Modernizing legacy Vaccine processes

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Imagination at work

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

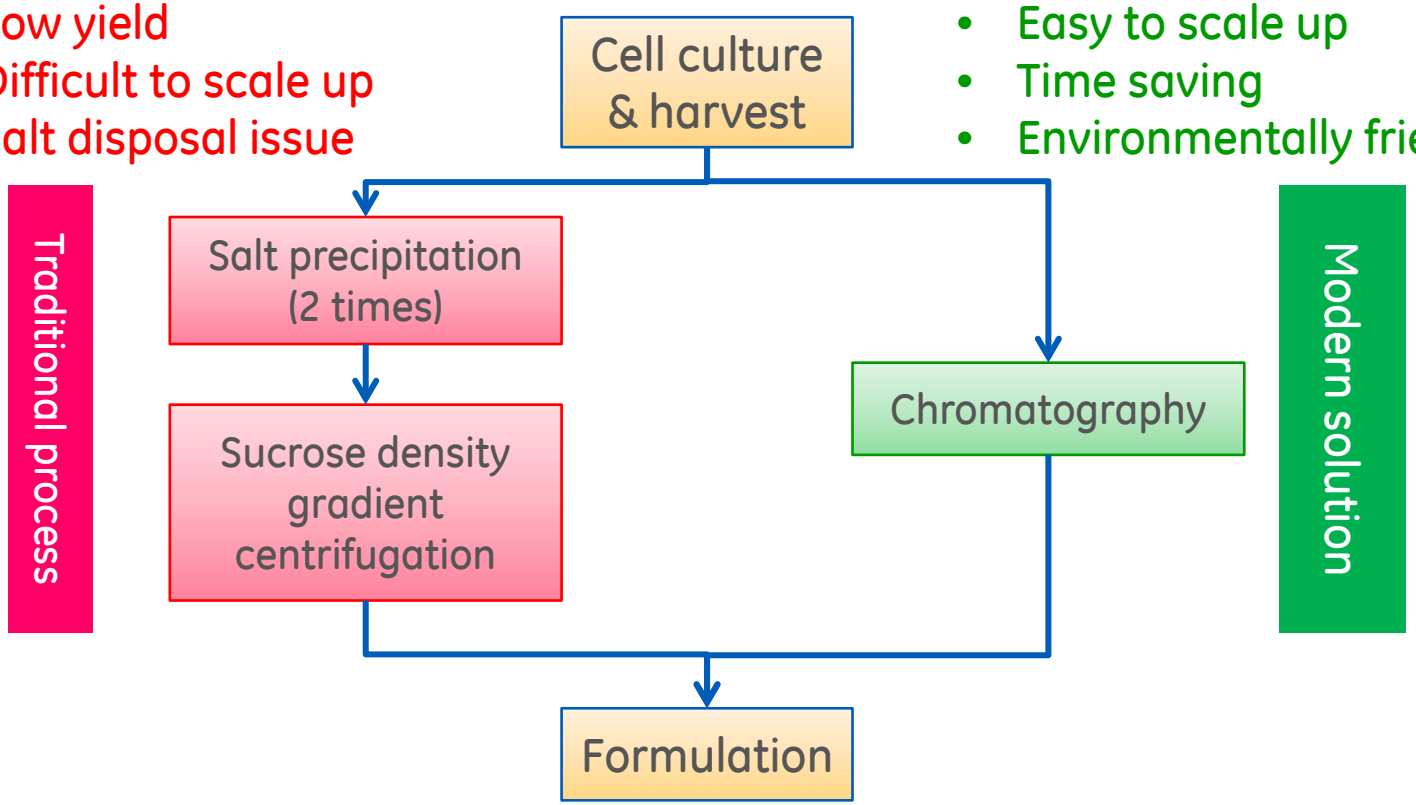
aP containing vaccines with reduced concentrations of the antigen have been formulated for use in adolescents and adults

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

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



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

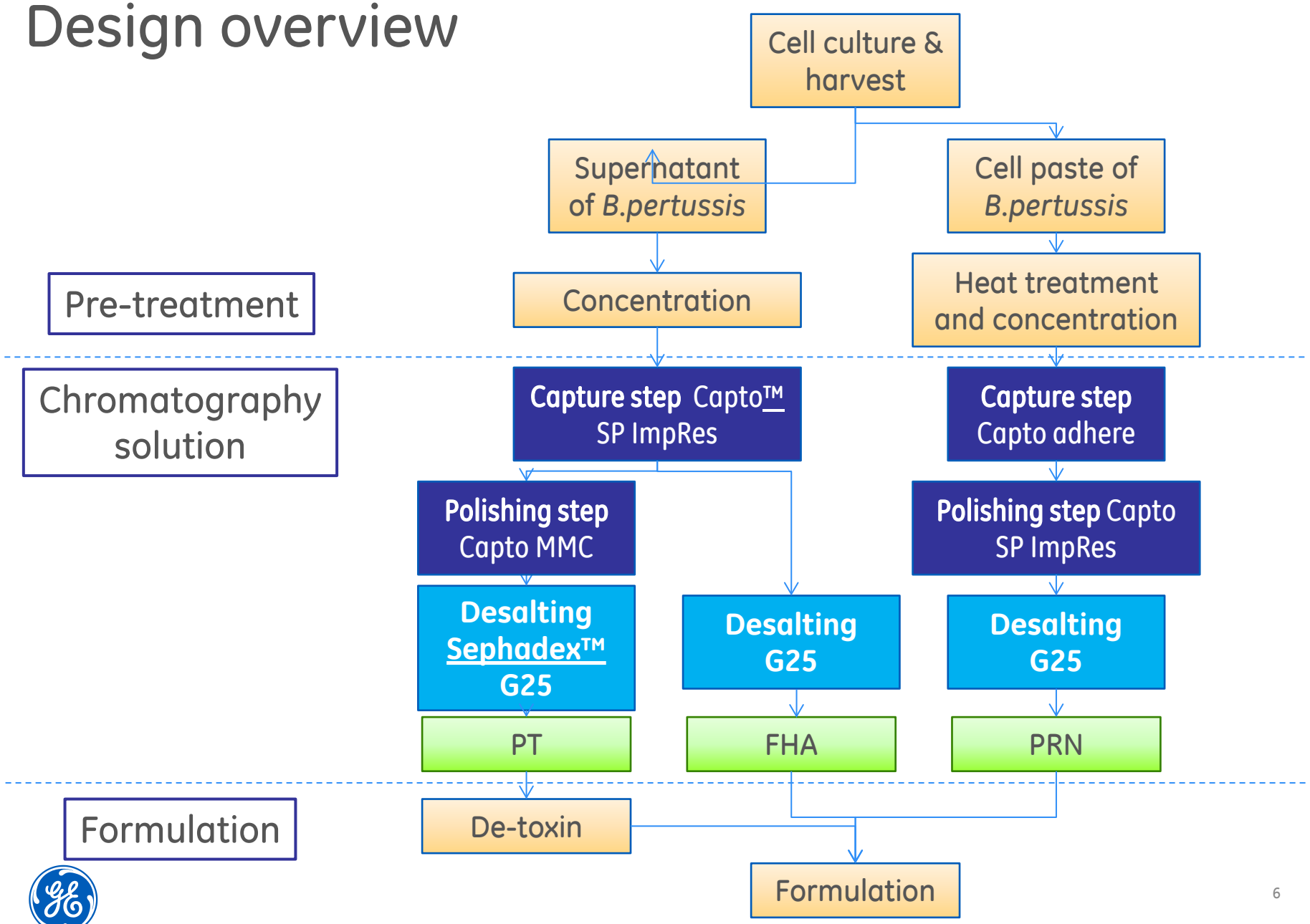
Develop a modern process for pertussis vaccine

