New Challenges in Virus Filtration: Continuous Manufacturing, Use of Virus Filter as an Upstream Barrier

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Overview

1. Asahi Kasei Bioprocess Business Units
2. Continuous Virus Filtration
3. Virus Filtration as an Upstream Barrier

1

Asahi Kasei Bioprocess Business Units

Asahi Kasei Bioprocess Portfolio

Asahi Kasei Bioprocess

PURIFICATION

PLANOVA™

Virus removal filters

BioOptimal™ MF-SL

Microfilters

Assurance Beyond Expectation

FLUID MANAGEMENT

Oligonucleotide Synthesis

Inline Buffer Formulation

Built for You™

BIOSAFETY TESTING SERVICES

VIRUSURE
Quality is no coincidence

bionique Testing Laboratories

Quality is no Coincidence

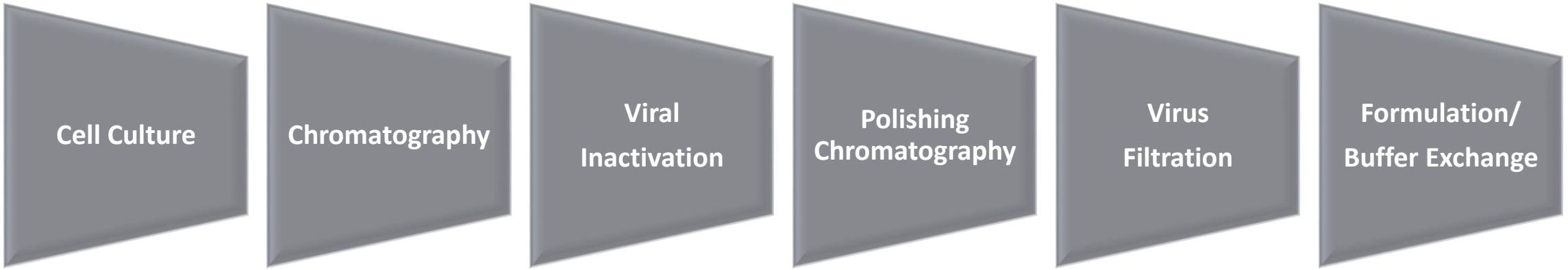
CONTRACT DEVELOPMENT and MANUFACTURING SERVICES

Bionova Scientific

2

Continuous Virus Filtration: Considerations for Implementation and Validation

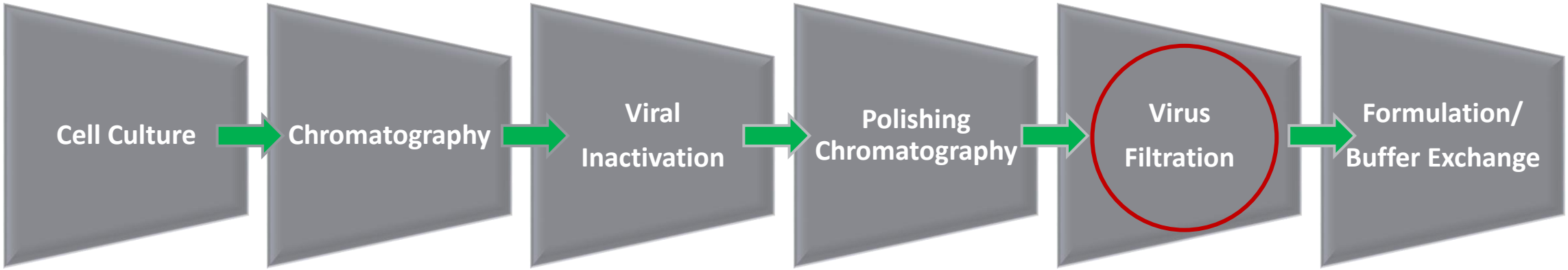
Batch vs. Continuous Bioprocessing



Batch mode:

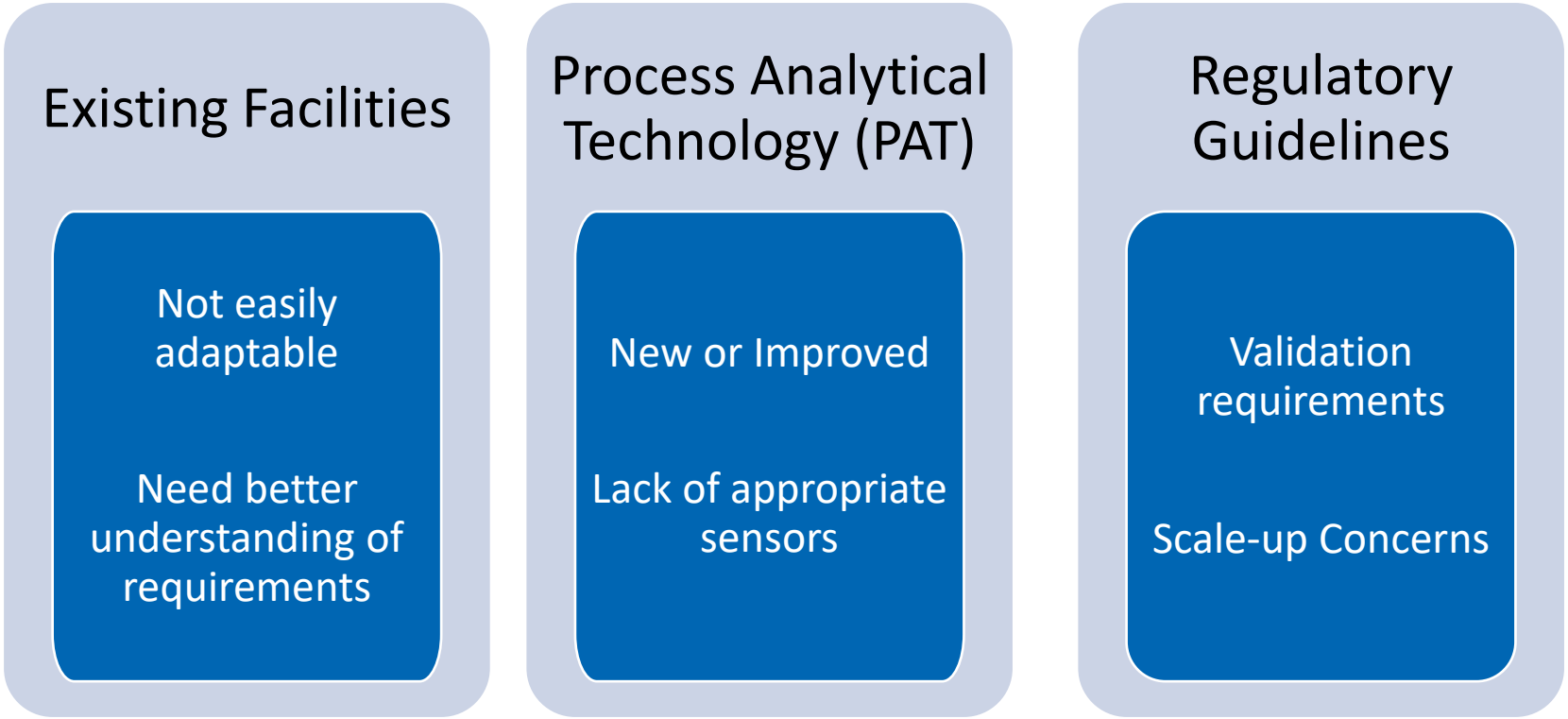
- 6- or 7-unit operations, requiring different manufacturing lines and teams
- When an issue occurs, easy to track back the problem
- Time consuming: one batch ends, another begins
- Prone to human error
- Costs associated with inefficiencies, losses and contaminations

