New trends in bioprocess of cell-based vaccines

Mats Lundgren, Customer Applications Director, GE Healthcare

Vaccine production technology trends

Vaccine bioprocess technology

- Platform technologies applied where possible (e.g., cell expansion on microcarriers and purification by chromatography)
- Single-use technologies and automated solutions
- Updated cell substrates—from eggs and diploid cells to continuous cell lines
- Live viral vector production—need for efficient platforms
- Process economy modelling implemented early in process development
- Focus on analytical technologies driven by increased regulatory requirements

Vaccine production today

Processes developed decades ago

Old cell substrates or eggs

Limited purification

Significant expertise required

Processes difficult to scale up

Fixed installations

Roller bottles

Unfavorable process economy

Low yields

Long process times

Labor-intense processes

Dedicated facilities

Increased regulatory requirements

Open handling

Batch variability

Serum supplementation

Vaccine production tomorrow

Processes developed decades ago

Platform cell lines

Efficient purification based on chromatography

Processes difficult to scale up

Scalable technologies enabled by, e.g., single-use technologies

Unfavorable process economy

Efficient and rational process design

Flexible facilites

Increased regulatory requirements

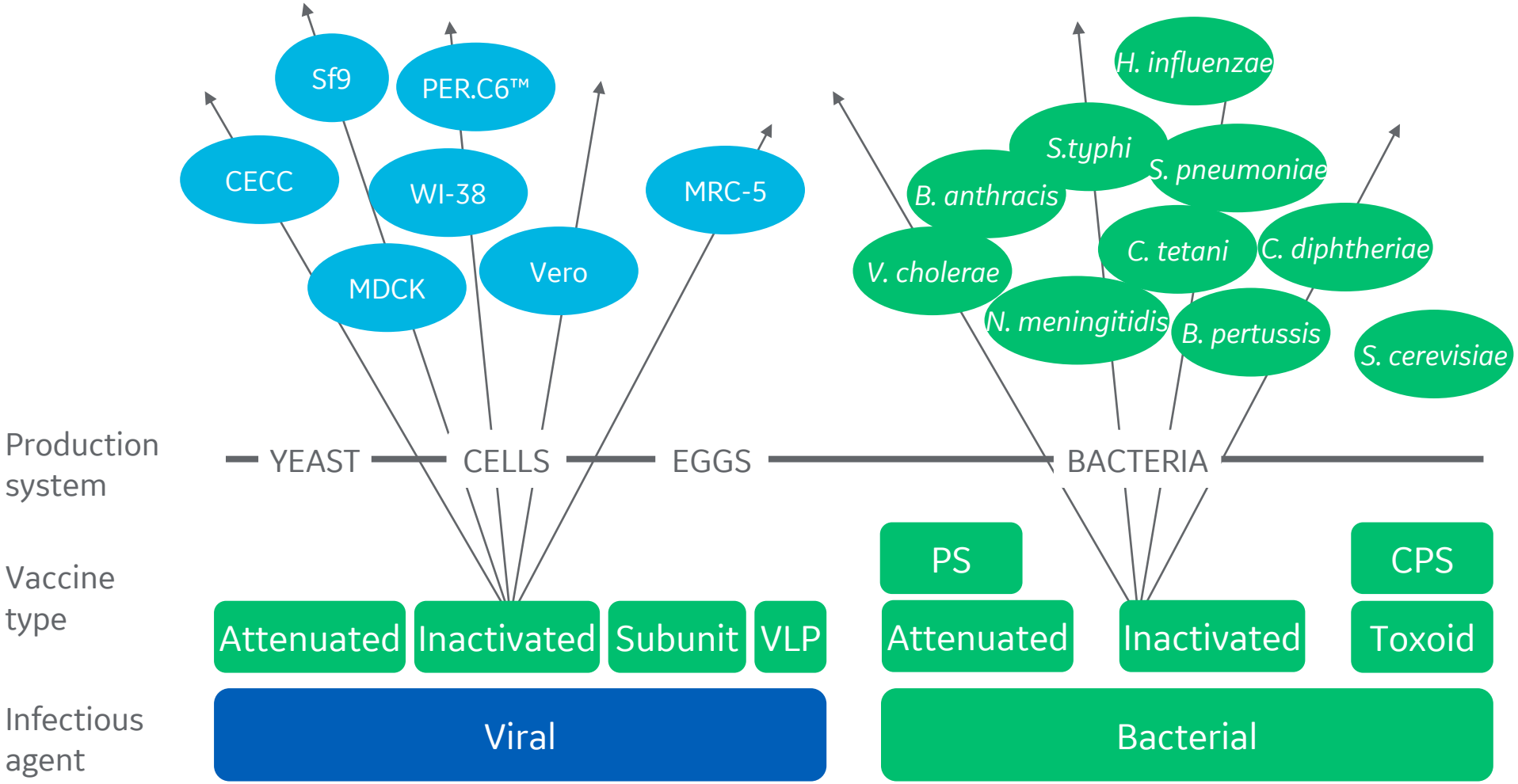
Closed handling

Quality by design (QbD)

