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Application Note

Virus Retention Performance of Viresolve® Pro Devices under a Range of Processing Conditions

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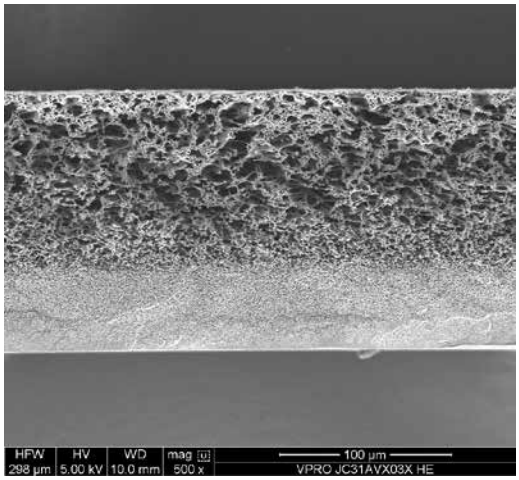

Figure 1: Transverse scanning electron micrograph (SEM) section of single layer of Viresolve® Pro membrane

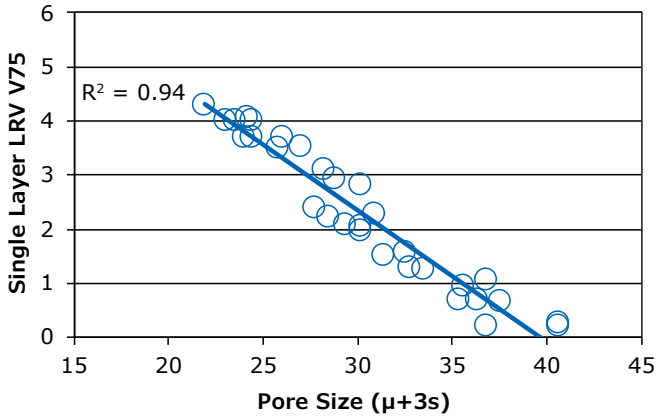

Figure 2: V75 ϕX-174 single-layer membrane LRV porosimetry pore size correlation. Membrane pore size reflects mean (µ) pore size measured by LLP + 3 standard deviations. The pore size distribution was fitted to a log-normal curve.

1) Overview

This document provides background information on virus filtration retention using Viresolve® Pro devices. The term device will be used when specifically describing the Viresolve® Pro virus filter. The terms used in this document are consistent with the Quality by Design (QbD) approach to improved process understanding[9,14,45]. This document is intended to consolidate a body of virus retention results on Viresolve® Pro devices and to communicate these results to help biologics manufacturers understand the parameters that might impact Viresolve® Pro device performance.

2) Background

Virus filtration is a critical operation in many biologic production processes and a key component of most viral safety strategies. A virus filter is a robust technology for removing viruses of different sizes. Virus removal across filters is based on the principles of size exclusion. Viresolve® Pro devices are released to a specification that provides at least 4.0 logs of small virus, such as parvovirus, removal.

This document provides information on virus retention performance of Viresolve® Pro devices and is framed in the context of a process design space within which the filters would be expected to operate. Scope is limited to operation of Viresolve® Pro devices and the information provided should not be extended to characterize performance of other virus filters. This information should be considered relevant if prefilters are used with Viresolve® Pro devices. Information summarized in this document increases process understanding with Viresolve® Pro devices and is intended to guide risk assessments associated with product characterization studies as outlined in ICH Q9.

Understanding the impact of the system, operating and process parameters on virus retention performance of the virus filter helps rank the risk posed by different process parameters, identify control strategies for high risk parameters, and guide validation study design.

Most studies in this document evaluated retention performance of small virus, e.g. parvovirus such as Minute virus of Mice (MVM), on Viresolve® Pro devices, as these represent perhaps the most challenging adventitious viral agents for removal based on size[15]. Some test conditions were evaluated using bacteriophage ϕX-174, an industry accepted model for parvovirus, which is used for quality release of Viresolve® Pro membrane and devices.

a. Size exclusion mechanism

Virus filters retain viruses predominantly by a size exclusion mechanism[7,11,23]. This means that retention levels of different size viruses is correlated to the pore size of the membrane in the filter. Viresolve® Pro devices contain two layers of asymmetric polyether sulfone (PES) membrane. Membranes are oriented in the device with more open pores on the upstream side, and the smaller, virus retentive pores on the downstream side of each membrane layer, Figure 1. The flow of liquid occurs through the open pores to the smaller, virus-retentive pores.

Measurement of virus membrane pore sizes can be performed by liquid-liquid porosimetry (LLP), which is analagous to gas-liquid porometry used for sterilizing-grade membranes[43,44]. Measurements of Viresolve® Pro membrane pore diameters using LLP confirms that the diameter of the virus retentive pores are smaller than a ~20 nm parvovirus, consistent with retaining viruses by size exclusion[3].

To correlate membrane pore size with viral retention, a series of experimental membranes of different pore sizes were developed, and their pore size was measured using LLP. These membranes were used to make custom devices containing a single layer of membrane and challenged with bacteriophage ϕX-174 in hIgG to 75% flow decay. Figure 2 shows the level of retention or log reduction value (LRV) in these devices tightly correlates with the membrane pore diameter. The R^2 value indicates that 94% of the variation in LRV is predicted by a linear dependence on pore diameter, consistent with virus retention occurring predominantly by a mechanism of size exclusion.

Another study predicted retention levels of different sized viruses on single layer membrane of specific pore size based on the principles of size exclusion. These predictions were compared to observed retention with custom Viresolve® Pro devices containing a single layer of this membrane. The closeness of observed to predicted results offers further support that viral retention occurs by size exclusion, Figure 3 [22].

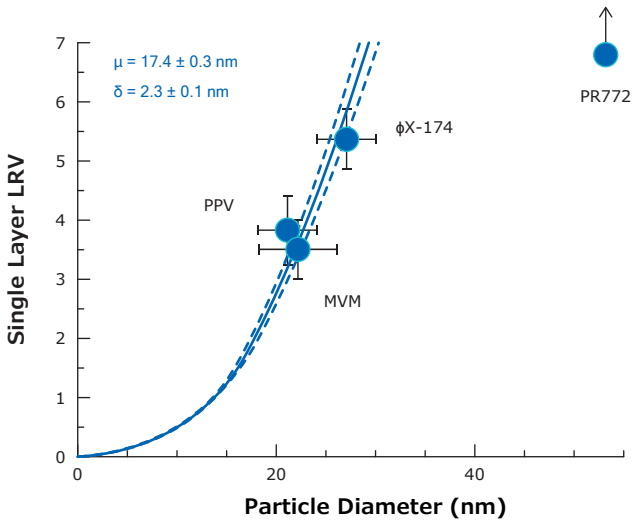

Figure 3: LRV of a single-layer of custom Viresolve® Pro devices vs. particle (virus) diameter. Observed (dots) and predicted (solid line) retention levels of different sized virus across custom single layer Viresolve® Pro devices

In summary, there is a large body of data supporting size exclusion as the principal mechanism of virus retention on Viresolve® Pro filters. This implies that the sole parameters determining retention are the mean and standard deviation of the membrane pore diameter distribution and the diameter of the virus.

Virus retention with some virus filters has been observed to be sensitive to filter plugging[4] and flow pauses, and these observations shaped some of the test strategy with Viresolve® Pro devices[1]. When a filter is 75% plugged (V75), it means that the flow through the filter is 25% of the initial flux. When particulates are present in the process fluid, smaller membrane pores are most likely to plug first, thus directing flow through the membrane's larger pores. If the size of the larger membrane pores exceeds the size of the virus, the virus is not retained by the membrane and LRV declines. Therefore, it is important to assess virus retention performance of filters under conditions where the membrane pores are fouled, at high flux decay.

During process pauses and depressurization, a virus that was actively retained by flowing fluid dragging it against a retentive pore is no longer held in place and becomes free to diffuse and find larger, less retentive pores it can pass through. In this situation a process pause can result in increased virus passage through a membrane and a decline in LRV. This effect of LRV decline is unique to specific operating conditions in some virus filters[32].

Studies with Viresolve® Pro devices show that retention performance does not appear to be sensitive to either flux decay or process pause under the range of conditions tested. These results confirm our understanding that viral retention is predominantly based on the principles of size exclusion.

b. Viresolve® Pro Device description

Virus filtration is a critical step in many biologics manufacturing processes to assure virus safety. Recognizing this, we have a multi-tiered approach to quality built into the manufacturing process of Viresolve® Pro devices to ensure a robust small virus claim of LRV ≥4.0 for the end user[38]. The manufacturing process was qualified over a controlled range of filter manufacturing parameters. In-process testing includes LLP to control membrane pore size, a proprietary binary gas integrity test to confirm absence of defects in 100% of devices before release from manufacturing [19,20,21], and virus retention testing of both membranes and devices. In addition, membrane lots for layering into devices are carefully selected to minimize differences in capacity between device lots[18]. The top (upstream) layer of membrane in the device drives capacity and will be referenced throughout the document. Manufacturing is compliant with an ISO 9001 quality system and is auditable by biomanufacturers.

