



# PDA Europe Training Course

**18-19 September 2018**

Asahi Kasei Bioprocess Europe  
**Cologne | Germany**



# All About Virus Filtration - A Practical Approach

**Tuesday, 18 September 2018**

**8:30 – 17:00**

<b>8:30</b>	<p><b>Welcome and Theory 1</b></p> <ul style="list-style-type: none"> <li>• Introduction into eukaryotic DS manufacturing process</li> <li>• Virus Filters in biopharmaceutical manufacturing</li> <li>• Sources of virus load</li> <li>• Reason/necessity for virus removal from DS</li> <li>• Brief overview of guidelines</li> <li>• Methods for virus removal</li> </ul>	<p>Sebastian Teitz Andrew Bailey</p>
<b>10:00</b>	<b>Coffee Break</b>	
<b>10:30</b>	<p><b>Hands-on 1: Set-up and Handling of Filters</b></p> <ul style="list-style-type: none"> <li>• Set-up in lab-scale: hands-on</li> <li>• Display of production scale filters</li> <li>• Integrity tests: hands-on</li> <li>• Demonstration of integrity test automation</li> </ul>	<p>Sebastian Teitz</p>
<b>12:30</b>	<b>Lunch Break</b>	
<b>13:30</b>	<p><b>Theory 2: Case Study</b></p> <ul style="list-style-type: none"> <li>• Up- &amp; Downscaling of a virus filtration step</li> </ul>	<p>Franz Nothelfer</p>
<b>15:00</b>	<b>Coffee Break</b>	
<b>15:30</b>	<p><b>Interactive Session: Designing a virus filtration process – assumption and points to consider</b></p> <ul style="list-style-type: none"> <li>• How to design a process</li> <li>• Calculating production costs</li> </ul>	<p>Sebastian Teitz</p>
<b>17:00</b>	<b>End of Day 1</b>	
<b>18:00</b>	<b>Networking Dinner</b>	

# Agenda

**Wednesday, 19 September 2018**

**8:30 – 15:30**

**8:30**      **Wrap-up Day 1**      Sebastian Teitz

**9:00**      **Theory 4:**      Sebastian Teitz

- Mechanistic principles of (Parvo-) Virus retention
- Virus filters as bioprocess subject – current hot topics (ATMPS, facility segregation, etc.)
- Challenges of implementing virus filtration into continuous manufacturing

**10:30**      **Coffee Break**

**11:00**      **Theory 5:**      Michael Lasse

- How to organize a virus clearance study
- Challenges in VC studies
- Historical data
- Case studies for VC studies

**12:30**      **Lunch Break**

**13:30**      **Interactive session:**      Sebastian Teitz  
**Pitfalls in the development of a virus filtration process**

- Bring your own case/topic/question/problem/challenge for discussion!

Participants have the opportunity to address real-life challenges during the implementation of a virus filtration process – from bench-top development through to commercial scale-up.

**15:00**      **Wrap-up, Q&A**      Sebastian Teitz

**15:30**      **End of Course**

# Speakers

**Dr. Sebastian B. Teitz,**  
Product Manager & Scientific Coordinator  
Asahi Kasei Bioprocess Europe  
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**Andy Bailey,**  
CEO  
Virusure GmbH,  
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**Franz Nothelfer,**  
Consultant at NotiConsult - Former Associate Director  
Protein Science at Boehringer Ingelheim Pharma  
franz.nothelfer@yahoo.de

**Dr. Michael Lasse,**  
Study Director Supervisor  
Charles River Biologics Testing  
michael.lasse@crl.com



# Brief Introduction of Asahi Kasei Bioprocess

Dr. Sebastian Teitz, Product Manager & Scientific Coordinator, Asahi Kasei Bioprocess Europe,  
s.teitz@akbio.eu, [www.ak-bio.com](http://www.ak-bio.com)

# Brief Introduction of Asahi Kasei Bioprocess

- Foundation: 1922; HQ: Tokyo; Employees: 25 000; Turnover: 19 B\$
- Our business fits within the Health Care segment of Asahi Kasei, a diversified manufacturer focused on the societal challenges of tomorrow.



*A range of reliable consumables, equipment and services, with a focus on virus filtration*



**BioOptimal™ MF-SL**  
microfilters for cell  
removal and clarification



**Cellufine™**  
chromatography media  
for IEX, Affinity, HIC and SEC



**Planova™** and  
**Planova™ BioEX**  
for virus removal



**Virus Filtration  
Systems**



**IBD™ Inline Buffer  
Dilution Systems**



**Chromatography  
Columns & Systems**



**Asahi  
Oligosynthesizer™**

## Services

- Consultancy on product screening, process development and optimization
- Virus clearance validation support
- Integrated equipment and automation



# Brief Introduction of Asahi Kasei Bioprocess

*Diverse locations for “always available” support and smooth business continuity*





# Brief Introduction of Asahi Kasei Bioprocess

- > 120 m<sup>2</sup> lab space in Cologne (Germany)
- **Seminars:** Planova, TFF Microfiltration, Chromatography
- **Optimization** of filtration performances, non GLP studies
- Demonstrations & training on **automatic skid/systems** (AGPTS, virus filtration skid, inline buffer dilution systems, chromatography columns...)

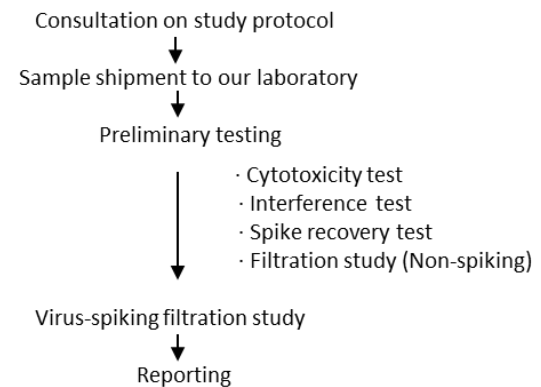


# Brief Introduction of Asahi Kasei Bioprocess

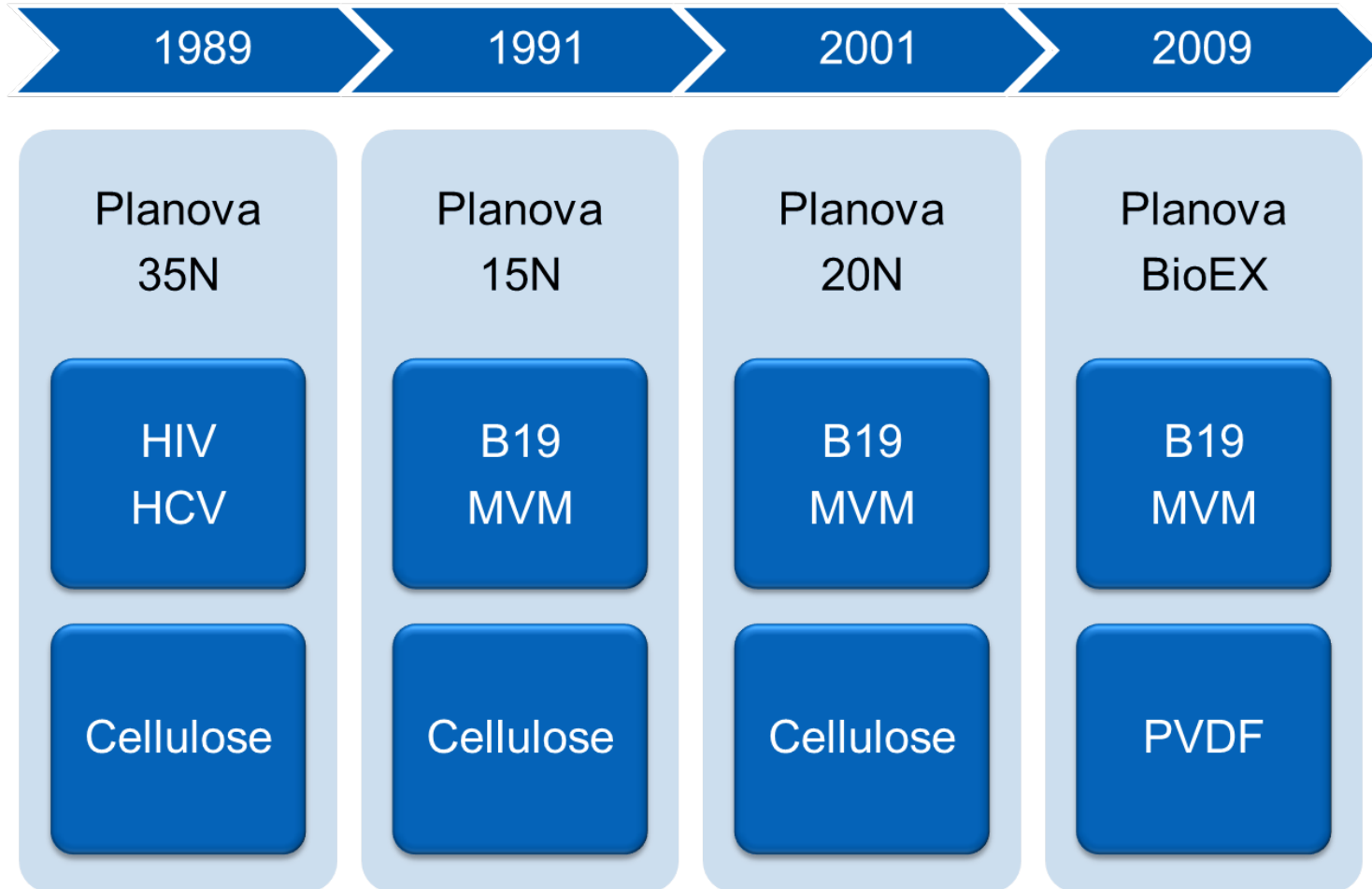
- Non-GLP viral clearance studies
- With PPV or phage PP7 in US, with PPV or MVM in Japan
- Advice on study protocols



Using samples from the planned process feed, our technical team will conduct non-GLP virus studies using stock virus developed by Asahi Kasei Bioprocess. Conducting these studies before the actual GLP virus validation effectively reduces the risk of failure in GLP studies.



