Vaccine Processing – an overview

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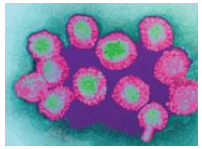
GE Healthcare Life Sciences

Imagination at work

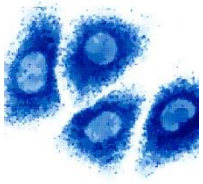
How Vaccines are manufactured

The Vaccines

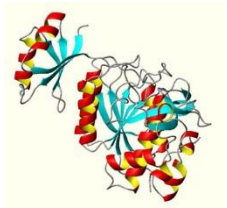
Bacteria based



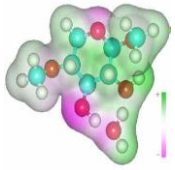
Virus based



Protein based



Polysaccharide based



DNA based

The Manufacturing process

Cell culture / Fermentation

⬇

Purification

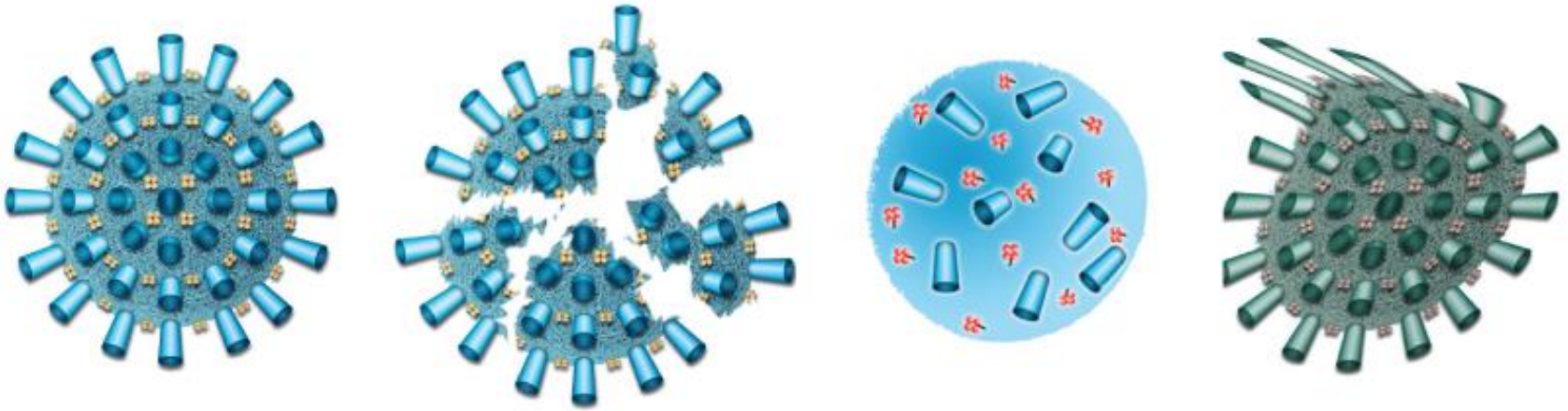
⬇

Fill and Finish

Analysis (QC/QA)

Number and order of the different steps depends on the specific vaccine production

Different types of marketed influenza vaccines



Whole virus | Split virus | Subunit | Live attenuated

The evolution of vaccine processes

1st generation processes:

Focus on upstream, optional inactivation

2nd generation processes:

Separations based on centrifugation, filtration

Currently developed processes:

Quality based approach: Quality by Design

Focus on process understanding of entire process incl. purification and virus safety

Outline of presentation

Cell substrates for virus production

Cell culture using Microcarriers

Downstream purification of vaccines

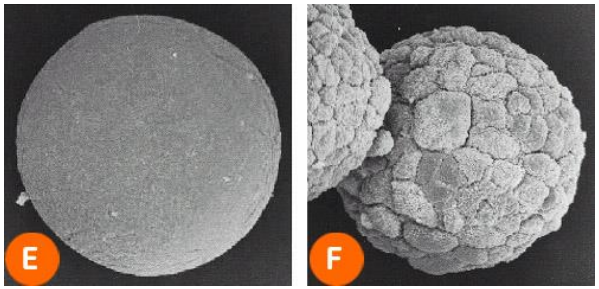
Modernizing legacy Vaccine processes

Conclusions

Cell substrates for virus production

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
  - Good virus propagation
  - Broadly and highly permissive
  - Scalable to high volume production



from: Pereira et al. Biotech Bioeng; 2004; 85; 5

MDCK and Vero cells

	MDCK	Vero
+	• Higher productivity • Technically easier • Less risk for propagation of adventitious viruses	• Platform cell line (can be used for several virus vaccines) • Good safety record • Used for several marketed vaccines
−	• Potential tumorigenicity/ oncogenicity • New cell substrate • Restricted to influenza	• Lower productivity • Technically challenging • Potential propagation of adventitious viruses

Virus safety

EP citation:

*"**Seed lots/cell banks**. The master seed lot or cell bank is identified by historical records that include information on its origin and subsequent manipulation. Suitable measures are taken to ensure that no extraneous agent or undesirable substance is present in a master or working seed lot or a cell bank."*

Cell culture using Microcarriers

Scale up of adherent cell cultures

Increase volume

Increase number of units

Genetic Engineering News, 2007

One 2500 L bioreactor with a carrier concentration of 3 g/L (Cytodex™ 1) provides the same surface area as 40 000 roller bottles (850 cm^2/bottle)

Viruses produced in microcarrier cultures

- Adenovirus
- Bovine rhinotrachteritis
- Endogenous C type
- Equine rhinopneumonitis
- Foot and mouth
- Group B arboviruses
- HAV
- Herpes
- Influenza
- Japaneese encephalitis
- Marek's
- Papova virus
- Polio
- Polyoma
- Pseudorabies
- Rabies
- RSV
- Rous sarcoma
- Rubella
- Sendai
- SV40
- Sindbis
- Small pox
- Vaccinia
- Vesicular stomatitis

