versatile technologies for vaccine manufacturing

DCVMN 17th Annual General Meeting
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Buenos Aires, Argentina

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Process Solutions

dcvmn
Developing Countries Vaccine Manufacturers Network

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The Economist Intelligence Unit Report
The Changing Biopharma Risk Equation

- Biopharma top growth strategies
- Top regions / countries of interest for expansion
- Types of products in development and which will be most disruptive
- ROI expectations on growth strategies
- Top of mind risks associated with growth strategies
- Approaches to managing risks
- Confidence in ability to execute

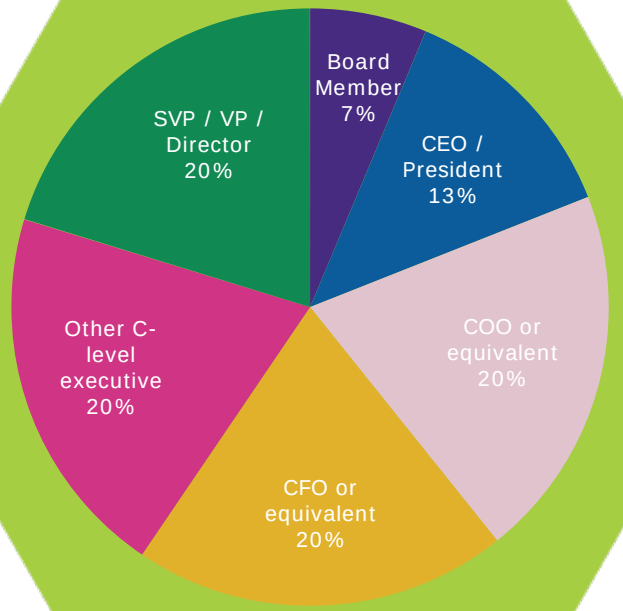
The Economist Intelligence Unit Survey Demographics

254 biopharma industry leaders

31% Europe
30% North America
29% Asia
10% Rest of World

9.8% Latin America (Brazil & Mexico)

25 questions



One Goal:
assess the balance between risk and growth in biopharma

LATAM Plans to Expand Beyond its Borders…

64% intend to **add production and/or development capacity or grow market share in EMEA & Asia**

Most important strategies for growth over the next five years:

52% **Expansion into new products**

40% **Expansion into new geographical markets**

while the rest of the world moves to LATAM…

52% intend to **add production and/or development capacity or grow market share in LATAM & Middle East/Africa**

Most important strategies for growth over the next five years:

40% **Expansion into new products**

30% **Expansion into new types of therapeutic categories**

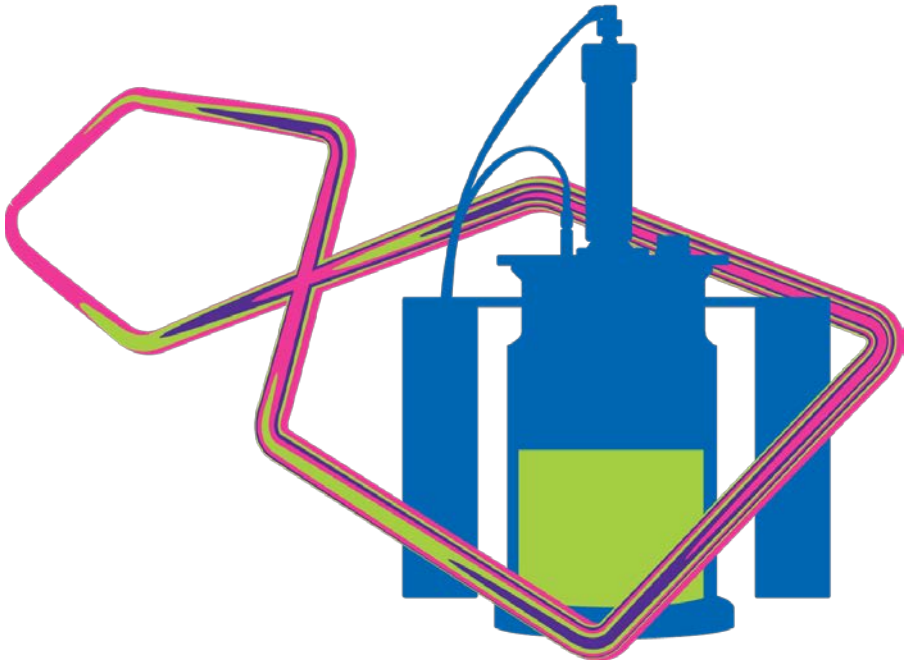
Vaccine Development & Manufacturing Strategy in LATAM

36%

Biotechs are already developing or plan to develop **vaccines**

40%

LATAM Biotechs plan to **partner with other pharmaceutical companies** to manufacture new drug and therapy products



30%

Global Biotechs plan to **invest in production facilities in emerging markets** to manufacture new drug and therapy products

LATAM: The Changing Biopharma Risk Equation

Manufacturing and Development of New Drug and Therapy Products

Risks will increase somewhat or significantly over the next five years:

LATAM

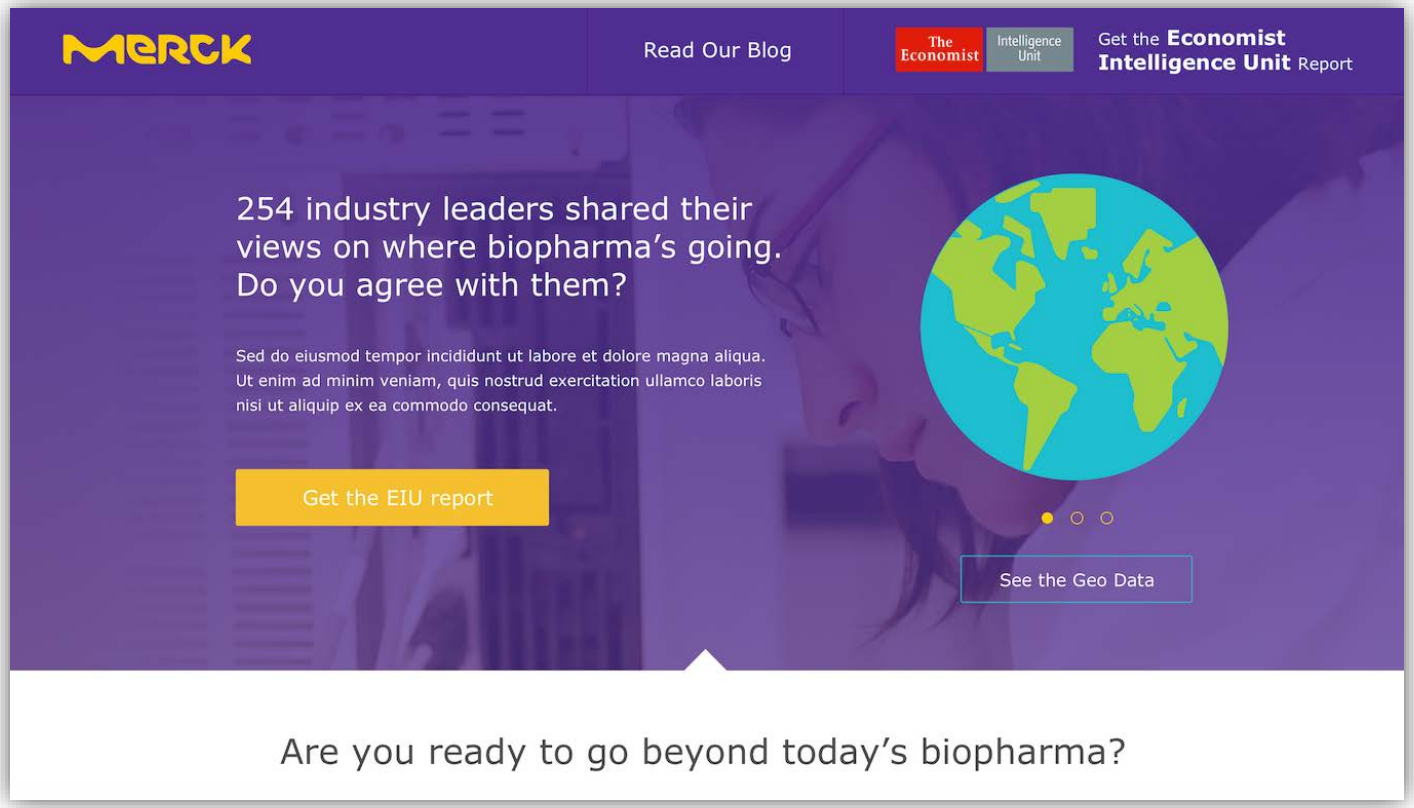
- **52%** controlling costs in development and production
- **44%** maintaining IP protection
- **44%** scaling up and supplying market demand

Vs.

GLOBAL

- **41%** controlling costs in development and production
- **40%** maintaining IP protection
- **36%** maintaining regulatory compliance

Get a Copy of the Report
www.gobeyondbiopharma.com



- Download the EIU report
- Explore the survey results through interactive infographics
- Stay engaged with updated blog content

production & purification of virus-like particle (VLP)-based vaccine

- Case study and testbed for modern vaccine scale-up and optimization
- VLPs
  Powerful platform for creation of new vaccines
  - Processes are currently quite diverse
- **Standardized platforms needed to accelerate vaccine development!**

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Production & Purification of VLP-Based Vaccine

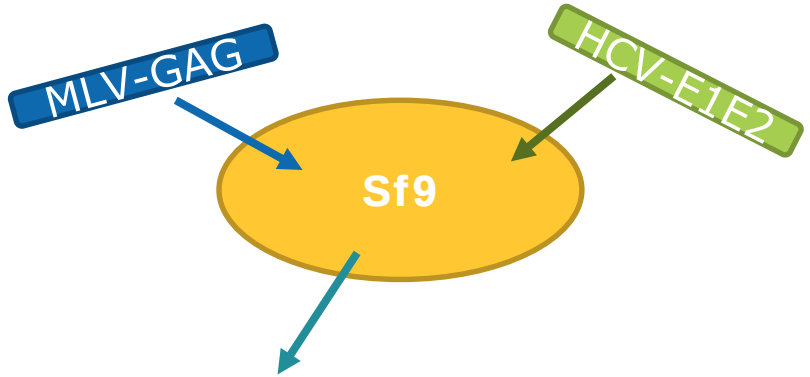
Work carried out in collaboration with iBET

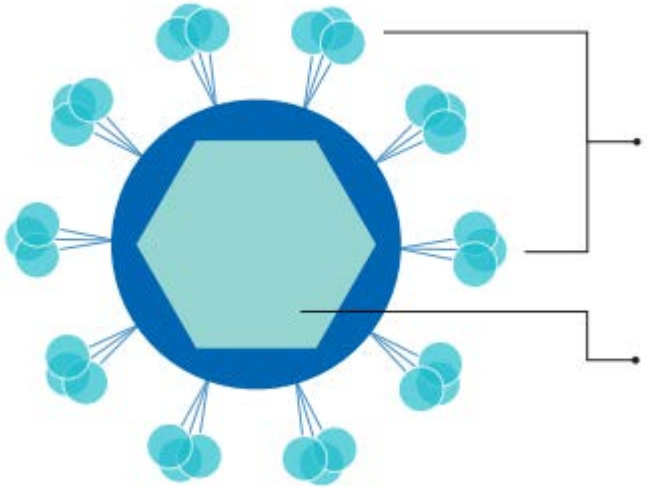
iBET: Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

