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

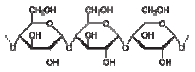
Vaccines

- Bacteria based

- Virus based

- Protein based

- Polysaccharide based



The manufacturing process

- Cell culture/ fermentation
- **Purification**
- Fill and finish
- Analysis (QA/QC)

E. Coli Bacteria from NIH Image Library
Influenza virus by Kat Masback.

Demands on vaccine purification

Safety and quality is priority

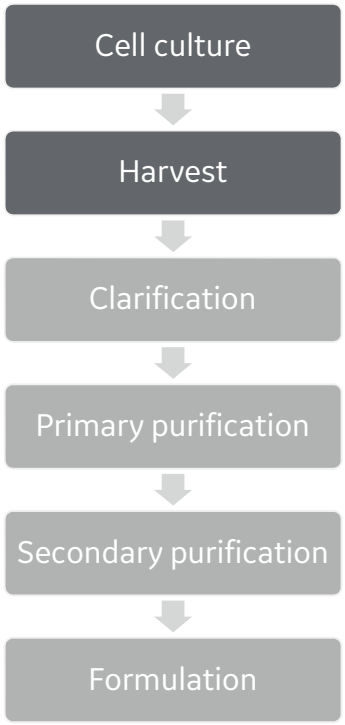
- Regulatory requirements
- Vaccine with no or minimal adverse effects
- Effective dose
- Stability
- Process control
- Reproducible process

Vaccine image by www.torange-pt.com.

Available technologies for downstream purification of vaccines

Release of target molecules

Process flow



Lytic virus

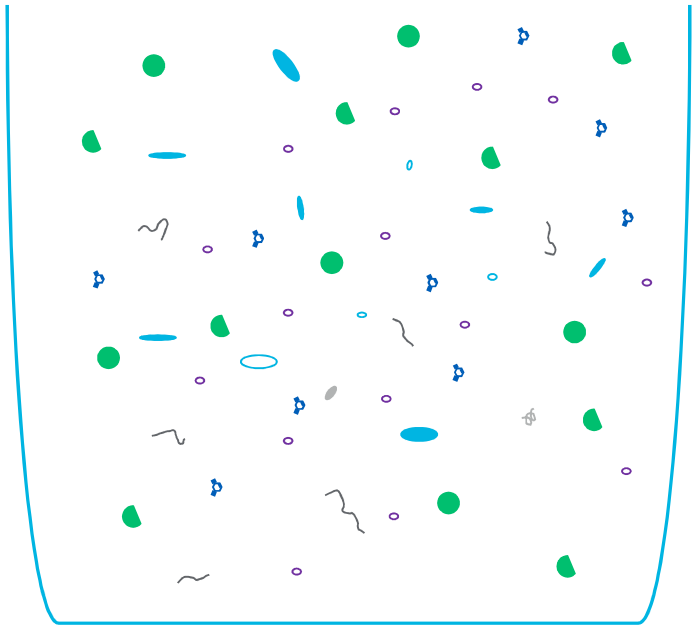
Non-lytic virus

- Detergent
- Mechanical disruption/homogenization
- Osmotic shock
- Freeze-thaw

Yeast- and bacteria-based vaccines

Impurity challenges after lysis

After cell lysis



Antigen (e.g. virus)