Chemically defined cell culture media

Cell culture and virus propagation

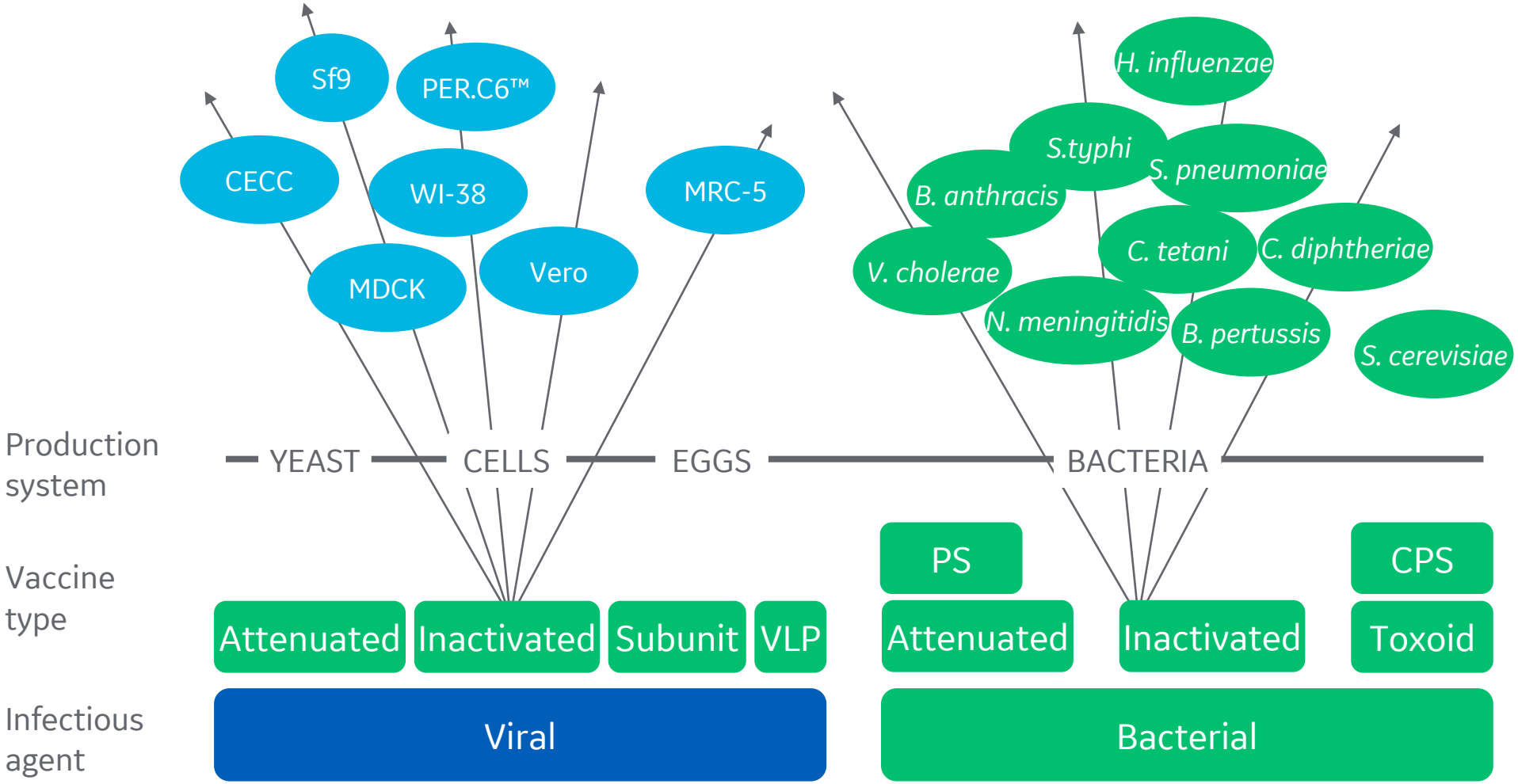
Cell substrates for vaccines



CPS = conjugated polysaccharides, PS = polysaccharides, VLP = virus-like particle

40 vaccines still to be developed

Where would this trend lead?



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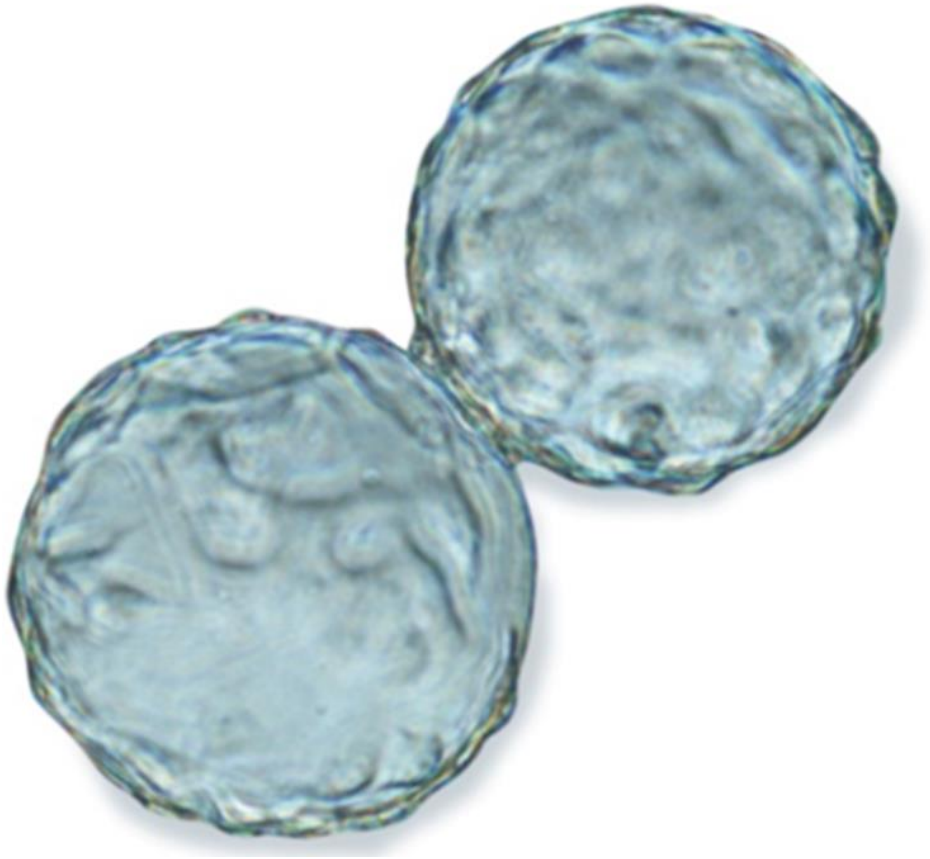
Selecting a cell line for virus production



Cell substrate evolution from primary to diploid to continuous cell lines

Modern options: Vero, MDCK, EBx, AGE, PER.C6™...

Requirements

- Suitable for GMP production
- Good safety track record
- Animal-origin free media preferred
- Good virus propagation
- Broadly and highly permissive
- Scalable to high-volume production

Cell culture medium and serum

Serum—ensure quality, traceability, and origin

Classical medium

Animal-origin free media

Complex media containing hydrolysates

Chemically defined media

Scale-up of adherent and suspension cells

Adherent cells

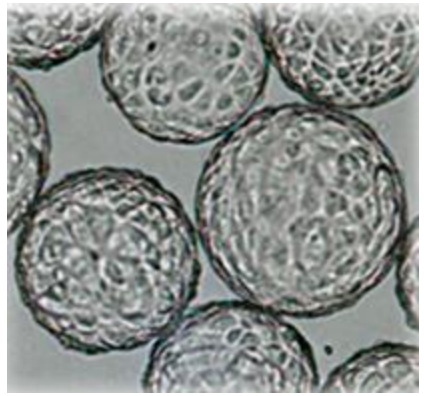
Cell growth is limited by surface area

Need enzymatic passaging

More complex scale-up

Higher virus production/cell

Microcarriers increase volumetric output by maximizing the surface to volume ratio for adherent cells



Suspension cells

Cell growth is limited by cell concentration in medium

Easier passage and scale-up

Lower virus production/cell

Introduction to Cytodex™ 1 and 3 Gamma microcarriers

Delivered gamma-sterilized and ready to use. Supplied dry to save storage space and facilitate transportation.

