Adapting Virus Filtration Operation to Continuous Processing Challenges

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Overview

1. The Pioneering Difference
2. Virus Filtration in Continuous Mode
3. Validation Strategies and Examples

1

The Pioneering Difference

Asahi Kasei Bioprocess Portfolio

Asahi Kasei Bioprocess

PURIFICATION

PLANOVA™

Virus Removal Filters

Assurance Beyond Expectation

BioOptimal™ MF-SL

Microfilters

FLUID MANAGEMENT

Oligonucleotide Synthesis

MOTIV™ Inline Buffer Formulation

Chromatography

Virus Filtration

Built For You™

BIOSAFETY TESTING SERVICES

VIRUSURE

Virus/Prion Clearance Studies

bionique

Mycoplasma Testing Services

Quality is no coincidence

BIOLOGICS DEVELOPMENT & MANUFACTURING

Bionova Scientific

End-to-end Process Development

GMP Manufacturing

Where concept becomes cure

2

Virus Filtration in Continuous Mode

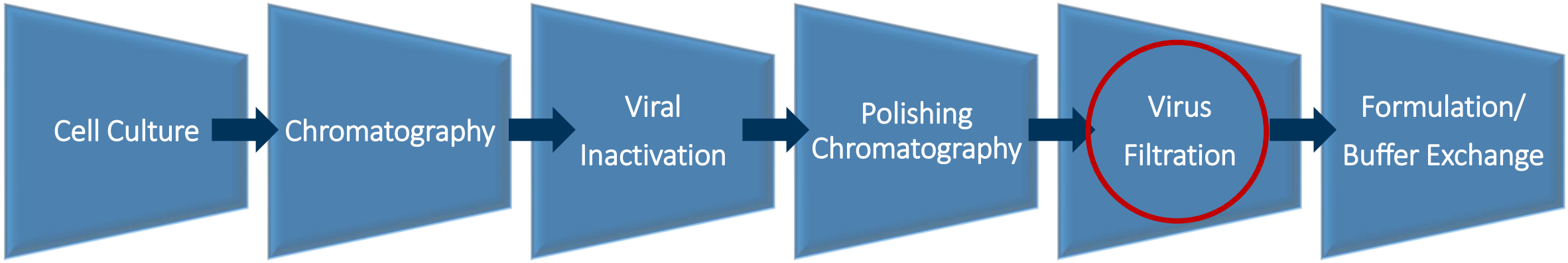
Virus Filtration

Batch mode

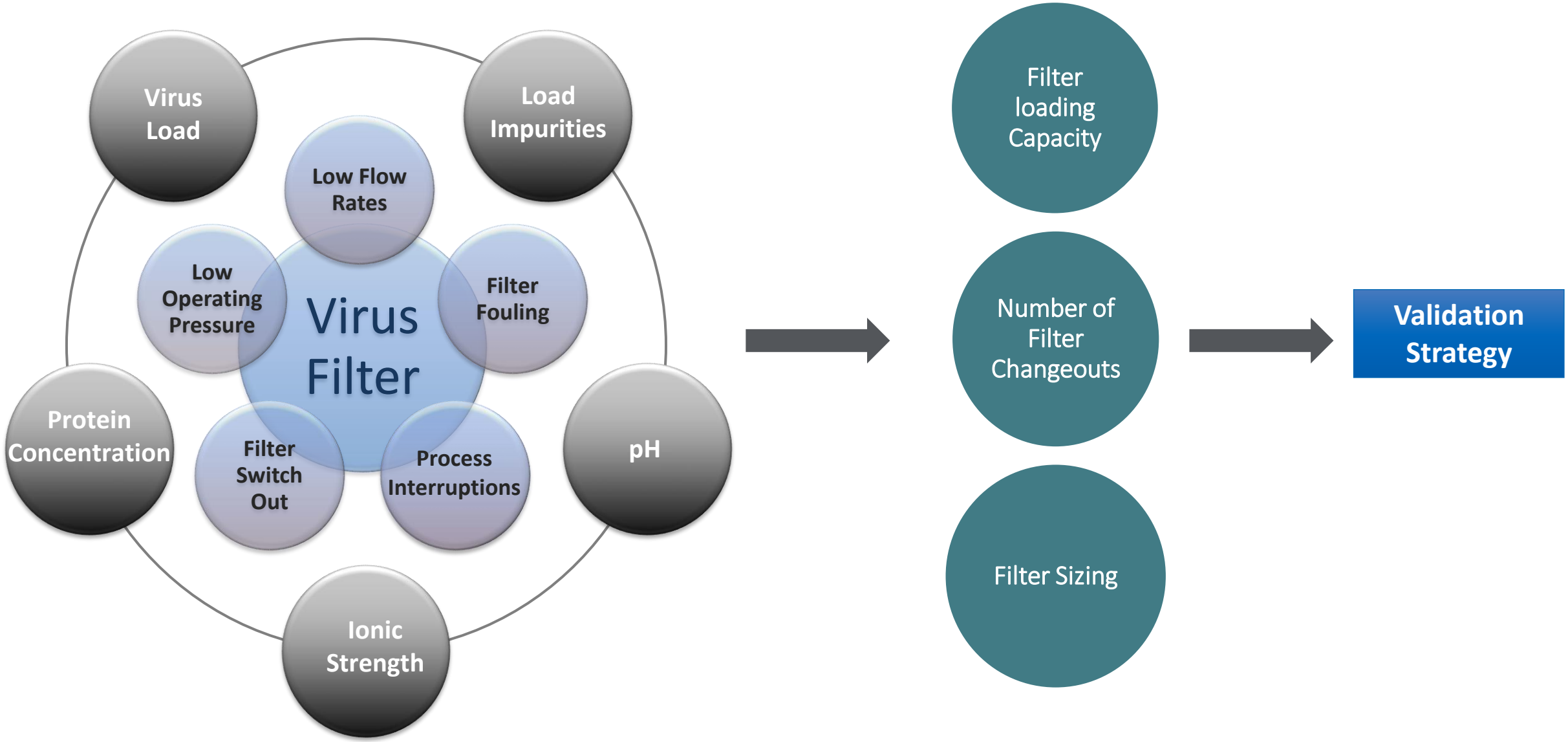
- High flux
- Constant pressure operation
- Throughout: 100-500 L/m^2
- Duration: up to 8 hours
- Homogeneous feedstock

Continuous mode

- Low flux
- Constant flow operation
- Throughput: ≥ 1000 L/m^2
- Duration: ≥ 3 days
- Homogeneous or variable feedstock



Understanding Virus Filtration in Continuous Mode



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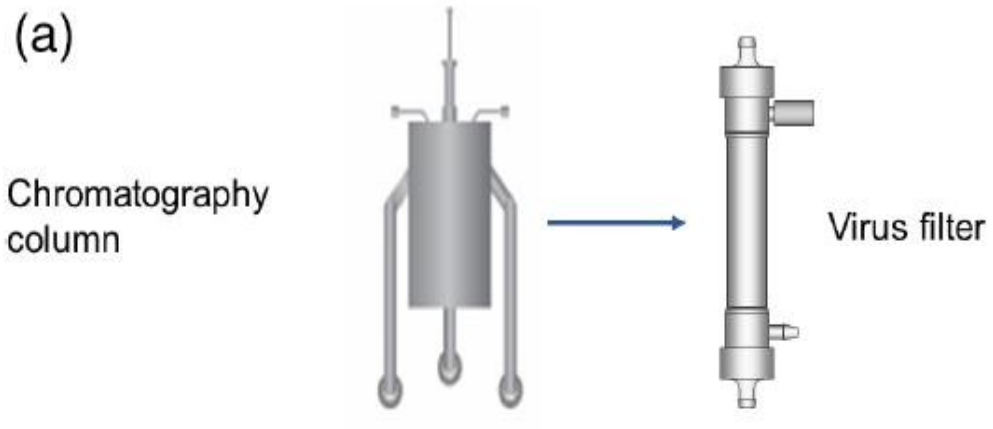
Validation Strategies and Examples

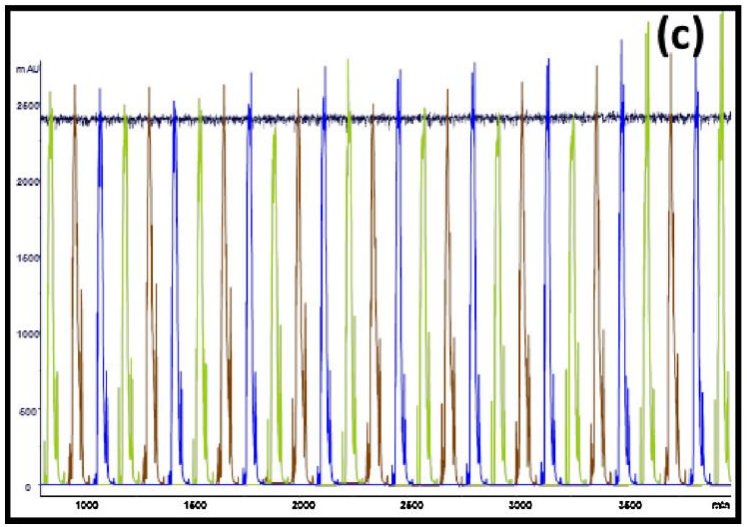
Validation Strategies



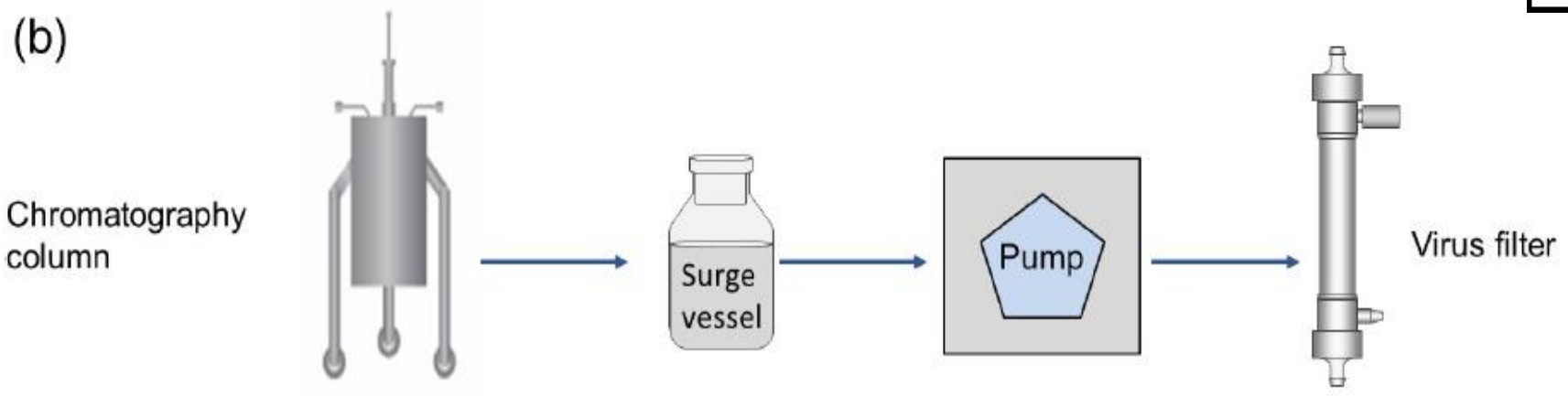
Without Surge Vessel:

- Heterogeneous feed
- Can achieve good viral clearance
- Significant challenges to implementation and validation

Output from a Continuous CEX Unit Operation



Godawat, *et al.*, 2015

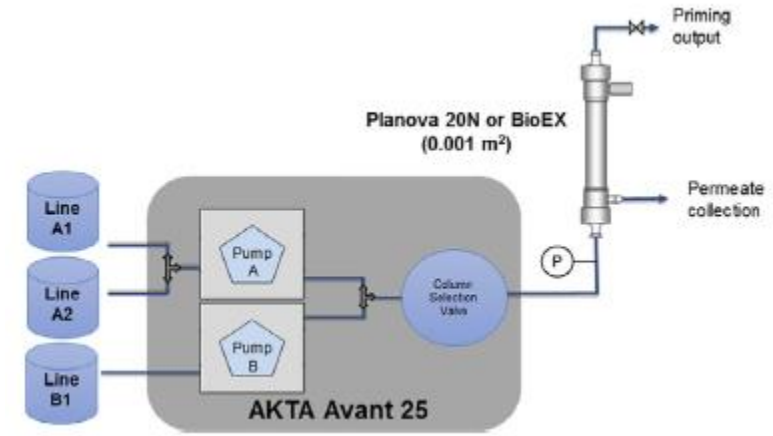


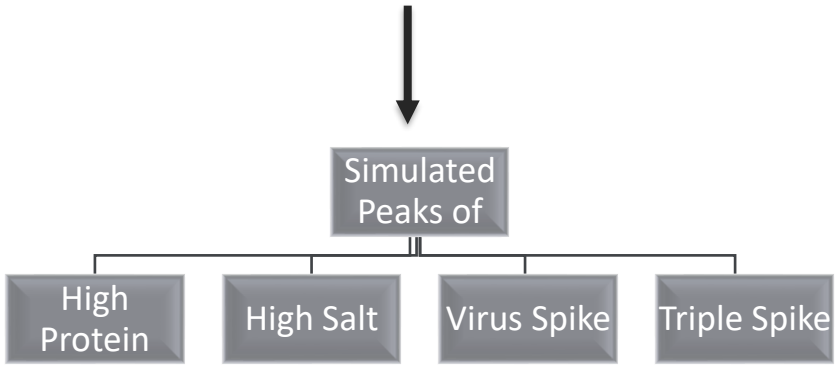
Advantages of Surge Vessel:

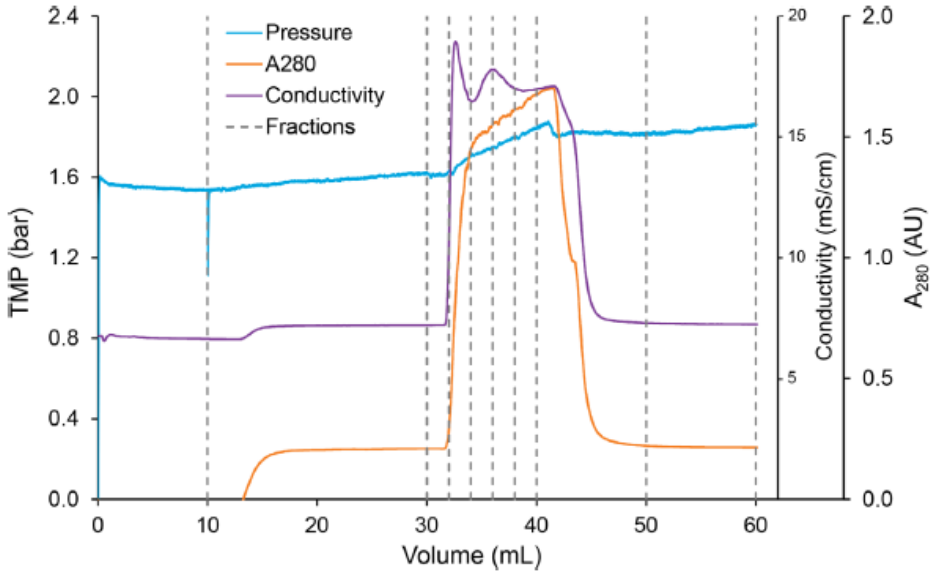
- Homogeneous feed
- Potential for batch validation
- Longer filter usage
- Mitigation for flow rate variations or deviations including process pauses

Kozaili et.al, Biotechnology Progress, December 2023

Effect of Load Variations on VF performance – Dynamic Load Model







Sample		Triple spike[a] Run 1	Run 2
Titer (log PFU/ml)	Load A	6.96	6.96
	Load B	7.85	7.85
LRV	Pre-spike	>6.96	>6.96
	Spike 1[b]	>6.85	>6.85
	Spike 2[b]	>6.85	>6.85
	Spike 3[b]	>6.85	>6.85
	Spike 4[b]	>6.85	>6.85
	Spike 5[b]	>6.85	>6.85
	Post spike 1	>6.96	>6.96
	Post spike 2	>6.96	>6.96
Total phage log PFU		9.03	

Load A:

- 1 mg/ml h-IgG, 50 mM acetate, 20 mM sodium chloride, pH 6.0.
- Target PP7 load: 10^7 PFU/ml.

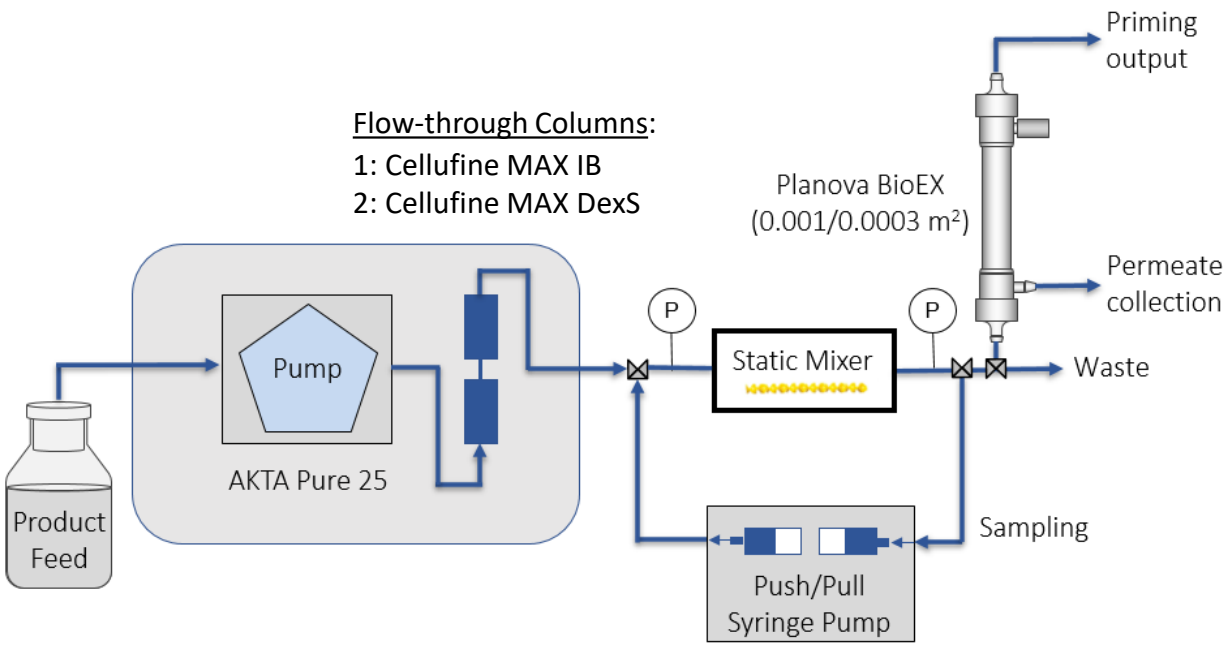
Load B (Triple spike):

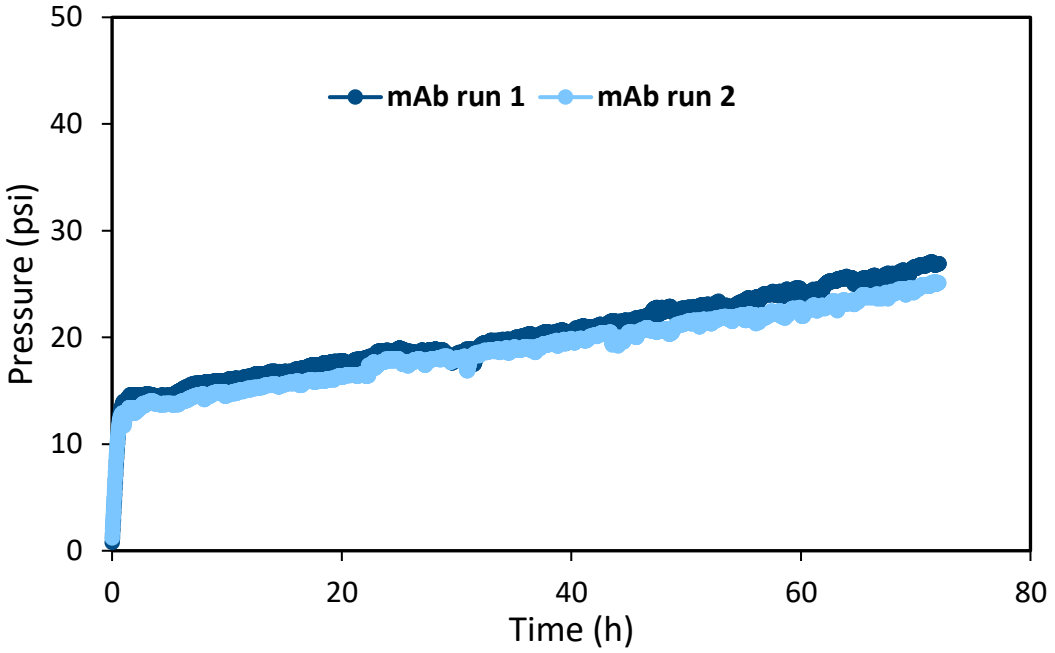
- 10 mg/ml h-IgG, 500 mM acetate, 20 mM sodium chloride, pH 6.0.
- Target PP7 load: 10^8 PFU/ml.

- ✓ Effective phage clearance is achieved with LRV ≥ 7.0.
- ✓ Challenges associated with inline spiking or extended spiking need further investigation.
- ✓ Is Parvovirus clearance comparable?

Lute et.al, Biotechnology Progress, January 2020

Inline Spiking Using Parvovirus





- Conditions:
  - Filter: 0.0003 m^2 BioEX
  - Protein: 2 g/L mAb
  - Buffer: 20mM Tris-Acetic Acid, 10 mS/cm, pH 6.5
  - PPV spike: 6 log TCID50/mL
  - AKTA pump flow rate: 0.17 mL/min (34 LMH)
  - Syringe pump push/pull flow rate: 3 µL/min
  - Filtration time: 72 h
  - Throughput: 2,400 L/m^2

mAb	Run 1	Run 2
Load titer (log $TCID_{50}$/mL)	6.25	6.38
LRV	≥ 5.5	≥ 5.7

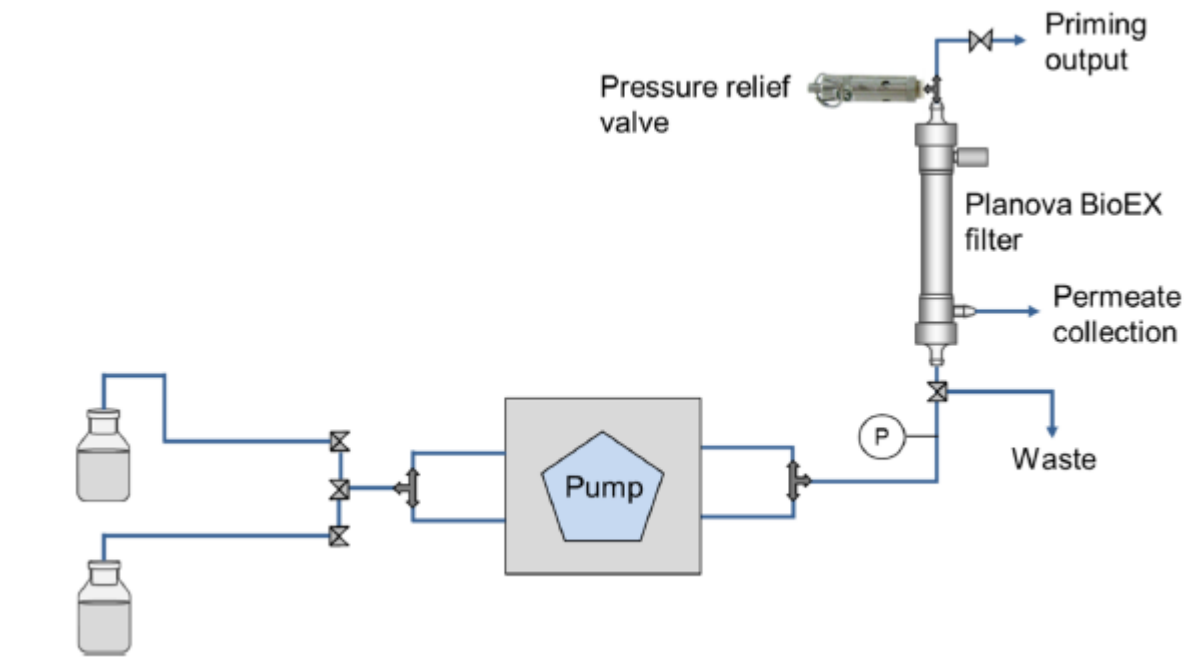
✓ Inline spiking is a validation option for stable viruses.
✓ More data needed for less stable viruses.

Virus Filters Can Withstand Continuous Processing Conditions

Study Goals: (collaboration with Merck)

- Perform virus-spiked filtrations for long durations, high throughput, and low flux
- Conditions:
  - Duration: ~ 6 days
  - Throughput: 1000 L/m2
  - Flux: 7 LMH
  - Filter: 0.001 m^2 Planova BioEX
  - Products: 4 mAbs
  - Virus spiking: ~6 log $TCID_{50}$/mL MVM

Molecule	Concentration (g/L)	pH	Conductivity (mS/cm)	Stability (days) RT	Stability (days) 4°C
mAb A	7.6	5.5	4	7	28
mAb B	10.6	4.9	15.8	7	28
mAb C	8.3	4.5	20	3	8
mAb D	6.3	5.4	22	7	14



Alternate between freshly spiked feed vessels as needed

Dual pump heads reduce pressure fluctuation

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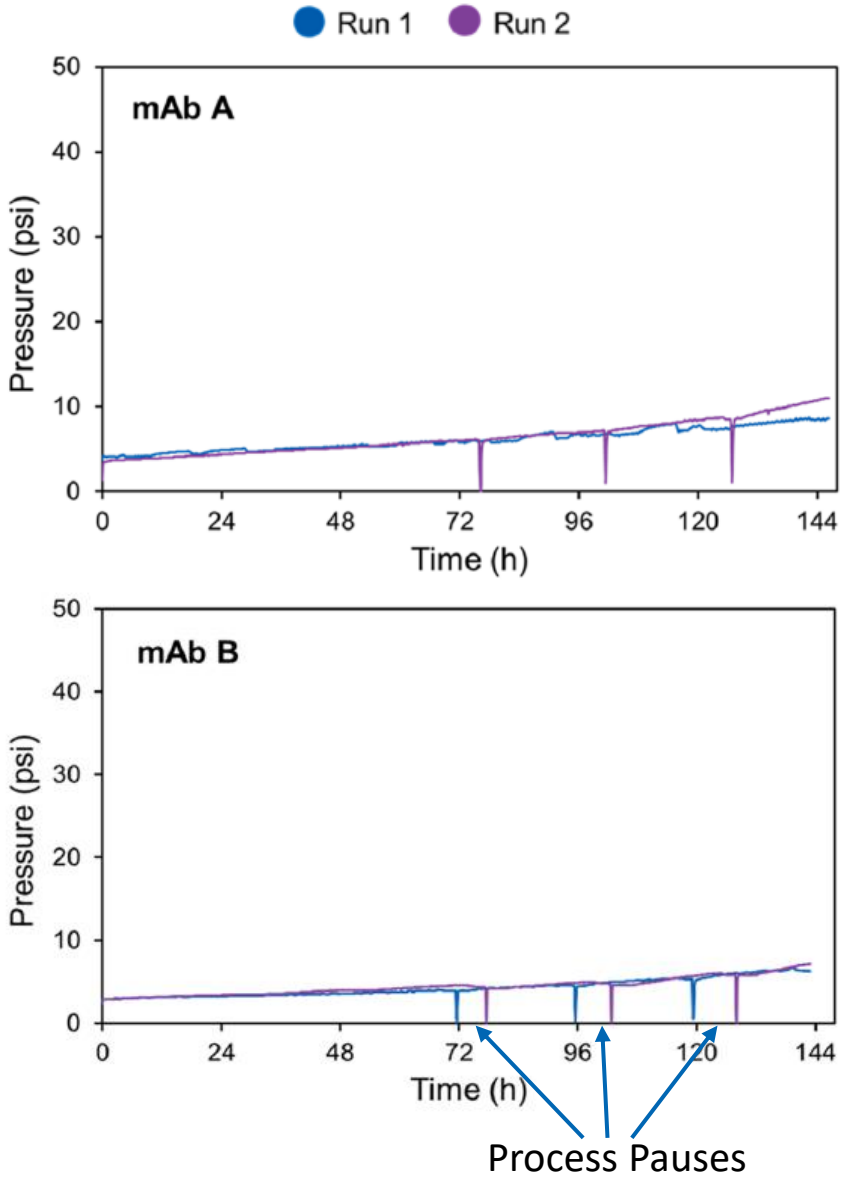
Continuous Virus Filtration of “Easy” mAbs



Molecule	mAb A		mAb B	
Run	1	2	1	2
Throughput (L/m^2)	1000		1000	
Run duration (days)	6		6	
Number of process pauses	None	3	3	3
MVM titer (log $TCID_{50}$/mL)				
Load	5.63	5.56	5.69	5.56
Day 1	0.63	0.75	≤0.50	≤0.50
Day 2				
Day 3				
Day 4	0.63	1.13	≤0.50	≤0.50
Day 5	0.63	0.88	≤0.50	≤0.50
Day 6	1.00	1.50	≤0.50	≤0.50
Pooled permeate	≤0.50	0.75	≤0.50	≤0.50
MVM LRV				
MVM LRV	≥5.1	4.8	≥5.3	≥5.1

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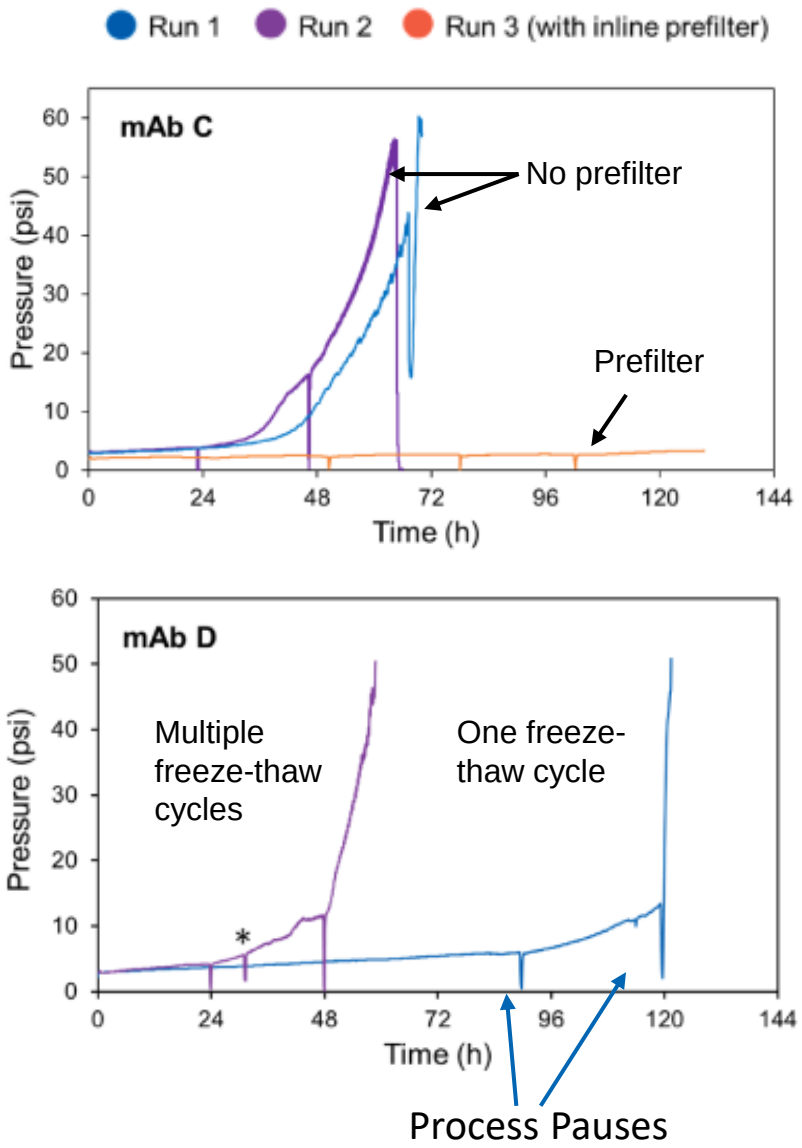
Continuous Virus Filtration of “Challenging” mAbs



Molecule	mAb C			mAb D	
Run	1	2	3	1	2
Throughput (L/m^2)	480	450	540	690	430
Run duration (days)	3	3	5	4	3
Number of process pauses	None	2	3	2	2
MVM titer (log $TCID_{50}$/mL)					
Load	5.75	5.79	5.53	5.81	6.04
Day 1	≤0.50	0.63	≤0.50	1.63	0.63
Day 2		1.38			1.25
Day 3		1.13	≤0.50		0.88
Day 4	-	-	≤0.50	1.50	-
Day 5	-	-	≤0.50	1.38	-
Day 6	-	-	-	-	-
Pooled permeate	-	0.88	0.63	1.63	0.88
MVM LRV					
MVM LRV	≥5.3	4.9	4.9	4.2	5.2

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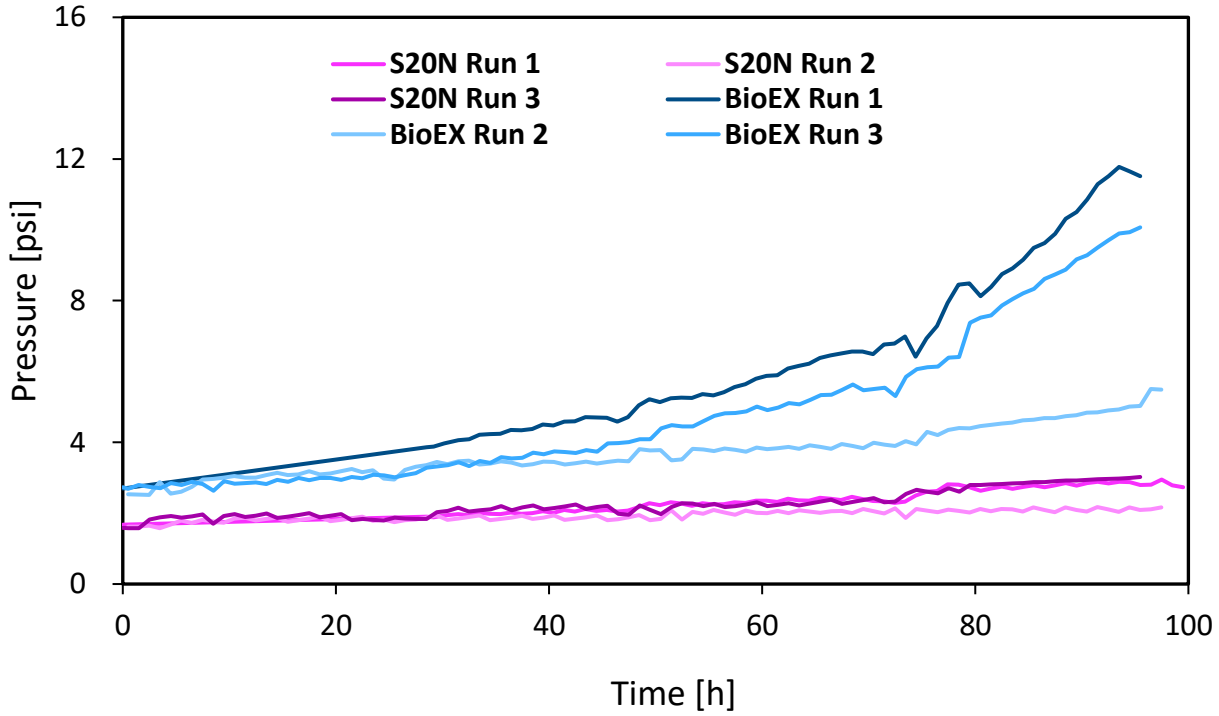
Planova S20N Performance Under Constant Flux

Asahi KASEI BIOPROCESS

Conditions:

- Molecule: 10 g/L mAb (internal)
- Buffer Solution: 25 mM acetate, 150 mM NaCl, pH 5.0
- Target throughput : 500 L/m^2
- PPV spike target: 6.5 log $TCID_{50}$/mL
- **Flux : 5 LMH**
- ~100 hours!



Filter Type	Run #	Filtrate PPV Titer (log_{10} $TCID_{50}$/mL)	LRV
Planova S20N	1	1.00	5.4
	2	0.75	6.1
	3	≤ 0.73	≥ 5.8
Planova BioEX	1	≤ 0.73	≥ 5.7
	2	2.00	4.8
	3	1.50	5.0

Important Considerations for Successful Continuous Virus Filtration

- Filter changeout strategy:
  - Reduced changeouts allow for increased operational flexibility
  - Reduced changeouts present a higher integrity risk
- Finding the balance:
  - Filtration duration (3-7 days)
  - Filter loading/ throughput (L/m^2)
  - Operating flux (LMH): can be product- dependent and affects pressure profile
- Fully vs. semi-continuous operation:
  - Fully continuous: added need for automation
  - Semi-continuous:
    - Following semi-continuous chromatography or automated upstream steps

Planova Filters in Continuous VF Application

- Planova BioEX can provide excellent MVM clearance during long duration filtrations at low flux
- Process pauses at low flux had little impact on MVM LRV
- For challenging feed streams, product-specific optimization may be needed:
  - Inline pre-filtration for unstable feed streams
  - Molecule-specific throughput (based on molecule stability or operation considerations)
  - Other filter options available to help with troubleshooting: Planova S20N

Acknowledgments

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Thank You