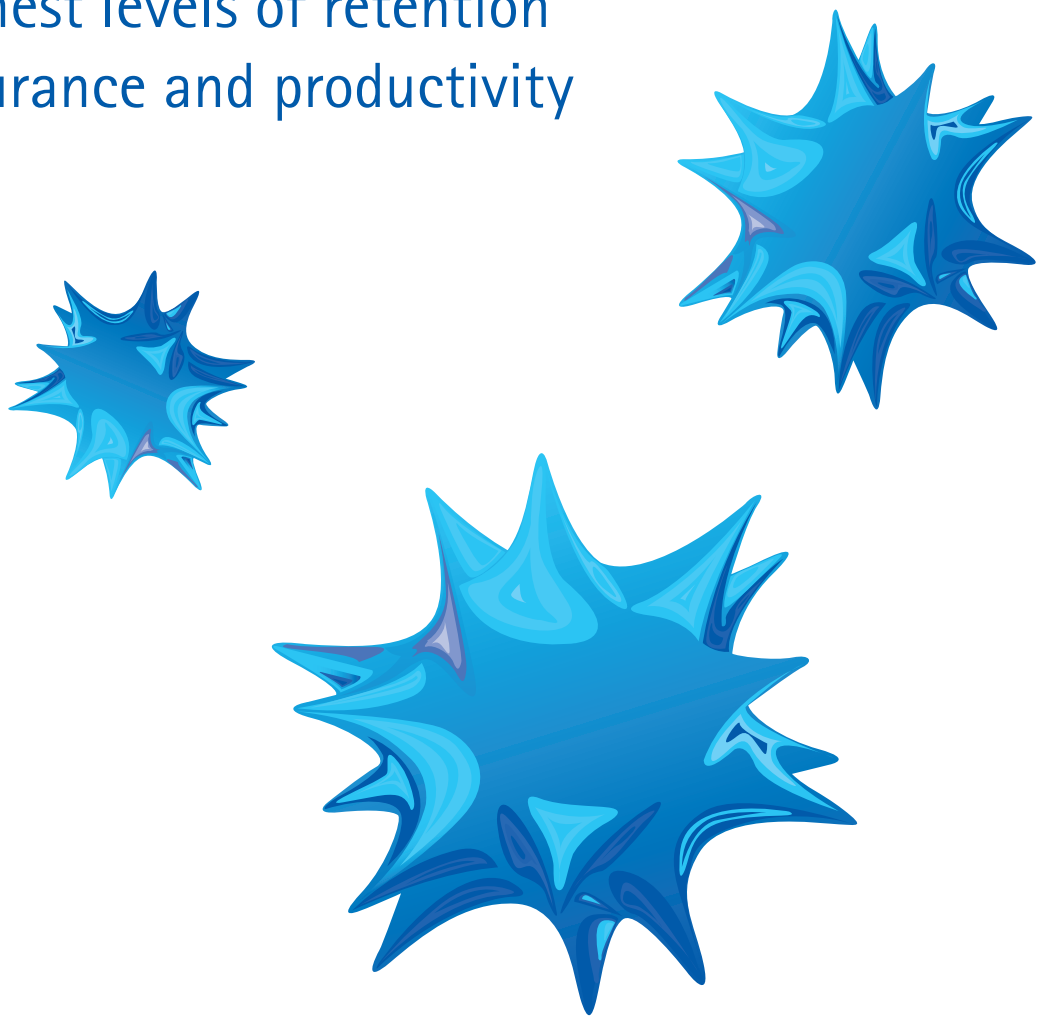
Viresolve® Pro Solution Performance Guide

The next generation parvovirus safety solution designed to provide the highest levels of retention assurance and productivity



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How to Use the Guide

The Viresolve® Pro Solution Performance Guide is a reference document to provide you with assistance in evaluating and validating Viresolve® Pro Devices for your virus safety solutions. Included in this guide are general guidelines on various performance aspects of virus filters that may be considered and evaluated by potential users of virus filters, such as the Viresolve® Pro Devices. Also included in this Performance Guide are summaries of a number of applications and case studies performed at Merck Millipore. These studies have been designed or selected for inclusion in this Performance Guide in order to provide you with a well-rounded overview of the device performance.

Results are intended as general examples and are not to be construed as product claims or specifications. While results included in this guide summarize outcomes and observations obtained in the specific applications studies with the particular model stream and experimental conditions, they may or may not be representative of results obtained by other feed streams or different test conditions, and should be confirmed by the end user using feed stream and process conditions representative of the specific application.

Robust Capacity

Product Consistency

Introduction

Consistent performance of virus filtration devices in the customer application is a critical expectation of its users. Therefore, performance consistency was a key development target during the development and validation of Viresolve® Pro membrane and Devices. But it is equally important to sustain consistent performance going forward, post-validation, as we continue to manufacture and sell Viresolve® Pro Devices over the years to come. We employ a number of complementary approaches to achieve this.

First, we have developed, validated and are performing a number of in-process and release tests used during the manufacture of the Viresolve® Pro membrane and Devices. These tests are performed using validated test methods and pre-defined sampling plans in order to measure and monitor critical performance attributes from membrane roll to roll, device to device, and lot to lot.

Second, we use information obtained from some of the critical membrane performance tests to selectively layer membrane in the final devices to reduce the overall performance variability.

Membrane Testing

Viresolve® Pro membrane process includes a number of in-process and lot release tests that are in place to assure product quality and consistency.

The following lot release testing are performed using a predefined sampling plan:

1. Bacteriophage φX-1741 retention test
2. Protein capacity test (using human-derived IgG (hIgG) as a model protein)
3. Water permeability test

1. Bacteriophage φX-174 Retention Test

The retention of φX-174 as a marker virus under controlled conditions provides evidence that the membrane meets or exceeds the minimum virus clearance target. The test is performed on samples from each membrane lot and roll based on a predetermined sampling plan. The test involves filtering a solution with φX-174 and human-derived IgG through membrane samples in a Micro Device format.

A filtrate grab sample is collected at 75% flow decay point from the initial buffer flux. The test offers the following benefits:

- Representative of the product application
- Foundation for measuring and tracking membrane performance as well as serving as a Quality Control test
- Consistent with the PDA Small Virus Task Force recommendations (PDA Technical Report No. 41, Virus Filtration, PDA *J Pharm Sci Technol Suppl*, Vol 59 No. S-2)

2. Protein Capacity Test

Given that the capacity performance of Viresolve® Pro Devices is primarily established during the membrane manufacturing process, Viresolve® Pro membrane lot release tests include a protein capacity test using hIgG as a surrogate protein marker at 0.1 mg/mL concentration in a 50 mM acetate buffer and 100 mM sodium chloride buffer. In this test, a solution with φX-174 and human-derived IgG is filtered through membrane samples in a Micro Device format. The capacity is measured at 75% flow decay point from the initial buffer flux and the membrane roll average capacity values must fall within the 2-sided specification.

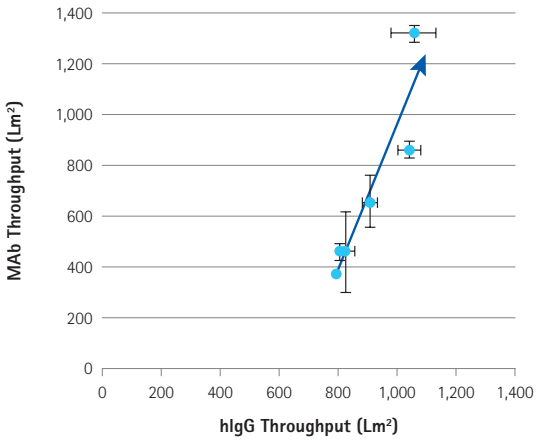
While membrane capacity results measured with hIgG are not predictive of the specific capacities

[1] φX-174 is a single-stranded RNA bacteriophage, approximately28 nm in diameter.

Robust Capacity

Figure 1.

Our protein capacity test method (hIgG) is consistent with that observed when tested with a customer's MAb



obtained from a particular user feed stream, which are a function of the virus filter, process stream characteristics and processing conditions, the data in Figure 1 demonstrates that general membrane ranking obtained using our protein capacity test method (hIgG) is consistent with that observed when tested with a customer's MAb. In this study, six different membrane samples with a range of hIgG capacity were tested in triplicate with both hIgG and a customer's MAb.

3. Water Permeability Test

Water permeability test is performed on Viresolve® Pro membrane samples to ensure consistent performance in the final application and to enable fast processing at the virus filtration unit operation. Samples of Viresolve® Pro membrane are tested for water permeability. In the test, single-layer membrane samples are tested at 25 psig and temperature normalized to 25°C.

Selective Layering*

Overview

Selective layering is the process of choosing the top and bottom layer based on the capacity specifications of membrane. Selective layering of the dual membrane layers provides a narrow range of membrane performance in Viresolve® Pro Devices.

Methods

Testing was performed in the Micro Device format and included capacity, φX-174 LRV and buffer flux testing. All layering order permutations were constructed and tested to prove that selective layering provides a narrow range of capacity, flux and LRV.

Results

Capacity is controlled primarily by the top layer of membrane in Viresolve® Pro Devices. Selection of top layer membranes with a narrow range of capacities results in a reduced capacity range and an increased mean capacity.

Flux is approximately the mean of the top and bottom layer membranes. Selective layering results in a reduced flux range, with the mean flux near the overall population mean.

LRV is additive of the top and bottom layer membranes. Selective layering narrows the LRV range.

Conclusions

This study shows, by selectively layering the dual membrane, we are able to provide a narrow range of membrane performance in Viresolve® Pro Devices.

*US 8,733,556 B2

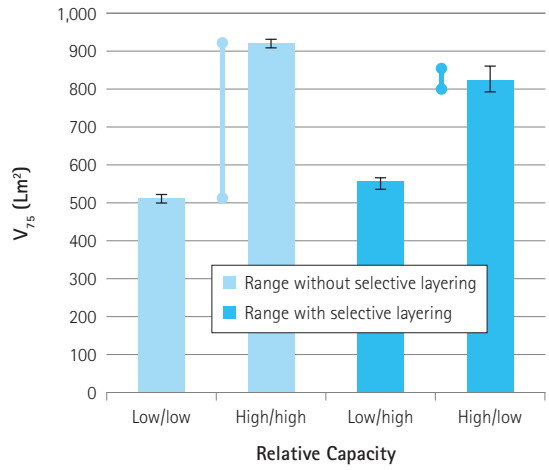


Figure 1.
Capacity of devices with layering of two membranes and the effect of selective layering

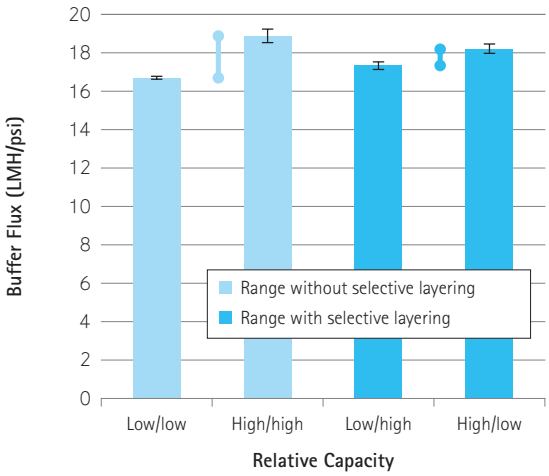


Figure 2.
Flux of devices with layering of two membranes and the effect of selective layering

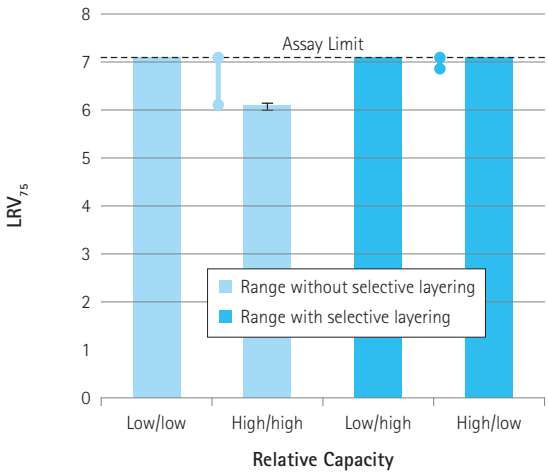


Figure 3.
LRV of devices with layering of two membranes and the effect of selective layering

Robust Capacity

Pre-conditioning

Introduction

Pre-treatment of feed streams has been shown to enhance the throughput of virus filters such as Viresolve® NFP and Viresolve® Pro Devices. The Viresolve® Prefilter provides performance enhancement for parvovirus filters such as Viresolve® NFP and Viresolve® Pro Devices. Pre-treatment of feed streams can remove plugging species, resulting in more favorable economics and more robust processing. Historically, the Viresolve® Pro Prefilter has given an average capacity enhancement of at least two fold over both the stand alone Viresolve® NFP and Viresolve® Pro Devices.

