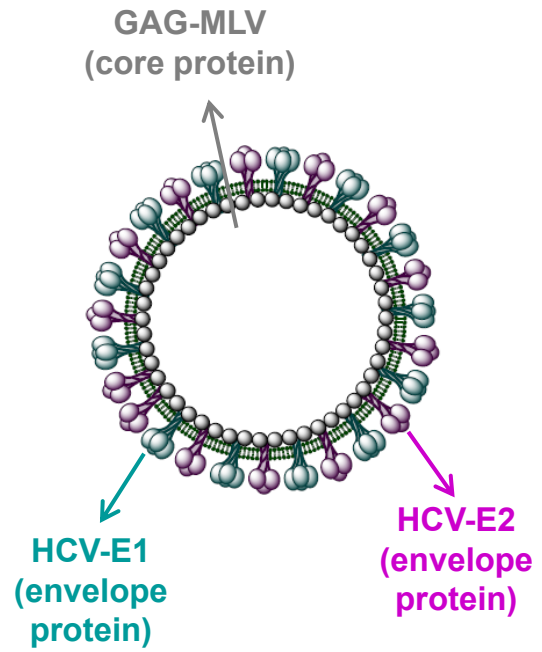


Microscopic evaluation of cells



Western blot analysis of VLPs using three markers





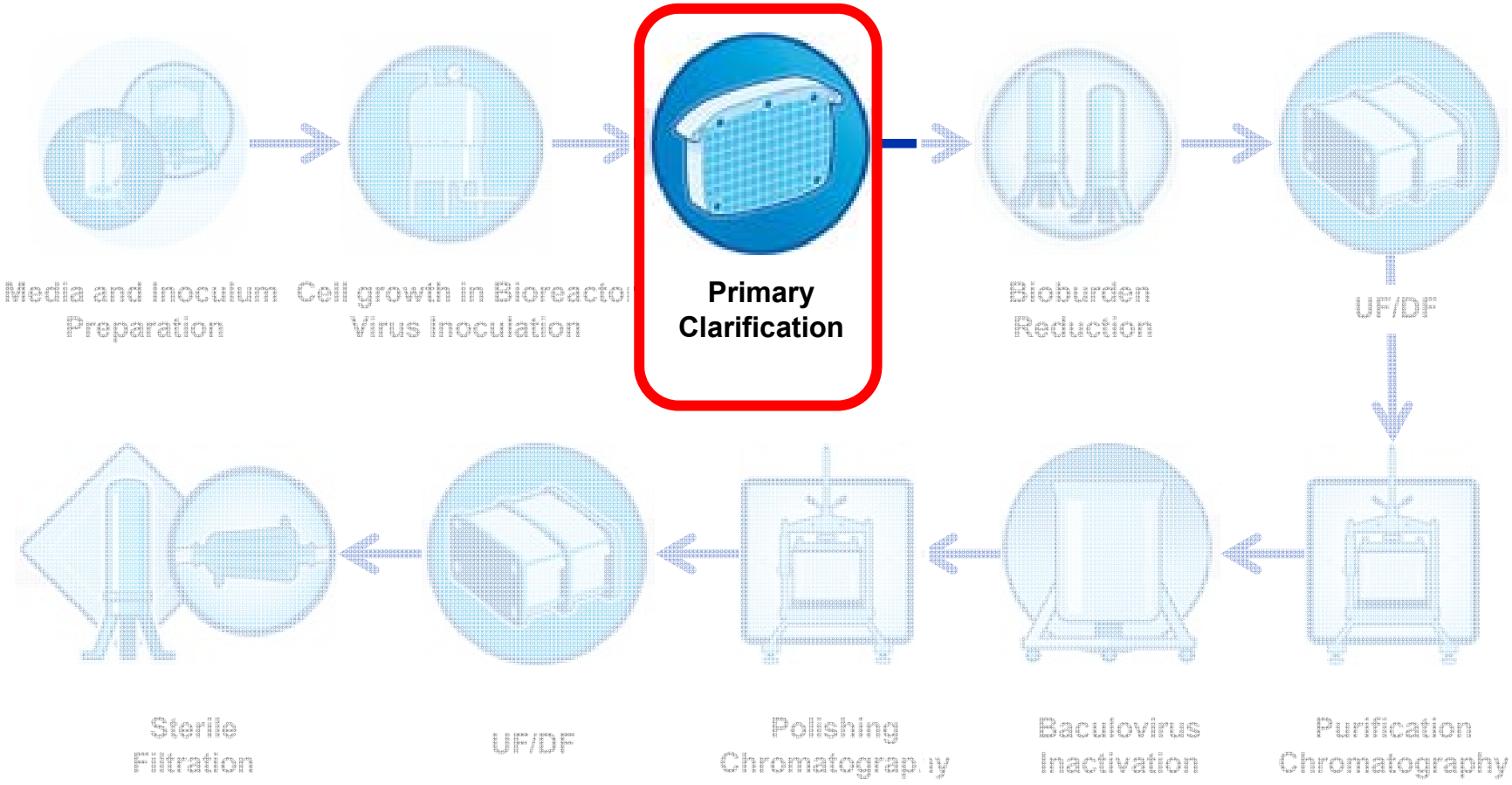
VLP
HepC V

Successful use of Mobius® bioreactor for VLP production

- Successful growth of Sf9 insect cells and infection with baculovirus for production of VLP vaccine using Mobius® 3L disposable bioreactor
- Comparable cell and VLP properties between disposable and glass bioreactors
- Reproducible performance of the disposable bioreactor was seen with identical results for three separate cell culture runs

Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform



Clarification

Centrifugation

- Lab models used early on
- Well suited for large-scale production
- High capital expense
- Shear

Depth filtration

- Well suited for smaller vaccine batches
- Easier to scale
- Lower cost
- Disposable
- Gentle treatment
- Simpler process development
- Wide choice of depth filters