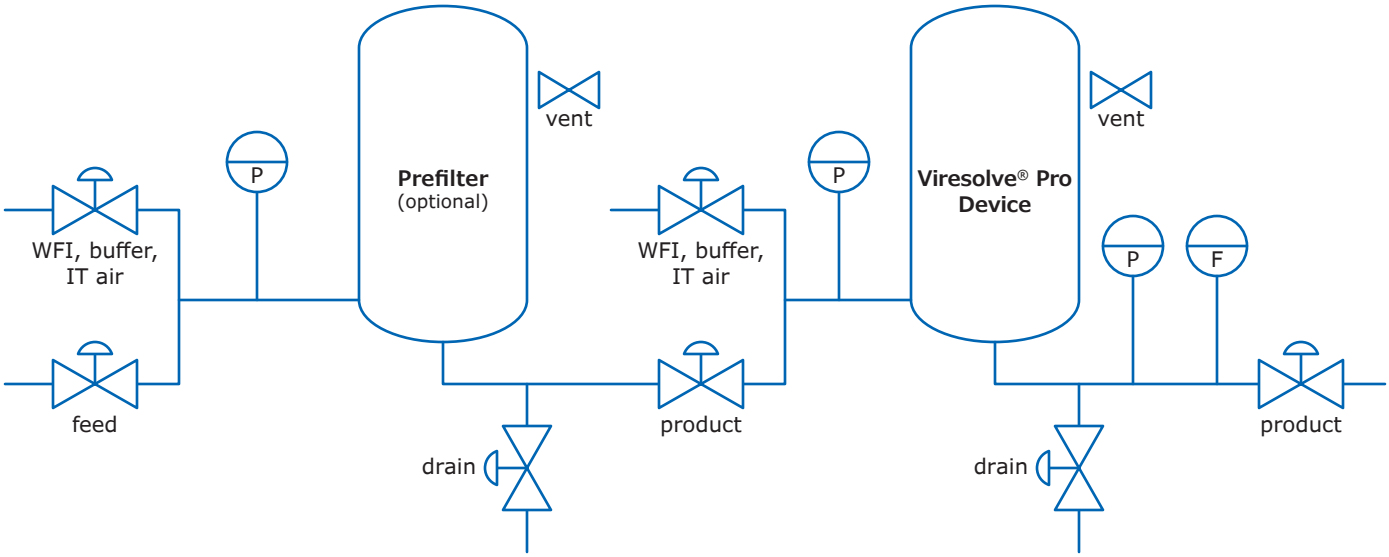

Figure 4: Generic Viresolve® Pro Filtration System Process Flow Diagram

3) System, Operation, and Process Parameters

A general Viresolve® Pro filtration system is presented in Figure 4. System components include an optional prefilter in series with the virus filter, inlet valves to control flushing (water for injection (WFI) or buffer), the flow of the feed solution, and compressed air for filter integrity testing. Outlet valves control feed flow, air venting, and draining. Pressure (P), and flow (F) sensors monitor the process. Generally, a volume or mass measurement tool is included to assess processing of the feed solution through the filter (not shown). Although system components might not be expected to affect virus retention performance of Viresolve® Pro devices, appropriate filter system design is critical to efficient filter operation.

Operation of Viresolve® Pro filtration devices involves a series of consecutive steps some of which are optional and tailored to individual operations (Table 1).

Process parameters that might affect viral clearance can be grouped into three distinct categories:

- virus filter feed
- filtration process operating conditions
- virus filtration device characteristics

The specifics of the filtration system design and the operating steps should be considered when evaluating the impact of specific process parameters on virus retention. Table 2 lists different process parameters with examples of operating ranges and potential sources of variation. The specific values shown represent a potential range for a manufacturing template.

Table 1: Generic Viresolve® Pro Device Process Operating Steps

Step	Description	Controls
Set Up	Virus device and prefilter* installation	Follow manufacturer recommendations
Set Up	Coupled or sequential virus device and prefilter flush	Pass/fail virus filter Normal Water Permeability (NWP) check Confirm virus filter NWP within 9-25 LMH/psi and stable across three sequential measurements
Set Up (optional)	Pre-use virus device integrity check, rewet	≤39.2 cc/min-m² air at 50 psig Confirm virus filter NWP within 9-25 LMH/psi and stable across three sequential measurements
Set Up (optional)	Virus device sanitize and flush	-
Set Up (optional)	Virus device equilibration	-
Processing	Feed processing	Check flow is greater than or equal to validated specification
Processing	Product recovery/buffer flush	-
Turnaround	Post-use virus device integrity	≤39.2 cc/min-m² air @50 psig

*Prefilter could be Viresolve® Pro Shield, Viresolve® Pro Shield H or Viresolve® prefilters as examples

Table 2: Process Parameters. Values shown are for illustrative purposes only.

Category	Process Parameter	Example Template Mfg. Range	Variation Causes
Virus Filter Feed	Protein concentration	0.5-25 g/L	Titer, flushes, chromatography cuts
	Conductivity	0.5-25 mS/cm	Buffer variability, dilution variations batch-to-batch, pH adjustment
	pH	4.5-8.0	Feed variability pH adjustment
	Temperature	10-30°C	Environmental controls HVAC, cleaning water temperature
	Aggregates	0-2%	Hold time, harvest variability, chromatography cuts
	Protein pI	6-9	Amino acid content
	Buffer species	Acetate, Phosphate, MES, Citrate, Histidine	Protein specific
Virus Filter Operating	Operating Mode	Constant pressure or flow	Plant preference
	Pressure	20-50 psig	Gauge error
	Processing endpoint: % flow decay	0-75%	Batch variability
	Processing endpoint: L/m^2 volumetric throughput	400-2000 L/m^2	Batch variability
	Processing endpoint: Kg/m^2 mass throughput	1-10 kg/m^2	Batch variability
	Process Interruption	0-5 hours	Batch variability
	Recovery Flush	0-100 L/m^2	Batch variability
	Pre-use caustic flush	0-50 L/m^2	Plant decision
Virus Filter Device	Device lots	Magnus 2.2 lot	Batch variability
	Integrity test value	0.5-0.784 $sccm/m^2$-psi	Batch variability, temperature, wetting

4) Systems for Viral Clearance Evaluation

This section describes the suitability of Viresolve® Pro Micro devices for evaluation of virus retention performance.

Process development studies and viral clearance evaluations of virus filters are typically performed with Viresolve® Pro Micro devices. Performance of these small-scale devices is intended to reflect the capability of large-scale manufacturing devices. Regulatory guidance requires the qualification of 'scale-down' models to represent the performance of the production scale process and that differences should be justified and potential impacts on results discussed[24]. Scalability from Micro to larger process scale devices was confirmed during development of Viresolve® Pro filters.

A schematic of a Viresolve® Pro Micro device configured for constant pressure operation is shown in Figure 5. The system can be configured to operate in constant flow operation by omitting the air valve/compressed air and inserting a feed pump and pressure gauge between the feed reservoir and the feed valve.

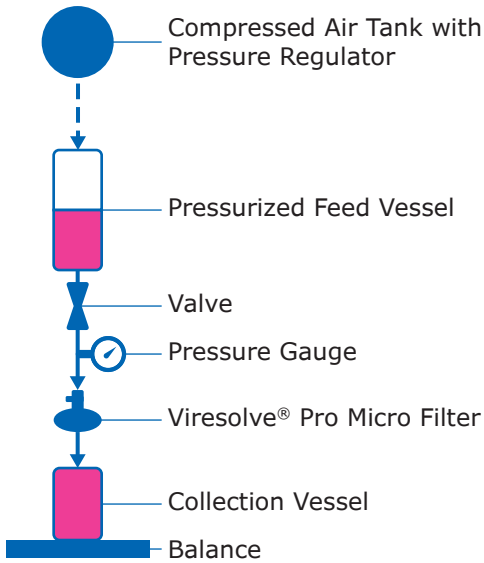

Figure 5: Typical Viresolve® Pro Micro device test system

Assessment of virus removal capabilities of Micro devices requires comparison of virus load (titer x volume filtered) in the virus spiked challenge stream, before processing over the device, with virus load in the filtrate solution, after processing over the device. Virus titers are expressed in logs and the difference in titer, which represents the clearance capability of the filter, is expressed as LRV. A pool LRV is based on the entire filtrate volume. A grab LRV is based on comparison of titers in the challenge solution and the grab sample collected over a short period of time. It is important to note that when no virus is detected in the filtrate sample, the calculated LRV is limited by the sensitivity of the assay and does not reflect the true virus clearance capability of the filter. In these situation, virus spike concentrations, filtrate assay volumes and sample dilutions can impact the LRV calculations, regardless of filter or virus type.

Generally, process steps that result in less than one log of viral clearance are not considered to significantly reduce virus. By contrast, demonstration of more than four logs of clearance in replicated studies is considered effective viral clearance[24].

Table 3 illustrates typical viruses used during validation studies of virus filters for monoclonal antibodies and recombinant proteins. For molecules entering phase I clinical trials, small virus retention must be evaluated. In calculating the overall virus safety, health authorities have allowed the use of the small virus LRV values to be applied to larger viruses[8]. For molecules entering late-stage clinical trials, virus filter clearance evaluations should include a range of viruses with different physico-chemical properties such as those listed in Table 3.

Table 3: Typical viruses used during validation studies

Virus	Family	Size (nm)
Murine Leukemia Virus (MuLv)	Retroviridae	80-110
Minute Virus of Mice (MVM)	Parvoviridae	18-26
Pseudorabies Virus (PRV)	Herpesviridae	120-200
Reovirus 3 (Reo3)	Reoviridae	60-80

Confirmation of Viresolve® Pro Device Scalability

This section summarizes two studies comparing retention performance of small and large-scale Viresolve® Pro devices. Each study has a one parameter experimental design with module type as the parameter. Results of these studies indicate virus retention on Micro devices accurately represents the retention performance of production scale Modus and Magnus devices when operated at the same conditions.

In the first study[37], replicate Viresolve® Pro Micro (n=45), Modus 1.1 (n=6) and 1.3 (n=6) and Magnus 2.1 (n=6) devices containing membrane lot M120607AVP5, were challenged with 24 g/L BSA in FA buffer pH 7.2 spiked with ϕX-174 at 30 psig. Grab samples were collected from the test devices at 75% flow decay (V75). LRVs are summarized in Figure 6.