Impurities

Process chemicals

DNA/RNA

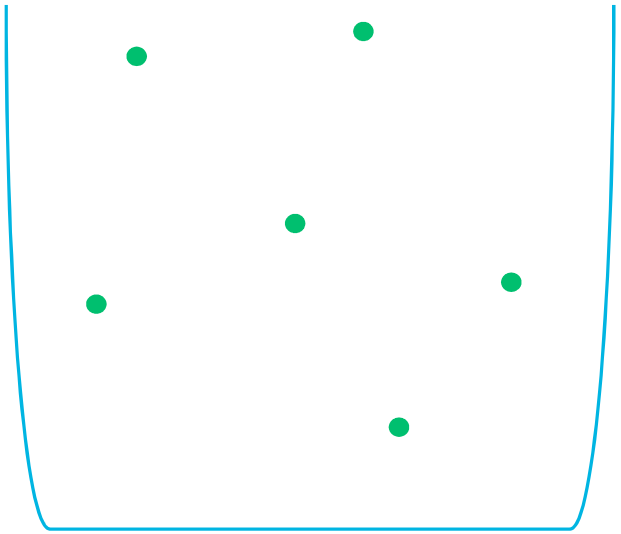
Proteins

Cell membranes/organelles/lipids

Antigen related impurities

Goal with purification

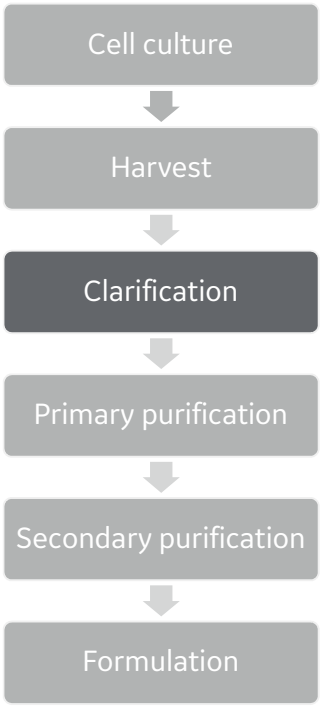
Purified sample



Antigen (e.g. virus) ●

Clarification

Process flow



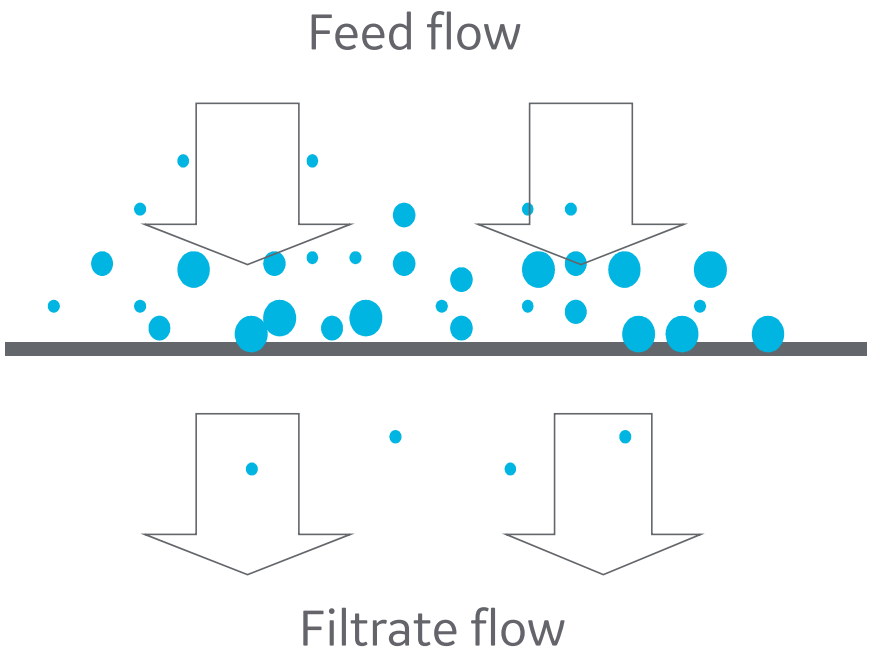
Available techniques

Filtration

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

Centrifugation

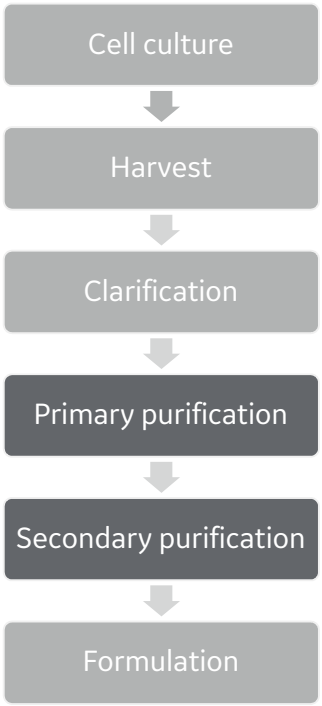
Normal flow filtration (NFF)



