Downstream Processing Techniques and Single Use Applications

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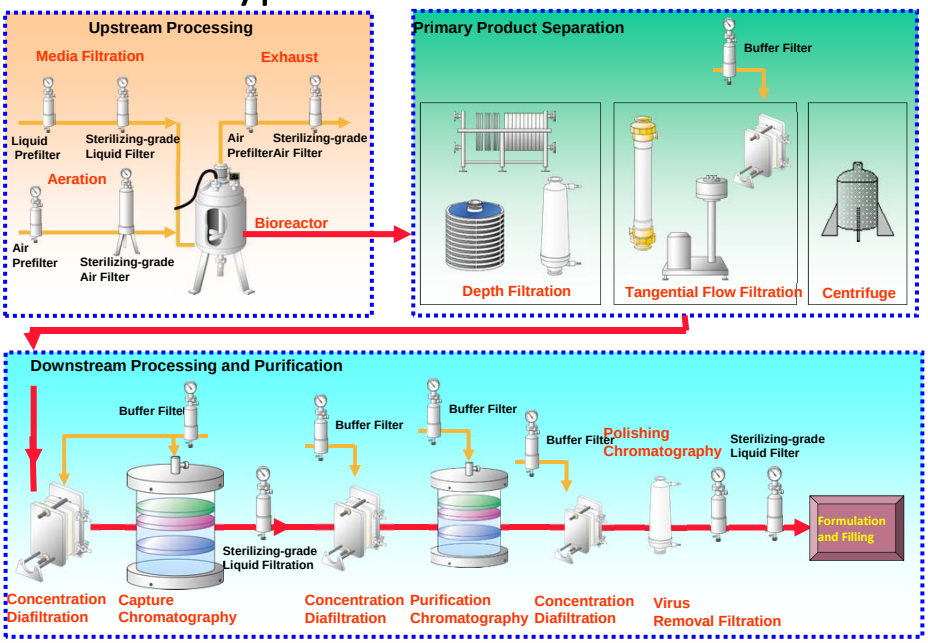
ISPE Connecting a World of Pharmaceutical Knowledge

Typical Biotech Process



Cell culture / Fermentation

Growth of appropriate cells, typically in a bioreactor or fermenter, to produce product of interest

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Primary Product Recovery

Primary Product Recovery or Primary Clarification is a:

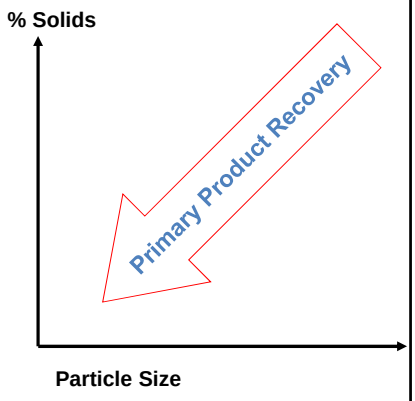
Solid : Liquid Separation

Whole Cells : Liquid Phase containing **product** + Cellular Debris

Product Transmission is KEY Objective

Separation Efficiency dependent on:

- Amount of Solids
- Particle Size
- Viscosity



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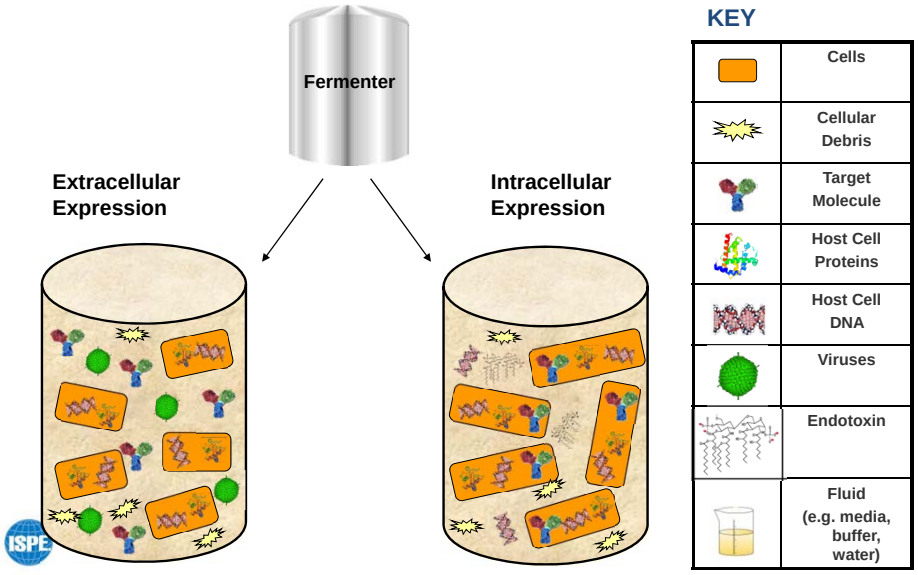
Product Location