Production & Purification of VLP-Based Vaccine

Insect Cell Expression System for VLP

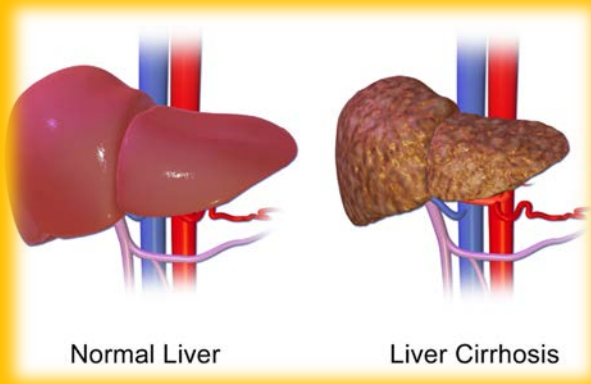
VLP produced in Sf9 insect cells co-infected with **MLV-GAG** and **HCV-E1E2** using **baculovirus**





ANTIGENS
E1 and E2 envelope glycoproteins from Hepatitis C virus

STRUCTURE
Capsid and envelope from retrovirus (murine leukemia virus)

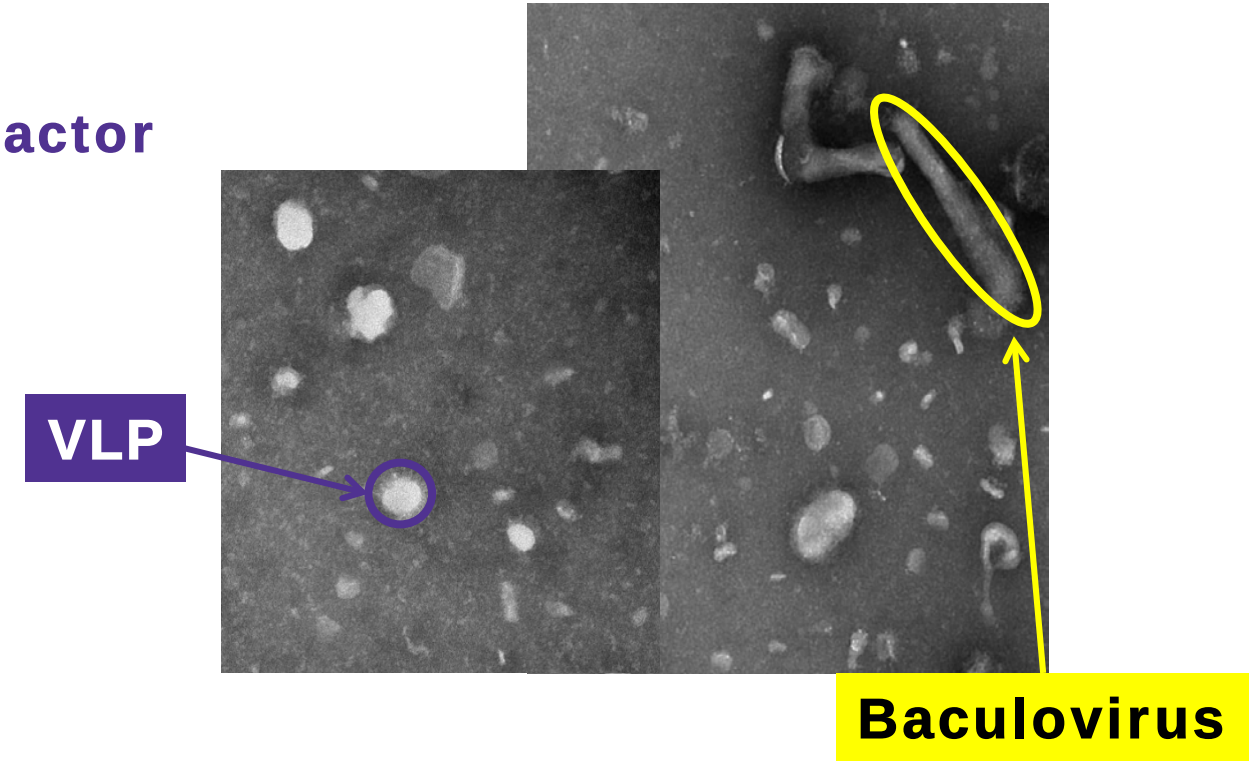


Hepatitis C

- 170 million people infected, over 350,000 deaths/year
- Causes cirrhosis and liver cancer
- Current therapies only partially effective, costly and poorly tolerated
- **No vaccine currently exists**

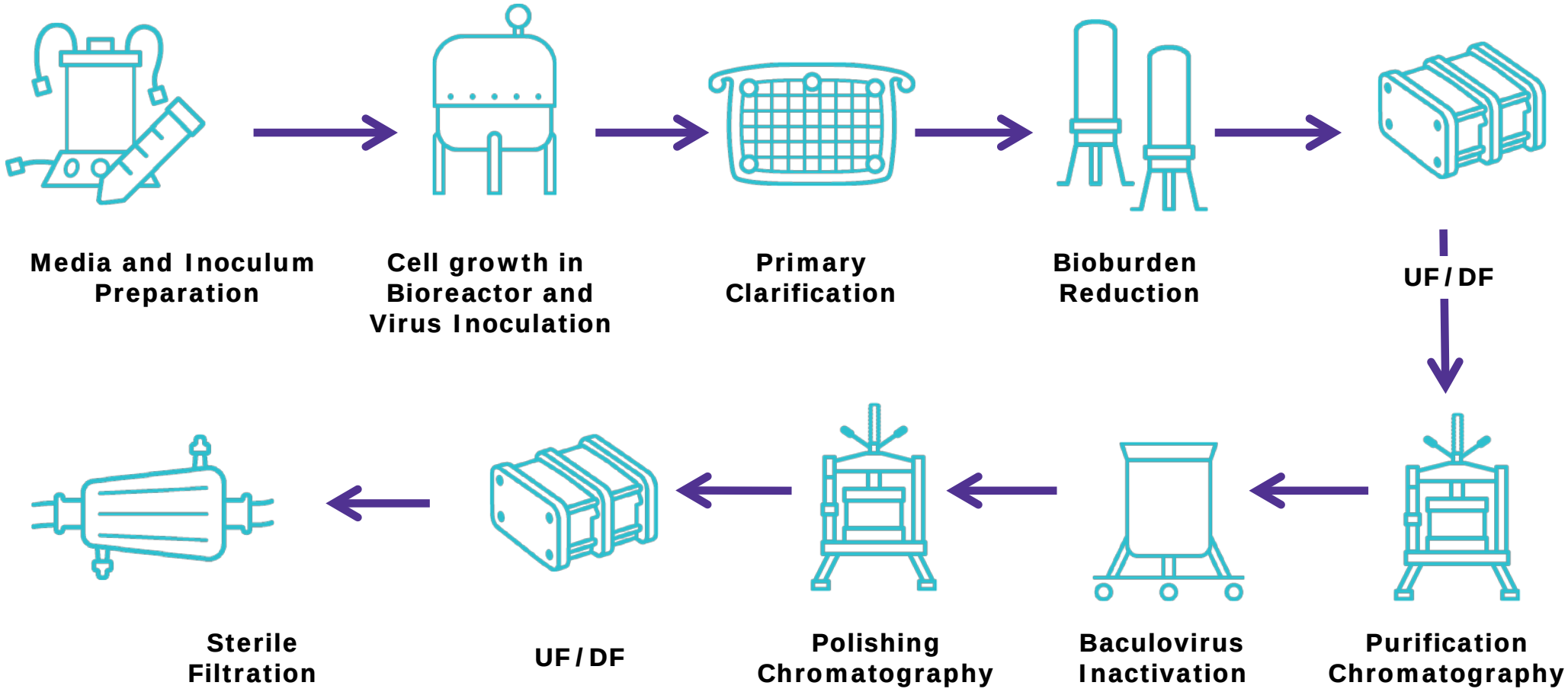
Production & Purification of VLP-Based Vaccine
Process Challenges

1. **Scalable production in bioreactor**
2. **Efficient purification**
3. **High recovery of VLP**
4. **Baculovirus clearance**