The Viresolve® Pro Shield provides an absorptive polishing step that enhances the capacity of the device by an average of a two-fold throughput. Results of the Viresolve® Pro Device capacity enhancement are shown in this section.

We were issued two patents that cover the use of adsorptive depth filters and charged or surface modified microfiltration (MF) membranes to remove aggregates from protein solutions in order to enhance performance of virus filters.

US Patent 7,118,675 B2 covers the use of adsorptive depth filters or charged or surface-modified microfiltration membranes in front of a viral filter to enhance the virus filter performance by removing aggregates from protein solutions.

US Patent 7,465,397 B2 involves the process of removing aggregates and viruses using a depth filter upstream of a viral filter in either a constant pressure or pump system. Adsorptive depth filters and modified microfiltration filters that allow for increased capacity and robustness of our virus filters are part of the Viresolve® offering.

As part of our continued goal to provide our customers with the right tools and technologies to perform effective and robust virus filtration, we provide customers with a free license to use the process with prefilters and viral filters purchased from us.

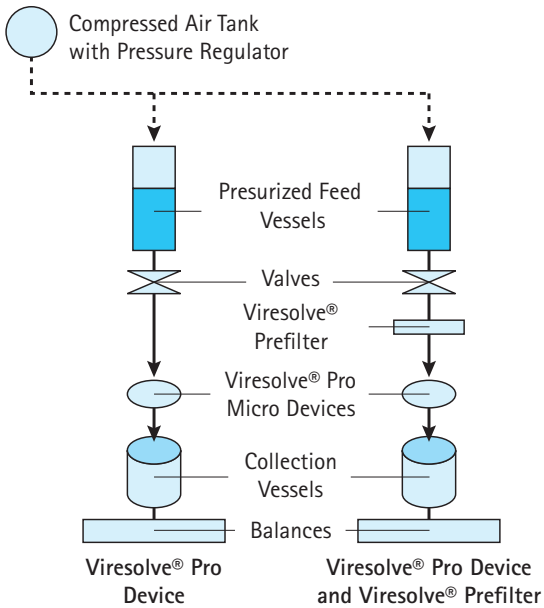


Figure 1.
Experimental setup

Viresolve® Pro Device Capacity Enhancement with Viresolve® Prefilter (VPF)

Overview

The objective of this study was to compare the performance of the Viresolve® Pro Devices with the use of a Prefiltration step prior to the retentive device. The primary focus of this study is the effect on capacity.

Materials and Methods

The Viresolve® Prefilter (VPF) combines the filtration benefits of cellulosic filtration media and diatomaceous earth. The composite media contains anionic, cationic and phobic functionality for retention of a wide variety of plugging impurities. In addition, a tight membrane layer has been added to reduce potential fiber shedding and to serve as a flow distributor.

Tests were carried out using customer MAbs either at our facilities or at customer sites, using Viresolve® Pro Micro Devices. In most cases, the tests were carried out using constant pressure of 30 psi (2 bar). Viresolve® Prefilter (5.0 cm^2) was run in line with Viresolve® Pro Micro Devices (3.1 cm^2). Figure 1 shows the experimental set up.

Results

The data also show that the average V_{75} capacity for Viresolve® Prefilter and Viresolve® Pro Devices increases in comparison to the Viresolve® Pro Devices alone. In general, the in-line prefilter increases capacity from an average of 7.5 to 13 kg/m^2. The data also shows that the prefilter further increase in capacity in the higher feed concentrations.

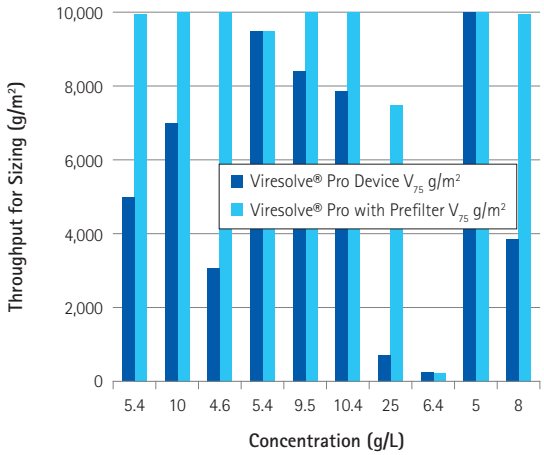


Figure 2.
Capacity for molecules tested on Viresolve® Pro Device alone first, and then with Viresolve® Prefilter and Viresolve® Pro Devices together

Robust Capacity

Viresolve® Pro Device Capacity Enhancement with Viresolve® Pro Shield.

Overview

The objective of this study was to compare the performance of the Viresolve® Pro Devices with the use of an adsorptive polishing filter step. The primary focus of this study is the effect on capacity.

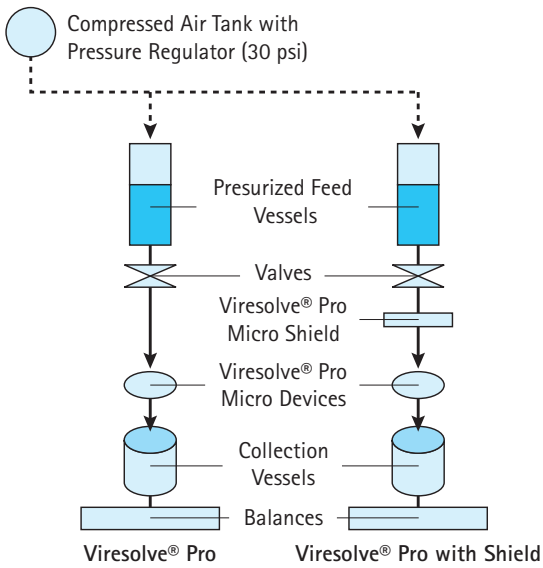


Figure 1.
Experimental setup

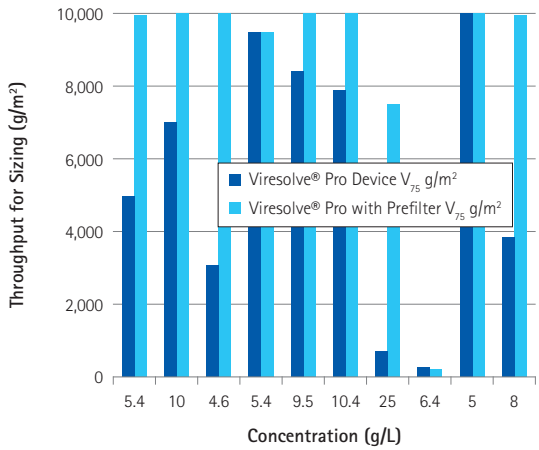


Figure 2.
Capacity for molecules tested on Viresolve® Pro Device alone first, then with Viresolve® Pro Shield and Viresolve® Pro Device together

Methods

Tests were carried out using customer MAbs either at our facilities or at customer sites, using Viresolve® Pro Micro Devices and Viresolve® Pro Micro Shields. In most cases, the tests were carried out using constant pressure of 30 psi (2 bar). The Viresolve® Pro Shield provides an adsorptive polishing step prior to the Viresolve® Pro Device. Viresolve® Pro Micro Shields (3.1 cm^2) were used in this study to run in line with the Viresolve® Pro Micro Devices (3.1 cm^2). Figure 1 shows the experimental set up.

Results

These data show that the average V_{75} capacity for Viresolve® Pro Device plus Viresolve® Pro Shield is approximately doubled compared with Viresolve® Pro Device alone. Throughput was sized based upon 75% flow decay or a four hour processing time when V_{75} was not reached. For certain molecules with high throughput, trials were flow-limited and for some other molecules, trials were limited by the amount of available feed stock. For these reasons data has been truncated at 10 kg/m^2. The purity of all the molecules tested is not well known; therefore, the capacities generated may be lower than expected with representative feed stock.

For eight MAb at 6.4 g/L concentration, the Viresolve® Pro Device plus Viresolve® Pro Shield did not offer improved capacity compared with the Viresolve® Pro Device alone. The buffer conditions of this molecule were pH 7.2 and high ionic strength (30 mS/cm), which would negate the charge mechanism of the adsorptive polishing step. For the majority of molecules tested, the buffer conditions (pH and conductivity) supported a significant performance enhancement by Viresolve® Pro Shield.

Process Optimization

Introduction

The capacity of Viresolve® Pro Devices may be affected by the conditions under which the filters operate. The location of the virus filtration step in the downstream process train, the concentration of the feed solution, the pH and conductivity of the feed solution, the operating pressure or flux, the volume of the product recovery flush and the use of a feed pre-treatment can be important considerations. Depending on the particular feed stream, these factors may influence the flux or capacity of the filters. Therefore, virus filters such as the Viresolve® Pro Device can be optimized with respect to the processing conditions in order to assure an economical and robust operation.

This section presents data demonstrating Viresolve® Pro Device viral retention and throughput performance for a range of various operating conditions.

Protein Concentration

Viresolve® Pro Device Performance in Presence of φX-174 and Different Concentrations of MAb 1

Overview

The objective of this study was to evaluate the performance of Viresolve® Pro Devices across a range of protein concentrations. MAb 1 at concentrations of 1.9, 5.7, 9.0 and 17.3 g/L was spiked with φX-174 virus. MAb 1 loading at 50% flow decay ranged 1.7–5.7 kg/m^2 among the four feed concentrations. Viral clearance was equal to or greater than 6.4 LRV for all concentrations. This study demonstrated an optimal capacity and robust virus retention across a range of MAb 1 concentrations.

Methods

All testing and assays were performed at our facility with four Viresolve® Pro Micro Devices at each concentration. All devices contained the same selective layering membrane combination.