❑ Pioneering nanofiltration technology since 1989





## Theory 1:

Introduction into eukaryotic DS manufacturing process

Virus Filters in biopharmaceutical manufacturing

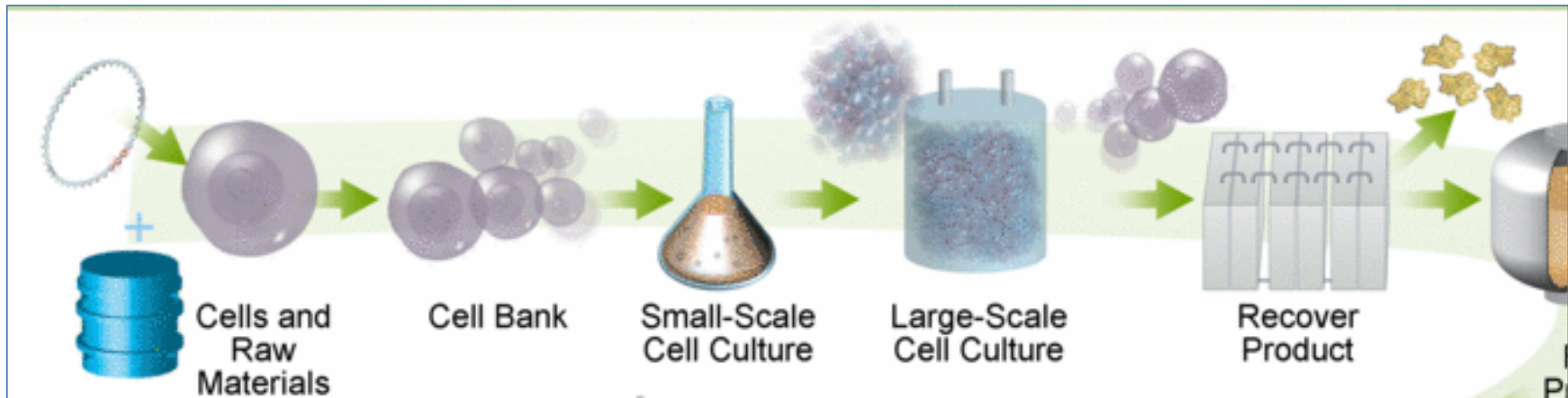
Dr. Sebastian Teitz, Product Manager & Scientific Coordinator, Asahi Kasei Bioprocess Europe,  
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# Disclaimer

***The presentation slides were prepared by the speaker in his personal capacity.***

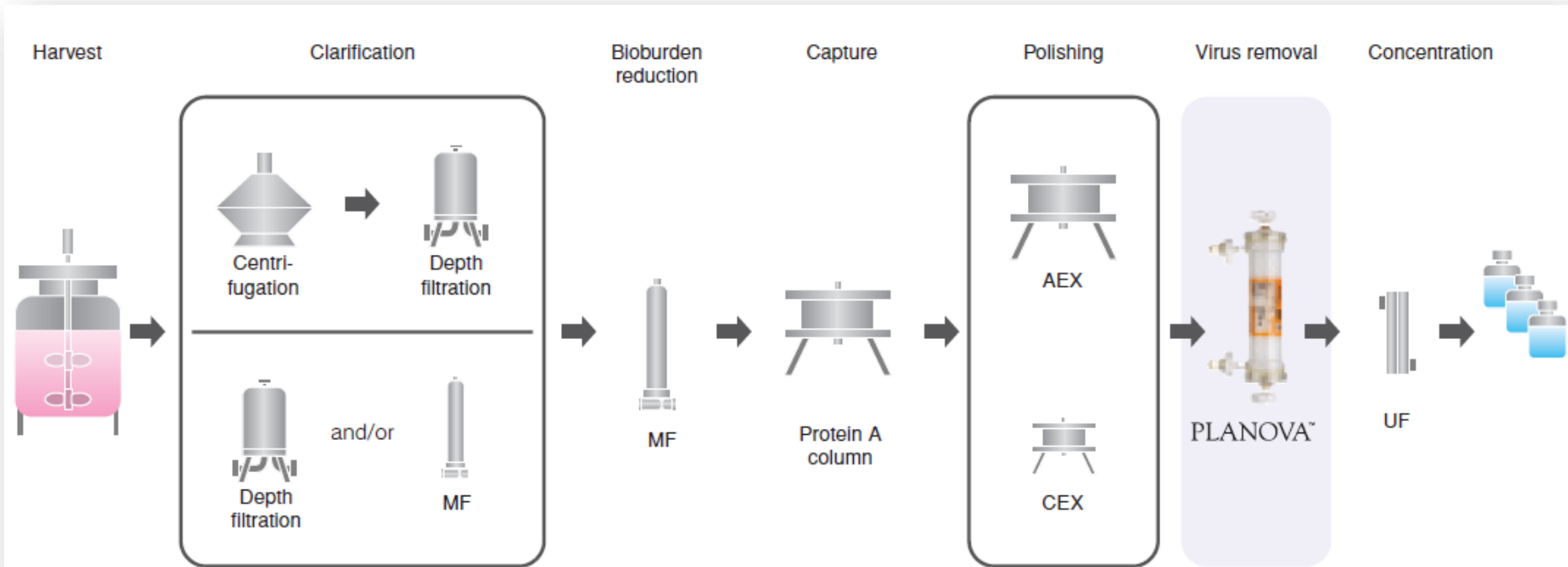
***The opinions expressed are the author's own and do not necessary reflect the view of Asahi Kasei Bioprocess.***

## *Eukaryotic Drug Manufacturing - USP*



Modified from: <http://www.sec.gov/Archives/edgar/data/732485/000110465910030452/g959514bci013.gif>

## *Eukaryotic Drug Manufacturing - DSP*



## *Eukaryotic Drug Manufacturing - The Challenge*

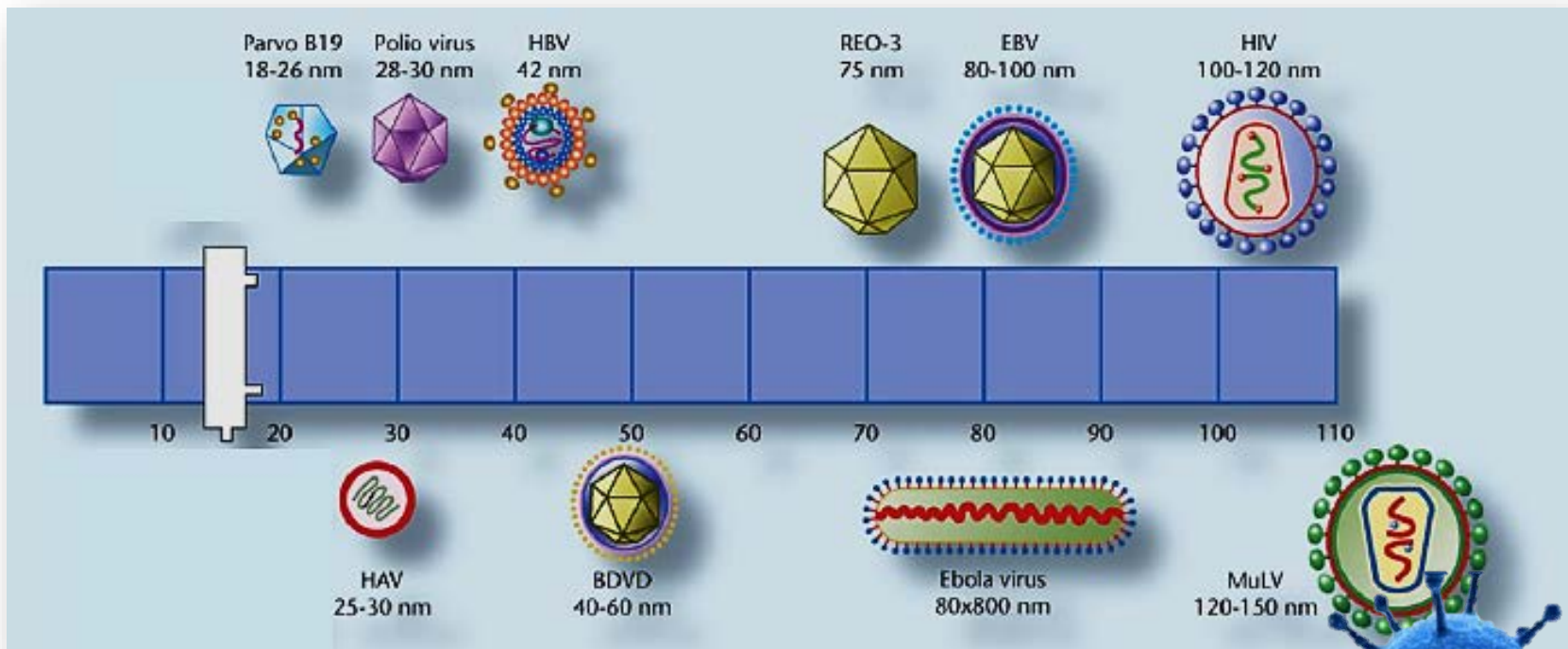


Image: MVM Virus



Image: Parvovirus B19

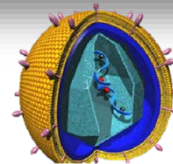


Image: Retrovirus

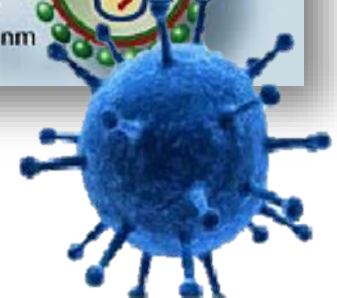
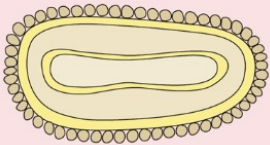














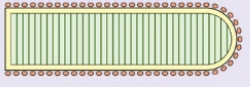
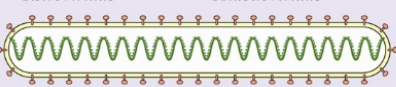






Image: Pseudorabies virus



# Introduction

	Enveloped	Nonenveloped
DNA	<p>dsDNA</p>  <p><i>Poxviridae, Chordopoxvirinae</i></p>  <p><i>Herpesviridae</i></p>  <p><i>Hepadnaviridae</i></p>	<p>dsDNA</p>  <p><i>Adenoviridae</i></p>  <p><i>Papovaviridae</i></p> <p>ssDNA</p>  <p><i>Parvoviridae</i></p>
RNA	<p>ssRNA</p>  <p><i>Coronaviridae</i></p>  <p><i>Paramyxoviridae</i></p>  <p><i>Bunyaviridae</i></p>  <p><i>Toroviridae</i></p>  <p><i>Orthomyxoviridae</i></p>  <p><i>Arenaviridae</i></p>  <p><i>Togaviridae</i></p>  <p><i>Flaviviridae</i></p>  <p><i>Retroviridae</i></p>  <p><i>Rhabdoviridae</i></p>  <p><i>Filoviridae</i></p> <p>100 nm</p>	<p>dsRNA</p>  <p><i>Reoviridae</i></p>  <p><i>Birnaviridae</i></p> <p>ssRNA</p>  <p><i>Picornaviridae</i></p>  <p><i>Caliciviridae</i></p>

