Monoclonal Antibody Synthesis Process – mAb-X

From the cell bank to the final product

cybernano

easyQBD

Goal : Deliver a safe, effective IgG1 drug product (mAb-X) from qualified cell bank

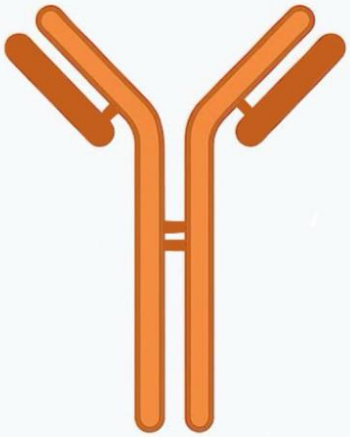
Description of Antibody Quality Attributes

Common product quality attributes for therapeutic antibodies

Disulfide bonds
Mis-paired / scrambled disulfides

Cyclization
N-terminal pyroglutamate

Integrity
Fragmentation, half-antibodies, free light chain



Glycosylation (Fc)
GOF/G1F/G2F, afucosylation, high-mannose, sialylation

Glycation
Non-enzymatic (reducing sugars)

Chemical modifications
Methionine oxidation isomerisation

C-terminal clipping
Lysine truncation

Higher-order structure
Aggregation, dimerization

Introduction

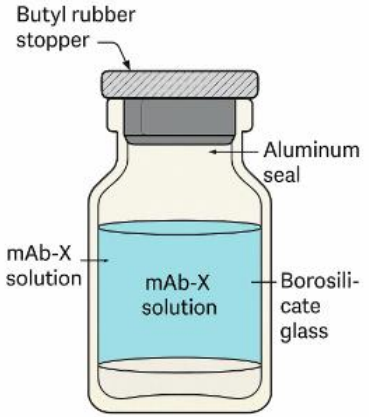
Quality Attributes of the Final Drug Product

Formulation :

Final Formulation of mAb-X

- IgG1 Antibody: 10 mg/mL
- Phosphate buffer: 10 mM
- NaCl : 150 mM
- Polysorbate 80: 0.01%
- pH: 6,0 ± 0.2
- Osmolality: ~ 290 mOsm/kg

Drug Product Packaging :



Stability

24 month at 2–8 °C ,

short-term: 30 days at 25 °C

Release specifications:

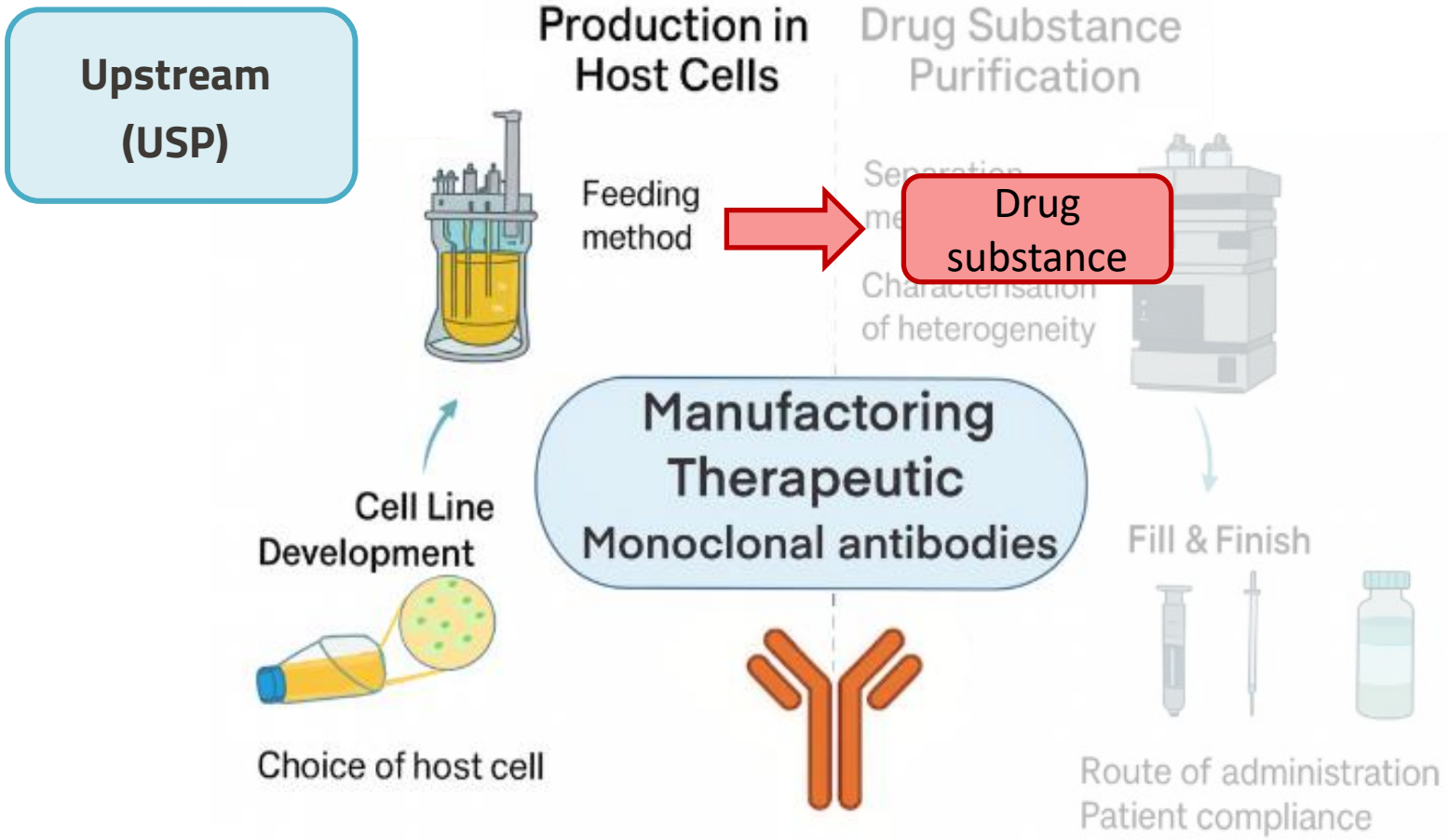
Purity ≥98%

Aggregates <1%

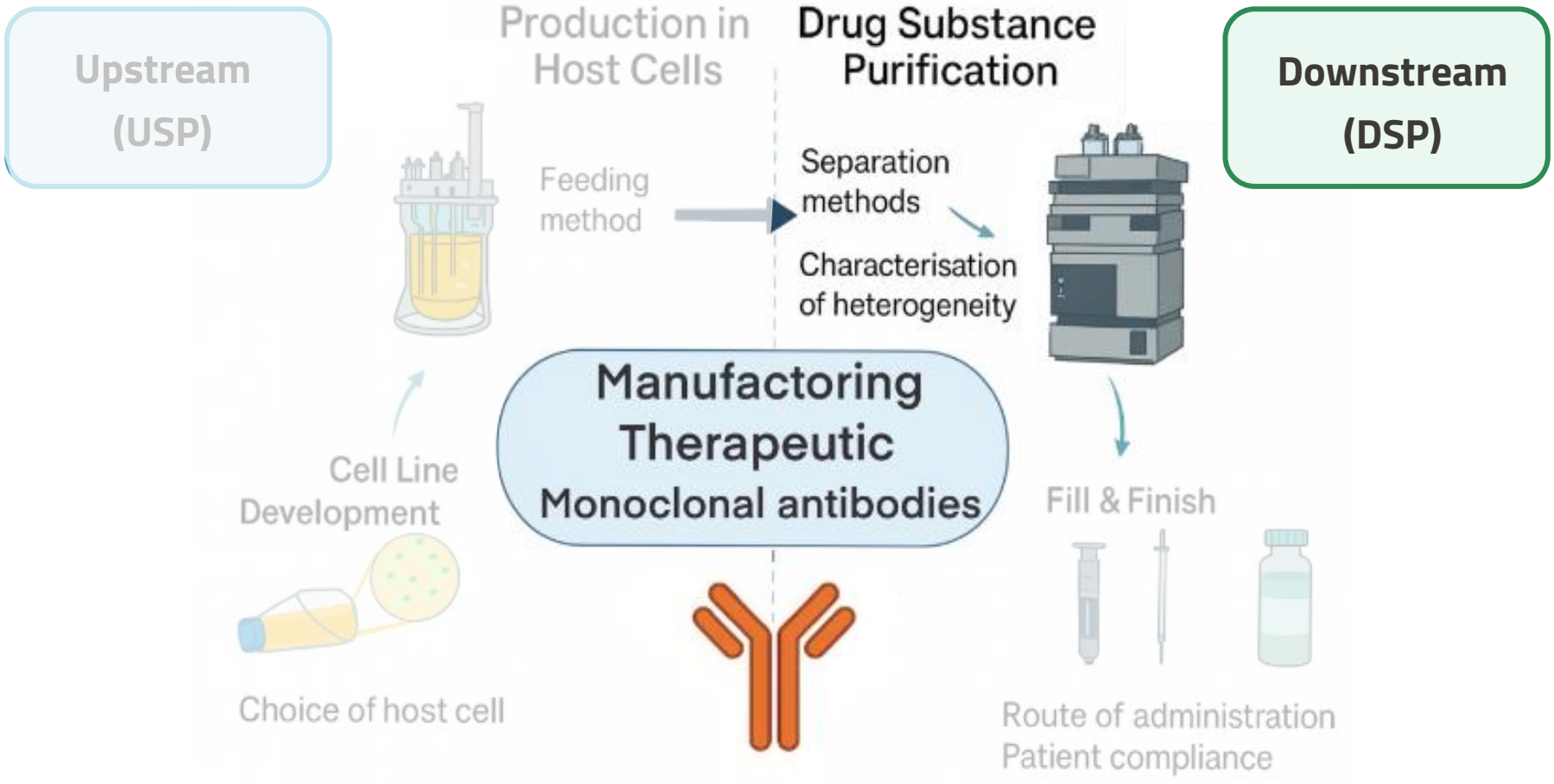
IC50 ≤15 ng/mL

HCP <5 ppm

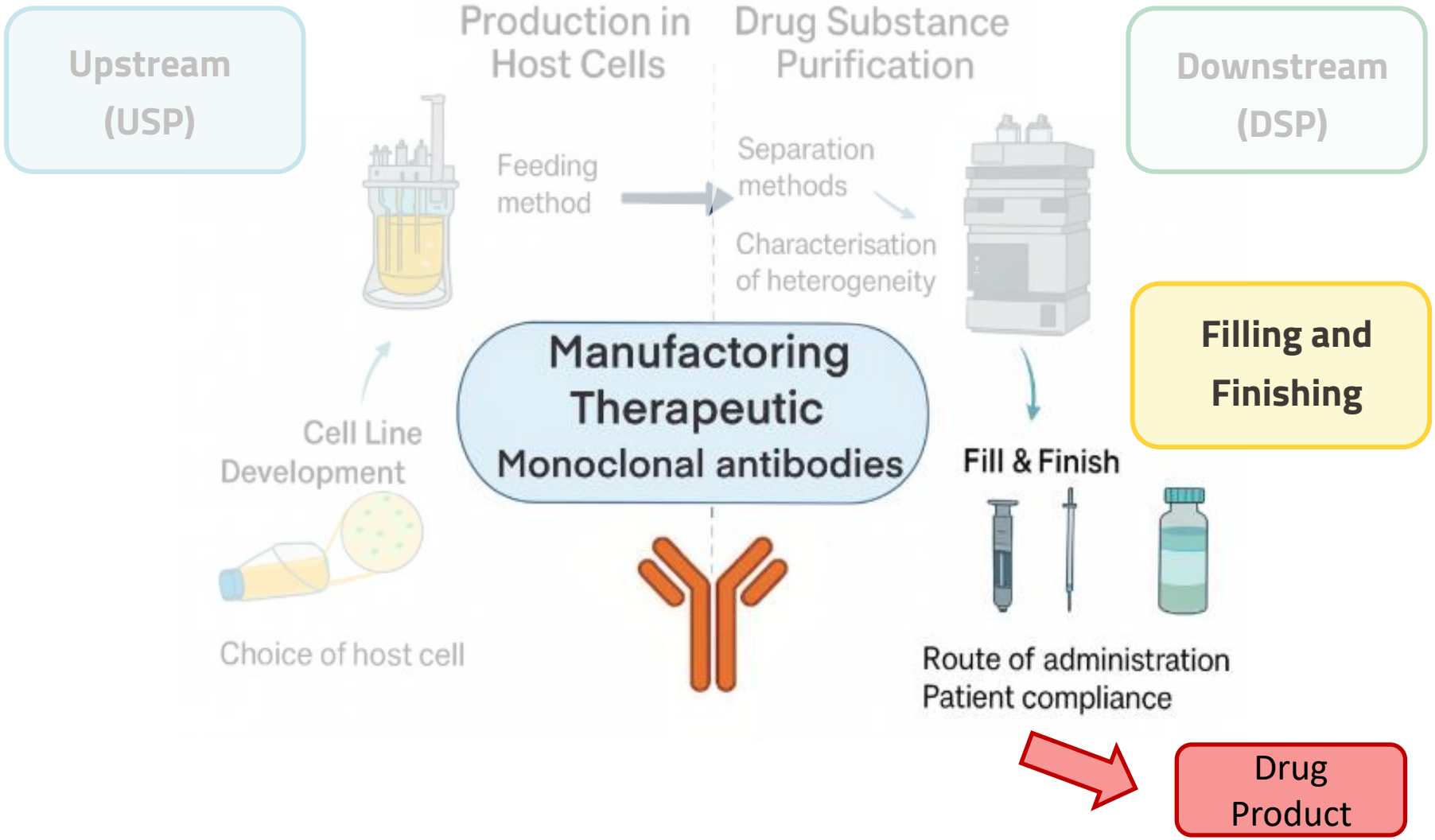
Global Process Flow



Global Process Flow



Global Process Flow



Introduction

Global Process Flow

Upstream (USP)

Production in Host Cells

Drug Substance Purification

Downstream (DSP)

Feeding method

Separation methods

Characterisation of heterogeneity

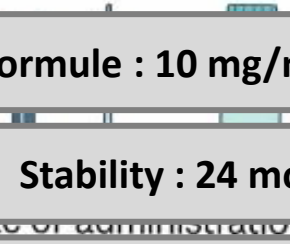
Manufactoring Therapeutic Monoclonal antibodies

