Vaccine downstream processing—an overview

DCVMN 10 March 2017
Outline

Vaccines overview
Demands on vaccine purification
Common techniques for vaccine purification
Example of a purification process
Summary
Vaccine overview
Vaccines and production

Vaccines

- Bacteria based
- Virus based
- Protein based
- Polysaccharide based

The manufacturing process

- Cell culture/fermentation
- Purification
- Fill and finish
- Analysis (QA/QC)
Demands on vaccine purification
Safety and quality is priority

- Regulatory requirements
- Vaccine with no or minimal adverse effects
- Effective dose
- Stability
- Process control
- Reproducible process
Available technologies for downstream purification of vaccines
Release of target molecules

**Process flow**

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

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

**Yeast- and bacteria-based vaccines**
Impurity challenges after lysis

After cell lysis

<table>
<thead>
<tr>
<th>Impurities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process chemicals</td>
</tr>
<tr>
<td>DNA/RNA</td>
</tr>
<tr>
<td>Proteins</td>
</tr>
<tr>
<td>Cell membranes/organelles/lipids</td>
</tr>
<tr>
<td>Antigen related impurities</td>
</tr>
</tbody>
</table>

Antigen (e.g. virus)
Goal with purification

Purified sample

Antigen (e.g. virus) •
Clarification

**Process flow**

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

**Available techniques**

**Filtration**
- Normal flow (NFF)
- Tangential flow (TFF)

**Centrifugation**
Normal flow filtration (NFF)

- Removal of cell debris and larger particulates
- Porosities from 0.2 to 20 µm
- Scalable
- Single-use technology
- Straightforward process set-up
- Not recommended for harvest with high particulate content
Purification

**Process flow**

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

**Available techniques**

- **TFF**
- Density gradient centrifugation
- Selective precipitation
- Chromatography
  - IEX, MM, AC, HIC, SEC
  - Bead format (packed bed)
  - Membrane format (capsule)

Chromatography techniques:
AC = affinity, HIC = hydrophobic interaction
IEX = ion exchange, MM = multi modal, SEC = size exclusion
**TFF**

**Basic principle**

- Sweeping effect clean filter surface
- Allow greater throughput on smaller surface area

**Schematic set-up**

- Feed reservoir (concentrate)
- UF or MF device
- Feed pressure
- Permeate (filtrate)

MF = micro filtration
UF = ultrafiltration
TFF

Flat sheet cassettes

Cassettes consists of sheet membranes
Concentration/diafiltration
Defined pore sizes
Reusable
Scalable

Hollow fiber filters

Hollow fiber cartridge consists of tubular fibers
Concentration/diafiltration
Microfiltration
Suitable for shear sensitive material
Possible to handle high particle loads (e.g., cell harvest)
Defined pore sizes
Reusable
Scalable
### Ion exchange chromatography (IEX)

<table>
<thead>
<tr>
<th>Anion exchange chromatography</th>
<th>Cation exchange chromatography</th>
<th>Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-) Negatively charged molecules binds to (+) positively charged ligands</td>
<td>(+) Positively charged molecules binds to (-) negatively charged ligands</td>
<td><img src="image" alt="Anion Cation Exchange Diagram" /></td>
</tr>
</tbody>
</table>
Hydrophobic interaction chromatography (HIC)

<table>
<thead>
<tr>
<th>Separation by hydrophobicity</th>
<th>Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic surfaces of proteins interact with the ligand in presence of salts</td>
<td><img src="image" alt="Diagram" /></td>
</tr>
<tr>
<td>High salt content enhances and low salt weakens the interaction</td>
<td></td>
</tr>
</tbody>
</table>
Size exclusion chromatography (SEC)

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

Sample injection

High molecular weight

Intermediate molecular weight

Low molecular weight

Absorbance

Injections

Columns
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 dextran

Principle
Chromatographic purification of large molecules can be challenging.

Pore size ~ 40–150 nm

- 1–7 nm proteins
- 25 nm polio virus
- 100 nm influenza virus

200 × 500 nm Pox virus

Flow-through chromatography recommended

Bind-elute chromatography possible
The pore size determines the properties

Chromatography beads
~ 2–6 min residence time

Membrane adsorbers
~ 2–60 s residence time

Pore size ~ 40–150 nm

Average pore size 3–5 µm

Virus ~100 nm
Core bead chromatography: host cell proteins and DNA fragments bind to the core and viruses stay in the void
Formulation

**Process flow**

- Cell culture
- Harvest
- Clarification
- Primary purification
- Secondary purification
- Formulation

**Available techniques**

- Buffer exchange
- TFF
- Chromatography
  - SEC
Process example
Process example inactivated polio vaccine (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

AIXE (FT)
DNA removal. Polio virus in flow through (FT)

Virus inactivation
formaldehyde

Formulation
Sterile filtration, mixing with other strains
Summary: robust downstream process can ensure high quality

Most vaccines have unique purification processes
Preferably use scalable techniques when developing new processes
Purification of particles in binding mode can be difficult with classic chromatography
Core bead chromatography suitable for purification of particles $> 700$ kDa