- Infectious agent
- Host (animals, plants, bacteria...)
- Enveloped & non-enveloped
- RNA & DNA
- Variety of shapes and sizes
- **virus removal steps contributing “significant” clearance**

- The viral safety of bio-therapeutic products is required by regulatory agents worldwide.
- Viral inactivation/ removal method is necessary in production process.
- “It is desirable to investigate the contribution of more than one production step for virus reduction and at least two orthogonal steps should be assessed”\*
- \* EMEA/ CHMP/ BWP/ 398498, 2005

- **Virus removal:**

Virus removal filtration: Hollow fiber, flat sheet

Chromatography: AEX, CEX, affinity

Precipitation: Ethanol, polyethylene glycol

- **Virus inactivation:**

Heat treatment: Dry-heat, pasteurization

Solvent/Detergent (SD): TNBP/ Triton X-100

Low pH: ~ pH 4

Chemical treatment: Caprylic acid

Irradiation: UV-C, gamma

## Why Virus Removal Filtration ?

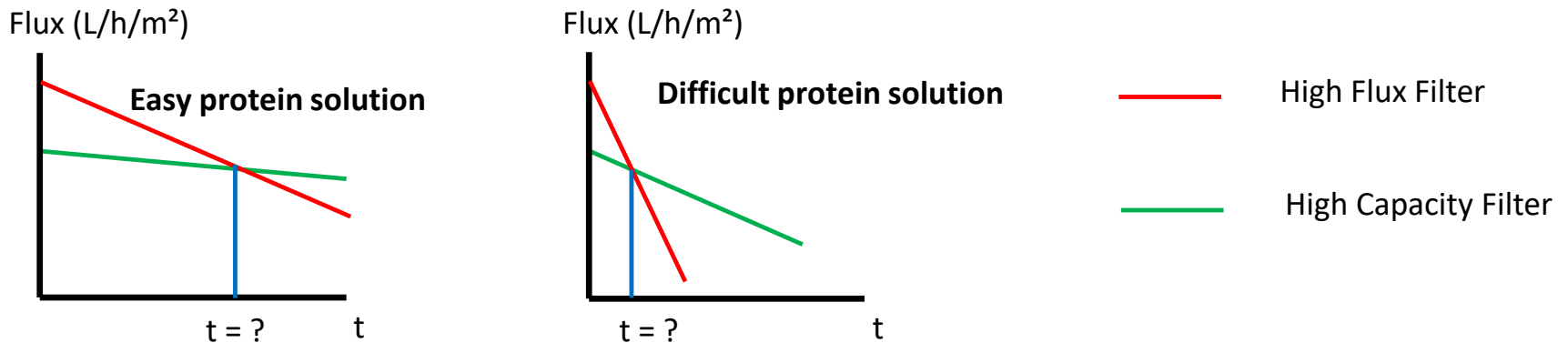
- “Simple” mechanism based on size exclusion
- Reproducible and reliable
- Not affected by the nature of virus or product
- No impact on chemical characteristics of the product solution
- Not requiring any stabilizers or other chemical agents
- Robust and consistent

# Virus Filters in Biopharmaceutical Manufacturing

- Planova 35N was first on the market in 1989: removal of retrovirus
- Since early 2000, several other nanofilters on the market
- 2 categories: “big virus” retentive filters, “small virus” retentive filters
- They differ from the material of the membrane (cellulose, PVDF, PS, PES) and by the design of the membrane (hollow fiber, flat sheet)
- 4 suppliers: Asahi Kasei Bioprocess, Merck, Pall, Sartorius

# Virus Filters in Biopharmaceutical Manufacturing

- Today, parvovirus removal filter is “the state of the art”!
- $\geq 4.0$  log removal of parvovirus
- 2 families of parvovirus removal filters ( $\sim 20$  nm pore size)



# Virus Filters in Biopharmaceutical Manufacturing

	<b>High Flux Nanofilter</b>	<b>High Capacity Nanofilter</b>
<b>Brand</b>	VPro (Merck) Virosart CPV & HF (Sartorius), Pegasus Prime (Pall)	Virosart HC (Sartorius), Pegasus SV4 & DV20 (Pall), Planova 20N & BioEX (Asahi)
<b>Prefiltration</b>	Special or adsorptive prefilters	Not or simple 0.2 or 0.1µm
<b>Filtration time</b>	Short	Average to long
<b>Price per m<sup>2</sup></b>	High to very high	Low to moderate
<b>Low concentration or “easy” protein solution</b>	Cost effective (very high average flux)	Cheaper if longer filtration time (no/small flux decay)
<b>High concentration or “difficult” protein solution</b>	Difficult, high flux decay	Moderate flux and flux decay

# Virus Filters in Biopharmaceutical Manufacturing

From supplier's websites:



VPro



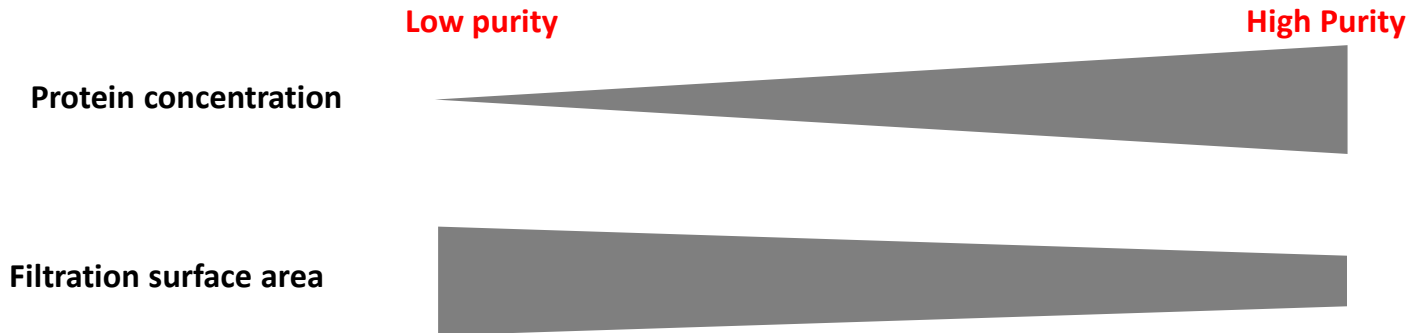
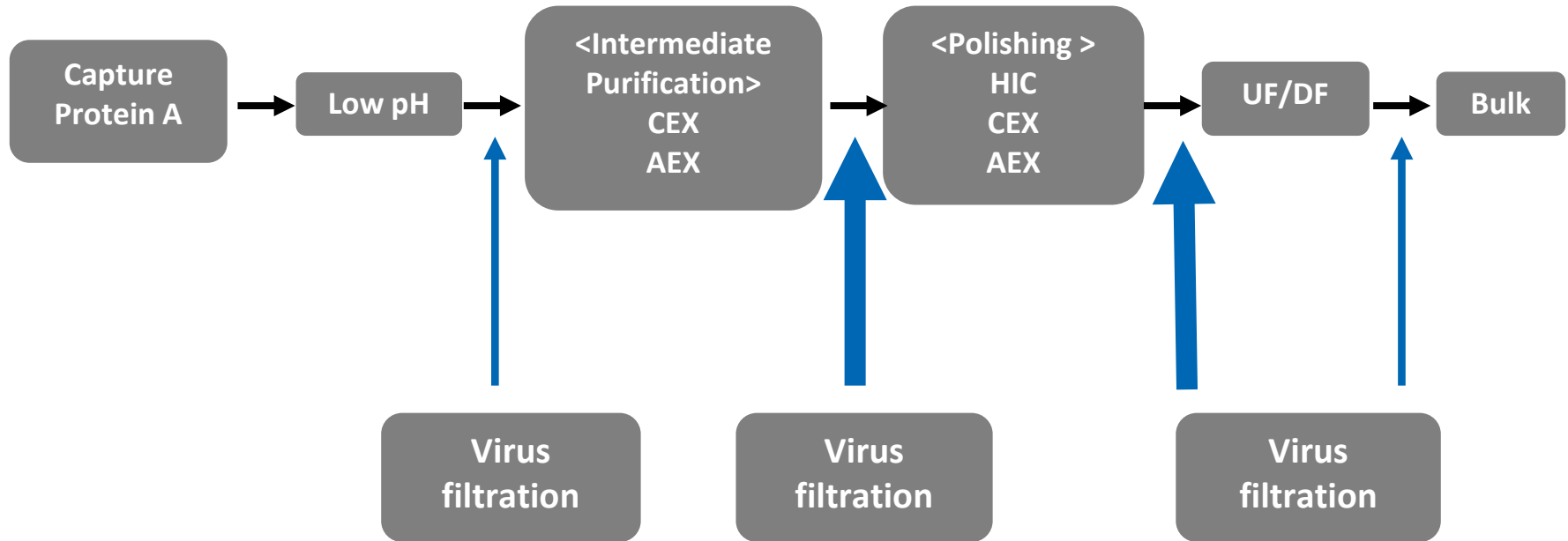
Pegasus Prime