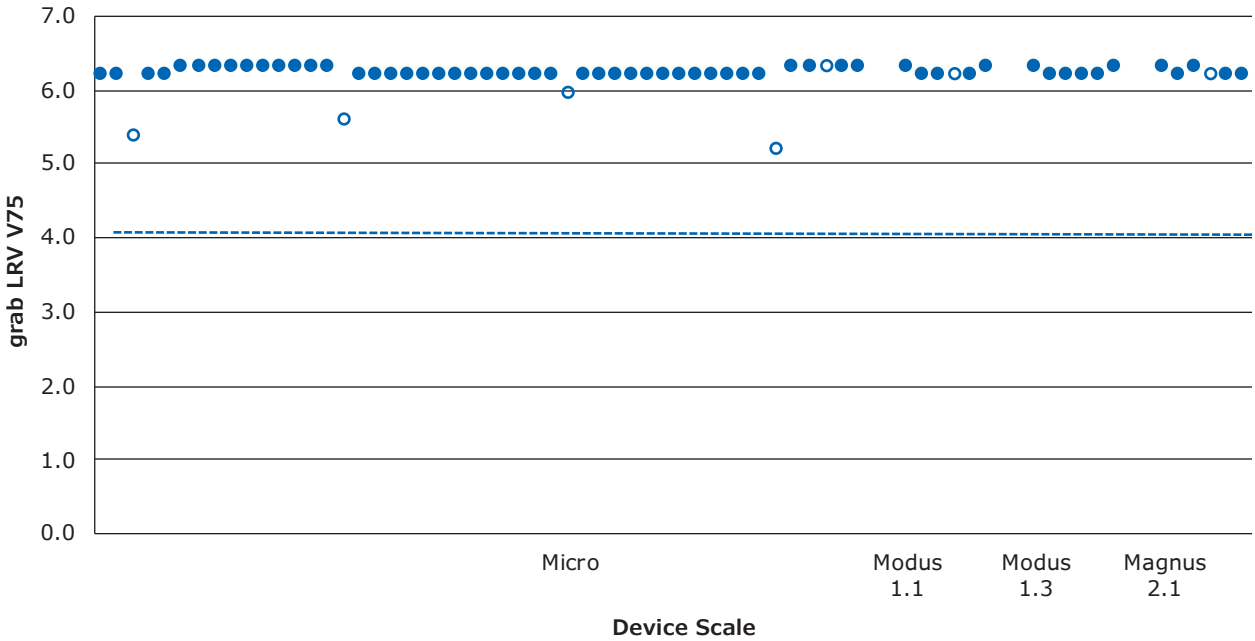

Figure 6. Individual device ϕX-174 LRV results from Viresolve® Pro Micro, Modus and Magnus devices. Open circles indicate detectable virus, solid circles indicate no virus was detected in the filtrate samples. The dotted line shows the target of LRV greater than or equal to 4.0.

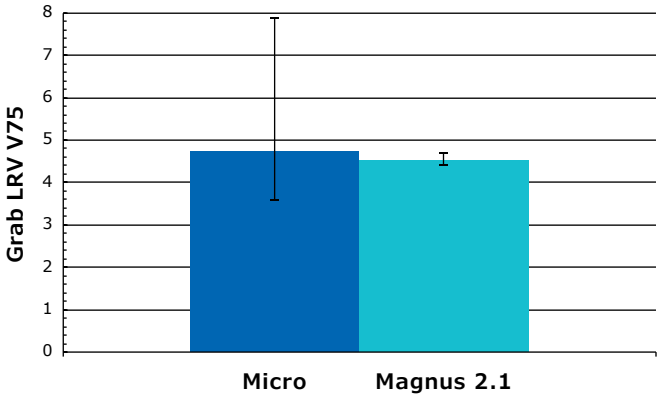

Figure 7: Open pore membrane scalability testing; grab LRV at 75% flow decay. Error bars indicate the standard deviation of the mean.

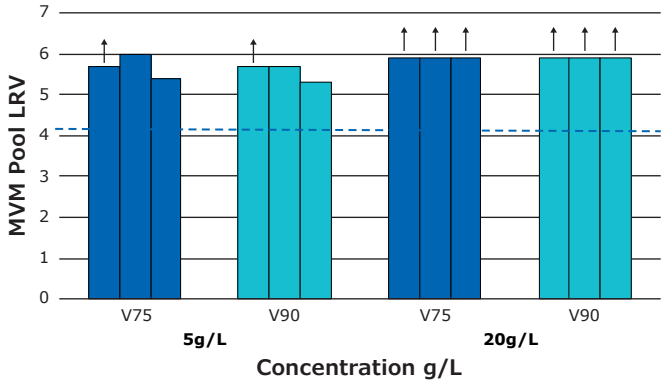

Figure 8. MVM retention in triplicate devices for two mAb concentrations at processing endpoints of V75 and V90. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

Many of the calculated LRVs are at the limit of assay sensitivity preventing a quantitative assessment of differences in performance between the device scales. However, virus retention was consistently higher than the target retention specification of greater than or equal to 4.0 logs and was not measurably affected by device size.

To quantitate potential differences in virus retention associated with device scale that could not be resolved using standard Viresolve® Pro devices, Micro (n=25) and Magnus 2.1 (n=3) custom devices were constructed with open-pore membrane below the specification for use in standard Viresolve® Pro devices. Devices containing two layers of open pore membrane (membrane lot, M022608AVP4) were challenged with 20 g/L BSA in FA buffer pH 7.2 spiked with ϕX-174 at 30 psig. Grab samples were collected from the test devices at 75% flow decay (V75) Figure 7.

Although overall viral retention of these custom devices containing open-pore membrane was lower than typical Viresolve® Pro devices, average virus retention of filtrate grab samples collected from the Micro and Magnus 2.1 devices was within 0.5 logs. These results confirm that virus retention of Micro devices represents retention performance of large scale production devices.

In summary, these studies demonstrate that virus retention of Viresolve® Pro Micro devices accurately represents the retention performance of production scale Modus 1.1, 1.3 and Magnus 2.1 devices.

5) Virus Retention Sensitivity to Process Parameters

A mechanism of viral retention based on size exclusion might suggest that the feed or operating parameters listed in Table 2 would likely not impact LRV. This section describes the results of controlled studies designed to evaluate the effects of individual parameters on viral retention. The results of the studies are summarized under three main categories dependent on the specific process parameter: A) Virus filter feed B) Virus filter operation, and C) Virus filter device.

a. Feed: Protein Concentration

Four studies, evaluated virus retention performance at a range of challenge feed concentrations and different filtration endpoints. Results demonstrate no differences in virus retention correlated with feed protein concentration from 2-25 g/L. No differences in retention were observed with increased filter fouling to 90% plugged (V90).

Study 1: mAb 02.1 at 20 g/L was diluted in buffer [10 mM Citrate, 135 mM NaCl pH5) to generate a solution of 5 g/L protein concentration. The 5 g/L and 20 g/L solutions were spiked with MVM and run at 30 psig to V90 endpoint, over triplicate Micro devices containing nominal membrane (membrane lot KC03A1V). Samples were collected from the filtrate pools at V75 and V90. This was a two-parameter experimental study design evaluating the effect of feed protein concentrations on virus retention at different flow decay points[40].

Figure 8 shows excellent MVM retention in all devices challenged with both 5 and 20 g/L solutions, with several devices at assay limit. All devices exceeded the specification of greater than or equal to 4.0 LRV confirming no effect of protein concentration on virus retention.

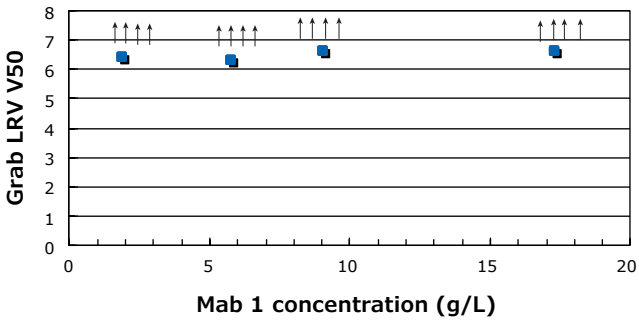

Figure 9: Individual Micro device ϕX-174 LRV (grab samples) vs. mAb1 concentration. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

Study 2: mAb 1 (17 g/L) was diluted in buffer (30 mM MES, 90 mM NaCl, pH 5.6), generating four feed solutions at protein concentrations ranging from 2-17 g/L[37]. Each solution was spiked with ϕX-174 and run at 30 psig to V50 endpoint through replicate Micro devices containing nominal membrane (membrane lot 112607AVP7). This was a one parameter experimental design study evaluating the effect of feed protein concentrations on virus retention.

Figure 9 shows complete retention in all devices demonstrating no measurable differences in virus retention at V50. These results indicate consistent virus retention with feed protein solutions ranging from 2 - 17 g/L.

Study 3: mAb 5 (25 g/L) was diluted in buffer (phosphate buffered saline, pH~7.4) generating four feed solutions at protein concentrations ranging from 2-25 g/L[37]. Each solution was spiked with MVM and run at 30 psig through Micro devices containing nominal membrane. Filtrate pool samples were collected at V25, V50 and V90. This was a two-parameter study design evaluating the effect of feed protein concentrations and processing endpoint (filter plugging) on virus retention. Table 4 summarizes the results.

Table 4: Impact of feed concentration and flow decay on MVM pool LRV. Results from individual Micro devices are shown

Feed Protein Concentration (g/L)	LRV at V25	LRV at V50	LRV at V90
2	≥5.8	N/A	≥5.8
7	≥6.1	≥6.1	5.8
15	≥5.8	≥5.8	≥5.8
15	5.5	5.3	4.8
25	≥6.0	5.4	5.7
25	≥6.0	≥6.0	≥6.0

Results show some measurable differences in retention between devices, but these appear to be unrelated to feed protein concentration; LRVs greater than 4.0 logs were measured at all protein concentrations tested. In addition, a sustained high level of MVM retention was observed with increased filter fouling to 90% plugging (V90) at all protein concentrations tested. These results indicate high level virus retention with feed solutions ranging from 2-25 g/L protein at plugging points out to V90, consistent with study 1.

Study 4: mAb 4 (15 g/L) was diluted in buffer (10 mM citrate, 140 mM , pH 5) generating two feed solutions of 10 and 15 g/L protein. These solutions were spiked with ϕX-174 and run through duplicate Micro devices containing nominal membrane (membrane M120607AVP-4) at 30 psig. Although the processing endpoint was different for each of the feed protein concentrations tested, the mass loading on the filter was consistent between the two protein concentrations. This was a one parameter study and evaluated the effect of feed protein concentration on virus retention. Table 5 summarizes the results. LRVs greater than 4.0 logs were measured at both 10 and 15 g/L protein concentrations.