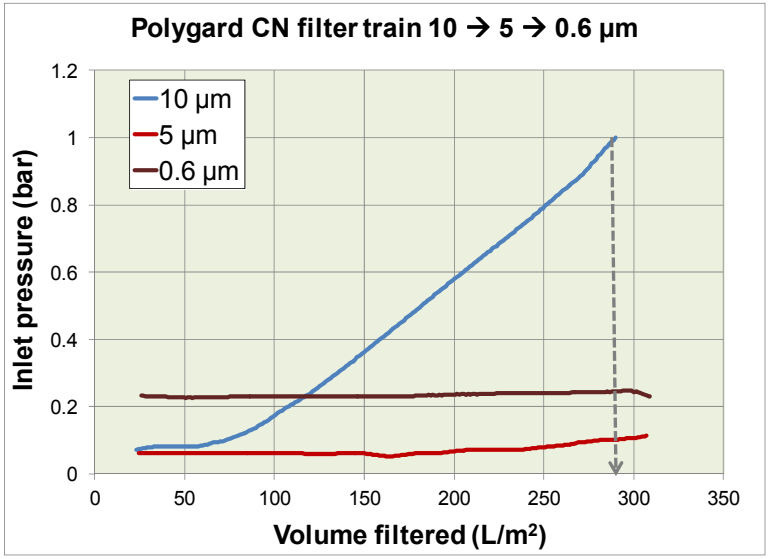
Clarification: throughput data

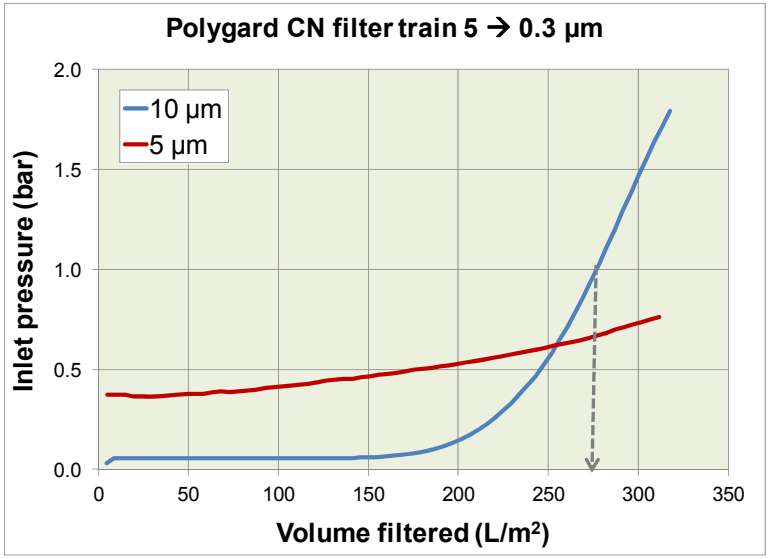
Disposable capsule filters

Polygard® CN, nominal pore sizes of 10, 5, 0.6 and 0.3 µm

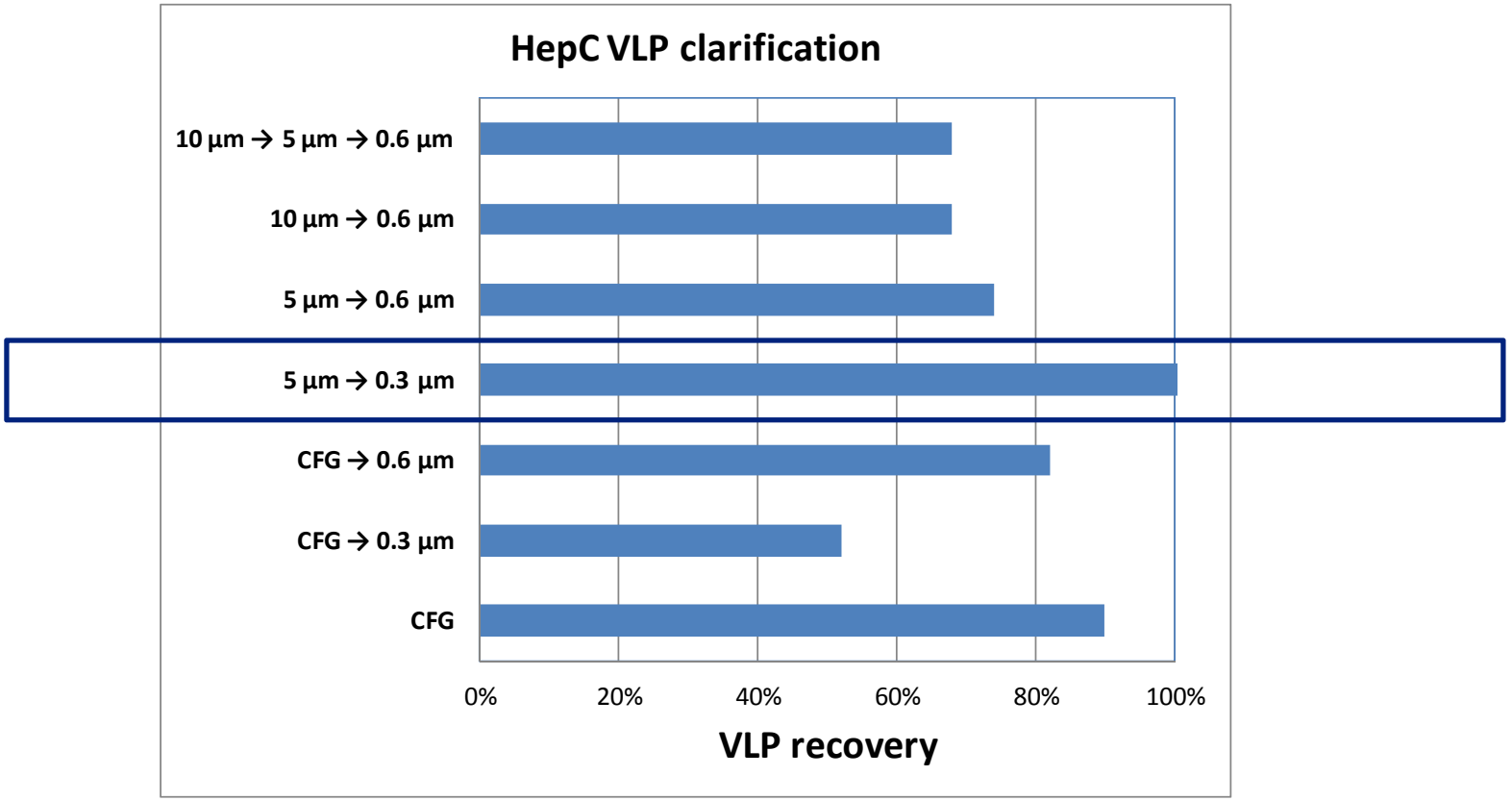
Pleated, all-polypropylene depth filters