Planova BioEX

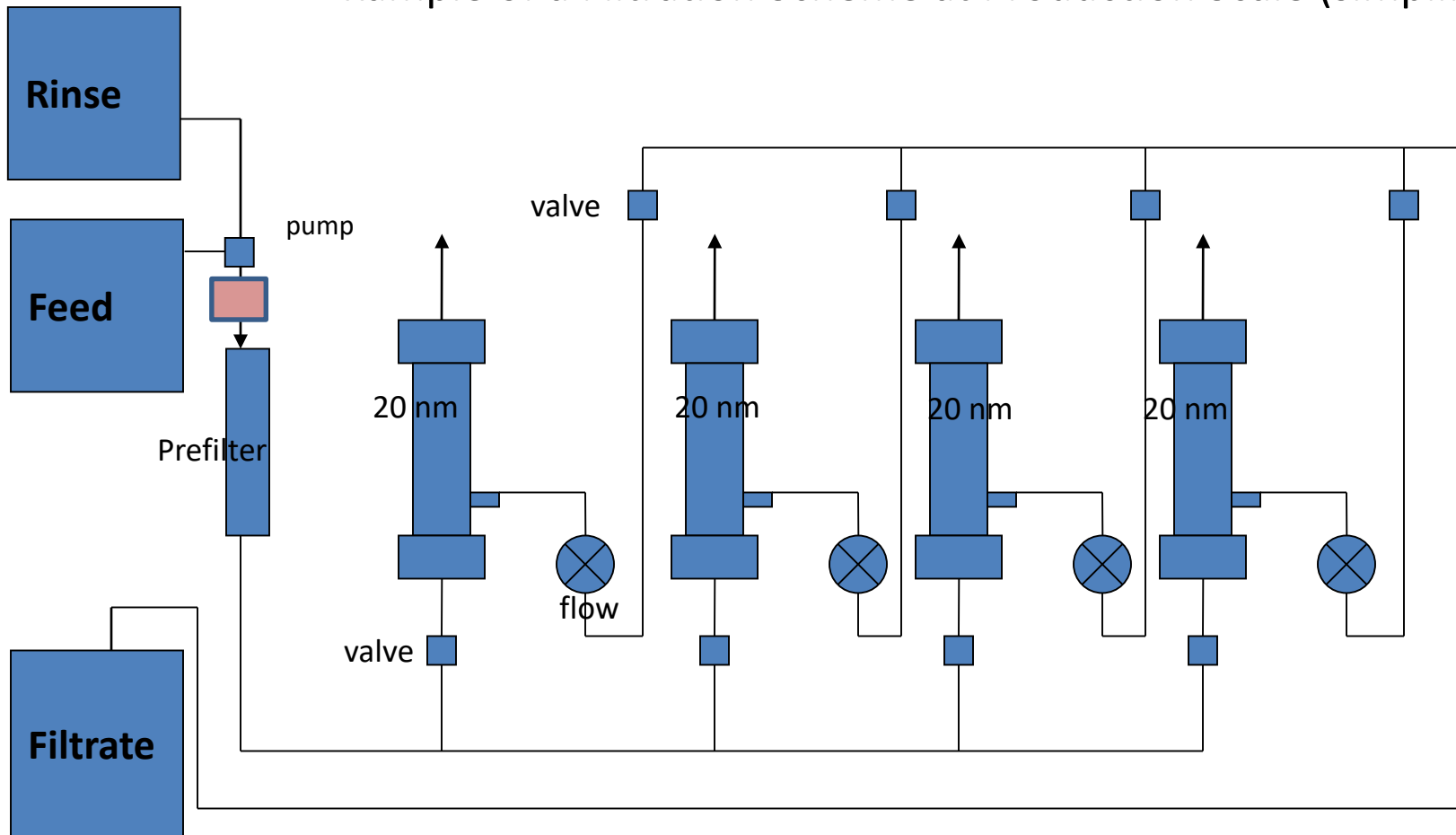


# Virus Filters in Biopharmaceutical Manufacturing



# Virus Filters in Biopharmaceutical Manufacturing

Example of a Filtration Scheme at Production Scale (simplified!)



*Modified from Dr. Dichtelmüller, Planova Workshop Rome, Nov 2013*

# Virus Filters in Biopharmaceutical Manufacturing

Example of a Filtration Scheme at Production Scale (simplified!)



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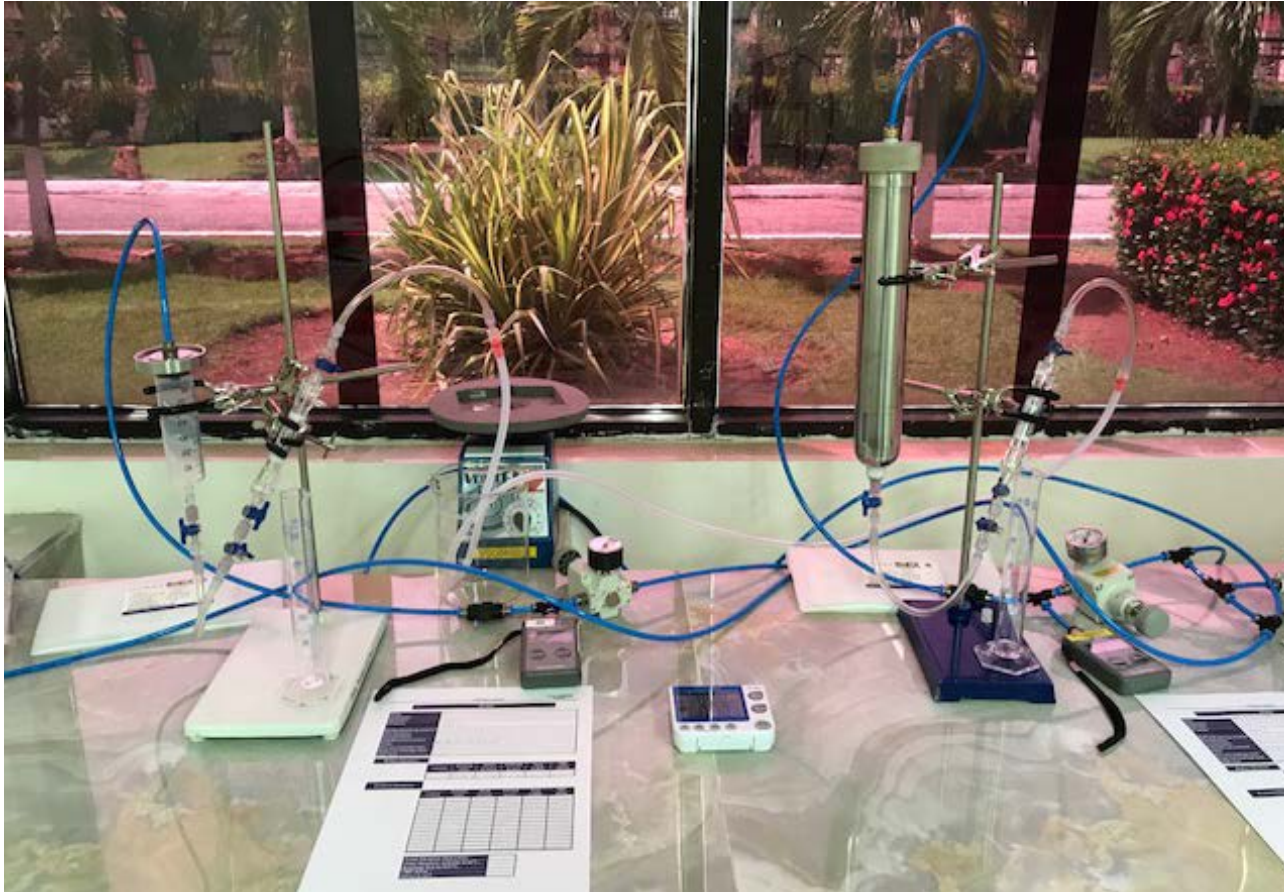
# Virus Filters in Biopharmaceutical Manufacturing

Automatic virus filtration systems (single use flow paths)



# Virus Filters in Biopharmaceutical Manufacturing

Everything starts from reliable lab scale studies ☺



# Questions ?





## Theory 1:

Sources of virus load, Reason/necessity for virus removal from DS, Brief overview of guidelines, Methods for virus removal

Andy Bailey, CEO, ViruSure GmbH, [andy\\_bailey@virusure.com](mailto:andy_bailey@virusure.com), [www.virusure.com](http://www.virusure.com)

# Questions ?





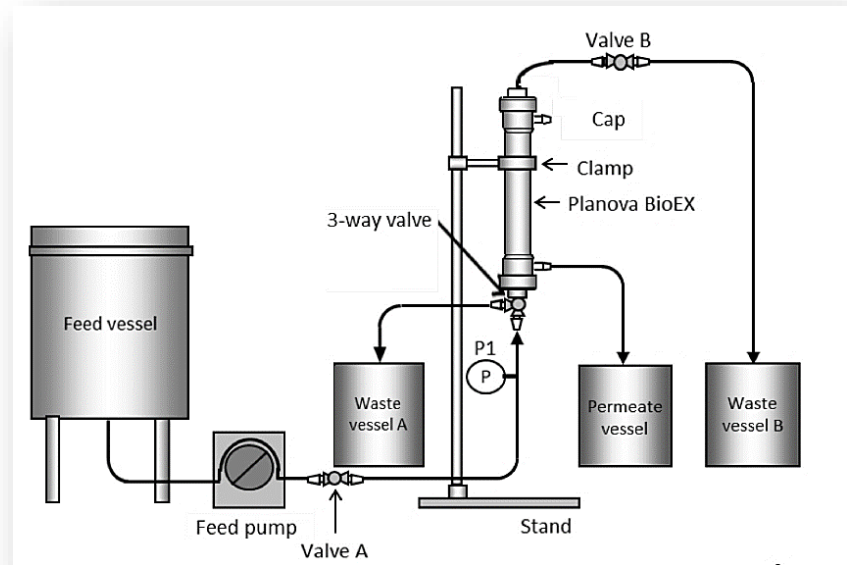
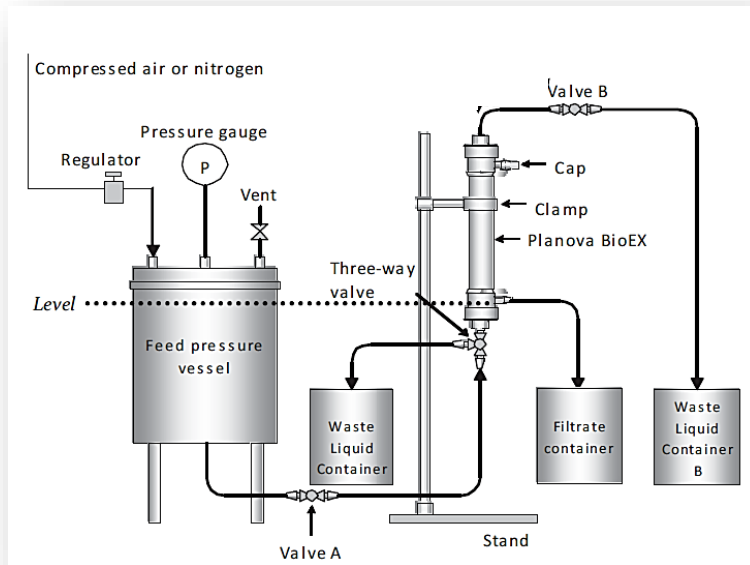