Cell culture media and serum

Serum - Ensure quality, traceability and origin

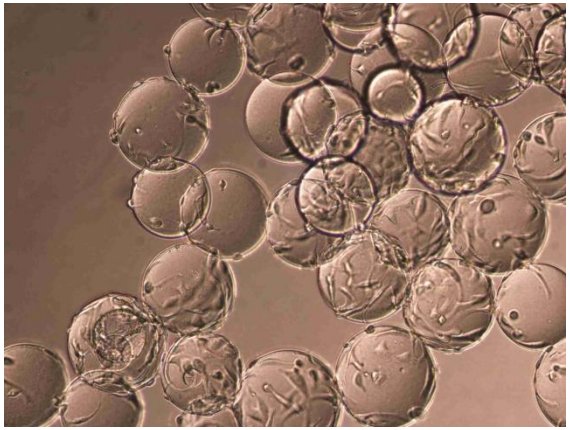
Classical media

Animal origin free media

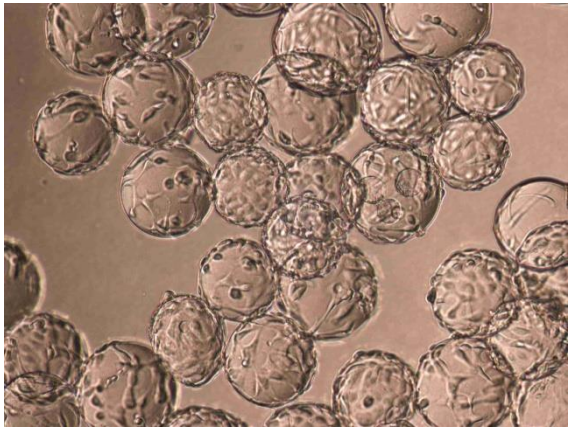
Complex media containing hydrolysates

Chemically defined media

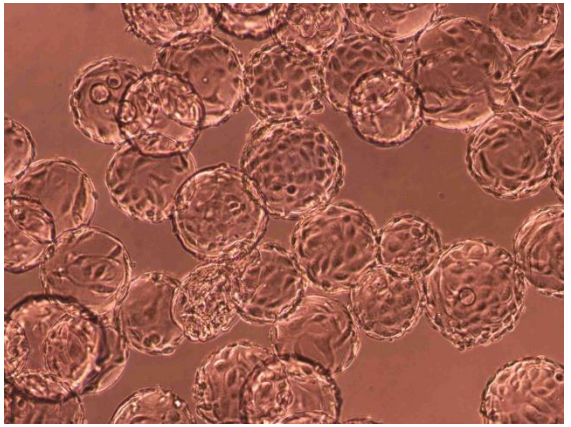
The effect of cell culture media



Medium 1



Medium 2



Medium 3

Bioreactors – Fixed vs Disposabled

Control and scalability

Stainless steel

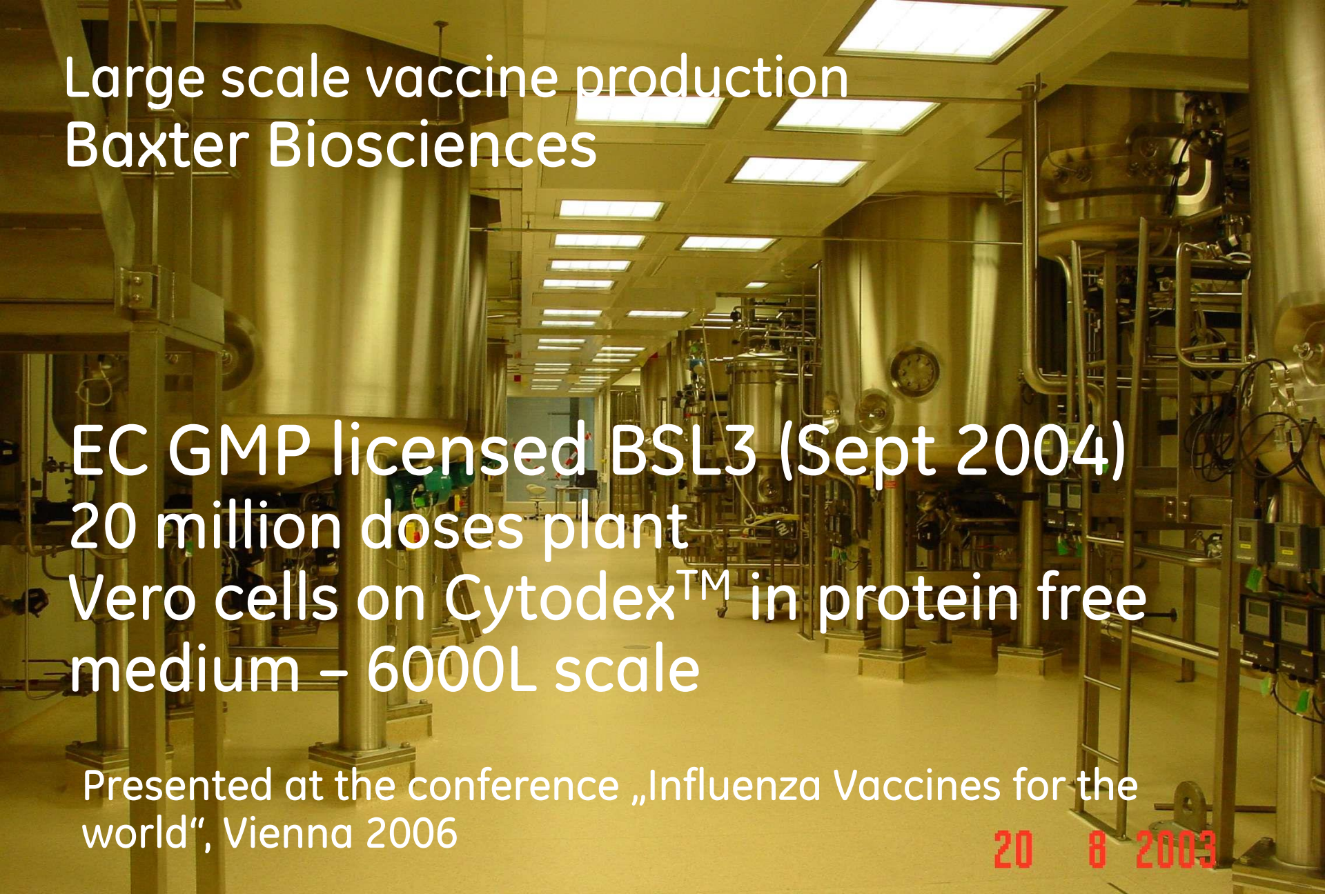
WAVE

10L

XDR

2000L


20 8 2003



Large scale vaccine production Baxter Biosciences

EC GMP licensed BSL3 (Sept 2004)
20 million doses plant
Vero cells on Cytodex™ in protein free medium – 6000L scale

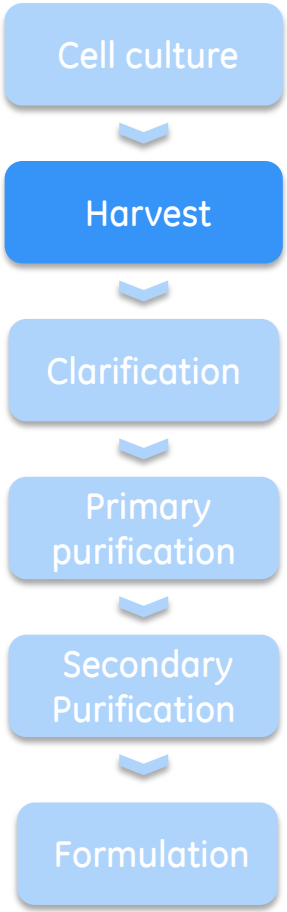
Presented at the conference „Influenza Vaccines for the world“, Vienna 2006

Downstream purification of vaccines

Downstream processing of viruses Available technologies

Harvest

- Lytic virus
- Non-lytic virus
  - Detergent
  - Mechanical disruption / Homogenization
  - Osmotic shock
  - Freeze-thaw

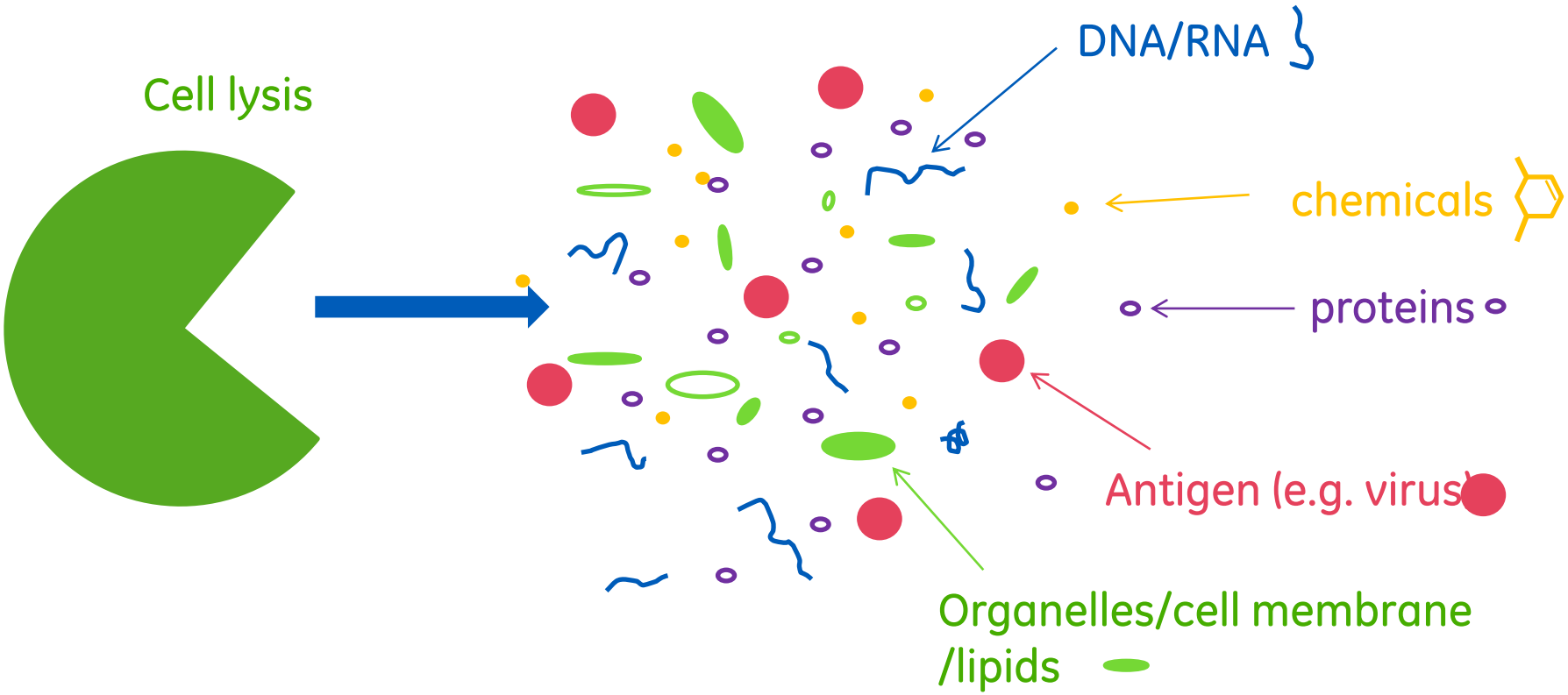


Safety and quality is priority

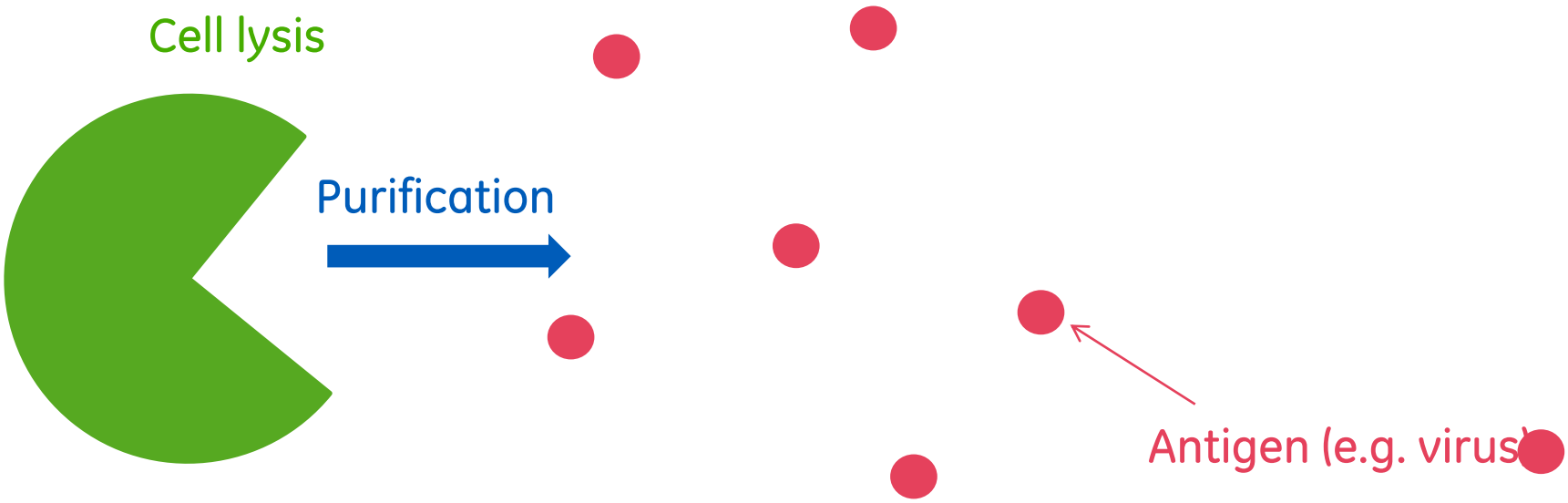
Regulatory requirements

- Safe vaccine with no or minimal adverse effects
- Effective dose
- Stability
- Process control
- Reproducable process