Filling and Finishing

Cell Line Development

Choice of host cell

Fill & Finish



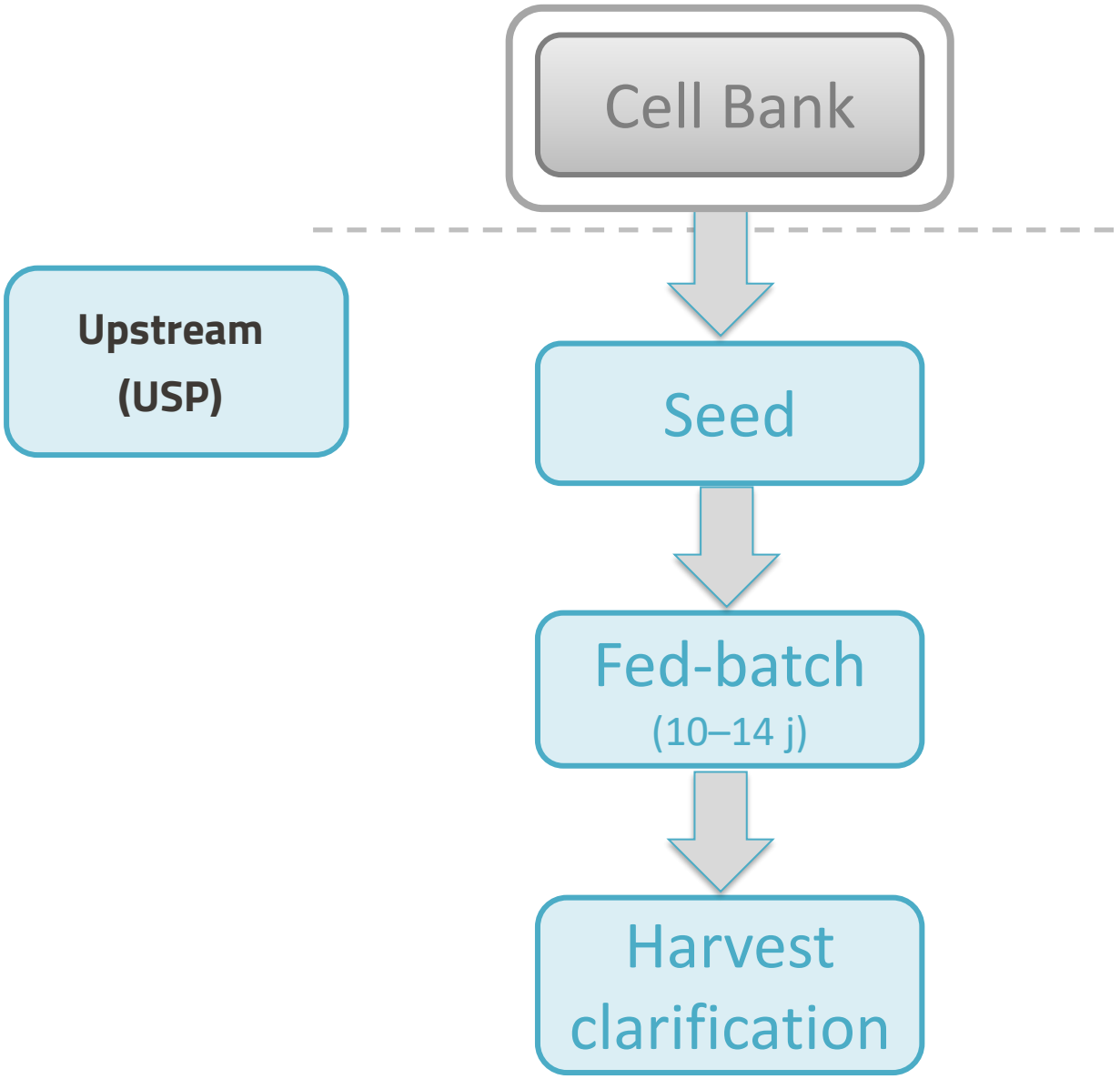
Formule : 10 mg/mL - pH 6.0±0.2

Stability : 24 month (2–8 °C)

Specs ex. :
Pureté ≥98 % - Agrégats <1 %
HCP <5 ppm - IC50 ≤15 ng/mL

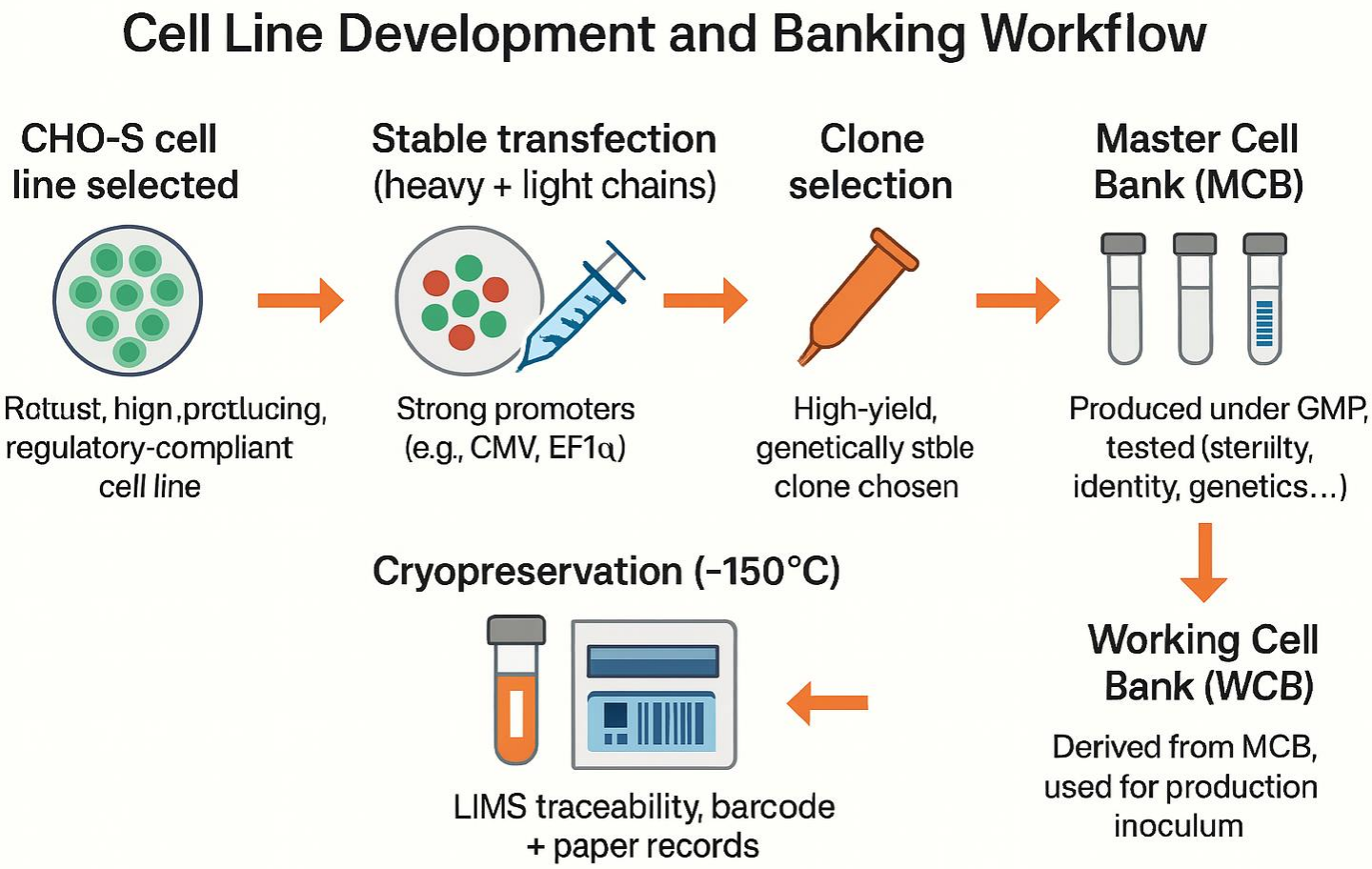
USP

Upstream Process Flow



USP

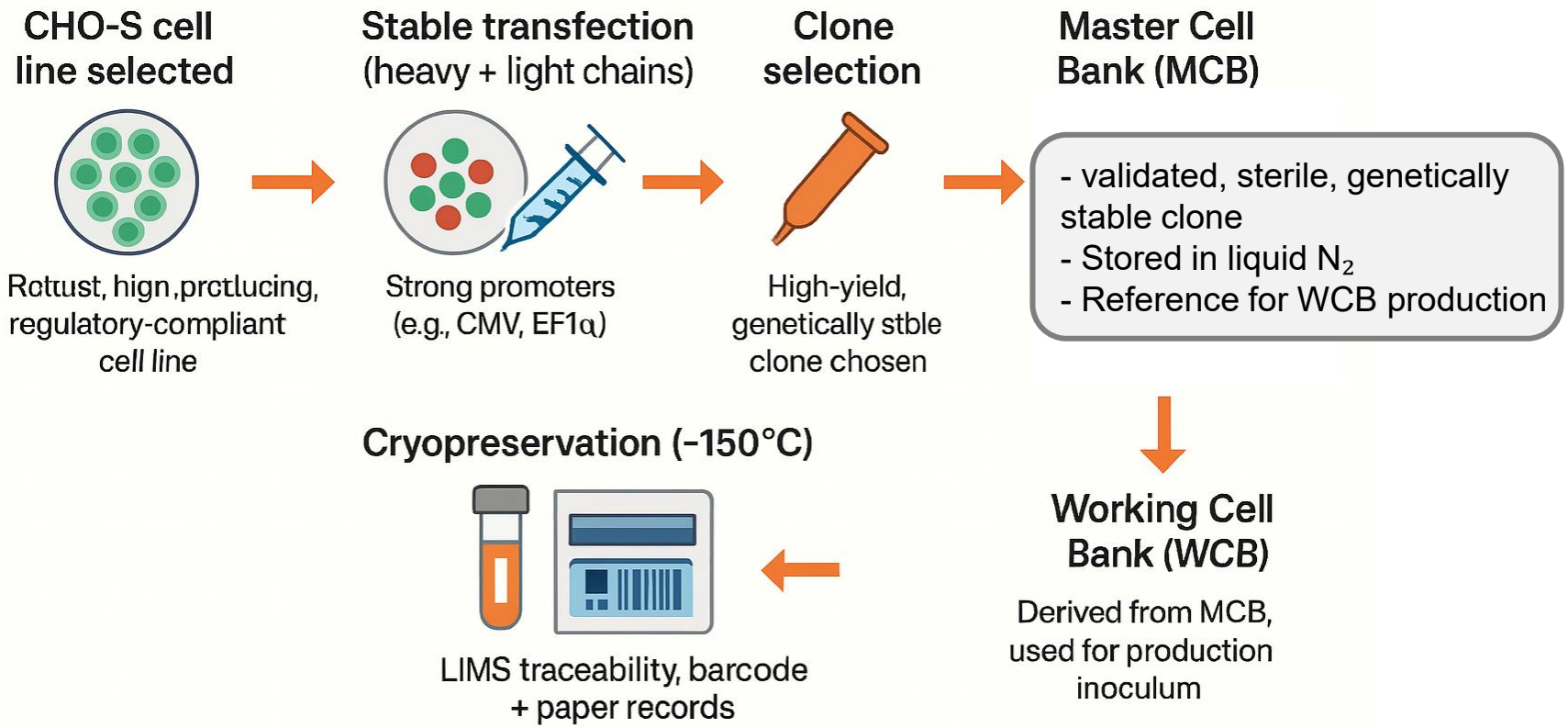
Before UPSTREAM Process



USP

Before UPSTREAM Process

Cell Line Development and Banking Workflow



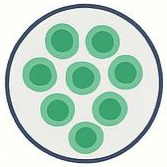
Tracked in the LIMS: unique ID, location and time-stamped history of cryotubes, **Quality Control**, and status

USP

Before UPSTREAM Process

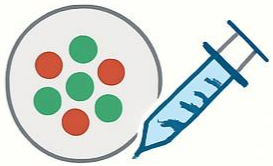
Cell Line Development and Banking Workflow

CHO-S cell line selected



Robust, high-producing, regulatory-compliant cell line

Stable transfection (heavy + light chains)

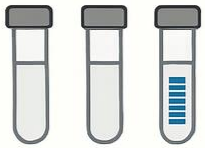


Strong promoters (e.g., CMV, EF1α)

Clone selection

High-yield, genetically stable clone chosen

Master Cell Bank (MCB)

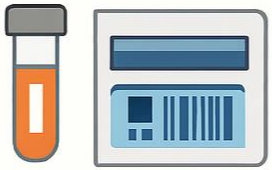


Produced under GMP, tested (sterility, identity, genetics…)

Working Cell Bank (WCB)

derived from MCB
Stored for production start

Cryopreservation (-150°C)

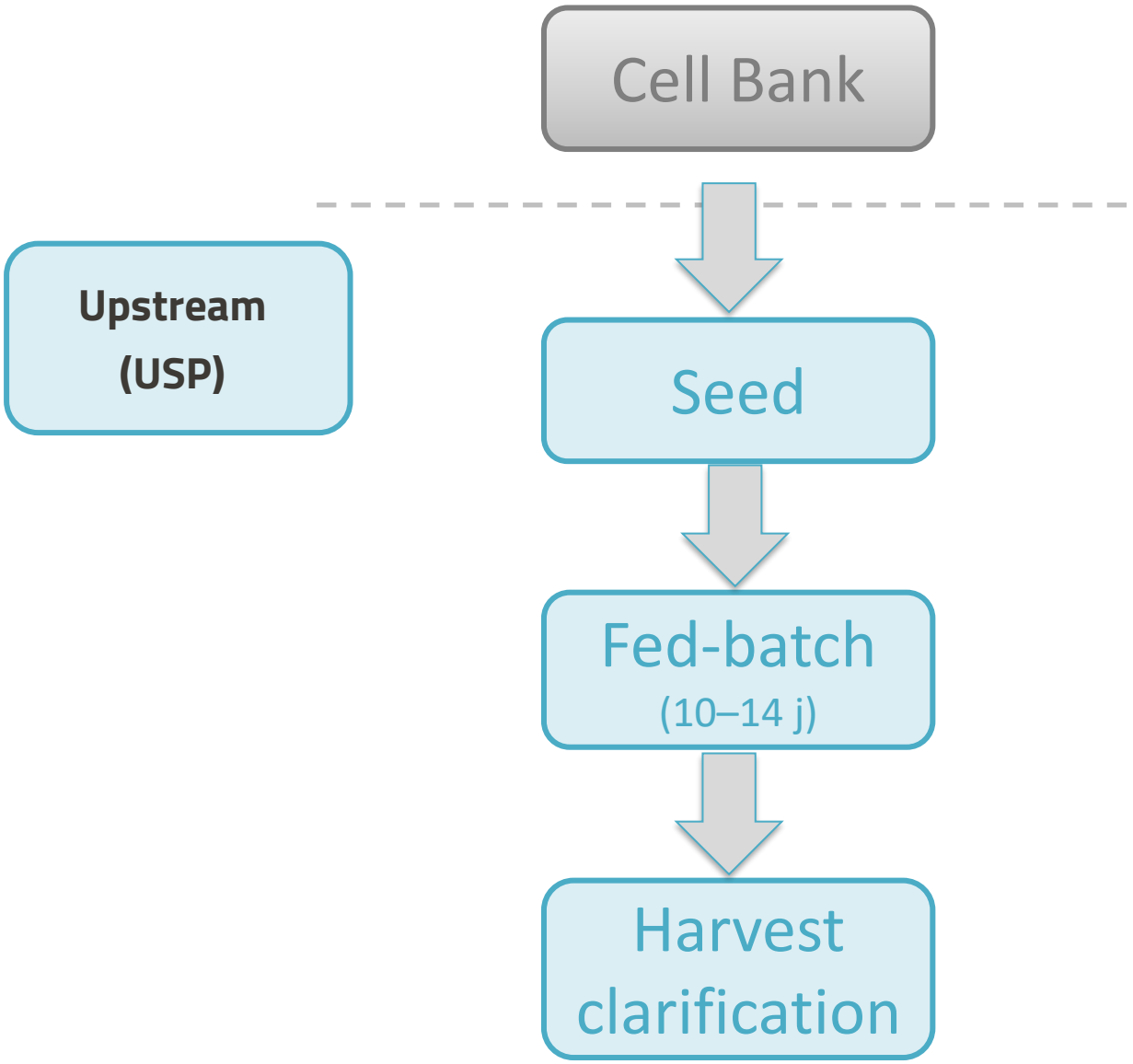


LIMS traceability, barcode + paper records

Tracked in the LIMS: unique ID, location and time-stamped history of cryotubes, **Quality Control**, and status