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

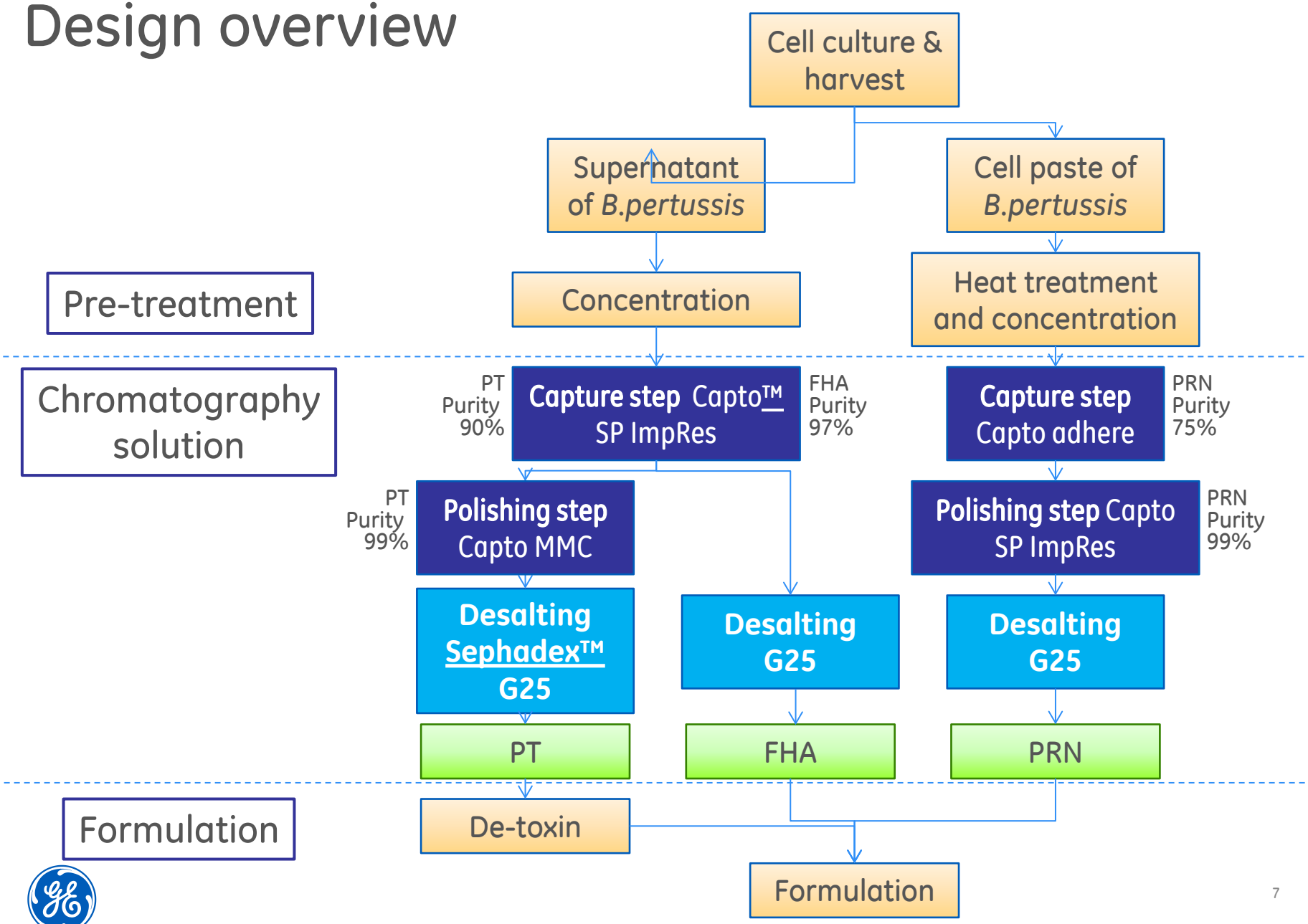
Yield >30%

Establish quantitative antigen determination using Biacore™ platform

Design overview



Design overview

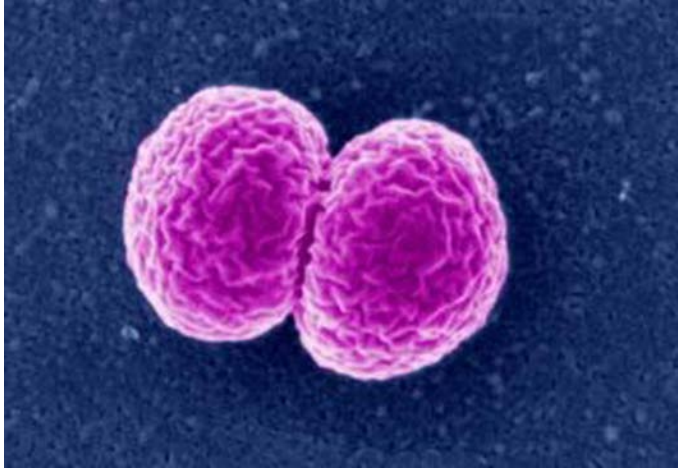


Process Highlights

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