Impurity challenges after lysis



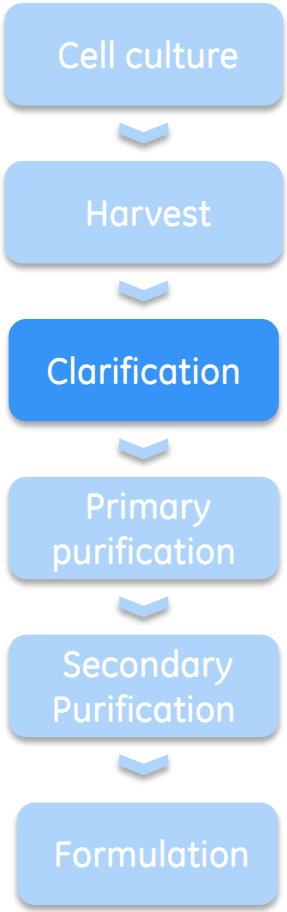
Goal with purification



Downstream processing of viruses
Available technologies

Clarification

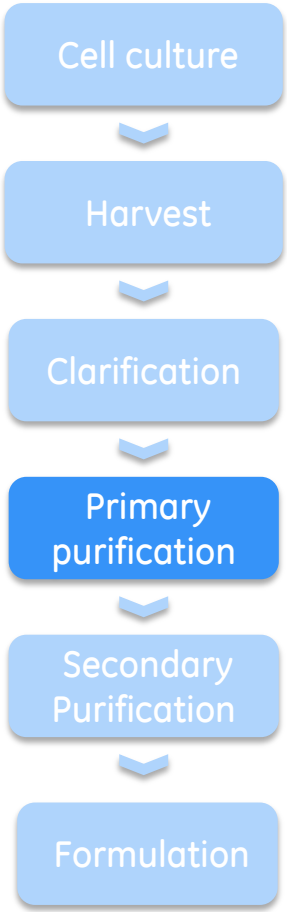
- Filtration
  - Normal flow
  - Tangential flow
- Centrifugation



Downstream processing of viruses
Available technologies

Primary purification

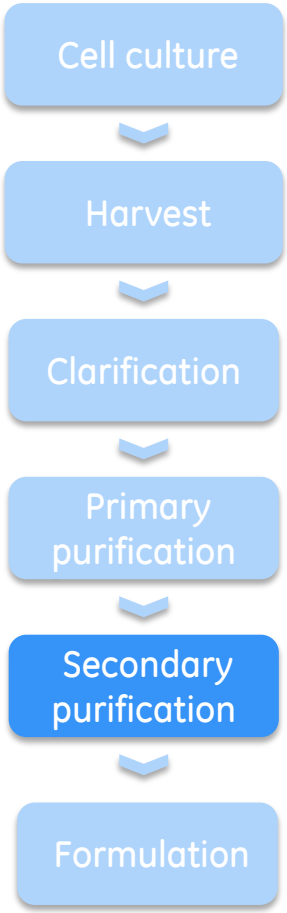
- Tangential flow filtration (TFF)
- Density gradient centrifugation
- Precipitation
- Chromatography



Downstream processing of viruses Available technologies

Secondary purification

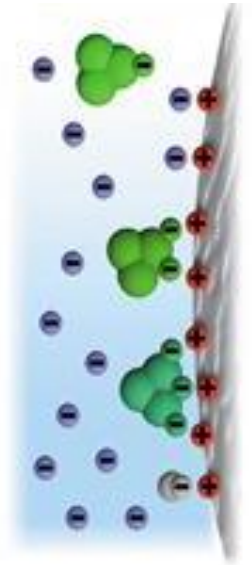
- Density gradient centrifugation
- Selective precipitation
- Chromatography
  - IEX, MM, AC, HIC, SEC
  - Bead format (Packed bed)
  - Membrane format (Capsule)