The Continuous Bioprocessing Promise



- Reduces or eliminates down time
- High-quality drug substance
- Flexible manufacturing allowing for faster production and reduction in drug shortages: higher efficiency
- Limited laboratory testing, standardized quality control with the help of PATs
- Reduced energy needs and waste

Challenges For Continuous Processing



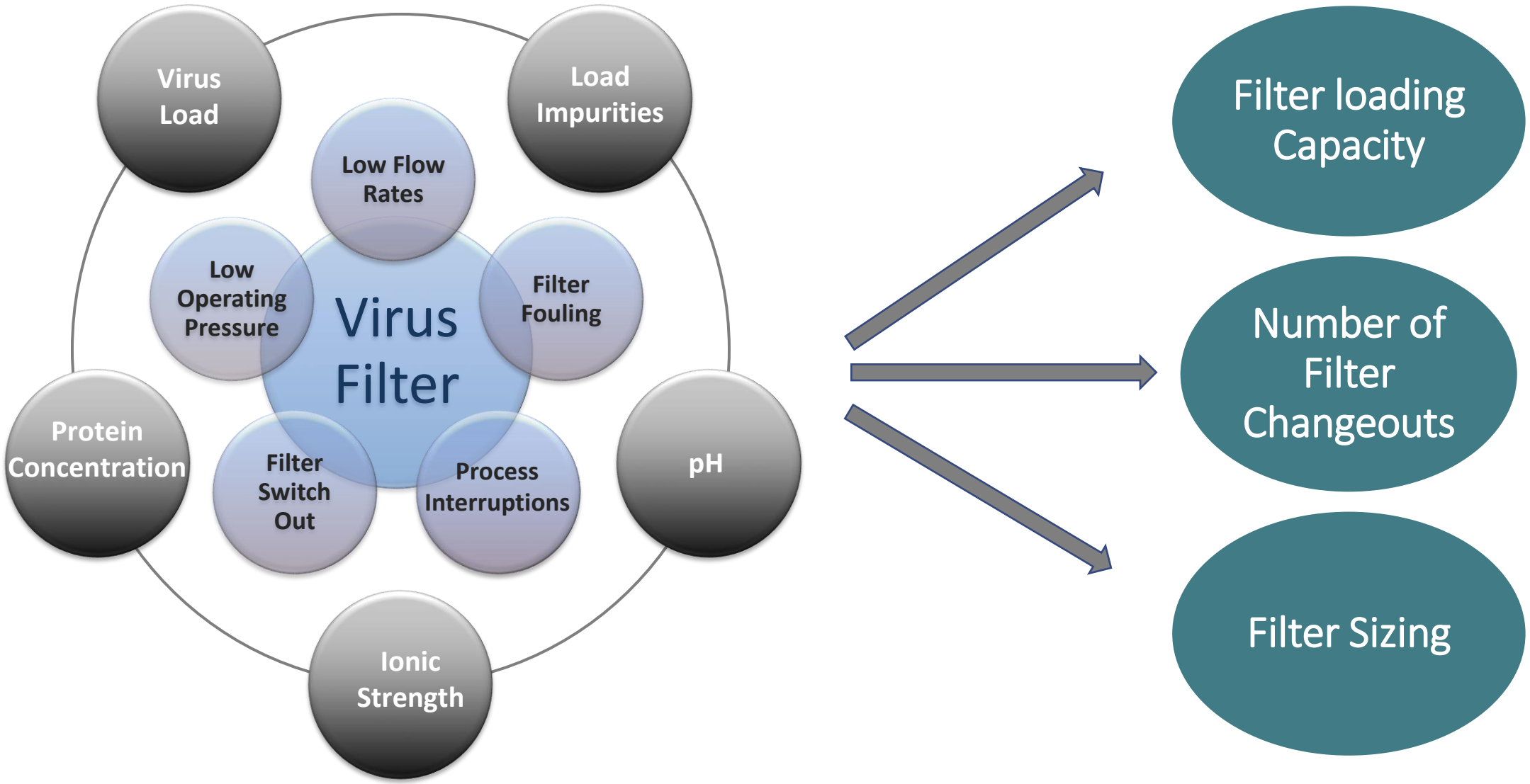
Virus Filter Sizing Considerations for Continuous Processing

- Planova 20N and BioEX virus filters lines include 4.0 m^2, 1.0 m^2, 0.1 m^2, 0,01 m^2 and 0.001 m^2. Planova BioEX virus filters are also available in 0.0003 m^2.
- Potential choice of filter switch out or oversizing and smaller numbers of filters used.

Planova 20N

Planova BioEX

Understanding The Design Space of a Virus Filter



How do virus filters work under continuous processing conditions?

How does continuous virus filtration impact viral clearance?

Feasibility of Long-Term Virus Filtrations

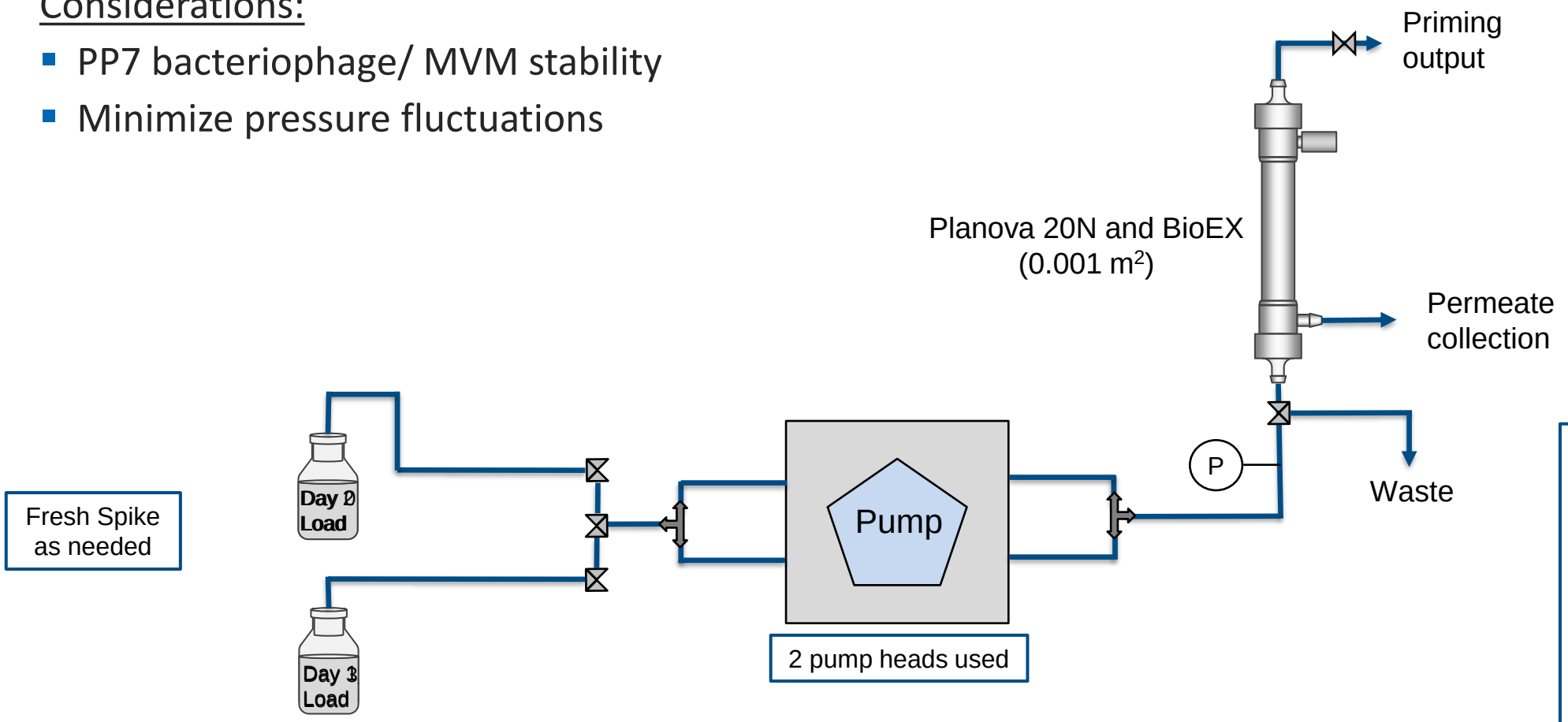
- Target: 4-5 days
- Low flow rates
- Low starting pressure
- High Loadings (L/m^2)