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Primary Product Recovery

Recovering product that has been produced by cell culture or fermentation

- **Clarification of cell / fermentation broths (removal of whole cells and cellular debris) to recover product (typically soluble)**
  – EXTRACELLULAR EXPRESSION
- **Concentration and recovery of whole cells prior to cell disruption / lysis to release product (soluble or insoluble)**
  - INTRACELLULAR EXPRESSION

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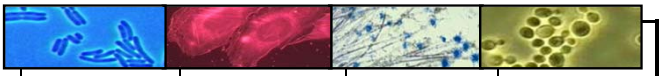
Commonly Encountered Cell Types



	Bacterial	Mammalian	Fungal	GMO Yeast
Example	*E. Coli*	CHO (Chinese Hamster Ovary)	*Penicillium*	*Pichia Pastoris*
Cell Size	0.5 – 0.8µm	10 - 100µm	3µm	14µm +
Potential Volumes	30,000 L	100 - 25000 L	100,000 L	30,000 L
Typical Cell Densities	2 – 5% w/v 20 – 50 g/l	1 – 5 x 10^7 cells /ml	40 – 50% w/v (400 – 500 g/l)	40 – 50% w/v (400 – 500 g/l)
Product Location	Mainly Intracellular Some Extracellular	Extracellular	Extracellular	Extracellular
Example	Antigen Binding Fragments (FAbs)	Monoclonal Antibodies	Antibiotics Industrial Enzymes	Small Peptides and proteins (e.g. Insulin)

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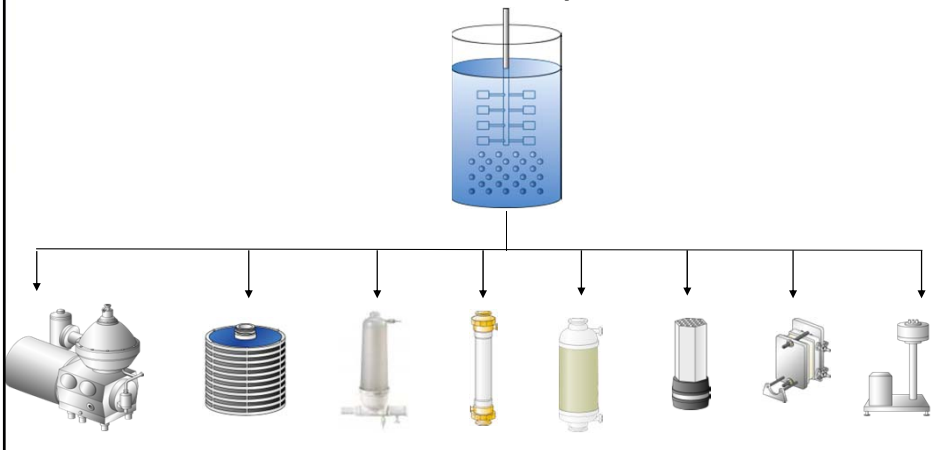
Technologies For Primary Product Recovery



Centrifugation | Depth filtration | DFF Capsule | Hollow fibres | TFF Capsule | Ceramic | Cassettes | VMF

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Biomolecule Purification Strategy

How do we purify the target molecule from this molecular 'soup'?