Table 5: Impact of feed concentration on ϕX-174 LRV. Results from individual Micro devices are shown

Feed Protein Concentration (g/L)	Final % Flow Decay	Final grab LRV	V75 Loading (kg/m^2)
10	75	≥5.1	5.0
10	75	≥5.1	5.0
15	90	≥5.2	5.6
15	90	≥5.3	5.6

b. Feed: Conductivity and pH

The impact of challenge feed conductivity and pH on virus retention was evaluated. No measurable differences in virus retention were identified that correlated with pH (pH 5-7) or conductivity (7.8-25.4 mS/cm). Additional results from non-controlled studies supported these observations and are summarized in Section 6; Meta-analysis of Retention Performance.

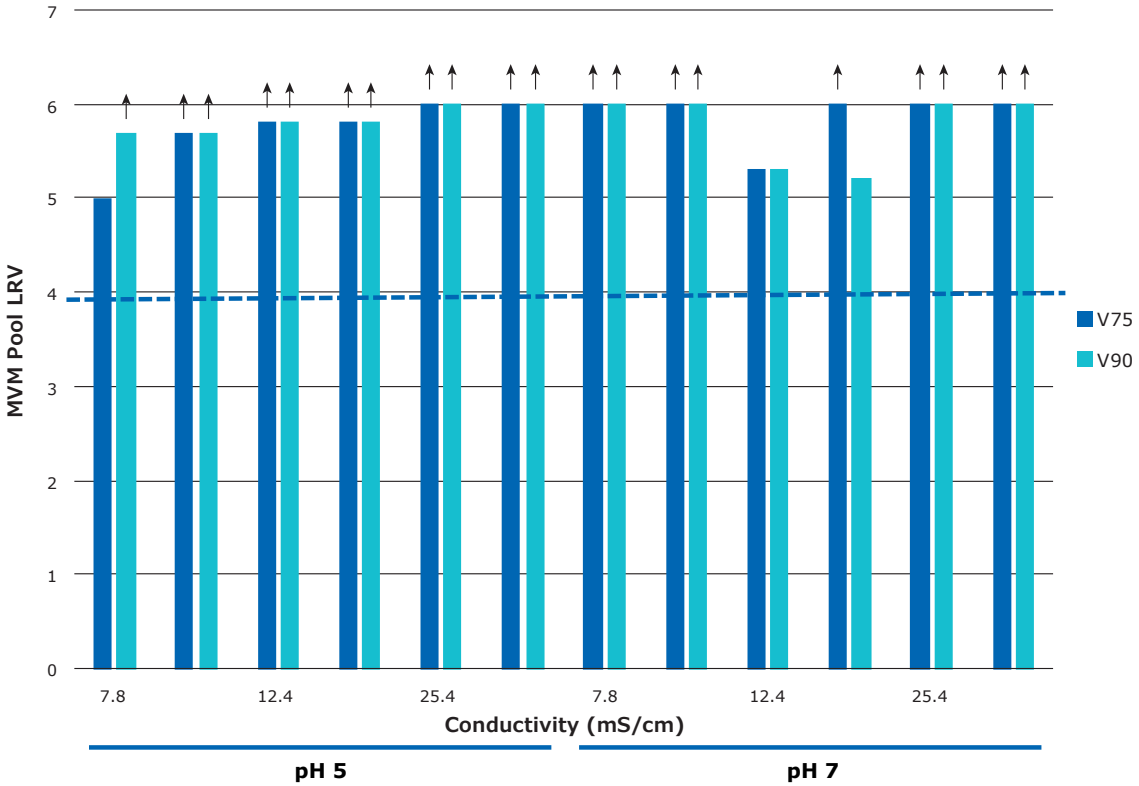

Figure 10. Individual Micro device MVM LRV including pH, Conductivity and % Flow Decay. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

Study 1: Human IgG (hIgG) at 0.1 g/L in 50 mM sodium acetate buffer was used to generate six feed solutions containing 50 mM – 250 mM sodium chloride at pH 5 or 7. Solutions were spiked with MVM and passed through duplicate Micro devices containing nominal membrane (membrane lot M060111AVP7) at 30 psig to V90 [28]. This was a three-parameter design: pH, conductivity, and % flow decay. Figure 10 summarizes the results.

Results show MVM LRVs of greater than 4.0 logs under all conditions tested, and most devices show complete virus retention at the test endpoint of V90. Although measurable differences in retention were observed in three of the test devices, these retention differences appear to be unrelated to the pH, conductivity condition or the processing endpoint. Overall, results indicate high level virus retention at pH 5-7 with feed solution conductivities of 7.8-25.4 mS/cm at plugging points out to V90.

c. Feed: Temperature

ϕX-174 retention over Viresolve® Pro Micro devices was evaluated at two operating temperatures and different pressures. Results demonstrate no impact of temperature (5-21°C) on ϕX-174 retention.

0.1 g/L hIgG in 50 mM sodium acetate buffer was spiked with ϕX-174 and passed through replicate (n=2-4) Micro devices containing nominal membrane (membrane lot M112607AVP7) at either at 5°C or 21°C. Processing at lower temperature results in reduced flux due to increased fluid viscosity, therefore studies were performed at different operating pressures under each temperature condition to normalize flux. Filtrate grab samples were collected at V75. This was a one parameter design evaluating operating temperature. The LRV results are summarized in Table 6.

Table 6: Individual Micro device ϕX-174 LRV vs. filtration temperature at different operating pressures. Results from individual Micro devices are shown.

Temperature	Pressure psig	Buffer Flux LMH	LRV			
5 °C	30.0	230	≥6.7	≥6.7	≥6.7	≥6.7
5 °C	50.0	350	5.7	≥7.1	≥7.1	6.6
21 °C	13.3	230	6.7	≥6.7	Not tested	Not tested
21 °C	21.5	350	≥7.3	≥7.3	≥7.3	≥7.3
21 °C	30.0	500	≥6.6	≥6.6	≥6.6	≥6.6

For the V75 filtrate samples, no virus was detected in 15 out of 18 test devices. In the three devices with detectable virus in the filtrate, two were operated at 5°C (at 50 psig) and one at 21°C (at 13.3 psig) suggesting virus passage was unrelated to operating temperature. In all test devices, under multiple operating temperature and pressure conditions, greater than 4.0 logs virus retention was observed, indicating no impact of temperature (5-21°C) on ϕX-174 retention.

d. Operation: Operating Pressure

Virus retention performance of Viresolve® Pro Micro devices was evaluated with two mAbs at three filtration operating pressures. No differences in retention were observed at operating pressures of 10-50 psig.

In the first study[37], mAb 2 (6 g/L) in buffer (30 mM MES, 90 mM NaCl, pH 5.6) was spiked with ϕX-174 and replicate devices (n=3) containing nominal membrane (lot M112607AVP6) were run at 10, 30 and 50 psig to a V50 endpoint. This is a one parameter study evaluating the effect of operating pressure on virus retention. Results are summarized in Table 7.

Table 7: Individual Micro device ϕX-174 grab LRV at V50 at different operating pressures

Operating Pressure (psig)	LRV
10	≥6.4
	≥6.4
	≥6.4
30	6.4
	5.7
	6.4
50	≥6.6
	≥6.6
	6.3

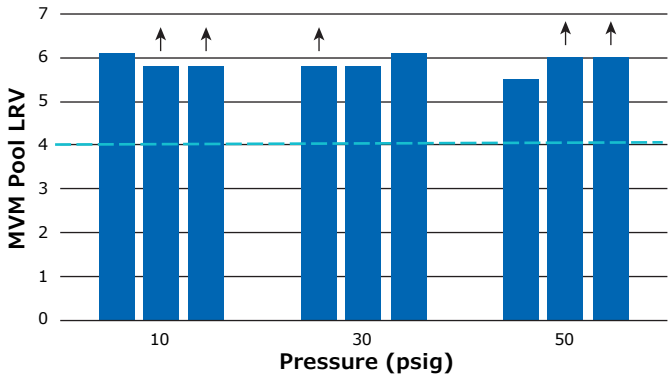

Figure 11. Individual Micro device LRVs for V75 pools. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

Results indicate five of the nine Micro devices showed no detectable virus in filtrate samples; of the four devices with detectable virus in filtrates, three were operated at 30 psig and the retention levels easily exceeded the greater than or equal to four-log specification. These results indicate no clear correlation between operating pressure (10-50 psig) and virus retention on Viresolve® Pro devices.

In the second study[40], mAb 2.1 (5 g/L) in buffer (10 mM Citrate, 135 mM NaCl pH 5) was spiked with MVM and replicate devices (n=3) containing nominal membrane (membrane lot KC26AV1) were run at 10, 30 and 50 psig to a V75 endpoint. This is a one parameter study evaluating the effect of operating pressure on virus retention. Results are summarized in Figure 11.

All devices exceeded the greater than or equal to four-log specification for retention; devices with detectable virus in the filtrate and completely retentive devices were observed under all conditions tested indicating no impact on virus retention from operating pressure (10-50 psig).