Filter area: 17 cm^2; Inlet flux: 988 LMH





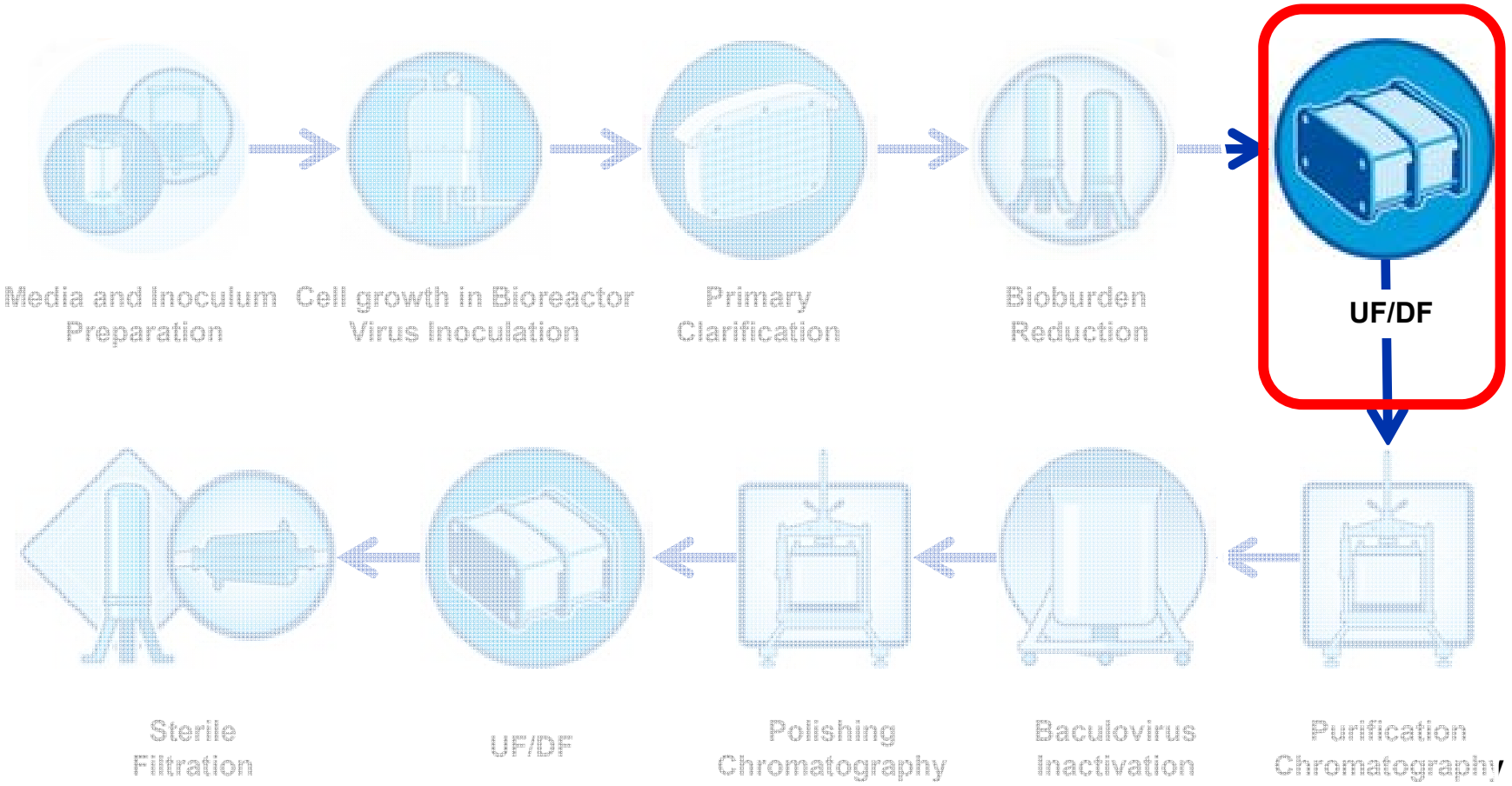
Clarification: recovery data



Unlike centrifugation, depth filtration resulted in ~70% DNA clearance

Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform



Concentration of clarified VLP harvest

Pellicon® cassettes

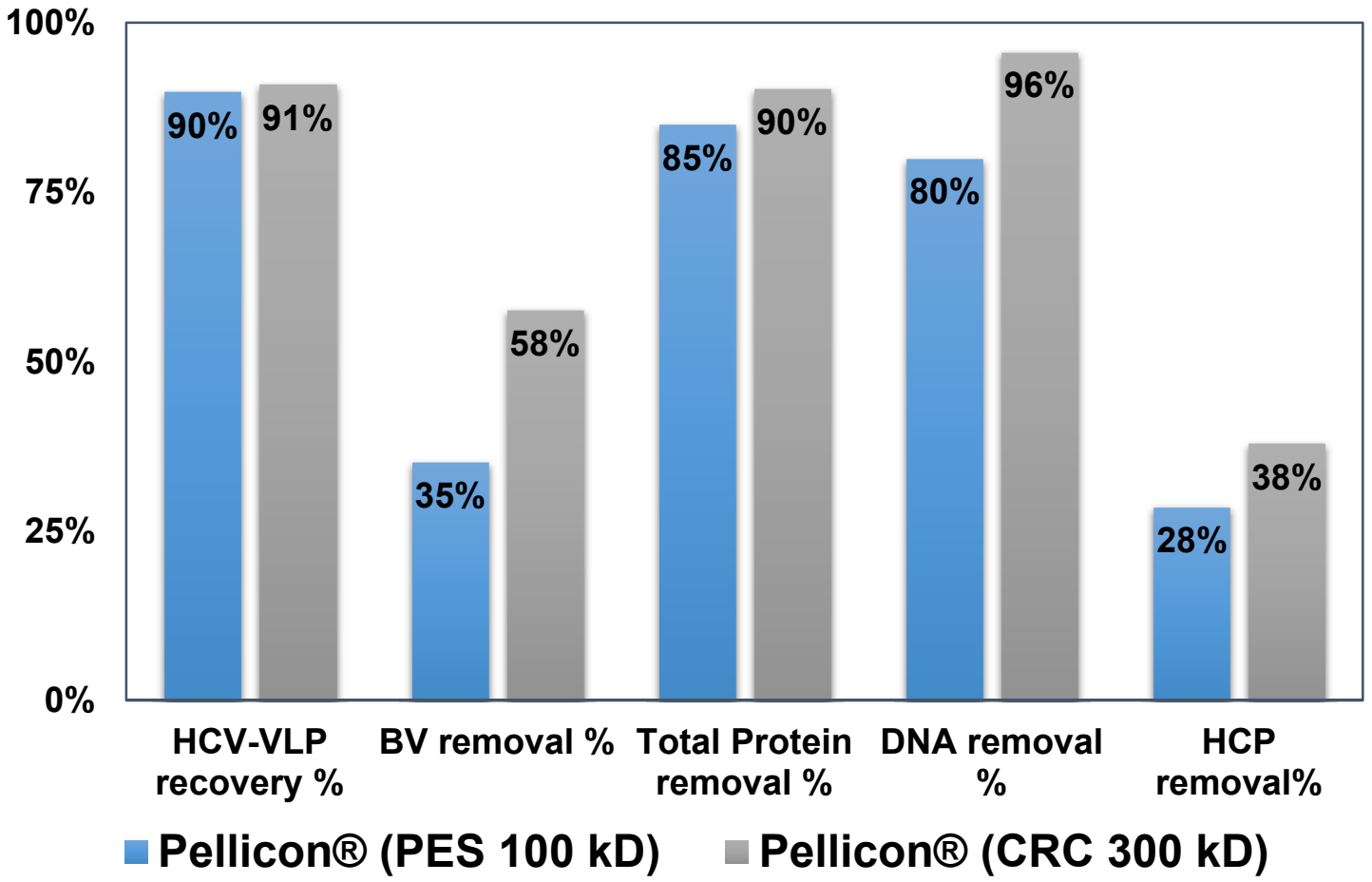
Two different ultrafiltration membranes

- 300 kD composite regenerated cellulose (Ultracel® membrane, “CRC”)
- 100 kD polyethersulfone (Biomax® membrane, “PES”)

Similar process conditions employed

- 4-5x concentration factor