Ion exchange chromatography

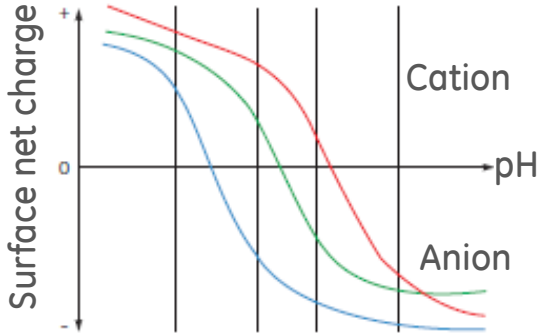
Anion exchange chromatography

- (-) Negatively charged molecules binds to (+) positively charged ligands

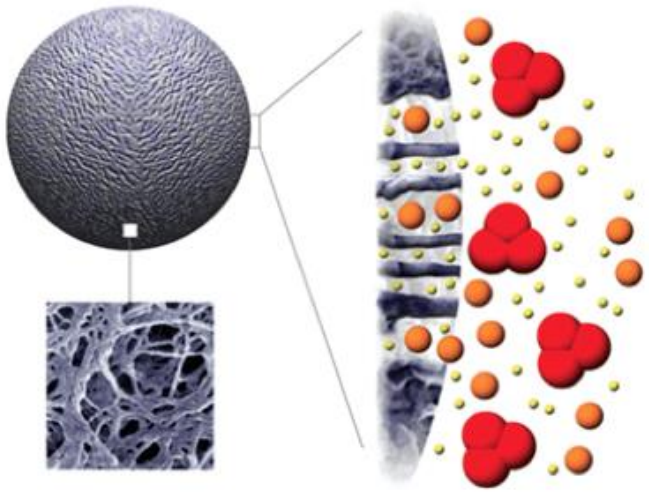


Cation exchange chromatography

- (+) Positively charged molecules binds to (-) negatively charged ligands



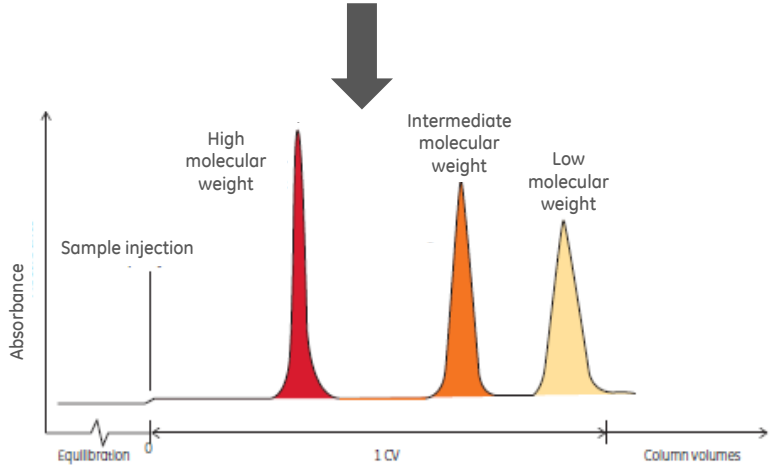
Size exclusion chromatography



Exluded from pores

Enter a fraction of the pores

Enter all pores



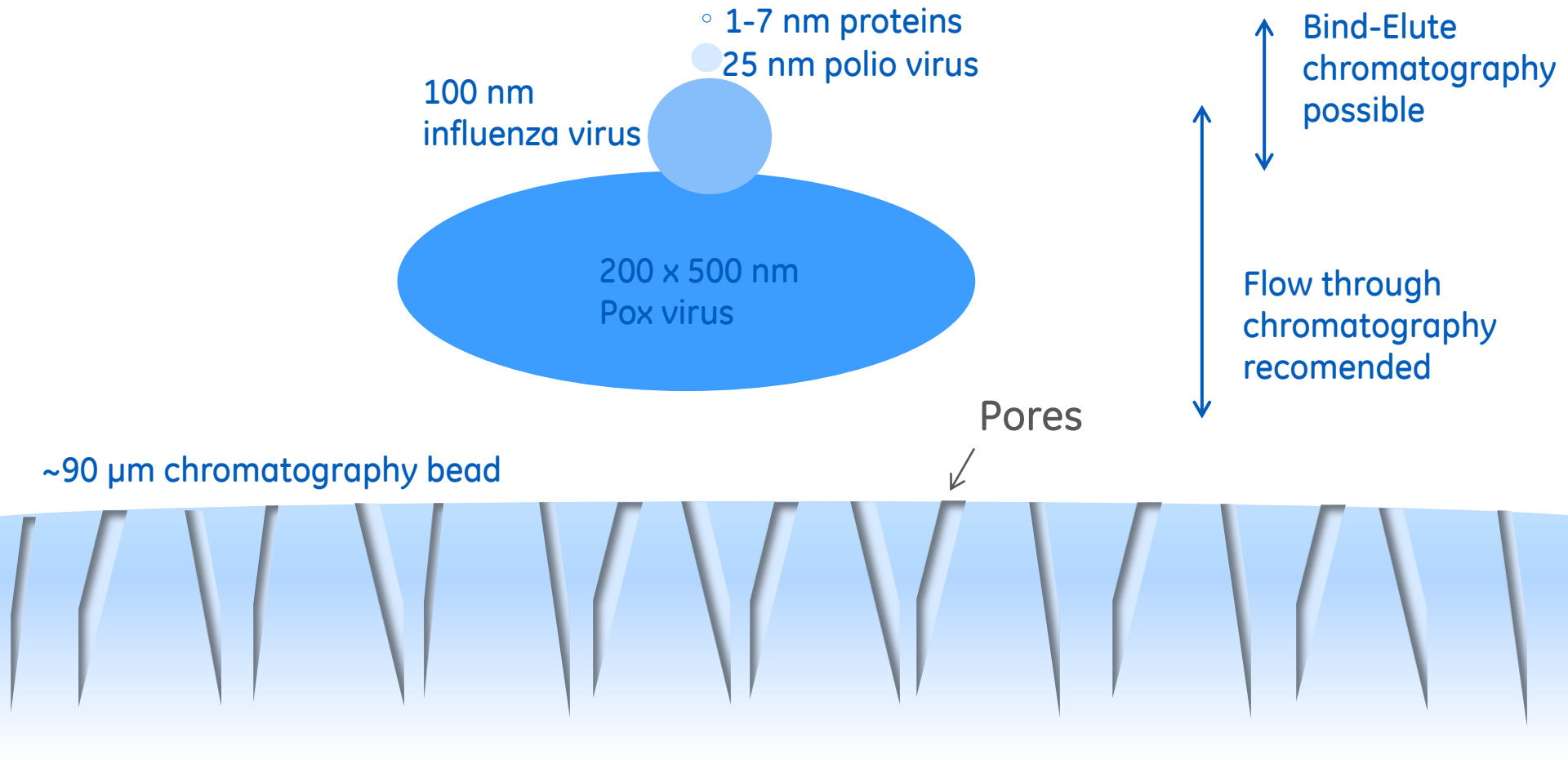
Affinity chromatography

Specific binding

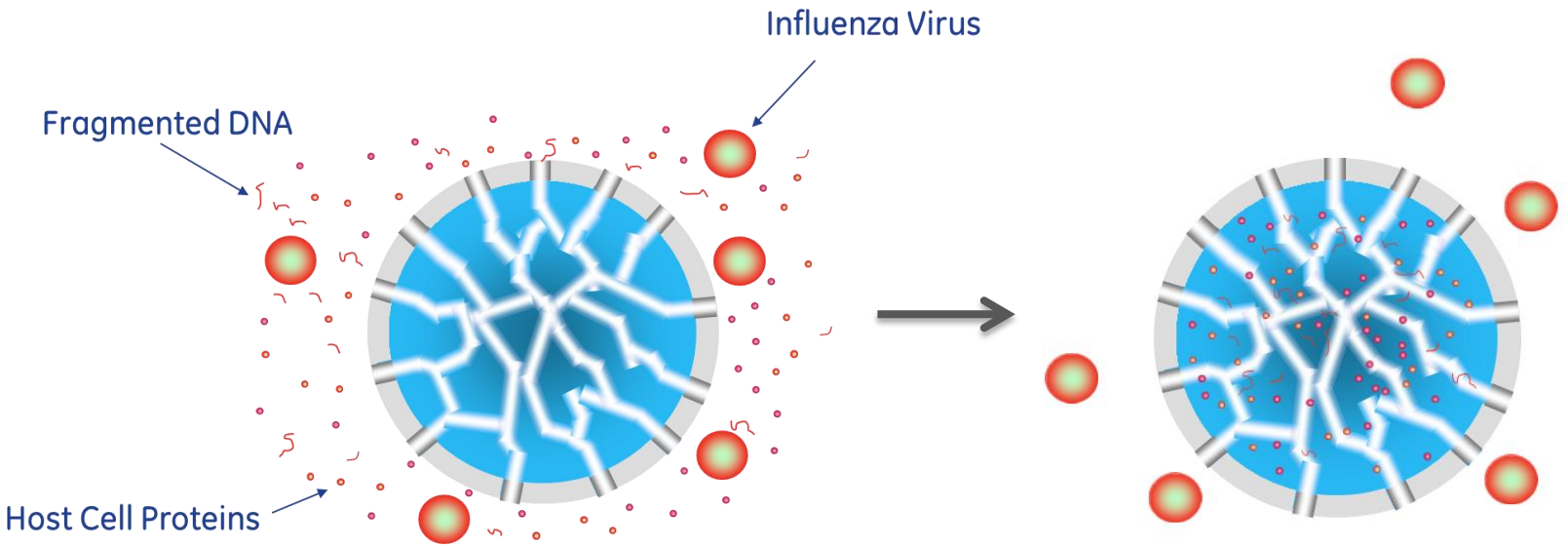
Few affinity resins available for vaccines

- Agarose based affinity resin for adeno associated virus
- Pseudo affinity resins for influenza
  - sulphated cellulose
  - sulphated dextrane

Chromatograpic purification of large molecules can be challenging



Core bead chromatography



- Host cell proteins and DNA fragments bind to the core and viruses stay in the void.

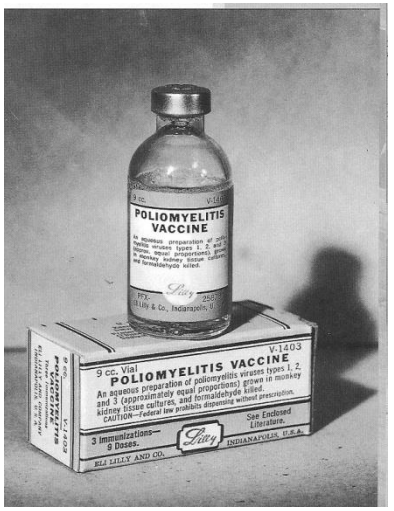
Process example

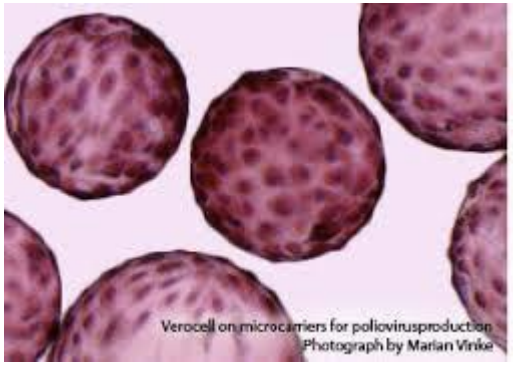
Polio IPV

Seed N-2 Cell expansion → **Seed N-1** Cell expansion → **Production bioreactor** Virus propagation → **Clarification NFF** Removal of cell debris and large particles → **TFF** Conc of polio virus → **SEC** Separation of polio virus from small molecular compounds → **AIEX (FT)** DNA removal. Polio virus in flow through → **Virus inactivation** formaldehyde → **Formulation** Sterile filtration, mixing with other strains

The history of Polio vaccine processes

- 1955: Inactivated Polio vaccine (IPV) launched (Salk Type)
- 1960: Attenuated Polio vaccine launched (Sabin type)
- 1960s: Collaboration between Prof. Van Wezel (RIVM/NVI Netherlands) and GE (former Pharmacia) around microcarrier cultures of primary monkey cells.
- 1970s: New IPV purification method using chromatography resins
- 1980s: Switch to Vero cell production
- 2010s: Updating the IPV processes using modern technology





Modernizing legacy Vaccine processes

A Modern Solution for Acellular Pertussis Vaccine

Whole-cell (wP) - Acellular Pertussis (aP)

wP Vaccines

70 year old technology based on killed *B. pertussis* strains

High protection efficiency ~78%

Associated with side effects and safety concerns

The reactogenicity of wP vaccine was thought to be too high to permit routine use in older children, adolescents and adults.

aP Vaccines

Introduced in 1990's

aP contain ≥1 of the separately purified antigens: pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae(FIM) type 2 and 3.

aP is now the dominant type in the industrialized world

aP containing vaccines with reduced concentrations of the antigen have been formulated for use in adolescents and adults

Ref: http://www.who.int/wer/2010/wer8540.pdf

Project Goal

Traditional process

Chinese pharmacopeia requirement and current situation

- Contain 2 antigens:
  Pertussis toxoid (PT),
  Filamentous Hemagglutinin (FHA)
- Purity >85% (SDS-PAGE)

Yield around 10%

Lack of stable antigen quantitative assay

Current Project

Develop a modern process for pertussis vaccine

- Contain 3 antigens:
  Pertussis toxoid (PT),
  Filamentous Hemagglutinin (FHA)
  Pertactin (PRN)
- Purity >95% (SDS-PAGE)