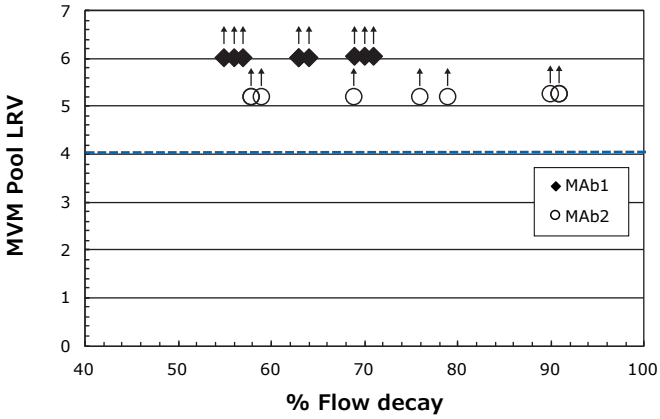

Figure 12a. Individual Micro device MVM pool LRV for mAb1 and mAb2 vs. % flow decay. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

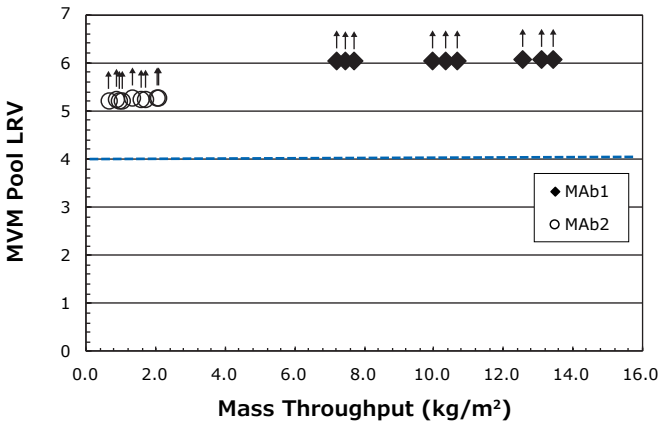

Figure 12b. Individual Micro device MVM pool LRV for mAb1 and mAb2 vs. mass loading (kg/m²). The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

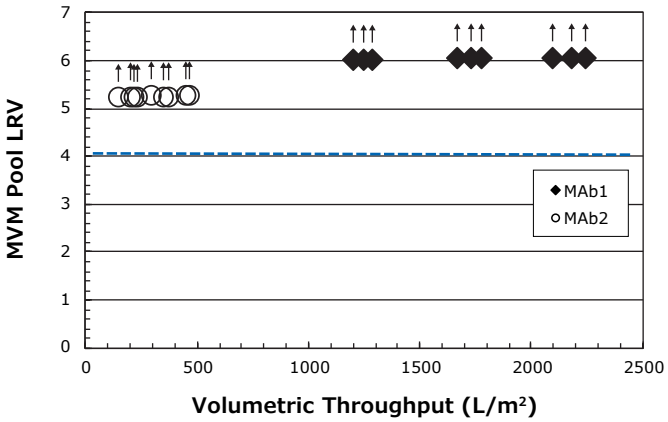

Figure 12c. Individual Micro device MVM pool LRV for mAb1 and mAb2 vs. volumetric loading (L/m²). The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

e. Operation: Processing Endpoint: % Flow Decay, Throughput (L/m²) or Mass Loading (kg/m²)

Processing endpoints can be defined by several different limits including degree of filter plugging (Vx), volumetric (L/m²) or mass throughput (kg/m²). Results demonstrate no impact of flow decay (up to V90), mass loading (2-20 kg/m²), or volumetric loading (up to 2200 L/m²) on MVM retention.

Study 1[37] evaluated retention of two mAbs (6 g/L of mAb1 in 30 mM MES, 90 mM NaCl, pH 5.6 buffer and 4.5 g/L of mAb2 in 50 mM MES, 50 mM NaCl, pH 5.5 buffer) spiked with MVM through replicate Viresolve® Pro Micro devices (membrane lot M112607AVP6) at an operating pressure of 30 psig. Retention results from filtrate pool samples are presented at different flow decay points (Figure 12a), as a function of the mass loading (Figure 12b) and as a function of the volumetric loading on the filter (Figure 12c). This was a two-parameter design: molecule and % flow decay.

No MVM was detected in any of the filtrate samples, even at V90, suggesting no correlation between filter fouling and a loss of MVM retention. Similarly, when either the volumetric throughput or the mass loading on each test filter were considered, increasing the volume processed from 0-2200 L/m² or loading up to 13 kg/m², did not impact MVM retention. In all cases MVM retention on Viresolve® Pro devices exceeded the target of greater than or equal to 4.0 log retention.

Study 2[37] evaluated retention of mAb 2.1 (5 g/L) in 10 mM Citrate, 135 mM NaCl pH 5, spiked with MVM on replicate Viresolve® Pro Micro devices containing three membrane lots (KC03A1V, KC27A1V and JG28AVP) at an operating pressure of 30 psig. Filtrate pool samples were collected at V75 and V90. This was a two-parameter design: % flow decay and membrane lot. Results are summarized in Figure 13.

Most of the samples collected from device filtrates were at assay limit and in the few devices with detectable virus, the levels of MVM were low and all calculated LRVs exceeded 4.0 logs of retention. No relationship was observed between % flow decay and MVM retention. No differences in retention were observed between devices containing the different membrane lots.

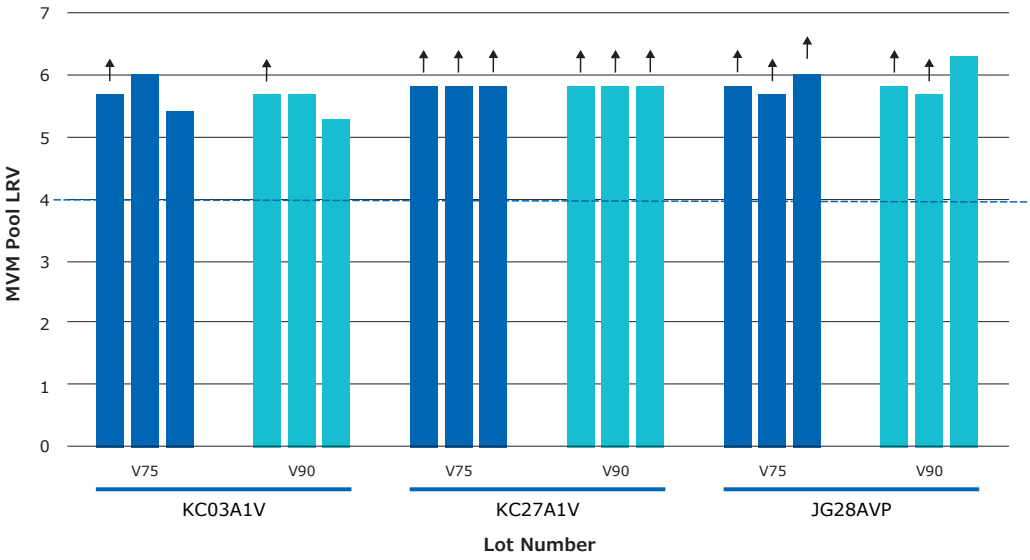

Figure 13. Individual Micro device MVM pool LRV at two flow decay points with three membrane lots. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

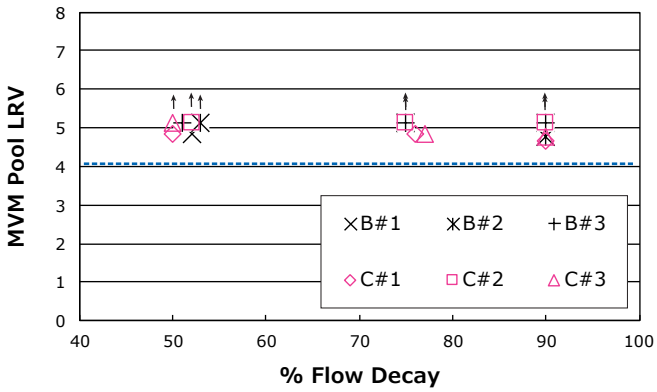

Figure 14: Individual Micro device MVM pool LRV in hIgG at increasing flow decay. The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

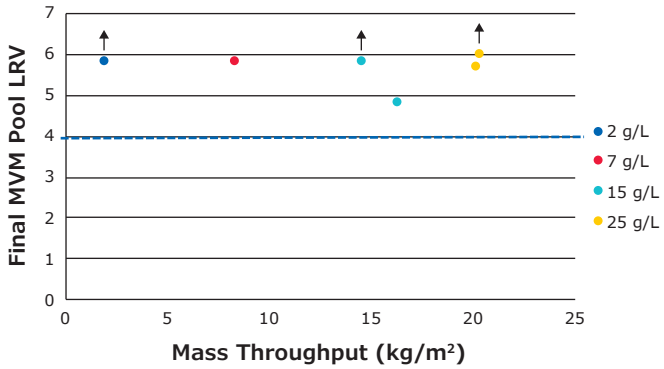

Figure 15: Individual Micro device MVM pool LRV vs. Mass loading (kg/m²). The dotted line shows the target of LRV greater than or equal to 4.0. Arrows indicate values were at assay limit.

Study 3[37]: 0.1 g/L of IgG in acetate buffer (50 mM sodium acetate, 100 mM NaCl, pH 5.0) was spiked with MVM and run through triplicate Micro devices containing two membrane lots (membrane lot B (M120607AVP1) and C (M120607AVP4)) to V90. Filtrate pool samples were collected at flow decay endpoints from V50-V90. This is a two-parameter experimental study evaluating membrane lot and % flow decay. Figure 14 summarizes the results.

Small differences in retention between devices at the different flow decay points can be attributed to variability in the test system. No difference in virus retention was identified between the two membrane lots and in all tests, greater than 4.0 logs MVM retention was measured.

Some of the studies previously described, Table 4 (feed concentration) also evaluated test endpoint as part of their design. Figure 15 shows MVM LRV at V90 and different mass loading from tests with different concentrations of mAb 5 in phosphate buffered saline, pH~7.4). These results suggest no relationship between MVM retention and mass loading between 2-20 kg/m².

In summary, the results of these studies indicate robust MVM retention at different processing endpoints on Viresolve® Pro devices. Multiple examples demonstrating either complete, or high levels of MVM retention, have been presented at V90 where flow rate over the filter is 10% of the initial buffer flow rate. Similarly, high levels of MVM retention are observed across a range of loading endpoints, from 2-20 kg/m² and from throughput endpoints up to ~2200 L/m². These results demonstrate that MVM retention is maintained during high volumetric and mass loading and under conditions where the filter is highly fouled.

f) Operation: Process Interruption – Recovery Flush and Operating Process Hold

Recovery Flush

When the processing endpoint is reached, most processes include a step in which Viresolve® Pro devices are flushed with a small volume of buffer to recover residual protein and maximize yield. Results indicate that a buffer flush does not measurably affect virus retention and that neither flush volume (10 L/m^2 -5% volumetric throughput) nor membrane lot affect the retention performance of Viresolve® Pro Micro devices.