- Loading: 72 L/m^2; Feed flux: 480 LMH; TMP: 1 bar; P_{feed}: 0.6-0.9 bar; P_{retent}: 1.1-1.4 bar

Concentration of clarified VLP harvest – results



Both membranes were fully retentive of the VLP

Polygard® CN depth filters and Pellicon® cassettes with Ultracel® membrane offered best results

Clarification

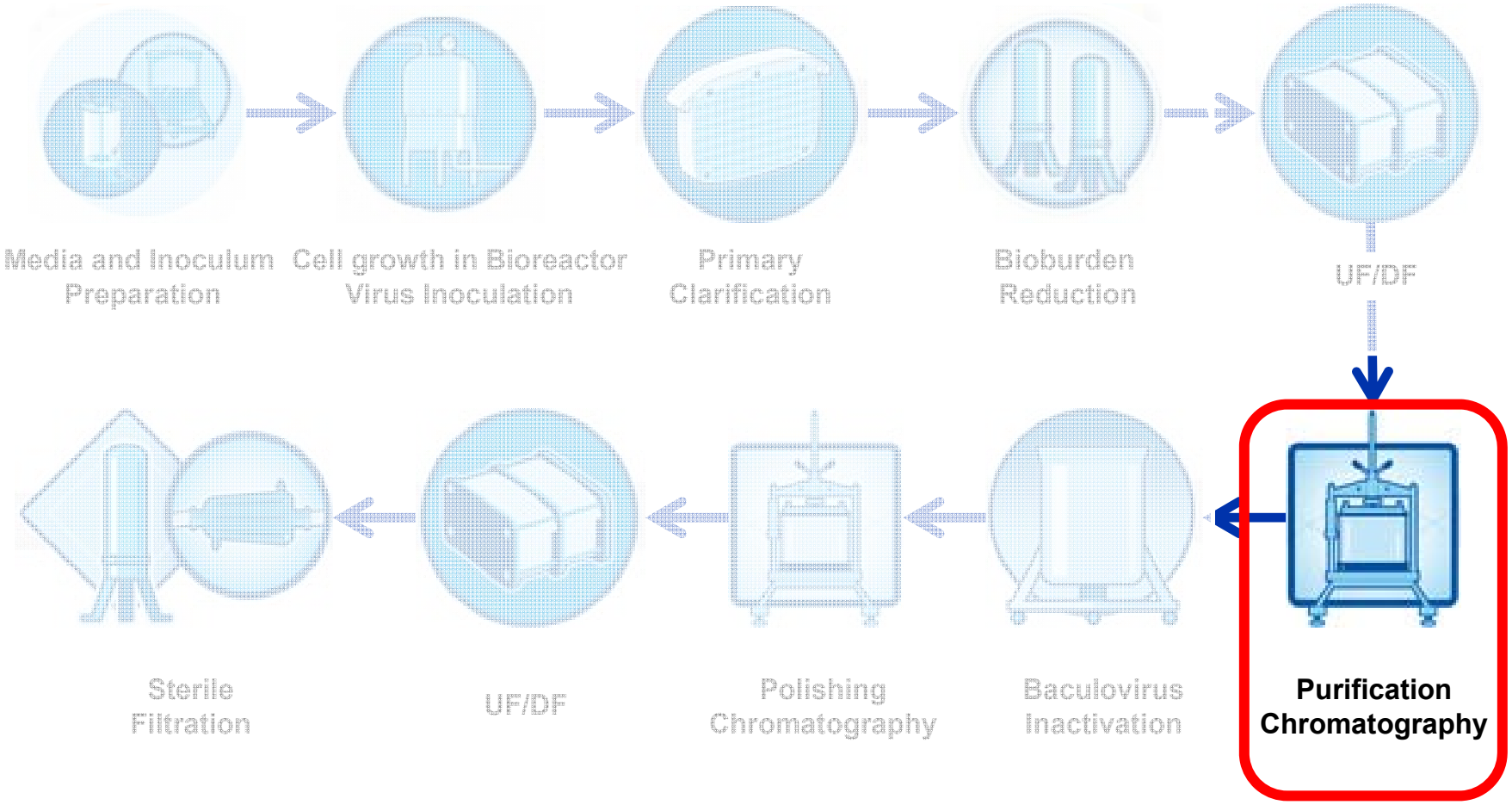
- Filter-only clarification train can be used without compromising recovery yield of VLPs.
- Filter cascade composed of a Polygard® CN 5 µm filter followed by a 0.3 µm depth filter showed the highest recovery of HCV-VLP, improving on centrifugation/2° depth filtration
- Moderate DNA removal with depth filtration was seen