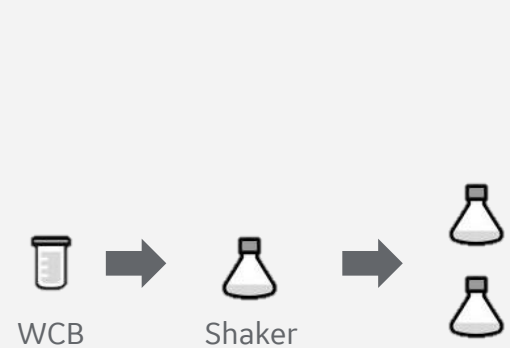
Conventional process:

Simplified process:

Adenovirus vector

AV vaccine production process

Upstream



Seed train
2–3 weeks

ReadyToProcess WAVE™ 25

Virus production

Downstream

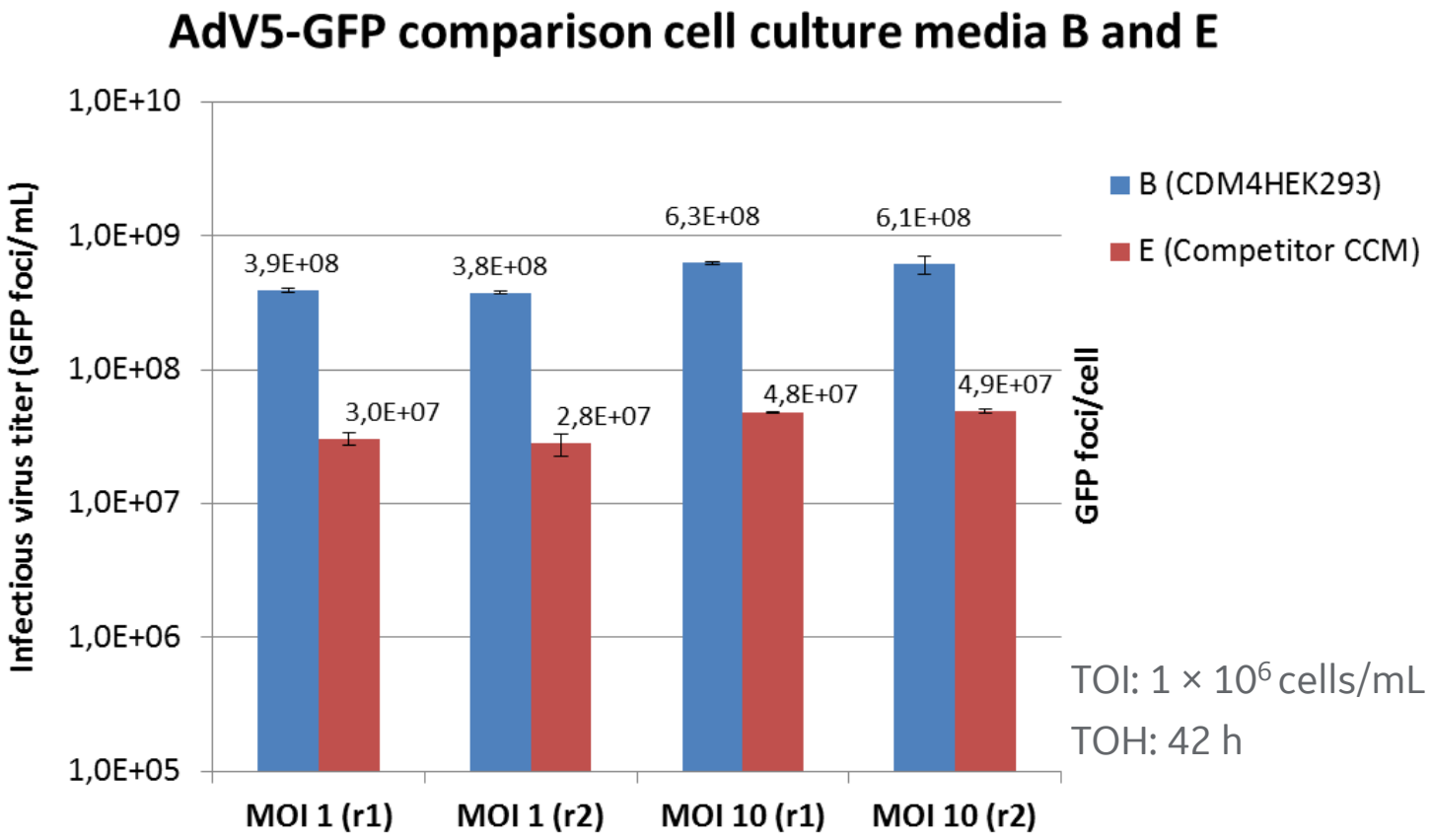
- **Cell lysis DNA fragmentation**
- **Clarification**
- **Conc. and buffer exchange**
- **Capture**
- **Polishing**
- **Conc. and buffer exchange**
- **Sterile filtration**

Analysis

Virus titer
% infected cells: flow cytometry
Virus infectious titer
$TCID_{50}$
Automated fluoresence microscopy
Total virus titer
qPCR
Nanosight™
Biacore™ system
Amersham™ WB system
Host cell
DNA: qPCR
Protein: ELISA
Protein pattern: Amersham WB system

$TCID_{50}$ = 50% tissue culture infective dose, WCB = working cell bank, WVSS = working viral seed stockc

AdV productivity in CCM B (CDM4HEK293) vs E (competitor)