An outside supplier provided the MAb 1 solution. The solution was concentrated using Pellicon® 2 Mini cassette (30 kDa) to achieve higher concentrations for this study. The concentrated solution was then serially diluted for final feed concentrations 1.9, 5.7, 9.0 and 17.3 g/L. MAb 1 concentrations were measured by OD_{280} readings. The solutions were spiked with φX-174 virus to a target titer of approximately 10^7 pfu/mL and then 0.22 µm filtered. Water and buffer flux were measured at 30 psig feed pressure. Four devices were challenged for each of the MAb 1 solution concentrations at 30 psig feed pressure. When the devices reached approximately V_{50}[2], grab and pool samples of the filtrates were collected. These samples were assayed using standard plaque assays to determine viral clearance.

Results

Water and buffer permeability values were consistent among all devices with less than 10% variability.

Average throughput at V_{50} results are summarized as a function of the MAb concentration in Figure 1. Throughput extrapolation to V_{50} was performed on some devices since V_{50} test endpoints were estimated during the testing. The flux decay for the individual devices are presented in Figure 2 as a function of mass throughput. Mass throughput was highest at 5.7 g/L concentration. Mass throughput was significantly limited at higher concentrations due to high fouling rates.

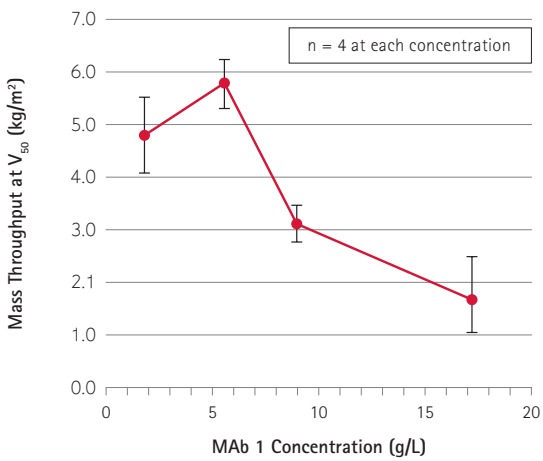


Figure 1.
Mass throughput of MAb 1 with φX-174 spike at four different protein concentrations

[2] V_{50} was selected due to limitations in amount of feed supplied.

Process Optimization

Figure 2.

Flow decay for individual Viresolve® Pro Micro Devices as a function of mass throughput

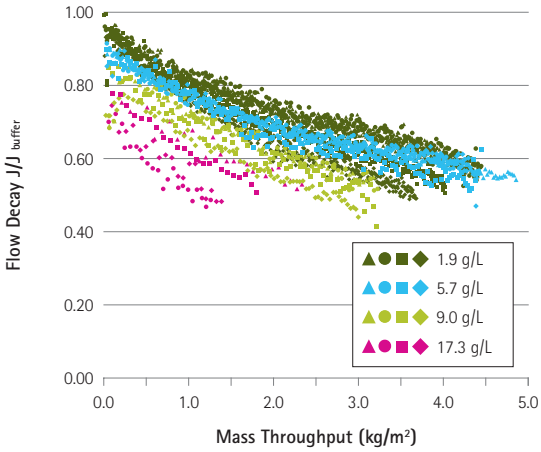


Figure 3.

φX-174 grab LRV as function of MAb 1 concentration

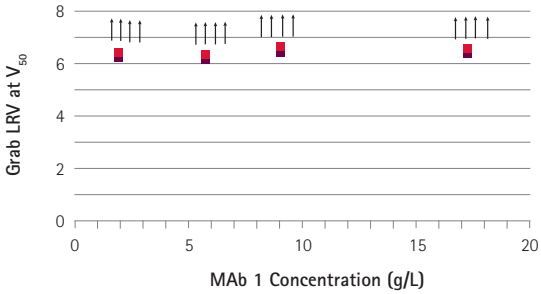
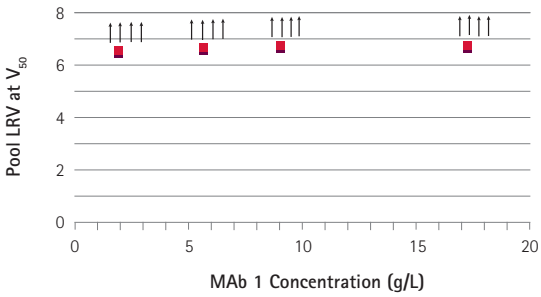


Figure 4.

φX-174 pool LRV as function of MAb 1 concentration



φX-174 LRV results for individual devices are shown in Figures 3 and 4. Grab and pool filtrate samples were collected between 43–53% flow decay. All LRV results were beyond the limit of detection (≥6.4 LRV). Robust viral clearance of Viresolve® Pro Solution was demonstrated as LRV values were independent of MAb 1 concentration.

Viresolve® Pro Device Performance in Presence of MMV and Different Concentrations of MAb 5

Overview

The objective of this study was to evaluate the performance of Viresolve® Pro Devices with a range of protein concentrations. MAb 5 was used as a protein stream at concentrations of 2, 7, 15 and 25 g/L with Mouse Minute Virus (MMV).

Actual mass loading ranged from 2.0–20.4 kg/m^2 across the four feed concentrations.

Viral clearance greater than four logs was observed across all conditions with several samples yielding no detectable virus in the filtrate pools. In all cases, no protein retention was observed by the membrane.

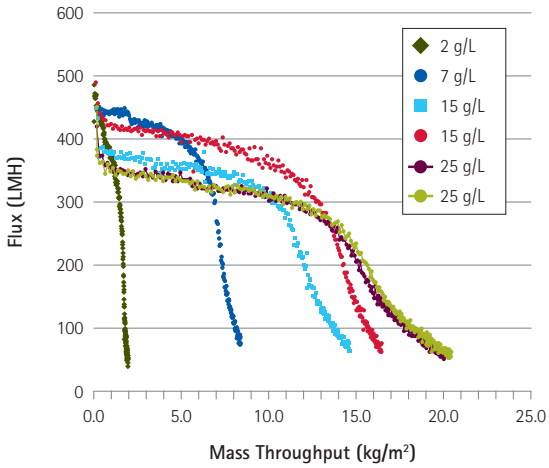


Figure 1.
Mass throughput of MAb 5 with MMV spike at different concentrations.

Methods

All testing and assays were performed at our facilities using Viresolve® Pro Micro Devices from the same membrane roll combination.

Feeds were supplied to us as 2, 7, 15 and 25 g/L solutions in a phosphate buffer. Before testing feeds were 0.22 µm filtered then prefiltered through a Viresolve® Prefilter prior to addition of virus. The Viresolve® Prefilter was run de-coupled from the Viresolve® Pro Devices. Viresolve® Pro buffer permeability was performed at 50 psi for two minutes,
and then at 30 psi for 15 minutes or until flow rate stabilized. Devices were challenged at 30 psi with protein solutions spiked with MMV to a target titer of 2 x 10^6 $TCID_{50}$/mL.

Samples were collected from the filtrate pools at designated volumetric throughput and samples were assayed for infectivity using the standard cell based $TCID_{50}$ assays. Protein levels were measured in all samples by OD_{280} readings.

Results

MMV LRV results and hydraulic data for individual Viresolve® Pro Micro Devices are presented in Table 1.

All devices were stopped after reaching 80–90% flow decay (approximately four hours after initiation of the test). Effective clearance of MMV was observed across all concentrations with LRVs greater than 4.8, indicating that retention by Viresolve® Pro Devices is not impacted by protein concentration.

Load (g/L)	Mass Throughput (kg/m^2)	LRV at 50% Target Volumetric Throughput	Final % Flow Decay	Final Pool LRV
2	2.0	N/A	93	≥5.8
7	8.4	≥6.1	86	5.8
15	14.6	≥5.8	87	≥5.8
15	16.3	5.3	89	4.8
25	20.2	5.4	88	5.7
25	20.4	≥6.0	90	≥6.0

Table 1.
Retention of MMV in MAb 5 solution at varying concentrations

Process Optimization

Viresolve® Pro Device Performance in Presence of MMV and Two Concentrations of MAb 4

Overview

The objective of this study was to evaluate the capacity and MMV retention performance of Viresolve® Pro Micro Devices with MAb 4 at two concentrations (10 and 15 g/L). Mass throughput achieved for the 10 and 15 g/L feed was 5.0 and 5.6 kg/m^2, respectively. No virus was detected in the filtrate pool samples, demonstrating robust viral clearance by Viresolve® Pro Devices at these relatively high-protein concentrations.

Methods

All testing and assays were performed at our facility using our virus stocks. MAb 4 protein solutions were supplied at 10 and 15 g/L concentrations. Duplicate devices for each protein concentration were challenged with feed containing MMV (spiked to a target titer of 4 x 10^6 $TCID_{50}$/mL). All testing was performed at 30 psig pressure. Filtrate pool samples were collected at the end of the test and assayed (2.5–3.2 mL volumes) for infectivity using standard cell-based $TCID_{50}$ assays.

Results

Effective clearance of MMV was observed at both protein concentrations with LRVs ≥5.1, indicating robust viral retention by Viresolve® Pro Devices. MMV LRV results and hydraulic data for individual Viresolve® Pro Micro Devices are presented in Table 1. Figure 1 shows that with MAb 4, mass throughput is similar at the two protein concentrations tested.

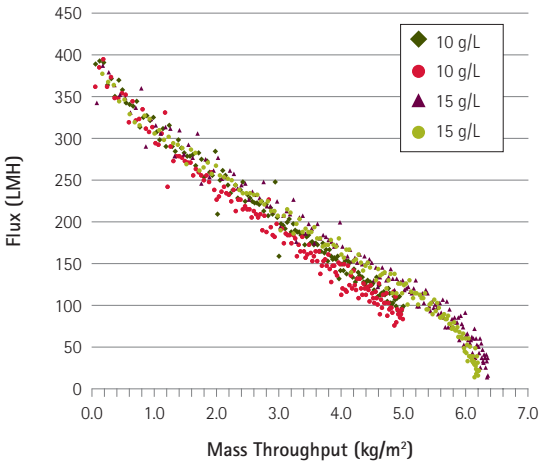


Figure 1.
Mass throughput of MAb 4 at two concentrations in presence of MMV spike

Table 1.
Performance of MAb 4 solution at two concentrations in presence of MMV

Feed Concentration (g/L)	V75 Throughput (kg/m2)	Final % Flow Decay	Final Pool LRV
10	5.0	75	≥5.1
10	5.0	75	≥5.1
15	5.6	96	≥5.2
15	5.6	95	≥5.3

Viresolve® Pro Device Performance at Different Constant Operating Pressures in Presence of MAb 1 and φX-174

Overview

The objective of this study was to characterize the capacity and virus retention performance of Viresolve® Pro Devices across a range of constant operating pressures. The feed stream contained MAb 1 protein and φX-174 as a model parvovirus. This study demonstrated enhanced capacity at higher pressure and robust retention across all pressures for the feed stream tested.

Methods

All testing and assays were performed at our facilities. Studies were performed with nine Viresolve® Pro Micro Devices made from the same membrane roll combination. All devices were wet with water at a minimum pressure of 30 psig. Water and buffer flux were measured at 10, 30 and 50 psig feed pressure. Devices were challenged at 10, 30 and 50 psig with a solution containing 6 g/L MAb 1 and φX-174 spiked to a target titer of approximately 10^7 pfu/mL. Devices were tested for retention of bacteriophage φX-174 by testing samples of the filtrate collected at V_{50}.