- 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



Available techniques

TFF

Density gradient centrifugation

Selective precipitation

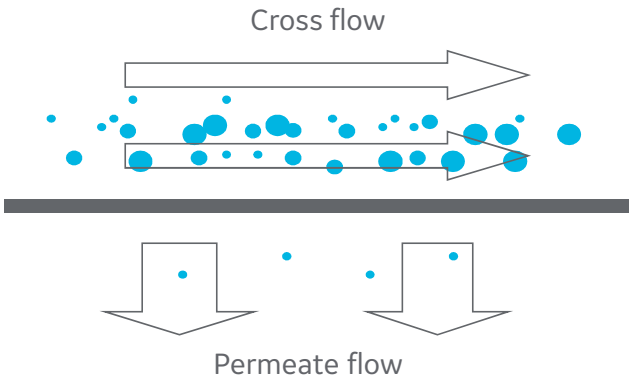
Chromatography

- 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

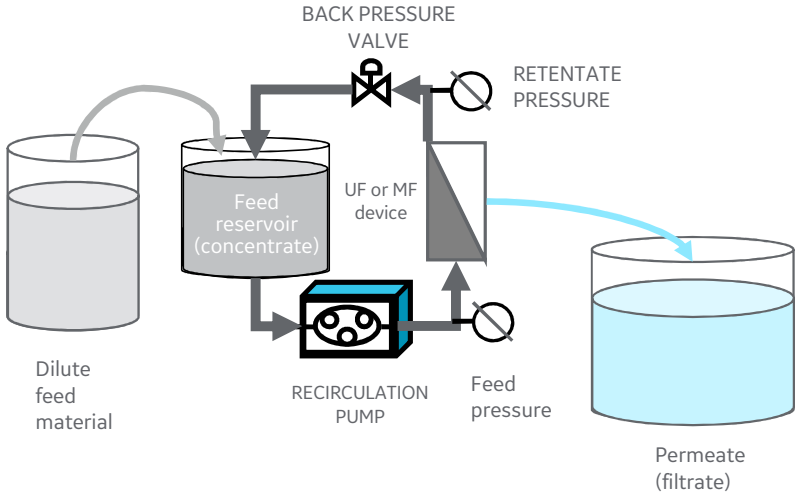
TFF

Basic principle



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

Schematic set-up



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)

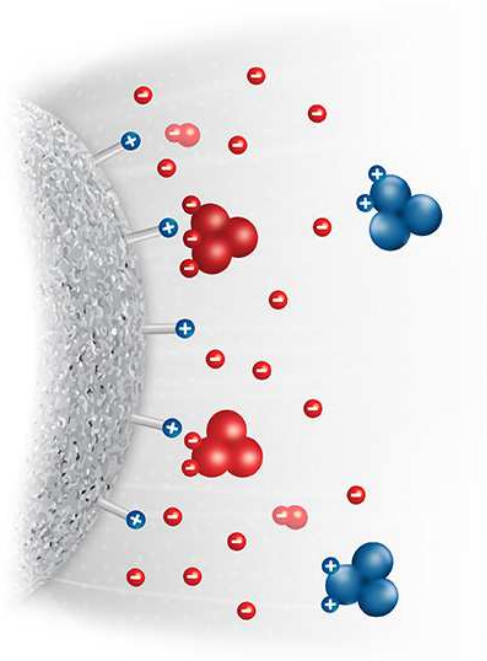
Anion exchange chromatography

(-) Negatively charged molecules binds to (+) positively charged ligands

Cation exchange chromatography

(+) Positively charged molecules binds to (-) negatively charged ligands

Principle



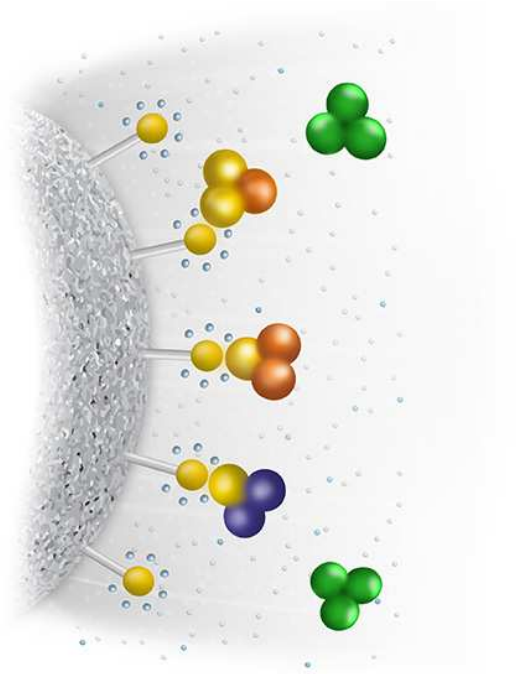
Hydrophobic interaction chromatography (HIC)

