Modernizing legacy Vaccine processes

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Imagination at work
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)
HA concentration at harvest was close to 12 µg/mL and the virus concentration was > 10⁹ infective units/mL
Purification Workflow

- **ULTA™ prime GF**
  - Microcarrier and cell debris removal
  - Adjustment of conductivity

- **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
Core bead chromatography, the principle

- Separation of substances with different features such as size, charge, and biological properties.
- The target product may bind or not, the important part is that it does the opposite of what most impurities do!

Highly porous particle, offering a huge surface for binding of proteins

Surface modified to enable selective binding of product or impurities

Spheric particle, 1/10th of a mm in diameter
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
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
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.

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

Traditional process:
- Cell culture & harvest
  - Salt precipitation (2 times)
  - Sucrose density gradient centrifugation

Modern solution:
- Cell culture & harvest
  - Chromatography
  - Formulation
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

- **Pre-treatment**
- **Chromatography solution**
- **Formulation**

1. **Cell culture & harvest**
   - Supernatant of *B.pertussis*
   - Cell paste of *B.pertussis*
   - Heat treatment and concentration

2. **Concentration**
   - Capture step Capto™ SP ImpRes
   - Polishing step Capto MMC
   - Desalting Sephadex™ G25
     - PT
     - De-toxin

3. **Capture step Capto adhere**
   - Polishing step Capto SP ImpRes
   - Desalting G25
     - FHA
     - PRN
   - Formulation
Design overview

Pre-treatment

Chromatography solution

Formulation

Cell culture & harvest

Supernatant of *B. pertussis*

Concentration

Heat treatment and concentration

Cell paste of *B. pertussis*

**Capture step** Capto™

SP ImpRes

**Capture step** Capto adhere

**Capture step** Capto™

SP ImpRes

**Polishing step** Capto MMC

**Desalting** Sephadex™

G25

**PT**

De-toxin

**FHA**

**PRN**

Purity 97%

Purity 75%

Purity 99%

Purity 99%

Purity 90%

Purity 99%

Formulation

Chromatography solution

**Desalting** G25

**Desalting** G25

**Desalting** G25

**Polishing step** Capto SP ImpRes

**Polishing step** Capto SP ImpRes

**Polishing step** Capto SP ImpRes

**Capture step** Capto adhere

**Capture step** Capto™

SP ImpRes

**Capture step** Capto™

SP ImpRes

**Polishing step** Capto MMC

**Desalting** Sephadex™

G25

**PT**

De-toxin

**FHA**

**PRN**

Purity 97%

Purity 75%

Purity 99%

Purity 99%

Purity 90%

Purity 99%

Formulation
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

Traditional process:
- Protein removal
- Endotoxin removal

Modern solution:
- Bacterial culture
- PS concentrate
- Nucleic acid removal
- Crude PS dissolve and SDC added
- Capto™ DEAE & Adhere FT mode
- Desalting

Flowchart:
- Add 1:10 Saturated sodium acetate
- Add cold phenol (three times)
- Add CaCl2, dialysis
- Centrifuge at 100000g
- Add 95% ethanol to 80% concentration
- Wash precipitation with ethanol and acetone, two times each
- Culture in bioreactor for 6-8 hours
- Harvest and inactivation using methanol
- Centrifuge
- Add cetavlon to precipitate PS
- Add CaCl2
- Add 95% ethanol to 25% concentration
- Add 95% ethanol to 80% concentration
- Precipitate and dry PS

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

**polysaccharide peak (OD206)**

**protein and DNA peak (OD280, OD260)**

**AIEX + HIC + SEC effect!**
Separation Flowchart

- Crude Polysaccharide
  - IEX+HIC
    - Flowthrough
    - Polysaccharide + Protein
      - SEC+IEX+HIC
        - Polysaccharide
        - Protein + DNA
    - Binding
      - DNA + Protein + Endotoxin
        - Waste
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

Crude polysaccharide serotype A, C, W, Y

Dissolve and SDC added

Capto™ DEAE & Adhere FT mode

Desalting

Formulation
Conclusions
Conclusions

By modernizing legacy vaccine processes there can be improvements in:

Yield
Quality
Scale-up
Cost efficiency
Less hazards
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