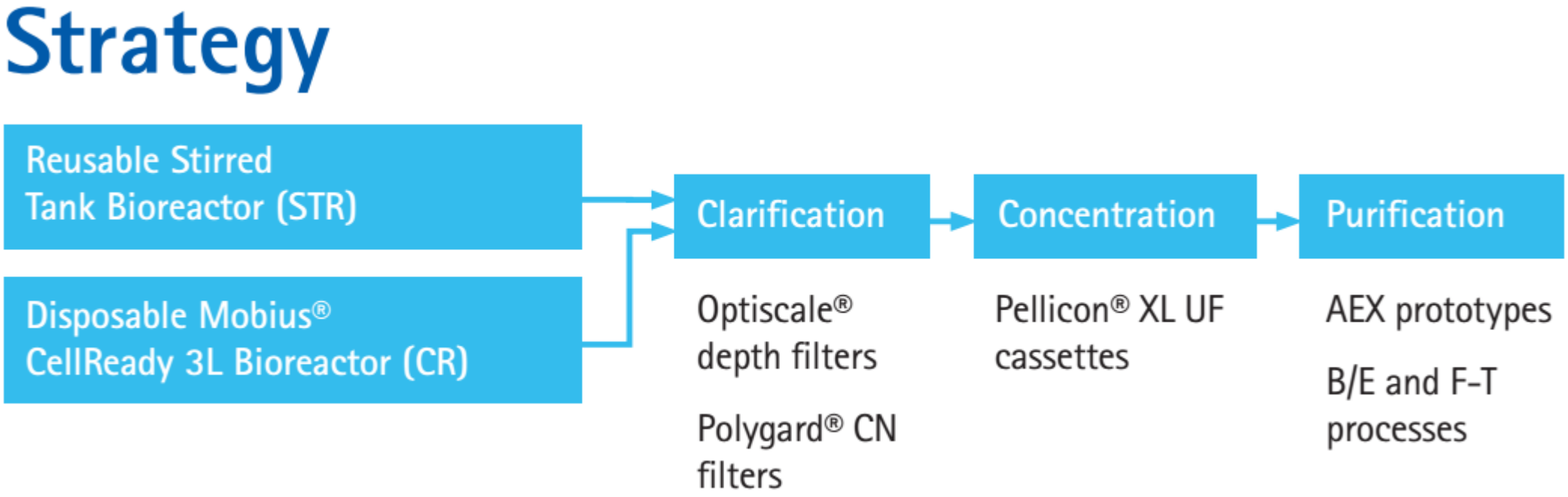


Production & Purification of VLP-Based Vaccine

Insect Cell / Baculovirus VLP Production Platform

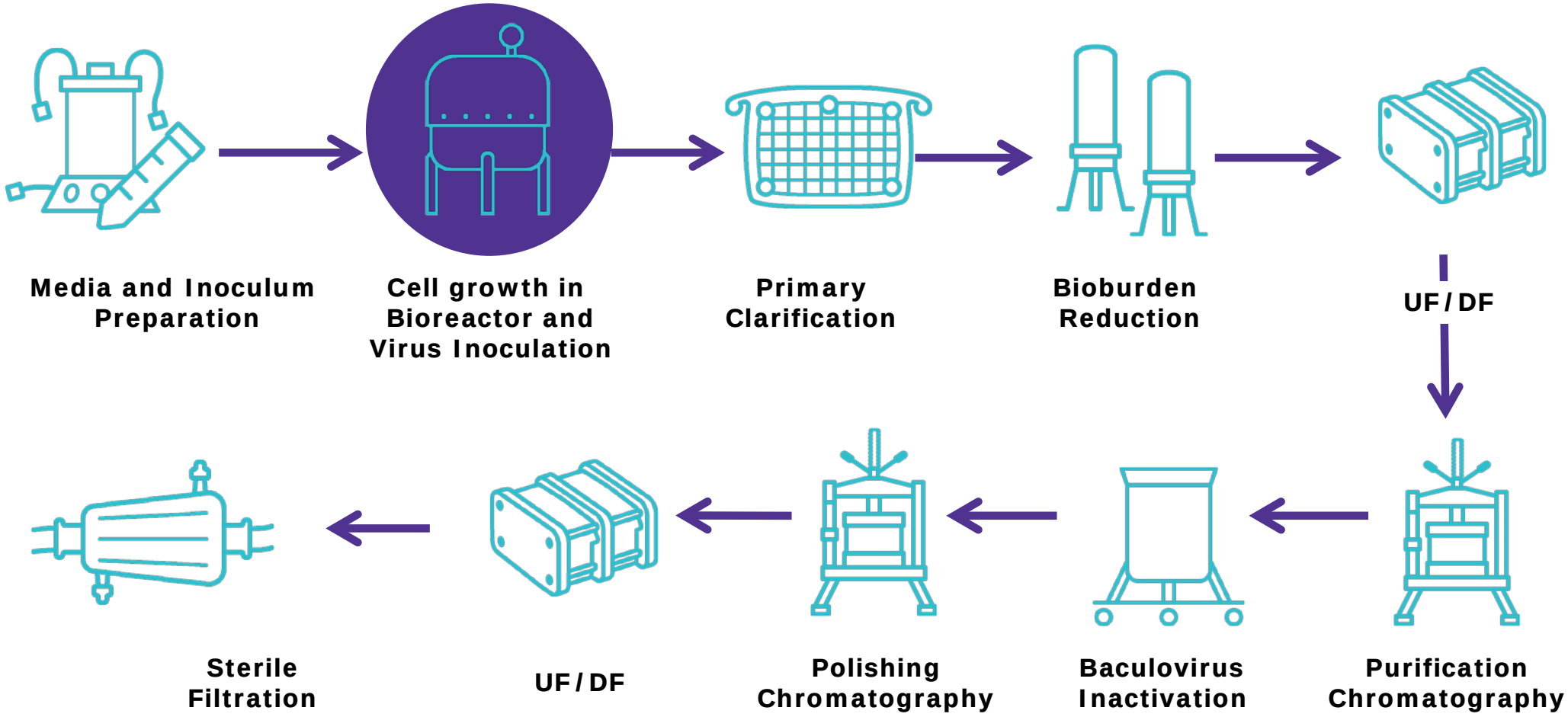


Production & Purification of VLP-Based Vaccine
Process Step Optimization



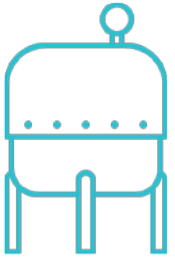
Production & Purification of VLP-Based Vaccine

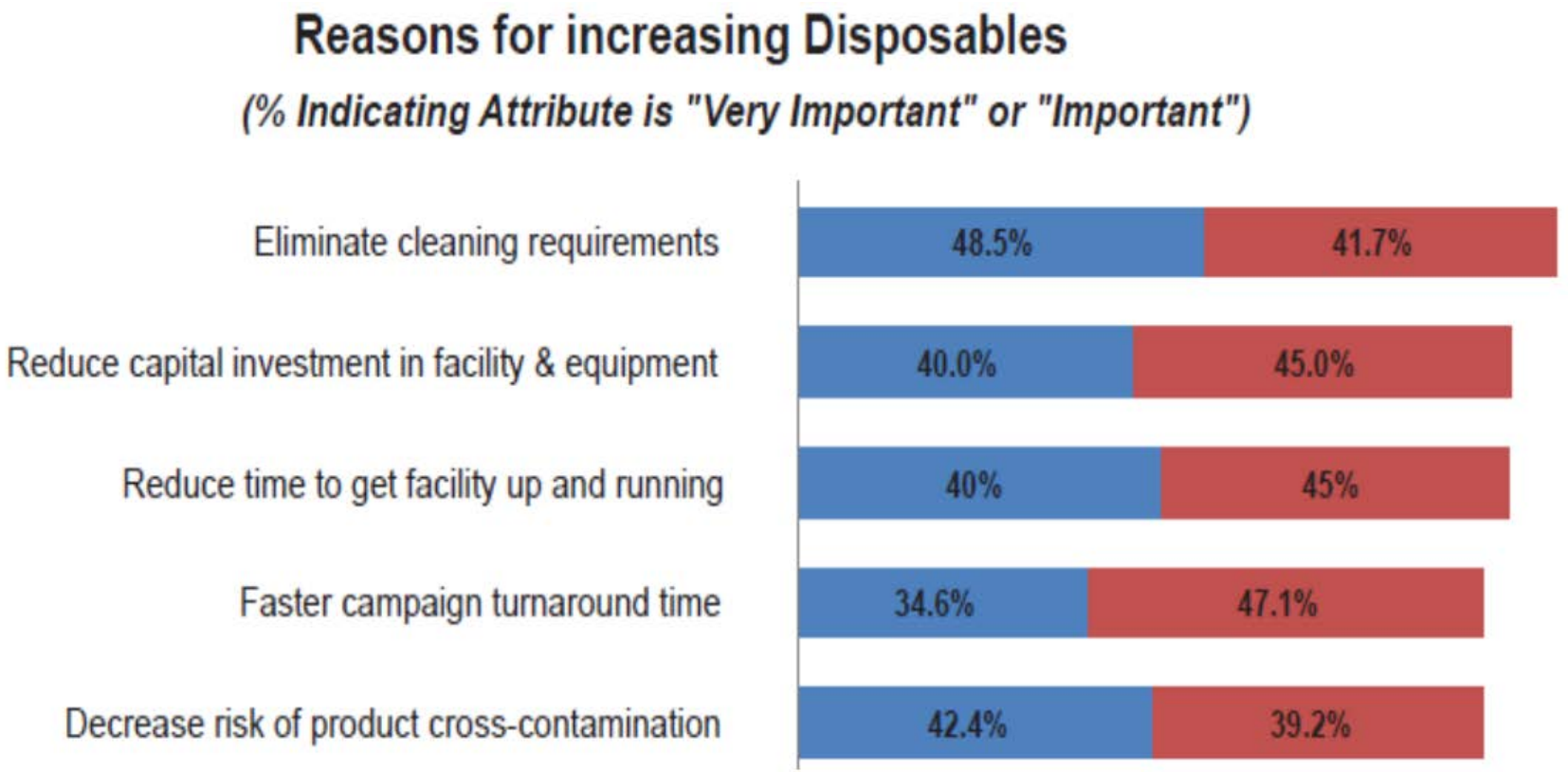
Establish Scalable, Single-Use Bioreactor Production



Production & Purification of VLP-Based Vaccine

Establish Scalable, Single-Use Bioreactor Production

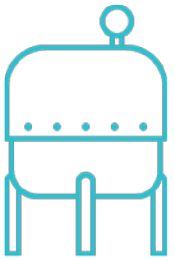




Source: 8th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production, April 2011

Production & Purification of VLP-Based Vaccine

Establish Scalable, Single-Use Bioreactor Production



Challenge #2
Leap to mfg scale

Challenge #1
Get on the path!

Ratio	3 L	50 L	200 L	1000 L	2000 L
Working Volume : Total Volume	0.8	0.8	0.8	0.8	0.8
Impeller Diameter : Vessel Diameter	0.6	0.3	0.3	0.3	0.3
Vessel Height : Vessel Diameter	1.8:1	2.0:1	2.0:1	2.0:1	2.0:1
Liquid Height : Vessel Diameter	1.4:1	1.7:1	1.6:1	1.6:1	1.6:1
Internal Baffle	No	Single (Paddle-Type)		Single (X-Type)	
Dual sparger (open pipe/ microsparger)	YES	YES	YES	YES	YES
Min – Max Working Volume (L)	1 – 2.4	10 – 50	40 – 200	200 - 1000	400 – 2000

Transition from Glass to Scalable Single-Use Bioreactor

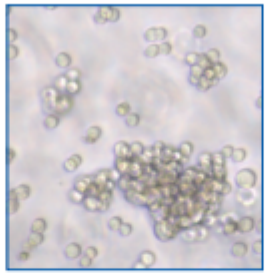
Initial challenges

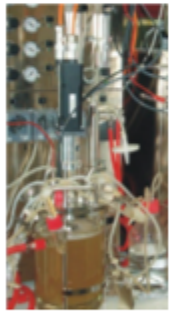
STR = Glass stirred tank
CR = Single-use bioreactor

CR Conditions:

27 °C, pO_2 = 30%
Agitation rate: 70–200 rpm
Impeller: 1 marine
Aeration rate: 0.01 vvm
Working volume = 2 L

Sf9 cells in CR

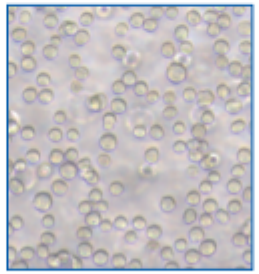


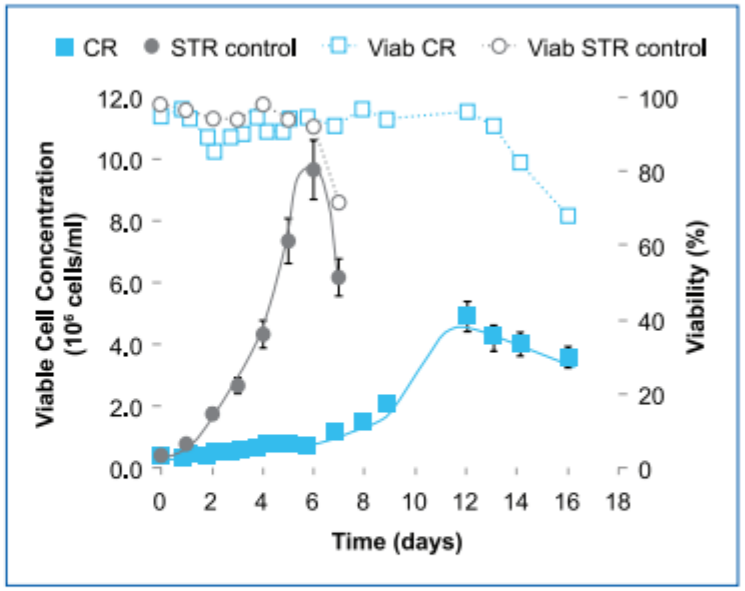


STR Conditions:

27 °C, pO_2 = 30%
Agitation = 70-250 rpm
Impeller: 2 six-blade rushton
Aeration rate: 0.01 vvm
Working volume = 2 L

Sf9 cells in STR





Observation of cell aggregates for unoptimized CR culture

Long lag-phase observed for CR culture

Transition from Glass to Scalable Single-Use Bioreactor

Process Optimization

STR = Glass stirred tank
CR = Single-use bioreactor

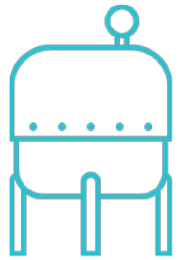


Table 1. Shear stress in the two bioreactors

Agitation Rate (rpm)	Shear stress (N/m^2)	
	STR	CR
70	**0.14**	0.05
90	0.20	0.07
110	0.27	0.09
130	0.34	0.12
150	0.43	**0.15**
170	0.51	0.18

