Vaccine Downstream processing – an overview

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May, 2015
Overview

- Vaccines overview
- Demands on vaccine purification
- Common techniques for vaccine purification
- Example of a purification process
- Summary
Vaccine Overview
How Vaccines are manufactured

The Vaccines

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

The Manufacturing process

- Cell culture / Fermentation
- Purification
- Fill and Finish
- Analysis (QC/QA)

Number and order of the different steps depends on the specific vaccine production

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Demands on vaccine purification
Safety and quality is priority

Regulatory requirements

- Safe vaccine with no or minimal adverse effects
- Effective dose
- Stability
- Process control
- Reproducable process
Downstream purification of vaccines
Downstream processing of viruses
Available technologies

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

Diagram:
- Cell culture
- Harvest
- Clarification
- Primary purification
- Secondary Purification
- Formulation
Impurity challenges after lysis

Cell lysis

- DNA/RNA
- chemicals
- proteins
- Antigen (e.g. virus)
- Organelles/cell membrane/lipids
Goal with purification

Cell lysis

Purification

Antigen (e.g. virus)
Downstream processing of viruses
Available technologies

Clariﬁcation
• Filtration
  – Normal ﬂow
  – Tangential ﬂow
• Centrifugation
Normal flow filtration

- Removal of cell debris and larger particulates
- Porosities from 0.2 - 20 µm
- Scalable
- Single-use
- Straight forward process set up
- Not recommended for harvest with high particulate content
Downstream processing of viruses
Available technologies

**Primary purification**
- Tangential flow filtration (TFF)
- Density gradient centrifugation
- Precipitation
- Chromatography
Tangential flow filtration

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

Hollow fiber filters

- Hollow fiber cartridge consists of tubular fibers
- Concentration/ diafiltration
- Microfiltration
- Suitable for shear sensitive material
- Possible handle high particle loads (ex cell harvest)
- Defined pore sizes
- Re-usable
- Scalable

Flat sheet cassettes

- Cassettes consists of sheet membranes
- Concentration/ diafiltration
- Defined pore sizes
- Re-usable
- Scalable
Downstream processing of viruses
Available technologies

Secondary purification
• Density gradient centrifugation
• Selective precipitation
• Chromatography
  – IEX, MM, AC, HIC, SEC
  – Bead format (Packed bed)
  – Membrane format (Capsule)
Ion exchange chromatography

Anion exchange chromatography
• (-) Negatively charged molecules binds to (+) positively charged ligands

Cation exchange chromatography
• (+) Positively charged molecules binds to (-) negatively charged ligands
Hydrophobic interaction chromatography

Separation by hydrophobicity

• Hydrophobic surfaces of proteins interact with the ligand in presence of salts

• High salt content enhance and low salt weakens the interaction
Size exclusion chromatography

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

Sample injection
High molecular weight
Intermediate molecular weight
Low molecular weight

Equilibration
1 CV
Column volumes

Absorbance

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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 dextrane
Chromatographic purification of large molecules can be challenging.

- 100 nm influenza virus
- 200 x 500 nm Pox virus
- 1-7 nm proteins
- 25 nm polio virus
- Pores

~90 µm chromatography bead

Bind-Elute chromatography possible

Flow through chromatography recommended
Core bead chromatography

- Host cell proteins and DNA fragments bind to the core and viruses stay in the void.
Process example

Polio 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

Virus inactivation
formaldehyde

Formulation
Sterile filtration, mixing with other strains

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Summary

• Robust downstream process can ensure consistent 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 of sufficient size
Thank you

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