Continuous Virus Filtration – Extended Processing Setup

Asahi KASEI BIOPROCESS

Considerations:

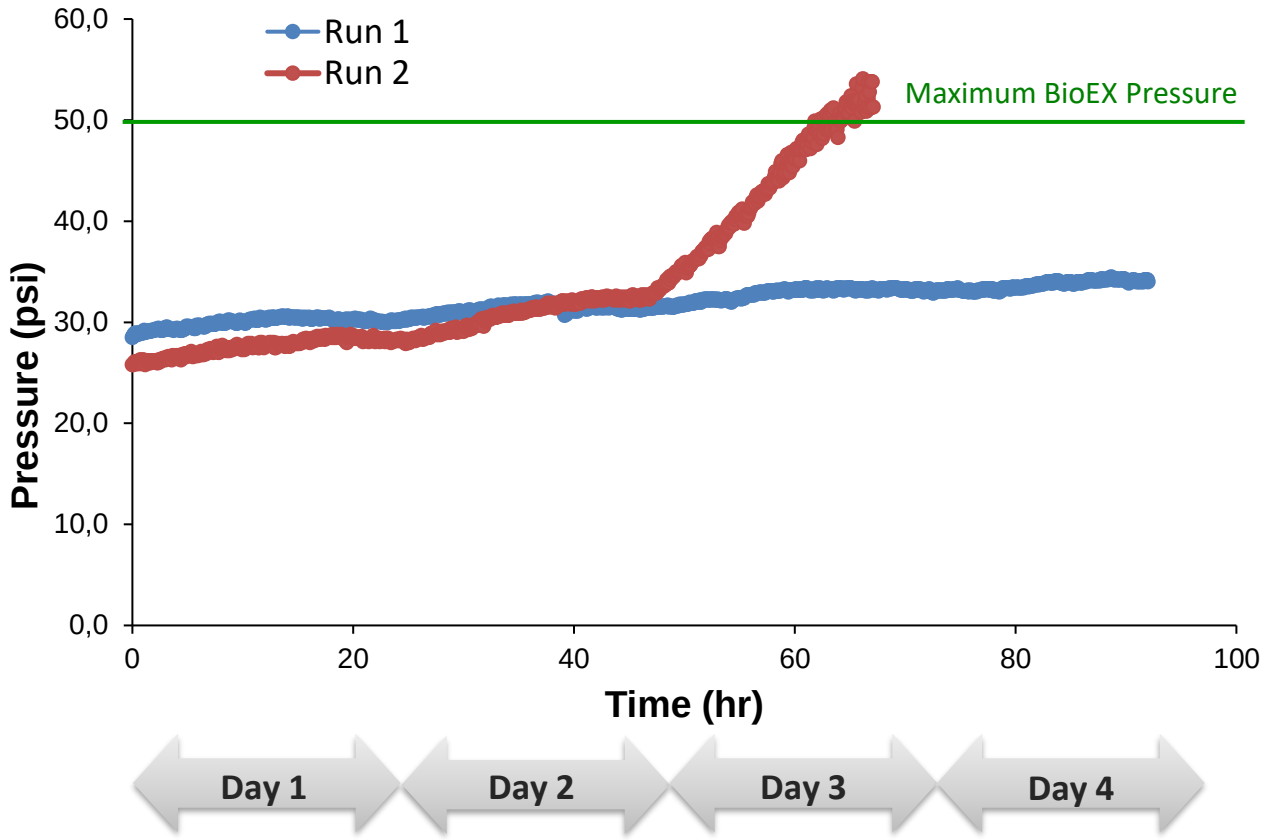
- PP7 bacteriophage/ MVM stability
- Minimize pressure fluctuations



Sample Collection:

- Day 1 Filtrate
- Day 2 Filtrate
- Day 3 Filtrate
- Filtrate Pool

Extended Processing Model – Planova BIOEX



Conditions:

- 0.025 g/L Human Gamma Globulin (HGG)
- 50 mM Acetate, 20 mM NaCl, pH 6.0
- Flow Rate: 1.2 mL/min
- Target Spike: 10^6 PFU/mL
- Flux = 72 LMH
- Throughput = 6,900 L/m^2

Lute et.al, Biotechnology Progress, January 2020

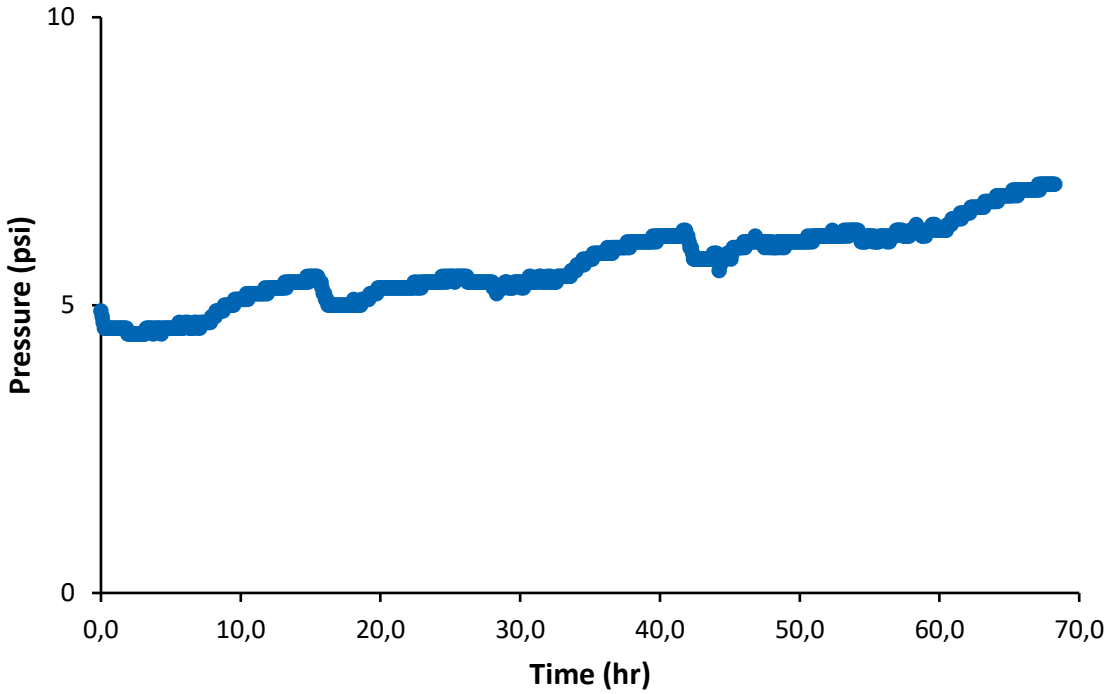
Extended Processing Model – Viral Clearance