New CR Conditions:
Agitation rate: **150 rpm**

	μ_{max} (h^{-1})	T_d (h)
CR	0.20	35
STR control	0.20	34

CR match STR hydrodynamic parameters for agitation rates above 150 rpm

✓ Enhanced cell growth in CR

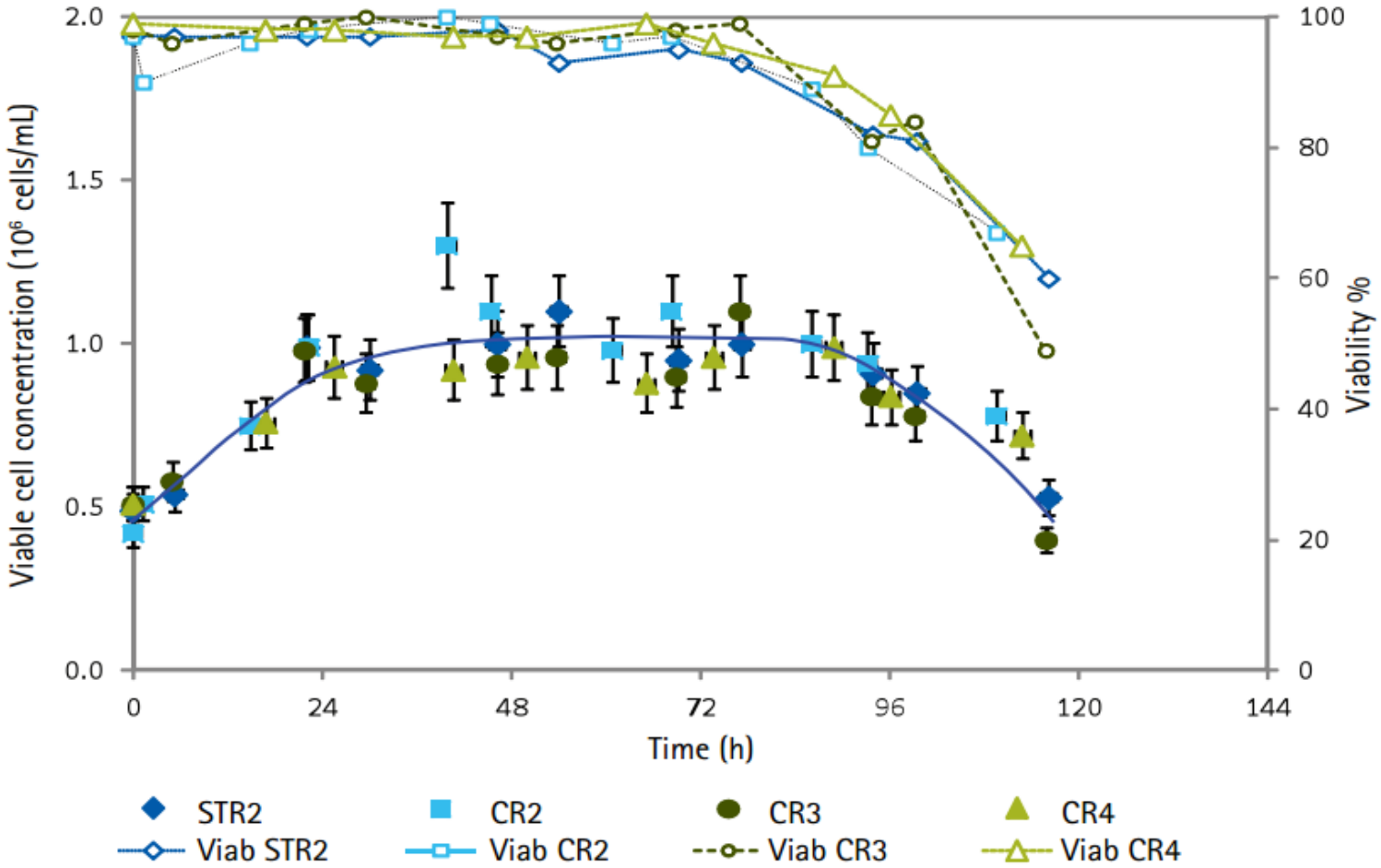
✓ CR and STR show similar growth profiles

Transition from Glass to Scalable Single-Use Bioreactor

Optimized Conditions

STR = Glass stirred tank
CR = Single-use bioreactor

Mobius® 3L Single-Use Bioreactor



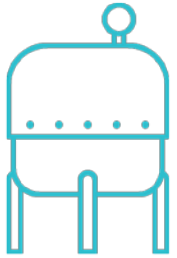
Optimized conditions

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

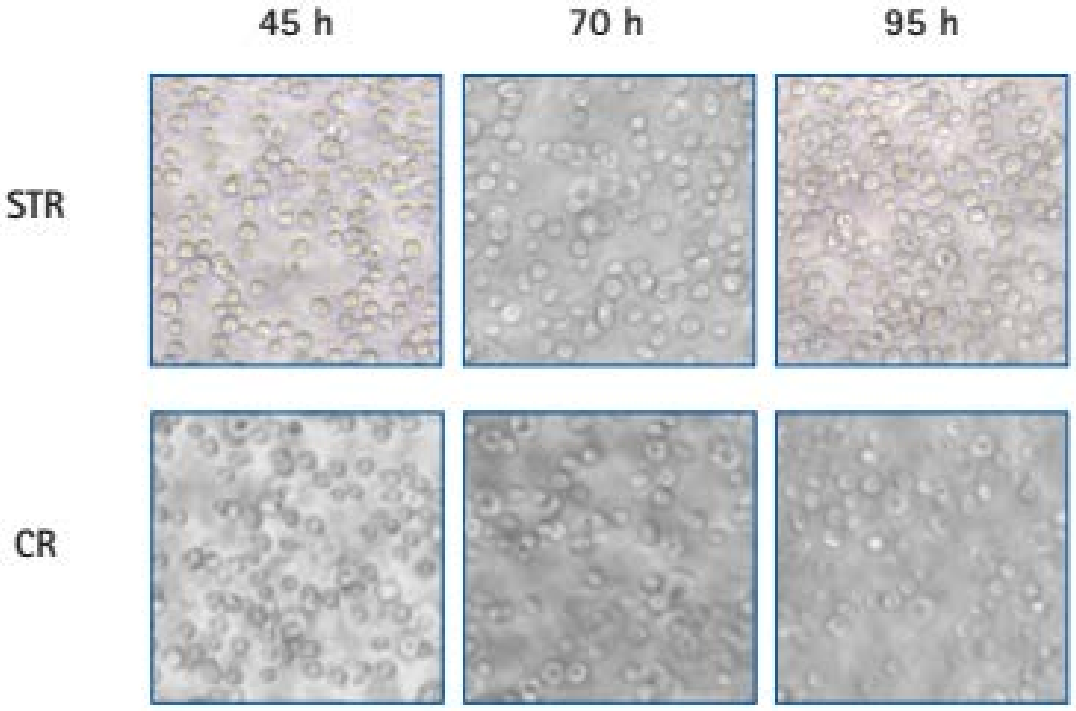
Transition from Glass to Scalable Single-Use Bioreactor

Sf9 Growth Profiles

STR = Glass stirred tank
CR = Single-use bioreactor

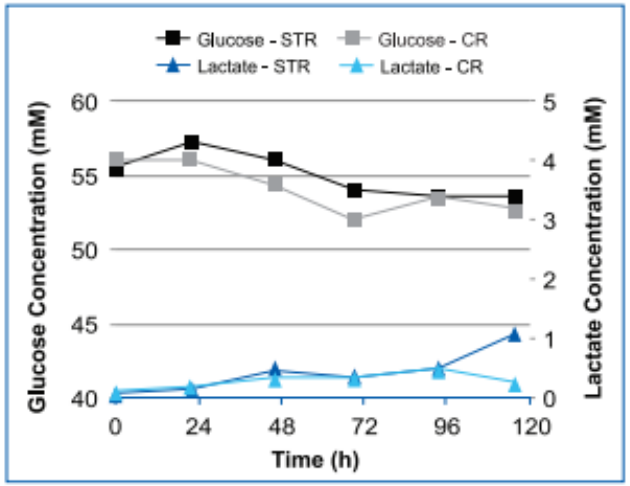


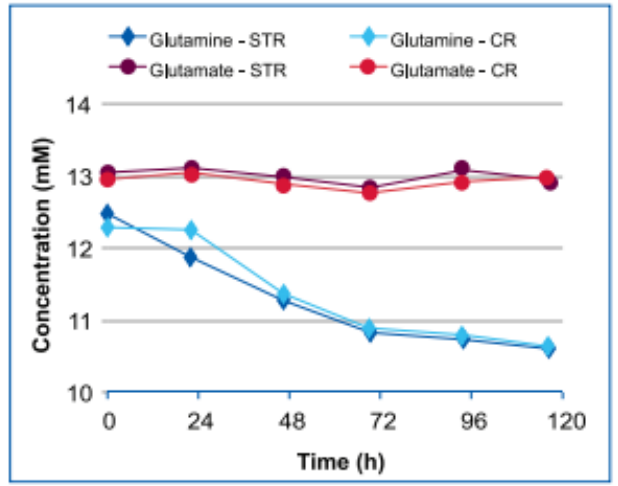
Images of Sf9 cell during culture in CR or STR



No formation of cell aggregates

Metabolite concentration in culture supernatants





Comparable metabolite profiles in STR and CR

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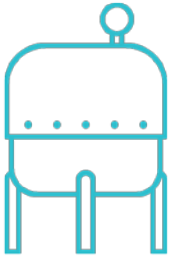
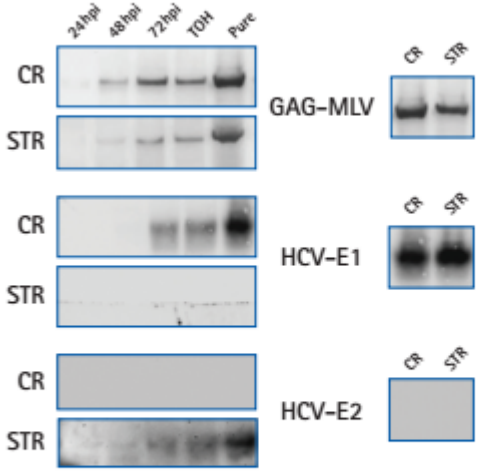
Transition from Glass to Scalable Single-Use Bioreactor

VLP-HCV Productivity in the Two Bioreactors

STR = Glass stirred tank
CR = Single-use bioreactor

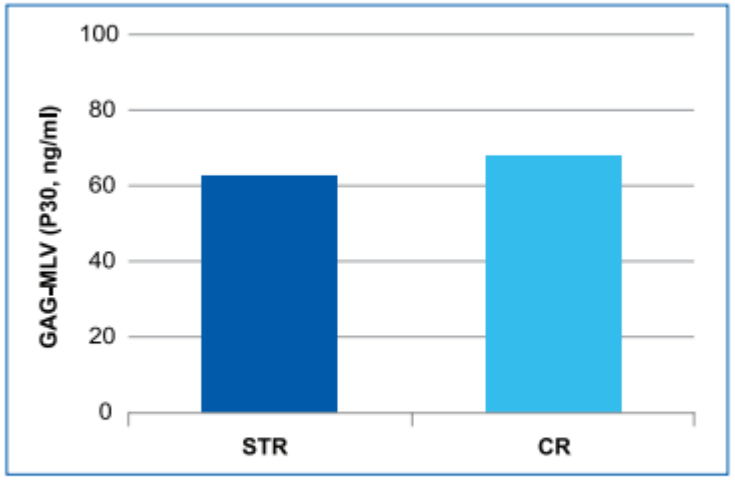


WB analysis of VLP-HCV production kinetics



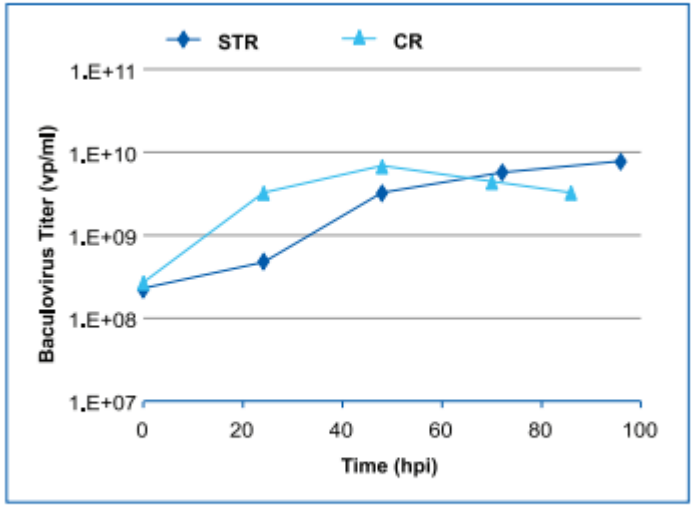
Similar GAG and HCV-E1E2 expression kinetics

