Considerations for Viral Filtration in an End-to-End Continuous Process

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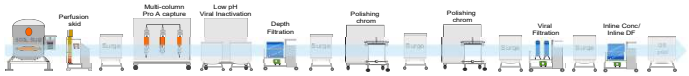

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INTRODUCTION

Integrated, end-to-end continuous processing presents challenges to the viral filtration operation:

- Higher filter loadings maximize filter utilization and minimize filter change-outs
- However, the larger filter formats used to prevent overloading and minimize change-outs can have very low filter pressures.
- Low pressures on the filter can exhibit virus breakthrough for some filters[1].

Table 1. Comparison of batch and continuous process for Filter A with an estimated permeability of 4LMH/psi

Operation Mode	Filter Size (m2)	Typical Flow rate (mL/min)	Expected Pressure (psi)	Filter Switch Frequency (Days)	Loading (L/m2)
Batch	1.0	1000	14	--	400
Continuous	1.0	60	0.9	4	346
				7	605
				15	1296
	0.3	60	3	4	1152
				7	2016
				17	4320

We sought to identify a robust virus filter that retains virus despite high load challenges and low operation pressures. High filter loadings and long process times create challenges in evaluating a filter's viral clearance capabilities.

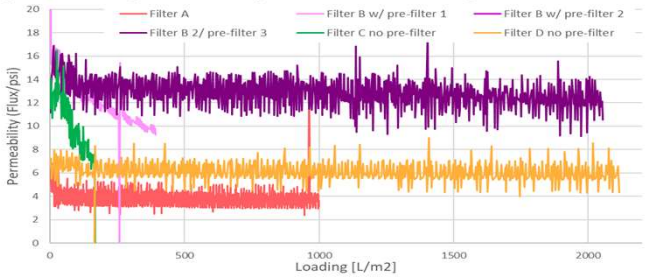
- Assessment of viral clearance should be evaluated over several days
- Additional viral clearance screening may be needed to achieve maximum throughput with robust viral clearance
- Novel spiking approaches may be needed
  - Higher virus spike % coupled with high loading could overload the filter causing non-representative fouling or virus breakthrough
  - Spiking too low many not adequately demonstrate the filter's ability to remove virus
- Surrogate virus spikes have potential to screen parameters outside traditional model virus approaches

FILTER SCREEN at HIGH mAB LOADING

To identify filters that can achieve high mAb loadings with little to no plugging, a filter screen was performed using mAb A alone (Figure 1).

- Fluxes and pressures consistent with batch operations were used to focus on loading.
- Flow rates were targeted that would achieve pressures typical for batch operation (15 – 30 psi, depending on the filter).
- Promising filter candidates will also be tested at flux levels more consistent with continuous processing

Figure 1. Screening of commercially available virus filters using mAB A



Filters A and D had little flow decay with no specialized pre-filters. Filter B had two pre-filter options where there was little flow decay. Filter C was only tested without a pre-filter due to long lead times of the pre-filter. Throughput may be improved with the addition of a pre-filter.

CONCLUSIONS AND NEXT STEPS

This work identifies multiple filter options that could be suitable for continuous processing, however, demonstrating viral clearance may lead to additional challenges. The use of surrogates for virus particles can give clues to filter behavior, however, the assay still needs optimization in terms of virus load. Alternatives to traditional virus spiking techniques such as a bracketed, integrity test approach can simplify viral clearance assessment while still demonstrating virus safety. Future work will include optimization of the surrogate virus spiking and testing techniques as well as testing other virus filters for robustness of virus clearance at low flux/pressure conditions.

REFERENCES

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SPIKING STUDIES

The use of similar-sized surrogate MMV particle as well as extended load duration were initially evaluated with Filter A due to the existence of in-house viral clearance data using MMV. Virus and virus surrogate spiking studies were performed with mAb A. Process parameters are captured in Table 2.

Table 2. Spiking Conditions – Surrogate particle load higher than MMV spiking to target ≥4 LRV if compete clearance was obtained

Spiking Condition	Process Equipment	Target Viral Load (Logs)	Duration (days)	Fractions	Process Pause (pressure release)
MMV	House air/ Pressure Vessel	8.5	1	1	No
MMV Surrogate – High Flow	Diaphragm pump w/ single use pressure sensors	10	1	2	Yes
MMV Surrogate – Low Flow	Akta Pure® system pump and pressure sessor	10	4	4	Yes

All spiked conditions show lower permeability than product alone. The permeability of the constant low flow case trends lower than the other cases, potentially due to the difference in pressure sensors or the low flow rate. Plugging is also observed in the constant low flow case, likely due to the high virus surrogate load.

Figure 2. Permeability profiles comparing MMV and surrogate MMV particle as well as impact of operating conditions (low flux vs. high flux)

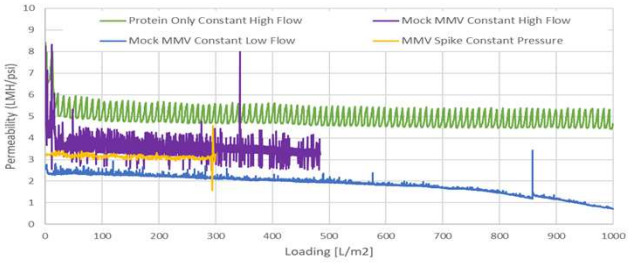

Table 3. LRV results of spiking MMV virus and Mock MMV at different operation conditions

Spiking Condition	Fraction 1		Fraction 2		Fraction 3		Fraction 4	
	Loading (L/m2)	LRV	Loading (L/m2)	LRV	Loading (L/m2)	LRV	Loading (L/m2)	LRV
MMV Virus	300	≥ 6.9	--		--		--	
Surrogate High Flow	300	1.7	190	1.1	--		--	
Surrogate Low Flow	278	0.6	293	0.3	284	0.3	278	0.0

The surrogate spiked runs show low LRVs, this may be due to overloading the filter with virus like particles, leading to breakthrough. Similar results have been reported previously with phage[2]. However, there is a trend of lower clearance with the lower flow rate. All fractions after fraction 1 experienced a pressure release, these fractions also trended lower in LRV.

ALTERNATIVES TO TRADITIONAL SPIKING STUDIES

To minimize non-representative filter fouling from high virus spikes over long loading durations, alternative spiking strategies, such as an integrity test approach, have been proposed[3]. A case study was performed using MMV and filter B with pre-filter 2 to evaluate four spiking strategies.

- Traditional high spike to achieve maximum clearance – Could lead to early filter fouling
- Lowering the virus spike concentration – Reduces fouling by virus spike but would limit LRV's achieved
- Bracketed (integrity test) – Spike a high concentration of virus and the beginning and end of product filtration to demonstrate the integrity of the filter. The intervening filtration operation is performed with just product alone to achieve the maximum product loading.
- Variable bracketed (integrity test) – Bracketed approach with a low percentage spike throughout the intervening product filtration to demonstrate virus removal through the entire operation

Table 4. MMV LRV results for alternative virus spiking strategies.

Spiking Strategy	Virus Spike %	Virus load ($log_{10}TCID_{50}$)	Pool LRV
High virus spike	1% in 720mL	8.48	≥ 5.94
Low virus spike	0.01% in 720mL	6.17	≥ 3.64
Bracketed	0.5% in 30mL, 0% in 60mL, 0.5% in 30mL	7.20	≥ 5.80*
Variable bracketed	0.5% in 30mL, 0.003% in 60mL, 0.5% in 30mL	6.79	≥ 5.01*

*LRV calculated using average of higher spike % brackets

These studies were performed over a 24-hour period. Throughput targets were met with the high virus spike; however, this may not be the case for longer load times. The low spike percentage did not demonstrate >4 logs of clearance due to the low amount of virus uses. However, all other strategies demonstrated adequate viral clearance.

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