USP

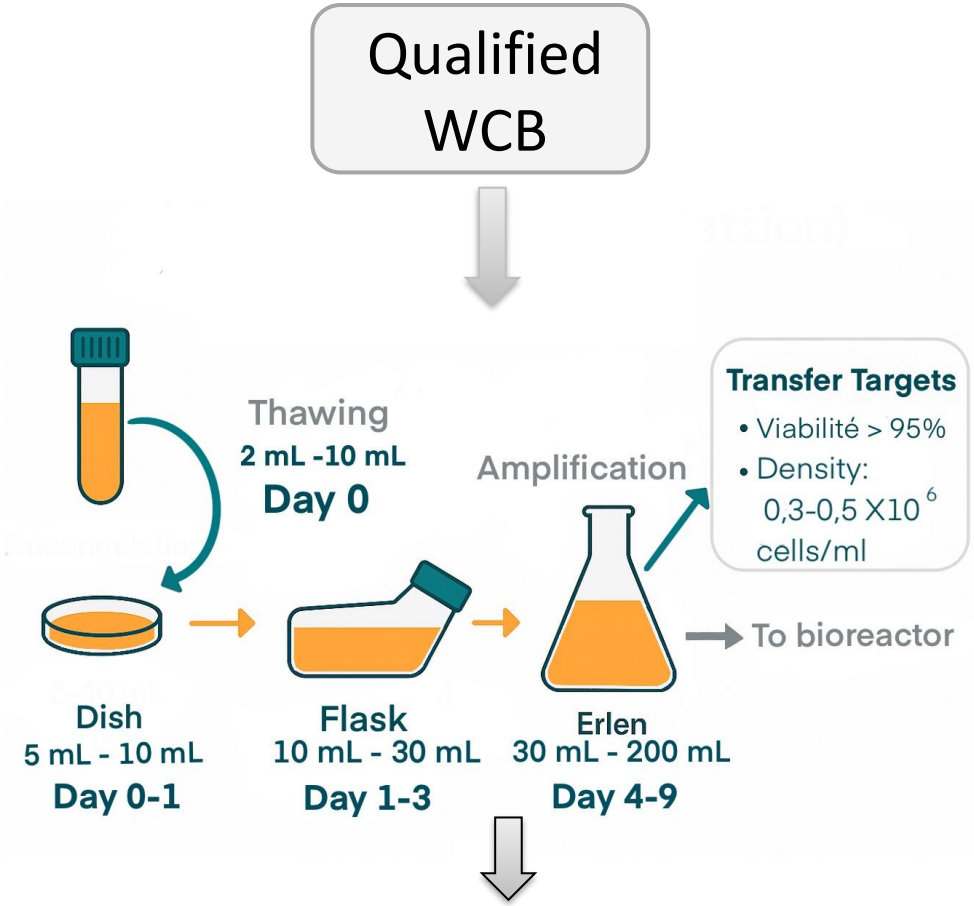
Upstream Process Flow



USP

Cell Bank Thawing and Pre-culture

Preparation of a viable and stable inoculum from the Working cell bank



Outputs : Viable inoculum for seed train

USP

Cell Bank Thawing and Pre-culture

Preparation of a viable and stable inoculum from the Working cell bank

Inputs:

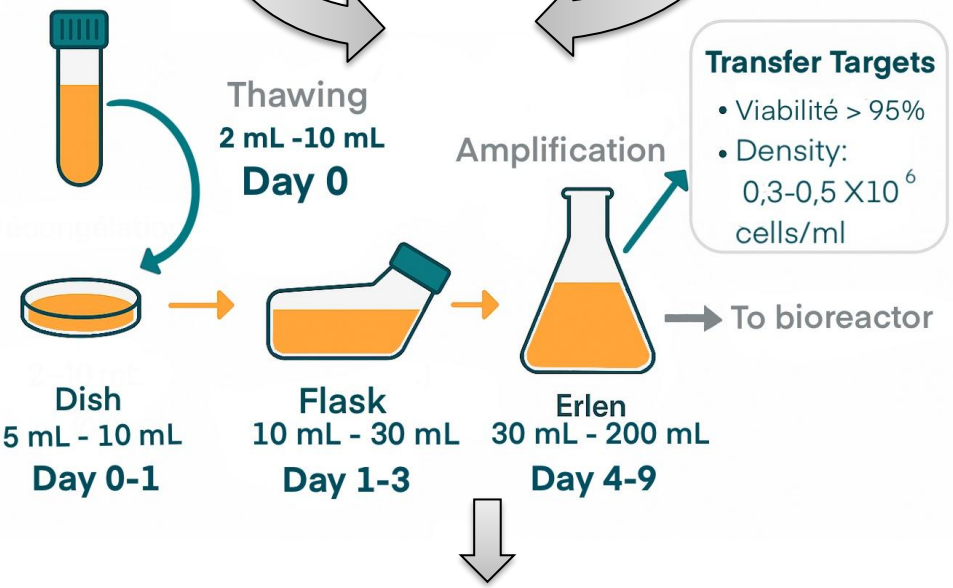
MA

Animal component–free medium
Feed and supplement

Qualified WCB

PP

post-thraw
temp, CO_2, pH, Feed schedule



Outputs : Viable inoculum for seed train

IPCs : viability, growth rate, morphology

USP

Cell Bank Thawing and Pre-culture

Preparation of a viable and stable inoculum from the Working cell bank

Inputs:

MA

Animal component–free medium

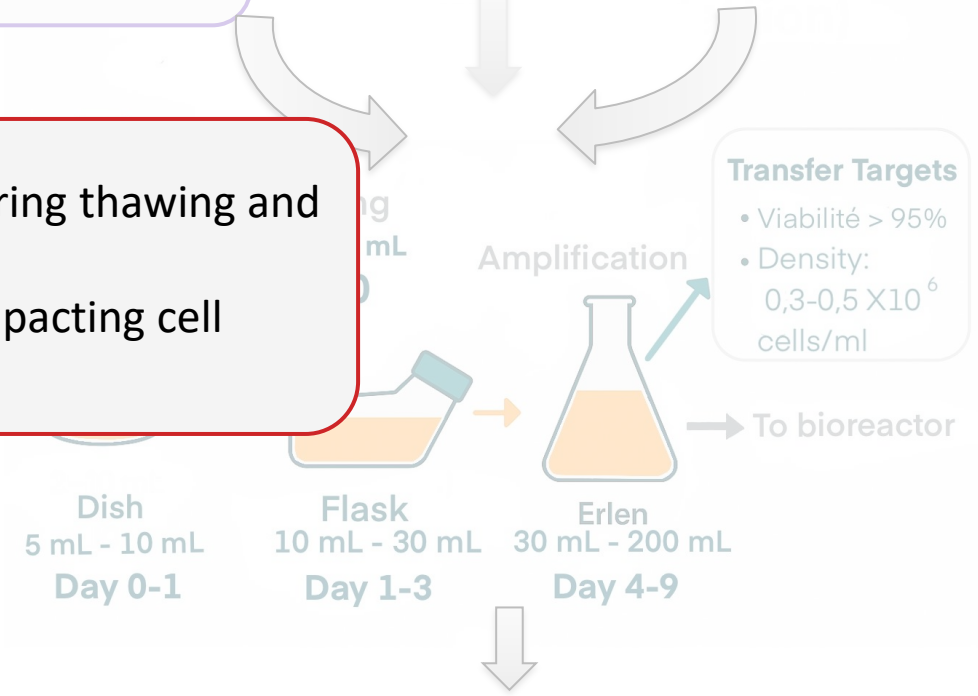
Qualified WCB

PP

post-thraw temp, CO_2, pH

Risks

- Contamination during thawing and handling
- Recovery stress impacting cell viability and growth



Outputs : Viable inoculum for seed train

IPCs : viability, growth rate, morphology

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Cell Bank Thawing and Pre-culture

Preparation of a viable and stable inoculum from the Working cell bank

Inputs:

MA

Animal component–free medium

Qualified WCB

PP

post-thraw temp, CO_2, pH

Risks

- Contamination during thawing and handling
- Recovery stress impacting cell viability and growth

Quality attributes

Sterility / Endotoxins
Cell identity (Genetic stability,
Appearance / morphology,
Viability)

Outputs : Viable inoculum for seed train

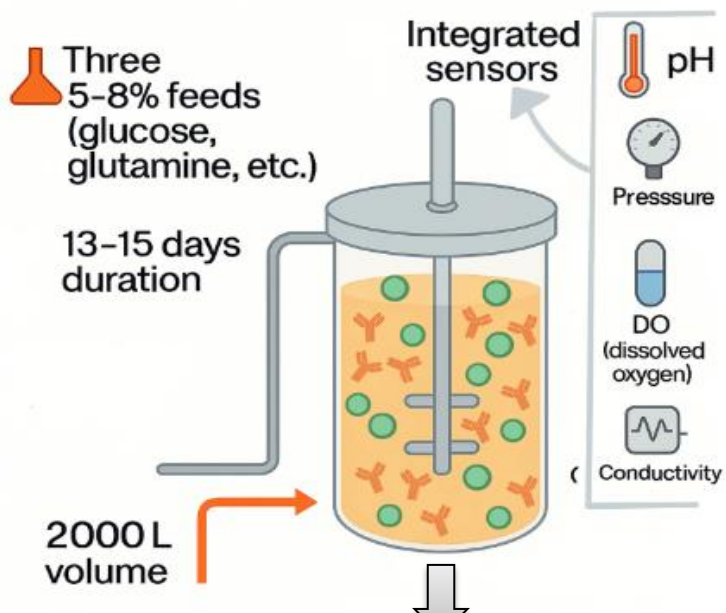
IPCs : viability, growth rate, morphology

USP

Production Fed-Batch (10–14 days)

High-titer IgG synthesis

Inoculum
from seed train



Outputs : Culture broth containing cells and secreted monoclonal antibody

USP

Production Fed-Batch (10–14 days)

High-titer IgG synthesis

Inputs:

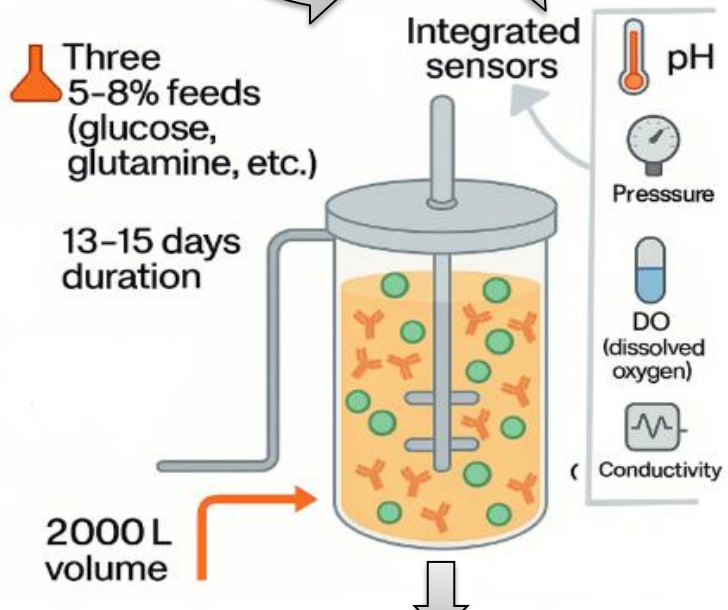
MA

Production medium
(chemically defined)
Feed solutions

Inoculum
from seed train

PP

pH, dissolved oxygen, temperature, feeding rate, agitation speed



Outputs : Culture broth containing cells and secreted monoclonal antibody

IPCs : Viability, titer, pH, and metabolite levels

USP

Production Fed-Batch (10–14 days)

High-titer IgG synthesis

Inputs:

MA

Production medium
(chemically defined)
Feed solutions

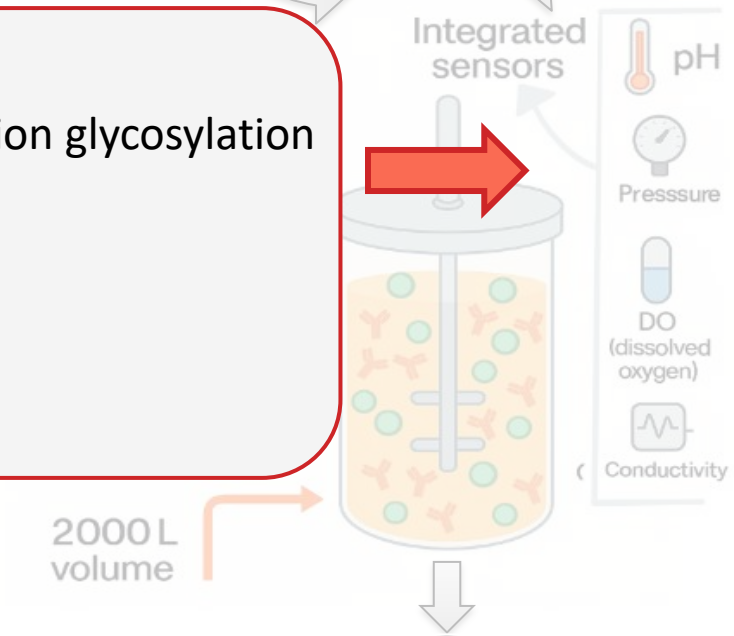
Inoculum
from seed train

PP

pH, dissolved oxygen, temperature, feeding rate, agitation speed

Risks

- pH or oxygen deviation glycosylation
- Nutrient depletion
- cell damage



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IPCs : Viability, titer, pH, and metabolite levels