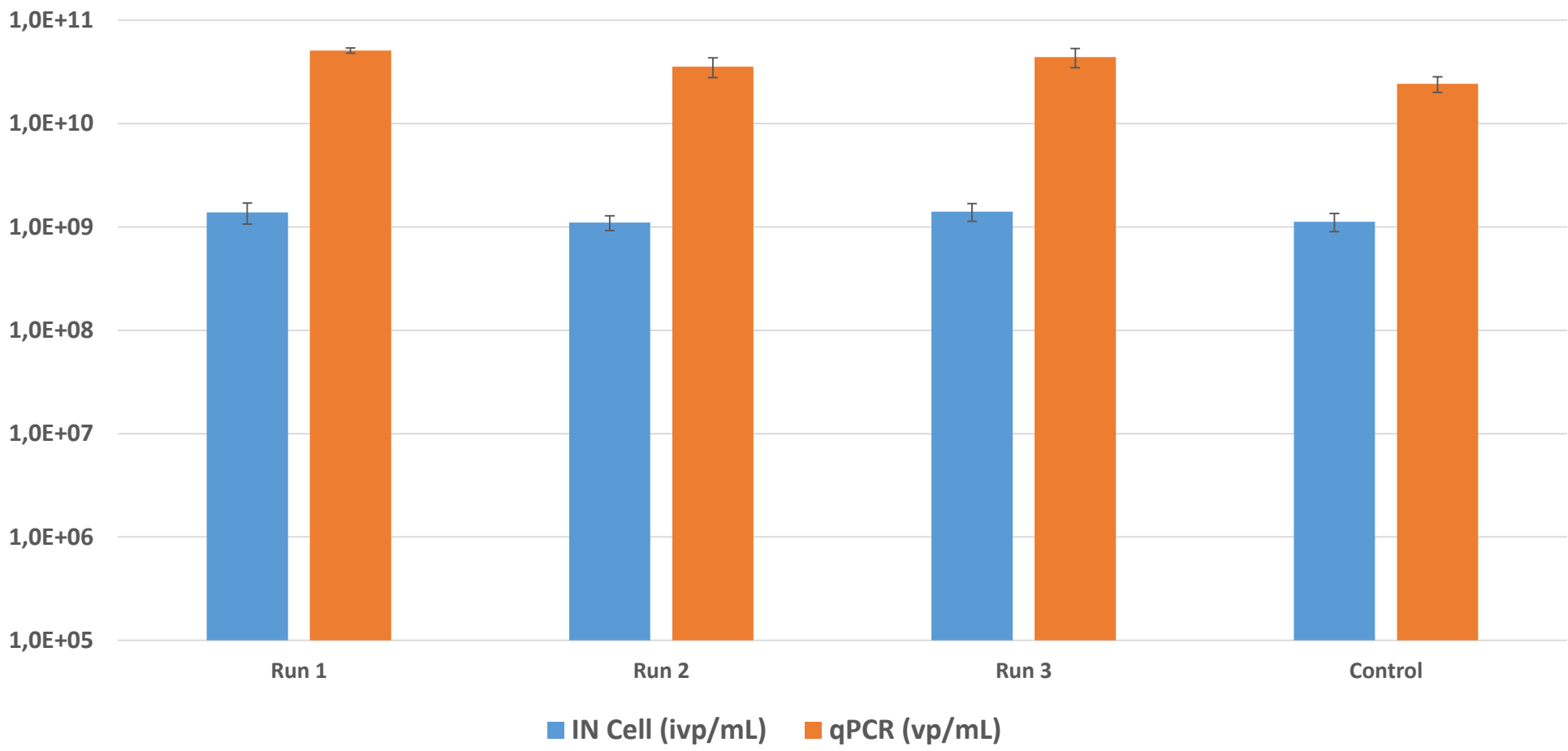
TOI: 1 × 10^6 cells/mL

TOH: 42 h

HyClone™ CDM4HEK293 media

AdV = adenovirus
CCM = cell culture medium
GFP = green fluorescent protein
MOI = multiplicity of infection
TOI = time of infection
TOH = time of harvest

Consistent adenovirus production in single-use Xcellerex™ XDR-10 bioreactor system



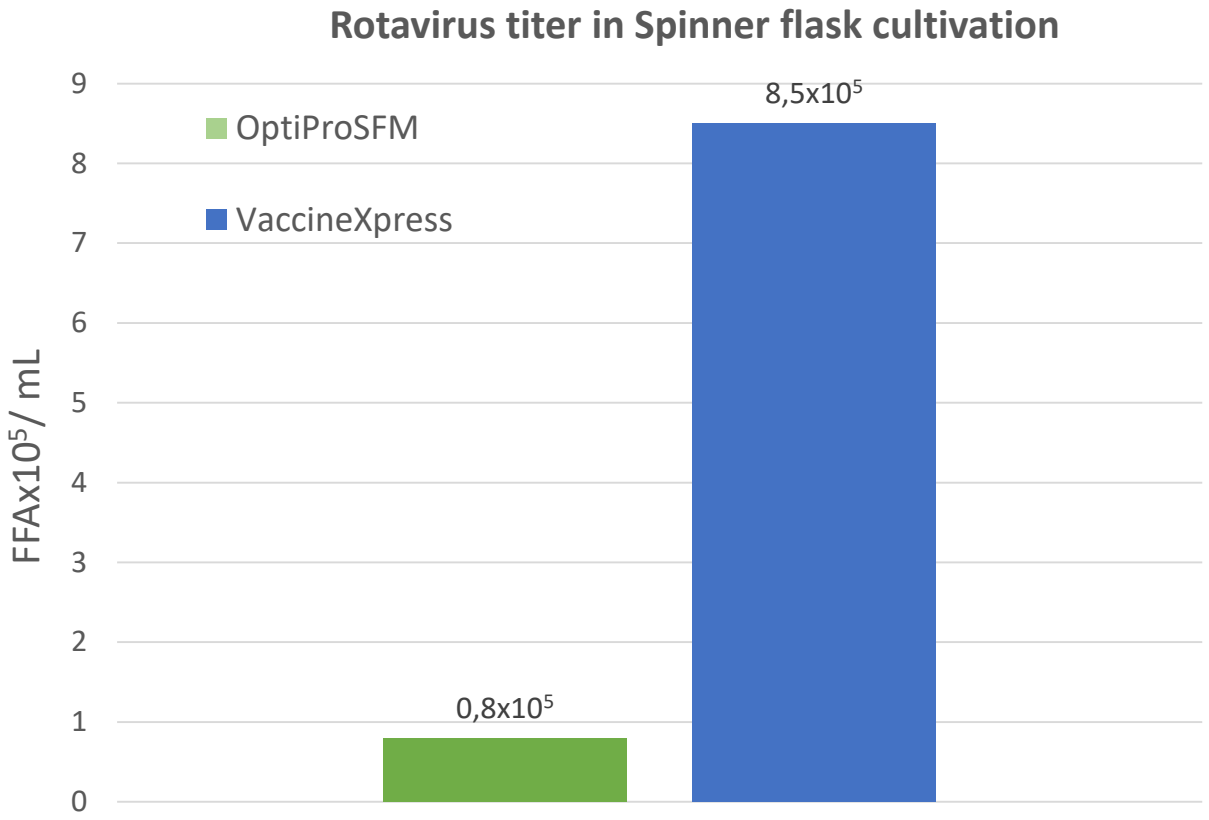
Rotavirus

Rotavirus vaccines

- Common cause of diarrheal disease in young children
- 200 000 deaths in children under 5 years of age annually, majority in Africa and Asia (data estimated from 2013)