Chromatography

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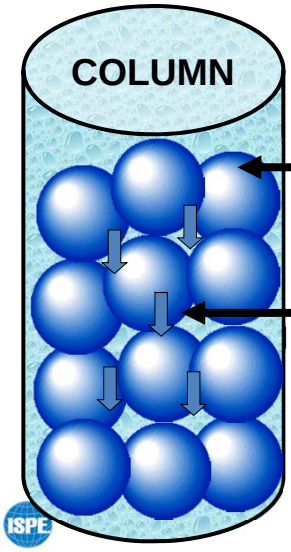
What is Chromatography?



A separation method consisting of a:

Stationary phase
(sorbent/gel/media/resin or membrane)

Mobile phase
(Process fluid stream/buffer)

Properties of mobile phase, stationary phase and molecule determine column residence time

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Chromatography Scales

Laboratory

Pilot

Production

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Key	ID	Typical Units
	Target Molecule	mg/mL
	Host Cell Proteins	ppm or ng/mg
	Host Cell DNA	pg/mL
	Viruses	LRV of virus particles
	Aggregates/ Misfolds	% or ppm
	Endotoxin	EU/mL
	Fluid (e.g. media, buffer, water)	L

Chromatography Purification

Process stream composition

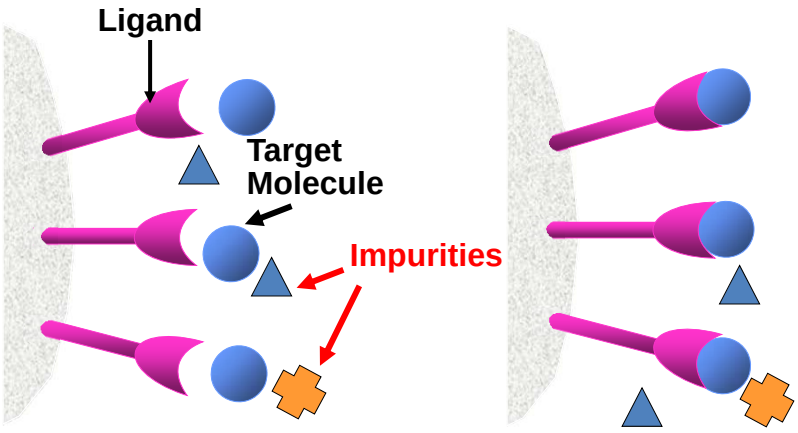
Out

Target Molecule >97%

Process-Related Impurities

- Impurities derived from the drug manufacturing process
  - Include Host Cell Proteins (HCPs), host cell DNA
  - Antibiotics, cell culture media components…
  - Column/filter extractables and leachables (protein A)
- **All must be removed during downstream processing, using various methods, including chromatography**
  - Require different strategies, according to the nature of the impurities, their concentration, and the target protein.

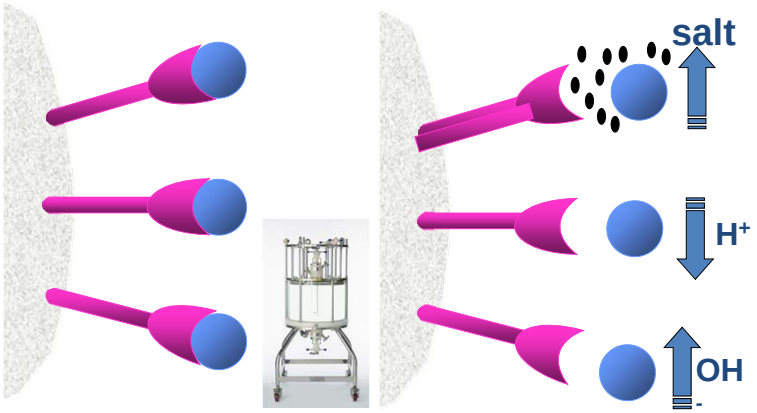
Affinity Chromatography
Protein-A



Target Molecure Bound at ~neutral pH

Affinity Chromatography

The specific molecule can be eluted by increasing salt concentration or change of pH (acidic)



Target Molecule
High Purity
Reduced Volume

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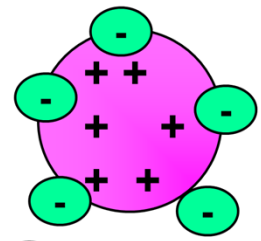
Ion Exchange Chromatography

