The future of cell based vaccine production

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Vaccine production technology trends

- 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

<table>
<thead>
<tr>
<th>Processes developed decades ago</th>
<th>Processes difficult to scale up</th>
<th>Unfavorable process economy</th>
<th>Increased regulatory requirements</th>
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</thead>
<tbody>
<tr>
<td>Old cell substrates or eggs</td>
<td>Centrifugation</td>
<td>Low yields</td>
<td>Open handling</td>
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<tr>
<td>Limited purification</td>
<td>Fixed installations</td>
<td>Long process times</td>
<td>Batch variability</td>
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<tr>
<td>Significant expertise required</td>
<td>Roller bottles</td>
<td>Labor-intense processes</td>
<td>Serum supplementation</td>
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<td></td>
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<td>Dedicated facilities</td>
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## Vaccine production tomorrow

<table>
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<th>Processes developed decades ago</th>
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<tbody>
<tr>
<td>Platform cell lines</td>
<td>Scalable technologies enabled by, e.g., single-use technologies</td>
<td>Efficient and rational process design</td>
<td>Closed handling</td>
</tr>
<tr>
<td>Efficient purification</td>
<td></td>
<td></td>
<td>Quality by design (QbD)</td>
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<tr>
<td>based on chromatography</td>
<td></td>
<td></td>
<td>Chemically defined cell culture media</td>
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</tbody>
</table>
Cell culture and virus propagation
Cell substrates for vaccines

Production system
- YEAST
- CELLS
- EGGs

Vaccine type
- Attenuated
- Inactivated
- Subunit
- VLP

Infectious agent
- Viral
- Bacterial

Infectious agent
- B. anthracis
- V. cholerae
- S. typhi
- N. meningitidis
- S. pneumoniae
- B. pertussis
- C. tetani
- C. diphtheriae
- H. influenzae
- S. cerevisiae

Production system
- Sf9
- PER.C6™
- WI-38
- Vero
- MRC-5
- CECC
- MDCK

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

CPS = conjugated polysaccharides, PS = polysaccharides, VLP = virus-like particle
40 vaccines still to be developed

Where would this trend lead?

Infectious agent

Vero, MRC-5, WI-38, MDCK, CECC

Production system

YEAST, CELLS, EGGS

Vaccine type

Attenuated, Inactivated, Subunit, VLP

**Viral**

**Bacterial**

CPS = conjugated polysaccharides, PS = polysaccharides, VLP = virus-like particle
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:**
- Dry Cytodex
- Weigh in
- Swell in buffer
- Sterilize
  - Autoclave (small scale)
  - Bioreactor (large scale)
- Drain buffer
- Add cell culture medium

**Simplified process:**
- Cytodex Gamma packages for 10, 100, and 1000 L cultures
- Add to bioreactor
  - Add cell culture medium
Adenovirus vector
AV vaccine production process

**Upstream**
- WCB
- Shaker flask
- Seed train
  - 2–3 weeks
- Xcellerex™ XDR-10
- WVSS
- 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 fluorescence 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 stock
AdV productivity in CCM B (CDM4HEK293) vs E (competitor)

**AdV5-GFP comparison cell culture media B and E**

<table>
<thead>
<tr>
<th>MOI 1 (r1)</th>
<th>MOI 1 (r2)</th>
<th>MOI 10 (r1)</th>
<th>MOI 10 (r2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9E+08</td>
<td>3.0E+07</td>
<td>6.3E+08</td>
<td>6.1E+08</td>
</tr>
<tr>
<td>3.8E+08</td>
<td>2.8E+07</td>
<td>4.8E+07</td>
<td>4.9E+07</td>
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</table>

**GFP expression at TOH**

**TOI:** 1 x 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)
72 h post Cytodex inoculation

Time of harvest

Competitor medium

VaccineXpress medium
Rotavirus can be propagated on Cytodex 1 Gamma using VaccineXpress medium.

Rotavirus titer in Spinner flask cultivation:
- OptiProSFM: $0.8 \times 10^5$
- VaccineXpress: $8.5 \times 10^5$

Competitor medium

VaccineXpress medium

Rotavirus expression (IN Cell)
Virus purification
Clarification

**Process flow**

1. Cell culture
2. Harvest
3. Clarification
4. Primary purification
5. Secondary purification
6. Formulation

**Available techniques**

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

- **Centrifugation**
**Purification**

**Process flow**

1. Cell culture
2. Harvest
3. Clarification
4. Primary purification
5. Secondary purification
6. Formulation

**Available 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
Application examples core beads

Influenza
- Egg-based
- Cell-based

Dengue, Zika, and other flaviviruses

Lentivirus

Adenovirus

Cytomegolaviruse

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

- **Upstream process**
  - Detergent

- **Nuclease treatment**
  - Clarification NFF

- **Sample conditioning**
  - TFF
  - ReadyToProcess Adsorber Q B/E
  - ReadyToProcess Adsorber Q FT
  - Capto Q ImpRes
  - ReadyToProcess Adsorber Q B/E
  - Capto Q ImpRes
  - ReadyToProcess Adsorber Q FT
  - Capto Q ImpRes
  - Nuclease treatment

- **Clarification NFF**
  - TFF
  - Capto™ Q ImpRes

- **TFF**
  - Q Sepharose™ XL
  - Sepharose 4 FF
  - TFF
  - Capto Core 700
  - TFF
  - ReadyToProcess Adsorber Q B/E
  - ReadyToProcess Adsorber Q FT
  - TFF
  - Capto Core 700
  - TFF
  - Capto Core 700
  - TFF

- **Capto Q ImpRes**
  - Capto Core 700
  - TFF
  - Capto Core 700
  - TFF

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

- Excluded from pores
- Enter a fraction of the pores
- Enter all pores

Sample injection

- High molecular weight
- Intermediate molecular weight
- Low molecular weight

Equilibration

Absorbance

Column volume (CV)

SEC = size exclusion chromatography
Productivity for SEC and core bead chromatography

**200-L scale**

- Core bead chromatography, SS column
- Core bead chromatography, SU column
- Size exclusion chromatography, SS column

**2000-L scale**

- Core bead chromatography, SS column
- Core bead chromatography, SU column
- Size exclusion chromatography, SS column

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 (2–3x) → Capture → Polishing → Drug substance → Drug product

- Vero cells grown on Cytodex™ microcarriers
- Seed train bioreactor (ReadyToProcess WAVE™ 25 system)
- Clarification (ReadyToProcess™ hollow fiber filters operated through the ÄKTA readyflux single-use filtration system)
- Final filtration (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)

Fast Trak services

Virus production
- Culture medium (HyClone™ SFM4MegaVir)
- Production bioreactor (Xcellerex™ XDR systems)
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