- Vaccines on the market: eg. Merck, GSK, Bharat and Lanzhou
- Limited efficacy in developing countries
- Live attenuated oral vaccines produced in Vero cells
- Vaccines produced by old technology in T-flasks / Roller bottles using animal derived components (serum and trypsin)

Rotavirus can be propagated on Cytodex 1 Gamma using VaccineXpress



Competitor medium

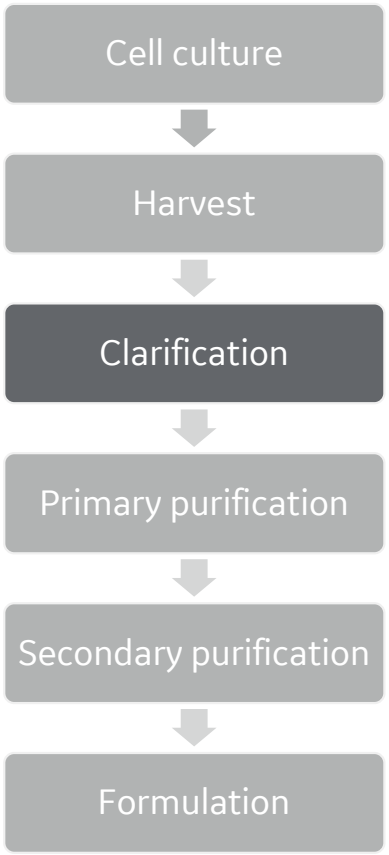
VaccineXpress medium

Rotavirus expression (IN Cell)

Virus purification

Clarification

Process flow



Avaliable techniques

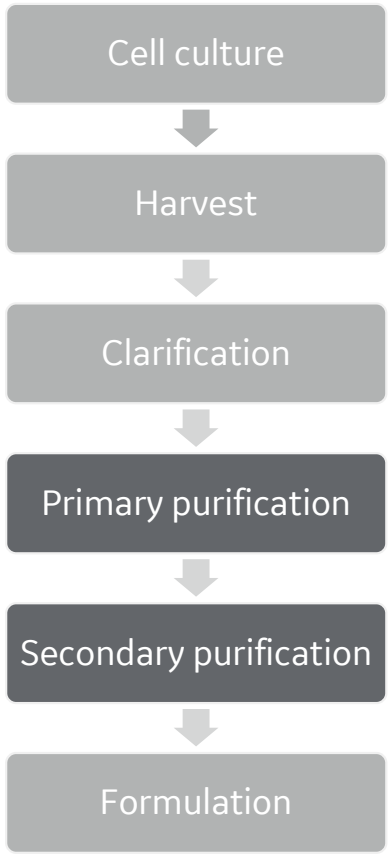
Filtration

- Normal flow
- Tangential flow (TFF)—hollow fiber filters

Centrifugation

Purification

Process flow



Avaliable techniques

TFF—hollow fiber filters

Density gradient centrifugation

Selective precipitation

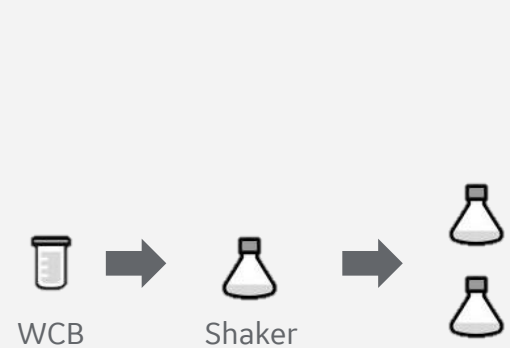
Chromatography

- IEX, MM, AC, HIC, SEC
- Resin format (packed bed)
- Membrane format (capsule), ReadyToProcess™ Adsorber Q

AC = affinity chromatography, HIC = hydrophobic interaction chromatography, IEX = ion exchange chromatography, MM = multimodal chromatography, SEC = size exclusion chromatography

AV vaccine production process

Upstream



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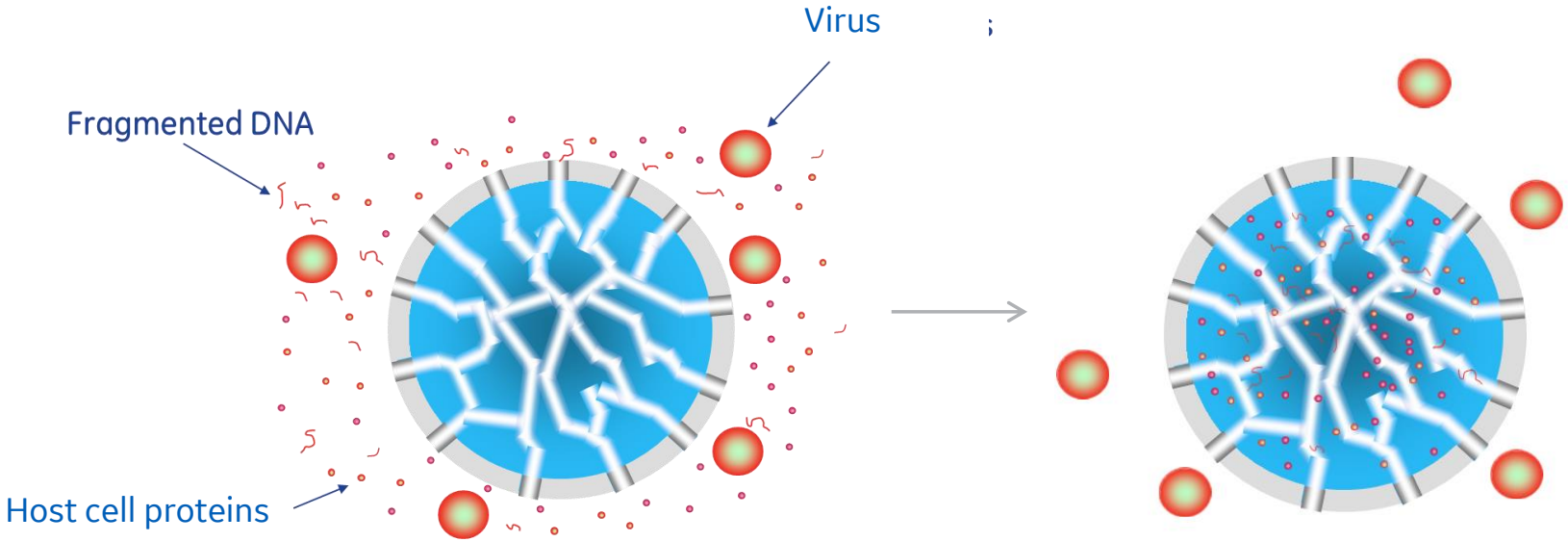
Core bead chromatography: host cell proteins and DNA fragments bind to the core and viruses stay in the void

Modern alternative to SEC

Easily scalable and suitable for single-use chromatography



SEC = size exclusion chromatography

Application examples core beads

Influenza

- Egg-based
- Cell-based

Dengue, Zika, and other flaviviruses

Lentivirus

Adenovirus

Cytomegolavirus

Respiratory syncytial virus

Poxvirus vectors

Polysaccharide conjugates

VLPs, etc., dependent on size

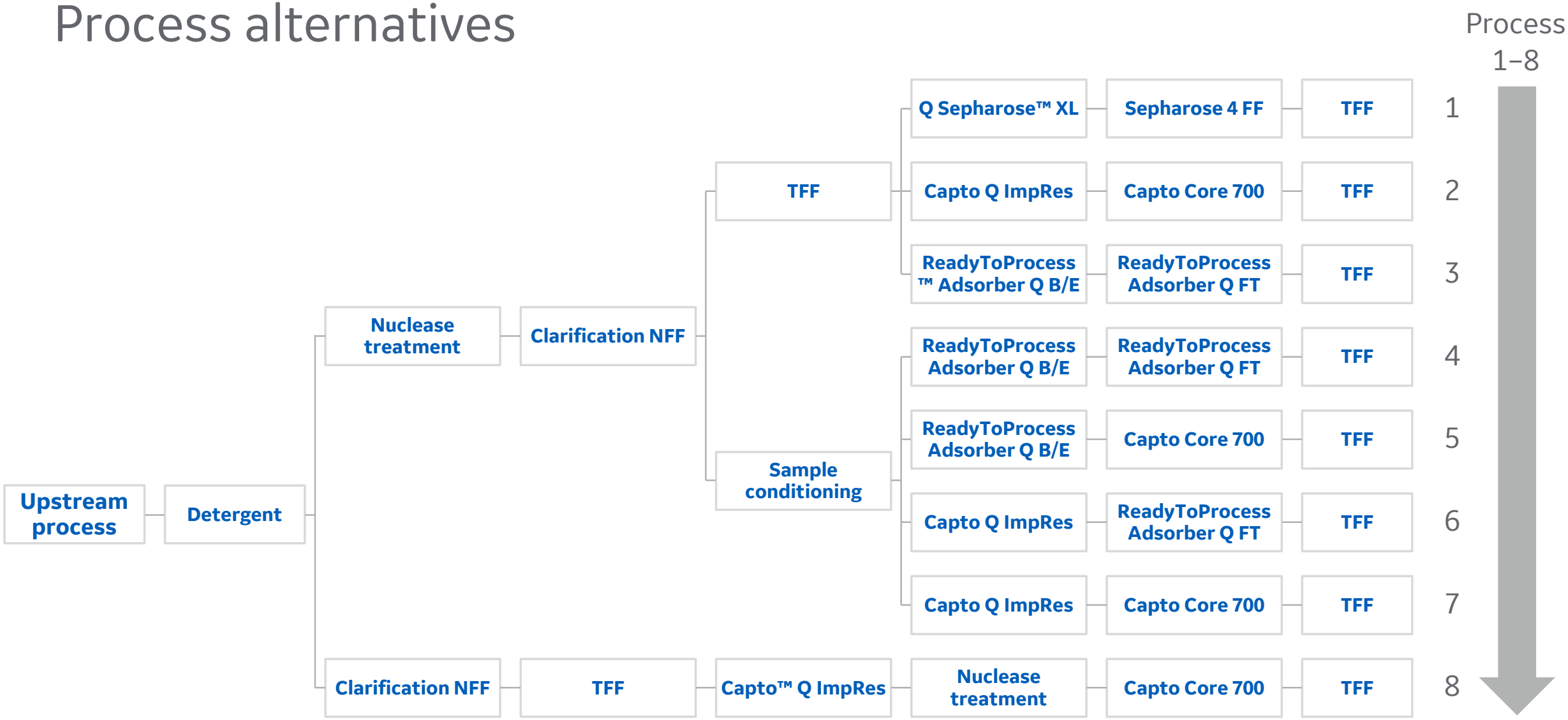
VLPs = virus-like particles

Cost breakdown of process steps and cost simulation of process alternatives

Economical considerations in early development

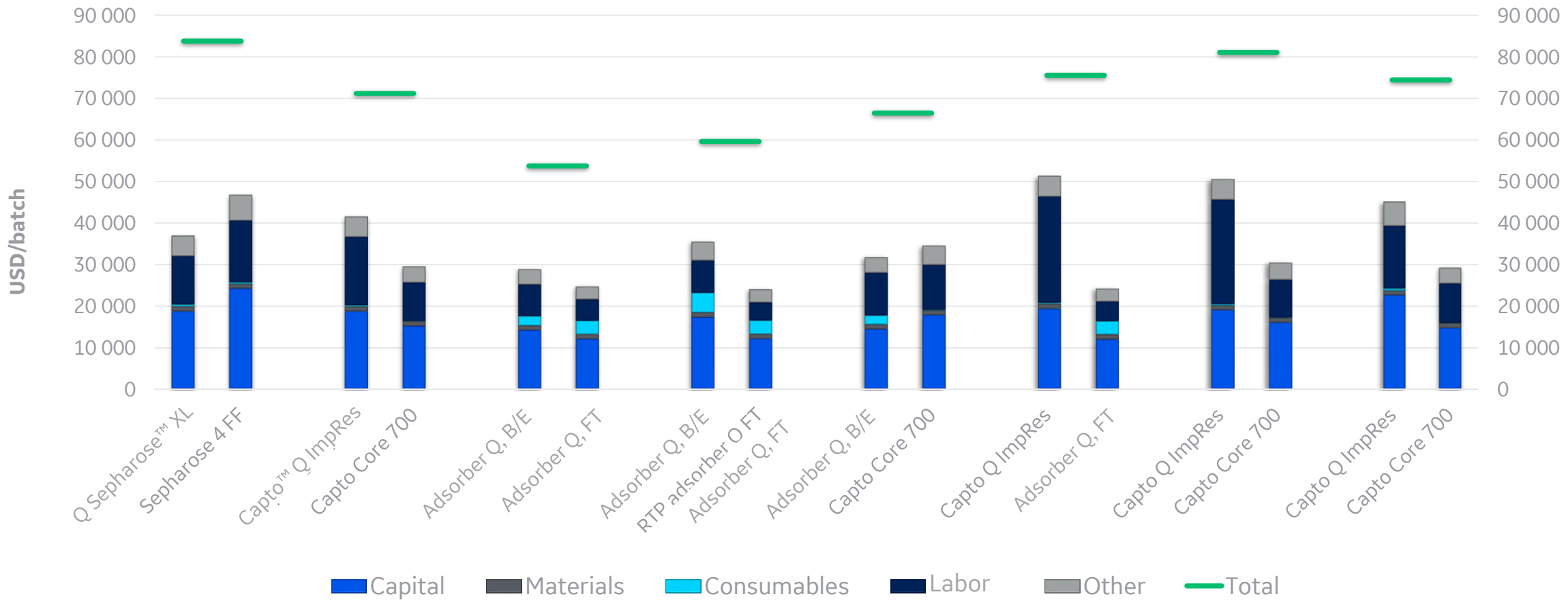
Litterature search	• Find unit operations for AV purification • Define suitable running conditions