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Feed solutions

Inoculum
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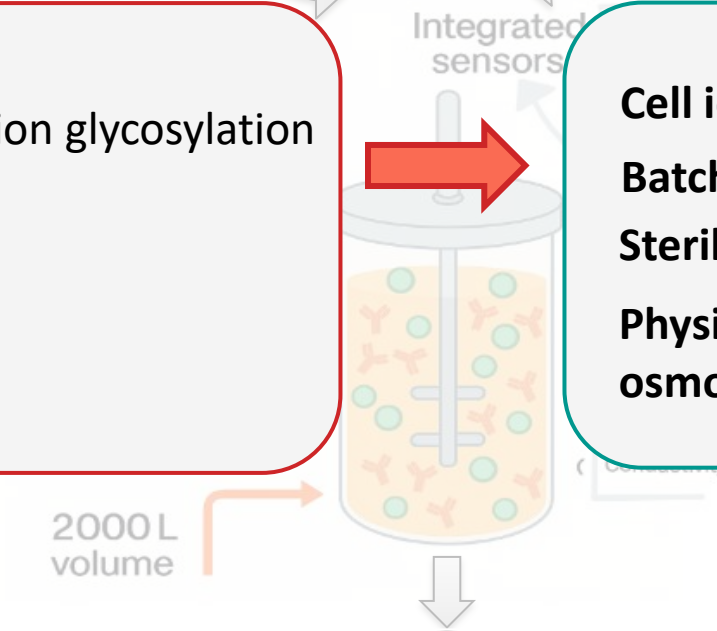
PP

pH, dissolved oxygen, temperature, feeding rate, agitation speed



Risks

- pH or oxygen deviation glycosylation
- Nutrient depletion
- cell damage

Quality attributes

Cell identity (Viability, appearance)

Batch homogeneity / Concentration

Sterility

Physico-chemical properties (pH, osmolality, conductivity)

Outputs : Culture broth containing cells and secreted monoclonal antibody

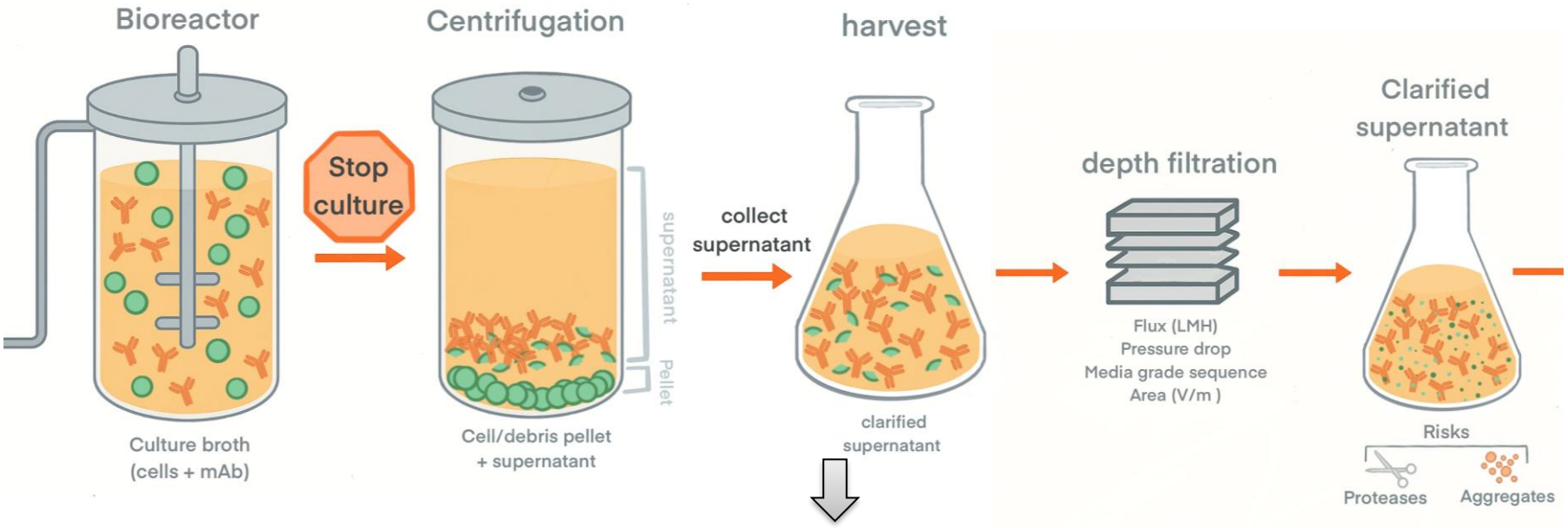
IPCs : Viability, titer, pH, and metabolite levels

USP

Harvest then Clarification

Stop the culture and preserve the product (supernatant).

Cell broth
from the bioreactor



Outputs : Supernatant containing monoclonal antibody and cell debris

USP

Harvest then Clarification

Stop the culture and preserve the product (supernatant).

Inputs:

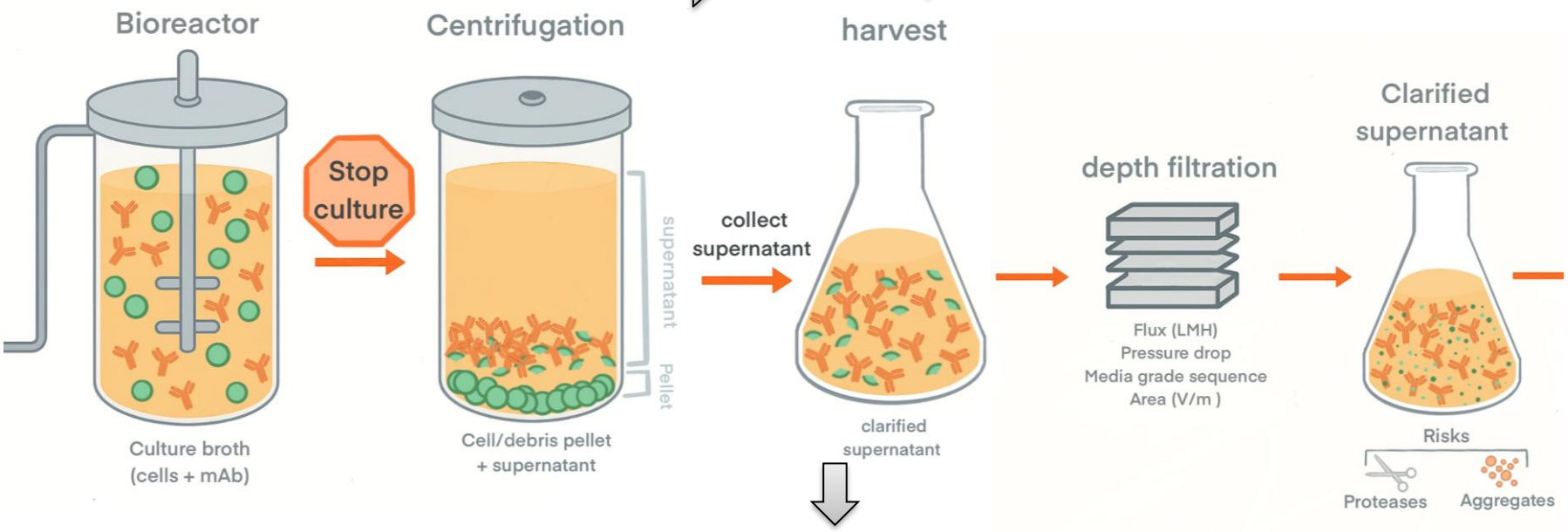
MA

Protease inhibitor
Antifoam agent
Acid/base solution
Filter material

Cell broth
from the bioreactor

PP

pH transition, dissolved oxygen, temperature, Transfer flow rate, Hold time, centrifugation speed



Outputs : Supernatant containing monoclonal antibody and cell debris

IPCs: Harvest timing, centrifugation parameters, transfer temperature, and clarification efficiency

USP

Harvest then Clarification

Stop the culture and preserve the product (supernatant).

Inputs:

MA

Protease inhibitor
Antifoam agent
Acid/base solution

Cell broth
from the bioreactor

PP

pH transition, dissolved oxygen, temperature, Transfer flow rate, Hold time

Risks

- Protease
- Aggregation formation
- Filter clogging
- Product loss during transfer or centrifugation

Outputs : Supernatant containing monoclonal antibody and cell debris

IPCs: Harvest timing, centrifugation parameters, transfer temperature, and clarification efficiency

USP

Harvest then Clarification

Stop the culture and preserve the product (supernatant).

Inputs:

MA

Protease inhibitor
Antifoam agent
Acid/base solution

Cell broth
from the bioreactor

PP

pH transition, dissolved oxygen, temperature, Transfer flow rate, Hold time

Risks

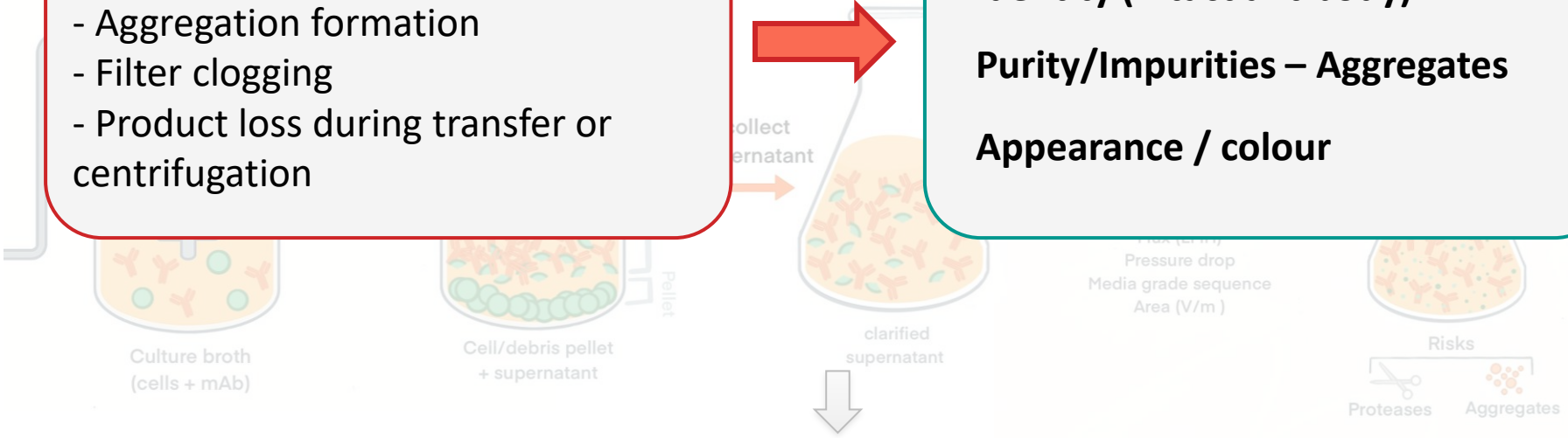
- Protease
- Aggregation formation
- Filter clogging
- Product loss during transfer or centrifugation

Quality attributes

Identity (Intact antibody)

Purity/Impurities – Aggregates

Appearance / colour

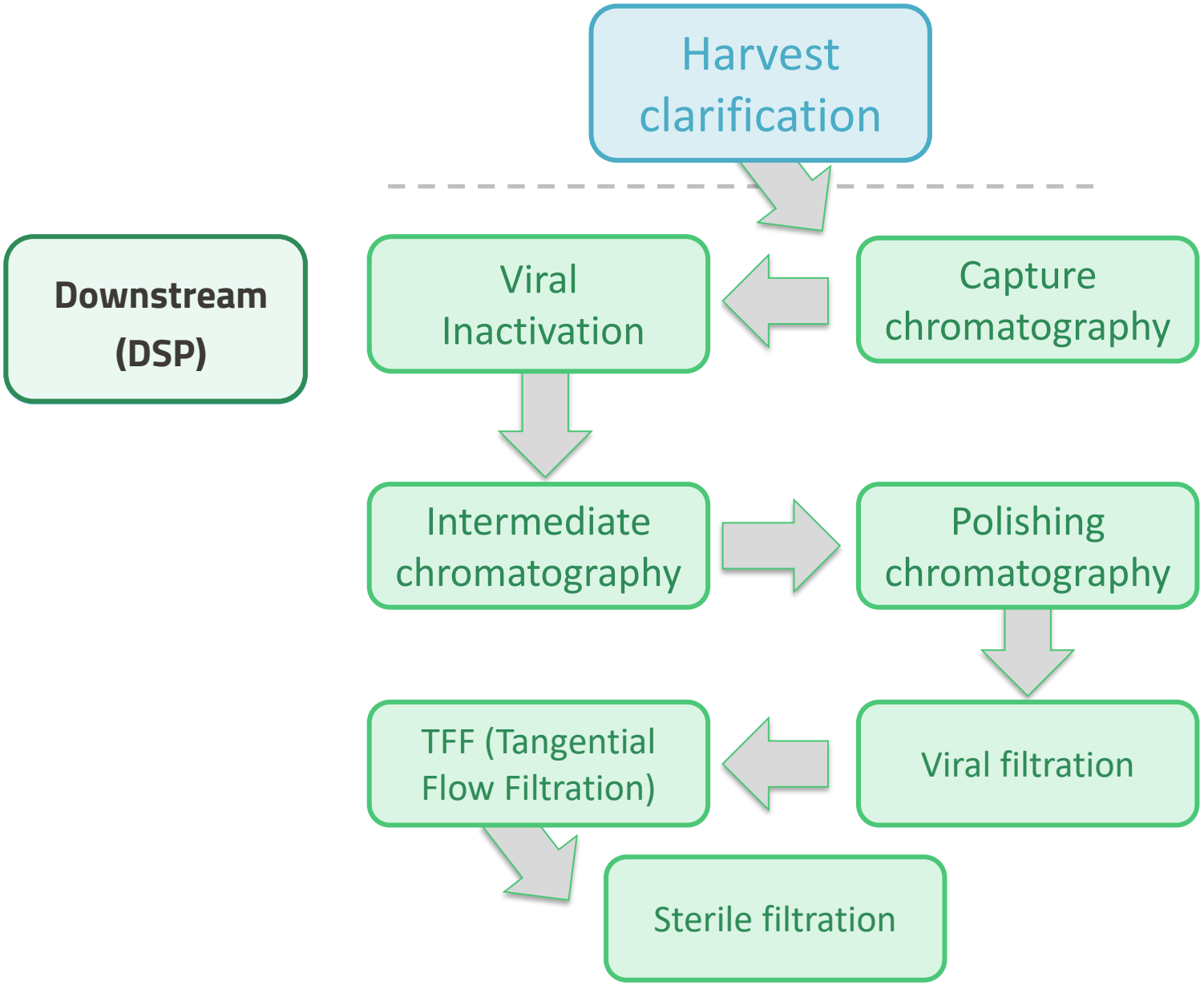


Outputs : Supernatant containing monoclonal antibody and cell debris

IPCs: Harvest timing, centrifugation parameters, transfer temperature, and clarification efficiency