Gag-MLV titer (P30) in the bioreactor harvested bulk



Equivalent productivity in STR and CR culture bulks

Baculovirus replication kinetics



Comparable baculovirus replication kinetics

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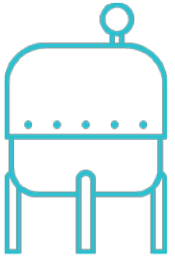
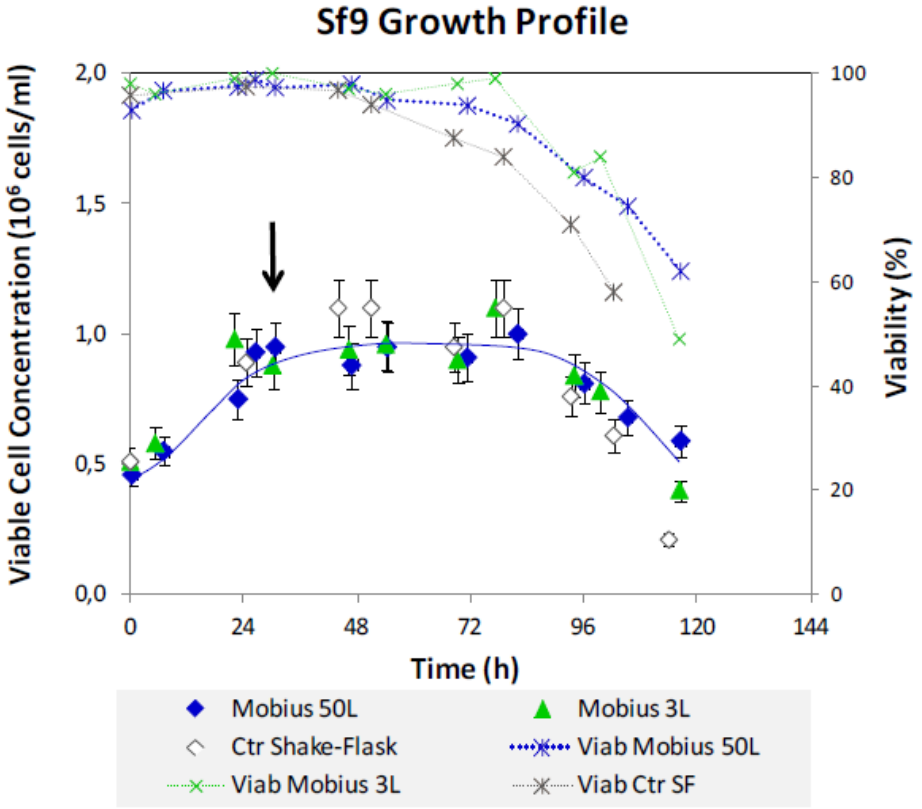
Production & Purification of VLP-Based Vaccine

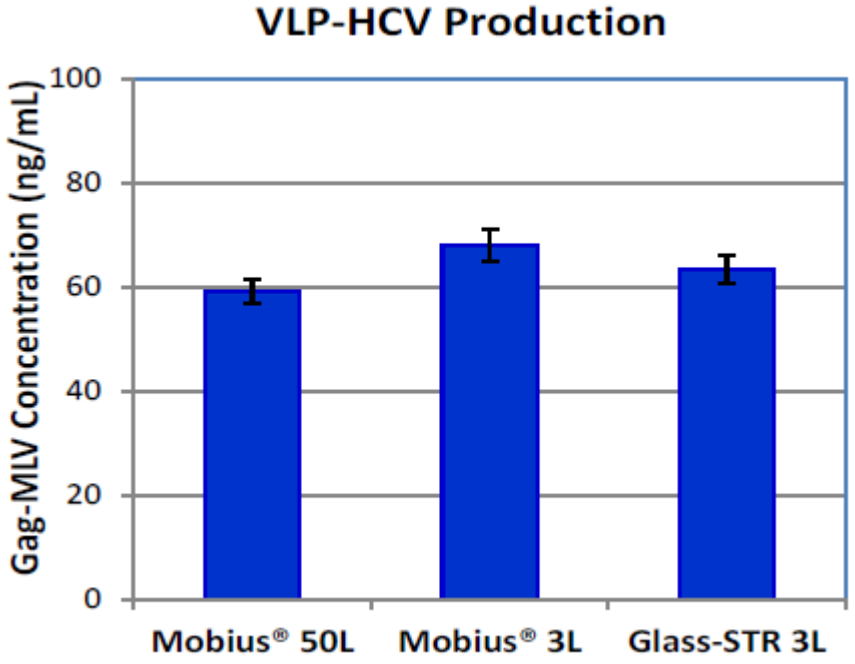
Single-Use Bioreactor, Scale-Up to 50L

STR = Glass stirred tank
CR = Single-use bioreactor





Growth kinetics of Sf9 cells in the Mobius® 50 L and 3 L bioreactors. The black arrow indicates the infection of the bioreactors and control shake-flask.



GAG-MLV titre in culture bulks of Mobius® 50L and 3L and in glass strirred tank bioreactor (Glass-STR).

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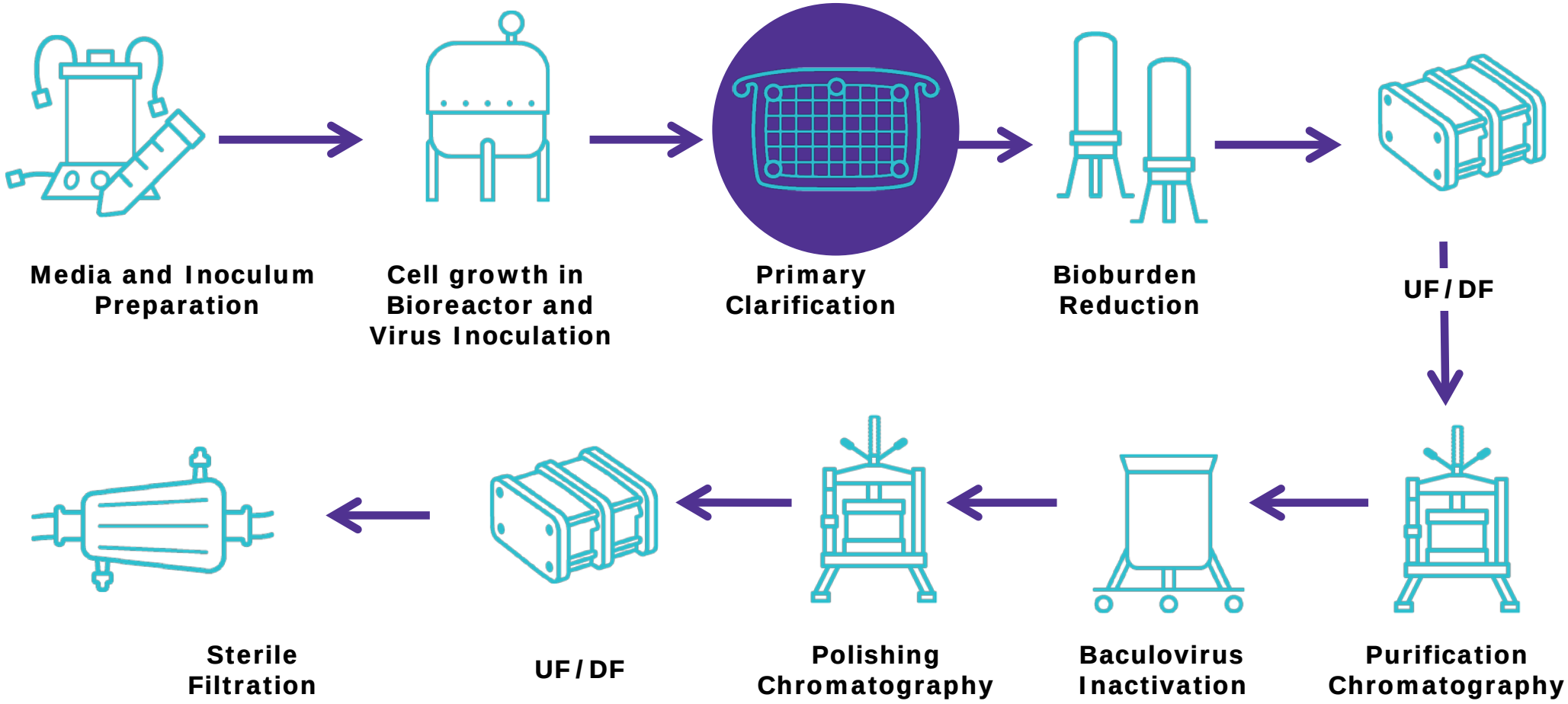
Production & Purification of VLP-Based Vaccine

Insect Cell / Baculovirus VLP Production Platform



Production & Purification of VLP-Based Vaccine
Clarification Optimization

Disposable capsule filters

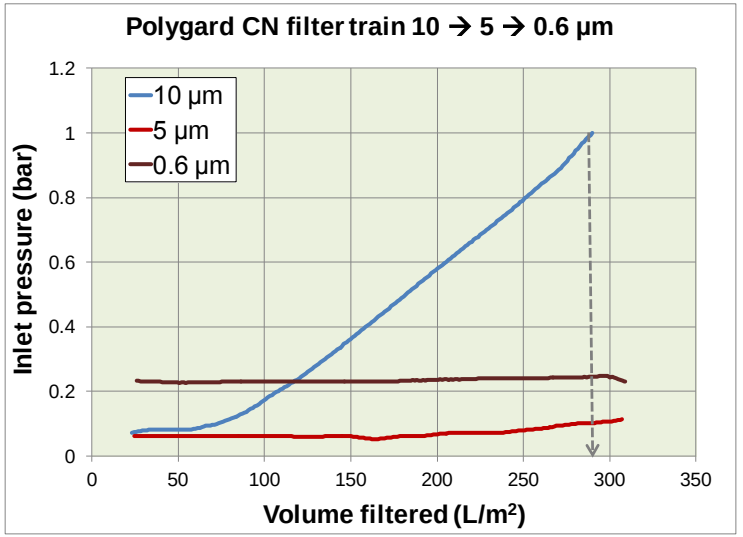
Eliminates the time and expense associated with assembling, cleaning, and validating stainless steel housings.