ANION exchanger
(Q or DEAE)

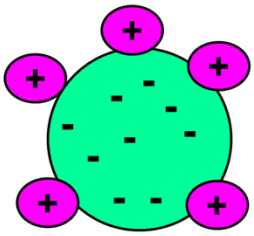


- = Cl^- or Molecule$^-$

AETMA or Diethylaminoethyl

Reduce HCP and Viruses

CATION exchanger
(S or CM)



+ = Na^+ or Molecule$^+$

Sulphonic acid or carboxymethyl

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Hydrophobic Chromatography (HIC)

Proteins Bind at High Salt Concentration and Elute at Low Salt Concentration

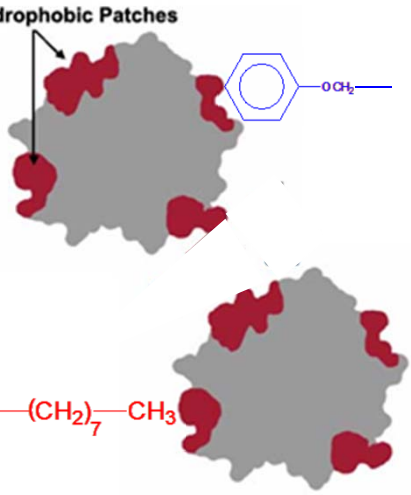
GE Healthcare

TOSOH

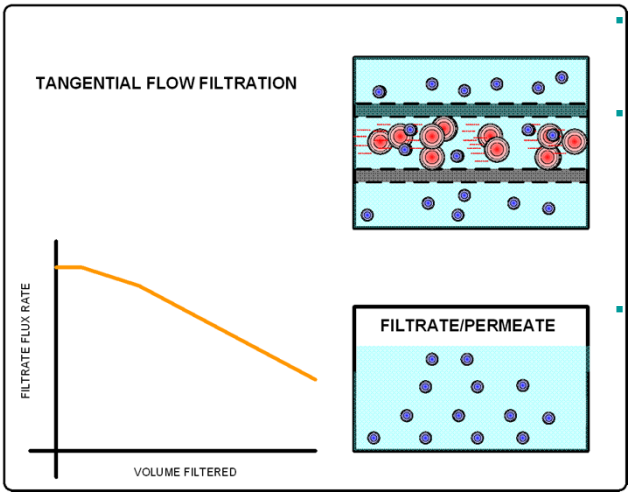
BIO-RAD

MERCK

$\left[NH_4^+ \right]_2 \left[O=S(=O)(-O^-)-O^- \right]$



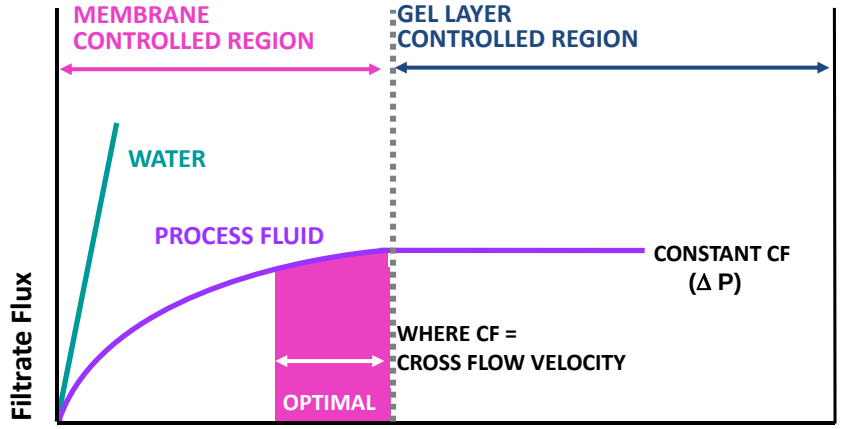
Tangential Flow Filtration



- As permeate (filtrate) volume increases...
- ... permeate flux decreases due to feed viscosity increase, but membrane has reduced fouling by crossflow action.
- Result: TFF allows processing of larger volumes with higher solids loading than DFF.