DSP

Downstream Process Flow

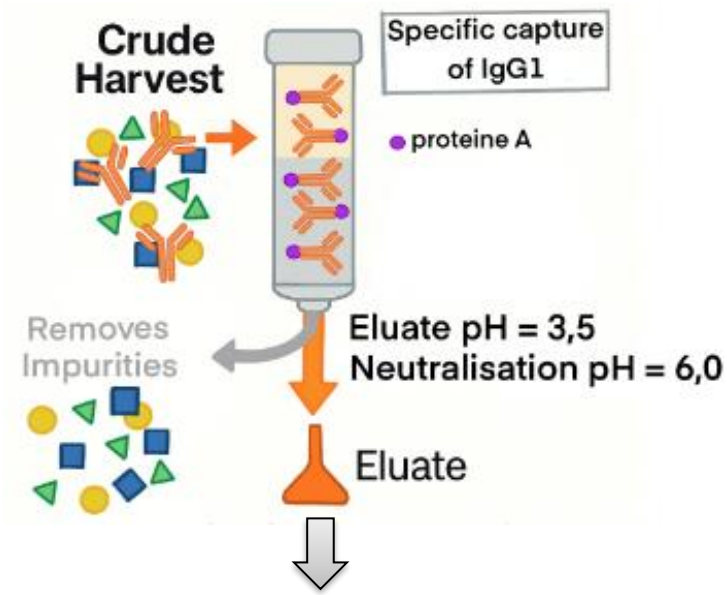


DSP

Capture chromatography - Protein A

To selectively capture and purify the monoclonal antibody (IgG)

Supernatant
mAb/cell debris

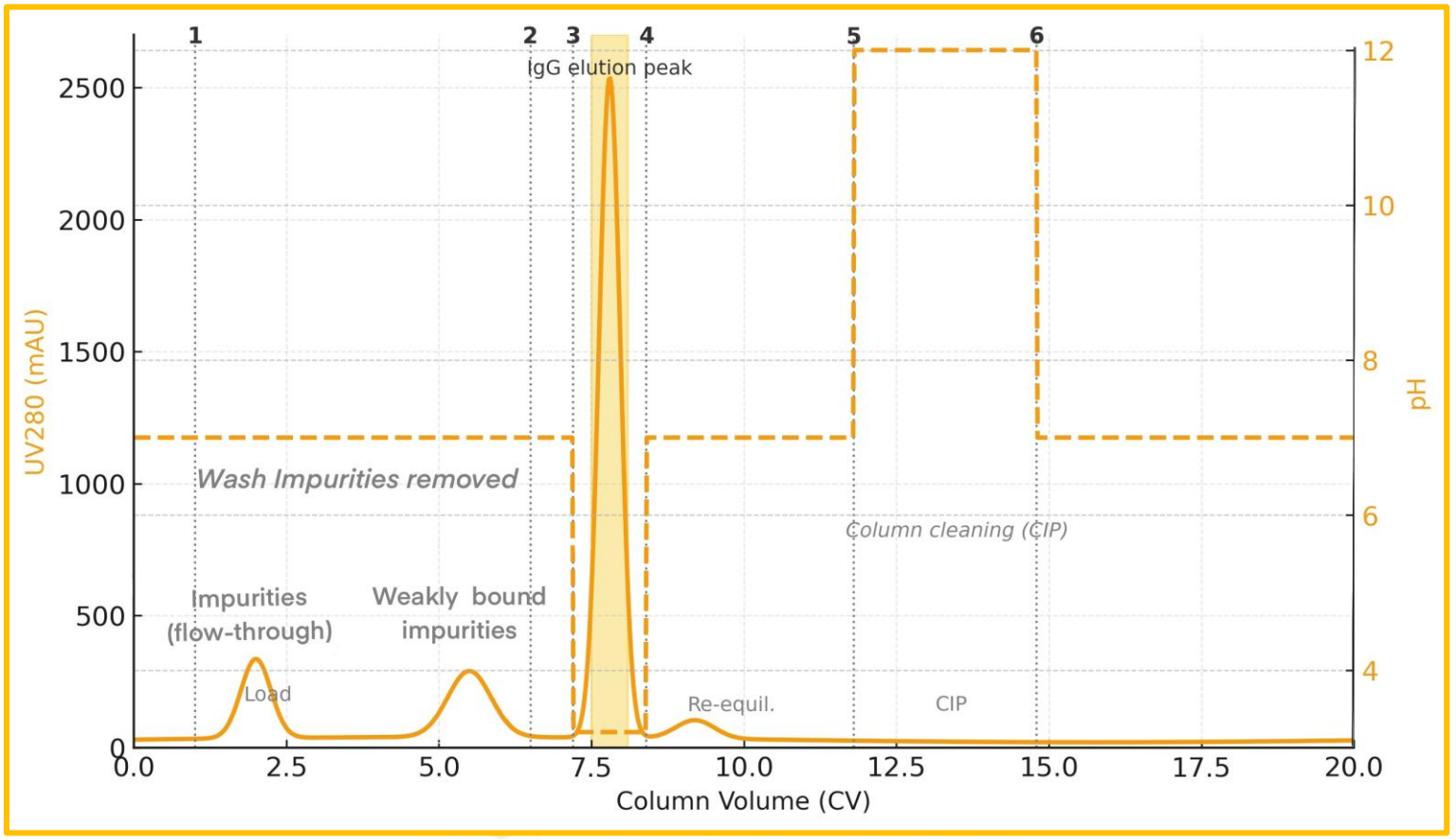


Outputs : Neutralized Protein A eluate containing purified monoclonal antibody

DSP

Capture chromatography - Protein A

To selectively capture and purify the monoclonal antibody (IgG)



Outputs : Neutralized Protein A eluate containing purified monoclonal antibody

DSP

Capture chromatography - Protein A

To selectively capture and purify the monoclonal antibody (IgG)

Inputs:

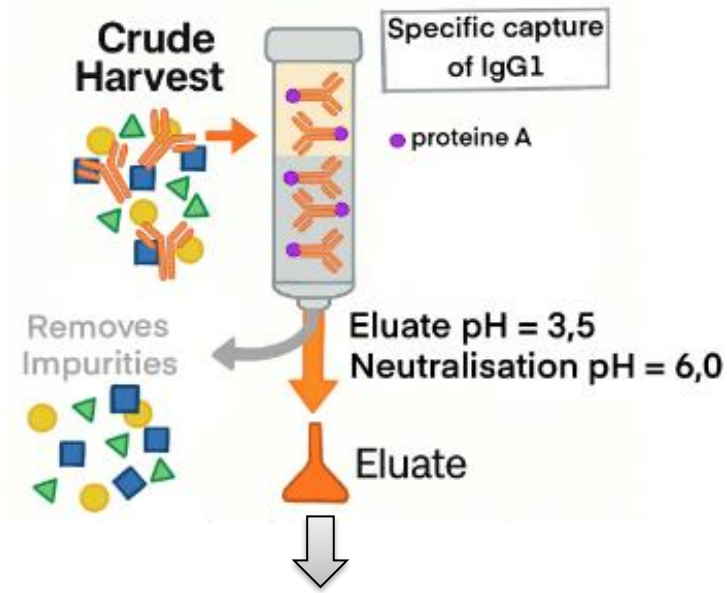
MA

Protein A resin
Buffer pH & conductivity
column material
Protease inhibitors

Supernatant
mAb/cell debris

PP

Loading density, flow rate, Wash and elution pH, and column pressure



Outputs : Neutralized Protein A eluate containing purified monoclonal antibody

IPCs: UV280 monitoring, eluate pH, conductivity, column pressure (ΔP), and protein concentration check

DSP

Capture chromatography - Protein A

To selectively capture and purify the monoclonal antibody (IgG)

Inputs:

MA

Protein A resin
Buffer pH & conductivity
column material
Protease inhibitors

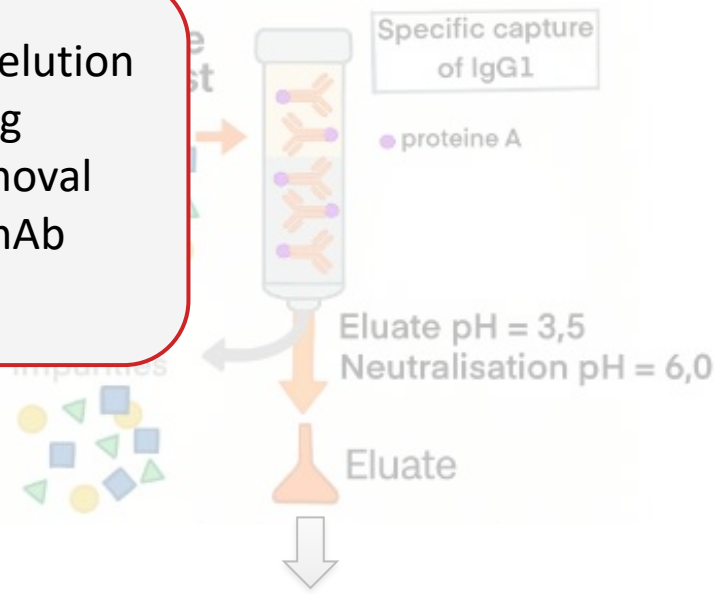
Supernatant
mAb/cell debris

PP

Loading density, flow rate, Wash and elution pH, and column pressure

Risks

- Aggregation during low-pH elution
- Incomplete antibody binding
- Insufficient HCP or DNA removal
- Over-acidification causing mAb denaturation



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IPCs: UV280 monitoring, eluate pH, conductivity, column pressure (ΔP), and protein concentration

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Buffer pH & conductivity
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Protease inhibitors

Supernatant
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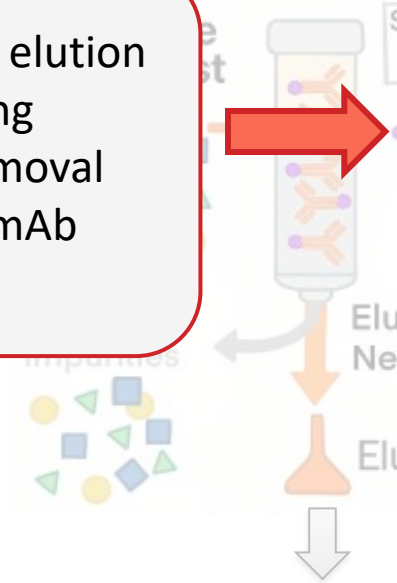
PP

Loading density, flow rate, Wash and elution pH, and column pressure



Risks

- Aggregation during low-pH elution
- Incomplete antibody binding
- Insufficient HCP or DNA removal
- Over-acidification causing mAb denaturation

Quality attributes

Purity / High recovery

Impurities (Low level of aggregates)

Process-related impurities

Outputs : Neutralized Protein A eluate containing purified monoclonal antibody

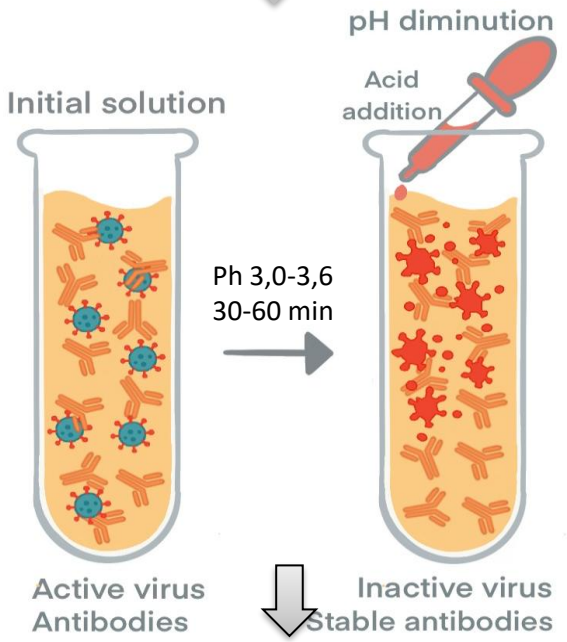
IPCs: UV280 monitoring, eluate pH, conductivity, column pressure (ΔP), and protein concentration

DSP

Viral inactivation (Low pH)

Inactivate enveloped viruses using an acidic pH

Eluate
mAb



Outputs : virus-inactivated mAb pool (pH 6.0–6.5)

DSP

Viral inactivation (Low pH)

Inactivate enveloped viruses using an acidic pH

Inputs:

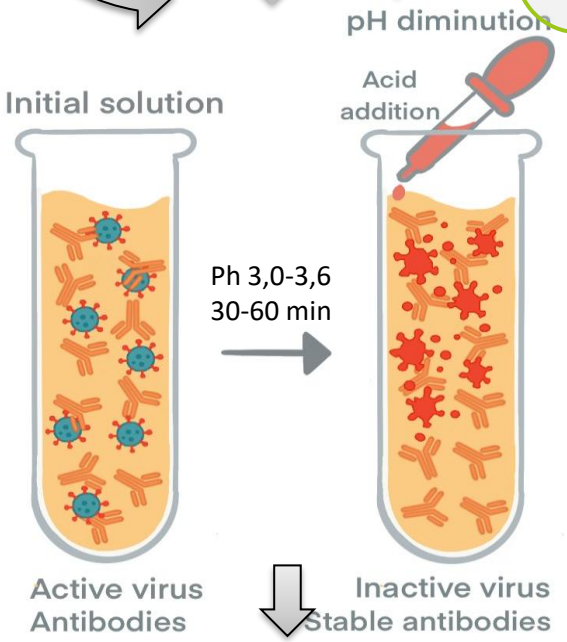
MA

Acid reagent
Base for neutralization
Contact materials

Eluate
mAb

PP

Target pH (typically 3.0–3.6), Hold time at low pH, Temperature during hold



Outputs : virus-inactivated mAb pool (pH 6.0–6.5)