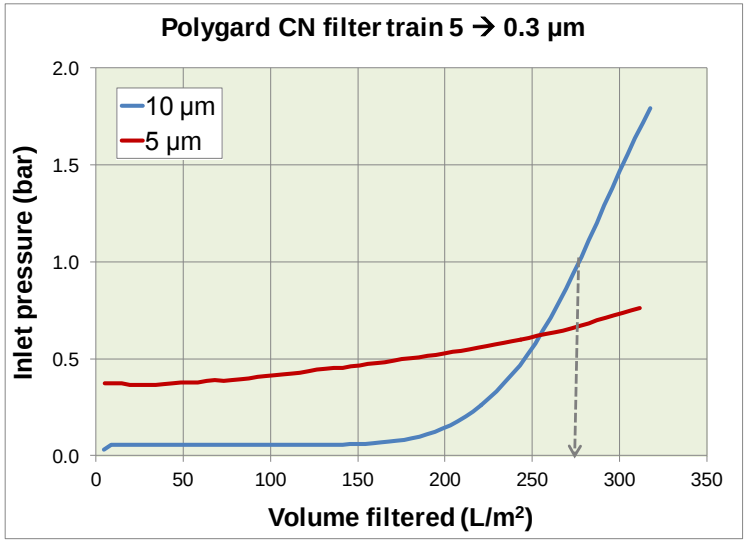
Replacement for centrifugation

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

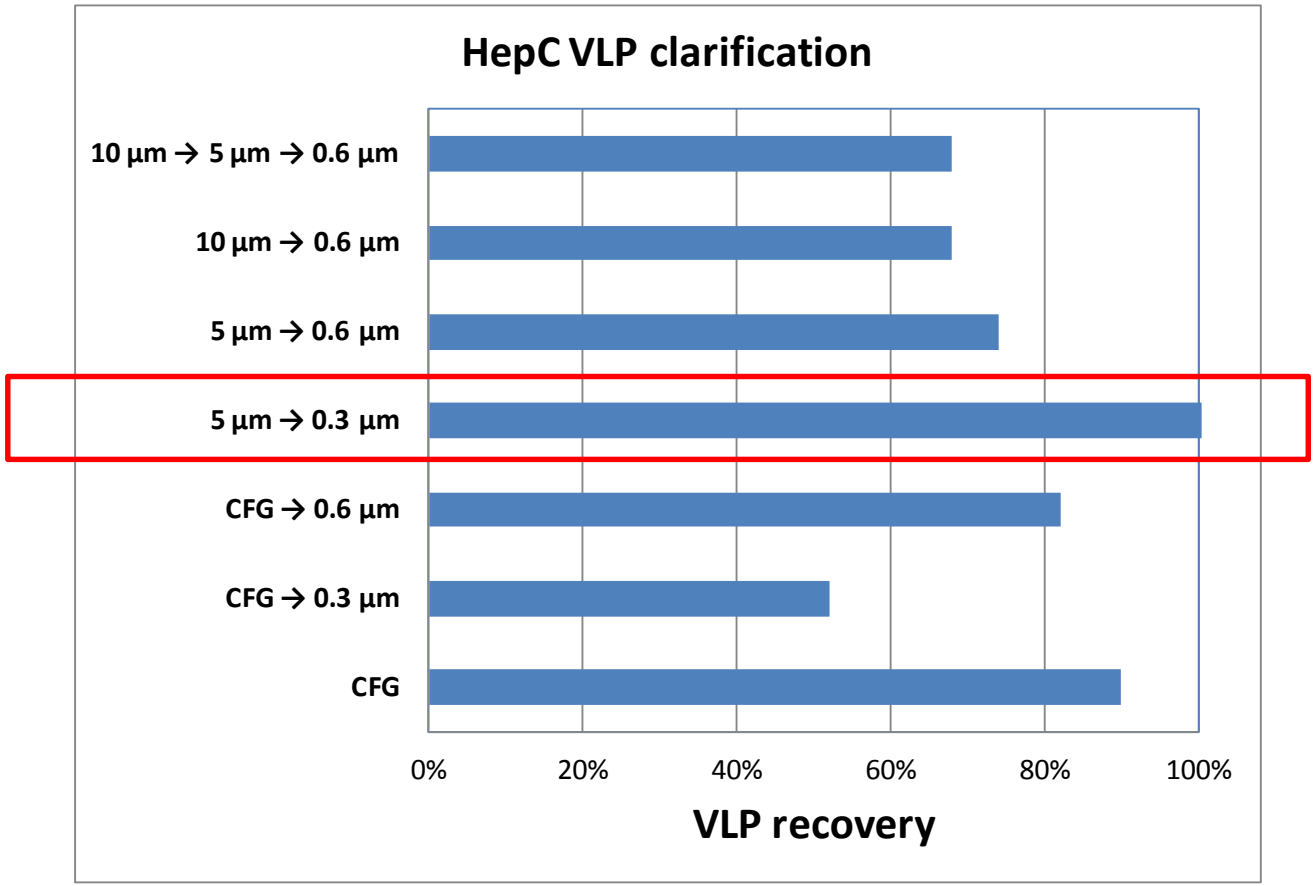
Pleated, all-polypropylene depth filters

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





Production & Purification of VLP-Based Vaccine
Clarification Optimization



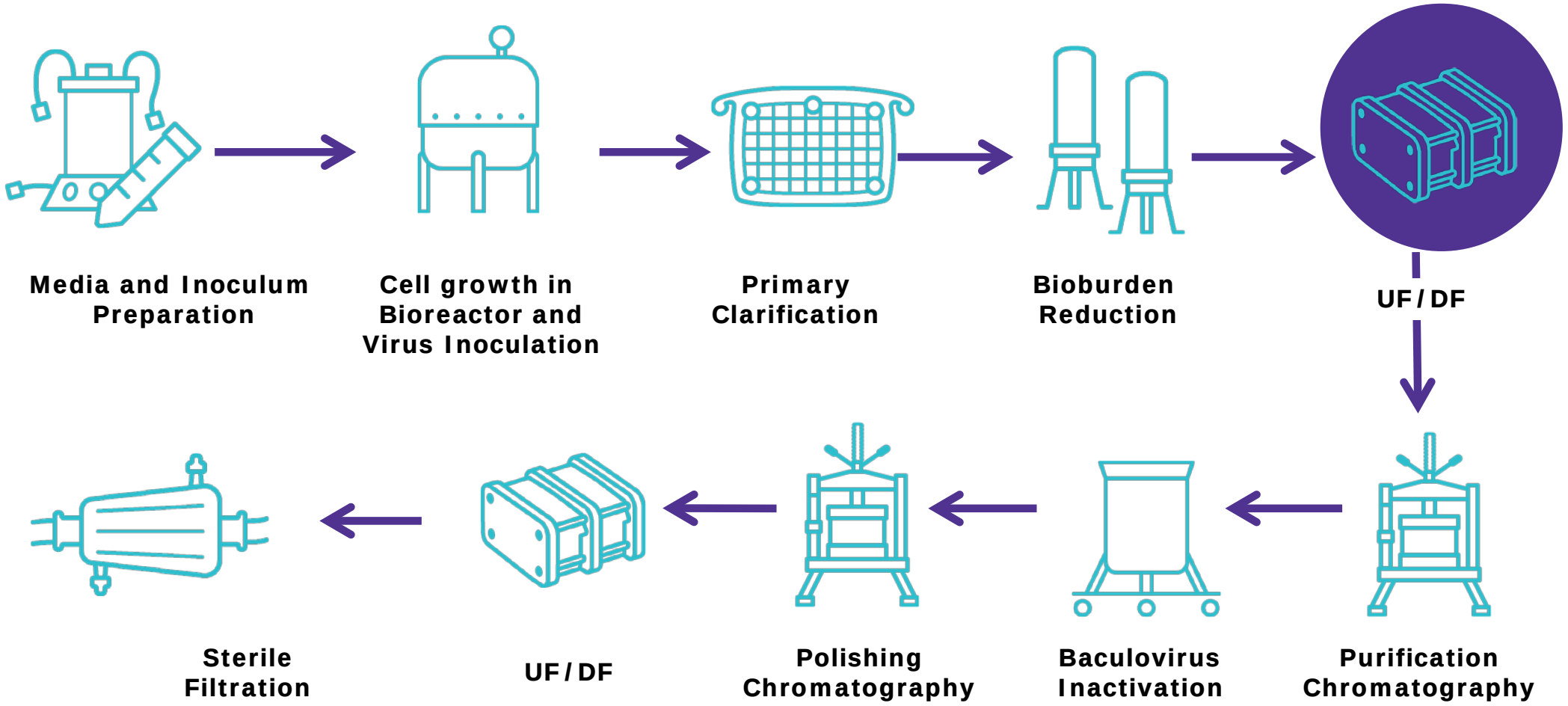
Unlike centrifugation (CFG),
depth filtration resulted in **~70% DNA clearance**

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

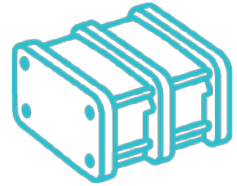
Production & Purification of VLP-Based Vaccine

Insect Cell / Baculovirus VLP Production Platform



Optimization of TFF for Concentration and Purification

Membrane Choices



	Cellulosic Membrane (e.g. Ultracel® membrane)	PES Membrane (e.g. Biomax® membrane)
Membrane	Composite regenerated cellulose (void free structure)	Hydrophilic modified polyethersulfone (void free structure)
Typical Available NMWL	3, 5, 10, 30, 100, 300, 1000 kD (new 1 kD in PD)	5, 8, 10, 30, 50, 100, 300, 500, 1000 kD
Relative protein binding	Ultra low (~0.1 g/m2) Use with any protein concentration (good with dilute)	Low –medium (~0.2 g/m2) Use with solutions > 0.1 mg/mL
pH Stability	2-13	1-14
Comments	▪ Organic solvent resistance ▪ High yield ▪ Very hydrophilic – use with solutions containing hydrophobic components (i.e. antifoams or detergents)	▪ Resistant to rigorous cleaning regimes (strong bases and acids) ▪ High flux