Flux Versus TMP

Critical for Proper Operation



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Tangential Flow Filtration

Microfiltration (MF)

- Pressure driven process where particulates (e.g. cells or cellular debris - waste) are retained on a basis of their physical size while small particles, small molecular weight species (product) & fluid/water pass through the membrane
- As fluid/water is removed the upstream is 'concentrated'
- Diafiltration in MF is used to 'wash' through more product to increase the yield
- 0.1 to 1 micron in general

Ultrafiltration - UF

- Pressure driven process where solutes (e.g. proteins) (Product) are retained on a basis of their molecular size while very small molecular weight species (e.g. salts) & fluid/water pass through the membrane (waste)
- As fluid/water is removed the upstream is 'concentrated'
- Diafiltration in UF is used to 'exchange buffers' to prepare for chromatography to maximize yield
- 0.01 to 0.1 micron
- 5-1000 kDa – MWCO (molecular weight cutoff)

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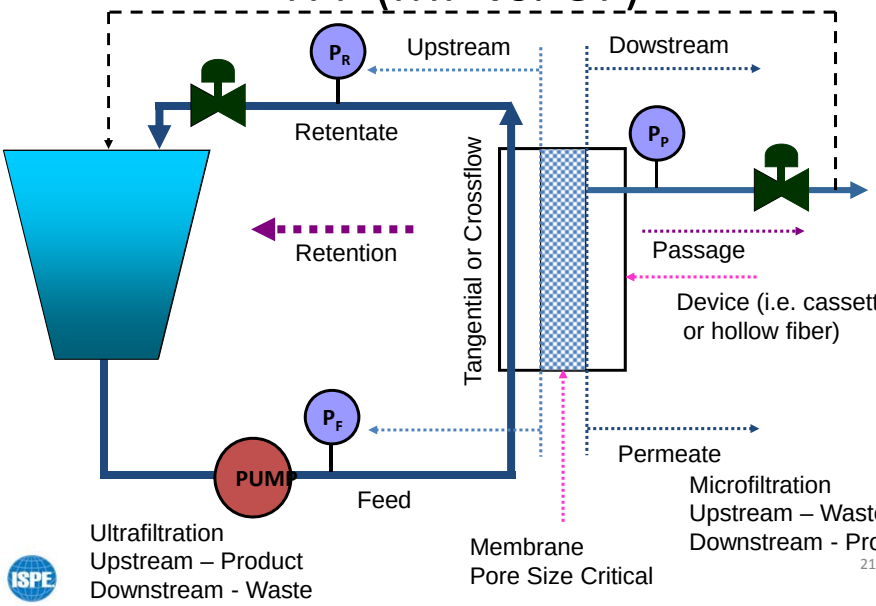
TFF (MF vs. UF)



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Single-Use TFF

- Single-Use Flow Path
- Automated
- Programmable
- Mobile
- Flexible
- Batch Reporting

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Tangential Flow Filtration – Microfiltration (MF)

- MF Objectives:

1. Transmit the product/protein through the membrane to a target yield (i.e. > 98% yield).
2. Retain unwanted waste material/particulate on the upstream side of the membrane.

- Note: Unwanted waste in downstream pool can be removed in successive steps… lost product (retained), is lost yield and is non-recoverable.

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Tangential Flow Filtration – Ultrafiltration (UF)

- UF Objectives:

1. Retain the product on the upstream side to be recovered as yield (i.e. >98% yield).
2. Prepare (via concentration and diafiltration) the upstream product for chromatography (maximize the efficiency of subsequent chromatography step) or formulation (exchanges product into ideal buffer for formulation operation).

- Note: lost product (passage or poor recovery), is lost yield.

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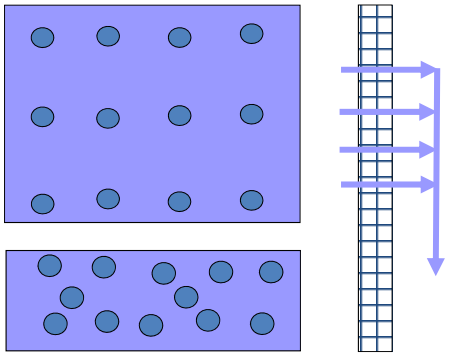
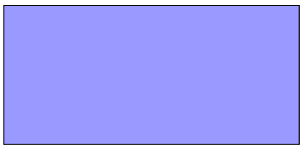
What is Concentration?