Study 1[37] evaluated ϕX-174 retention in 0.1 g/L of hIgG in acetate buffer (50 mM sodium acetate, 100 mM NaCl, pH 5.0) over replicate Micro devices containing 3 membrane lot combinations (A-M120607AVP-4a, B-M120607AVP-4b, C-M112607AVP-4) at 30 psig. Tests were continued to V75 (650 L/m^2) endpoint where pool samples were collected. At this point, the devices were briefly depressurized and the inlet valve to the test devices was turned to enable flushing with unspiked buffer. Test devices were flushed with 10 L/m^2 buffer, followed by an additional flush corresponding to 5% volumetric throughput (32.5 L/m^2) buffer. This was a two-parameter design with membrane lot and flush volumetric throughput; results are shown in Table 8.

Table 8: Individual Micro device ϕX-174 pool LRV in IgG vs. recovery flush throughput

Viresolve® Pro Membrane	Pre-Flush V75 Pool LRV	Post 10 L/m^2 Buffer Flush LRV	Post 5% Volumetric Throughput LRV
A	≥6.3	≥6.3	≥6.3
	≥6.3	≥6.3	≥6.3
	≥6.3	≥6.3	≥6.3
B	≥6.3	≥6.3	≥6.3
	≥6.3	≥6.3	≥6.3
	≥6.3	≥6.3	6.3
C	≥6.3	≥6.3	≥6.3
	≥6.3	6.3	≥6.3
	≥6.3	≥6.3	≥6.3

Results show no measurable differences between the test conditions, with all results exceeding the target of 4.0 logs retention. Neither membrane lot nor flush volume measurably impacted ϕX-174 retention.

Study 2[37] evaluated MVM retention in 0.1 g/L of hIgG in acetate buffer (50 mM sodium acetate, 100 mM NaCl, pH 5.0) at 30 psig using replicate Micro devices containing membrane lots B and C described above. Tests were continued to V90 (650 L/m^2) endpoint where pool samples were collected. Micro devices were briefly depressurized and flushed 10 L/m^2 unspiked buffer, followed by an additional flush corresponding to 5% volumetric throughput (32.5 L/m^2) buffer. This was a two-parameter design with membrane lot and flush volumetric throughput; results are summarized in Table 9.

Table 9: Individual Micro device MVM pool LRV vs recovery flush with two membrane lots

Viresolve® Pro Membrane	Pre- Flush V90 Pool LRV	Post 10 L/m^2 Buffer Flush LRV	Post 5% Volumetric Throughput LRV
B	≥5.2	≥5.2	≥5.2
	4.9	≥5.2	4.9
	≥5.2	4.8	≥5.2
C	≥5.2	≥5.2	≥5.2
	≥5.2	≥5.2	≥5.2
	4.7	4.6	4.7

Some devices showed complete MVM retention and others had detectable virus in the filtrate samples before and following the buffer flush. In all cases, MVM retention easily exceeded the 4.0 logs retention target and there was no clear relationship between flush volume and MVM retention for either membrane lot tested.

Study 3[37] evaluated retention of MVM in 4.5 g/L of mAb2 in buffer (50 mM MES, 50 mM NaCl, pH 5.5) at 30 psig using replicate Micro devices containing membrane lots A and B described above. Tests were continued to V90 (190 L/m^2) endpoint where pool samples were collected. Micro devices were briefly depressurized, and the inlet valve was turned to enable flushing with a volume of unspiked buffer corresponding to 5% volumetric throughput (9.5 L/m^2). This was a two-parameter design with membrane lot and flush. Results are summarized in Table 10.

Table 10: Individual Micro device MVM pool LRV vs recovery flush with two membrane lots

Viresolve® Pro Membrane	Pre-Flush V90 Pool LRV	Post-Flush LRV
A	≥5.9	≥5.9
	≥5.9	≥5.9
	≥5.9	≥5.9
B	≥5.9	≥5.9
	≥5.9	5.1
	≥5.9	≥5.9

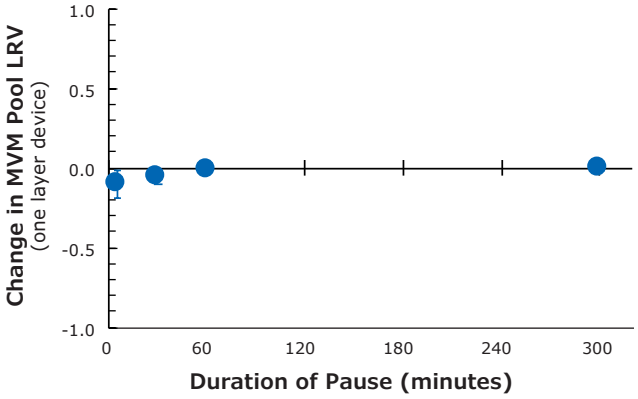

Figure 16: Differences in MVM retention pre and post pause pools following pauses of different durations. Error bars indicate the standard deviation of the mean.

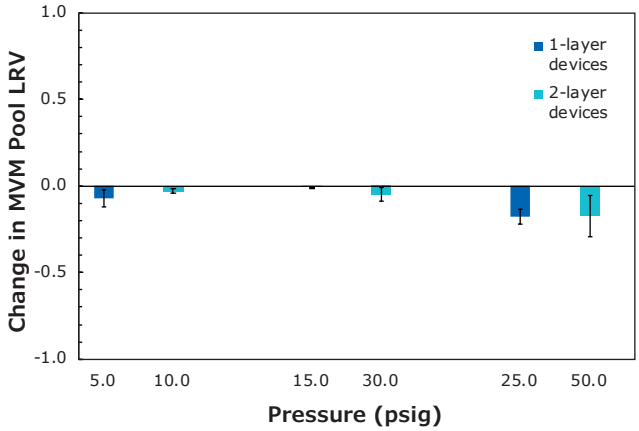

Figure 17: Differences in MVM Retention under different operating pressures following process pause. Error bars indicate the standard deviation of the mean.

As with the previous studies, complete MVM retention was observed in the majority of test devices and, under all conditions, the target of 4.0 logs MVM retention was maintained following buffer flush. One device replicate showed a measurable change in retention following buffer flush although two additional devices containing the same membrane lot did not show any measurable change in MVM retention.

In summary, the results of these studies indicate that buffer flush under most conditions, does not measurably affect virus retention and that neither flush volume (10 L/m^2 -5% volumetric throughput) nor membrane lot affect the retention performance of Viresolve® Pro Micro devices.

Operating Process Hold

Operating process holds are often either planned or unplanned pauses in processing. Several reports describe examples where retention performance of some virus filters is impacted by these short duration pauses and depressurizations. The impact of such pauses on virus retention with Viresolve® Pro devices was evaluated using custom Micro devices containing one layer of membrane, as well as standard devices, containing two layers of membrane. Results indicate no changes in MVM retention associated with the duration of pause (0-300 mins) and operating pressure (10-50 psig). No changes in retention performance were identified, even if processing was paused when the membrane was highly fouled (V90). These results demonstrate robust MVM retention performance during process pause and depressurization events.

Study 1: Custom Micro devices containing one layer of membrane (lot M042312AVP), rather than the two layers of membrane in typical Viresolve® Pro Micro devices were used to measure differences in retention following process pause. Acetate buffer was spiked with MVM and replicate devices were run at 15 psig to 600 L/m^2 at which point processing was stopped and the system depressurized for defined durations. Tests with custom devices were run at 15 psi to maintain similar flow rates to tests with standard Viresolve® Pro devices run at 30 psi. Following the pause, runs were continued, and devices flushed with unspiked buffer to ~ 50 L/m^2. Samples were collected from the filtrate fractions before and after the pause and assayed for titer to calculate changes in retention pre and post pause in LRV, Figure 16. This was a was a one parameter study design evaluating pause duration.

Results indicate no change in MVM retention with pause and depressurize durations of 5-300 minutes.

Study 2: Custom Micro devices containing a single layer of membrane (membrane lot JJ27AVP06), and standard Viresolve® Pro Micro devices containing two layers of membrane (membrane lot JJ27AVP06) were challenged with MVM spiked buffer at various operating pressures. Replicate devices for each test condition were run to 600 L/m^2 at which point processing was paused for 60 mins and the system depressurized. Following the pause, runs were resumed, and the devices flushed with unspiked buffer to 50 L/m^2. Tests with standard Viresolve® Pro devices were run at 10, 30 and 50 psig, and tests with the custom single layer devices were run at 5, 15 and 25 psig to achieve similar flow rates to the standard Viresolve® Pro devices. Samples were collected from the filtrate fractions before and after the pause and assayed for titer to calculate differences in LRV, Figure 17. This was a two-parameter study design evaluating pause and operating pressure.

Results indicate changes in MVM retention following a process pause of up to 60 mins were less than 0.5 logs. These results demonstrate process pause under operating pressures of 10-50 psig does not impact virus retention on Viresolve® Pro devices.

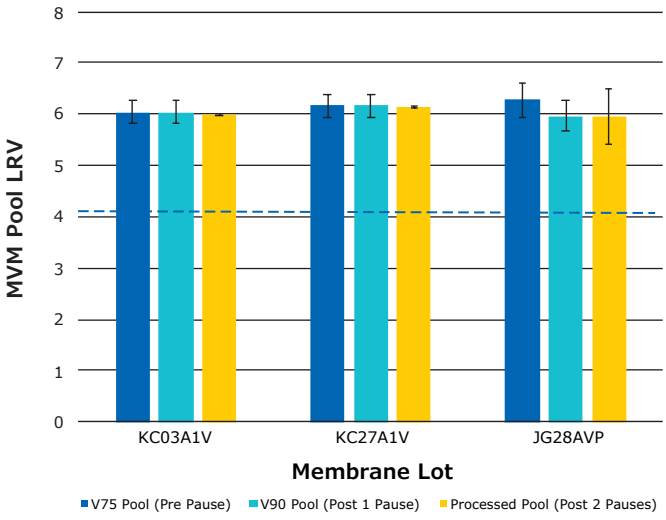

Figure 18. Average MVM LRV with different membrane lots following two process pauses. The dotted line shows the target of LRV greater than or equal to 4.0. Error bars indicate the standard deviation of the mean.