Separation by hydrophobicity

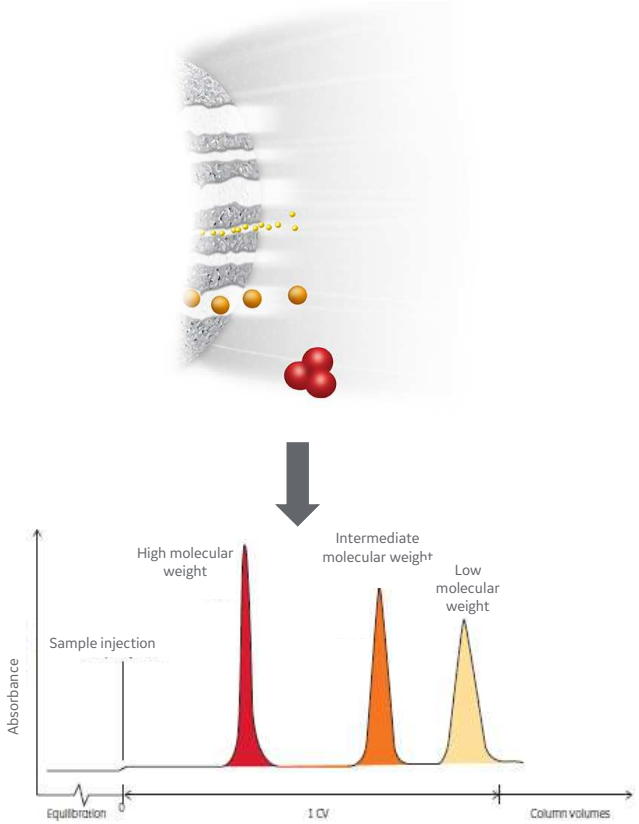
Hydrophobic surfaces of proteins interact with the ligand in presence of salts

High salt content enhance and low salt weakens the interaction

Principle



Size exclusion chromatography (SEC)



Exluded from pores

Enter a fraction of the pores

Enter all pores

Affinity chromatography

Specific binding

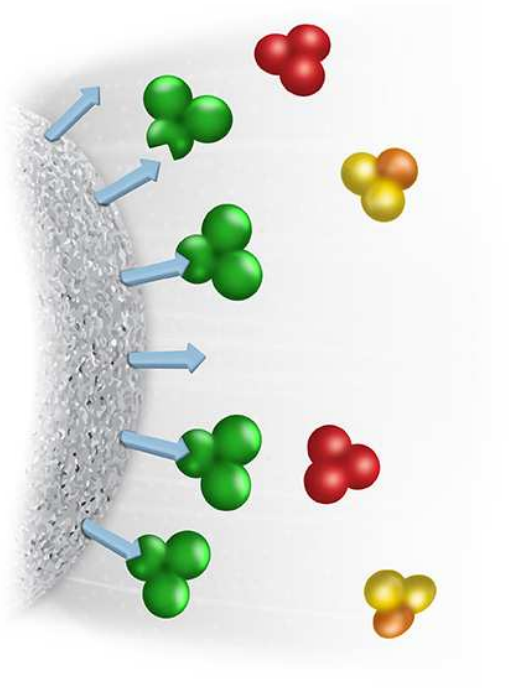
Few affinity resins available for vaccines