Results

Water and buffer permeability values were constant (less than 10% variability) across the range of operating pressures tested.

Filtrate flux as a function of volumetric throughput for each device, are presented in Figure 1. The initial flux was directly proportional to the pressure. Higher operating pressures corresponded to higher flux measured through the filtration process.

Average capacity results (V_{50}) for each pressure are shown in Figure 2. Capacity at 10 and 30 psig were comparable, but capacity was 20% higher on average at 50 psig compared to 30 psig. This relationship between pressure and capacity may be molecule-specific and should be evaluated on a case-by-case basis.

φX-174 retention results for grab samples at V_{50} are presented in Table 1. Robust virus retention (>5 logs) was observed at all pressures tested.

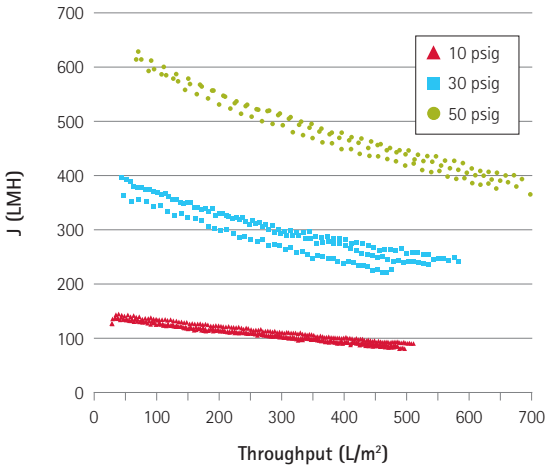


Figure 1.
Filtrate flux as a function of volumetric throughput at different pressures for each device

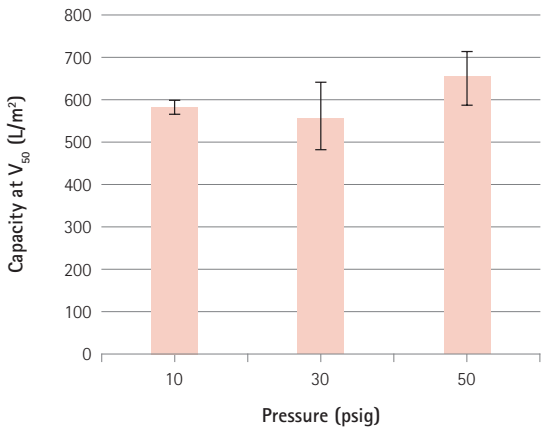


Figure 2.
Average capacity (V_{50}) at each operating pressure

Pressure (psig)	LRV_{50}
10	≥6.4
	≥6.4
	≥6.4
30	6.4
	5.7
	6.4
50	>6.6
	>6.6
	6.3

Table 1.
φX-174 retention values for grab samples at V_{50}

Viresolve® Pro Device Performance at Different Pressures in Presence of MAb 3 and φX-174

Overview

The objective of this study was to demonstrate improved capacity of Viresolve® Pro Micro Devices at increased pressures while maintaining robust viral retention. MAb 3 was used as a protein stream and φX-174 as a parvovirus model. This study demonstrates robust retention and capacity of φX-174 by Viresolve® Pro Devices at 10 and 50 psi operating pressure at V_{90}.

Methods

All testing and assays were performed at our facilities. φX-174 studies were performed with two Micro Devices from the same membrane roll combination. Permeability of devices was measured at 10 and 50 psi. Devices were challenged at 10 and 50 psi with 25 g/L MAb solution spiked with φX-174 to a titer of 5×10^7 pfu/mL. Grab samples from the filtrate were taken at 10 L/m^2 and again when the filters reached approximately V_{90}. An additional sample was taken from each filtrate pool at V_{90}.

Results

φX-174 LRV results for individual Micro Devices are presented in Table 1.

Virus was not detected in any of the filtrate samples of the test run at 10 psi. However, virus was detected in the V_{90} grab and pool samples from the 50 psi test.

Higher operating pressure resulted in higher flux with faster fouling of the membrane. Robust retention of bacteriophage was observed, even at 90% flow decay.

Table 1.
Performance of MAb 3 solution at two concentrations in presence of φX

Pressure (psig)	Final Throughput (L/m^2)	Final % Flow Reduction	Mass Loading (kg/m^2)	V_{90} Grab LRV	V_{90} Pool LRV
10	43.0	91	1.08	≥7.2	≥7.2
50	54.0	93	1.35	6.7	7.2

Product Recovery Flush

Retention of φX-174 and MMV Following Recovery of hIgG Using Viresolve® Pro Devices

Overview

The objective of this study was to demonstrate robust viral retention of Viresolve® Pro following flushing with buffer. Human IgG was used as a model protein stream and MMV and φX-174 as parvovirus models. This study demonstrates robust viral retention of Viresolve® Pro Micro Devices during IgG product recovery.

Methods

All testing and assays were performed at our facilities. φX-174 studies were performed with nine Micro Devices from three different membrane roll combinations (further referred to as A, B or C). MMV studies were performed with six Micro Devices from two membrane roll combinations (further referred to as B or C). Buffer flux was measured at 30 psi feed pressure. Devices were challenged at 30 psi with a solution containing 0.1 g/L hIgG and φX-174 at a target titer of 10^7 pfu/mL until approximately V_{75}, corresponding to a mean throughput of approximately 650 L/m^2. For MMV testing, devices were challenged with approximately 10^6 $TCID_{50}$/mL MMV until approximately V_{90}, corresponding to a mean throughput of approximately 550 L/m^2. When the end of the test was reached, the valve upstream of each Micro Device was closed, and the feed source was switched to buffer. The upstream valves were then opened and the Micro Devices flushed with buffer. Flush samples were collected in the same vessel as the filtrate and samples from this combined pool were collected at 10 L/m^2 and 5% of volumetric throughput. Samples were assayed for presence of bacteriophage by plaque assay or MMV (~1.6 mL assay volume) using cell based $TCID_{50}$ assays.

Results

φX-174 LRV results for individual Micro Devices are presented in Table 1. No virus was detected in any of the pool samples for seven of the nine devices tested; a single plaque was detected in the filtrate pools for the remaining two devices following recovery of the hIgG.

Table 1.
Retention of φX-174 following recovery of hIgG solution

	Replicate Collection Points		
	φX/IgG Filtration	Buffer Flush	
Viresolve® Pro Membrane Roll Combination	V_{75} LRV	LRV at 10 L/m^2 Flush	LRV at 5% Volumetric Throughput
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

Table 2.
Retention of MMV following recovery of hIgG solution

	Replicate Collection Points		
	MMV/IgG Filtration	Buffer Flush	
Viresolve® Pro Membrane Roll Combination	V_{90} LRV	LRV at 10 L/m^2 Flush	LRV at 5% Volumetric Throughput
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

MMV LRV results following product recovery are presented in Table 2. Most devices showed no detectable virus in filtrate pools after flushing with buffer and those with measurable virus in the filtrate pools, showed LRV values equal to or greater than 4.6.

These results confirm robust viral retention during recovery of hIgG following filtration.

Table 1.
Retention of φX-174 in MAb 2 solution (pool samples)

Viresolve® Pro Membrane Roll Combination	V_{90} LRV	Post Recovery LRV
A	≥5.1	≥5.1
	≥5.1	≥5.1
	≥5.1	≥5.1
B	≥5.1	≥5.1
	≥5.1	≥5.1
	≥5.1	≥5.1

Table 2.
Retention of MMV in MAb 2 solution (pool samples)

Viresolve® Pro Membrane Roll Combination	V_{90} LRV	Post Recovery 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

Retention of φX-174 and MMV Following Recovery of MAb 2 Using Viresolve® Pro Devices

Overview

The objective of this study was to demonstrate robust viral retention of Viresolve® Pro Micro Devices following flushing with buffer. MAb 2 was used as a protein stream, and MMV and φX-174 as parvovirus models. This study demonstrated robust viral retention of Viresolve® Pro Device at extended flow decay and also following recovery of MAb product.

Methods

All testing and assays were performed at our facilities. φX-174 and MMV studies were performed with at least two Viresolve® Pro Micro Devices each from two different membrane roll combinations (further referred to as A or B). Buffer flux was measured at 30 psig feed pressure. Devices were challenged at 30 psig with MAb 2 containing φX-174 at a target titer of 10^6 pfu/mL or 2.5 x 10^6 $TCID_{50}$/mL MMV until approximately V_{90}. Mean volumetric throughputs for the φX-174 and MMV tests were 292 and 186 L/m^2 respectively. When the end of the test was reached, samples were collected from the filtrate pools, and the valve upstream of each Micro Device was closed and the feed source was switched to buffer. The upstream valves were then opened and the Micro Devices flushed with buffer. Flush samples were collected in the same vessel as the filtrate. This combined pool was sampled after the Micro Devices had been flushed with a volume corresponding to 5% of volumetric throughput. Samples were assayed for presence of bacteriophage by plaque assay or MMV (~4.8 mL sample volumes) using cell based $TCID_{50}$ assays.

Results

φX-174 LRV results for pool samples from individual Micro Devices are presented in Table 1. No virus was detected in any of the filtrate pool samples.

MMV LRV results for pool samples from individual Micro Devices are presented in Table 2. No virus was detected in any of the V_{90} pools. Most of the Micro Devices had no detectable virus in the filtrate pool following buffer flush however a low level of virus was detected in one filtrate pool.

Viral Clearance

Introduction

Manufacturers of biological drug products are required to incorporate sufficient virus clearance steps into their manufacturing processes to ensure that their products are virus contaminant-free[1]. In this section, data is presented on both mammalian and bacteriophage viral clearance studies using multiple MAb feed streams. The effect of different virus preps and % spikes on throughput and virus retention is also examined, while a separate study demonstrates that the Viresolve® Pro Devices meet the acceptance criteria for the small virus retentive filter test recommended by the Parenteral Drug Association (PDA). Discussion is included around the topic of virus validation using Viresolve® Pro Devices, including key considerations, study design, interpretation of results, and alternative spiking strategies. Through the use of virus spikes and our liquid-liquid porometry (LLP) test, the size exclusion principle is demonstrated as the Viresolve® Pro Solution's primary mechanism for viral clearance. Additionally, data is presented on select competitive virus filters for comparison.

References

1. "Ensuring Regulatory Compliance: Validation of Virus Filtration", Merck Millipore, Lit. No. AN1650EN00.

Filtration Endpoint: V_{75} versus V_{90}

Viresolve® Pro Retention of MMV in Presence of MAb Solutions

Overview

The objective of this study is to demonstrate robust retention of Viresolve® Pro Devices across a range of flow decay endpoints using MMV. Two commercial MAbs, designated MAb 1 and MAb 2, were used as representative low and high-fouling protein streams, respectively. No virus was detected in filtrate pools demonstrating robust retention of MMV by Viresolve® Pro Devices, up to V_{90}. Complete retention was observed to mass throughput in excess of 12 kg/m^2 for MAb 1 and up to 2 kg/m^2 for MAb 2.