Concentration:

- Reduction of initial volume to increase the concentration (i.e. protein) per liter of material which is withheld by the membrane.



Concentration speeds up chromatography by reducing the volume to be processed.



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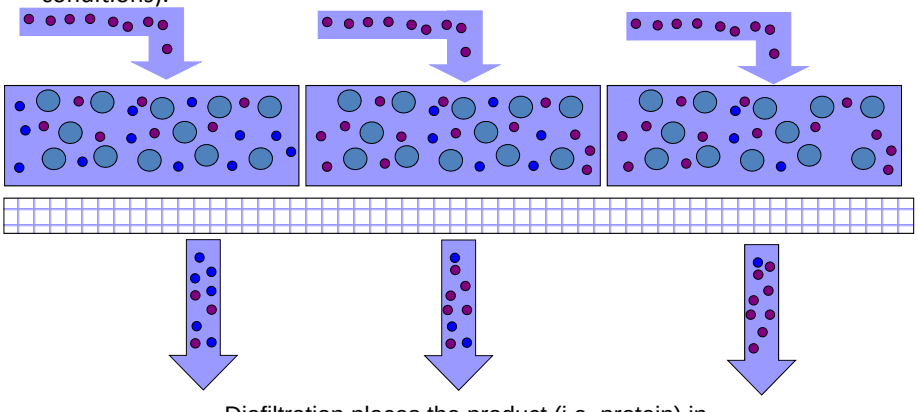
What is Diafiltration?

Diafiltration (DF):

- Exchange of buffer in which product is held to alter conditions (i.e. salt or pH conditions).



Diafiltration places the product (i.e. protein) in the ideal buffer conditions to optimize chromatography.

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Continuous Versus Discontinuous DF

Diafiltration Volumes	Continuous Percent removal (100% permeable)	Discontinuous 2X Percent removal (100% permeable)
1	63	50
2	86	75
3	95	88
4	98.2	94
5	99.3	96.9
6	99.7	98.4
7	99.9	99.2

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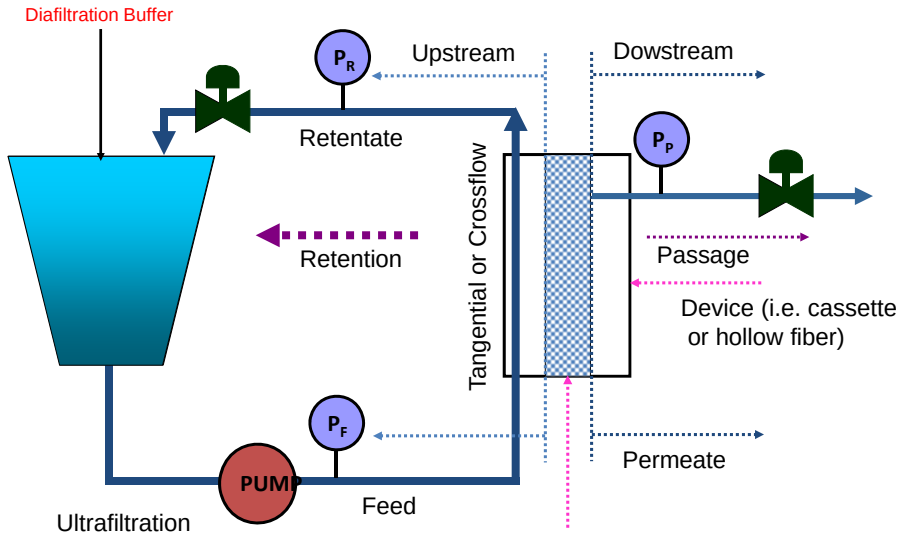
TFF Diafiltration



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TFF Downstream Examples

TFF Example 1 – Post-Protein A or Low pH VI

- TFF diafiltration to modify buffer and pH to optimize load conditions for Ion Exchange chromatography.

TFF Example 2 – Post-Cation Exchange

- TFF diafiltration to lower conductivity of MAb eluted in 300mM NaCl from Cation Exchange chromatography from 30-60 mS/cm to 5 mS/cm to optimize load conditions for Anion Exchange chromatography.