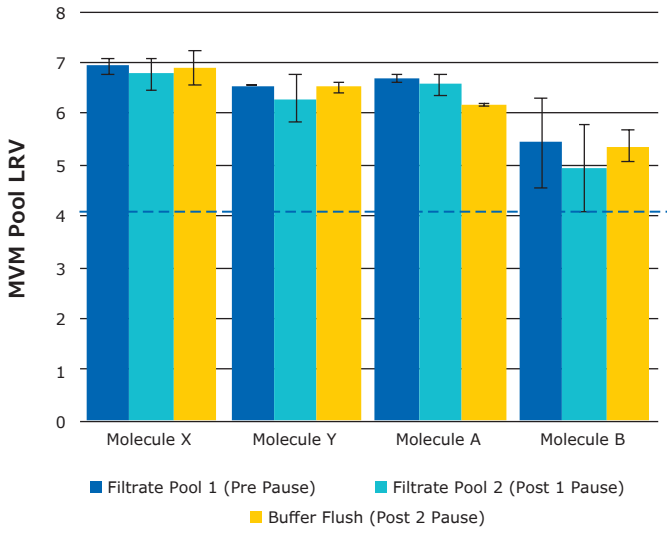

Figure 19. Average MVM LRV following two process pauses. The dotted line shows the target of LRV greater than or equal to 4.0. Error bars indicate the standard deviation of the mean.

Study 3: In this study 0.1 g/L hIgG in 50 mM sodium acetate buffer was spiked with MVM and run at 30 psig through replicate Viresolve® Pro Micro devices containing three lots of nominal membrane (membrane lot KC03A1V, KC27A1V and JG28AVP). Processing was continued until V90 at which point the spiked challenge solution was replaced with unspiked buffer for a product recovery step. Two process pauses each of 30 minutes duration were introduced during processing: one following sampling at V75 and the other at V90 before the product recovery or buffer flush step. During each of these pauses, the test systems were completely depressurized. Samples were collected from the filtrate fractions before and after each pause and assayed for titer. Filtrate pool LRVs before pause and after each pause are shown in Figure 18. This was a three-parameter study design evaluating membrane lot, processing endpoint, and process pause parameters[40].

Results indicate virus retention of at least 4.0 logs in Micro devices with three membrane lots following two process pauses (and depressurizations) of 30 minutes each. Differences in mean pool LRVs at the various sampling points differ by less than 0.3 logs. These results indicate that pauses in processing of up to 30 minutes do not impact MVM retention even when the membrane is extremely fouled.

Study 4: Four molecules of differing concentrations (4-20 g/L) and buffer conditions were spiked with MVM and run at 50 psig through replicate Viresolve® Pro Micro devices containing nominal membrane (device Lot C1MA16074). Processing was continued until 600 L/m^2 (molecules X and Y) or 1000 L/m^2 (molecules A and B). Two process pauses, each of 10 minutes duration, were included in each processing run: one at 50% throughput, and the other at 100% throughput before the buffer flush. During each pause, the system was depressurized. Samples were collected from the filtrate fractions before and after each pause and assayed for titer. The filtrate pool LRVs before pause and after each pause are shown in Figure 19. This was a two-parameter study design evaluating molecule type and pause parameters[25,27].

Results indicate at least four logs retention with all molecules following one or two process pauses. In all cases differences in LRV before and after pause were small and within the expected level of test variability. These results indicate that the introduction of one or two short-duration (10 mins) pauses in processing did not impact MVM retention in a range of molecules of different concentrations and buffer conditions.

In summary, the results of studies with custom devices containing a single membrane layer indicate no changes in MVM retention associated with the duration of pause (0-300 mins) and operating pressure (10-50 psig). Furthermore, results with standard Viresolve® Pro Micro devices containing two layers of membrane indicate no changes in retention, even if processing was paused when the membrane was highly fouled (V90). These results demonstrate robust MVM retention with Viresolve® Pro Micro devices during process pause and depressurization events.

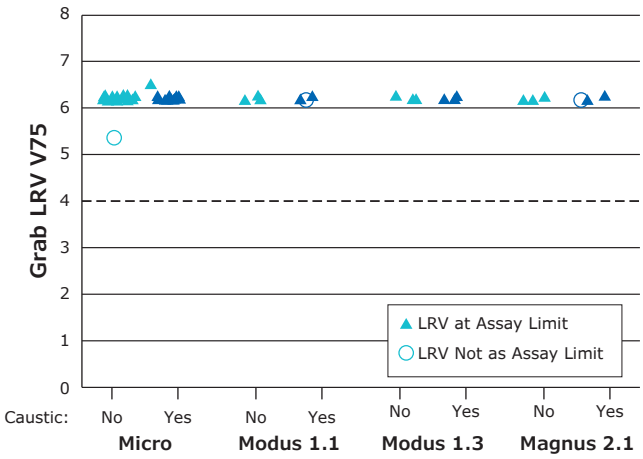

Figure 20. ϕX-174 LRV from Viresolve® Pro Micro, Modus and Magnus devices following caustic treatment. Open circle indicates detectable virus, closed triangles indicate assay limit. The dotted line shows the target of LRV greater than or equal to 4.0.

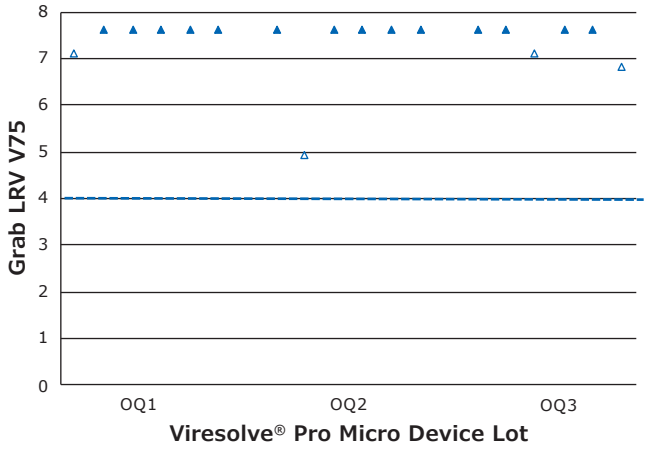

Figure 21a. ϕX-174 grab LRV in 3 lots of Micro devices. Results from individual devices are shown. Open triangles indicates detectable virus, triangles indicates assay limit. The dotted line shows the target of LRV greater than or equal to 4.0.

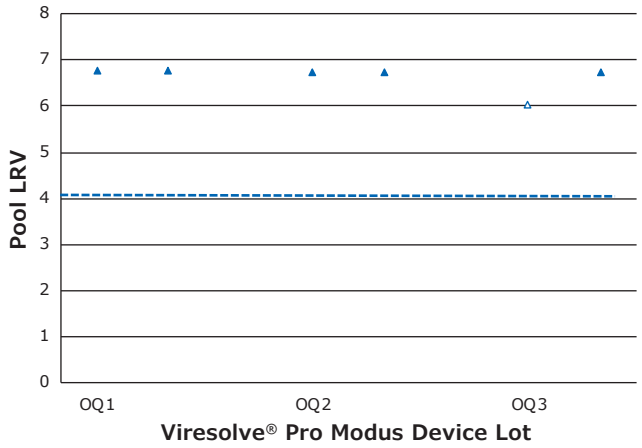

Figure 21b. ϕX-174 pool LRV in 3 lots of Modus devices. Results from individual devices are shown. Open triangle indicates detectable virus, triangles indicates assay limit. The dotted line shows the target of LRV greater than or equal to 4.0.

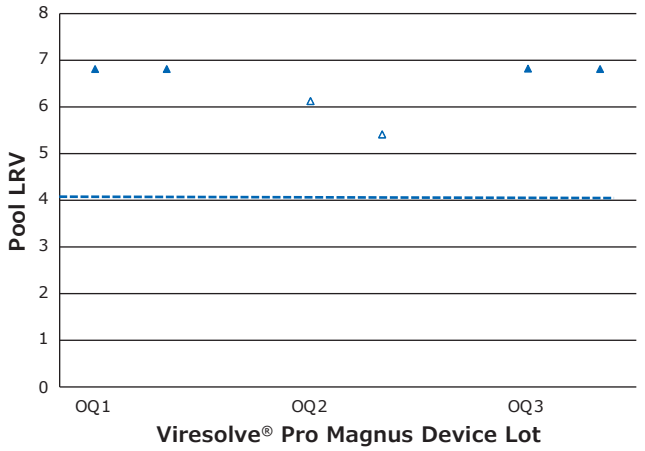

Figure 21c. ϕX-174 pool LRV in 3 lots of Magnus devices. Results from individual devices are shown. Open triangle indicates detectable virus, triangles indicates assay limit. The dotted line shows the target of LRV greater than or equal to 4.0.

g) Operation: Pre-use Caustic Flush

Virus filtration is not a sterile operation, but Viresolve® Pro devices are caustic stable and can be sanitized with 0.5N NaOH before use to reduce bioburden. Studies indicate no measurable changes in virus retention of Viresolve® Pro Micro, Modus and Magnus devices following caustic treatment.

Study 1: Viresolve® Pro devices (Micro, Modus and Magnus) containing nominal membrane (membrane lot M120607AVP5) were caustic sanitized with 0.5N NaOH (for 1h dynamic and 16 hours static) then challenged with ϕX-174 in 24 g/L BSA in FA buffer pH 7.2 at 30 psig. Filtrate grab samples were collected at V75 and LRVs were compared to LRVs from non-caustic treated devices. This was a two-parameter study design evaluating caustic treatment and device scale[37]. Results are summarized in Figure 20.

No measurable differences in retention for any device scale were observed following caustic treatment. Results indicate at least four logs of virus retention from both caustic and non-caustic treated devices.

h) Device: Consistency of Membrane and Device Performance

Each membrane and device lot are tested for virus retention using bacteriophage ϕX-174. During validation of the Viresolve® Pro device manufacturing process, it was demonstrated that at least four logs of small virus removal could be achieved in each lot of both Viresolve® Pro membrane and devices. Moreover, Viresolve® Pro devices were shown to consistently achieve greater than or equal to 4.0 logs of virus removal at all device scales.