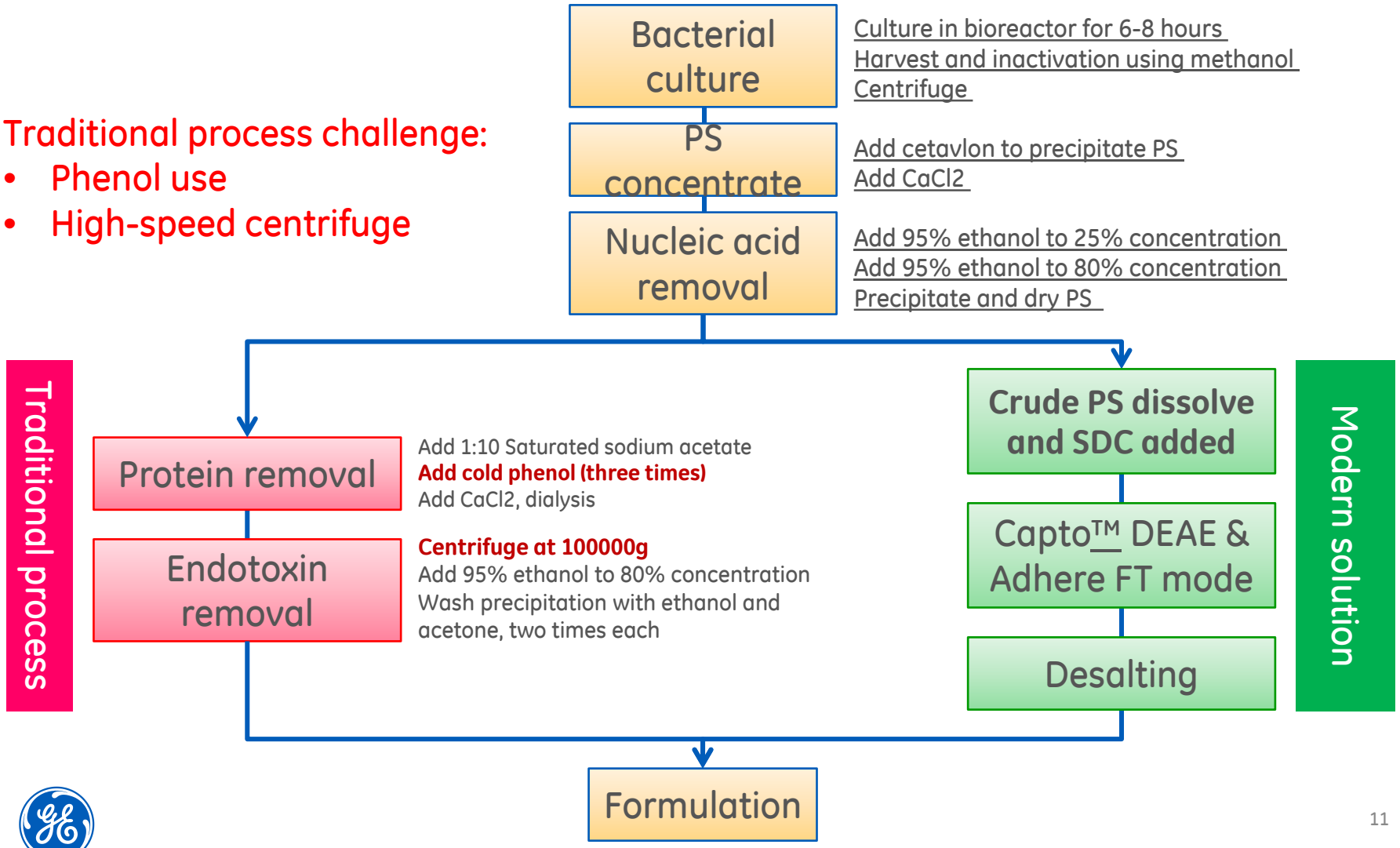


13 clinically significant serotypes. A, B, C, W-135, Y responsible for 90% of global cases

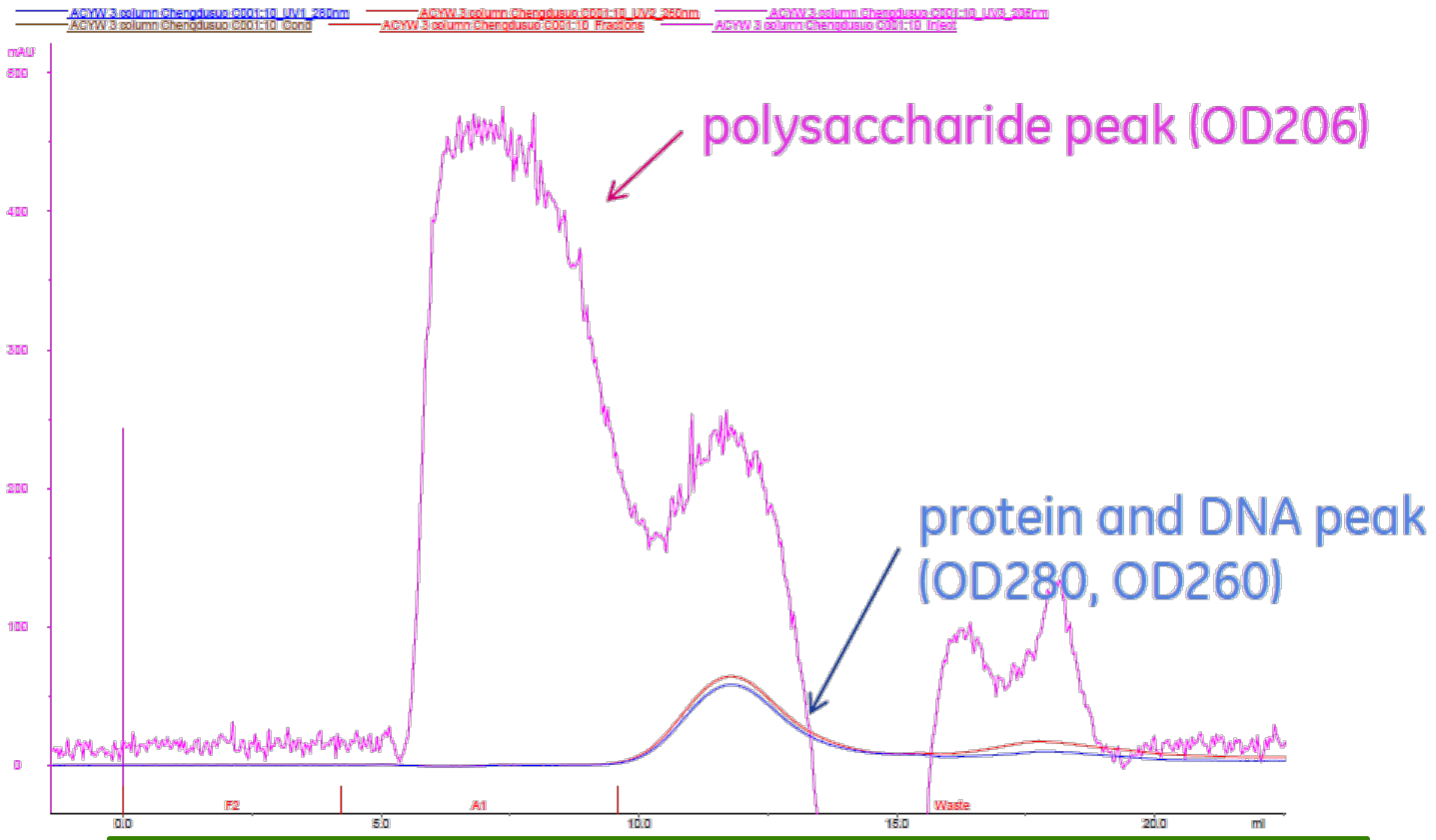
Vaccine for A, C, W, Y are produced using capsular polysaccharide (PS), conjugant technology to enhance immunogenicity

Traditional Process vs. Modern Solution

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

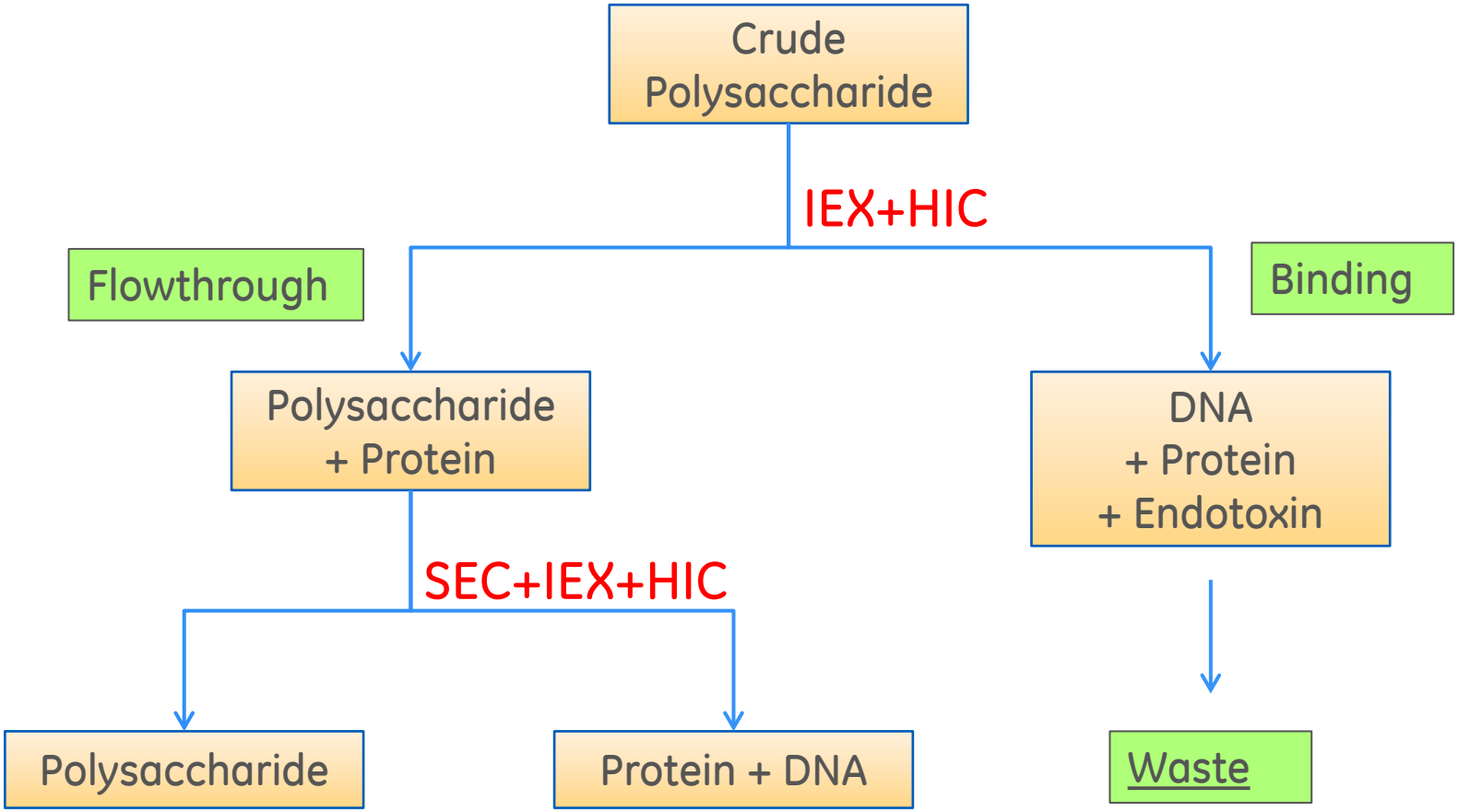


Typical Result From Upgraded Process 1x Capto DEAE + 2x Capto Adhere



AIEX + HIC + SEC effect!

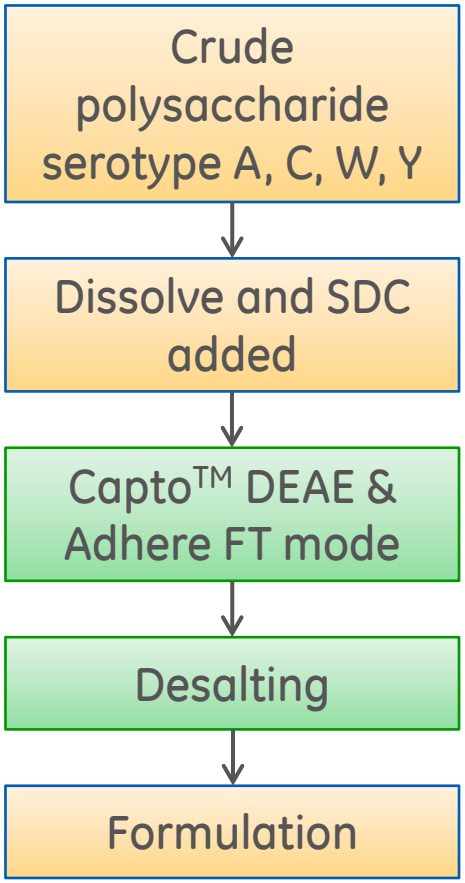
Separation Flowchart



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

Virus seed stock 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."*

Influenza process overview

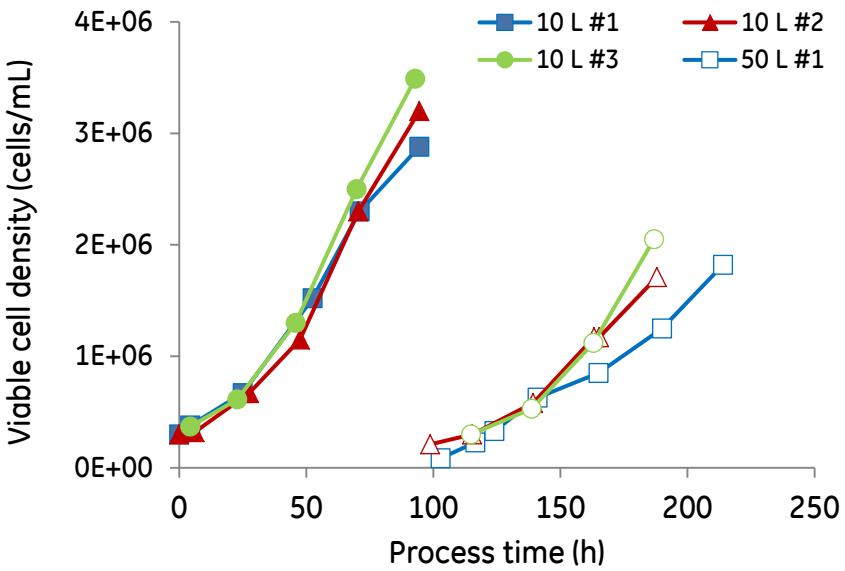
Scale-up from small scale to pilot scale in single-use format

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

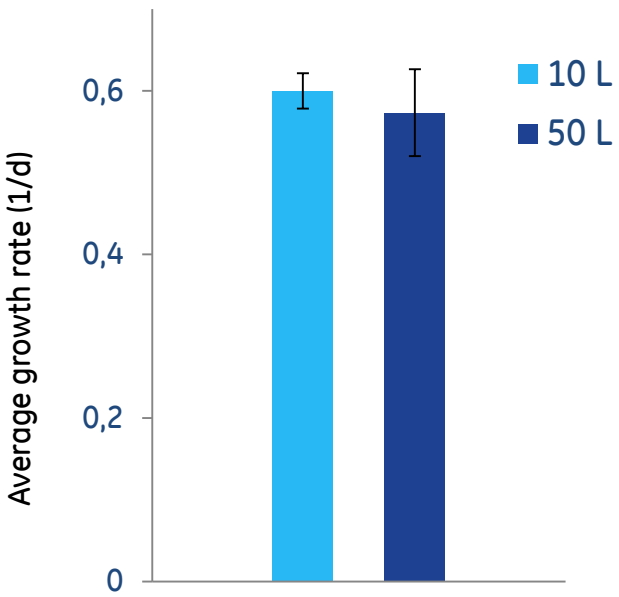
Downstream purification in flow-through chromatography mode with Capto™ Q and Capto Core 700 chromatography media (resins)

Cell growth in single-use bioreactor stage

Cell concentration



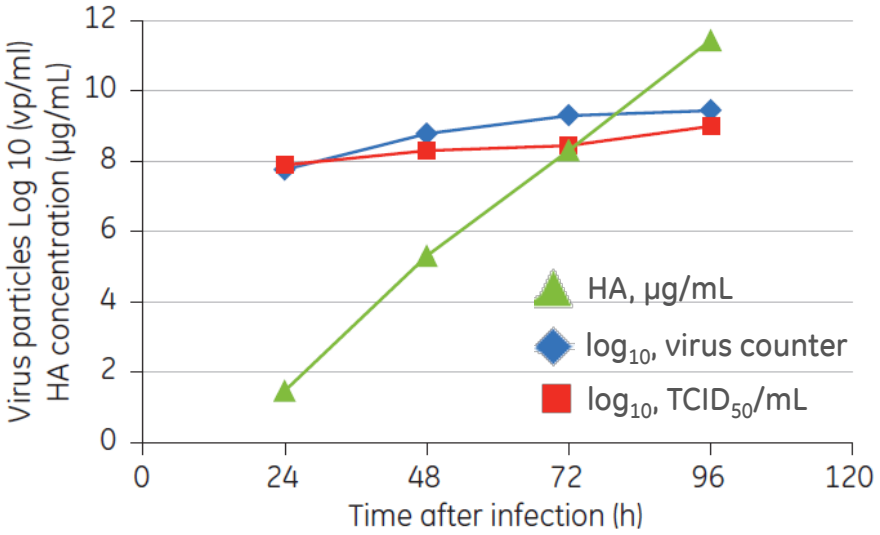
Average growth rate



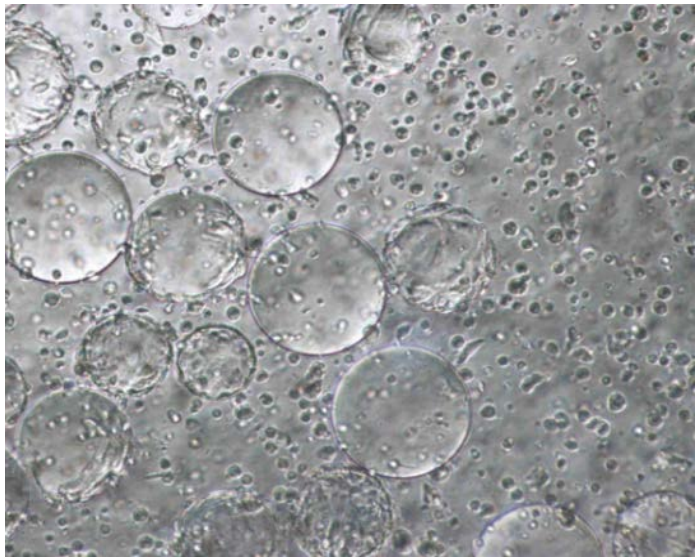
Bead to bead transfer was successful and cell growth was comparable at 10 L and 50 L scale

Virus growth kinetics

HA concentration and virus titer during culture

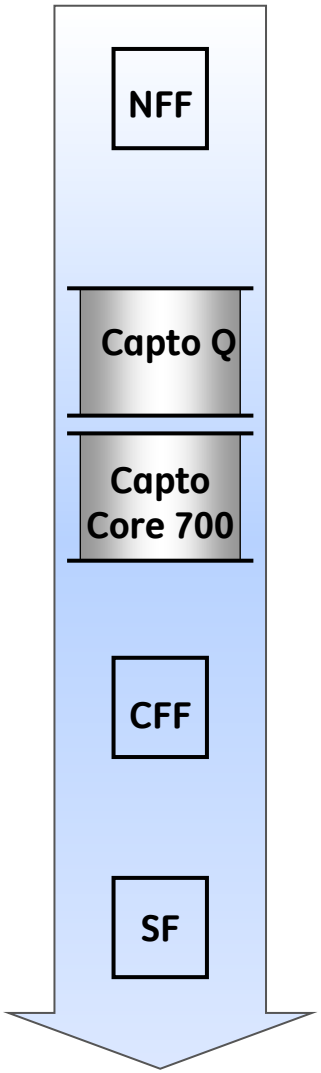


Cell morphology at time of harvest (96 h)



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

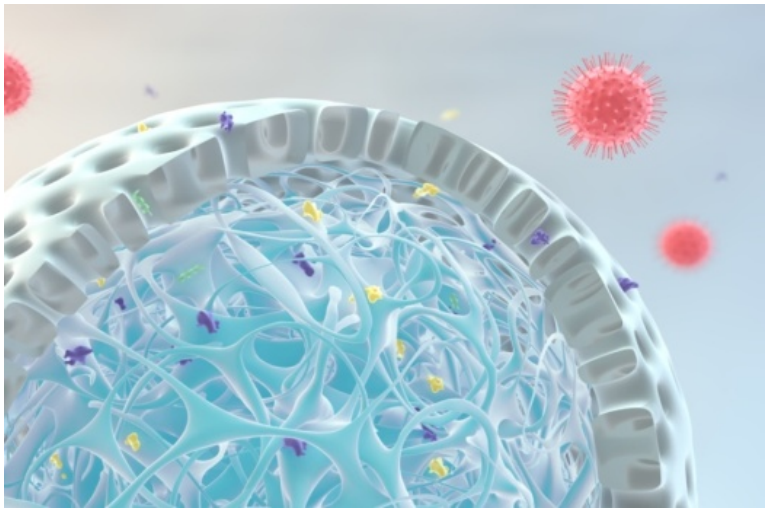
Capto™ Q – Flow through
Reduction of DNA and host cell proteins

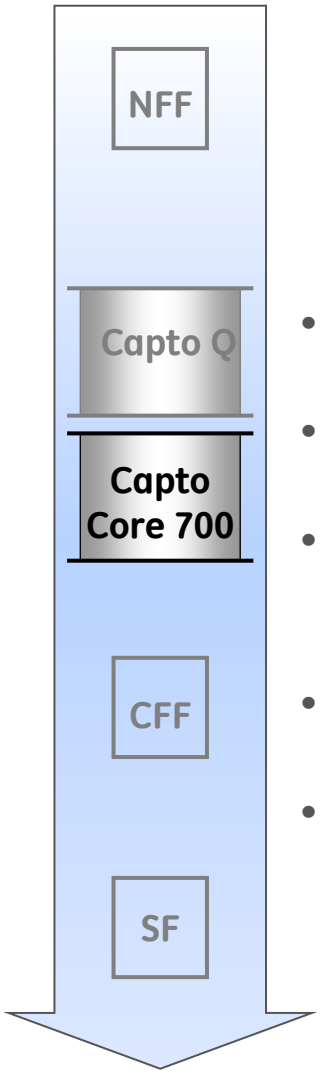
Capto Core 700 – Flow through
Reduction of host cell proteins

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

ULTA pure HC
Sterile filtration

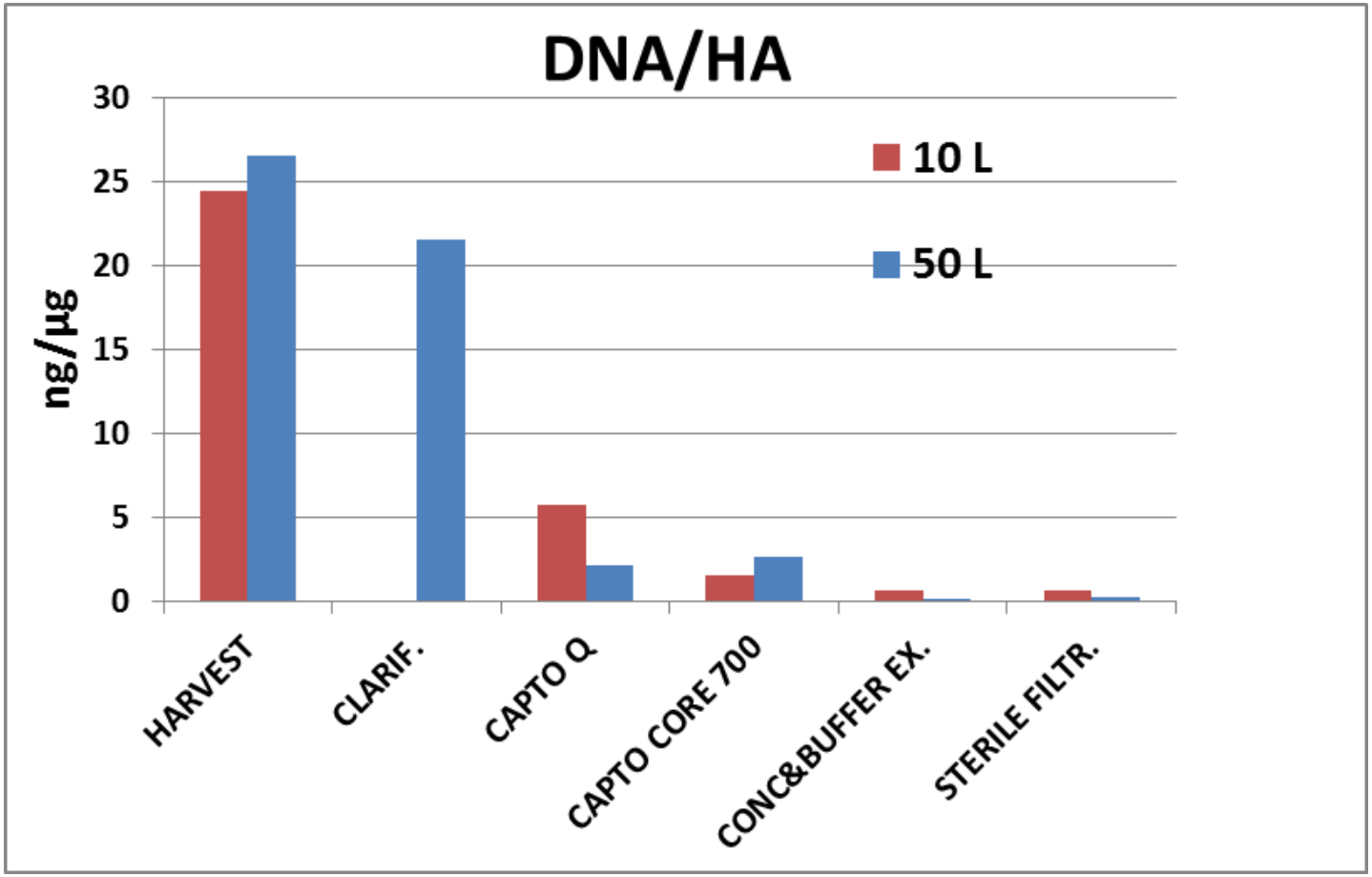
Capto™ Core 700





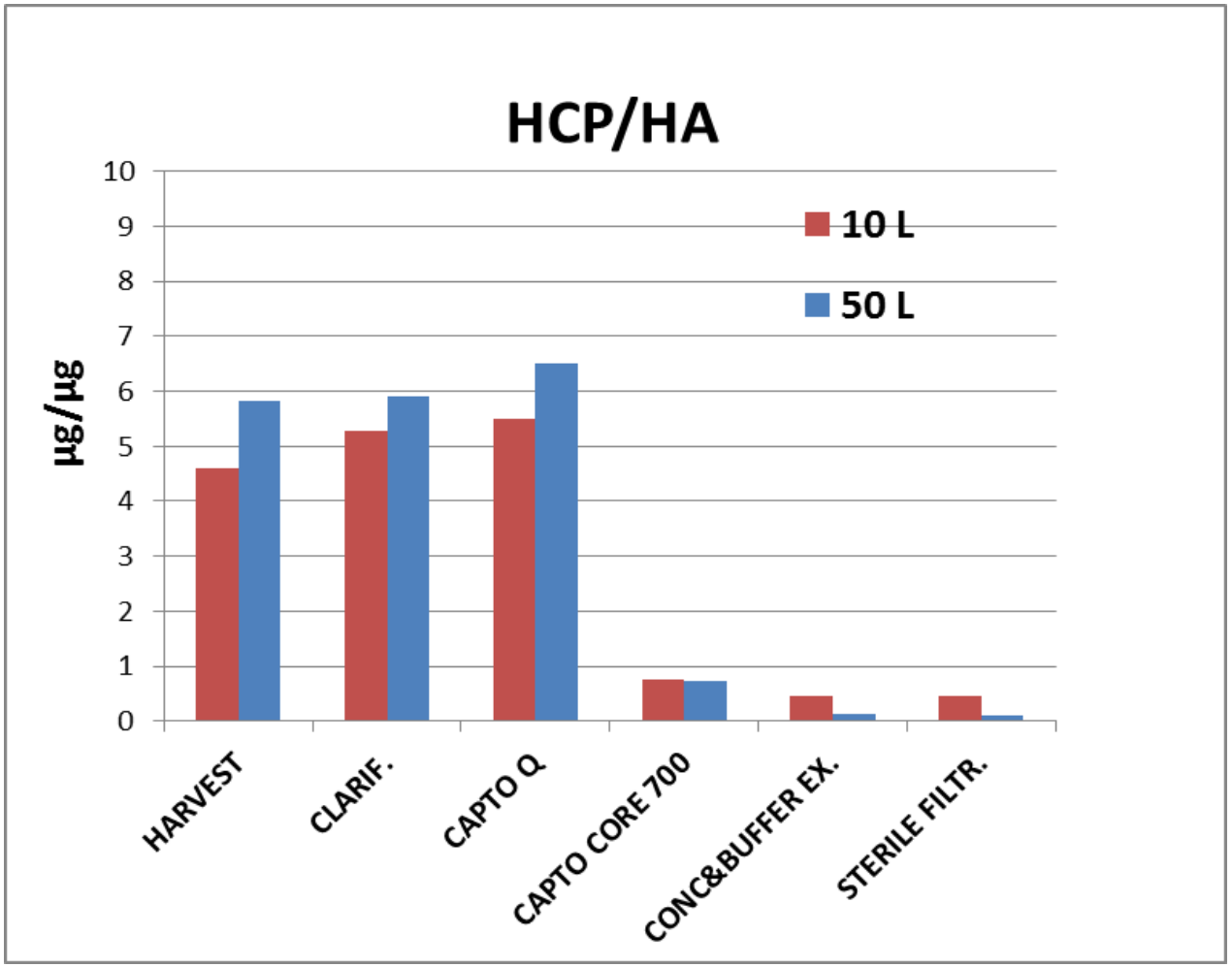
- Shell: Cross-linked native agarose
- Inner core ligand: Octylamine
- Large entites pass outside of the bead while small entities (< 700 kDa) are captured in the core
- Binding not affected by salt , pH or common buffer systems
- Enables flexible unit or serial operation

Purification results



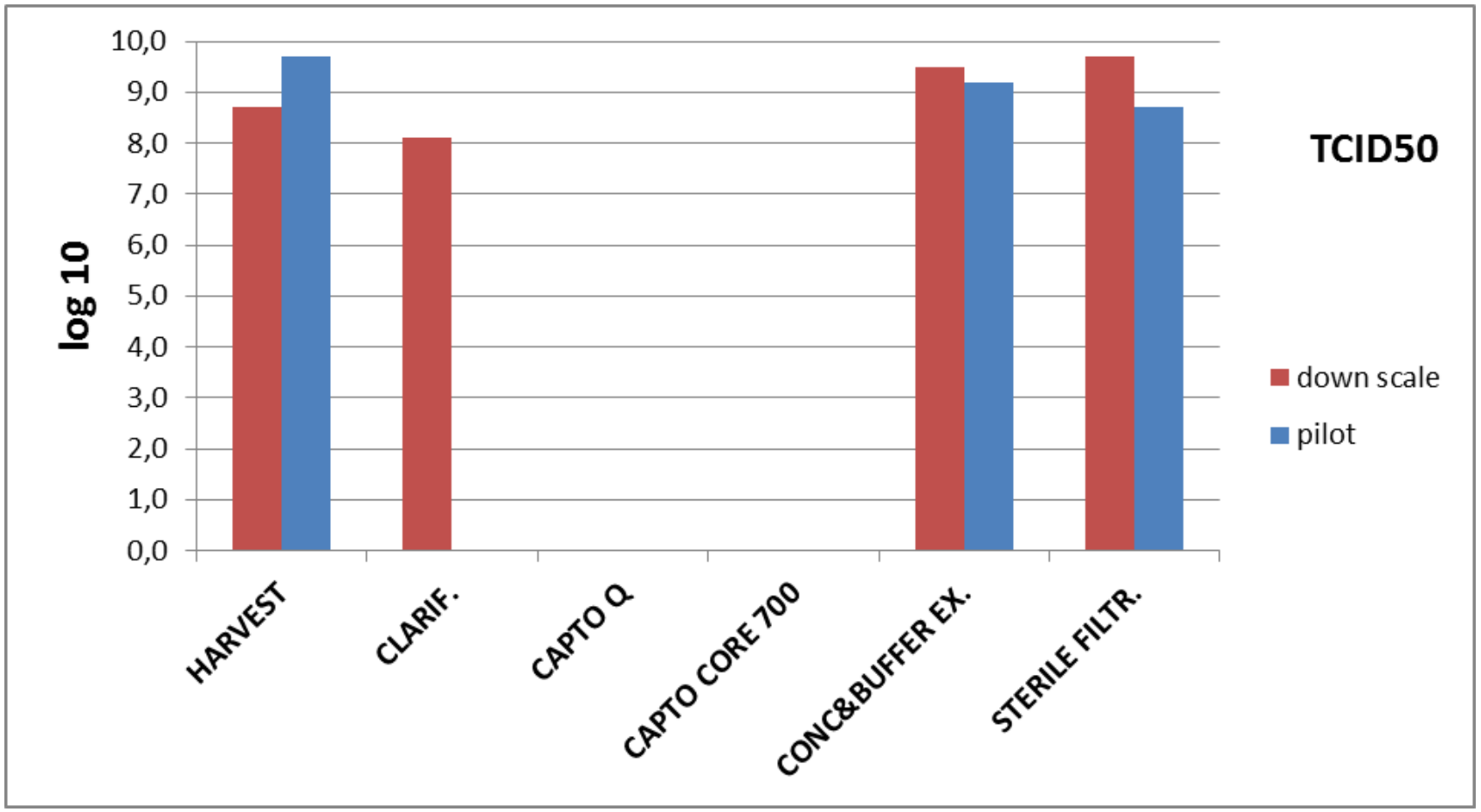
Capto™ Q: Reduces host cell DNA

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

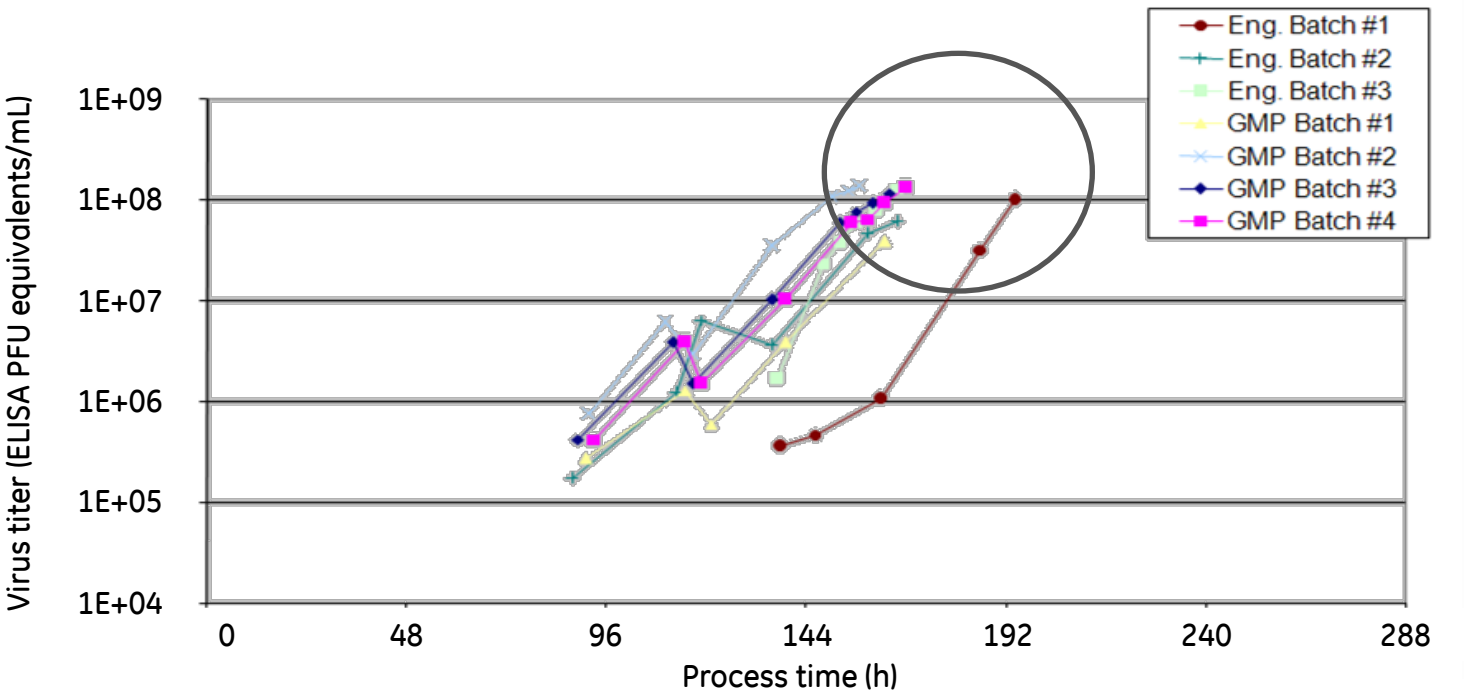
Vero cells (WHO-10-87)

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

Yellow fever virus 17D

Virus production drain down refeed

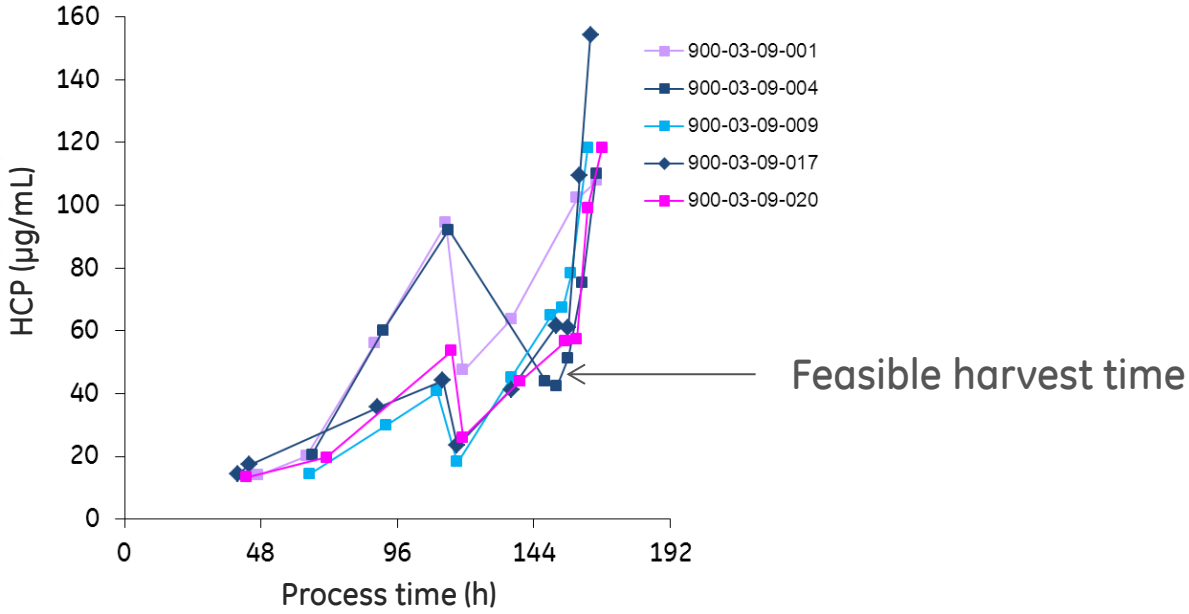