Methods

All testing and assays were performed at a Contract Testing Organization (CTO). Three Micro Devices from a single membrane roll combination were used for each MAb. Buffer flux was measured at 30 psig feed pressure. Devices were challenged at 30 psig with MAb 1 (6 g/L) or MAb 2 (4.5 g/L) and MMV (from a CTO) at a minimum titer of 4 x 10^6 TCID50/mL. For MAb 1, samples were collected from the filtrate pool at three points between approximately V_{50} and V_{70} (sampling at V_{90} was limited by feed availability). For MAb 2, samples from the filtrate pools were collected at approximately V_{50}, V_{75} and V_{90}. Each 4 mL sample collected was assayed by cell-based $TCID_{50}$ assays to determine retention of MMV.

Viral Clearance

Figure 1.
Retention of MMV in MAb solutions (pool samples)

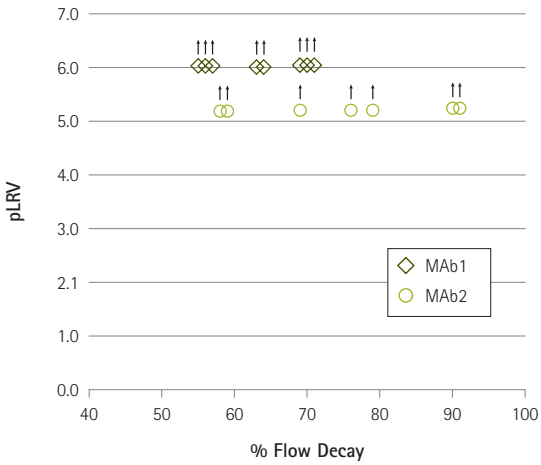
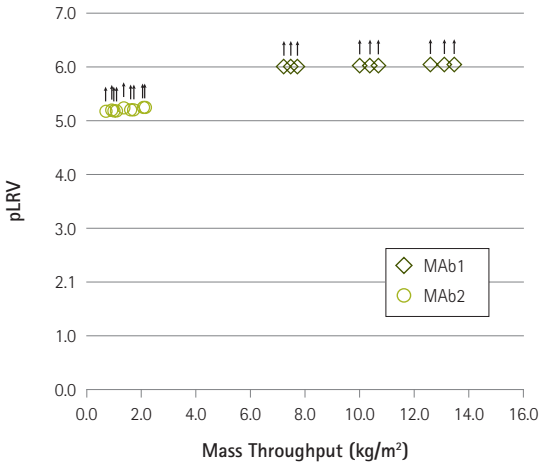


Figure 2.
Retention of MMV as a function of mass throughput (pool samples)



Results

MMV LRV results for individual Micro Devices are presented in Figure 1. No virus was detected in any of the filtrate pool samples. Complete retention was observed to mass throughput in excess of 12 kg/m^2 for MAb 1 and up to 2 kg/m^2 for MAb 2, as seen in Figure 2.

Viresolve® Pro Retention of φX-174 and MMV in Presence of hIgG

Overview

The objective of this study was to demonstrate robust retention of Viresolve® Pro Devices across a range of flow decay endpoints. Human IgG was used as a model protein stream and MMV and φX-174 as parvovirus models. This study demonstrates robust retention of φX-174 and MMV by Viresolve® Pro Devices, up to V_{90}.

Methods

All testing and assays were performed at our facilities. φX-174 and MMV studies were performed with six Micro Devices, each from two different membrane roll combinations, designated as B and C. Buffer flux was measured at 30 psi feed pressure. Devices were challenged at 30 psi with a solution containing 0.1 g/L hIgG and a minimum titer of 10^7 pfu/mL φX-174 or 10^6 $TCID_{50}$/mL MMV. Devices were tested for either retention of bacteriophage φX-174 or MMV. Samples of the filtrate were collected at approximately V_{50}, V_{75} and V_{90}.

Results

φX-174 LRV results for individual Micro Devices are presented in Figure 1. No virus was detected in any of the filtrate grab samples and pool samples (not shown). MMV LRV results for grab and pool samples for the individual devices are presented in Figures 2 and 3 respectively. No virus was detected in V_{75} grab samples for 5 of the 6 devices and in V_{90} grab samples for 2 devices. Of the devices that showed passage of MMV, grab LRVs were greater than 4.5 through V_{90}. Pool LRVs were greater than 4.5 for all devices through V_{90}.

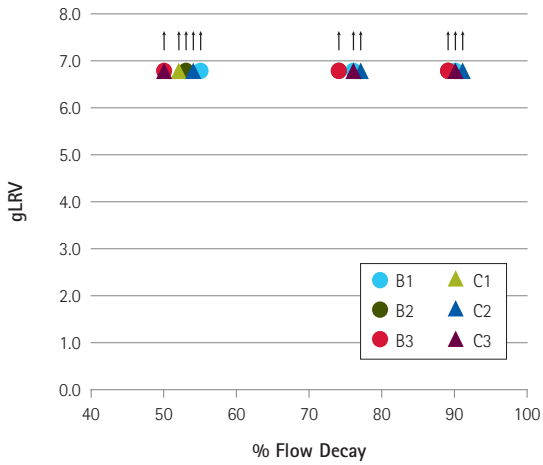


Figure 1.
Retention of φX-174 in hIgG solution

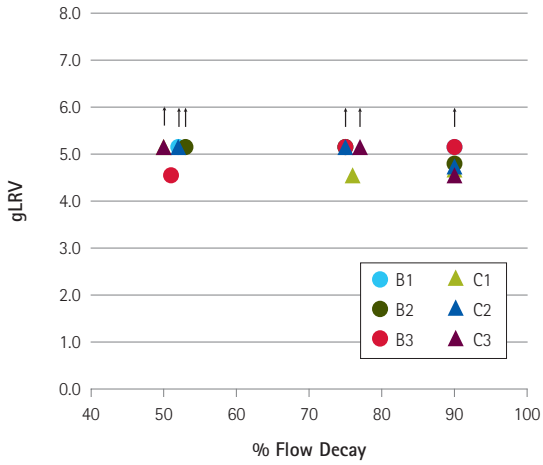


Figure 2.
Retention of MMV in hIgG solution (grab samples)

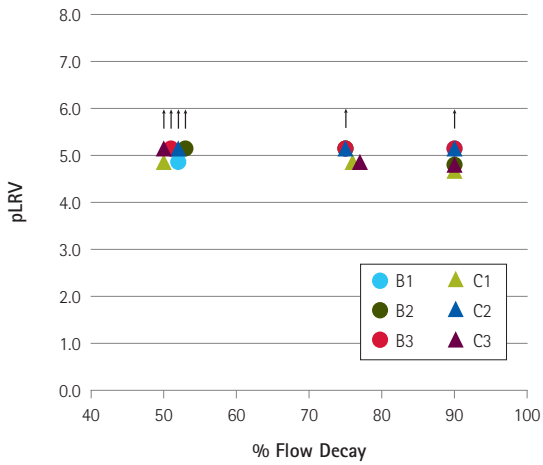


Figure 3.
Retention of MMV in hIgG solution (pool samples)

Viral Clearance

Figure 1.
Retention of φX-174 in MAb 2 solution (pool samples))

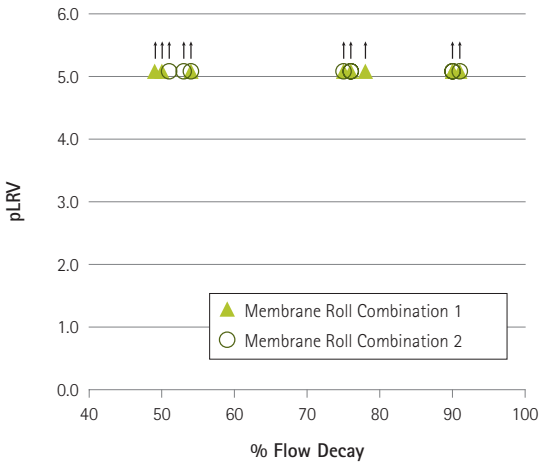
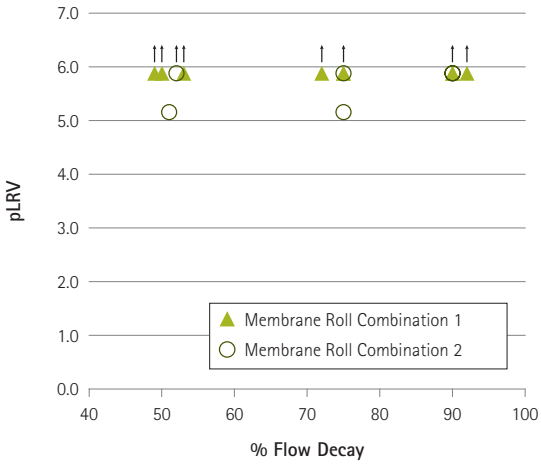


Figure 2.
Retention of MMV in MAb 2 solution (pool samples)



Viresolve® Pro Retention of φX-174 and MMV in Presence of MAb

Overview

The objective of this study was to demonstrate robust retention of Viresolve® Pro Devices across a range of flow decay endpoints using MMV and φX-174 as parvovirus models. A high fouling, commercial MAb designated MAb 2 (~4.5 g/L) was used as a model protein stream. This study demonstrates robust retention of φX-174 and MMV by Viresolve® Pro Devices, up to V_{90}.

Methods

All testing and assays were performed at our facilities. φX-174 and MMV studies were performed with six Micro Devices each from two different membrane roll combinations.

Buffer flux was measured at 30 psig feed pressure. Devices were challenged at 30 psig with a solution containing MAb 2 and with a target titer of 10^6 pfu/mL φX-174 or 2.5 x 10^6 $TCID_{50}$/mL MMV. Samples of the filtrate were collected at approximately V_{50}, V_{75} and V_{90} and tested for the presence of bacteriophage φX-174 by plaque assay or MMV using cell based $TCID_{50}$ assays.

Results

φX-174 LRV results for individual Micro Devices are presented in Figure 1. No virus was detected in any of the filtrate pool samples.

MMV LRV results for pool samples from individual Micro Devices are presented in Figure 2. No virus was detected in any of the V_{90} pool samples however, a low level of virus was detected in V_{50} and V_{75} samples from one device, but not in the V_{90} sample from the same device. Data from one of the devices tested with MMV is not shown owing to a known contamination of the filtrate pool.

Different Virus Preparation and Different Viruses

Impact of Virus Spike on Performance of Viresolve® Pro Devices

Overview

The objective of this study was to evaluate the impact of virus spike preparations from two independent CTO's on performance of Viresolve® Pro Micro Devices. Two commercial MAbs, designated MAb 1 (6 g/L) and MAb 2 (4.5 g/L), were evaluated with MMV and XMuLV virus preparations supplied by the two test laboratories. Following preliminary testing, the study was performed with complete retention of MMV and XMuLV by Viresolve® Pro Micro Devices. Working closely with the CTO virus validation laboratory prior to the validation study helped assure a successful outcome.

Methods

All testing was performed at two independent test laboratories (A and B), and was part of a larger study to evaluate mammalian virus retention by Viresolve® Pro Devices in the presence of commercial MAb solutions. Studies were performed with a single lot of Viresolve® Pro Micro Devices. Water and buffer flux were measured at 30 psi feed pressure. Devices were challenged at 30 psi with a solution containing MAb 1 or MAb 2, and different levels of either MMV or XMuLV virus spike. All spike levels are expressed as percentages of total volume. Baseline runs without virus spike were also performed at each test laboratory. The volumetric throughputs of the various tests were compared to determine the relative impact of virus spike as compared to unspiked baseline on performance of the two MAb solutions with Viresolve® Pro Micro Devices. In most instances, single devices were tested; however, when replicate devices were evaluated, mean values are shown with the standard deviation.