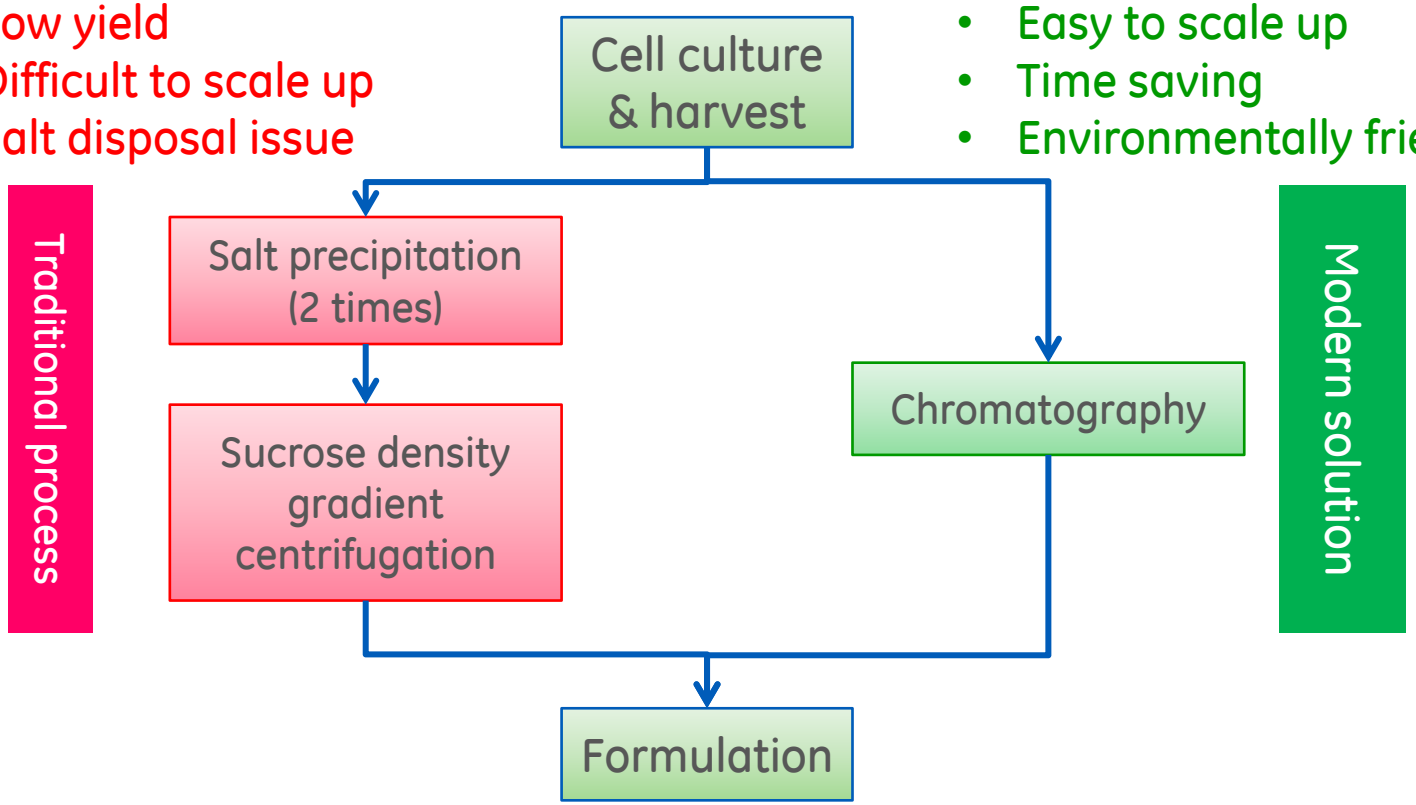
Yield >30%

Establish quantitative antigen determination using Biacore™ platform

Traditional Process vs. Modern Solution

Challenges
- Time consuming
- Unable to purify separate antigens
- Low purity
- Low yield
- Difficult to scale up
- Salt disposal issue

Advantages
- Able to purify separate antigens
- High yield
- High purity
- Easy to scale up
- Time saving
- Environmentally friendly

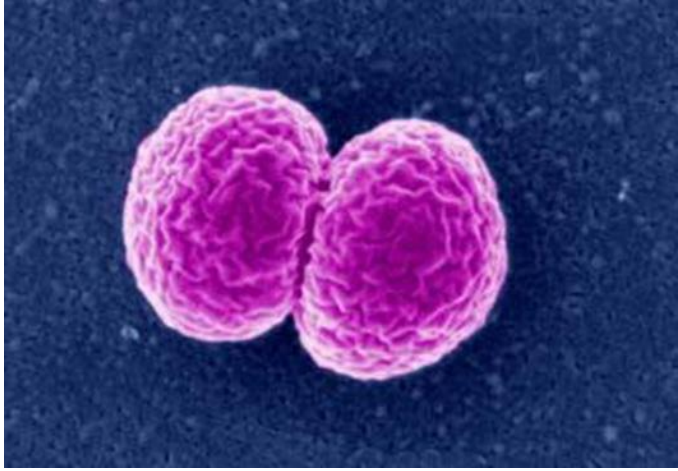


Process Highlights

1. Modern process to produce PT, FHA & PRN using bioprocess friendly, easily scalable, new generation chromatography platform.
2. Environmentally friendly.
3. Increase purity from 85% to >95%.
4. Reduce manufacture time from month to days.
5. Recovery increased from 10% to 30%.
6. Establish a sensitive, stable platform using Biacore to quantify PT & FHA.

Modern Process for Meningococcal Vaccine

Meningococcal Vaccine

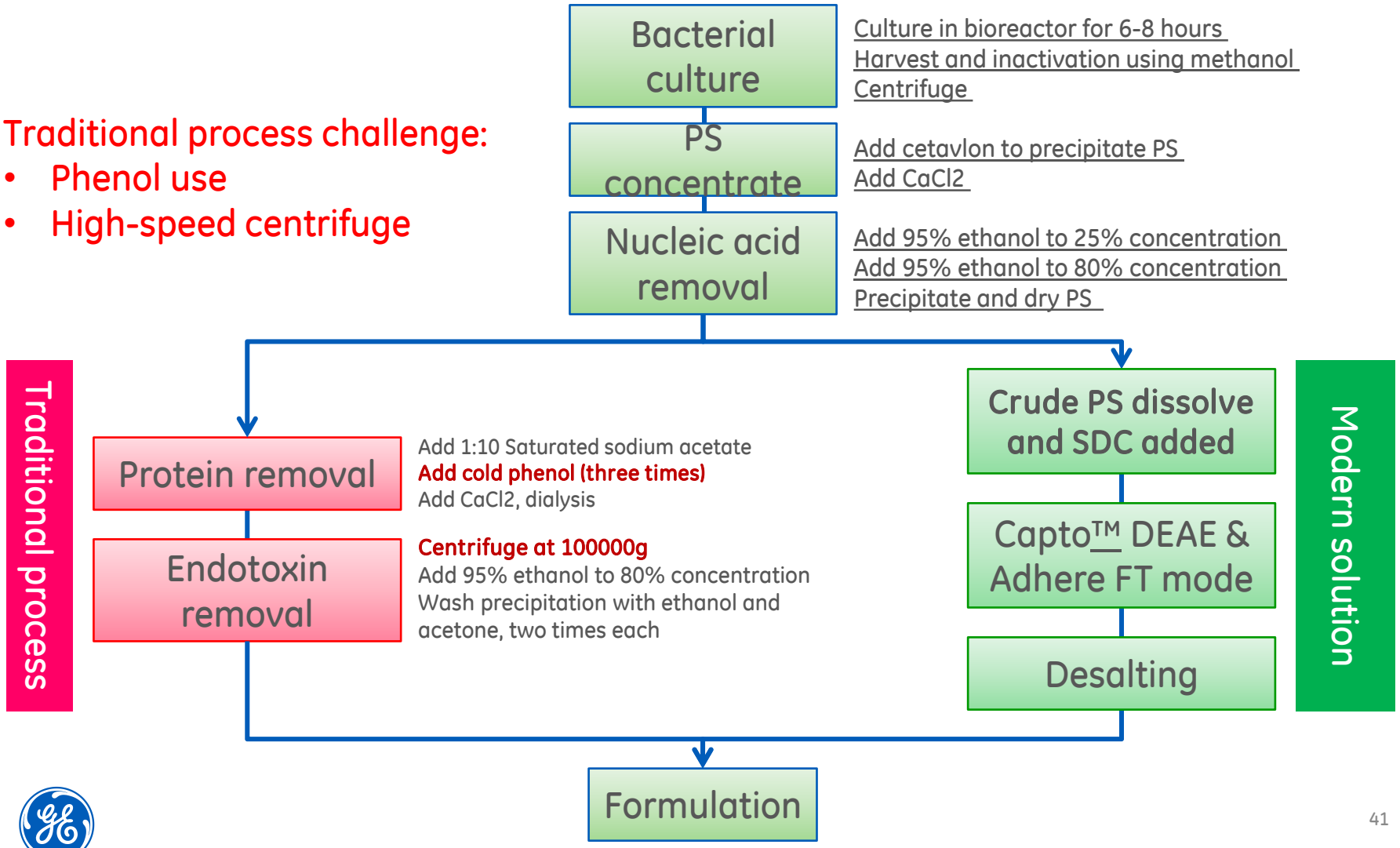


13 clinically significant serotypes. A, B, C, W-135, Y responsible for 90% of global cases

Vaccine for A, C, W, Y are produced using capsular polysaccharide (PS), conjugant technology to enhance immunogenicity

Traditional Process vs. Modern Solution

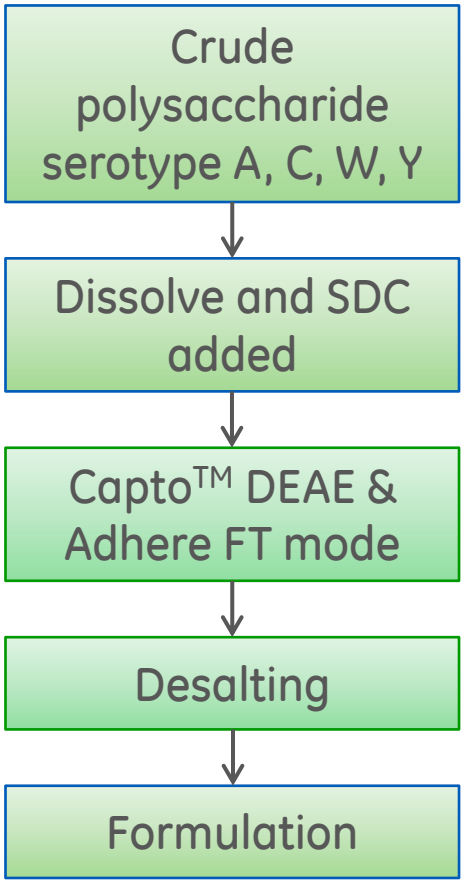
Traditional process challenge:
- Phenol use
- High-speed centrifuge



Modern solution for Meningococcal Vaccine A,C,W,Y

Advantages vs. traditional process:

- No phenol use in process, benefit environment & operator's health & safety
- Easy to scale up
- Simple flow-through mode
- All 4 serotypes using same process
- Protein/DNA/endotoxin in products meet requirement