Agarose-based affinity resin for adeno associated virus

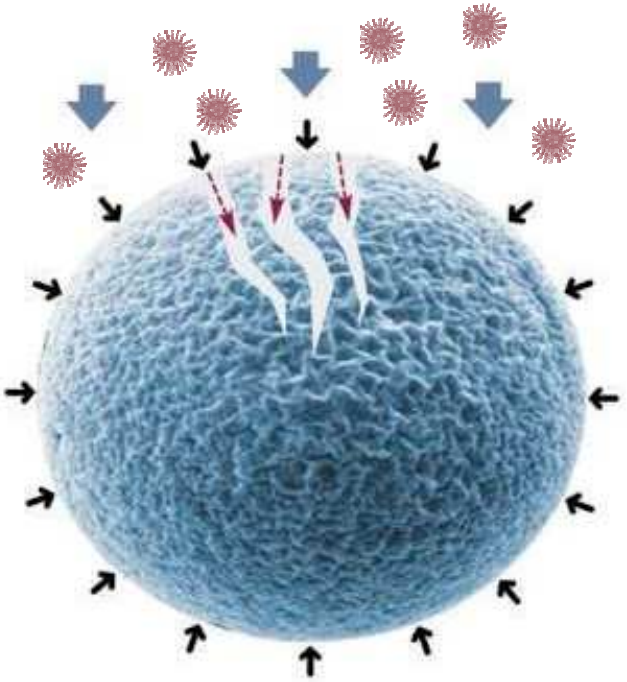
Pseudo affinity resins for influenza

- 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

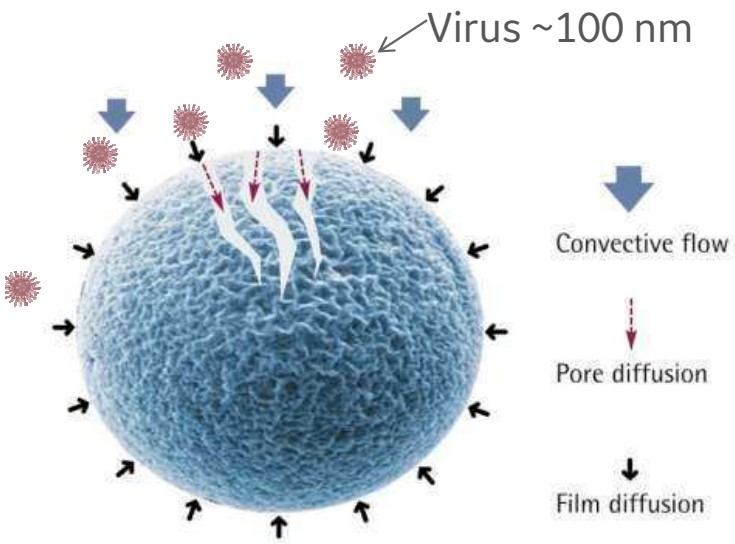
Bind-elute chromatography possible

Flow-through chromatography recomended

The pore size determines the properties

Chromatography beads

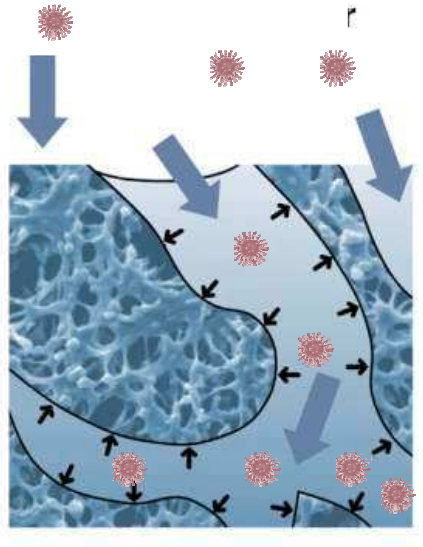
~ 2–6 min residence time



Pore size ~ 40–150 nm

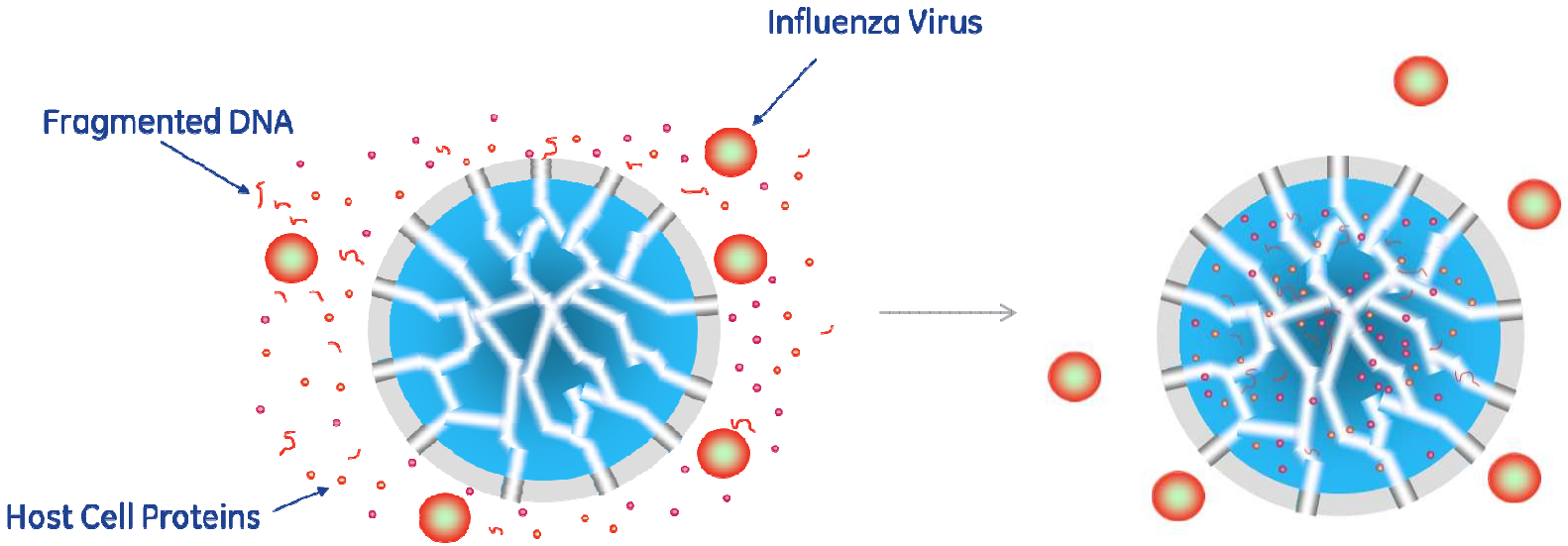