## **Hands-on 1: Set-up and Handling of Filters**

Set-up in lab-scale: hands-on, Display of production scale filters,  
Integrity tests: hands-on, Demo of integrity test automation

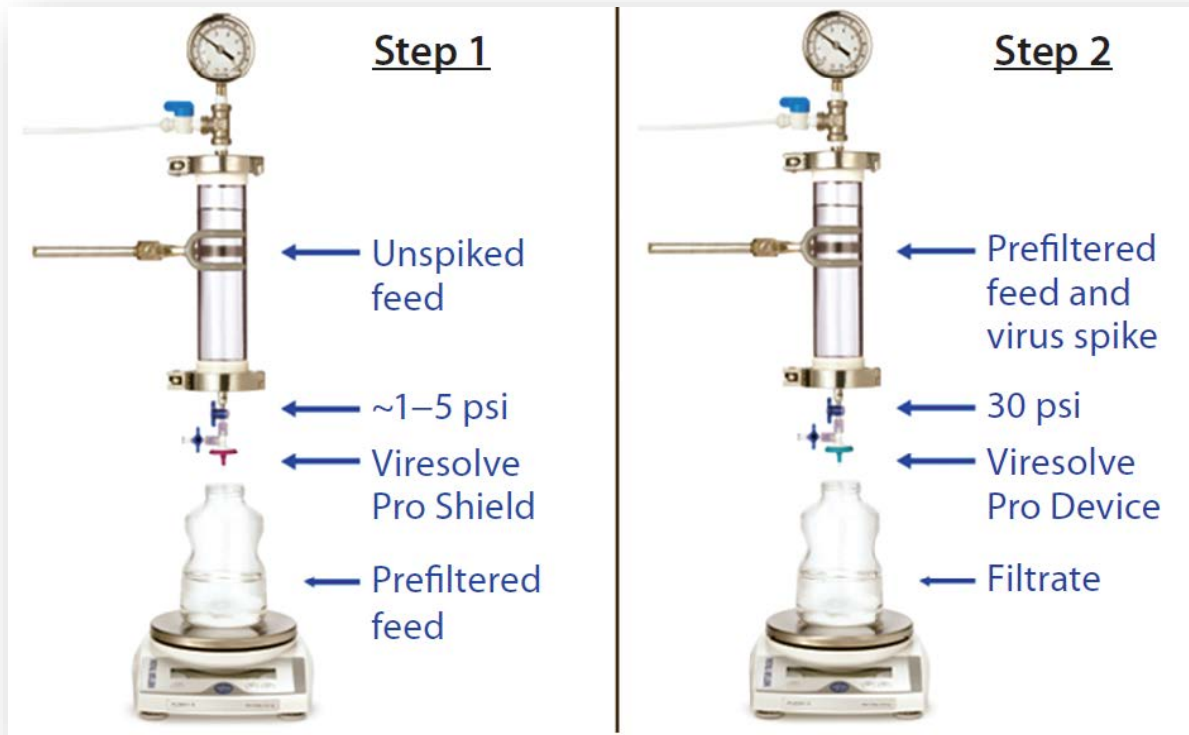
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# Set-Up in Lab-Scale



« Same » set-up at manufacturing scale

# Set-Up in Lab-Scale



*From the Article "Artifacts of Virus Filter Validation" published in Bioprocess International*

## 1 - Pre-use IT

## 2 - Assembly and Setting of pressure or pump flow rate

## 3 - Pre-washing with water and/or buffer solution

To remove substances leached out from the membrane and to saturate the inside of the filter with washing solution **(to remove air)**.

## 4 - Filtration of the protein solution

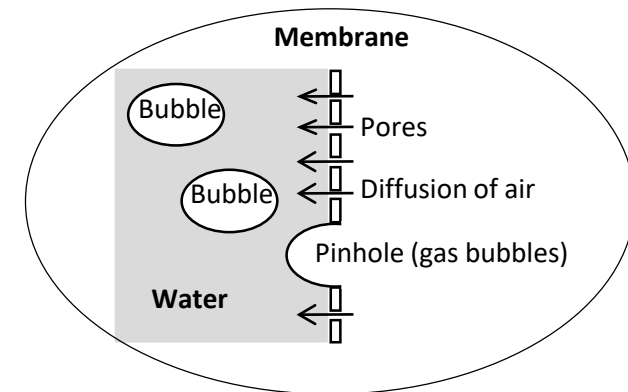
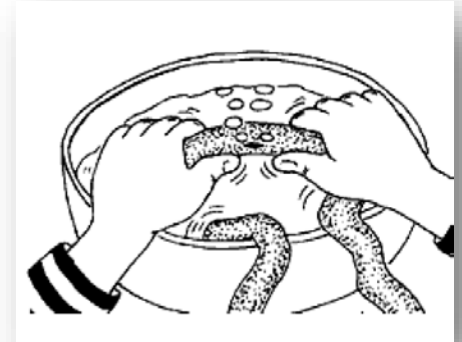
## 5 - Post-washing

To improve the recovery rate by pushing out the proteins remaining in the piping or the filter after the filtration process by using the pre-washing solution.

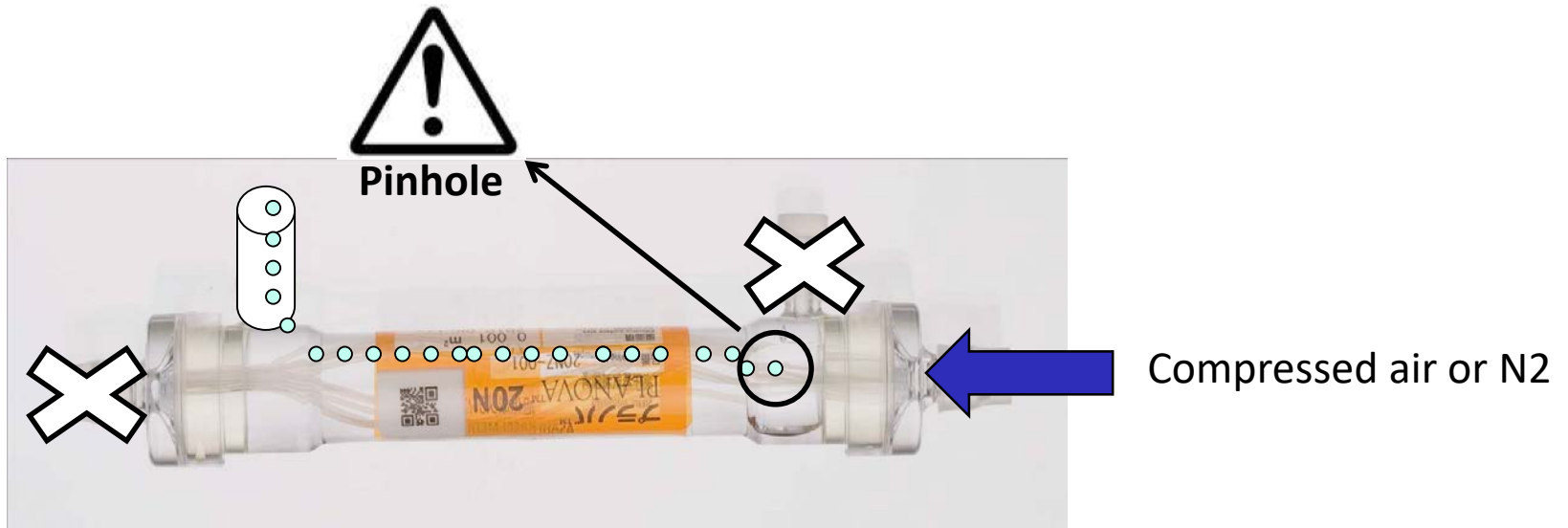
## 6 - Post-use IT

## 1 - Pre-use IT:

- **Why:**
  - Free from pinholes?
  - Other gross membrane damages?
- **How:**
  - ✓ Visual/manual:
    - Investigation if air bubbling (Planova) or gas flow (other nanofilters)
  - ✓ Automated: air diffusion measurement
    - Planova Leak Tester (Planova only)
    - Palltronic, Sartocheck or Integritest



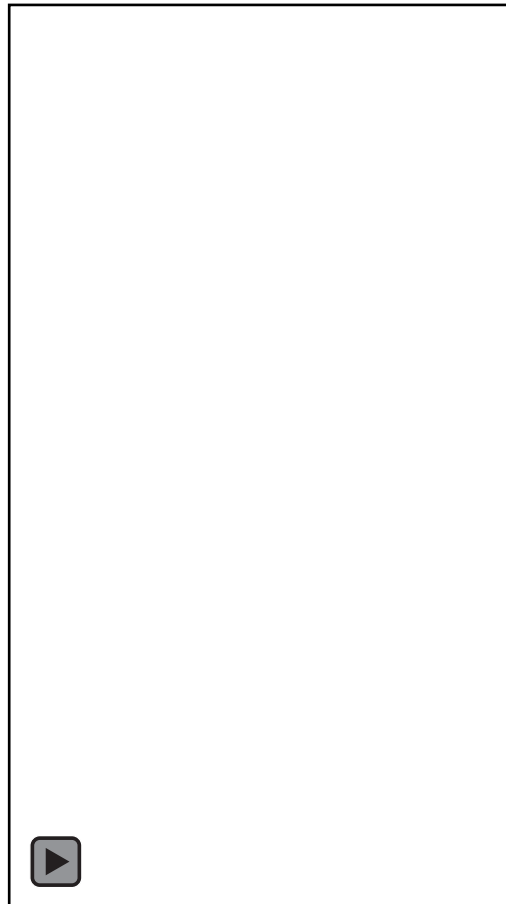
## 1 - Pre-use IT:



Planova N:        **P = 98 kPa**  
Planova BioEX:    **P = 343 kPa**

**Important:** The filtrate side of the filter must be filled with water

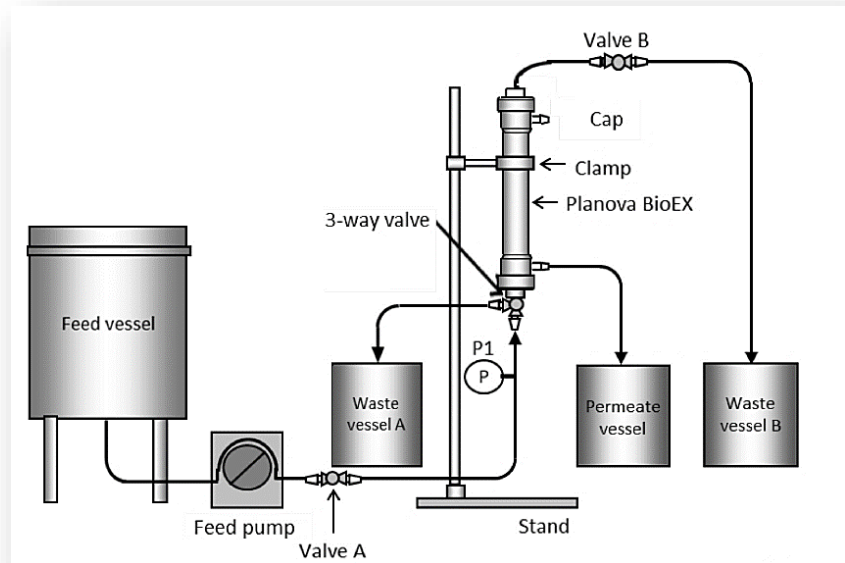
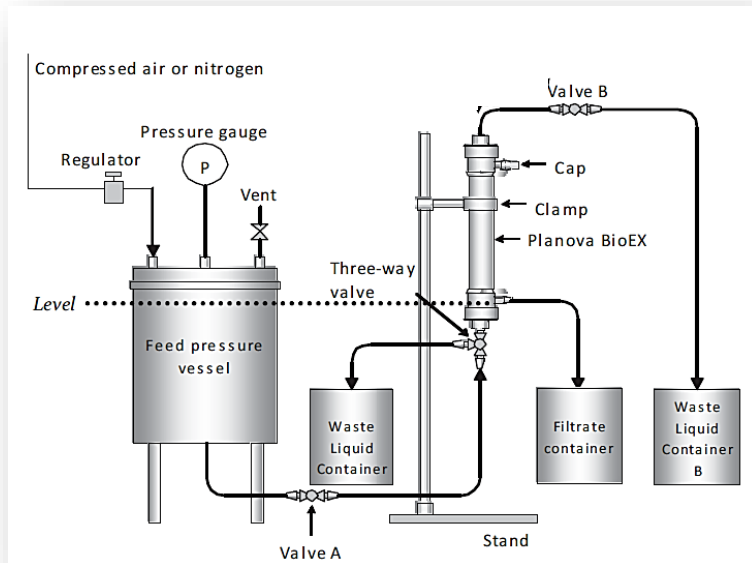
## 1 - Pre-use IT:



✓ 15 µm pinhole

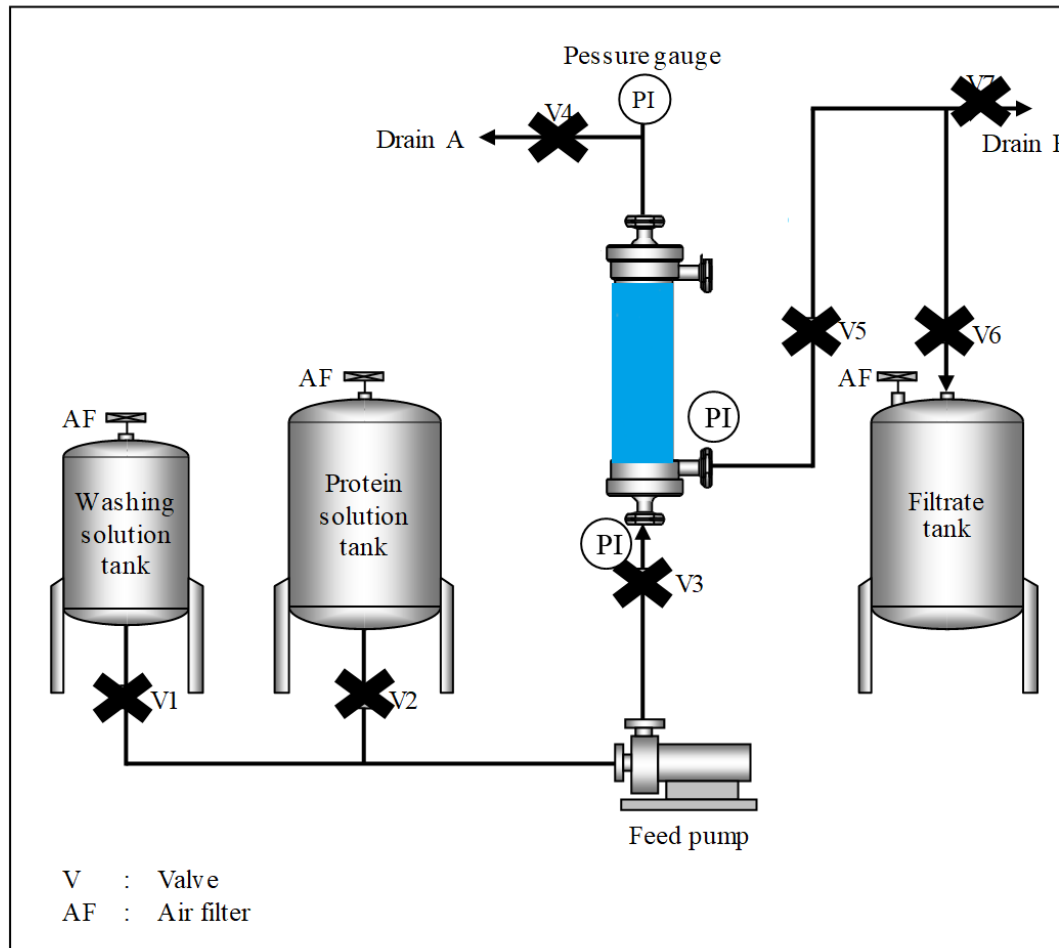
✓ 1 bar

## 2 - Assembly and Setting of pressure or pump flow rate



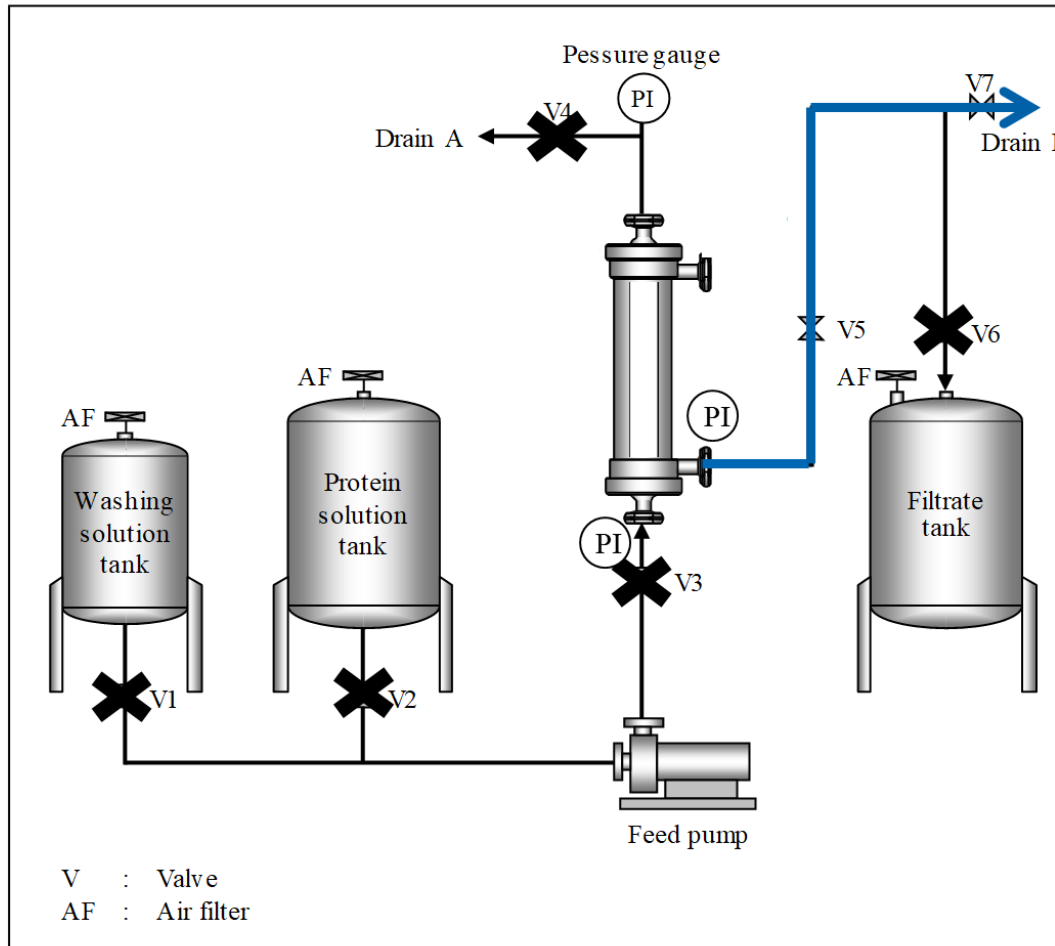


## 3 - Pre-washing with water and/or buffer solution - Preparation



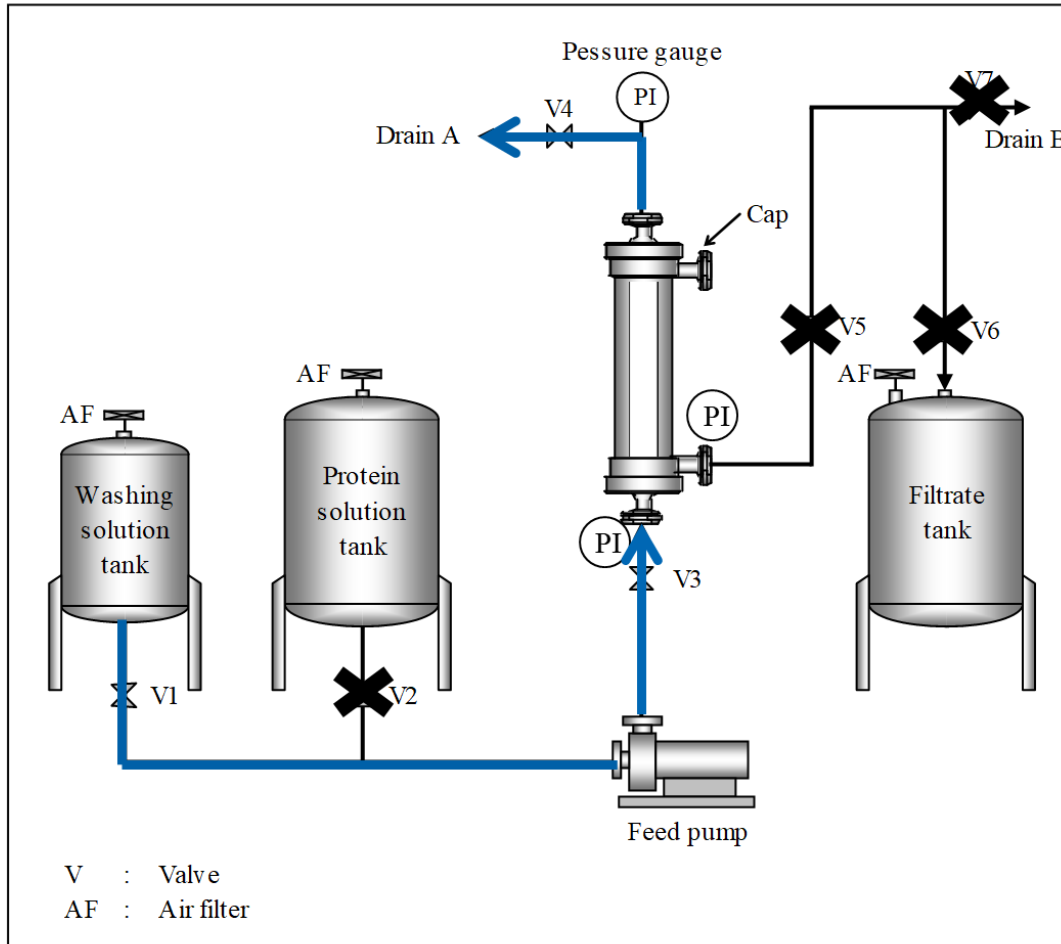
- Open the cap of the top filtrate outlet
- Drain the filtrate side of the filter

## 3 - Pre-washing with water and/or buffer solution - Preparation



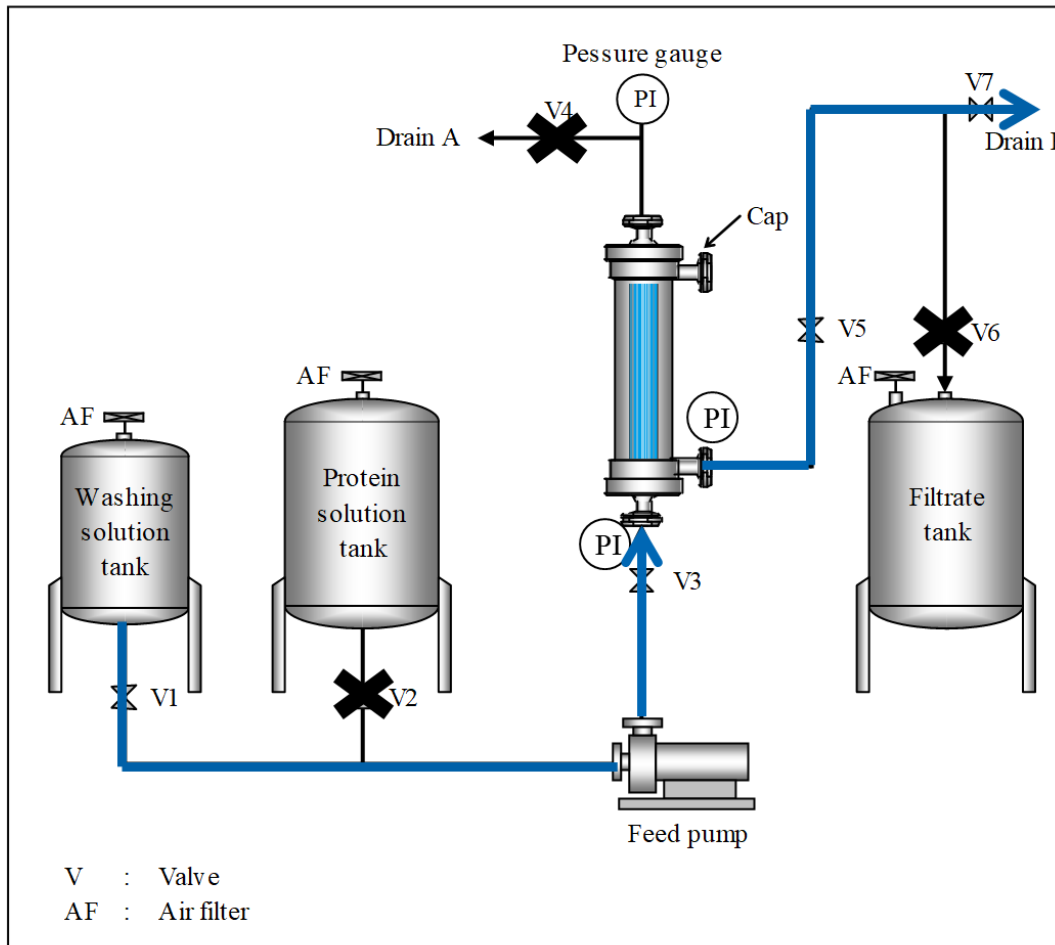
- Open the cap of the top filtrate outlet
- Drain the filtrate side of the filter

## 3 - Pre-washing with water and/or buffer solution



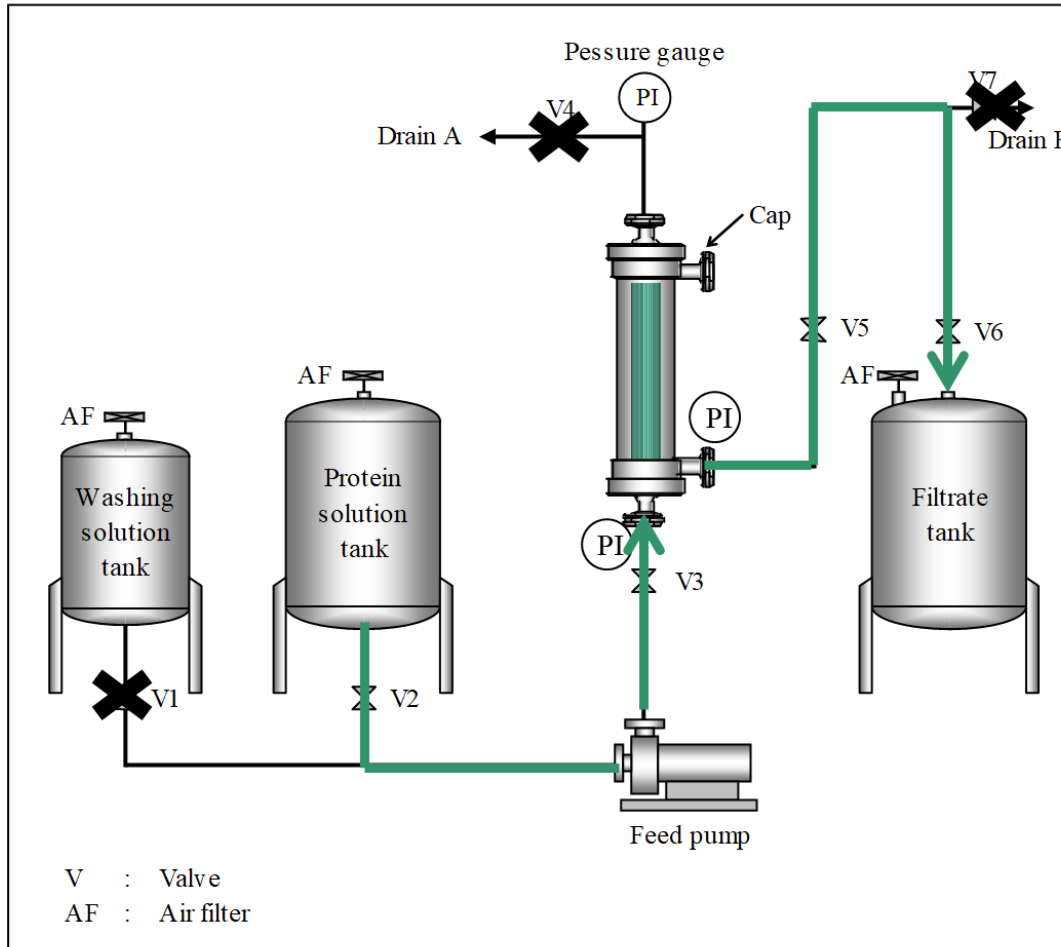
- **Inlet Pressure:**  
< 30 kPa
- **Volume:**  
Refer to SOP.  
Different for each surface area.  
Independent on pore size.

## 3 - Pre-washing with water and/or buffer solution



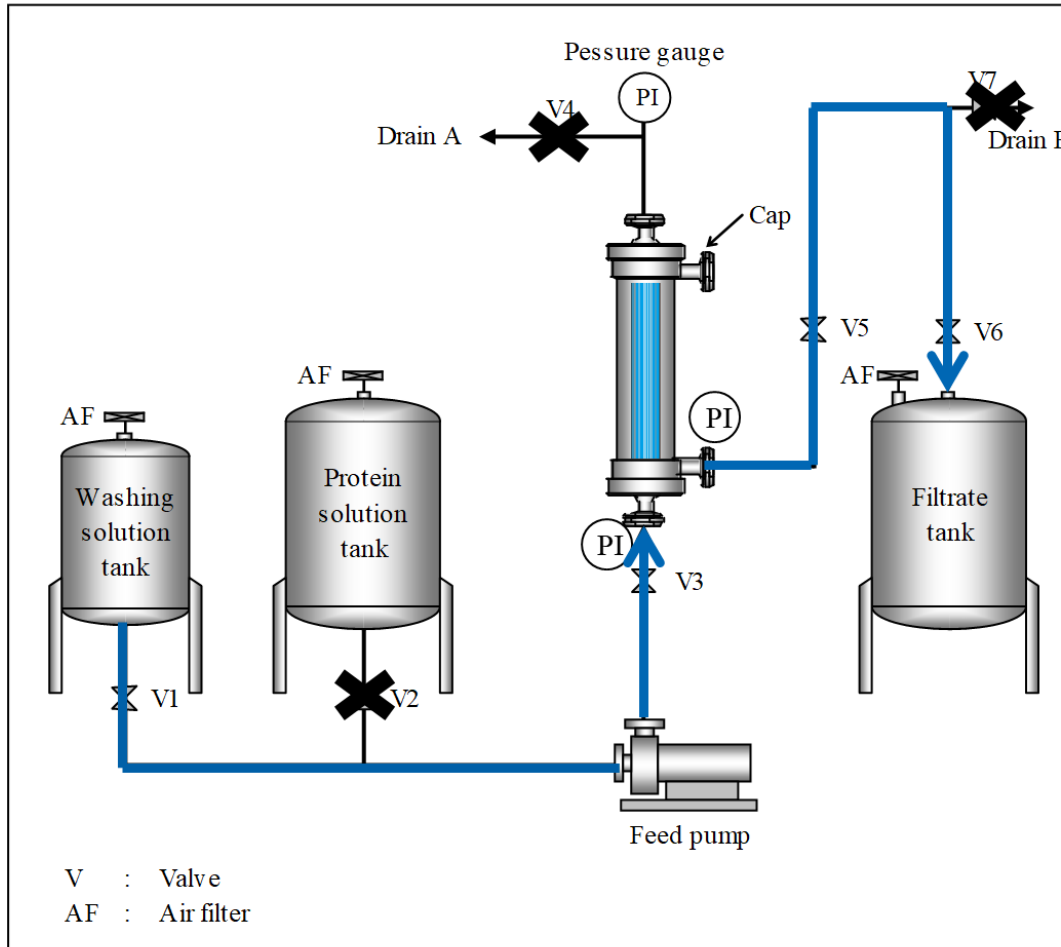
- **TMP:**
  - < 98 kPa (Planova N)
  - < 343 kPa (Planova BioEX)
  
- **Volume:**
  - Refer to SOP. Different for each surface area.
  - Independent on pore size.