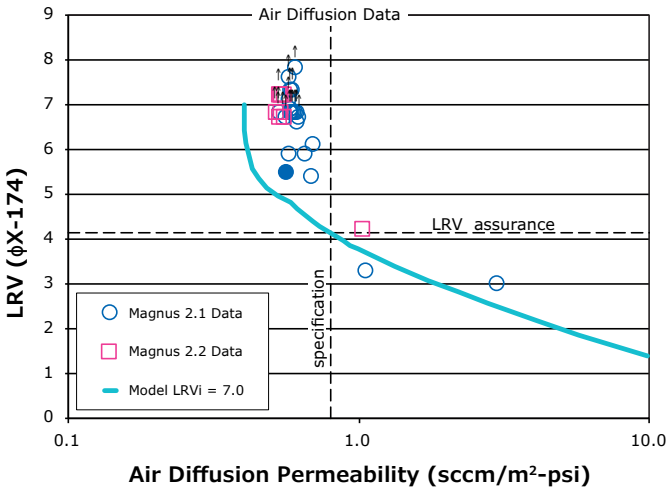

Figure 22. Small virus retention (<30nm) on Viresolve® Pro devices. Open circle indicates detectable virus, closed circle indicates assay limit. The dotted line shows the target of LRV greater than or equal to 4.0.

Study 1: At the time of the Viresolve® Pro filter validation, the retention of ϕX-174 bacteriophage was evaluated in multiple lots of Micro, Modus and Magnus devices. All devices were challenged at 30 psig with at least 10^7 pfu/mL of the ϕX-174 bacteriophage [36]. Micro devices were challenged with spiked 0.03 g/L IgG in acetate buffer (50 mM sodium acetate, 100 mM NaCl, pH 5.0) and grab LRV samples were taken at V75. Modus and Magnus devices were challenged with spiked acetate buffer to a throughput of 10 L/m². Retention results are shown in Figure 21 a, b and c.

Results indicate that at least 4.0 logs of virus retention was consistently achieved across device scales and membrane lots. Viresolve® Pro membrane and device lots are tested for ϕX-174 retention before release. All membrane and device lots must demonstrate LRV greater than or equal to 4.0 for quality release.

i) Device: Post Use Integrity Test

Integrity testing can be performed before use to reduce risk of a non-integral device being used in the filtration operation. Post-use integrity testing is mandatory to confirm the filtration device operated as expected. The integrity test filtrate air flow is an output of the integrity test and is commonly described as a test value. It would be expected that virus filter vendors have comprehensive data packages demonstrating a defined level of virus retention for an expected integrity test value. This test value is not a process input parameter or a quality or process attribute. However, since a failure to meet the integrity test specification directly impacts the retention of the virus filter, the integrity test value can be considered a critical process parameter.

A study was performed with Viresolve® Pro Magnus devices, challenged with ϕX-174 virus in acetate buffer to a throughput of 10 L/m². This was a one parameter design evaluating device size. Figure 22 shows the correlation between ϕX-174 retention and the pre-use air diffusion rates.[19] The air diffusion model predicts that air diffusion permeability greater than the specification of 0.8 (standard cubic centimeters per minute per square meter per psi (sccm/m²-psi)) indicates a large defect which will correlate to lower retention. The air diffusion test can be used to distinguish between an integral and a non-integral device, but cannot distinguish between an integral device predicted to give an LRV of 5 with one predicted to give an LRV of 7. The air diffusion permeability for these devices, if they are integral, will be the same.

The results confirm that the air diffusion test specification for Viresolve® Pro devices provides reliable assurance of LRV ≥4.0. Air flow values higher than the specification (0.8 sccm/m²-psi) indicate defects in the device which result in lower LRV. Below the specification, the LRV is insensitive to changes in air diffusion.

6 Meta-analysis of Retention Performance

An internal database containing results of 498 small viral clearance evaluations using Viresolve® Pro devices was analyzed using a similar approach to published reports [34,35,48]. These clearance evaluations were not systematic controlled studies of the filtration design space, but rather represent a composite of many filtration conditions executed by multiple biomanufacturers at different testing laboratories using virus preparations prepared using a range of purification methodologies. Importantly, the results represent a range of Viresolve® Pro devices operating conditions and include different types of molecules, protein concentrations (0–42 g/L), buffer pH (4.5-8.5), conductivity (1–75 mS/cm), operating pressures (10-50 psig), different viruses (21-30 nm), with over 40 different lots of Viresolve® Pro Micro devices, Figure 23.

The vast majority (85%) of clearance test results with small virus had no detectable virus in the filtrate with clearance levels limited by assay sensitivity. Approximately 15% of reported test results had detectable virus in the filtrate and in 490/498 cases the calculated LRV was greater than or equal to 4.0 logs retention. The specifics of individual evaluations are not available for review, and it is possible that some of these results with lower than expected retention levels could be attributed to specific experimental conditions or problems during execution of the clearance evaluations. These results were not repeatable, and they were included here for completeness. Analysis of the specific studies did not reveal any relationship between virus retention levels and molecule type, protein concentration, buffer pH, conductivity or operating pressures [16].

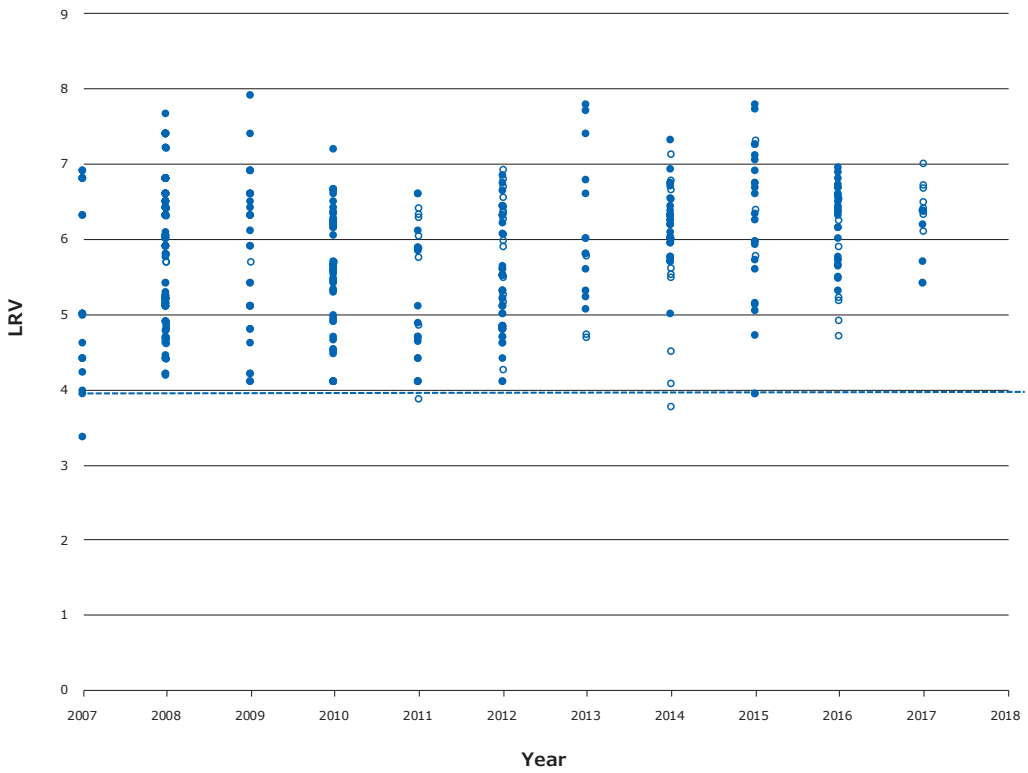

Figure 23. Small virus retention (<30nm) on Viresolve® Pro devices. Open circle indicates detectable virus, closed circle indicates assay limit. The dotted line shows the target of LRV greater than or equal to 4.0.

Table 11: Parameter Test Range Summary

	Process Parameter	Range Tested (controlled)	Meta Analysis Range
Virus Filter Feed	Protein concentration	2-15 g/L	0-42 g/L
	Conductivity	8-25 mS/cm	1-90 mS/cm
	pH	5.0-7.0	4.5-9.0
	Temperature	5-21°C	Not Tested
	Aggregates	Not Tested	Not Tested
	Protein pI	Not Tested	Not Tested
	Buffer species	Not Tested	Not Tested
Virus Filter Operation	Pressure	10-50 psig	9-50 psig
	Processing endpoint: % Flow Decay	25-90%	0-95%
	Processing endpoint: L/m^2 Volumetric Throughput	2.0-20.4 Kg/m^2	0.5-25.0 kg/m^2
	Processing endpoint: Kg/m^2 Mass throughput	10-2200 L/m^2	10-2100 L/m^2
	Pocess Interruption	0-300 minutes	Various
	Recovery Flush	0-50 L/m^2	Various
	Pre-use caustic flush	0-17 hrs; 0-0.5N NaOH; room temperature	Not Tested
Virus Filter Device	Device Size	0.00031-1.53 m^2	0.00031 m^2
	Device Lots	20	40
	Integrity Test value	Not Tested	Not Tested

7 Summary

This data set, in combination with the previously described studies, confirms Viresolve® Pro devices meet the small virus retention claim of LRV ≥4.0. Further, the broad range of conditions examined demonstrate that virus retention appears insensitive to all feed and operating parameters over the wide operating ranges tested. These results agree with other reported analyses which did not identify any critical process parameters for filtration operation using Viresolve® Pro devices[46,48].

This document is intended to consolidate a body of virus retention results on Viresolve® Pro devices to help biologics manufacturers understand the parameters that might impact Viresolve® Pro device performance. Table 11 summarizes the parameters tested and the ranges evaluated in both controlled studies and meta-analysis. These parameters and ranges tested may not represent the process conditions for every biomanufacturer, however the results indicate robust virus retention under a broad range of many different feed, operating and filter conditions. Following extensive testing, no specific parameter was identified that compromised retention capabilities of the filtration devices[46,49]. In all cases virus retention of Viresolve® Pro devices exceeded the target four log retention specification.

It is hoped that this package provides supporting documentation which can be used by biologics manufacturers to simplify risk assessments for filtration processes implementing Viresolve® Pro devices and streamline clearance evaluations with these filters.

8 References

This section contains regulatory and scientific presentations, publications, and communications that support the approach and conclusions of this document.

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