Live Influenza virus production

Influenza process overview

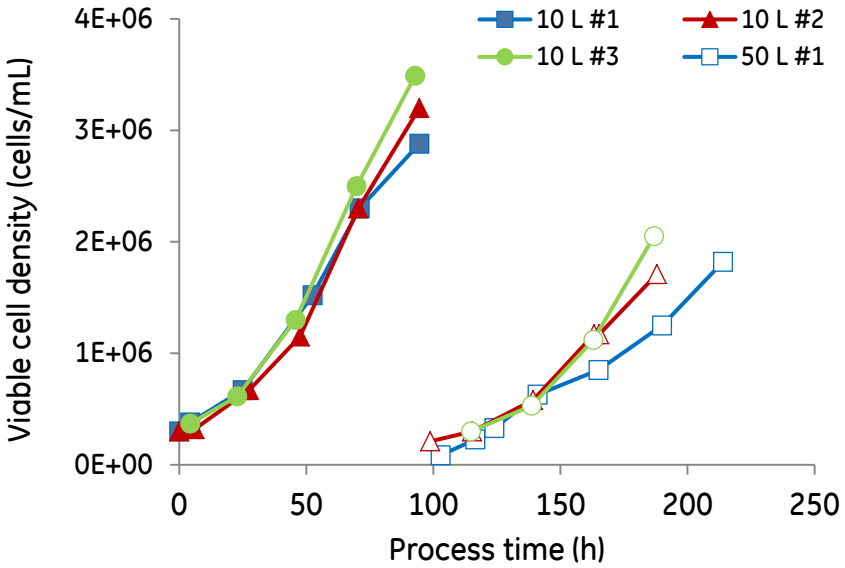
Scale-up from small scale to pilot scale in single-use format

Comparison of culture performance in 10 L and 50 L microcarrier culture in rocking bioreactors

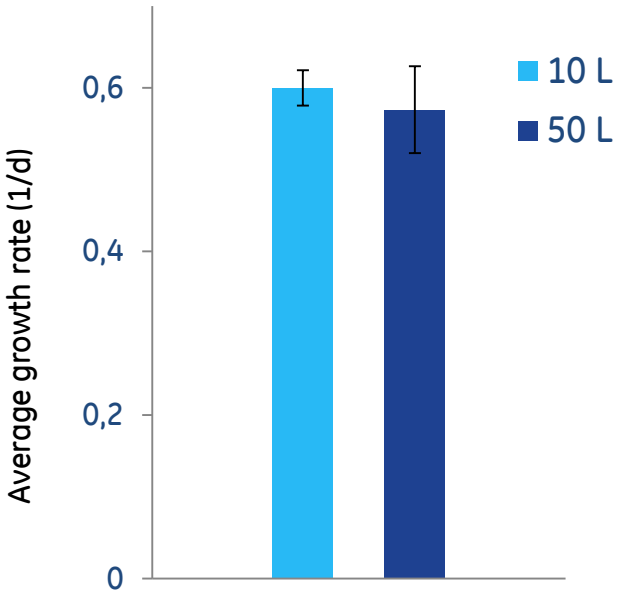
Downstream purification in flow-through chromatography mode with Capto™ Q and Capto Core 700 chromatography media (resins)

Cell growth in single-use bioreactor stage

Cell concentration



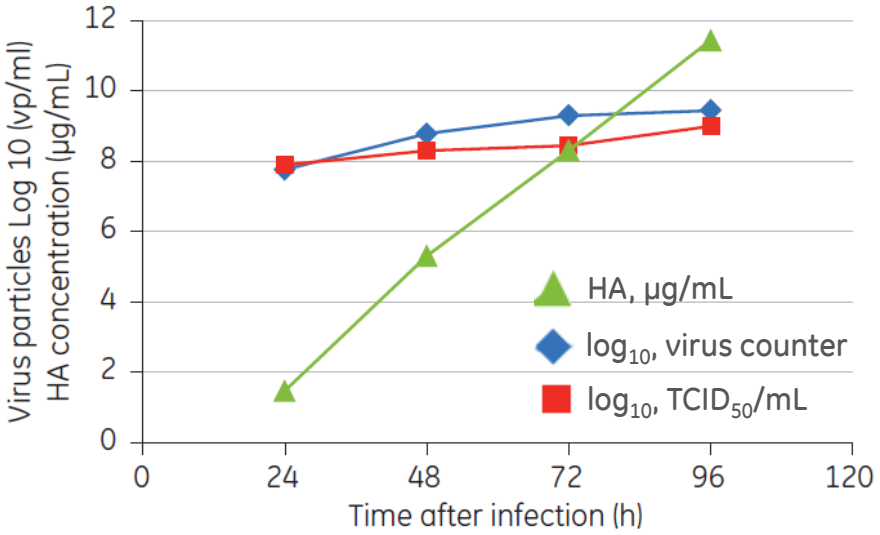
Average growth rate



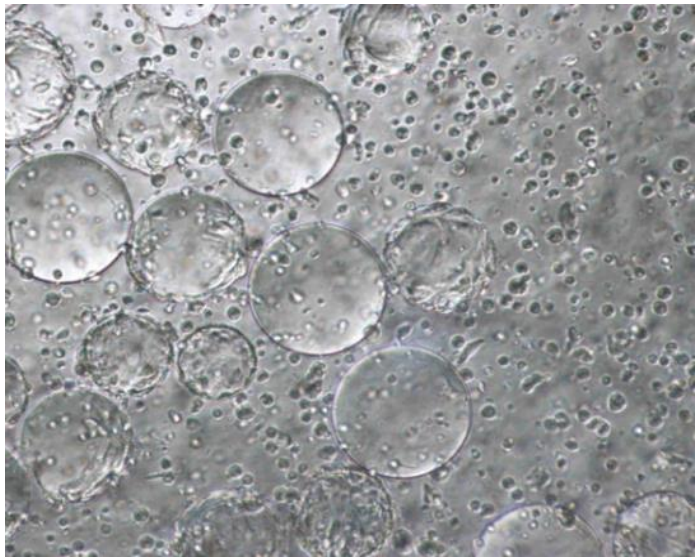
Bead to bead transfer was successful and cell growth was comparable at 10 L and 50 L scale

Virus growth kinetics

HA concentration and virus titer during culture

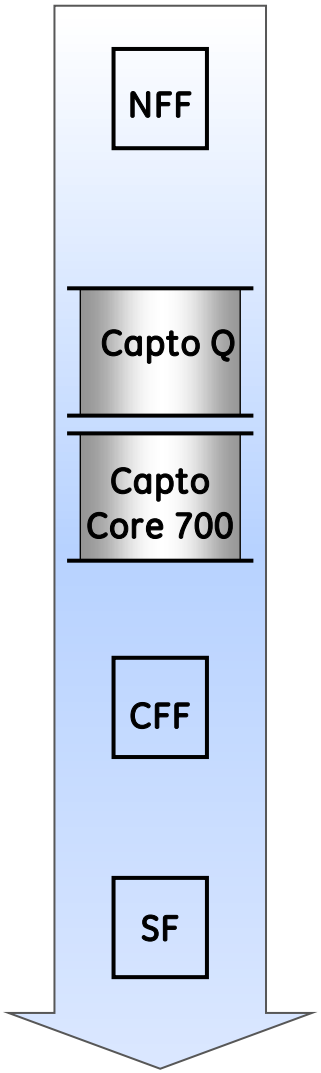


Cell morphology at time of harvest (96 h)



HA concentration at harvest was close to 12 µg/mL
and the virus concentration was > 10^9 infective units/mL

Purification Workflow



ULTA™ prime GF
Microcarrier and cell debris removal
Adjustment of conducitvity

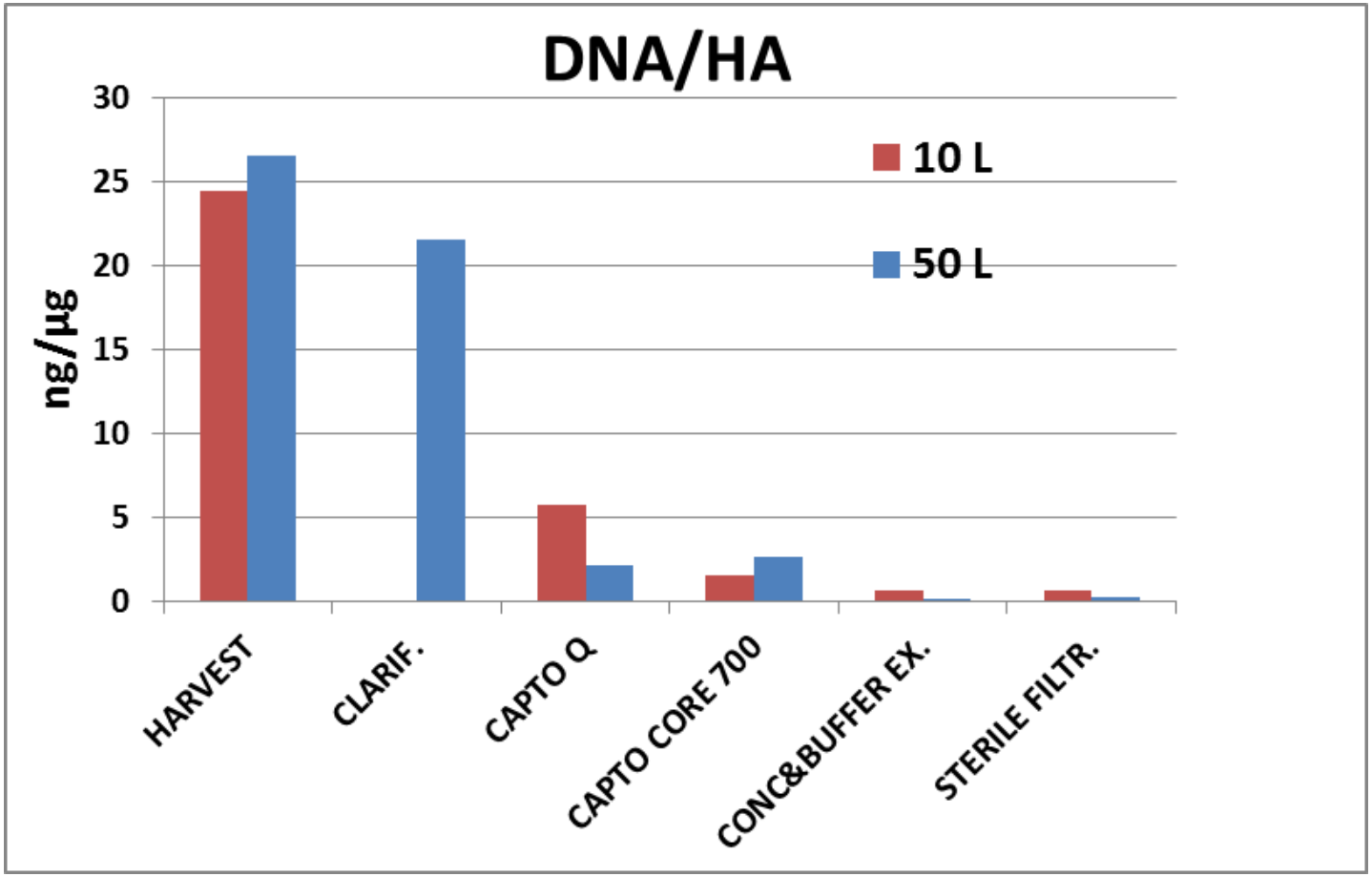
Capto™ Q – Flow through
Reduction of DNA and host cell proteins