## 4 - Filtration of the protein solution



- **TMP:**  
As pre-determined  
< 98 kPa (Planova N)  
< 343 kPa (Planova BioEX)
- **Flow rate:**  
As pre-determined  
< buffer flow rate
- **Volume:**  
As validated during study
- **Stop:**  
If max volume or max TMP or max filtration time

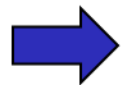
## 5 - Post-washing



- **TMP:**  
< 98 or 343 kPa
- **Flow rate:**  
To keep TMP  
< 98 or 343 kPa
- **Volume:**  
As validated during Virus  
Clearance  
Study

## 6 - Post-use IT:

**Regulatory requirement** to enhance reliability and safety of the virus removal filter:

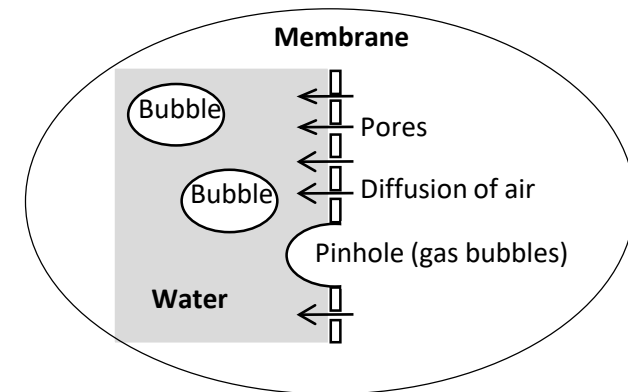
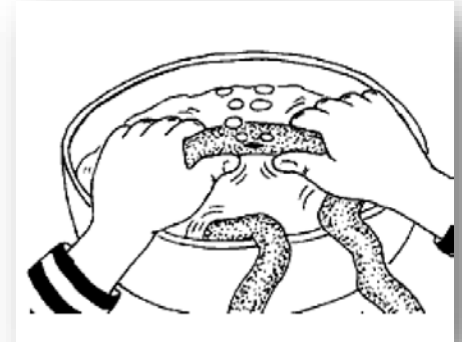


**Question to answer :**

**Is the filter unchanged after the product filtration process?**

## 6 - Post-use IT:

- **Why:**
  - Free from pinholes?
  - Other gross membrane damages?
- **How:**
  - ✓ Visual/manual:
    - Investigation if air bubbling (Planova) or gas flow (other nanofilters)
  - ✓ Automated: air diffusion measurement
    - Planova Leak Tester (Planova only)
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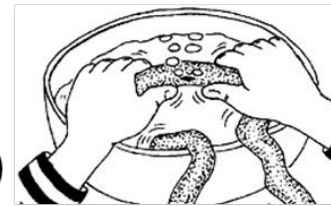


## 6 - Post-use IT:

Planova offers 2 **complementary** Integrity Tests (IT) :

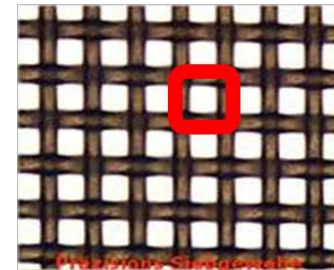
- **Pre/Post-use Leakage Test (LT)**

➔ Detection of large defects (pinholes)



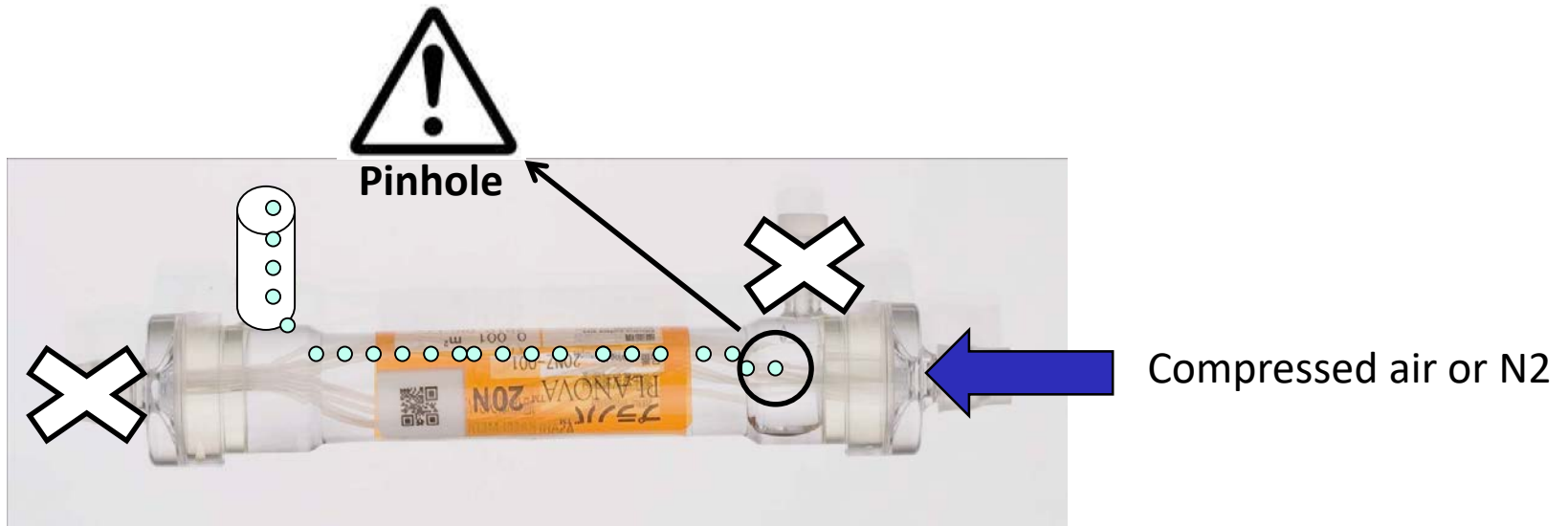
- **Asahi Gold Particle Test (GPT)**

➔ Control of the pore size distribution



***Only LT for Planova BioEX (PVDF hollow fibers)***

## 6 - Post-use IT: Leakage Test



Planova N:            **P = 98 kPa**  
Planova BioEX:    **P = 343 kPa**

**Important:** The filtrate side of the filter must be filled with water

## 6 - Post-use IT: Air Diffusion Rate (ADR)

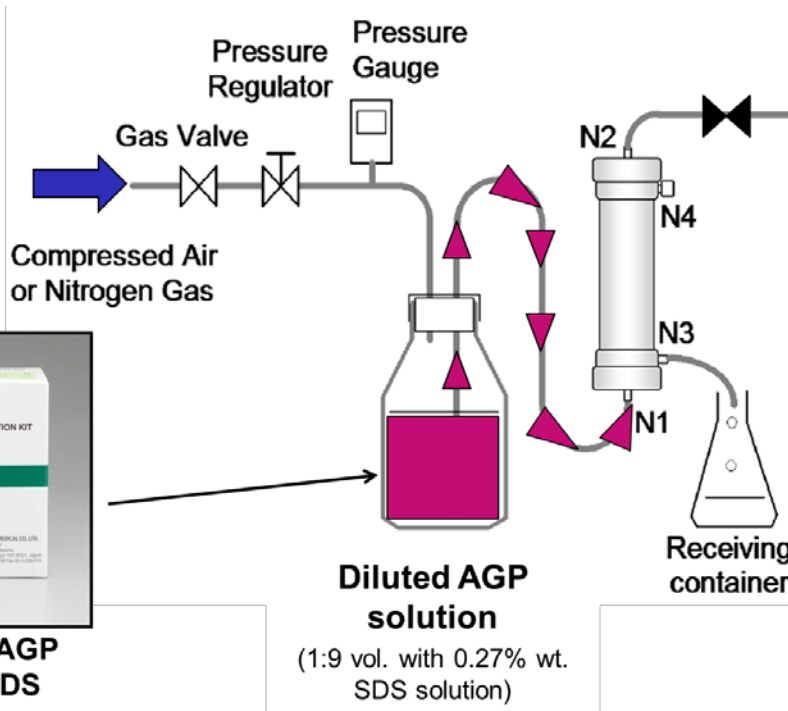


## 6 - Post-use IT: Gold Particle Test

- **Objective:** to validate no change in the pore size distribution.
- **Principle:** filtration of Gold Particles (AGP) solution (pink color) simulating a target virus.
- **Measurement:** difference of absorbance between the AGP feed solution and the filtrate.
- **Result:** if the AGP removal rate is in the specification range, the filter has passed successfully GPT.

**Protein washing + AGP Filtration: < 30 min**

## 6 - Post-use IT: Gold Particle Test



$$\Phi_i = \log_{10} \frac{A_{max}}{(A - A_{pvp} - A_{wm})}$$

- A<sub>max</sub>**: absorbance of diluted AGP (feed)
- A**: absorbance of the filtrate
- A<sub>pvp</sub>**: absorbance of PVP contained in the AGP solution (value given in AGP's COA)
- A<sub>wm</sub>**: mean absorbance of water

## 6 - Post-use IT: Gold Particle Test



Automatic AGP Test

# Questions ?