Process modeling in Biosolve™	• Set up different process alternatives • Investigate different production scales
Evaluation of results	• Identify economically feasible unit operations to evaluate experimentally
Process development	• Start to experimentally evaluate low cost alternatives • Evaluate only high cost alternatives if needed for required purity

Process alternatives



B/E = bind-elute mode, FT = flow-through mode, NFF = normal flow filtration,

Contributing cost factors



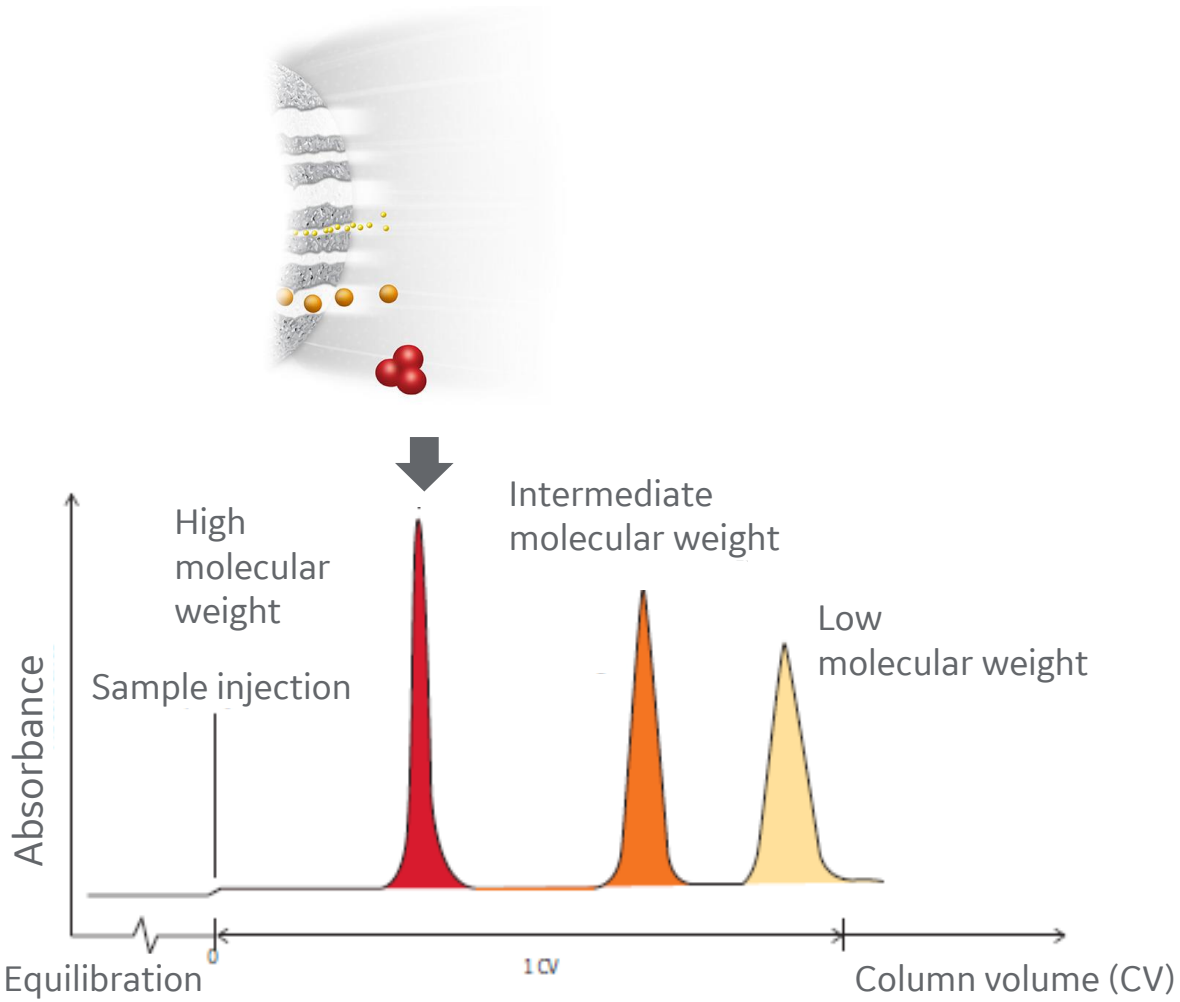
Evaluation of productivity for modernizing a vaccine process with a different purification technique

Study objectives

Evaluate the effect on productivity
by replacing a SEC step with a core bead chromatography step in a vaccine process at different production scales