Capto Core 700 – Flow through
Reduction of host cell proteins

ReadyToProcess™ hollow fiber
Concentration, buffer exchange and removal of DNA and host cell proteins

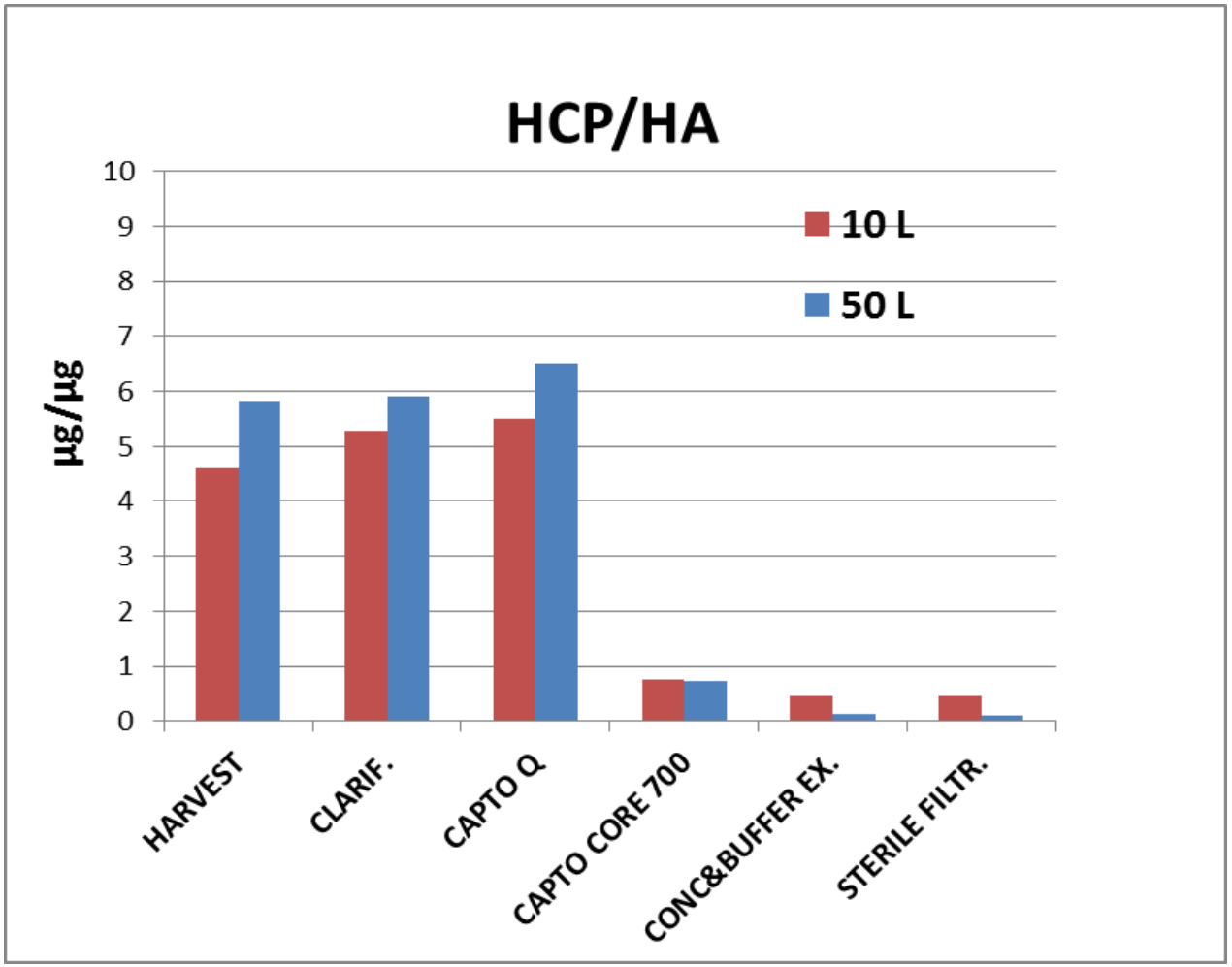
ULTA pure HC
Sterile filtration

Purification results



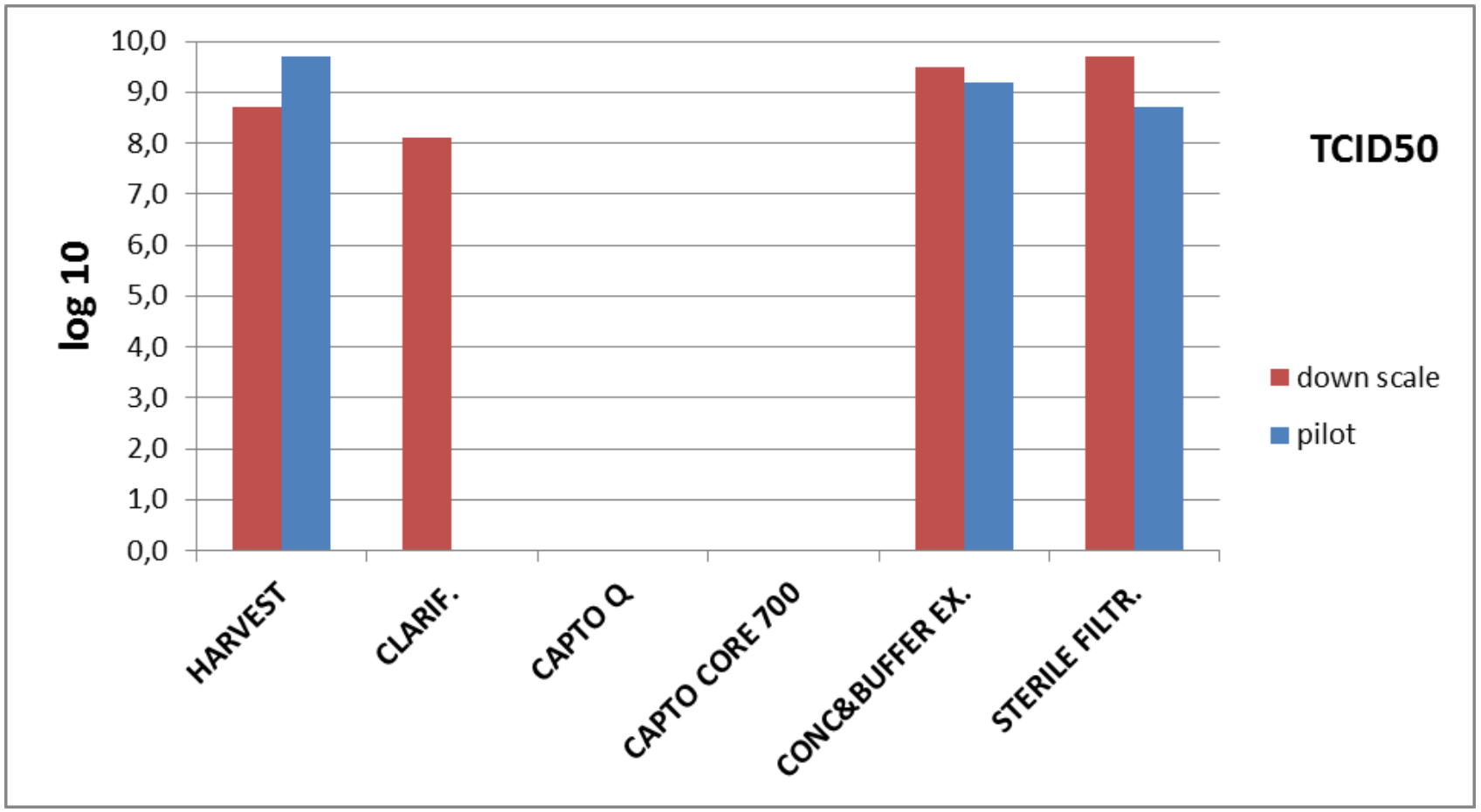
Capto™ Q: Reduces host cell DNA

Purification results



Capto™ Core 700: Reduces host cell protein

Virus infectivity



Process does not impair virus infectivity

Process summary

Estimation of doses per liter harvest, compared with WHO guidelines for protein and DNA impurities in influenza vaccine

	Split-inactivated vaccine[1]	Nasal LAIV[2]
Scale-up output/L harvest	175 doses á 15 µg HA	3075 doses á 10^7 $TCID_{50}$ units
Harvest volume to produce 10^6 doses	5760 L	325 L
Protein impurity[3]	30 µg protein/15 µg HA	1.5 µg protein/10^7 $TCID_{50}$ units
DNA impurity[4]	3.0 ng/15 µg HA	0.15 ng/10^7 $TCID_{50}$ units

[1] Split-inactivated vaccine contains 3 strains á 15 µg/HA (e.g., 3 × 15 = > 45 ug HA/dose á 0.5 mL).