UF/DF

- Pellicon® cassette with 300 kD regenerated cellulose membrane offered the best combination of recovery and purification

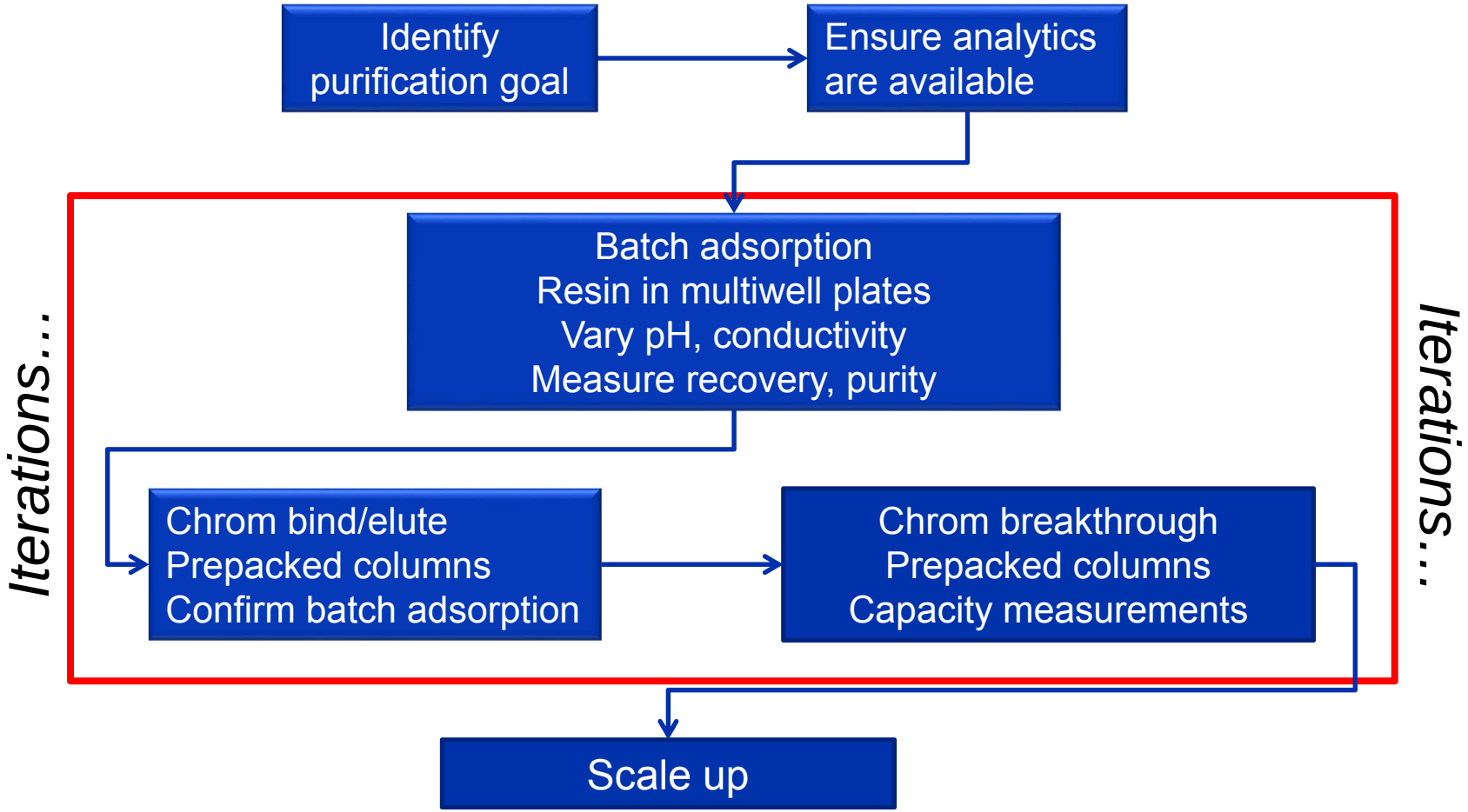
Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform

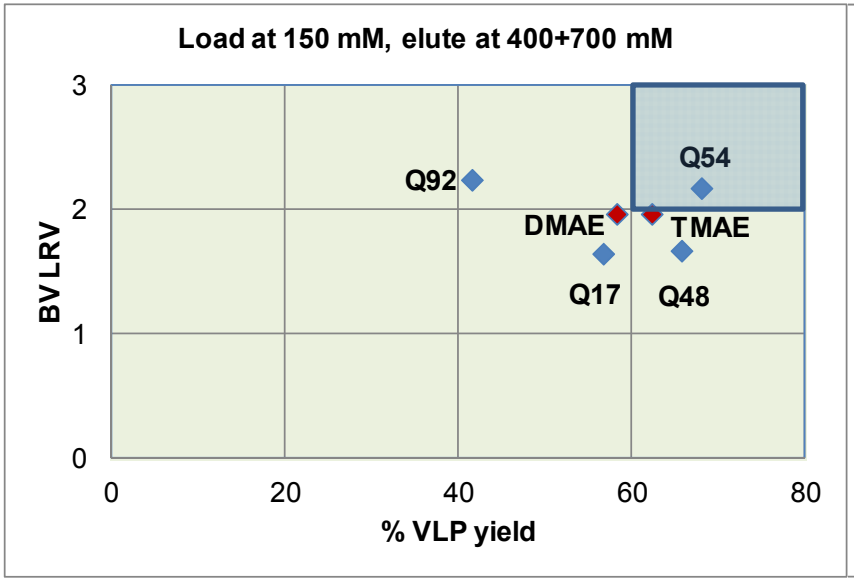


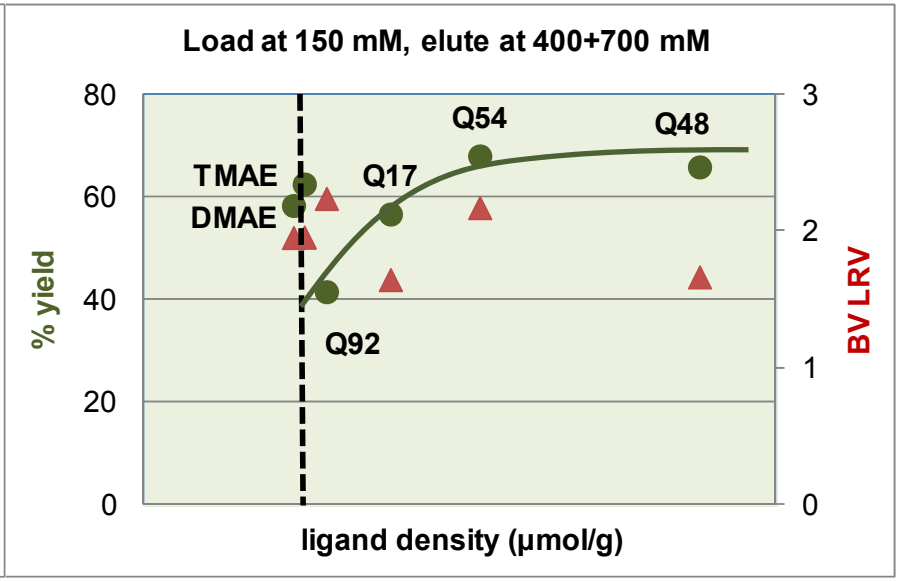
Purification strategy

Anion exchange chromatography (AEX) resins used



Batch adsorption experiments (bind-elute)