IPCs : pH setpoint, hold time, mixing uniformity (no pH gradients), neutralization

DSP

Viral inactivation (Low pH)

Inactivate enveloped viruses using an acidic pH

Inputs:

MA

Acid reagent
Base for neutralization
Contact materials

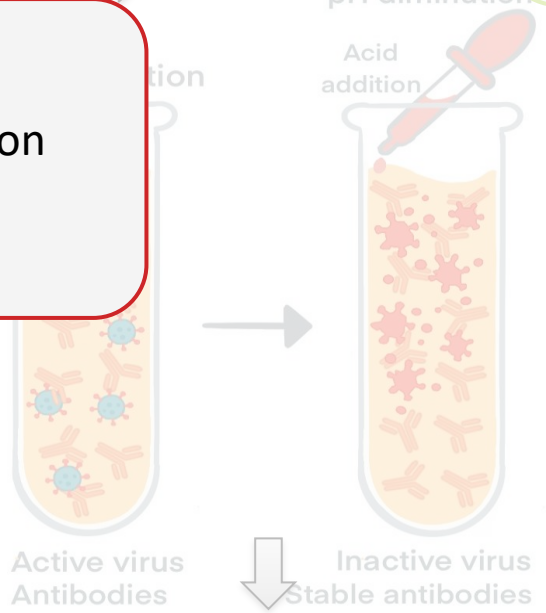
Eluate
mAb

PP

Target pH (typically 3.0–3.6), Hold time at low pH, Temperature during hold

Risks

- Antibody aggregation
- heterogeneous viral inactivation
- product degradation
- mAb instability denaturation



Outputs : virus-inactivated mAb pool (pH 6.0–6.5)

IPCs : pH setpoint, hold time, mixing uniformity (no pH gradients), neutralization

DSP

Viral inactivation (Low pH)

Inactivate enveloped viruses using an acidic pH

Inputs:

MA

Acid reagent
Base for neutralization
Contact materials

Eluate
mAb

PP

Target pH (typically 3.0–3.6), Hold time at low pH, Temperature during hold

Risks

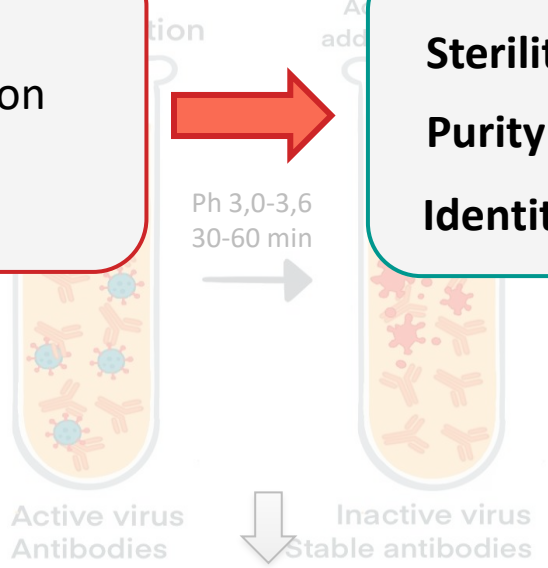
- Antibody aggregation
- heterogeneous viral inactivation
- product degradation
- mAb instability denaturation

Quality attributes

Sterility / Endotoxins

Purity / Impurities (aggregates <1%)

Identity / Conformation / Stability



Outputs : virus-inactivated mAb pool (pH 6.0–6.5)

IPCs : pH setpoint, hold time, mixing uniformity (no pH gradients), neutralization

DSP

Polishing chromatography - CEX

Polish the mAb by removing charged impurities

Inputs:

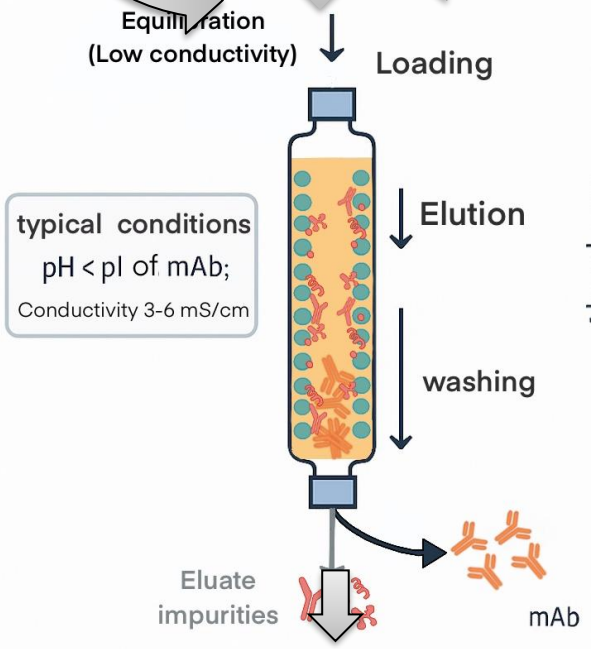
MA

Buffer composition
mAb concentration
Resin type and ligand

virus-inactivated mAb pool

PP

conductivity and pH setpoints, flow rate, Column pressure



Outputs : Purified mAb pool – impurities removed, pH/conductivity on spec.

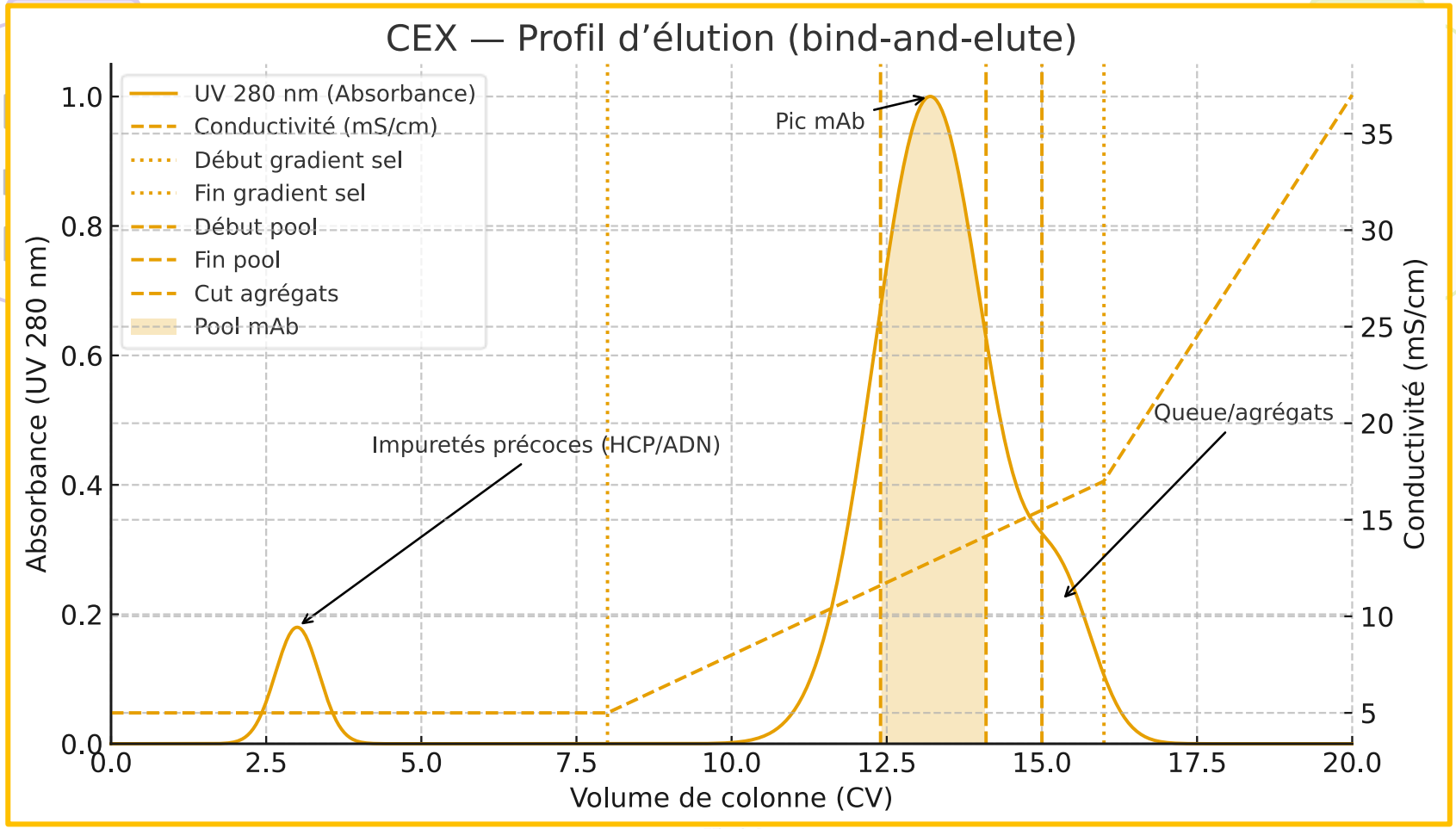
IPCs : conductivity and pH, UV280 monitoring, column pressure , confirmation of pool pH/conductivity

DSP

Polishing chromatography - CEX

Polish the mAb by removing charged impurities

Inputs:



impurities mAb

Outputs : Purified mAb pool – impurities removed, pH/conductivity on spec.

IPCs : conductivity and pH, UV280 monitoring, column pressure , confirmation of pool pH/conductivity

DSP

Polishing chromatography - CEX

Polish the mAb by removing charged impurities

Inputs:

MA

Buffer composition
mAb concentration
Resin type and ligand

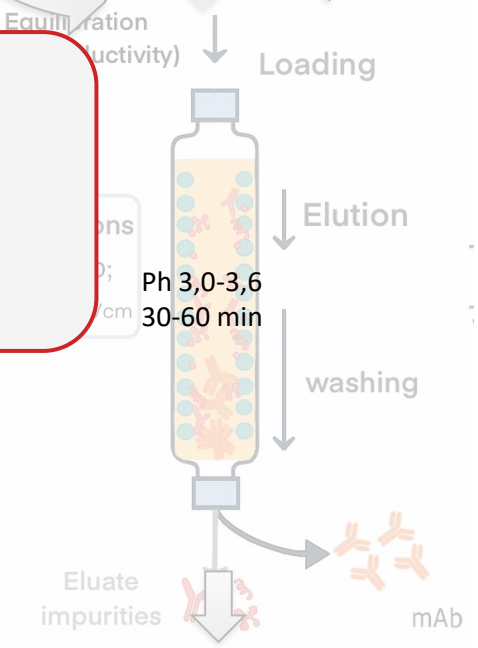
virus-inactivated mAb pool

PP

conductivity and pH setpoints, flow rate, Column pressure

Risks

- Poor pool selection
- Column fouling
- mAb instability denaturation



Outputs : Purified mAb pool – impurities removed, pH/conductivity on spec.

IPCs : conductivity and pH, UV280 monitoring, column pressure , confirmation of pool pH/conductivity

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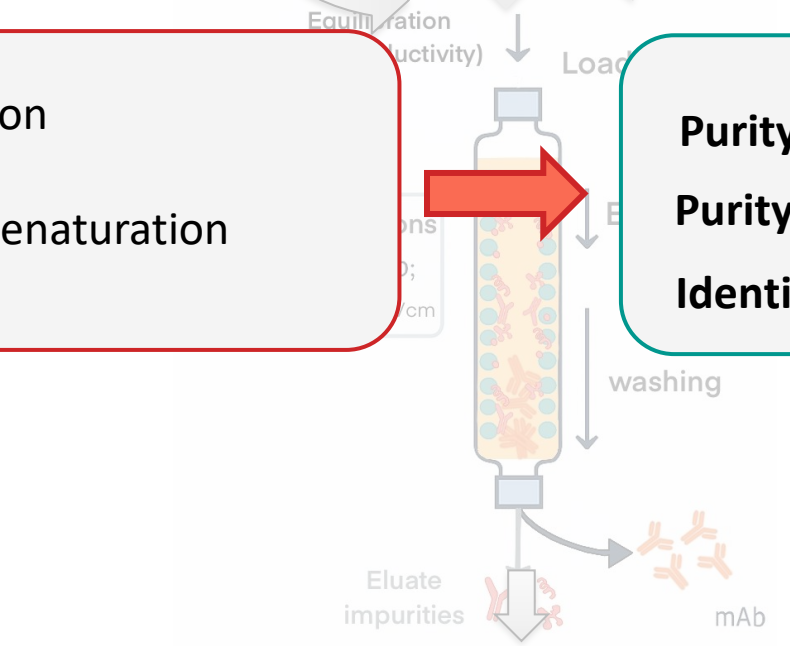


Quality attributes

Purity / Impurities

Purity / Impurities – Aggregates <1%

Identity – Conform sequence

Outputs : Purified mAb pool – impurities removed, pH/conductivity on spec.