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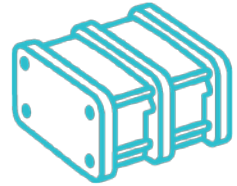
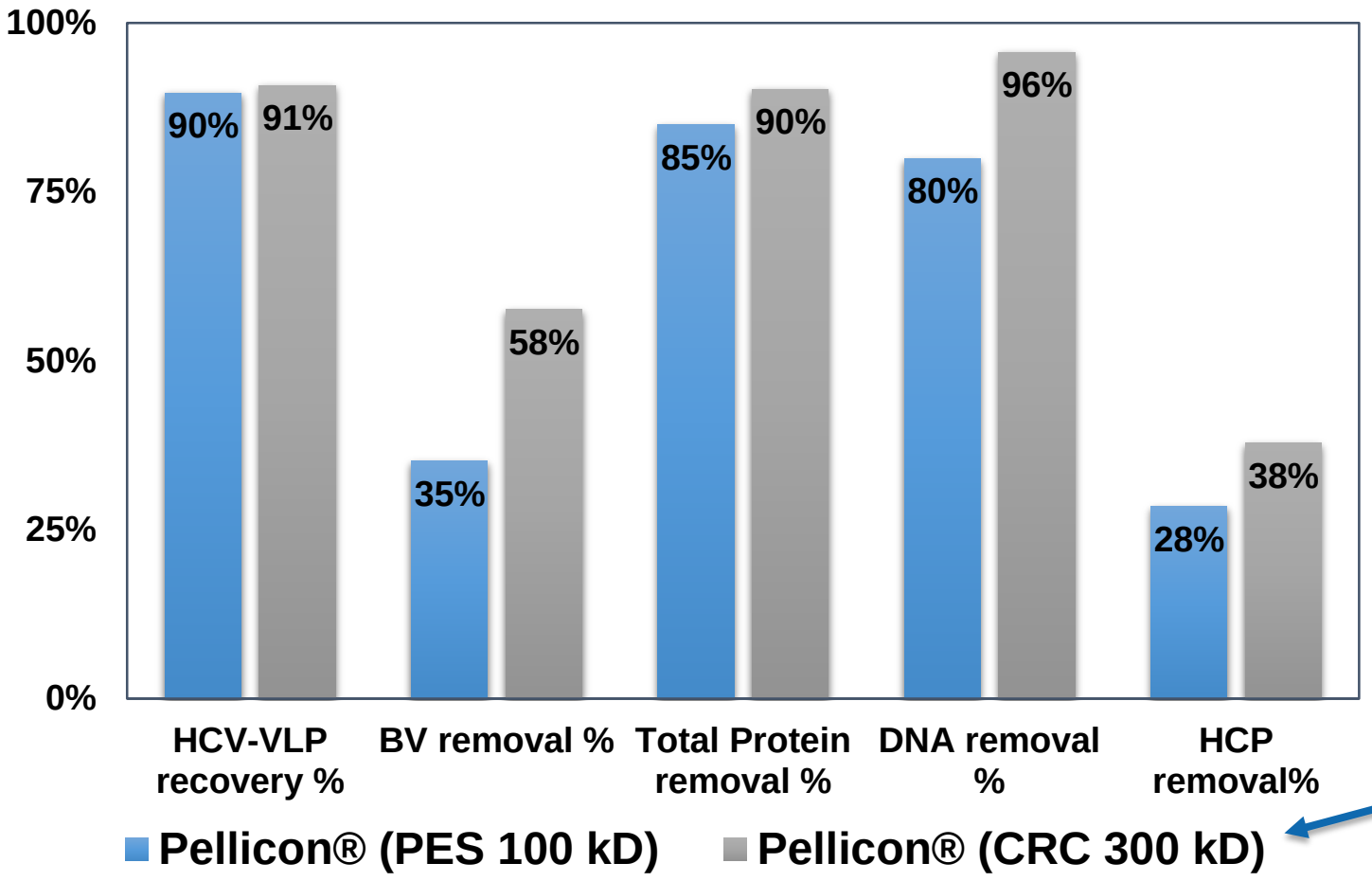
Optimization of TFF for Concentration and Purification

Purification Results





UF / DF

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

4-5X concentration achieved

Better removal of baculovirus, DNA, and host-cell protein!

Both membranes were fully retentive of the VLP

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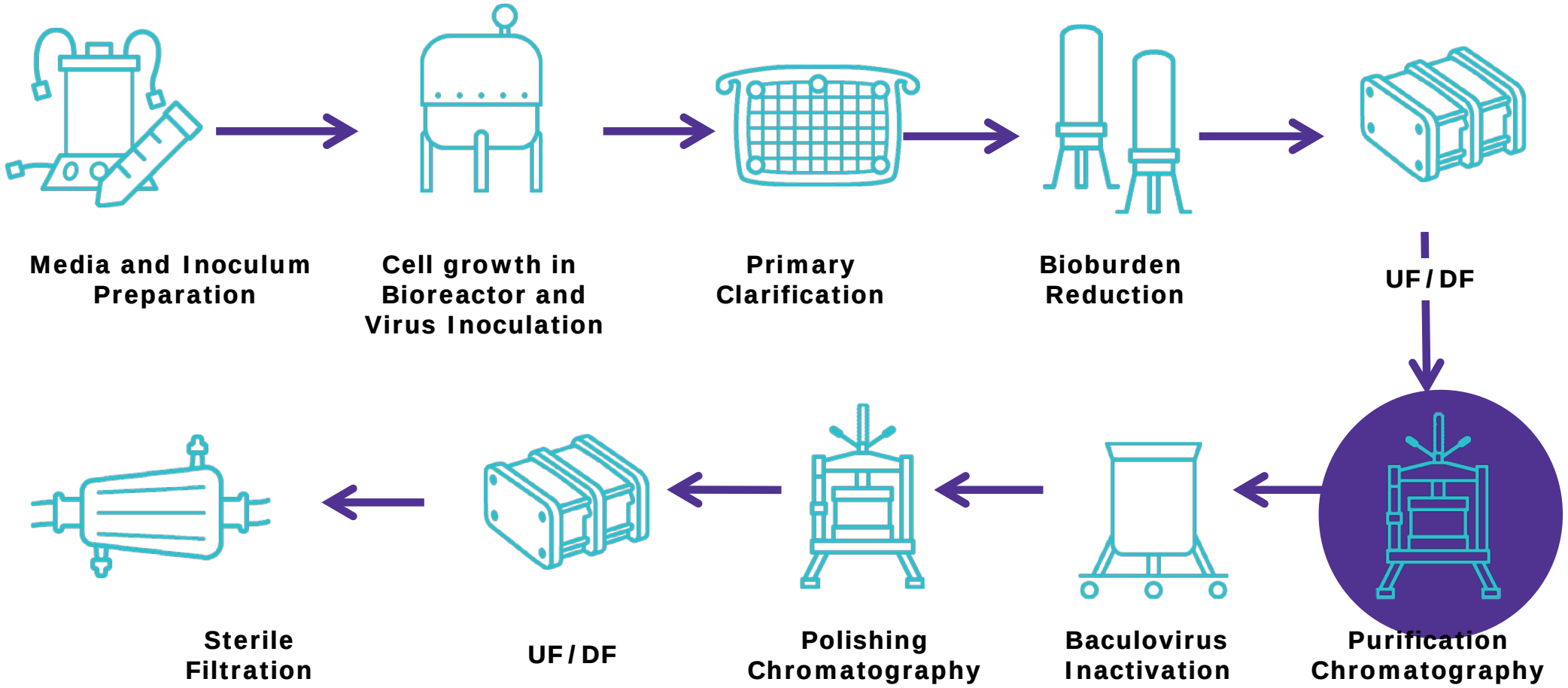
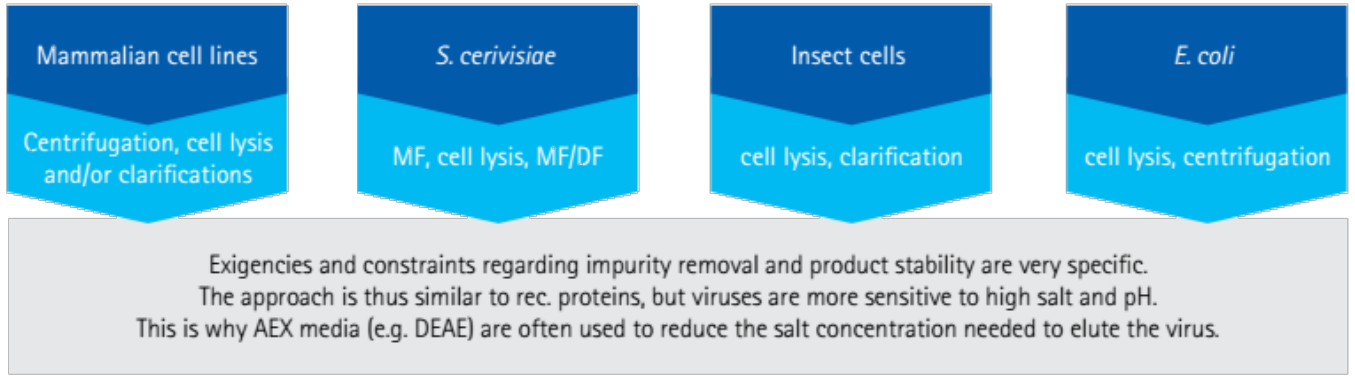
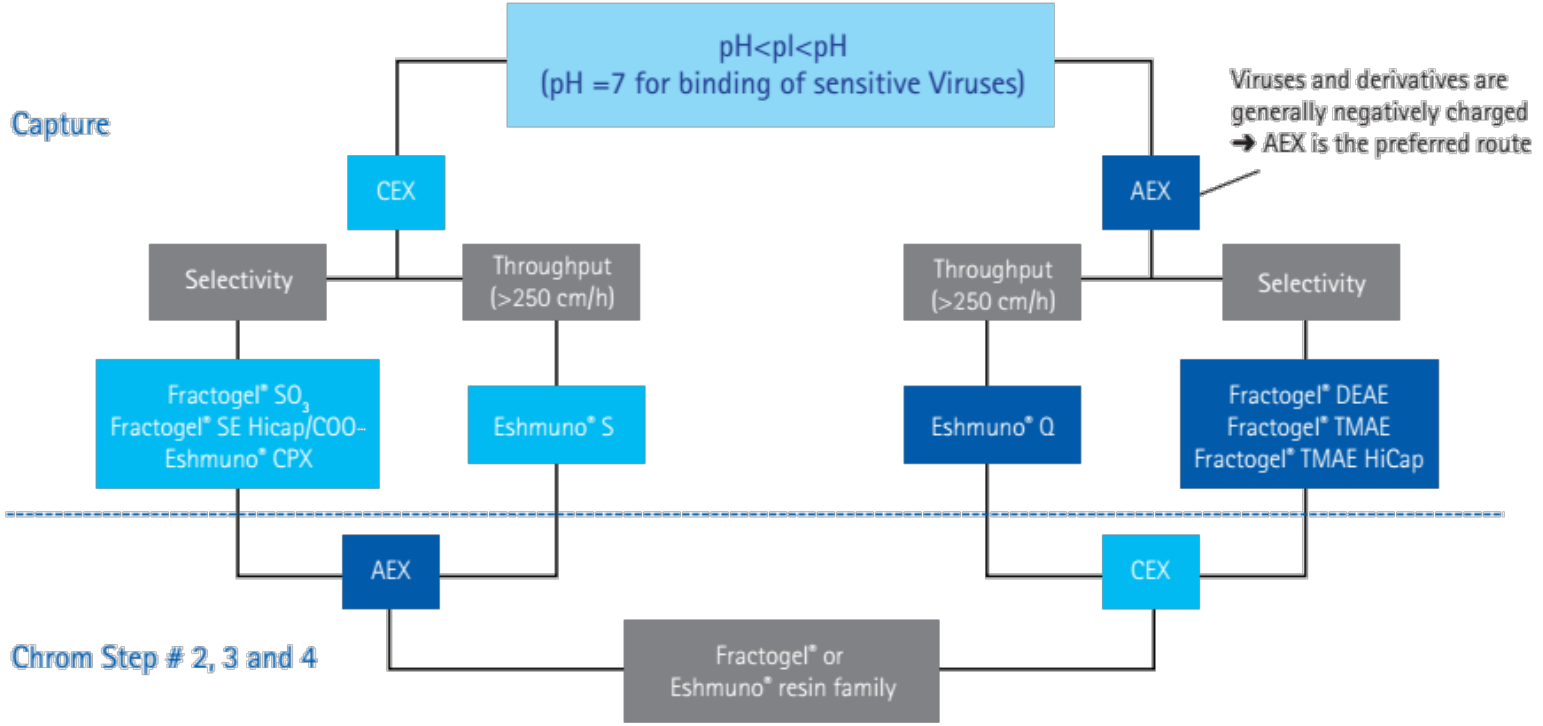
Production & Purification of VLP-Based Vaccine