SEC = size exclusion chromatography

Principle of SEC



Exluded from pores

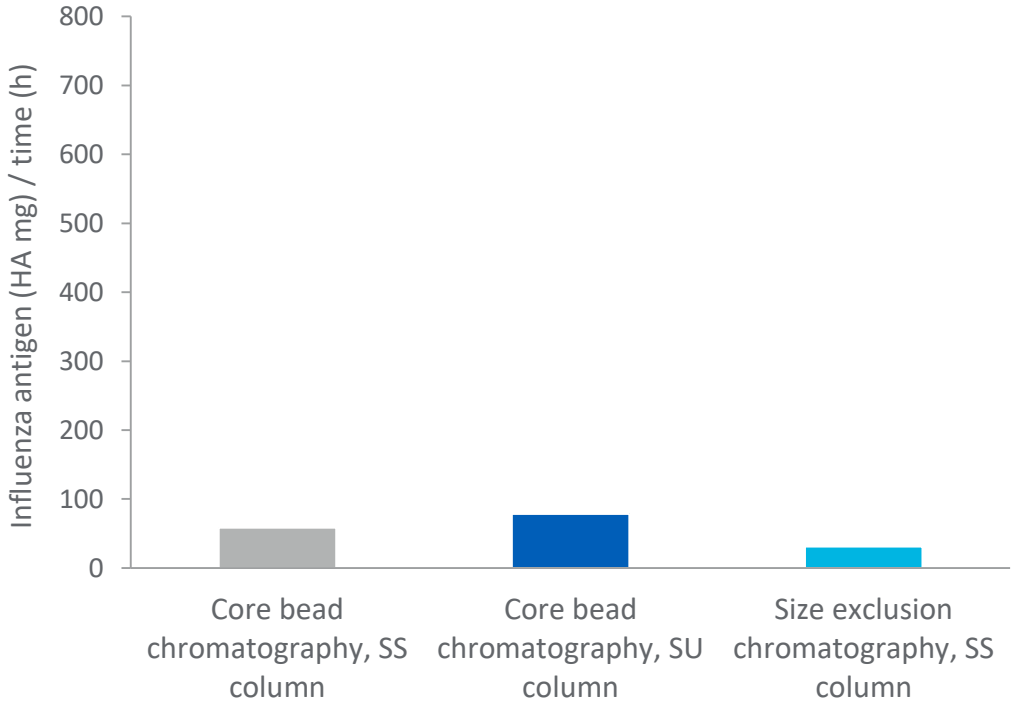
Enter a fraction of the pores

Enter all pores

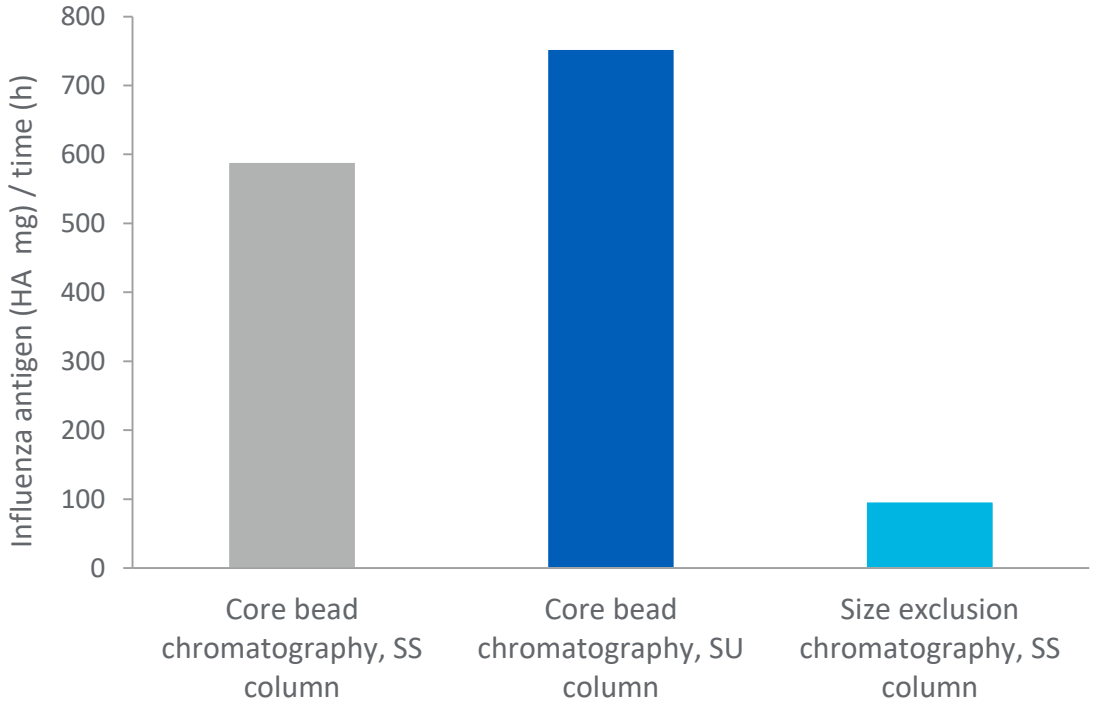
SEC = size exclusion chromatography

Productivity for SEC and core bead chromatography

200-L scale



2000-L scale



HA = hemagglutinin, SEC = size exclusion chromatography, SS = stainless steel, SU = single-use

Conclusion

- Paradigm shift for vaccine production—from lab bench process to rational design incorporating process economy calculations early
- A combination of single-use membrane and resin technologies seems to yield beneficial economy overall
- Core bead technology can increase productivity as compared to SEC

End-to-end vaccine manufacturing solutions

Molecule design | Cell design and selection | Cell culture | Product recovery (2–3×) | Capture | Polishing | Drug substance | Drug product

Vero cells
grown on Cytodex™ microcarriers

Seed train bioreactor
(ReadyToProcess WAVE™ 25 system)

Virus production

Culture medium
(HyClone™ SFM4MegaVir)

Production bioreactor
(Xcellerex™ XDR systems)

Clarification
(ReadyToProcess™ hollow fiber filters operated through the ÄKTA readyflux single-use filtration system)

Chromatography
(ReadyToProcess columns operated through the single-use ÄKTA™ ready chromatography system)

Final filtration
(ReadyToProcess hollow fiber filters operated through the ÄKTA readyflux single-use filtration system)

Fast Trak services

Acknowledgement

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Elisabeth Wallby

Åsa Hagner McWhirter

Eva Blanck

Åsa Lagerlöf

gelifesciences.com

For local office contact information, visit **gelifesciences.com/contact**

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden