Modernizing legacy Vaccine processes

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GE Healthcare Life Sciences
A Modern Solution for Acellular Pertussis Vaccine
<table>
<thead>
<tr>
<th><strong>wP Vaccines</strong></th>
<th><strong>aP Vaccines</strong></th>
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<tbody>
<tr>
<td>70 year old technology based on killed <em>B. pertussis</em> strains</td>
<td>Introduced in 1990’s</td>
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<tr>
<td>High protection efficiency ~78%</td>
<td>aP contain ≥1 of the separately purified antigens: pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae (FIM) type 2 and 3.</td>
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<tr>
<td>Associated with side effects and safety concerns</td>
<td>aP is now the dominant type in the industrialized world</td>
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<td>The reactogenicity of wP vaccine was thought to be too high to permit routine use in older children, adolescents and adults.</td>
<td>aP containing vaccines with reduced concentrations of the antigen have been formulated for use in adolescents and adults.</td>
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</table>
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

Cell culture & harvest

Salt precipitation (2 times)

Sucrose density gradient centrifugation

Formulation

Chromatography

Modern solution
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
Design overview

Pre-treatment

Chromatography solution

Formulation

Cell culture & harvest

Supernatant of *B. pertussis*

Concentration

Capture step Capto™ SP ImpRes

Desalting Sephadex™ G25

PT

De-toxin

Polishing step Capto MMC

PT Purity 99%

Capture step Capto adhere

Desalting G25

FHA

Polishing step Capto SP ImpRes

Desalting G25

PRN

Heat treatment and concentration

Cell paste of *B. pertussis*

Capture step Capto adhere

Desalting G25

PRN

Purity 75%

Capture step Capto SP ImpRes

Desalting G25

PT

Purity 90%

Purity 97%

Purity 99%

Purity 99%

Formulation

FHA

Purity 97%

PRN

Purity 99%

PT

Purity 90%

PT

Purity 99%
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**

**Formulation**

**Modern solution**

- **Bacterial culture**
- **PS concentrate**
- **Nucleic acid removal**

- **Crude PS dissolve and SDC added**
- **Capto™ DEAE & Adhere FT mode**
- **Desalting**

**Traditional process** steps:
- Centrifuge at 100,000g
- Add 95% ethanol to 80% concentration
- Wash precipitation with ethanol and acetone, two times each
- Add cold phenol (three times)
- Add CaCl\(_2\), dialysis

**Modern solution** steps:
- Culture in bioreactor for 6-8 hours
- Harvest and inactivation using methanol
- Centrifuge
- Add cetavlon to precipitate PS
- Add CaCl\(_2\)
- Add 95% ethanol to 25% concentration
- Add 95% ethanol to 80% concentration
- Precipitate and dry PS
Typical Result From Upgraded Process: $1\times$ Capto DEAE + $2\times$ Capto Adhere

- Polysaccharide peak (OD206)
- Protein and DNA peak (OD280, OD260)

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

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

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

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>HA concentration (µg/mL)</th>
<th>log₁₀, virus counter</th>
<th>log₁₀, TCID₅₀/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>24</td>
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<td>48</td>
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<td>72</td>
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<td>96</td>
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<tr>
<td>120</td>
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HA concentration at harvest was close to 12 µg/mL and the virus concentration was > 10⁹ infective units/mL

Cell morphology at time of harvest (96 h)
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
Capto™ Core 700

- Shell: Cross-linked native agarose
- Inner core ligand: Octylamine
- Large entities 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

<table>
<thead>
<tr>
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<th>Split-inactivated vaccine</th>
<th>Nasal LAIV</th>
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<td>Scale-up output/L harvest</td>
<td>175 doses á 15 µg HA</td>
<td>3075 doses á 10^7 TCID_{50} units</td>
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<tr>
<td>Harvest volume to produce 10^6 doses</td>
<td>5760 L</td>
<td>325 L</td>
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<tr>
<td>Protein impurity³</td>
<td>30 µg protein/15 µg HA</td>
<td>1.5 µg protein/10^7 TCID_{50} units</td>
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<tr>
<td>DNA impurity⁴</td>
<td>3.0 ng/15 µg HA</td>
<td>0.15 ng/10^7 TCID_{50} units</td>
</tr>
</tbody>
</table>

1 Split-inactivated vaccine contains 3 strains á 15 µg/HA (e.g., 3 x 15 = > 45 µg 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\textsubscript{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\textsubscript{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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