Results

Figure 1 shows the impact of XMuLV spike from two independent test laboratories, on performance of MAb 1 with Viresolve® Pro Micro Devices. Baseline performance of the MAb 1 was similar irrespective of test laboratory. The addition of virus spike at 0.1% (v/v) did not change the flux profile as compared to the unspiked feed; however, the addition of 1% (v/v) XMuLV spike from both labs reduced flux,

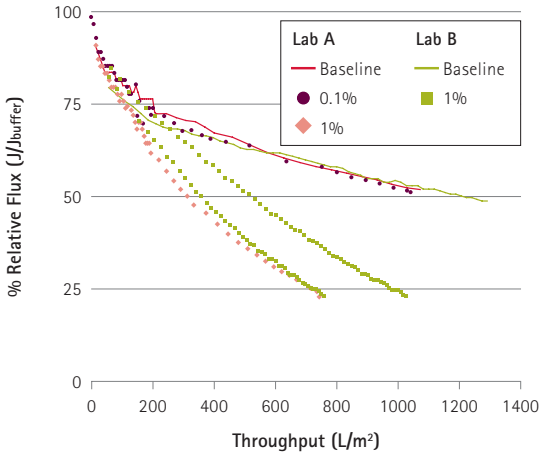


Figure 1.
Impact of XMuLV virus spike on volumetric throughput of MAb 1

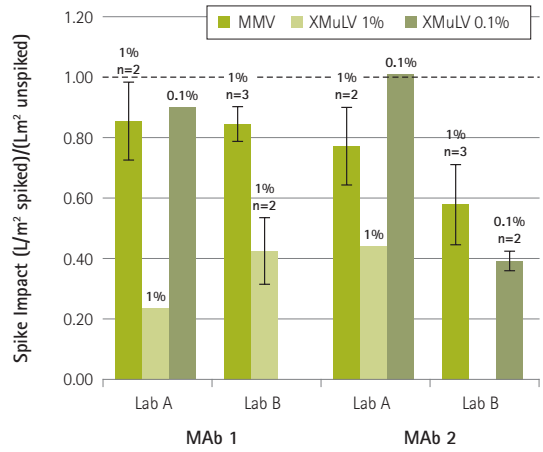


Figure 2.
Impact of virus spike on volumetric throughput of MAb 1 and MAb 2

Viral Clearance

Table 1.
Virus Retention with Viresolve® Pro Micro Devices

MAb	Virus	% Spike (v/v)	Test Lab	Average % Flow Decay	Pool LRV at Collection Point Device replicates		
Mab 1	MMV	1	A	53	≥4.8	≥5.0	
	MMV	1	B	70	≥6.0	≥6.0	≥6.0
	XMuLV	1	A	77	≥5.1		
	XMuLV	1	B	77	≥4.0	≥4.0	
Mab 2	MMV	1	A	85	≥5.4	≥4.4	
	MMV	1	B	75	≥5.2	≥5.2	≥5.2
	XMuLV	0.1	A	70	≥4.1		
	XMuLV	0.1	B	82	≥3.5	≥3.5	

indicating increased fouling as a consequence of addition of spike.

As a general phenomenon, the extent of the impact of spike on performance is dependent on virus spike, the laboratory providing the virus spike, and MAb solution. Figure 2 shows the impact of virus spikes from two different sources on throughput of MAb 1 and MAb 2. In this figure, the throughput of the various spiked feeds is expressed as a proportion of throughput of baseline unspiked feed.

MAb 1 comparisons are shown at V50; data for MAb 2 is shown at V70. Where replicate devices were tested, the number of replicates is noted. Baseline performance of each MAb is expressed as 1.00 and is shown as a dotted line.

Since the addition of the virus spike may alter the fouling characteristics of the feed stream, it is critical to determine the minimum spike that will permit sufficient throughput and also deliver target LRV. When optimum spike levels are identified, LRV can be estimated based on virus titer, cytotoxicity dilutions and assay volume of the filtrate before the validation study proceeds.

These data demonstrate the importance of pre-studies at the virus testing lab to determine the likely impact of the virus spike on the throughput before validation. With the appropriate design, throughput targets and high levels of LRV clearance can be achieved.

The results of these viral clearance studies are summarized in Table 1. No virus was detected in any filtrate sample, demonstrating that, with minimal optimization, Viresolve® Pro Micro Devices offer a robust viral clearance solution.

As no virus was detected in any filtrate samples, LRV determinations were based on titer values at the assay limit of detection.

PDA Small Virus Filter Nomenclature Test

PDA Small Virus Retentive Filter Rating Test on Viresolve® Pro Devices

Overview

The objective of this study was to demonstrate that Viresolve® Pro Devices meet the acceptance criteria for the small virus retentive filter test recommended by the Parenteral Drug Association (PDA)1.This test specifies a minimum LRF (Log Reduction Factor) of four (4) for PP7 bacteriophage and at least 90% transmission of Human IgG (hIgG). In addition, each filter must pass the vendor-specified integrity test. In this study, each device was conforming, demonstrating that Viresolve® Pro Devices achieve a rating of PP7-LRF4.

Methods

All testing and assays were performed at the FDA laboratory in Silver Spring, MD, following the test protocol recommended by the PDA. The protocol contains two portions—a test to measure virus retention and a test to measure protein transmission. For each of these two tests, three Viresolve® Pro Micro Devices were tested from each of three different membrane roll combinations, for a total of nine devices for each test. Before each device was tested, the water and buffer flux were measured at 30 psig.

For the virus retention portion of the test, devices were challenged at 30 psig with a solution containing 1 g/L of bovine serum albumin (BSA), a minimum titer of 10^7 pfu/mL PP7, and a minimum titer of 10^4 pfu/mL of PR772 (both viruses obtained from FDA). Sample titers were determined by the FDA using an infectivity assay. Grab samples were collected at 25 L/m^2 throughput and pool samples were collected at 50 L/m^2 throughput.

For the protein transmission portion of the test, devices were challenged at 30 psi with a solution containing 2 g/L of hIgG. Grab samples were collected at 30 L/m^2 throughput and the protein concentration was determined spectroscopically from the sample absorbance at 280 nm wavelength.

Results

No virus was detected in the filtrate grab and pool samples of all devices challenged with PP7 and PR772. The reported LRV was ≥7.4 for PP7 and ≥4.3 for PR772. Transmission of hIgG was 95% or greater for all devices tested. Based on these results, Viresolve® Pro Devices meet all of the nomenclature acceptance criteria for the PDA Small Virus Filter: PP7-LRF4.

Validation

Background/Requirements: Viral Clearance Validation

Overview

The overall aim of a virus spiking study is to assess the effectiveness of the chosen unit operation in clearing viruses from the feed stream. The nature of a virus spiking study depends on two main factors: the source of the drug product and the development phase. Biopharmaceutical products that are cell-line derived (such as CHO and NS0) will be challenged with viruses that are endogenous to that cell line or represent similar characteristics. Early stage products may only be tested for retrovirus clearance whereas late stage clinical products are usually tested more extensively using a panel of 4–5 viruses. Manufacturers aim to demonstrate LRVs between 4 and 6 per unit operation, with a total of 12 to 15 throughout the entire process2.

Study Design and Key Considerations

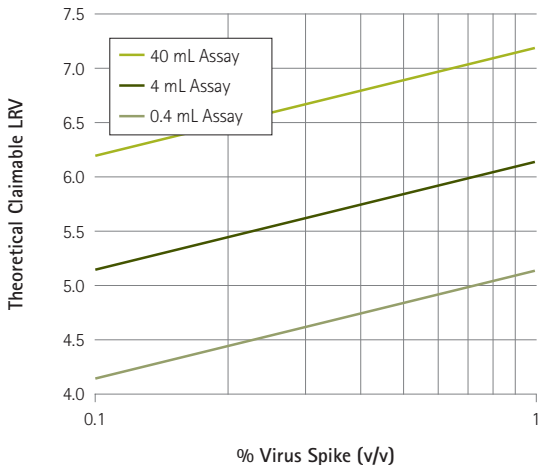
For the virus filtration step, the target throughput is typically determined through process development studies with scale-down Viresolve® Pro Micro Devices. The objective of these studies is to demonstrate achievable throughput with representative feed stock. Additional studies could be executed to maximize target throughput through the optimization of pre-treatment strategies, operating pressure/flux, protein concentration, and product recovery. To ensure success at the virus validation lab, this work should be completed prior to the validation study.

The small-scale validation study, typically performed with Micro Devices from Viresolve® Pro Validation kits, is intended to reflect the viral clearance capability of the large-scale manufacturing process, typically performed with Viresolve® Pro Magnus Devices. Therefore, it is important to scale down the key operating parameters that have an effect on viral clearance and filtration performance, such as throughput, operating pressure/flux, process time, protein concentration, feed quality, and device. Viresolve® Pro Validation kits provide Micro Devices with multiple membrane lots, which may be used to ensure that the scale-down devices are representative of large-scale manufacturing devices.

Viral Clearance

Figure 1.

Theoretical impact of % virus spike and filtrate assay volume on claimable LRV (assumes 10^8 virus $TCID_{50}$/mL and feedstock volume of 200 mL)



Since the addition of currently available virus spikes may alter the fouling characteristics of the feed stream, the minimum spike should be determined that will permit sufficient throughput while also delivering the target LRV. This can be estimated based on the virus titer, any cytotoxicity as identified by the validation lab, and the chosen filtrate assay volume. If no virus is detected in the filtrate, increasing the filtrate assay volume will increase the claimable LRV, allowing for a corresponding reduction in virus spike, as shown in Figure 1.

Once at the validation lab, the first step is to confirm the baseline performance of the feedstock on Viresolve® Pro Micro Devices without the addition of the virus spike. Ideally, it should match the performance obtained in process development. If any reduction in performance is observed due to shipping, age, or freeze/thaw effects, then additional pre-treatment methods can be used to restore the feed to its original state before the addition of the virus spike.

The second step is to examine the actual effect of various % virus spikes on the throughput of Viresolve® Pro Micro Devices. If one or more of the runs meet the targets for both throughput and LRV as predicted earlier, then it is possible to use those conditions to proceed with the validation study. However, if the throughput is limited with the required % virus spike, then several options or a combination thereof can be considered:

- Use a higher % spike to meet the target LRV and accept a lower throughput
- Use a lower % spike to meet the target throughput and accept a lower LRV
- Assay larger volumes to reach the target LRV
- Use a higher titer virus stock from the validation lab
- Use an alternative virus spiking method [3, 4, 5]

Once the final strategy is settled upon, the validation study can be conducted. The outcome of the study is governed by the target throughput, target LRV and study cost.

Conclusion

In order to implement Viresolve® Pro Devices into a biopharmaceutical manufacturing process, virus validation studies are required to document adequate viral clearance. Therefore, proper study design is critical to ensuring its success, including scale-down validity, feedstock quality, % virus spike, and assay volume. We can provide technical expertise and support to expand on the key considerations highlighted here.