TFF Example 3 – Prep for Formulation

- TFF diafiltration to exchange to neutral buffer to enable formulation of final product.

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Viral Clearance by Solvent/Detergent Inactivation

- Organic Solvent tri-(n-butyl) phosphate (TnBP)
- Detergent (Tween 80, Triton X-100)
- Generally Done following Protein-A capture
- Effective for lipid enveloped viruses
- Solvent enhances aggregation reaction between viral lipid coating and detergent

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Viral Clearance by Low pH Viral Inactivation

- Lower pH ~3.5 to 4.0 depending on protein of interest
- Done following Protein-A capture
- Denature enveloped viruses
- Target protein should be resistant to denaturation from low pH for at least 2 hours.

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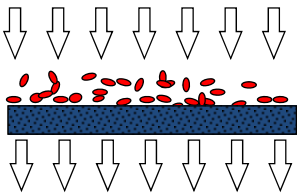
Viral Clearance by Direct Flow Filtration

- Broad capability based on size exclusion
- Specific 'Robust' step
- Biological activity of product is maintained
- Viral components are removed
- Non-contaminating
- Easily validated



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Single-Use
Viral Filtration or Low pH Inactivation

- Single-Use Flow Path
- Automated
- Programmable
- Mobile
- Flexible
- Batch Reporting

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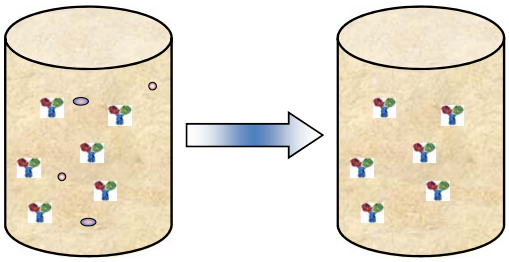
Sterile Filtration – Bulk Fill

Primary Objectives
Transmission of target molecule
Removal of any bacterial contaminant

Purified Product



Bioburden CFU / 100mL

Process stream composition

Key	ID	Typical Units/ measures
	~~Cells~~	~~Cell/mL; NTU; viability~~
	~~Cellular Debris~~	~~NTU~~
	Target Molecule	Titre; mg/mL
	~~Host Cell Proteins~~	~~ppm~~
	~~Host Cell DNA~~	~~pg/mL~~
	~~Viruses~~	~~LRV of virus particles~~
	Fluid (e.g. media, buffer, water)	L

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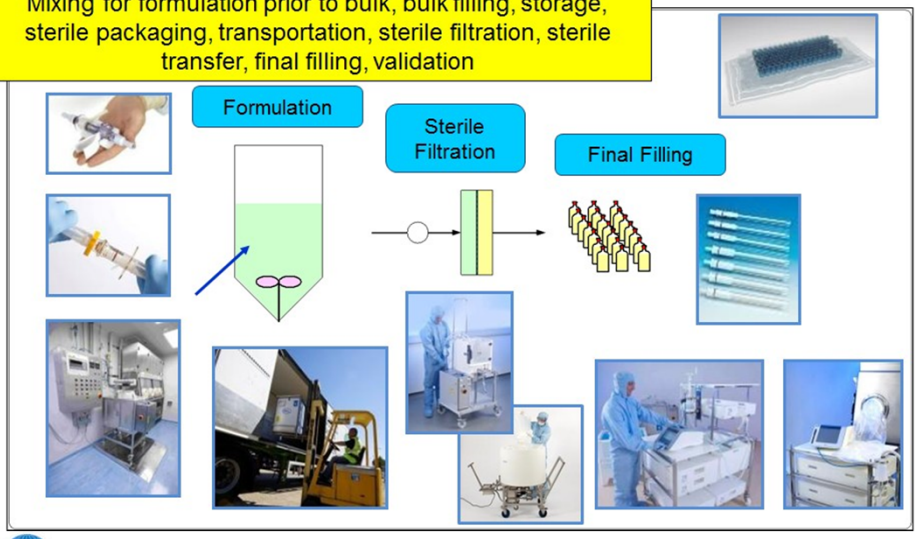
Single-Use Applications for Formulation & Filling