Sample	Log Titer (PFU/mL)	
	BioEX – Run 1	BioEX – Run 2
Load Range	5.9- 6.0	4.2- 4.3
Day 1 Filtrate	≤ 0.78	≤ 0.78
Day 2 Filtrate	≤ 0.78	≤ 0.78
Day 3 Filtrate	≤ 0.78	≤ 0.78
Day 4 Filtrate	≤ 0.78	≤ 0.78
Filtrate Pool	≤ - 0.22	≤ - 0.22
LRV	≥ 6.1	≥ 4.5

Long-term continuous virus filtrations can achieve acceptable virus removal

Lute et.al, Biotechnology Progress, January 2020

Case Study – Low Pressure Challenge on Planova BioEX using MVM



Sample	(log $TCID_{50}$/mL)
Load	5.9
Filtrate Fraction 1	≤ 0.5
Filtrate Fraction 2	0.8
Filtrate Fraction 3	≤ 0.5
Filtrate Pool	0.6
LRV	**5.3**

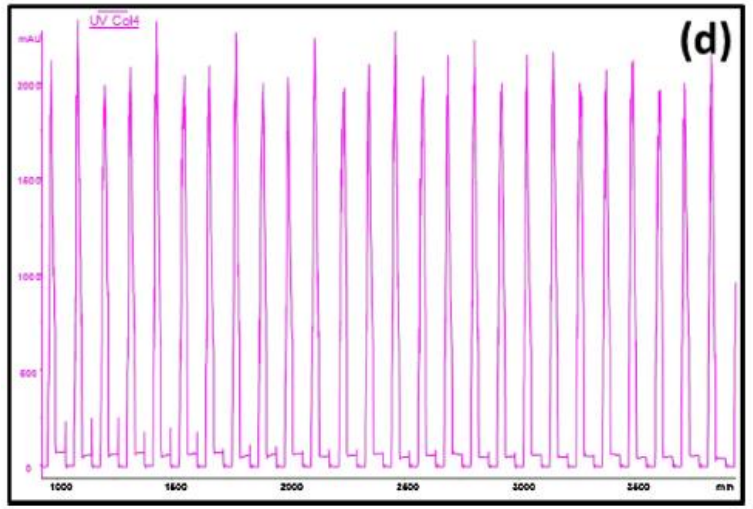
- Flux: 7.2 LMH (0.12 mL/min)
- Throughput: 500 L/m^2 (3 days)
- Target MVM spike: 10^6 log $TCID_{50}$/mL

High LRV with low flow/pressure filtration on BioEX

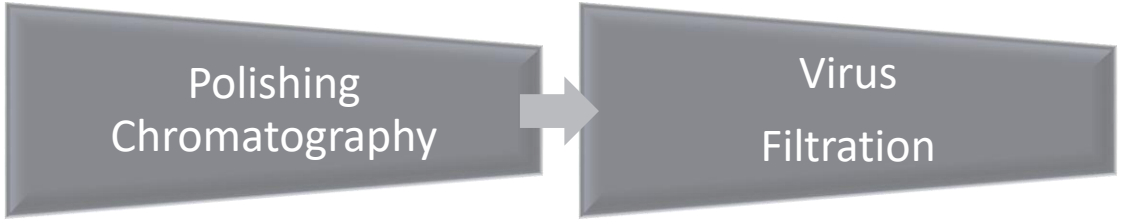
William Rayfield, adapted from 2020 Planova Virtual Symposium

Understanding the Effects of Load Variations on Virus Filter Performance

- Batch size: may be defined by the capacity of the filter used
- Filter capacity: largely dependent upon load
- Virus filter (VF) load variations:
  - **Load concentration**
  - **Salt**
  - **Virus spike**
  - **pH**
  - **Impurities**



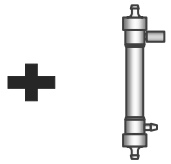
Godawat, et. al, J. Biot., 2015



Dynamic Load Model

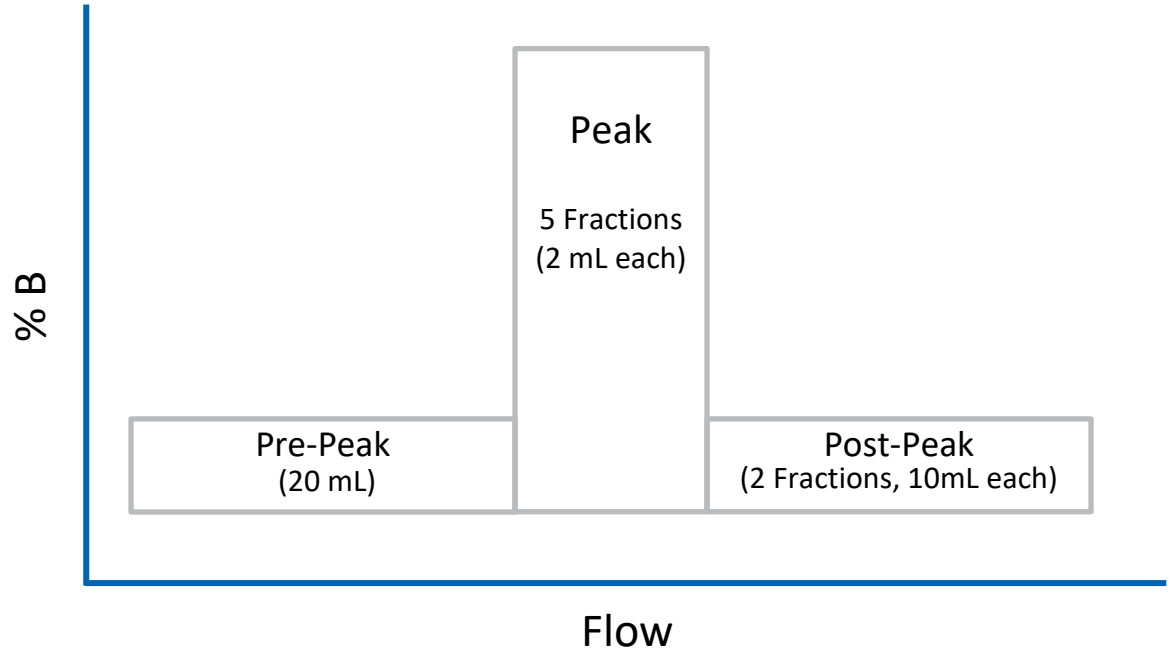
AKTA
(GE Healthcare)

Planova 20N or BioEX
(Asahi Kasei Bioprocess)



Simulated Peaks of

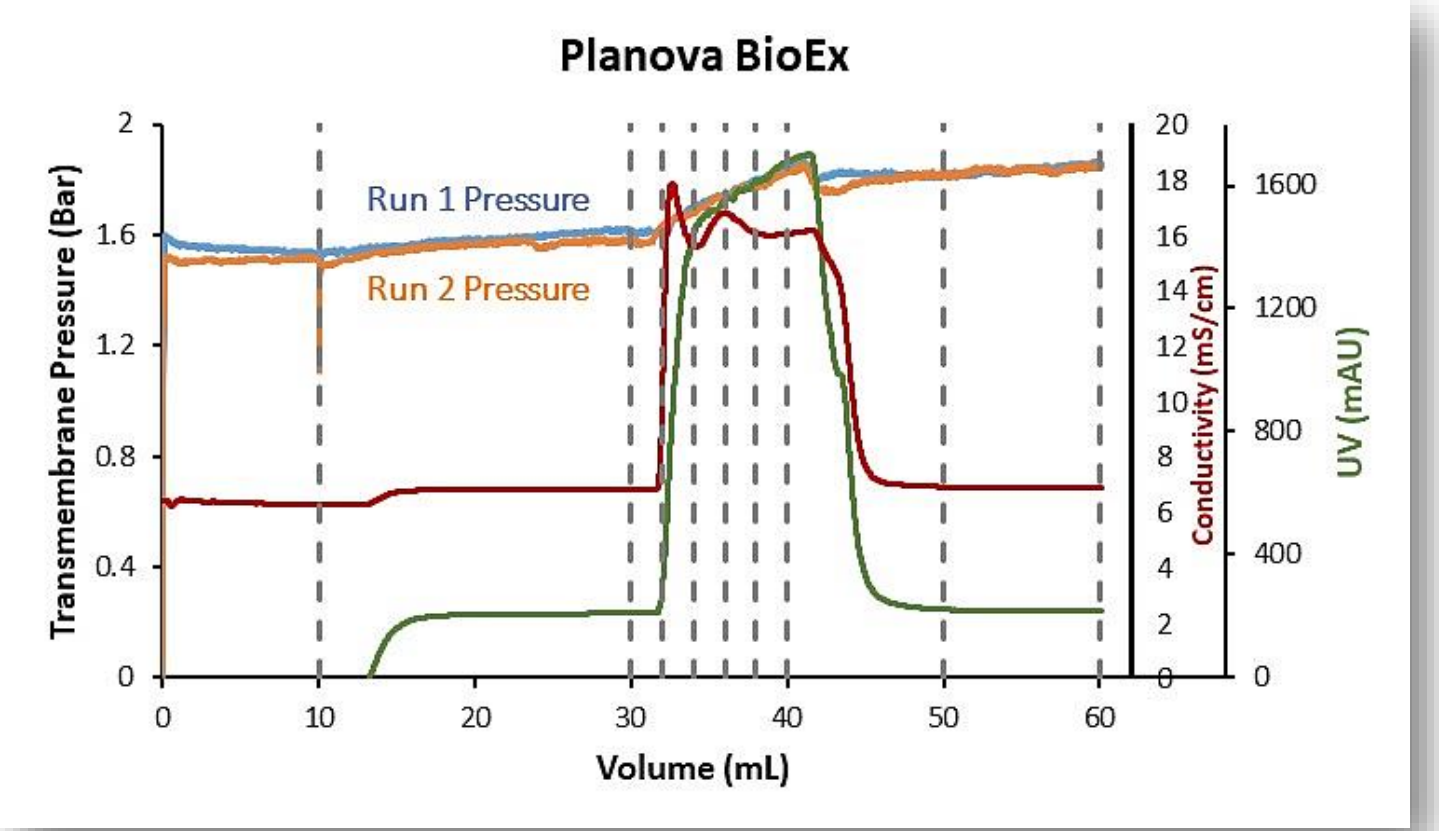
- High Protein
- High Salt
- Virus Spike
- Triple Spike



Load A: Baseline Conditions

Load B: Same as A with variable(s)

Effect of Protein, Salt and Virus Spike on Planova BioEX



Sample	Run 1	Run 2
Load (log PFU/mL)		
Load A	7.0	7.0
Load B	7.9	7.9
LRV		
Pre-Peak	> 7.0	> 7.0
Peak Fraction 1	> 6.9	> 6.9
Peak Fraction 2	> 6.9	> 6.9
Peak Fraction 3	> 6.9	> 6.9
Peak Fraction 4	> 6.9	> 6.9
Peak Fraction 5	> 6.9	> 6.9
Post-Peak Fraction 1	> 7.0	> 7.0
Post-Peak Fraction 2	> 7.0	> 7.0
Total PP7 Log PFU	9.0	

Effective Virus Clearance is achieved when filters are run under recommended conditions

Lute et.al, Biotechnology Progress, January 2020

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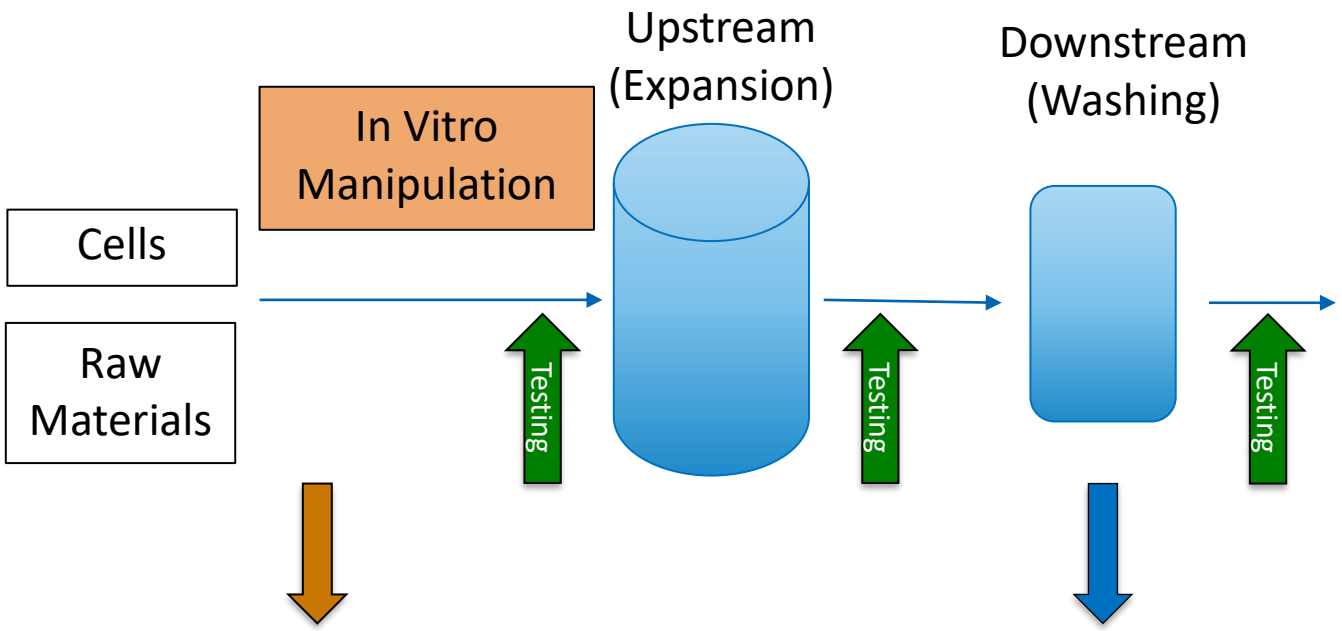
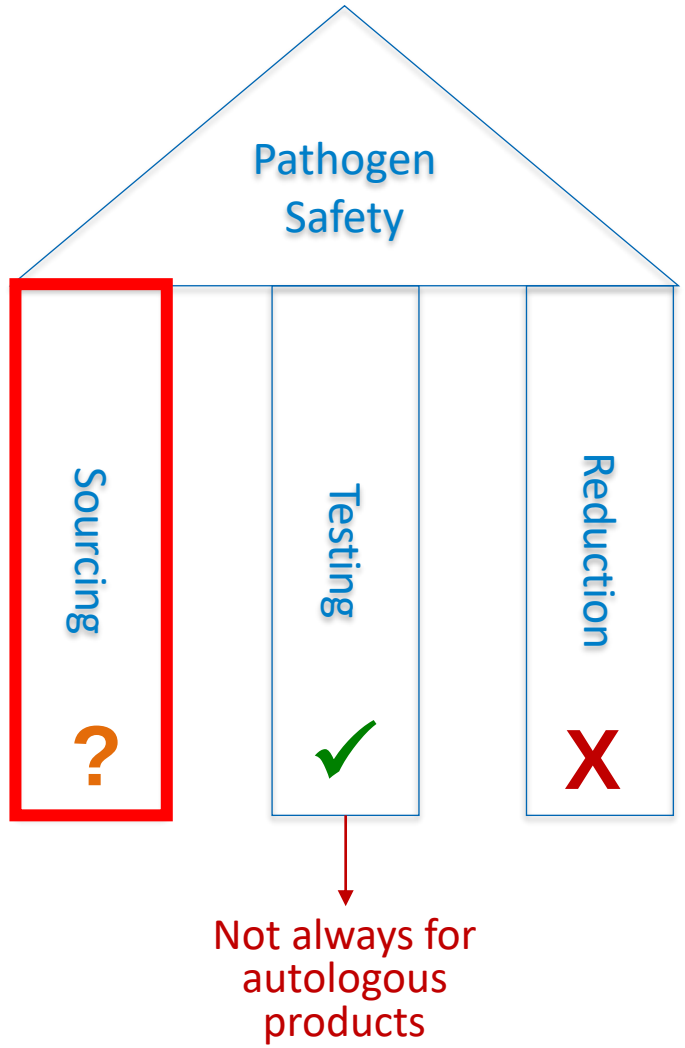
Virus Filtration as an Upstream Barrier

Pathogen Safety – Cell Therapy Products



- Not well-defined
- Animal-derived
- Little experience

- Little or no viral clearance capability
- Virus can propagate



Virus Filtration for CGTs

- CGT processes can have high risk and minimal virus removal capability
- Virus filtration is highly effective and robust at removing viral contaminants
- BUT: Some CGTs are too big to pass through virus filters
- How can Virus Filtration be used to improve pathogen safety of other CGTs?
  - ✓Downstream processing for select gene therapy products
  - ✓Upstream barrier

Contamination Events on Upstream

Many contamination events are believed to come from raw materials.

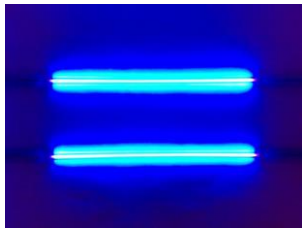
Sources of Virus Contaminations in Raw Materials

Virus Contamination	Virus Family	Enveloped	Size (nm)	Source
Cache Valley Fever Virus	Bunyaviridae	Yes	80-100	Fetal Bovine Serum
Blue Tongue Virus	Reoviridae	No	65-75	Fetal Bovine Serum
Blue Tongue Virus	Reoviridae	Pseudo-enveloped	40	Possible insect transmission in testing lab
Bovine Viral Diarrhea Virus	Flaviviridae	Yes	40-70	Fetal Bovine Serum
Vesivirus 2117	Caliciviridae	No	35-40	Unknown
Equine Rhinitus A Virus	Picornaviridae	No	25-30	Equine Serum
Minute Virus of Mice	Parvoviridae	No	18-24	Non-Animal Raw Material
Circoviridae	Circovirus Type I	No	17	Porcine Trypsin

Barbara Potts, Amer. Pharma. Rev., 2011 (excerpted)

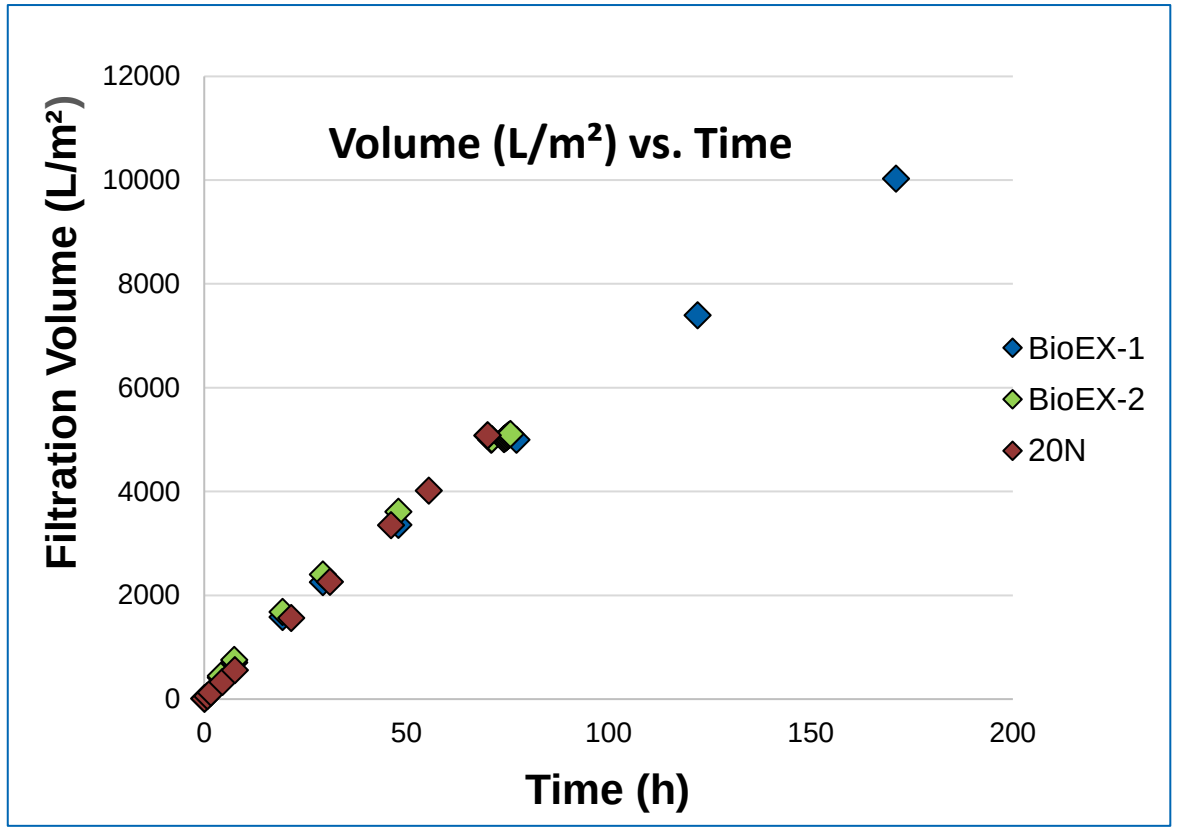
Other potential exposure to contaminants:

Upstream barriers



Irradiation	**HTST (High-Temperature Short Time)**	**UVC**	**Virus Filtration**
Pros: • Highly effective • Cost	Pros: • Cost (large scale)	Pros: • Point-of-use	Pros: • Highly effective • Scalability • Ease of use • Much experience
Cons: • Not point-of-use • Material impact	Cons: • High capital costs • Large footprint • Material impact	Cons: • Scalability • Virus-dependent • Material impact	Cons: • Cost • Requires filterability

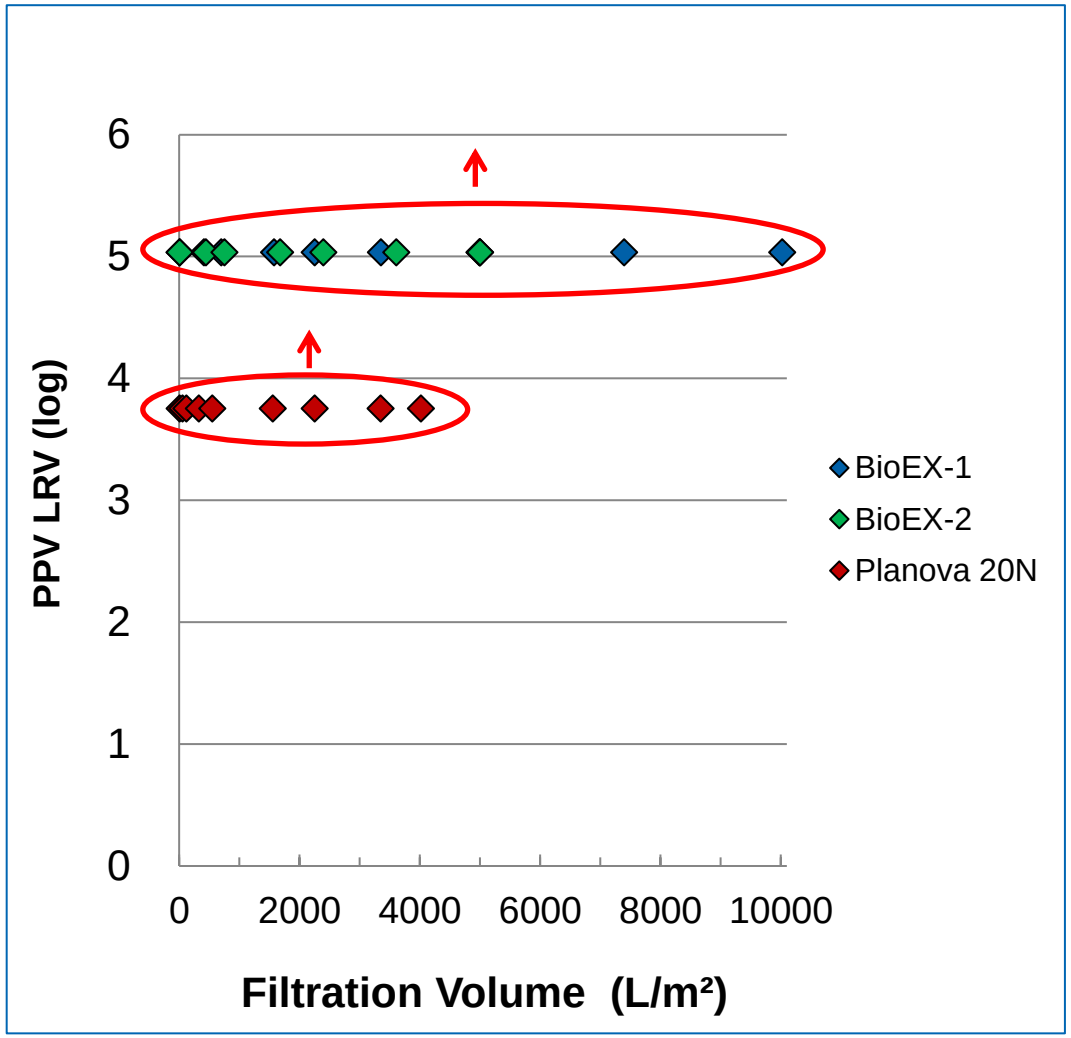
CD-CHO Media Filtration



- ✓ No impact of the virus spike on Filtration Volume
- ✓ **Consistent** performance
- ✓ **20N:**
  2 000 L/m² in 1 day
  5 000 L/m² in 3 days
- ✓ **BioEX:**
  same as 20N
  + 10 000 L/m² in 7 days