References

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Cleanliness and Sanitization Methods

Introduction

Viral clearance is not a sterile unit operation, but bioburden control is necessary within manufacturing processes. For users who would like to sanitize devices prior to use, Viresolve® Pro Devices are caustic stable and can be sanitized with 0.5 N NaOH prior to use. This allows for assurance of bioburden reduction in the viral clearance step.

Caustic Flush Procedure

Overview

This section describes the recommended caustic and flush procedure for Viresolve® Pro Devices.

Methods

Initial wetting and flushing can be performed with only 50 L/m^2 of wetting fluid or for two minutes at 2.1 bar(d) (30 psid).

Flow 0.5 N NaOH at room temperature through the device for up to 60 minutes at 0.7 to 4.1 bar(d) (10 to 60 psid). The caustic solution maybe left in the device for a static soak at room temperature for up to 16 hours.

Rinse the device after sanitization with (150 L/m^2) of flushing fluid. Conductivity of the filtrate maybe monitored to reduce the flushing volume as required.

Quantitation of Microbe Inhibition within Spiked Viresolve® Pro Modus 1.1 Devices upon Exposure to Sodium Hydroxide

Overview

Experiments were designed to assess the effectiveness of a caustic sanitization treatment on degree of inhibition of spiked microbes within the feed channels of Viresolve® Pro Modus 1.1 Devices. A 16-hour static exposure to 0.5 M NaOH treatment was assessed in this study. The static caustic treatment was successful in reducing the number of spiked microbes by acceptable target log reduction values with calculated log reductions of ≥8 for bacteria, ≥6.4 for vegetative yeast, and ≥5.1 for mold spores.

Study Limitations

This study did not assess the action of a full flushing regime with water and caustic combinations on the ability to remove spiked bioburden from the feed channels, nor did it address sanitization of any bioburden that may have been downstream of the membrane. It is incumbent upon each customer to validate sanitization efficacy of unit operations using their own equipment, piping, water source, and inherent bioburden.

Methods

Microbe challenge preparation:
Test organisms recommended by the current U.S. Pharmacopeia (USP 31:NF26 S2) Chapter 51, Antimicrobial Effectiveness Testing, were purchased commercially from ATCC®, or derived from characterized Merck Millipore internal sources.

- *Aspergillus brasiliensis* ATCC 16404 spores (Ab)
- *Candida albicans* ATCC 10231 (Ca)
- *Staphylococcus aureus* ATCC 6538 (Sa)
- *Pseudomonas aeruginosa* ATCC 9027 (Pa)
- *Escherichia coli* ATCC 8739 (Ec)

Immediately prior to device spiking, bioburden was introduced into each 1 L bottle of Phosphate Buffered Saline (PBS) or 0.5 M NaOH test solution as follows:

- 1 mL each of the *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* suspensions
- 0.5 mL *A. brasiliensis* spore suspension

Spiked solution was slowly pumped into the feed port of eight pre-weighed Modus Devices and feed channels were allowed to fill with the fluid with the vent valve open until spiked fluid visibly exited the valve. The mass of each spiked and clamped device was then measured, and devices sat at room temperature for 16 hours prior to bioburden recovery.

To recover microbes at the end of each static hold period, the devices were flushed with sterile Desorption Medium (DM). All DM was recovered from the feed channels using compressed air and stored refrigerated (2–8 °C) until the

Table 1.
Treatment, exposure time and calculated LRV of each test organism based on bioburden recovered for each test device

	Exposure	Spike	LRV *Ec*	LRV *Sa*	LRV *Pa*	LRV *Ca*	LRV *Ab*
Test Solution	Time (hours)	mass (g)	cfu/device	cfu/device	cfu/device	cfu/device	cfu/device
0.5 M NaOH only	16	35.1	0	0	0	0	0
0.5 M NaOH	16	39.1	8.3	8.2	8.2	6.4	6.7
0.5 M NaOH	16	58.9	8.5	8.4	8.4	6.6	6.9
0.5 M NaOH	16	39.8	8.3	8.2	8.2	6.4	6.7

microbes were plated for enumeration on Tryptic Soy Agar (TSA) plates (the yeast and mold used in this study grow on TSA medium). Plates were incubated at 35 (±2.5) °C to slow the growth of the *A. brasiliensis* and to mitigate the overgrowth of any bacteria that may have survived the caustic treatment.

The microbial spike/challenge for each device was determined from the titer of the microbe solutions and the volume of challenge solution per device. The bioburden recovered from each test device was enumerated, and the Log Reduction Value (LRV) calculated based on the number of known microbes spiked into each device.

Results

No bacteria or yeast were recovered from the caustic test solutions indicating that all three bacteria species, in addition to the yeast, were completely killed by the caustic treatment of Modus Devices using 0.5 M NaOH for 16 hours. Calculated LRVs are reported in Table 1. By contrast, *Aspergillus brasiliensis* spores were recovered from two out of three test devices, indicating the caustic treatment did not result in complete kill of these fungal spores. As expected, no bacteria were recovered from the non-spiked control device.

Conclusion

Sodium hydroxide is a potent sanitant used for inactivation of a variety of bacteria, yeast, and molds, but its effects on spore forming microbes (both bacterial or fungal) are highly dependent on concentration, exposure time and temperature. The results of this study confirm effectiveness of sodium hydroxide for kill of bacteria and yeast in Viresolve® Pro Modus Devices and demonstrate that treatment with 0.5 M NaOH for 16 hours results in 5.1–6.7 log reduction of *A. brasiliensis* spores.

Integrity Testing

Introduction

Our multi-tiered approach (as described under Product Consistency) for assuring virus retention includes: process and product validation, membrane release tests, device release tests, device 100% integrity tests, and end-user validation and integrity tests[1]. In this section, the manufacturing and end-user integrity tests are described. End-user integrity testing (the air diffusion test) can be accomplished either manually or with automated integrity test instrumentation. Equivalency between the two methods was demonstrated using our Integritest® 4 system.

Integrity Testing Multi-Tier Strategy

Overview

Manufacturers of biopharmaceuticals are required to characterize the ability of key process steps to clear viruses. Typically, the entire manufacturing process is qualified to attain a cumulative virus reduction factor, which significantly contributes to the documentation of virus safety. In many of these manufacturing processes, a virus retentive filter is utilized to achieve a robust and effective virus clearance step.

The key to the implementation of virus filtration is the assurance of virus retention and device integrity. The Viresolve® Pro Solution is based on a highly retentive parvovirus membrane, packaged in a range of devices well suited for customers' process development, validation and production stages.

A critical aspect of the Viresolve® Pro Solution is to present users with an industry-leading level of virus clearance assurance by offering effective performance, as well as integrity testing methods. This section provides a summary of our multi-tier approach to assuring virus filter performance and integrity, which comprises complementary steps taken by us and the end user.

Methods

Tier 1. Process and Product Validation

Membrane process validation must occur before the membrane is used in the manufacturing of filtration devices. Device process validation occurs before finished devices can be released to manufacturing and shipped to end users. A validated process provides assurance that the manufacturing process consistently yields product that meets predefined quality attributes. As part of the process validation, each of the critical manufacturing parameters has been identified. Tight manufacturing windows have also been assigned to ensure continued control over the manufacturing process. Viresolve® Pro Devices are manufactured in our state-of-the-art manufacturing facility, which adheres to cGMP, and whose Quality Management System is approved by an accredited registering body to the appropriate ISO® 9001 quality standards.

Tier 2. Membrane Release Tests

The Viresolve® Pro membrane process includes the following lot release tests using a sampling plan based on guidelines defined in ANSI/ASQ/Z1.4:

- Bacteriophage φX-174 retention test
- Protein capacity test (using human IgG as a model protein)
- Water permeability test
- Visual inspection of membrane

Tier 2.1 Membrane Release Test - Bacteriophage φX-174 Retention

The retention of φX-174 marker virus under controlled conditions provides evidence that the membrane meets or exceeds the minimum virus clearance target. This test is performed on each membrane lot and roll based on a

Integrity Testing

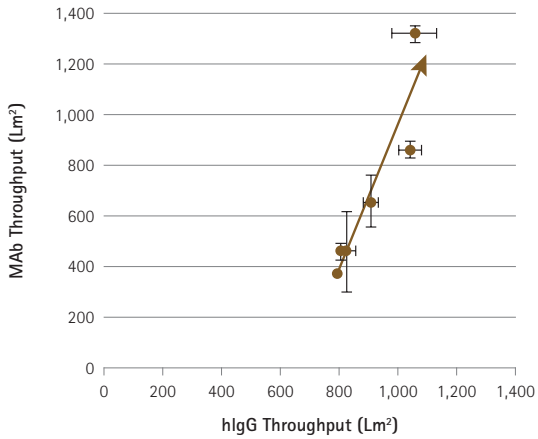


Figure 1.
Our protein capacity test method (hIgG) is consistent with that observed when tested with a customer's MAb

sampling plan designed to adhere to guidelines form ANSI/ASQ/Z1.4. The test involves filtering φX-174-human-derived IgG (hIgG) solution through the membrane samples in a Micro Device format. A filtrate grab sample is collected at 75% flow decay point from the initial buffer flux.

This test offers the following benefits:

- Represents the product application
- Serves as a Quality Control test as well as a foundation for measuring and tracking membrane performance
- Adheres to the guidelines set forth in the PDA Small Virus Filter Task Force (PDA Technical Report No. 41, Virus Filtration, PDA *J Pharm Sci Technol Suppl* Vol 59 No. S-2)

Tier 2.2 Membrane Release Test - Protein Capacity

Consistent protein capacity performance for a given process is a critical expectation from virus end users. Given that capacity performance is primarily established during the membrane manufacturing process, Viresolve® Pro membrane lot release tests include a protein capacity test using hIgG as a surrogate protein marker at 0.1 mg/mL in 50 mM acetate buffer. The hIgG molecule exhibits similar biochemical and physical properties to other mammalian-derived antibodies typical in customer applications, as shown in Figure 1.

In this test, a φX-174 and hIgG solution is filtered through the membrane samples. The capacity is measured to a 75% flow decay point (from initial buffer flux) and the results are normalized using a control membrane. The membrane roll average throughput values must fall within the two-sided specification.

Tier 2.3 Membrane Release Test - Water Permeability

Water permeability, along with protein capacity and virus retention, is an important performance measurement and is tested and controlled to ensure consistent product performance in the final application and to enable fast processing at the virus filtration unit operation. Samples of Viresolve® Pro phobic membrane are tested for water permeability as an in-process test prior to membrane hydrophilization. After hydrophilization, hydrophilic (final version) membrane is tested for water permeability as part of the membrane release testing using a predefined sampling plan. In the test, single layer membrane samples are tested at 25 psig and temperature normalized to 25 °C. Hydrophobic membrane is first prepared by wetting in alcohol and exchanging in water while hydrophilic membrane is wetted in water.

Tier 3. Viresolve® Pro Device Release Tests

The lot release testing of Viresolve® Pro Devices includes:

- Bacteriophage φX-174 retention test
- Housing burst test
- In-Process manufacturing controls

Tier 3.1 Bacteriophage φX-174 Retention Test

Samples from each Viresolve® Pro Device lot are tested for the ability to retain the φX-174 marker virus. This test confirms that the device manufacturing process is free of systematic issues that could compromise the inherent retention performance of the Viresolve® Pro membrane established during the membrane manufacturing process and verified during the membrane release testing.