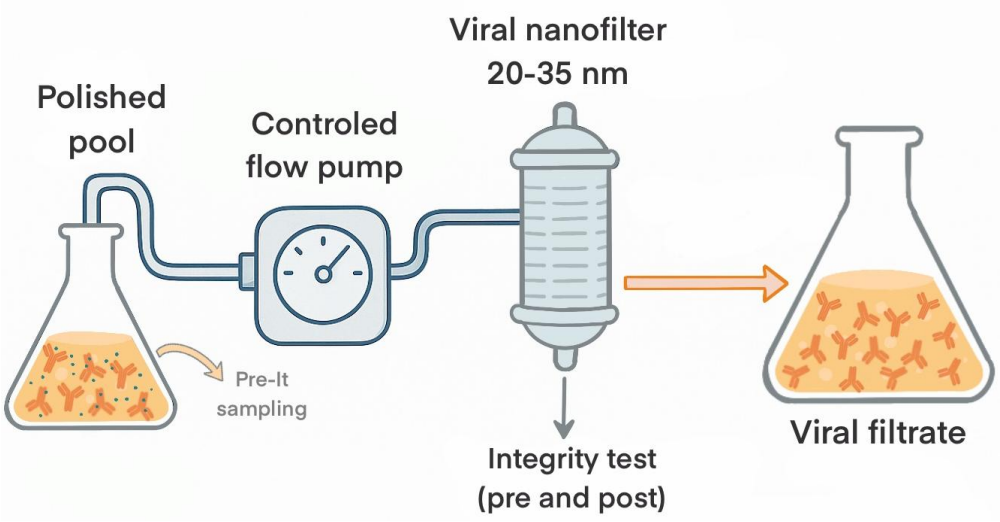
IPCs : conductivity and pH, UV280 monitoring, column pressure , confirmation of pool pH/conductivity

DSP

Viral filtration

Remove potential viral contaminants from purified mAb

Purified
mAb pool



Outputs：Purified mAb filtrate – virus and aggregates removed

DSP

Viral filtration

Remove potential viral contaminants from purified mAb

Inputs:

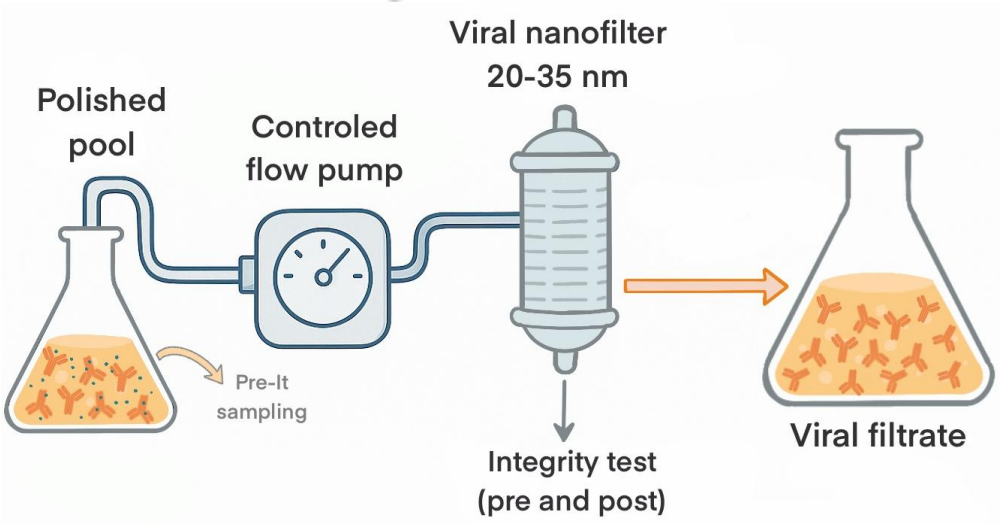
MA

Filter membrane pore size (20–35 nm)
Buffer composition

Purified mAb pool

PP

Transmembrane pressure, flow rate, filtration time



Outputs : Purified mAb filtrate – virus and aggregates removed

IPCs : Flow rate monitoring, differential pressure, filter integrity (pre and post), conductivity, pH.

DSP

Viral filtration

Remove potential viral contaminants from purified mAb

Inputs:

MA

Filter membrane pore size (20–35 nm)
Buffer composition

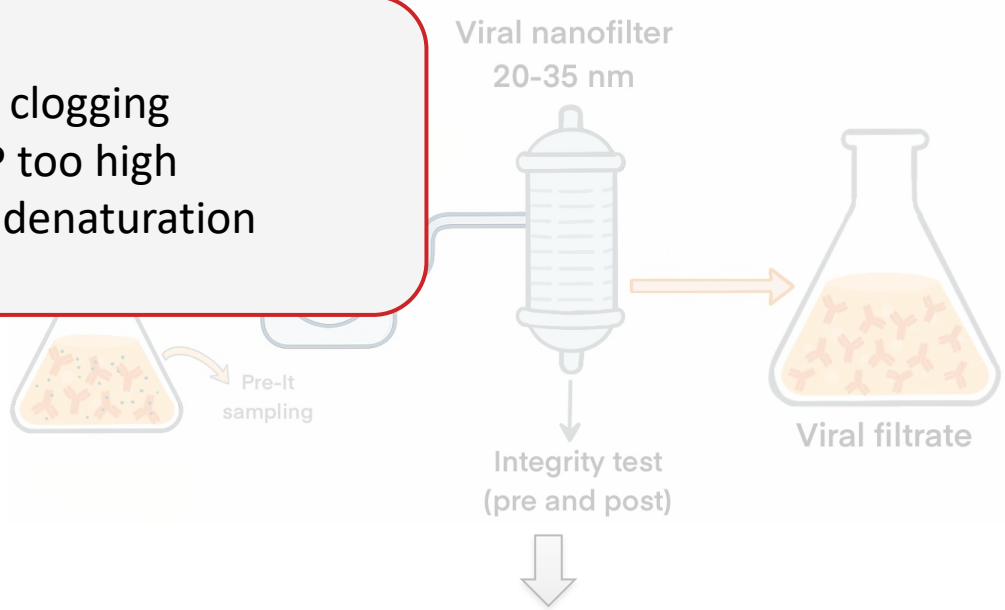
Purified mAb pool

PP

Transmembrane pressure, flow rate, filtration time

Risks

- Filter fouling or clogging
- Filter bypass ΔP too high
- mAb instability denaturation



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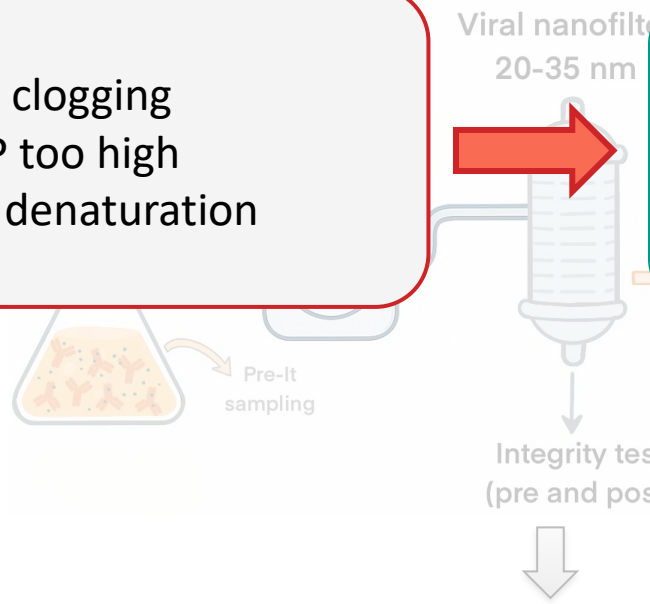


Risks

- Filter fouling or clogging
- Filter bypass ΔP too high
- mAb instability denaturation

Quality attributes

Sterility / Endotoxins

Purity / Impurities

Appearance / Colour

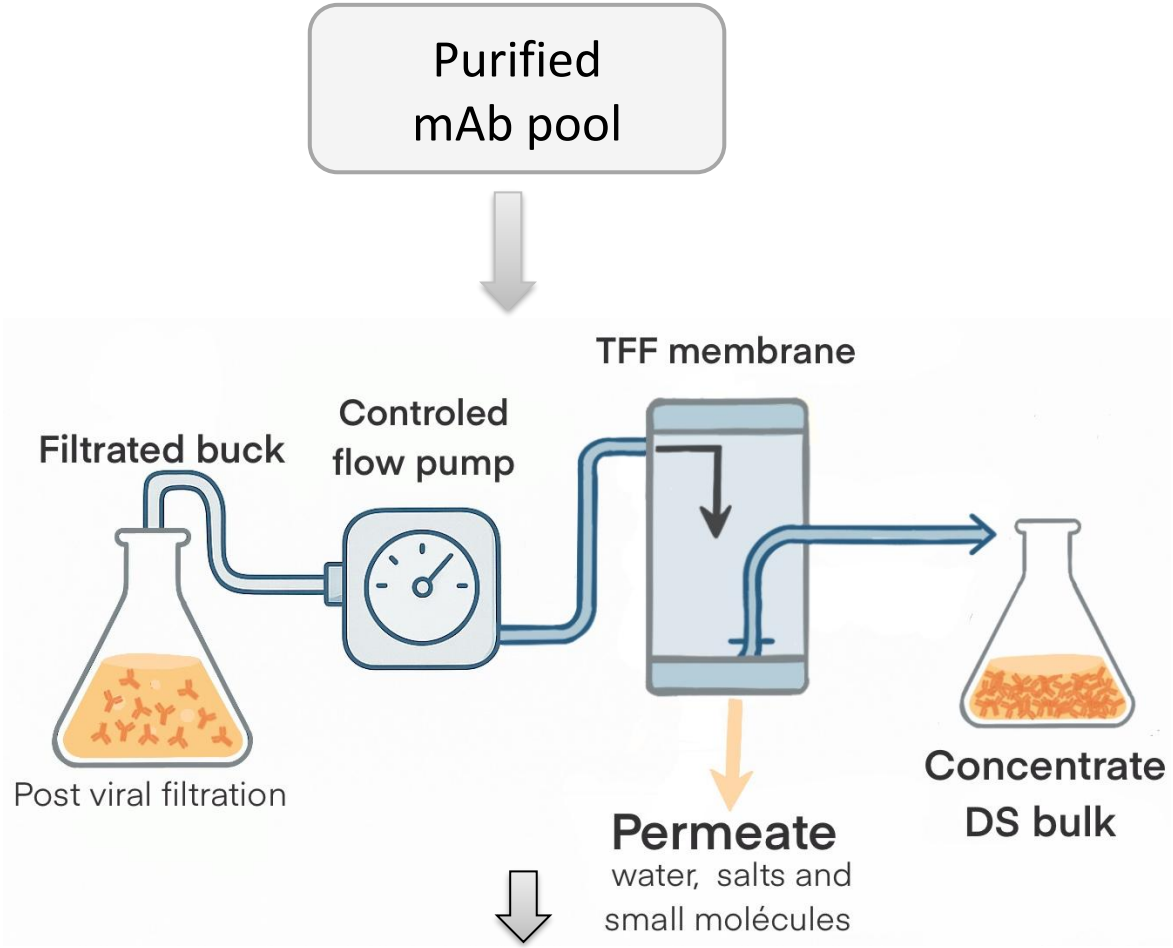
Outputs : Purified mAb filtrate – virus and aggregates removed

IPCs : Flow rate monitoring, differential pressure, filter integrity (pre and post), conductivity, pH.

DSP

UF/DF — Tangential Flow Filtration

Concentrate the mAb and exchange into the formulation buffer



Outputs : concentrated mAb, buffer-exchanged to formulation buffer

DSP

UF/DF — Tangential Flow Filtration

Concentrate the mAb and exchange into the formulation buffer

Inputs:

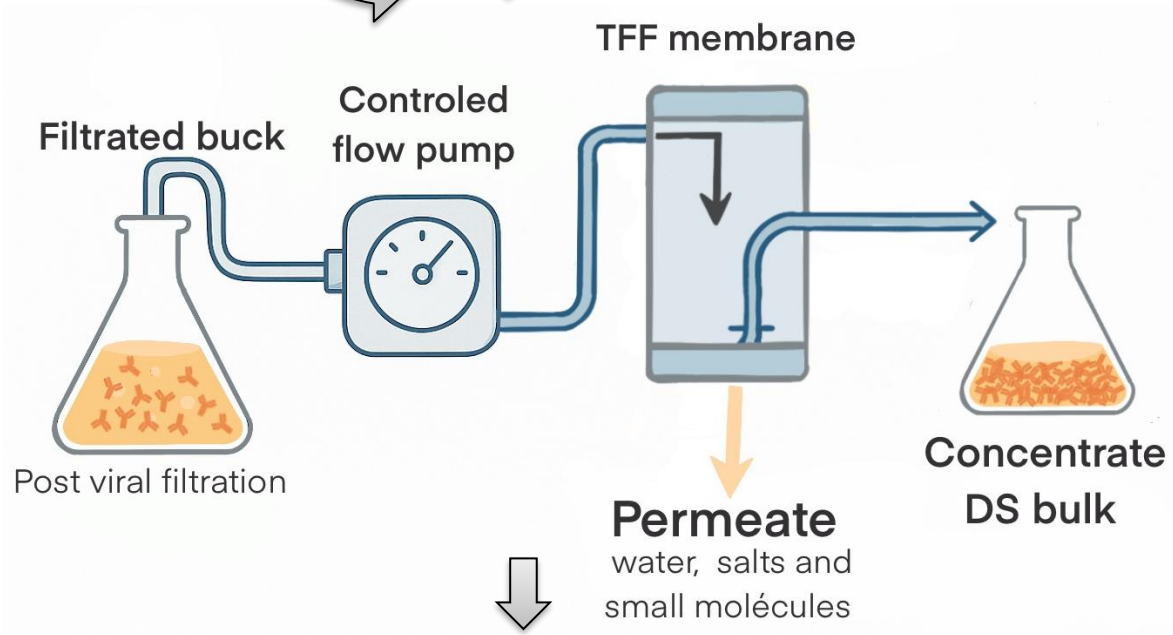
MA

TFF membrane
Buffer composition
Contact materials

Purified
mAb pool

PP

TMPress setpoint &
limits, flow rate,
Concentration factor



Outputs : concentrated mAb, buffer-exchanged to formulation buffer

IPCs : UV280 monitoring, TMP, permeate flux, pH/conductivity, and turbidity check.