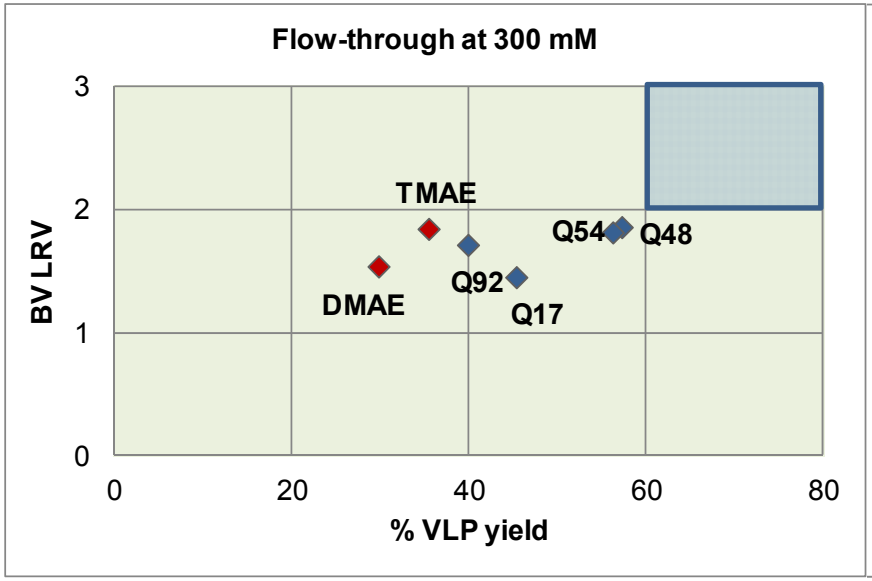


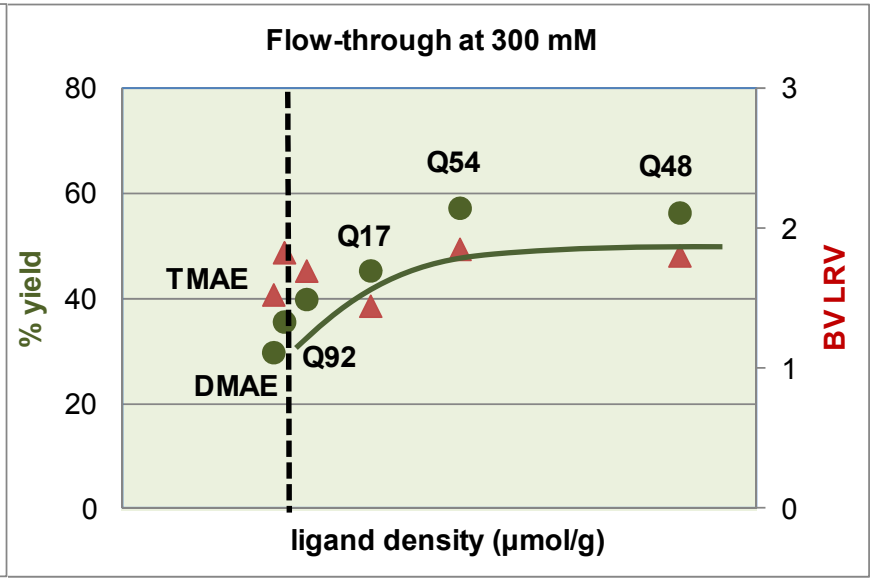
Fractogel® – red
Eshmuno® – blue

Fractogel® – dotted line

- Fractogel® and two Eshmuno® prototypes approach target of 2 BV LRV
- Yield increases with increasing ligand density for Eshmuno® prototypes

Batch adsorption experiments (flow-through)





Fractogel® – red
Eshmuno® – blue

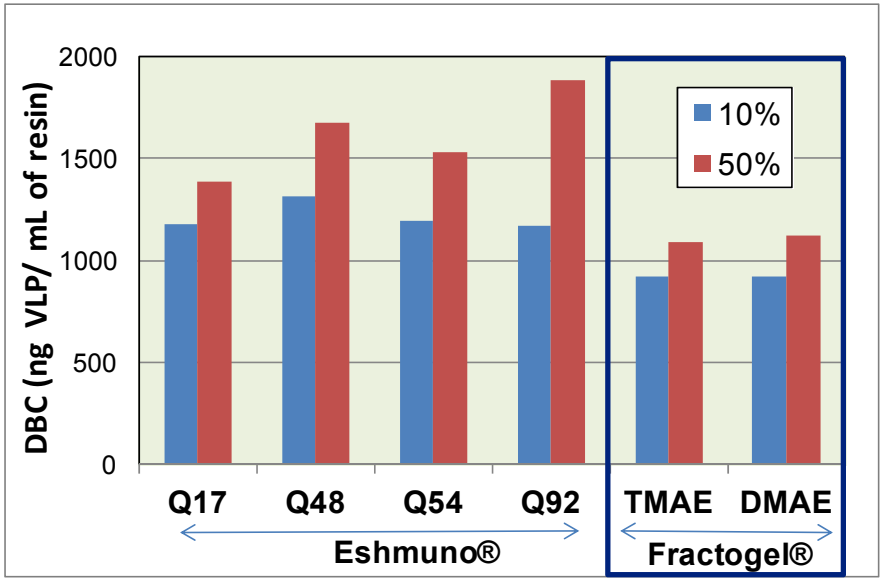
Fractogel® – dotted line

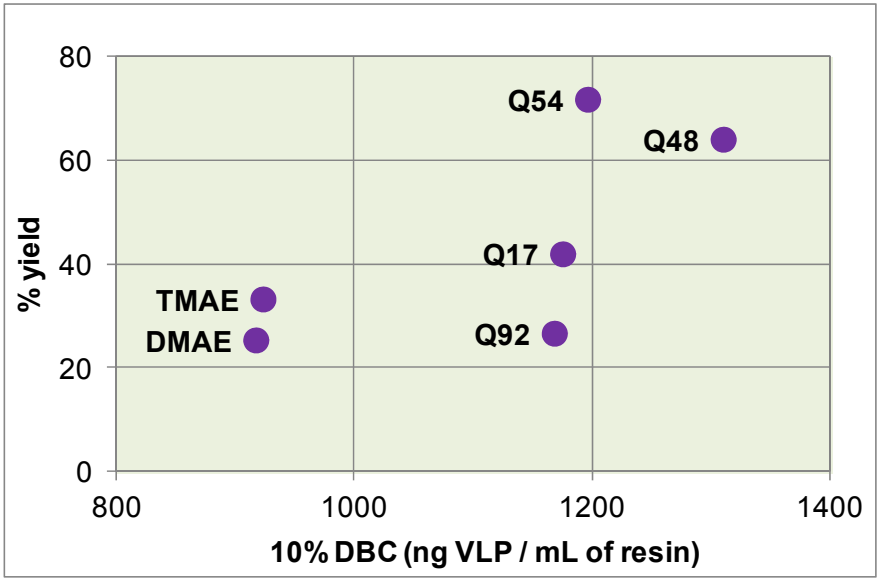
- Inadequate performance in pure flow-through mode; Similar trends with ligand density

Adopted strategy: collect the flow-through fraction, then wash/elute the resin to recover more material

Column experiments

Breakthrough curves for dynamic binding capacity

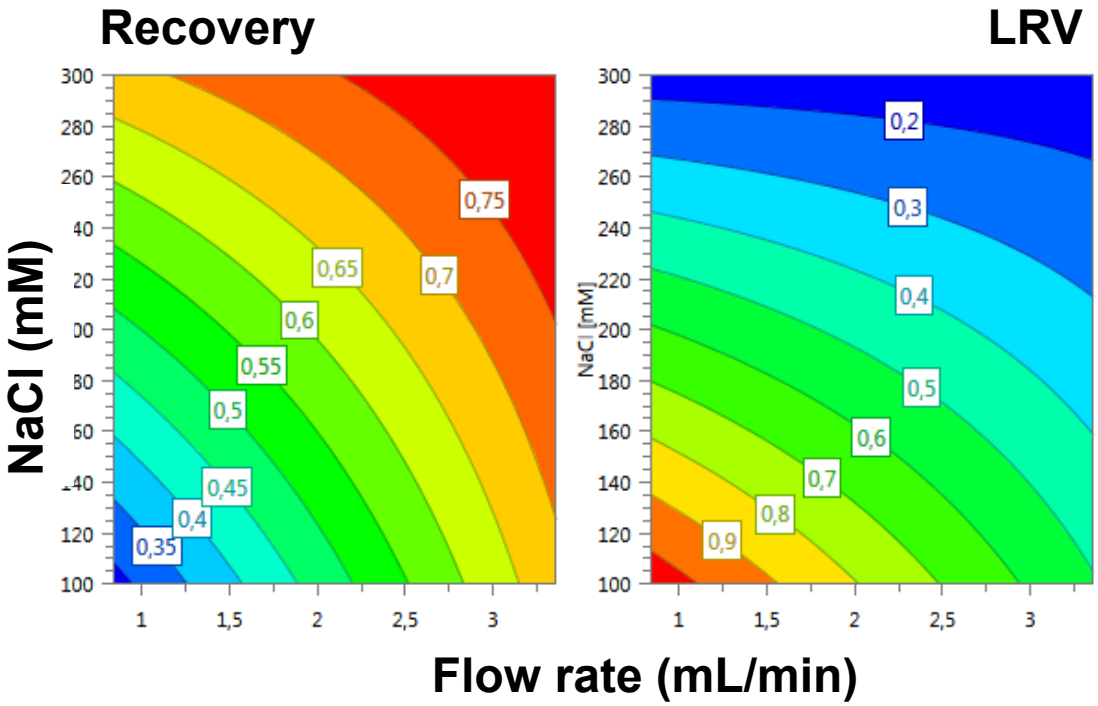




- 10% dynamic binding capacity ranges at 900-1300 ng VLP / mL of packed resin
- The Eshmuno® series has about 30% higher DBC compared to Fractogel®

DOE of flow-through conditions: Fractogel® TMAE

Inputs: load NaCl (100/200/300 mM) and flow rate (100/200/400 cm/hr)
Responses: % VLP recovery and BV LRV



Higher flow rate
OR
Higher load conductivity

Higher recovery
AND
Lower BV LRV

Successful purification of VLPs using Fractogel® and Eshmuno® AEX chromatographic resins

- Successfully purified VLPs using Fractogel® TMAE commercial resins and Eshmuno® QPX prototype resins
- Yield of >60% with ~2 LRV baculovirus can be achieved with a flow-through/wash purification strategy for both resins
- Options to increase recovery or purification depending on product value by varying process conditions

Optimum performance achieved

	Traditional lab process	New scalable process
Purity		
Baculovirus clearance	94%	97.6%
DNA clearance DNA		99.9%
HCP clearance HCP		82%
Recovery by P30 ELISA		
VLP recovery VLP	< 10%	~ 65%

Typical VLP-based vaccine process

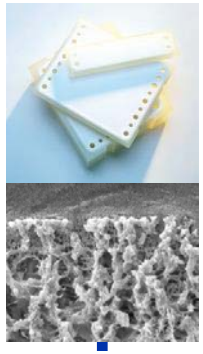
Insect cell / baculovirus VLP production platform

Media and Inoculum Preparation

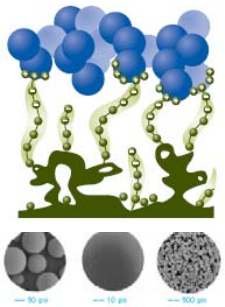
Mobius® Bioreactor

Polygard®-CN 5.0→0.3 µm filters

Bioburden Reduction



Pellicon® Ultrafiltration cassettes with Ultracel® 300 kD membrane

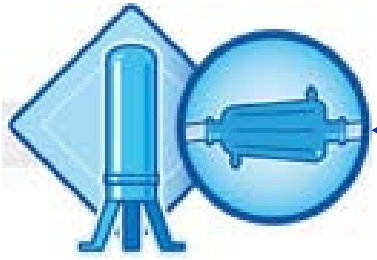


Fractogel® AEX resins

Baculovirus Inactivation

Polishing Chromatography

UF/DF



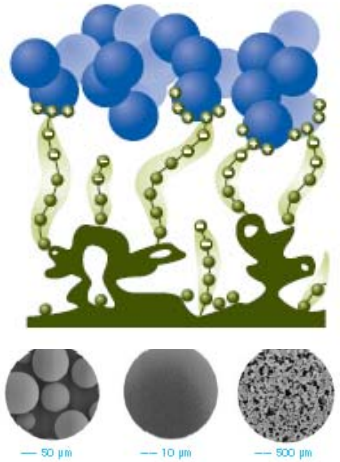
Sterile Filtration

Typical VLP-based vaccine process

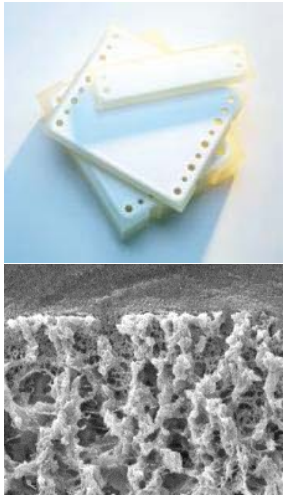
Insect cell / baculovirus VLP production platform

Mobius®
Bioreactor

Polygard®-CN
5.0→0.3 µm filters



Fractogel®
AEX resins



Pellicon®
Ultrafiltration
cassettes with
Ultracel® 300 kD
membrane

Summary

- Successfully used Mobius® 3L disposable bioreactor for production of VLP-based vaccine in insect cell culture system
- Optimized downstream processing using Polygard® CN 5.0→0.3 µm depth filters followed by UF/DF using Pellicon® cassette with Ultracel® 300 kD membrane
- Purified VLP by using Fractogel® resins and Eshmuno® QPX prototypes
- Integrated all the above components to achieve recovery and impurity clearance in line with requirements

Team and acknowledgments

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