Mixing for formulation prior to bulk, bulk filling, storage, sterile packaging, transportation, sterile filtration, sterile transfer, final filling, validation



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Bulk Fill System
Single-Use

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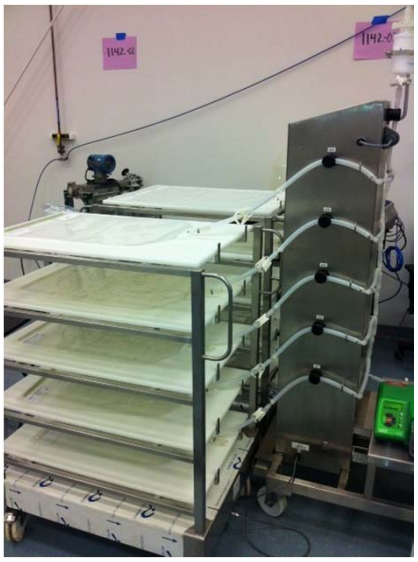
Bulk Fill System
Single-Use



- Single-Use
- Automated
- Programmable
- Mobile
- Flexible
- Batch Reporting

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Single-Use Applications

- **Process safety, robustness and automation**
  - Fully automated process control, monitoring and reliability in manufacture
  - Reproducible process performances, scalable solutions
- **Single-use flow paths**
  - Eliminates risk of batch or cross contamination
  - Eliminates cleaning requirements
  - Reduces validation time and costs
- **Ease-of-use / Flexibility**
  - Installation, operation, disassembly
  - Ready-to-use solutions, with reduced pre-use conditioning
- **Process economics**
  - Significant savings in capital, materials, labor & facility operating costs
  - Increases productivity and enhanced resource allocation

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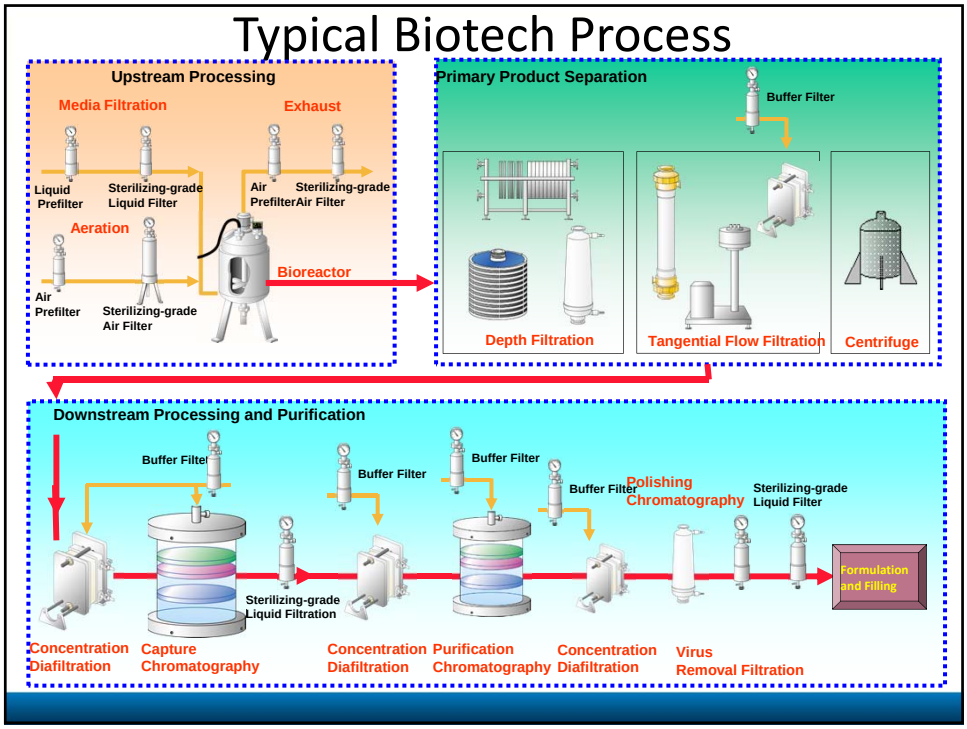

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