Membrane adsorbers

~ 2–60 s residence time



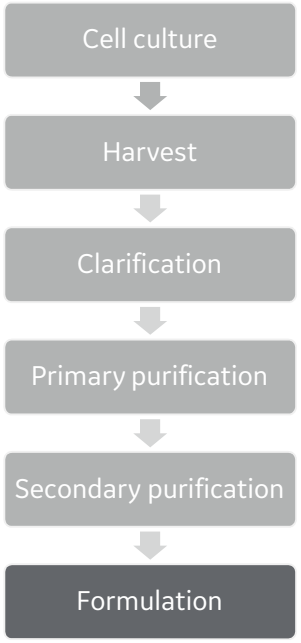
Average pore size 3–5 µm

Core bead chromatography: host cell proteins and DNA fragments bind to the core and viruses stay in the void



Formulation

Process flow



Available techniques

Buffer exchange

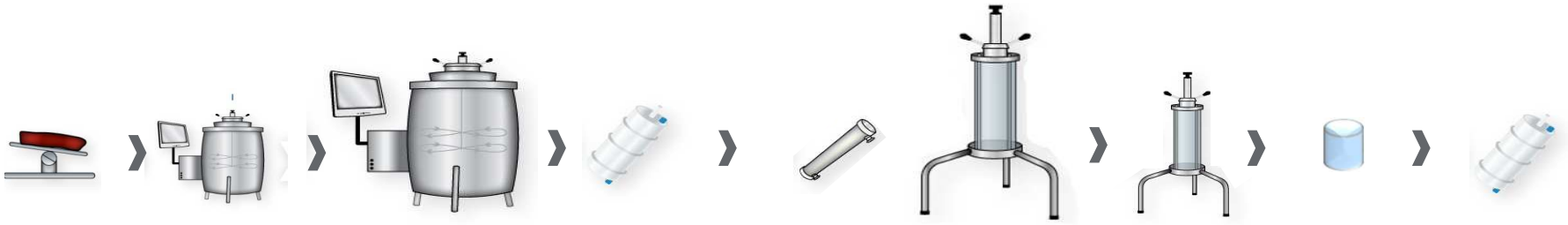
TFF

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

AIEX (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

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden