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Application Note

Continuous Virus Filtration

Robust Retention using Serial Filtration at High Area Ratio

Summary

Virus filtration is a critical viral clearance step in monoclonal antibody (mAb) production. However, the shift towards intensified or continuous processing to enhance productivity means that retention performance characteristic of typical batch operations needs to be verified under these new operating conditions. Several reports in the literature report lower parvovirus retention on virus filters operated at lower flux. Developing solutions to address this challenge is crucial for implementation of advanced manufacturing methodologies. One potential solution is serial filtration where two filters of different filtration areas are arranged in series. These studies assessed the feasibility of this approach with the Viresolve® Pro Device and a filtration area ratio of 13:1 for first: second stage filters.

Studies at pilot scale used Viresolve® Pro Device Modus 1.3 and 1.1 formats as first and second stage filters respectively. At an operating flux of 5 LMH across the first stage filter, MVM retention across the serial filtration train was calculated as LRV ≥6.7; no virus was detected downstream of the filters, even following process interruptions. Similar studies were performed at bench scale using manifolded Viresolve® Pro Device Micro 40 formats upstream of a single Micro 40 format to create the 13:1 filtration area. Retention performance of the bench scale devices was aligned with that of the pilot scale devices; cumulative MVM retention across the filtration train resulted in LRVs of ≥6.4 and confirm that the bench-scale is representative of pilot scale performance.

These studies highlight one solution to a challenge reported for several virus filters and offers an option for integrating existing technologies into continuous operations to maintain process safety.

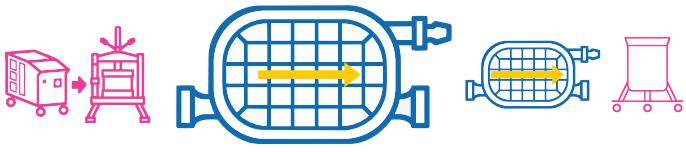


Figure 1: Serial virus filtration at high filter area ratio: Yellow arrows indicate direction of flow.

Overview

Virus filtration is a critical operation in monoclonal antibody (mAb) and recombinant protein processing, serving as a dedicated viral clearance step that removes virus through size exclusion. This operation is crucial to achieving viral safety of drug products for administration to patients. As biomanufacturers transition towards intensified or continuous processing to improve productivity, adapting batch unit operations to a continuous can be challenging. Retention performance of virus filters that show robust parvovirus retention under high flux conditions typical of batch processes, may be compromised under low flux operating conditions.[1,2,3,4,5,6] While the impact of low flux virus filtration is variable across published studies, the potential impact across many current commercial virus filters is emphasized with these worst-case reported log reduction values (LRVs) shown in **Table 1**.

Table 1. Low flux conditions reported to influence parvovirus retention on commercially available virus filters.

Virus filter	Flux range (High: low)	LRV at low flux	Change in LRV from high to low flux condition	Source Citation
P	N/A : 0.3 LMH	3.5	N/A	1
V	40 : 5 LMH	3.2	≤-2.5	2
N	40 : 5 LMH	2.4	-1.9	2
H	40 : 5 LMH	2.0	≤-3.6	2
A	1.1 : 0.19 bar	2.9	-2.6	3
B	2.8 : 0.69 bar	4.2	-0.8	3

The universal nature of the loss in retention at lower flux is attributed to Brownian motion contributing to the overall movement of virions within the pore structure of the filter membrane and subsequent diffusion of virus particles through larger membrane pores. Understanding these dynamics, and developing solutions to mitigate the challenge, is essential to support advanced manufacturing strategies. One such solution to circumvent this loss in virus retention is so-called asymmetric 'serial filtration', where two virus filters are connected in series[7,8], **Figure 1**.

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Serial filtration can be configured at a high area ratio, in which 'first stage' filter has a larger filtration area than the 'second stage' filter. This high area ratio provides the retention assurance needed while minimizing the costs associated with an additional filter.

The goal of these studies was to evaluate serial filtration at both bench and pilot scales for Viresolve® Pro Device and identify process conditions where effective virus clearance was maintained, even under low flux operating conditions.

Materials and Methods

Challenge Tests of Virus Filters at Different Operating Flux

Viresolve® Pro Device Micro 40 filters were challenged with parvovirus, minute virus of mice (MVM, 18–24 nm) at 6.3 log $TCID_{50}$/mL or with the retrovirus, xenotropic murine leukemia virus (XMuLV, 80–110 nm) at 6.5 log $TCID_{50}$/mL spiked in a model feed (0.07 g/L human immunoglobulin (hIgG) in 50 mm sodium acetate, 100 mM sodium chloride buffer pH 5). All single stage virus filtration virus challenge tests were run at constant flux conditions ranging from 5–117 L/m^2/h (LMH) using a syringe pump; filter devices were tested at 1 or 2 different fluxes each, staggered to cover the full range with duplicate devices tested at each flux point. Filtrate fractions were collected after each filter was equilibrated at the target flux for a minimum of 25 L/m^2.

Virus titers were determined using $TCID_{50}$ assay (50% tissue culture infectious dose assay). The virus log reduction value (LRV) was calculated according to the equation below. Virus challenge was held for duration of study to account for any infectious titer loss due to viability decay.

LRV = log (Titer feed/ Titer filtrate)

Challenge Tests of Virus Filters Connected in Series

Performance of the serial filtration train was evaluated at both bench and pilot scales at constant flux conditions. For all tests, inline pressure sensors measured the inlet and interstage pressures to assess filter permeability.

For the bench-scale testing, 13 Viresolve® Pro Devices, Micro 40 filters, were manifolded in parallel as the 'first stage' filter connected in series to a single Viresolve® Pro Device Micro 40 filter as the 'second stage' filter. The model feed (0.05 g/L hIgG in 50 mM sodium acetate, 100 mM sodium chloride buffer pH 5) was processed over the filtration train continuously at 5 LMH ('first stage' flux) for 200 hours using a peristaltic pump. At 0, 72, and 144 hours, feed was spiked with MVM (7 log $TCID_{50}$/mL) and used to challenge the devices for 4 hours then switched over to the non-spiked feed for remainer of the run[9]. To simulate low flux conditions, processing was paused for 1 hour at 76, and 148 hours into the run. The same operating conditions were conducted simultaneously at pilot-scale as part of a side-by-side scalability study.

The pilot-scale study was run using Viresolve® Pro Devices Modus 1.3 (0.22 m^2) and Modus 1.1 (0.017 m^2) formats, **Figure 2**, with a 13:1 membrane area ratio between the first and second stage filters. The model feed (0.05 g/L hIgG in 50 mM sodium acetate, 100 mM sodium chloride buffer pH 5) was processed over the serial filtration train at 5 LMH ('first stage' flux) for 200 hours using a peristaltic pump. At 0, 97, and 169 hours the serially connected devices were challenged with MVM spiked feed (7 log $TCID_{50}$/mL) for 4 hours then switched to non-spiked feed for the remainder of the run. To simulate worse-case low flux conditions, processing was paused for 1-hour intervals at 96, 145, and 168 hours into the run.

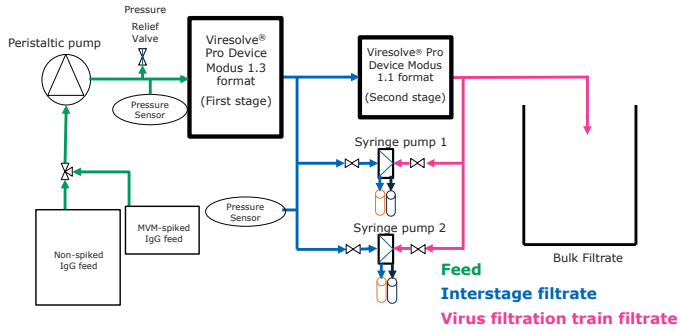


Figure 2. Serial virus filtration train set-up

For both pilot and bench-scale tests, first and second stage filtrate samples were collected using syringe pumps (operating at <12% of system flowrate). Bulk filtrate samples were collected every 24 hours, after process interrupts, and during virus challenge. Filtrate pools were collected, concentrated using Centricon® Plus-70 100 kDa filter devices, to increase the sensitivity of the virus detection assay, then assayed for titer. MVM titers were determined using $TCID_{50}$ assay. MVM clearance at any point in the test was based on the log ratio of total virus challenge: total virus in the filtrate. Additional technical information on these studies is reported elsewhere.[8]

Results and Discussion

Impact of Low Flux on Virus Retention

Retention performance of Viresolve® Pro Devices Micro 40 format was assessed under constant flux operating conditions ranging from 5–117 LMH. Above 60 LMH, there was no measurable impact of flux on MVM retention, and all test devices exceeded the ≥4.0 log MVM retention specification. Below 60 LMH, the retention specification was achieved, but slightly lower LRVs were observed, while at exceptionally low flux conditions (5–7 LMH) the retention specification was not reached. Irrespective of operating flux, complete XMuLV retention was observed in all test devices with LRVs ≥6.1.

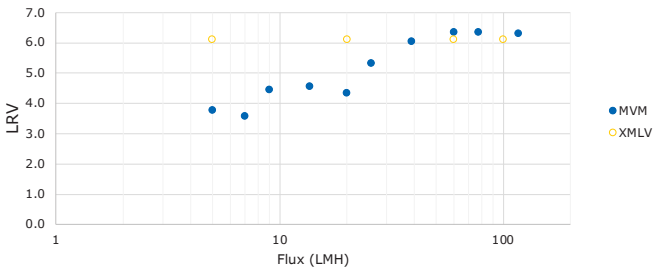


Figure 3. The effect of flux on Viresolve® Pro Device Micro 40 formats viral clearance over a range of operating flux. The line between 5 and 60 LMH is a linear fit to that subset of the data. Above 60 LMH, the horizontal line shows the assay limit of the test. Relative filter permeability decay was limited (≥75% relative permeability by end of run).

These single-stage virus filtration results support reports that low levels of parvovirus can pass through virus filters such as the Viresolve® Pro Device at low operating flux. When flux is higher than 10 LMH, the expected ≥4.0 log retention is consistently achieved. In general, above 60 LMH, no virus is detected downstream of the Viresolve® Pro Device and retention is at assay limit. In these instances, the stronger convective force of the process fluid keeps virions trapped within the retentive pathways in the membrane, preventing them from passing through the membrane. Retrovirus retention is not impacted by low operating flux as these viruses are considerably larger than, and therefore cannot pass through, the retentive pores of the virus filter membrane.

Retention of retrovirus is not measurably impacted by operating flux, but under low operating flux conditions, particularly below 50 LMH, MVM retention may be compromised.

Modelling Serial Virus Filtration

Serial virus filtration was explored as a means of mitigating the impact of parvovirus retention at low flux. This approach uses two stages of virus filters with a high area ratio between the first and second stage filters hence the description of asymmetric virus filtration. Modelling expected performance of virus filters connected in series, specifically throughput capacity and virus retention, required considering several key operating and process parameters including filter permeability, effective filtration area, pressure drop between filtration stages, assumed fouling mechanism (e.g., intermediate blocking), and the operational flow rate. Together, these factors influence how the system behaves over the duration of the filtration operation.

The results from single-stage filtration studies shown in **Figure 3** were used to predict expected retention of serial virus filtration operations. Worst case assumptions were made for retention at extended flow decay to include any impact of filter fouling.

Figure 4 shows modeled predictions of filter productivity for both single stage and serial filtration operations for a 10 L/hour flow rate and an intermediate blocking model feed loaded to a 90% filter permeability decay or 138 kPa pressure whichever occurred first. Single stage filtration with larger Viresolve® Pro Device Magnus formats enabled maximum productivity and long changeout times, but with reduced virus retention performance. When smaller Modus formats were used at the same 10 L/hour flow rate, effective virus retention was achieved, but the tradeoff was lower productivity and more frequent filter changeouts.

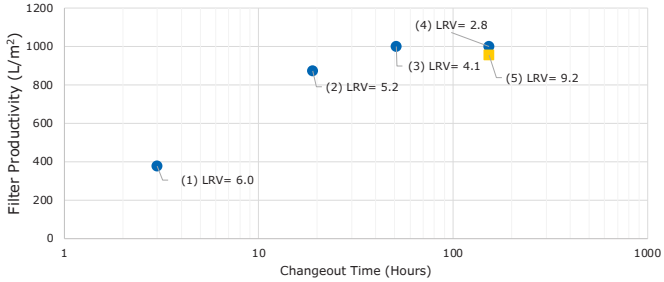


Figure 4. Simulated performance of single stage and serial virus filtration using Viresolve® Pro Devices of different sizes operated at constant flux. Each datapoint is a Viresolve® Pro Device configuration for the simulated conditions: **(1)** Modus 1.2, **(2)** Modus 1.3, **(3)** Magnus 2.1, **(4)** Magnus 2.2, **(5)** serial virus filtration using Magnus 2.2 into Modus 1.2.

When serial filtration was modeled, the tradeoff between productivity, filter changeout time and virus retention was resolved. Connecting the Viresolve® Pro Device Magnus 2.2 format (first stage) in series to a Viresolve® Pro Device Modus 1.2 format (second stage) (**Figure 4**), was predicted to maintain a long filter changeout time of 153 hours with only 5% reduction in productivity and increase MVM retention to LRV 9.2.

Modelling performance of a serial filtration train predicts extended operation time, high productivity and high virus retention across the filtration train.

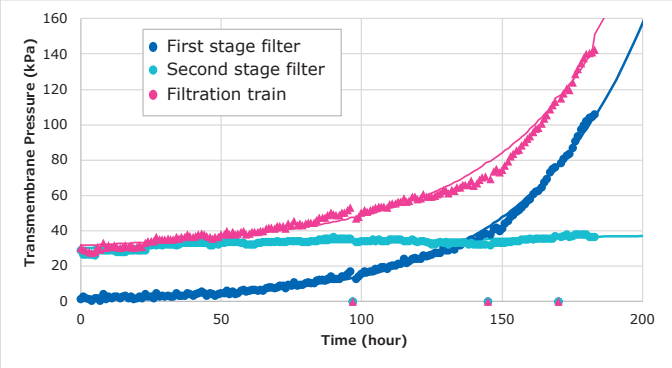
Demonstrating Performance of Serial Virus Filtration

The performance of a serial virus filtration train was evaluated experimentally using a Viresolve® Pro Device Modus 1.3 format (0.22 m^2) as the stage 1 filter and a Viresolve® Pro Device Modus 1.1 format (0.017 m^2) as the stage 2 filter. This configuration resulted in a 13:1 membrane area ratio for the first: second stage filters.

Tests were run at constant flux of 5 LMH and initially the pressure across the serial filtration train was dictated by the transmembrane pressure (TMP) across the second stage filter (**Figure 5a**). However, as the run proceeded, TMP increased across the entire filtration train, mainly due to fouling and TMP increases across the first stage filter. By contrast, the second stage filter had minimal change in TMP throughout the run as it was protected from fouling components of the feed stream by the stage 1 filter. Using the intermediate plugging model stream[10] to fit the data, the blocking constant for the first stage filter was more than 100 times higher than for the second stage filter. Although the second stage filter was initially responsible for the filtration train pressure, the filtration endpoint was determined by the TMP across the first stage filter.

The filtration train pressure reached 138 kPa when the throughput across the first stage filter was 890 L/m² at 98% flux decay Stage 1 (**Figure 5b**). The pressure across the second stage filter limited the throughput capacity of the first stage filter by ~8%.[8]

A



B

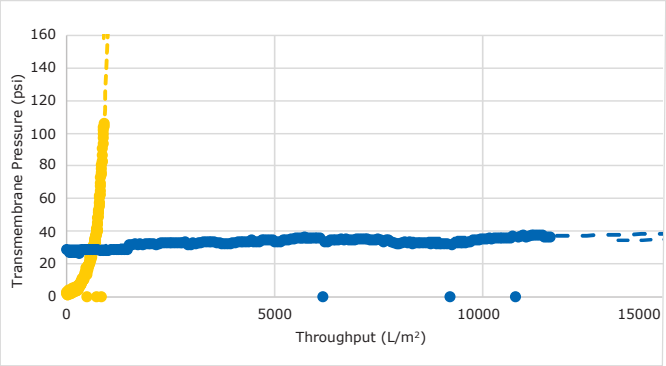


Figure 5. Serial Virus filtration (13:1 membrane area ratio) transmembrane pressure at constant flow rate (1.1 L/h) as a function of filtration time **(A)** and volumetric throughput **(B)**. The first stage filter was Viresolve® Pro Device Modus 1.3 format (5 LMH, 0.22 m²) and second stage filter was a Viresolve® Pro Device Modus 1.1 format (65 LMH, 0.017 m²). Solid lines are fit curves using an intermediate blocking model.

MVM retention performance of each of the virus filters in this serial configuration was assessed throughout the run. The first stage Viresolve® Pro Device Modus 1.3 format operating at 5 LMH effectively retained MVM with LRV of 4.6 at the end of the run (**Figure 6**). However, when the serial filtration train at 13:1 membrane area ration was run at the same 5 LMH (first stage) operating flux, no MVM was detected in the second stage filtrate, including immediately after each process interruption. MVM retention was notably improved using the serial filtration train with calculated cumulative retention and LRV ≥6.7 by the end of the run.

Results confirmed the robust retention performance of the serial virus filtration train at pilot scale using Viresolve® Pro Devices Modus formats at a high area ratio under continuous low flux conditions with process interruptions.

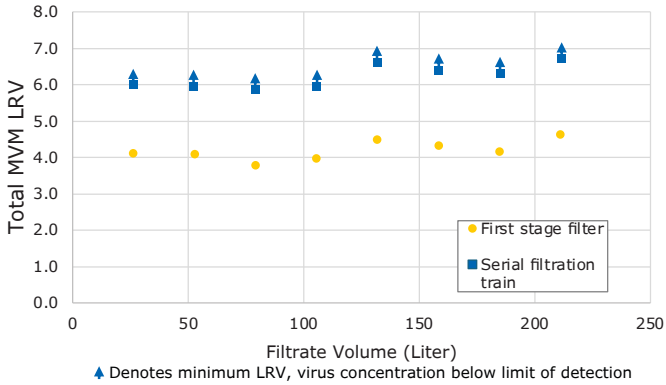


Figure 6. Serial virus filtration MVM retention performance at pilot scale (13:1) operating at 1.1 L/hr (first stage: 5 LMH, Viresolve® Pro Device Modus 1.3 format, second stage: 65 LMH, Viresolve® Pro Device Modus 1.1 format).

Confirming Benefits of Serial filtration at Bench Scale

Demonstrating representative performance of the serial virus filtration strategy at bench-scale is critical to viral clearance evaluations. The serial filtration approach for low flux operations was evaluated at both bench and pilot scales with filtration trains of different total membrane areas but with same membrane area ratio (13:1) between first and second stage filters.

Bench-scale testing was achieved by manifolding 13 Viresolve® Pro Device Micro 40 filters in parallel as stage 1 filters, connected in series to a single Viresolve® Pro Device Micro 40 filter. Pilot-scale testing used a Viresolve® Pro Device Modus 1.3 filter connected to a Viresolve® Pro Device Modus 1.1 filter, **Table 2**.

Table 2. Filters used for Bench and Pilot Scale Serial Filtration Train Evaluations

Scale	First Stage Filters (membrane area)	Second Stage Filters (membrane area)	Area Ratio
Bench	13 Viresolve® Pro Device Micro 40 (13 x 0.34 cm²)	1 Viresolve® Pro Device Micro 40 (0.34 cm²)	13:1
Pilot	1 Viresolve® Pro Device Modus 1.3 Format (0.22 m²)	1 Viresolve® Pro Device Modus 1.1 Format (0.017 m²)	13:1

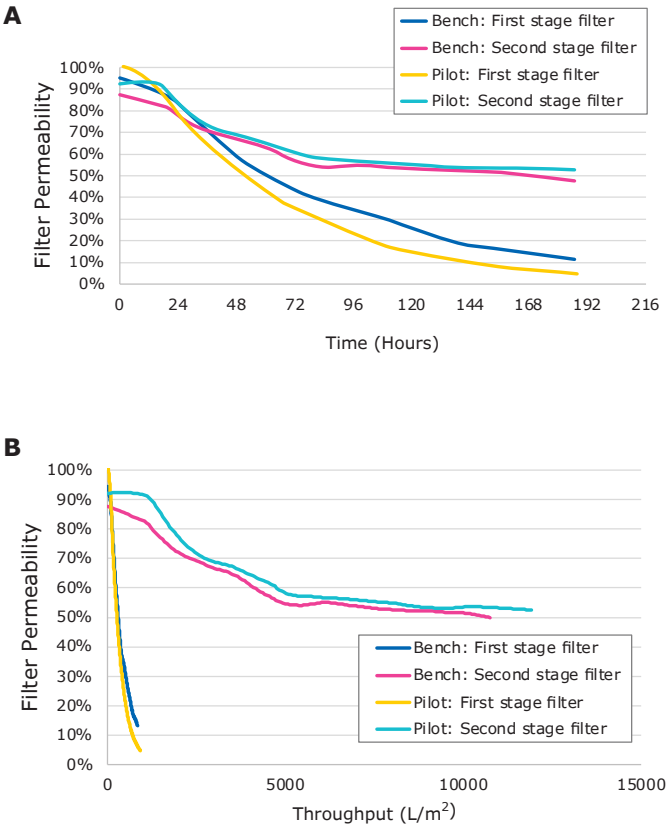


Figure 7. Relative filter membrane permeability at two operating scales for both stages of the serial filtration train (13:1) at constant flux (first stage flux of 5 LHM, second stage flux of 65 LMH) (A: as a function of time, B: as a function of throughput).

Figure 7 shows consolidated filter permeability data from the scaling study and **Table 3** summarizes throughput capacities and filter permeability decay curves of the bench and pilot scale studies. At both scales, the highest levels of membrane fouling were observed in the first stage filters; with the second stage filters experiencing less than 50% permeability decay. These results highlight the close alignment in performance of the filtration trains at different scales running under a 5 LMH low flux operating condition.

Table 3. Serial Virus Filtration Train Performance

	First Stage Filter Device		Second Stage Filter Device	
Scale	**Bench**	**Pilot**	**Bench**	**Pilot**
Throughput capacity (L/m^2)	835	920	10,752	11,898
Maximum difference in relative filter permeability over time	11%		9%	

Figure 8 shows retention performance of the filtration trains: MVM clearance the first stage virus filters operating at 5 LMH, were 3.8 LRV (Bench) and 4.0 LRV (Pilot) at the end of the runs. This retention at low flux (~5 LMH) is similar to the serial filtration studies shown previously.

Additionally, based on a 95% probability, no MVM was detected in the filtrate pools after processing over either the pilot scale or the bench scale serial filtration trains, even following multiple process interrupts. The cumulative MVM retention resulting across each filtration train resulted in calculated LRVs of ≥6.4.

Filtrate samples were concentrated to increase the sensitivity of the titer assay to detect virus passage. Using this method, a low level of virus was detected in the filtrate of the pilot scale filtration train, resulting in calculated LRVs of 7.9 for the pilot-scale and LRV ≥8.0 for the bench scale study. All virus retention studies indicate close alignment in retention performance at both bench and pilot scales.

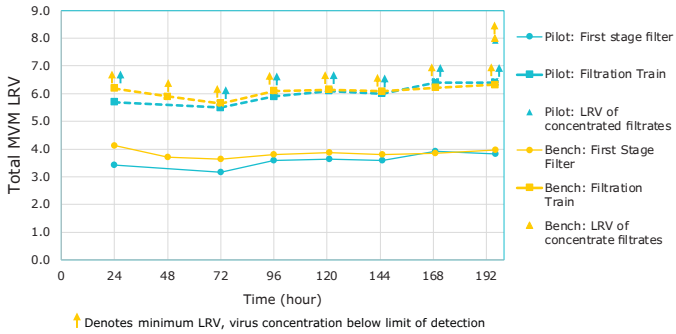


Figure 8. Scalability of serial virus filtration (13:1) parvovirus retention performance at constant flux (first stage flux of 5 LHM, second stage flux of 65 LMH).

These data confirm close alignment in throughput performance and robust virus retention using serial virus filtration at high area ratio and demonstrate the bench scale model is representative of pilot scale performance.

Conclusions

Any biomanufacturing process must include a virus control strategy to ensure patient safety. Removal of virus contaminants during processing is a critical component of that strategy, with virus filtration being crucial element due to its effective clearance of viruses through size exclusion.

As biomanufacturers transition towards intensified and continuous manufacturing, the performance of critical unit operations, such as virus filtration, needs to be re-assessed in the context of a new operating paradigm.

Operating at the low flux typical of continuous operations, has been reported to compromise MVM retention performance of virus filters. To address this, a serial virus filtration approach with two stages of virus filters at high area ratio was evaluated at both bench and pilot scales. Results confirmed:

- Below operating flux of 10 LMH virus retention of the Viresolve® Pro Device Micro 40 filters may be compromised. Retrovirus removal is not measurably affected by low operating flux.
- When Viresolve® Pro Device Modus filters are configured in a serial filtration train at high area ratio, robust virus retention and throughput capacity performance can be achieved at low operating flux, even following process interruptions.

- Serial virus filtration with Viresolve® Pro Devices at high area ratio at both bench and pilot scales provides consistent throughput and retention performance and confirm the bench scale model with Micro 40 filters is representative of larger pilot scale process performance with Modus filters.

These data support the serial virus filtration approach and provide an option for biomanufacturers to integrate existing technologies into intensified and continuous operations while maintaining performance and safety.

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