[2] Comparison is based on a commercially available specification for a nasal LAIV. A dose of 0.2 mL contains 10^7 fluorescent focus units, which is assumed to be equal to $TCID_{50}$ titer.

[3] WHO guideline for protein impurity: max. 100 µg protein/strain

[4] WHO guideline for DNA impurity: < 10 ng DNA/dose = 3.3 ng DNA/15 µg HA.

Assuming a recovery of 25% for the overall process and a dose requirement of 10^7 $TCID_{50}$, more than 1.5 million doses of monovalent live attenuated influenza vaccine could be produced from a 50 L cell culture

Yellow fever virus propagation – from eggs to cells

GMP manufacturing of viral vaccine

Xcellerex™ XDR-50 bioreactor

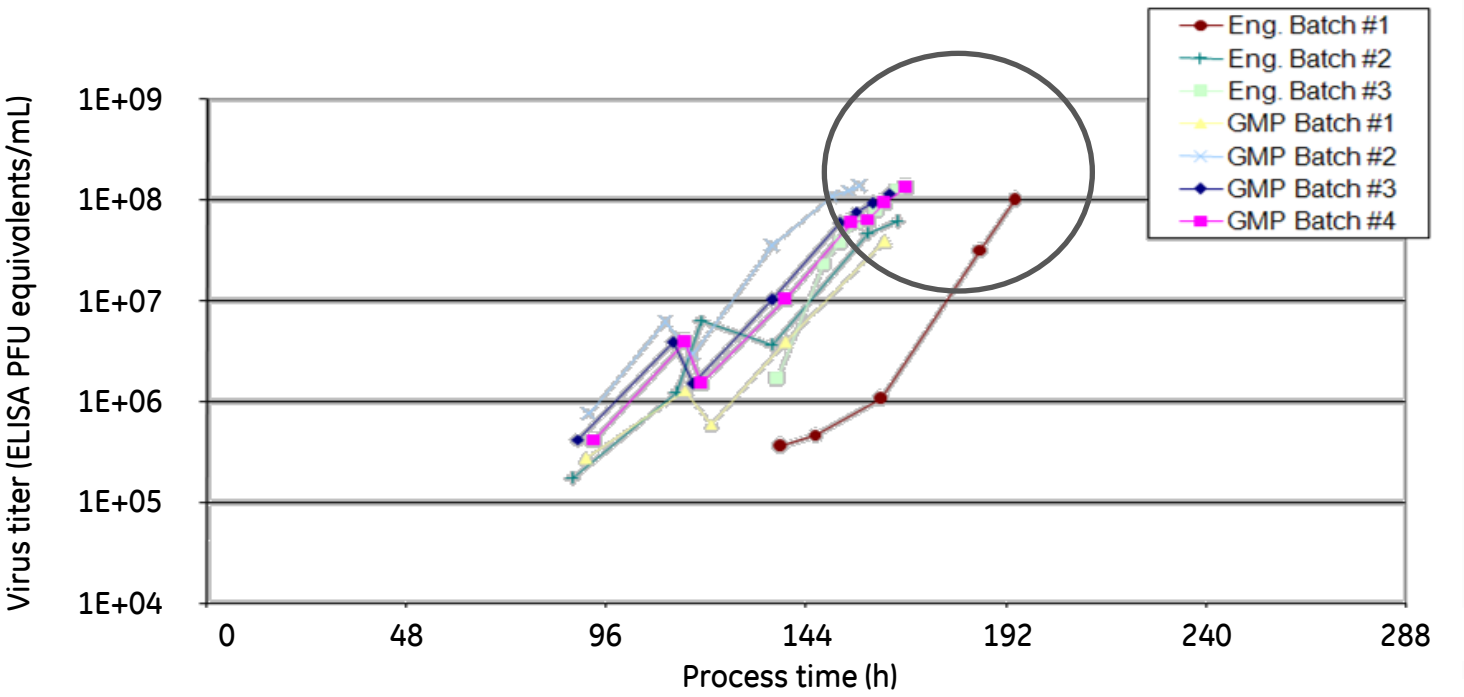
Vero cells (WHO-10-87)

- Cytodex™ 1 microcarrier
- Serum free, animal component-free medium

Yellow fever virus 17D

Virus production drain down refeed

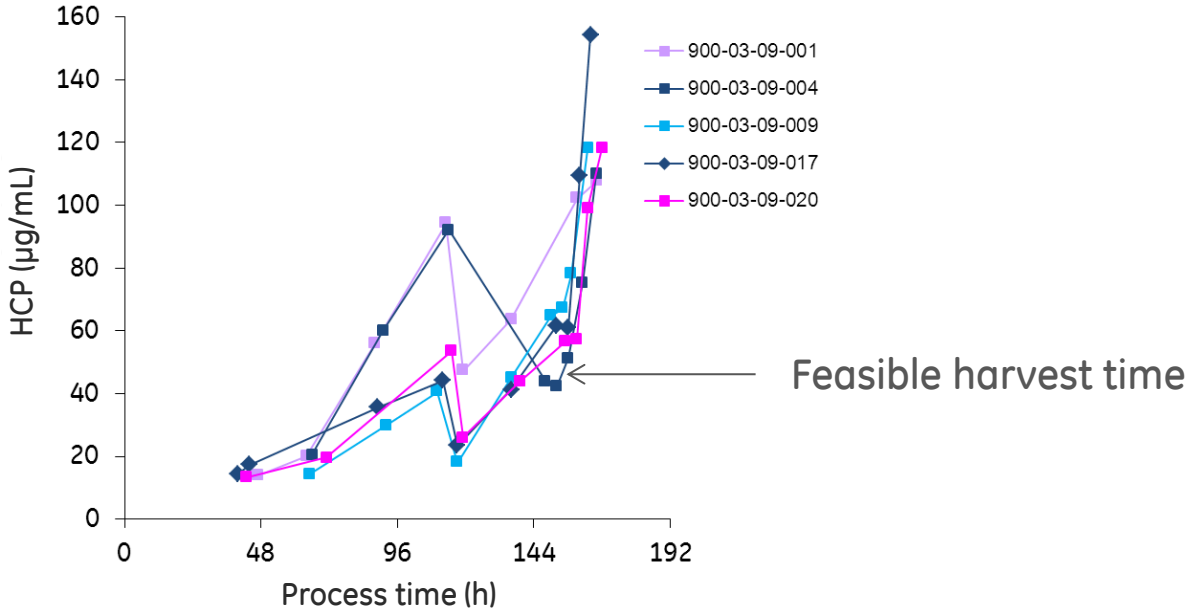
PFU equivalents from Eng and GMP bioreactor runs



The process consistency was high and virus titers were similar between runs

Virus propagation and release of HCP

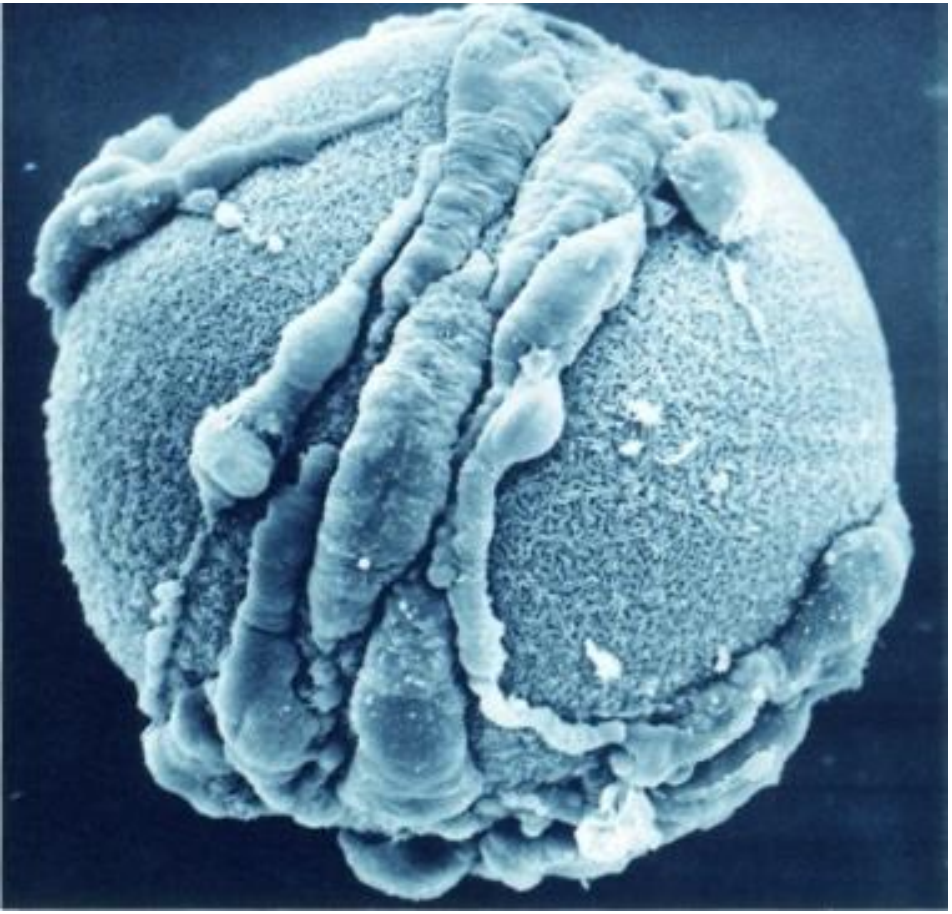
HCP content after ELISA analysis



A feasible time for harvest is before the HCP peaks, to facilitate downstream processing

Conclusions

Conclusions



By modernizing legacy vaccine processes there can be improvements in:

- Yield
- Quality
- Scale-up
- Cost efficiency
- Environmental impact

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Thank you!

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