Insect Cell / Baculovirus VLP Production Platform

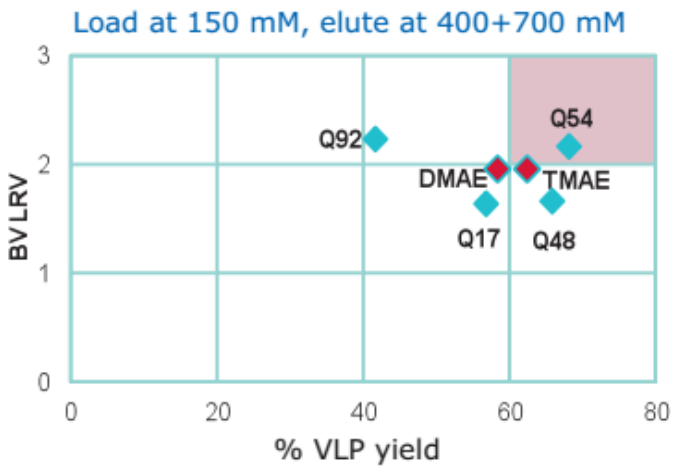


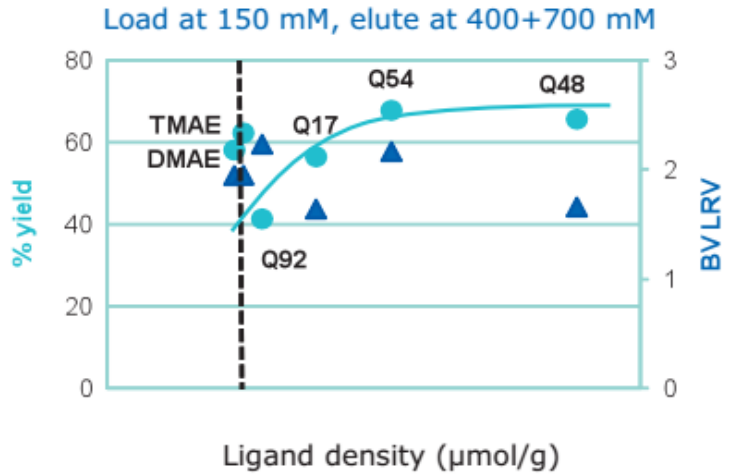
Purification strategy
Resin Selection





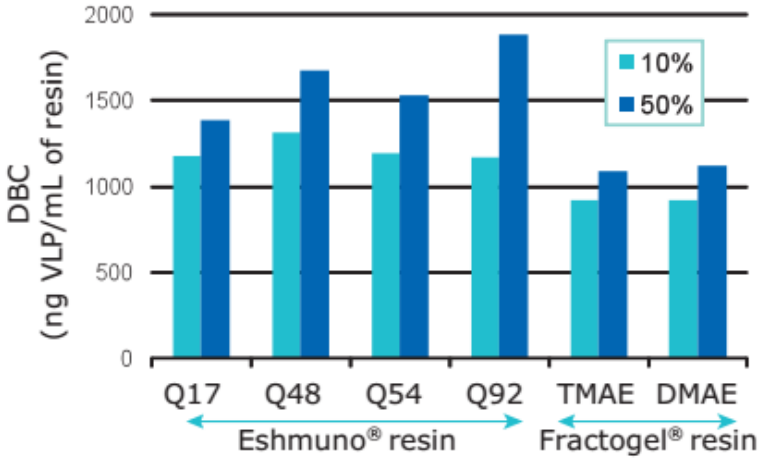
Anion Exchange Chromatography (AEX) for VLP Purification
Resin Selection

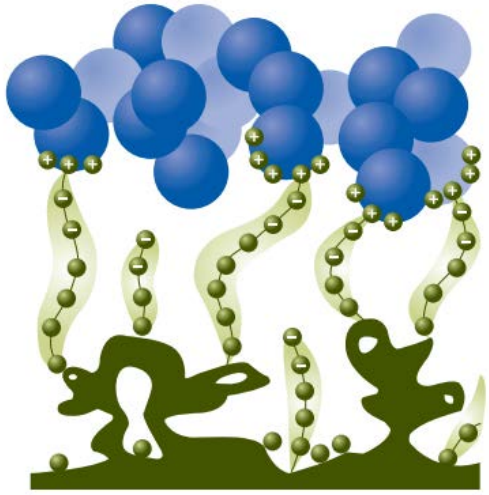




Fractogel® resin – red; Eshmuno® resin – blue

Fractogel® resin – dotted line





Tentacle morphology
increases surface area and virus binding capacity

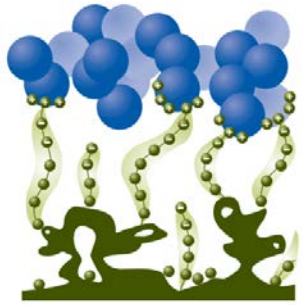
Anion Exchange Chromatography (AEX) for VLP Purification

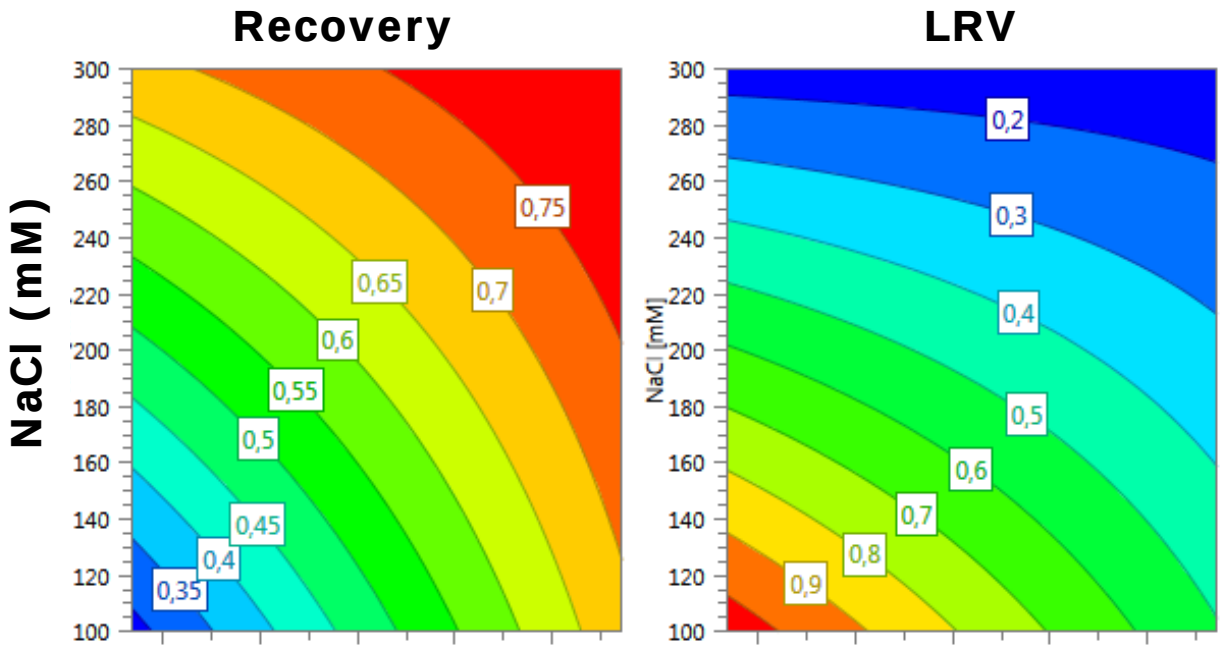
Separation of VLP from Baculovirus

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

Fractogel® TMAE

Anion Exchange Resin





Higher flow rate
OR
Higher load conductivity

AND

Higher recovery
Lower BV LRV

VLP-Based Vaccine Process

Optimal Purification Balance

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

Recovery

Purification

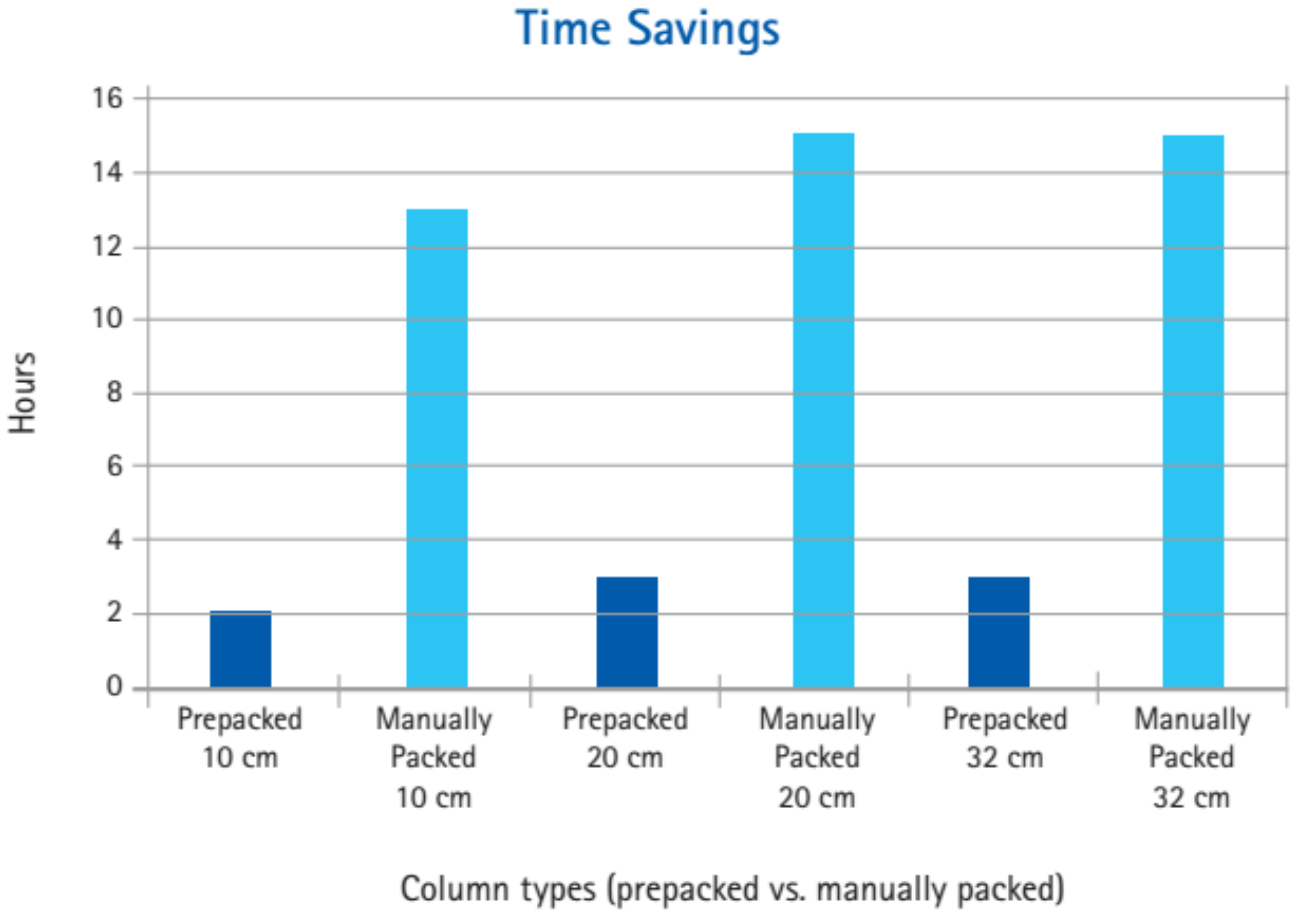
MERCK

VLP-Based Vaccine Process

Pre-Packed Chromatography Columns Add Flexibility

- Elimination of labor-intensive packing
- No capital investment
- No hardware to clean
- Fast setup and qualification

Chromabolt® Prepacked Chromatography Columns 10cm, 20cm, 32cm



Summary

1. Successfully used Mobius® 3L **disposable bioreactor** for production of VLP-based vaccine in insect cell culture system

2. 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**

3. Purified VLP by using Fractogel® **commercial AEX resins**

4. Integrated all the above components to create **a fully scalable process** that meets **recovery and impurity clearance** requirements.

Thank you

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