PFU equivalents from Eng and GMP bioreactor runs



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

Virus propagation and release of HCP

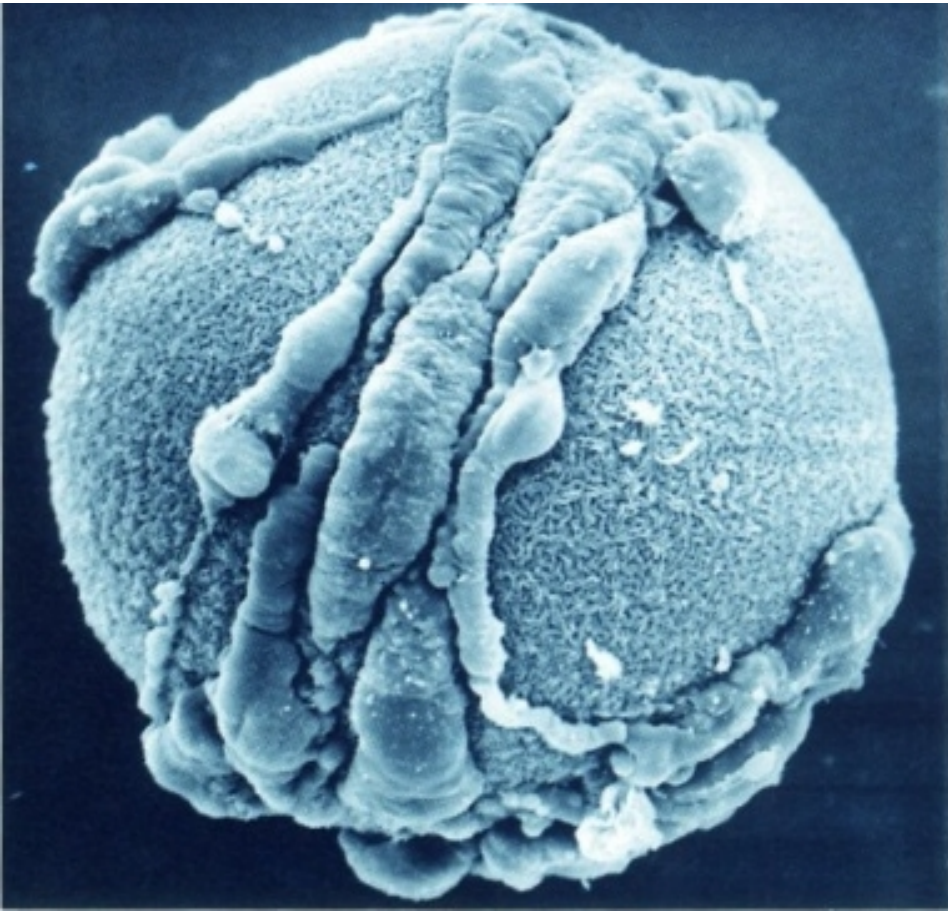
HCP content after ELISA analysis



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

Less hazards

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