DSP

UF/DF — Tangential Flow Filtration

Concentrate the mAb and exchange into the formulation buffer

Inputs:

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TFF membrane
Buffer composition
Contact materials

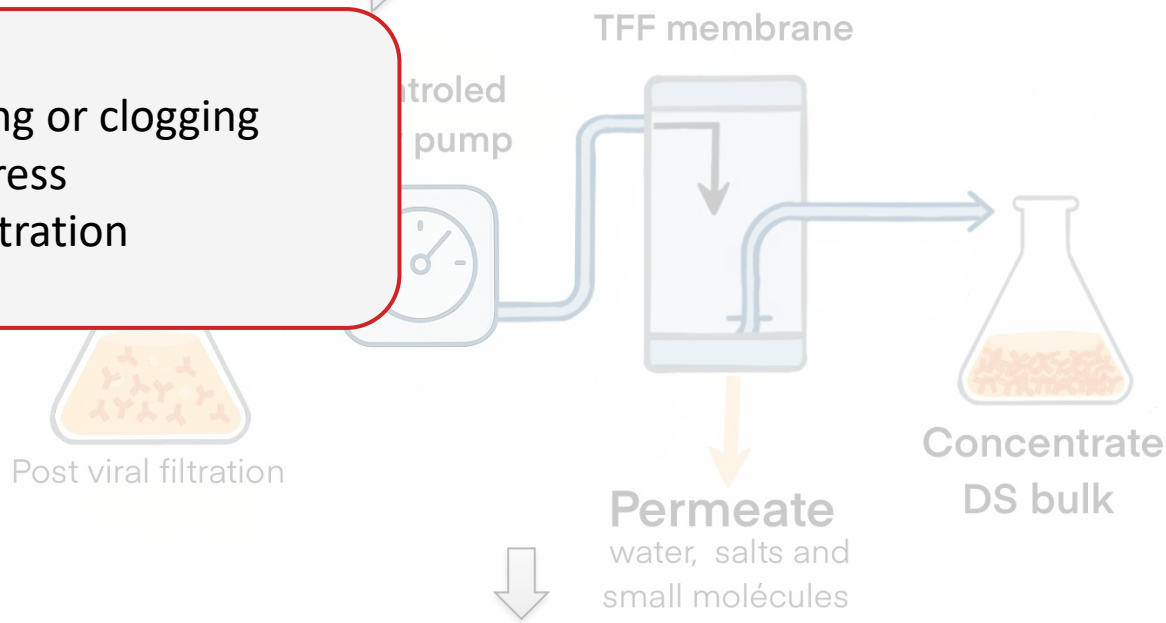
Purified
mAb pool

PP

TMPress setpoint &
limits, flow rate,
Concentration factor

Risks

- Membrane fouling or clogging
- concentration stress
- Insufficient diafiltration
- Antibodies loss



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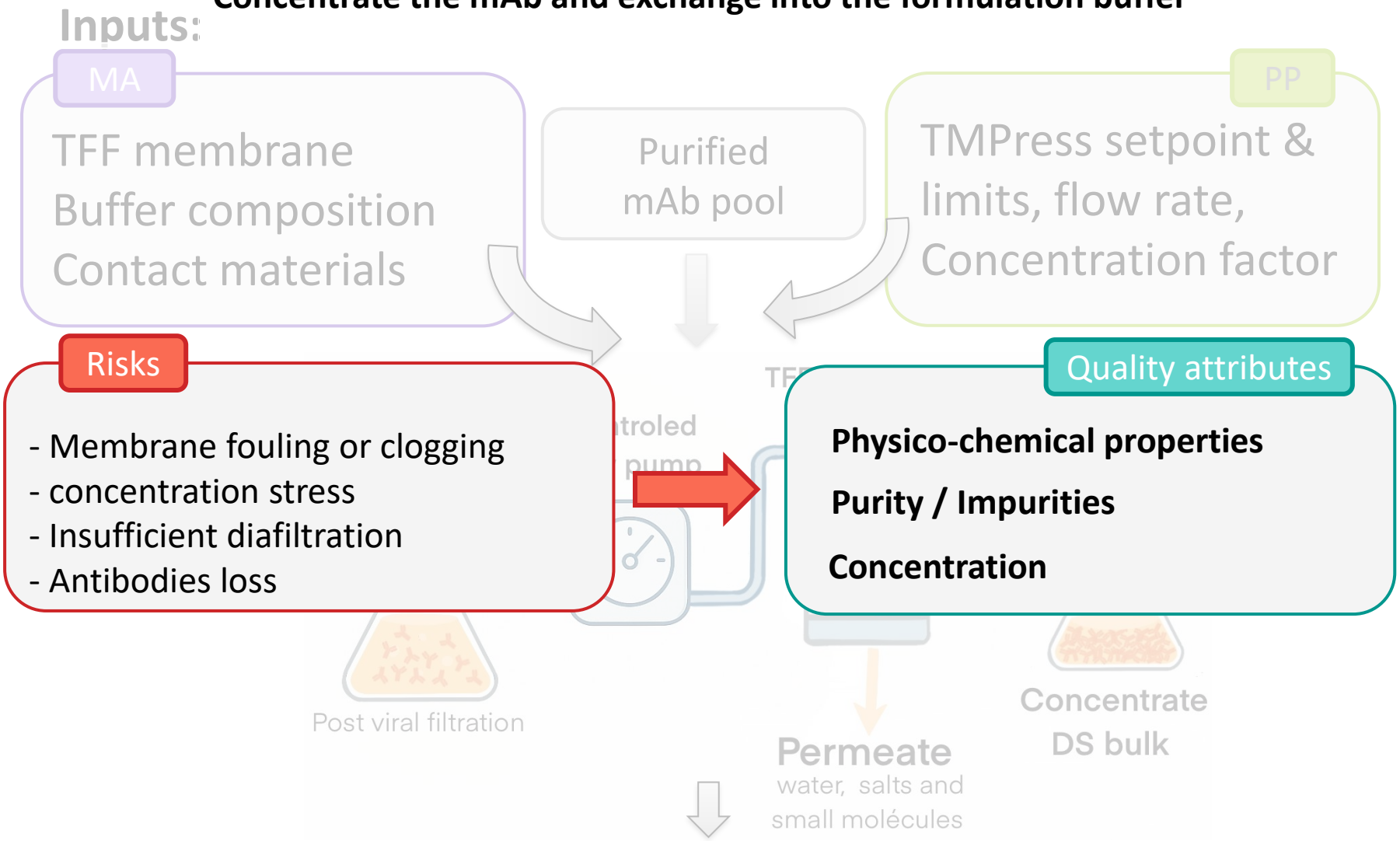
UF/DF — Tangential Flow Filtration

Concentrate the mAb and exchange into the formulation buffer

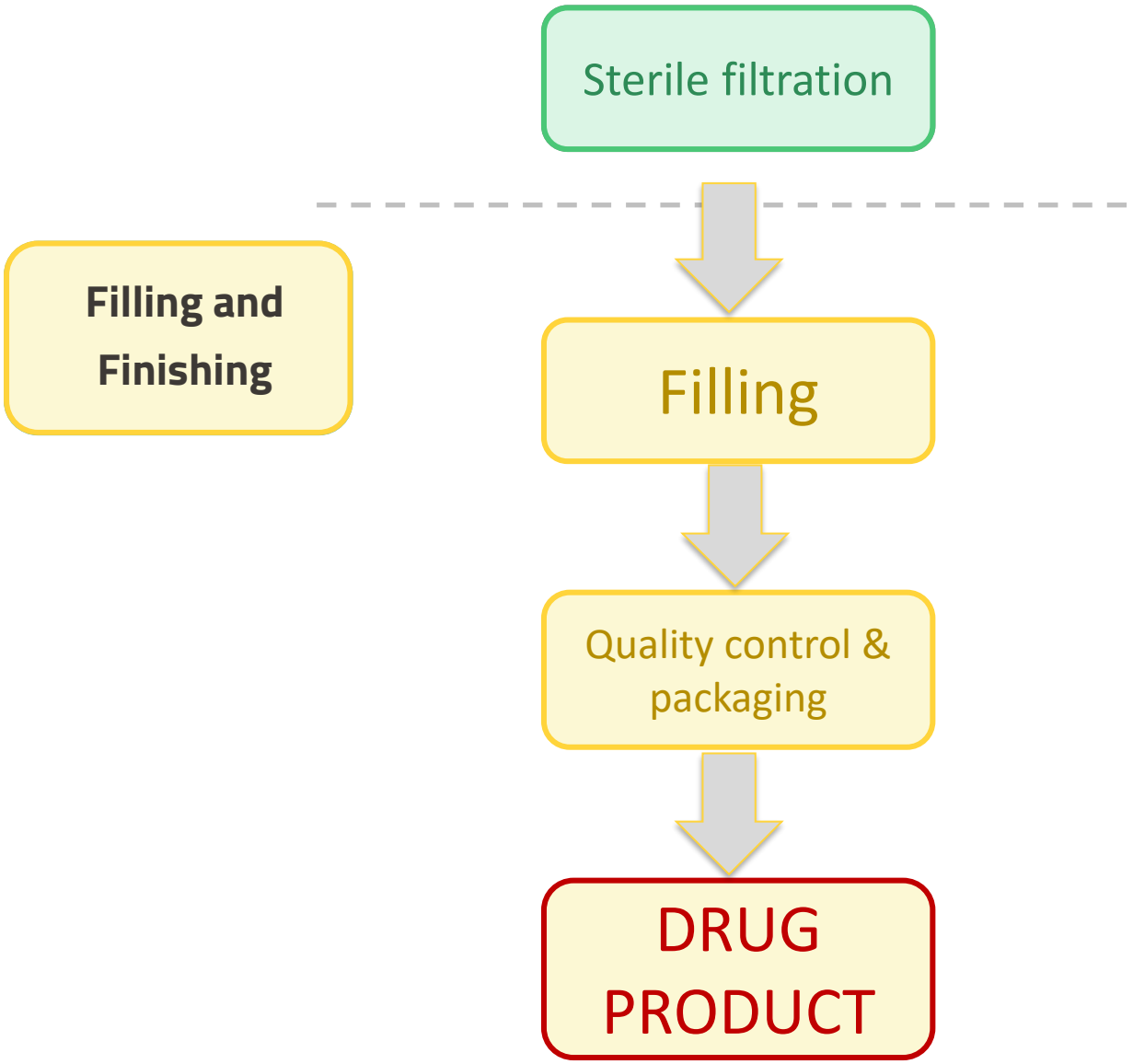


Outputs : concentrated mAb, buffer-exchanged to formulation buffer

IPCs : UV280 monitoring, TMP, permeate flux, pH/conductivity, and turbidity check.

F&F

Fill and Finish Process Flow



F&F

Remplissage & Conditionnement

Perform aseptic filling, stoppering, crimping, and visual inspection

Inputs:

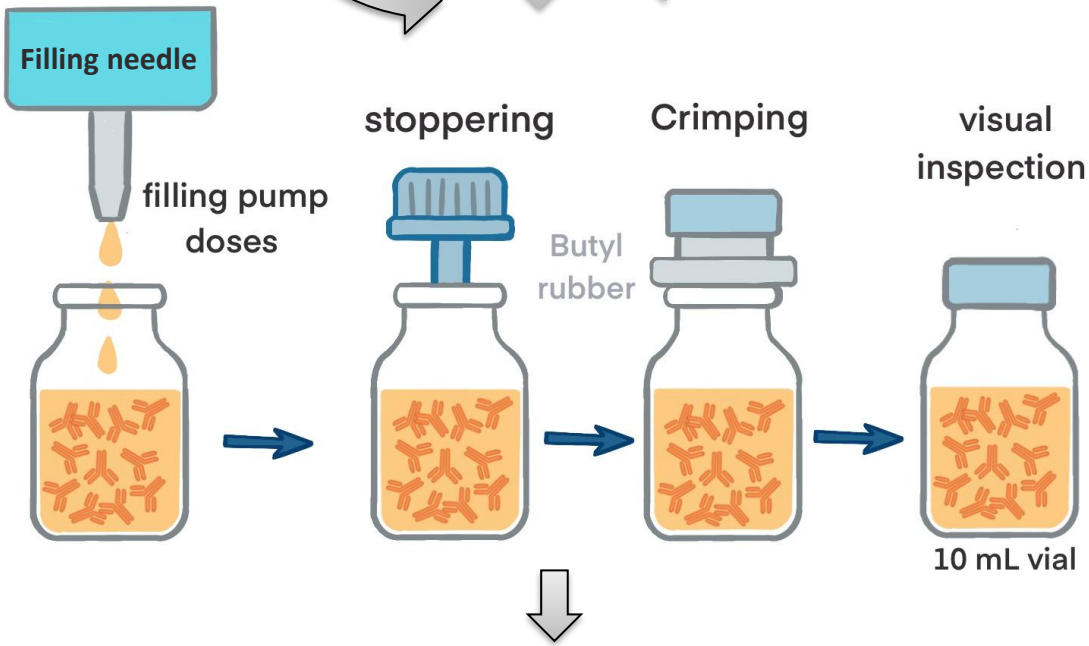
MA

TFF membrane
Buffer composition
Contact materials

concentrated mAb
In formulation buffer

PP

TMPress setpoint & limits, flow rate, Concentration factor



Outputs : concentrated mAb, buffer-exchanged to formulation buffer

IPCs : UV280 monitoring, TMP, permeate flux, pH/conductivity, and turbidity check.

F&F

Remplissage & Conditionnement

Perform aseptic filling, stoppering, crimping, and visual inspection

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Buffer composition
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concentrated mAb
In formulation buffer

PP

TMPress setpoint & limits, flow rate, Concentration factor

Risks

- Membrane fouling or clogging
- concentration stress
- Insufficient diafiltration
- Antibodies loss

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Perform aseptic filling, stoppering, crimping, and visual inspection

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In formulation buffer

PP

TMPress setpoint & limits, flow rate, Concentration factor

Risks

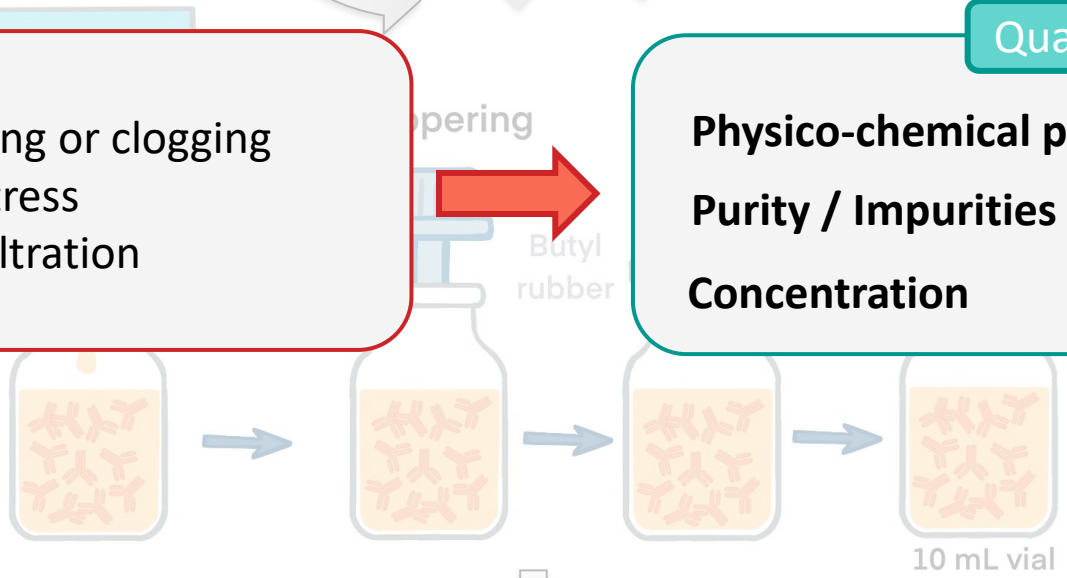
- Membrane fouling or clogging
- concentration stress
- Insufficient diafiltration
- Antibodies loss

Quality attributes

Physico-chemical properties

Purity / Impurities

Concentration



Outputs : concentrated mAb, buffer-exchanged to formulation buffer

IPCs : UV280 monitoring, TMP, permeate flux, pH/conductivity, and turbidity check.

Ce qui change en clinique

- Contraintes GMP/supply : hold times qualifiés, QP release, chaîne du froid
- Sécurité virale : Viral Inactivation qualifiée, filtrations virales + integrity tests
- Culture en circuit fermé
- CEX/AEX : critères de pool HCP/ADN/agrégats
- DP : media fill, intégrité fermeture, étiquetage clinique