Konstantin Agolli, Asahi Kasei, BioInnovation 2016, Berlin, February 10th, 2016

CD-CHO Media Filtration

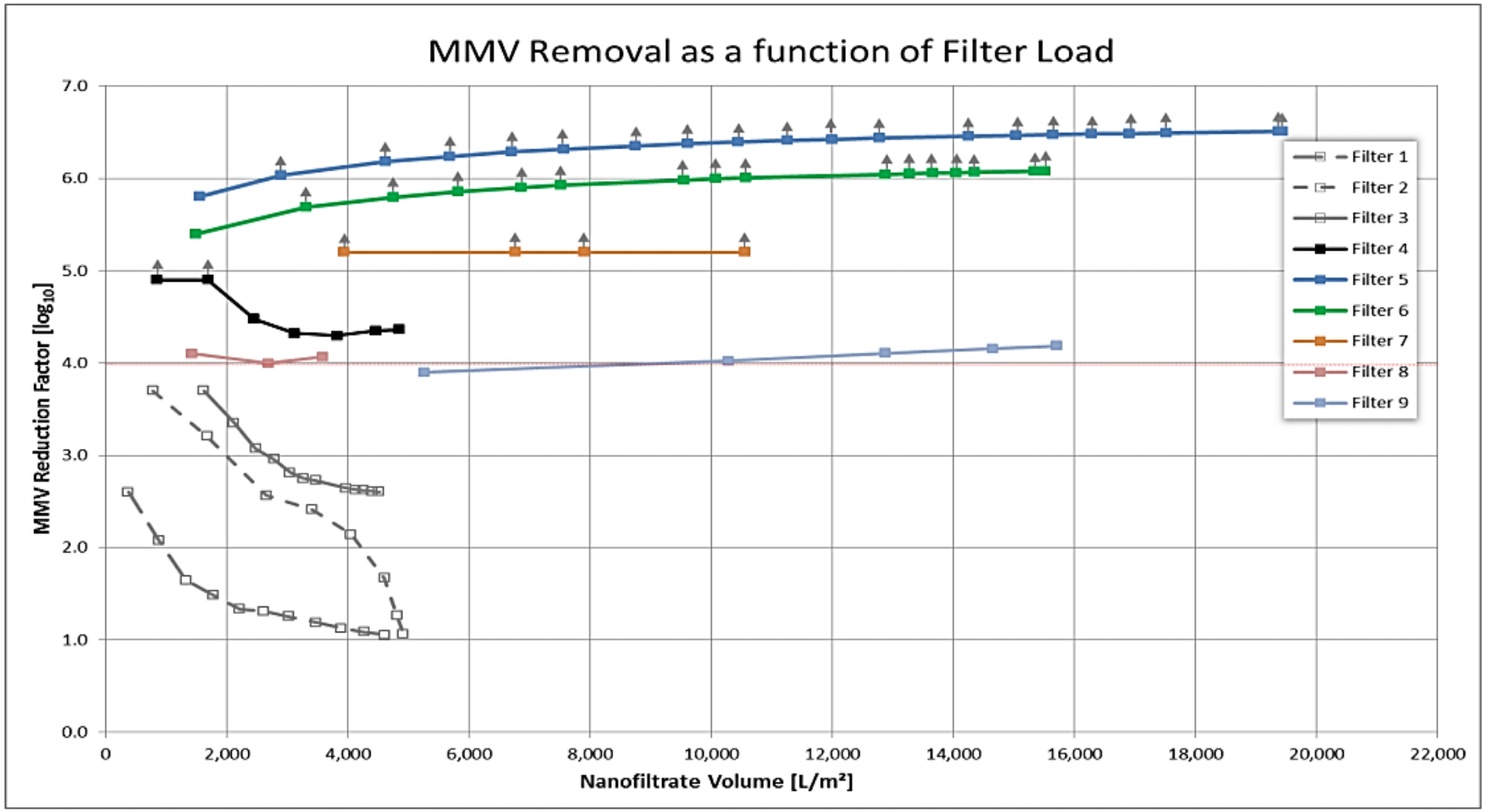


- ✓ **No virus detected (↑)**
- ✓ Difference in PPV LRV is due to differences in assay sensitivity

Konstantin Agolli, Asahi Kasei, BioInnovation 2016, Berlin, February 10th, 2016

Case Study - Takeda

Virus filtration can be effective for large volume media treatment



Andreas Wieser, Shire, 20th Planova Workshop, Prague, 2017

Filtration Costs

How expensive is upstream virus filtration?

Assumptions:

60 LMH

~ 7 000 € /m²

Media Volume (L)	Duration (hr)	Filtration Area (m²)	Cost/batch (€)	
12 000	4	50	350 000	
12 000	24	8,3	58 333	Longer virus filtration
200	3	1,1	7 800	Smaller volumes for CGTs
10	2	0,8	2 250 *	Especially for autologous cell therapy

For smaller volumes, media filtration can be very feasible!

* Price €/m² higher for small size filters

Treatment Of Media Components

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Virus Filtration of Bacterial Fermentation Media Components

Nr.	Media type	Concentration [g/L]	Volume per 4000 L scale [L]	PN20			BioEX		
				Flow	Average flux [L/h/m2]	Area for 4000L scale [m2]	Flow	Average flux [L/h/m2]	Area for 4000L scale [m2]
1	Glucose Feed	>100	>200	decrease	<10	>50	constant	10-100	>10
2	Vitamin solution	<50	<20	constant	10-100	<0.1	constant	>100	<0.1
3	Salt solution	>100	20-200	constant	10-100	0.1-0.5	constant	10-100	<0.1
4	Amino acid stock	<50	20-200	constant	10-100	<0.1	constant	10-100	<0.1
5	Tetracycline - alcohol	<50	<20	decrease	10-100	<0.1	blocked	n.a.	n.a.
6	Tetracycline - water	<50	<20	constant	10-100	<0.1	decrease	>100	<0.1
7	IAA solution	<50	20-200	constant	10-100	0.1-0.5	blocked	n.a.	n.a.
8	Tace elements solution	>100	<20	constant	10-100	<0.1	decrease	>100	<0.1
9	Kanamycine Solution	50-100	<20	constant	10-100	<0.1	constant	>100	<0.1
10	Fe-sulfate-stock	50-100	<20	constant	10-100	<0.1	constant	>100	<0.1
11	Inducer	50-100	<20	constant	10-100	0.1-0.5	constant	10-100	<0.1
12	Media solution	<50	20-200	constant	10-100	0.1-0.5	constant	>100	<0.1
13	Sterile addition	>100	>200	decrease	10-100	>0.5	constant	>100	>0.5
14	Fe-chloride stock	>100	<20	constant	10-100	<0.1	constant	>100	<0.1

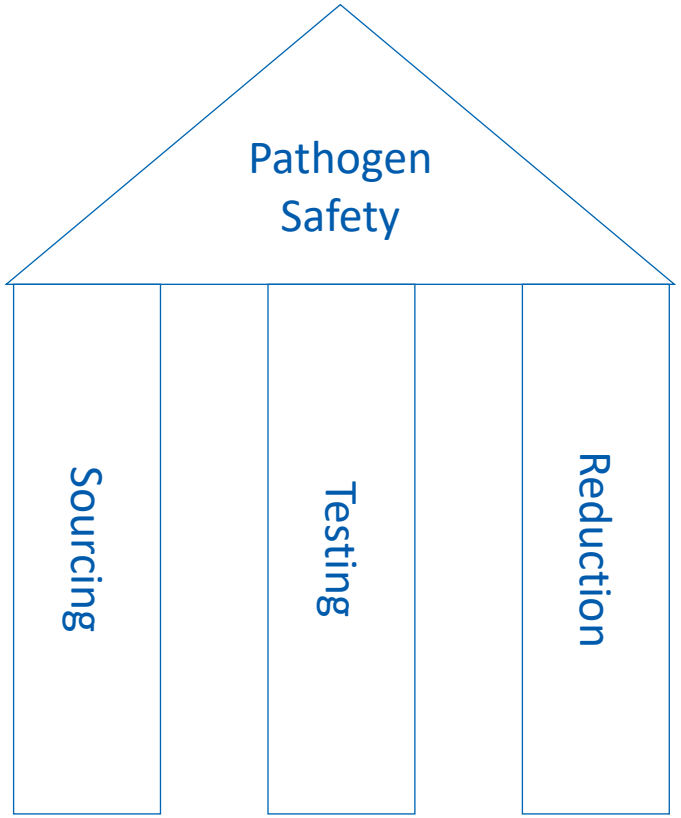
Simon Haidinger, Boehringer Ingelheim, 18th Planova Workshop, Athens, 2015

Summary - 1

- Virus filtration is applicable/adaptable to continuous processes
- Virus filters are robust enough to withstand process challenges
- Several validation options are available
- Virus filters are scalable, it’s a matter of understanding the design space

Summary - 2

- Risks for viral contamination are large
  - ✓ Higher risk raw materials
  - ✓ Reduced viral clearance capability
  - ✓ Less overall manufacturing experience
- Virus filtration is the most effective and robust virus removal option
- Incorporating virus filtration into manufacturing processes early in development will significantly advance patient safety of CGT products



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감사합니다 Gracias Danke

Благодаря 谢谢 Tack धन्यवाद Dziękuję

Спасибо Thank You Obrigado

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ありがとうございました Teşekkür ederim