Tier 3.2 Housing Burst Test

Samples from each Viresolve® Pro Device lot are tested to determine the maximum pressure the device can withstand. During this test, the device is filled with water and the pressure inside of the device is increased at a fixed rate until failure occurs. The specification requires that the samples maintain integrity up to a pressure that corresponds to twice the rated pressure.

Tier 3.3 In-Process Manufacturing Controls

In addition to the device release tests, the following in-process manufacturing controls are used:

- Luster sensors performed during membrane bonding operations confirms correct membrane orientation

- Visual system after the membrane bonding step (for Viresolve® Pro Modus and Magnus Devices) confirms that appropriate membrane/plate bond has been achieved
- Thermal imaging systems verify that the correct thermal energy is being applied to all portions of the bonding surfaces
- Electronic servo-controlled motors are utilized to position the parts during heat, welding and cooling portions of the bonding cycle

Tier 4. Device 100% Tests

The Viresolve® Pro Devices are subject to the following 100% device tests:

- Pressure hold test
- Water flux test
- Air/water diffusion test
- Binary Gas Test (BGT)

Tier 4.1 Pressure Hold Test

The pressure hold test assures that the interface between the interior and exterior of the device is leak-free to ensure that the device will not leak during usage. It does not challenge filter integrity. During this test, the device is brought up to no less than 1.5 times the rated pressure using compressed air and allowed to stabilize. The internal volume is then isolated and the internal pressure is carefully monitored for a specific period of time.

Tier 4.2 Water Flux Test

Water flux is a measure of the internal resistance to water flow. All Viresolve® Pro Modus and Magnus Devices are wet challenged with water at a constant pressure and the flow rate is measured and compared to the specification.

Tier 4.3 Air/Water Diffusion Test

All Viresolve® Pro Magnus and Modus Devices are integrity tested for air/water diffusion using a test method that is similar to the air/water diffusion test recommended for pre- and/or post-use testing by the end user. The air/water diffusion test can provide up to 4.5–5 virus log reduction value (LRV) assurance for devices with membrane of the proper pore size distribution.

Tier 4.4 Binary Gas Test (BGT)

Our BGT method is an innovative, highly sensitive device test that utilizes a two component gas mixture in which there is a large difference in permeability between the two gases across a wetted membrane and concentration is measured on the downstream side. A deviation from the expected concentration is an indication of the presence of a defect that could negatively affect the virus retention capabilities of the device. The BGT is performed on 100% of Viresolve® Pro Magnus and Modus Devices, as well as Viresolve® Pro Micro Devices intended for virus validation studies. The addition of BGT to our multi-tier approach has further strengthened the virus retention assurance delivered by the Viresolve® Pro Solution

Tier 5. End User Validation and Testing

Tiers 1–4 provide assurance that the Viresolve® Pro Solution meets its performance specifications in terms of virus retention and device integrity. Due to this extensive testing, the user is responsible for only ensuring that the filters have not been damaged during shipping, and are installed, tested and utilized correctly.

Air/Water Diffusion Test

We recommend an air/water diffusion test as an optional integrity test, with following benefits:

- Ease of use: water wetted test, simple procedure, non destructive test, results in approximately 40 minutes
- Accurate: downstream flow meter or Integritest® 4 Automated Integrity Tester
- Sensitive to the type of defects that could be introduced during shipping, handling, installation and use
- Similar to the test performed by us, which can facilitate device failure investigations

An end user's integrity test may be performed prior to or after use, utilizing either a downstream air flow rate measurement with a flow meter or an integrity test instrument. We recommend the Integritest® 4 instrument, which is designed to accurately measure air/water diffusion values for Viresolve® Pro Devices. Please consult the User Guide for specific instructions for device installation, testing and use.

Integrity Testing

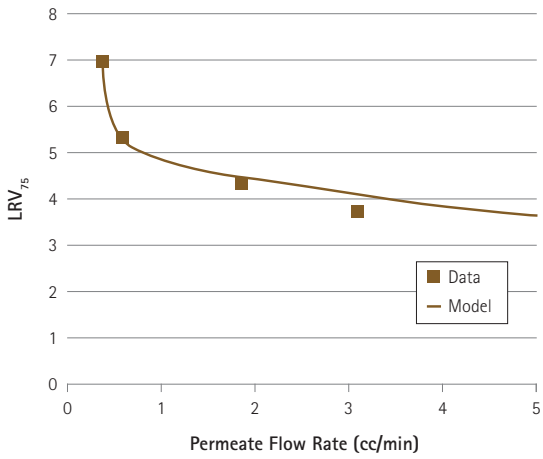


Figure 2.
Air diffusion rates and virus retention performance of Viresolve® Pro Devices fabricated with controlled defects

The 50 psig (3.45 Bar) air/water diffusion test was selected because of its suitability for detection of the type of defects that could be introduced during an accidental shipping issue, improper handling or processing of the Viresolve® Pro Modus and Magnus Devices.

Figure 2 shows an example of the air diffusion rates and virus retention performance of Viresolve® Pro Devices that were fabricated with controlled defects. At the steep portion of curve in the 5–6 LRV range, small defects will result in significant effects on LRV but will have minor effects on air diffusion rate. These small defects, if present, will be detected by using the 100% BGT and, therefore, would not be released for shipping to end users. Presence of larger defects that are more likely to occur during shipping, handling and use will result in elevated air diffusion flow rates as illustrated at the right side of the curve knee. Devices with defects in this range can be detected by the end user using the recommended air/water diffusion test.

Results

From membrane manufacturing to filter use, our multi-tier approach for ensuring virus retention and integrity of Viresolve® Pro Devices creates a continuum of filter integrity testing. This approach places responsibility on the filter manufacturer to document the production of: (1) membrane that consistently provides robust virus clearance and (2) integral filter devices that house this membrane.

The end user is then responsible for auditing the filter manufacturer to verify compliance to effective quality systems. As a result, users can be assured that filters will be produced and tested in a controlled manner. During day-to-day operations, the end user's primary responsibility is to ensure that filter integrity has been maintained from receipt to use.

Procedure Comparability

Manual Integrity Testing

Overview

The integrity test may be performed manually using a graduated cylinder inverted in a vessel of water or a downstream mass flowmeter.

Methods

Manual integrity testing may be performed before and after device use. The following procedure was performed post device use, after product recovery.

1. Flush WFI or Milli-Q® water through the device installation at a minimum of 0.7 bar(d) (10 psid) for a minimum of 10 L/m^2
2. Connect process air line to the inlet side of the device.
3. Increase air pressure to 3.4 bar(d) (50 psid)
4. Drain water through the outlet line. After the filtrate flow is reduced to slow dripping, start a timer and allow the system to stabilize for at least 20 minutes
5. Measure the diffusion flow rate using a downstream mass flow meter or inverted cylinder. If using the inverted cylinder, place the outlet line into a vessel of water. Wait until a few air bubbles are seen exiting the filtrate tubing. Start measuring the diffusion flow rate using an inverted graduated cylinder for one to five minutes
6. Compare the test results to the specifications
7. Release the pressure in the feed vessel
8. Disconnect the device

If the installation fails the integrity test, rewet the device at 2.1 bar(d) (30 psid), ensuring proper venting. Although wetting at 0.7 bar(d) (10 psid) may be adequate, wetting at 2.1 bar(d) (30 psid) ensures a fully wetted membrane.

Note: Integrity testing introduces air into the device. If integrity testing the device prior to use, ensure the device is fully wetted after the integrity test by wetting with 50 L/m^2 of WFI or buffer after the integrity test.

Results

Compare the test results to the specifications printed on each certificate of quality shipped with the device.

Integrity Testing of Viresolve® Pro Device with Integritest® 4 System Installed with Service Pack 4

Overview

The Integritest® 4 automatic integrity tester is routinely used in pharmaceutical production to test the integrity of sterilizing or virus filters. To address the integrity test of the brand new Viresolve® Pro Devices, a new software update to Integritest® 4 instrument, Service Pack 4, was developed and released in February 2009.

The key update in Service Pack 4 is the addition of a pre-pressurization step so that the wetting liquid can be removed out of the downstream of the Viresolve® Pro Device effectively.

The pre-pressurization step in Service Pack 4 includes three parts:

- Pressurizing the Viresolve® Pro Device to 55 psig for 15 minutes
- Depressurizing it to 0 psig for 3 minutes
- Pressurizing it to 55 psig for 10 minutes.

The complete list of changes in Service Pack 4 can be found in the validation report at our document control system (document number: 00017307-SATR). Validation methods and results are summarized in this report.

The validation results indicated that the accuracy of Integritest® 4 instrument installed with Service Pack 4 was within 0.1 mL/min (when flow rate <1 mL/min) or 10% for the air diffusion test of Viresolve® Pro Devices. The validation results also showed that the reproducibility was within 0.1 mL/min or 10%. The reproducibility is defined as the standard deviation (when diffusion flow rate <1 mL/min) or the coefficient of variance, which is the ratio of the standard deviation to average Integritest® 4 instrument value.

Methods

Table 1 lists the Viresolve® Pro Devices used in the validation of Integritest® 4 instruments with Service Pack 4. It includes all types of commercial-scale Viresolve® Pro Devices. Seven stacks of Magnus Devices, the largest configuration of Viresolve® Pro holder, are also included in the validation. All the devices were wetted with water at 30 psig for 30 minutes. In the validation, each diffusion test was repeated three times with a simultaneous manual test. The initial state of the Viresolve® Pro Devices is dry, which simulates the initial state of a brand new Viresolve® Pro Device. The devices were not dried but rewetted between the repeat tests. The diffusion test pressure is 50 psig.

Two Integritest® 4 instruments and two sets of Viresolve® Pro Devices, Table 1, were used in the validation. One set of Viresolve® Pro Devices was tested on one Integritest® 4 instrument and the other set on the second Integritest® 4 instrument. The results for the full configuration (seven Magnus Devices) are reported separately since one of the seven is a Magnus 2.1 Device and the remaining six are Magnus 2.2 Devices.

The manual diffusion flow rate was measured by mass flow meters. Four mass flow meters (0–10 mL/min, 0–50 mL/min, 0–100 mL/min, and 0–1000 mL/min) were used in the validation to measure the diffusion flow rate at their respective flow rate range.

Results

Tables 2 and 3 list the Integritest® 4 diffusion flow rates compared to the simultaneous measured manual flow rates. The test accuracy and reproducibility are also shown in the tables. The accuracy target of Integritest® 4 instrument for Viresolve® Pro Device air diffusion test is 0.1 mL/min for flow rates of 1 mL/min or less or 10% of flow rates larger than 1 mL/min. Due to the low flow rate for Viresolve® Pro Modus 1.1 Device, the accuracy is calculated by absolute method and other devices by relative methods. It should be noted that traditionally the accuracy of Integritest® 4 instrument is defined as the difference between the manual and Integritest® 4 instrument average value of three consecutive repeats. It is not meant to be the difference of every single run.

The reproducibility is also calculated in two ways, as listed in Tables 2 and 3. Standard deviation method is used when the diffusion flow rate of Viresolve® Pro Device is less than 1 mL/min. Otherwise the coefficient of variant is used for the reproducibility. This procedure is the traditional method used in our Automatic Integrity Tester Validation Guide, Lit. No. VG1014EN00.

The data in Tables 2 and 3 confirm that Integritest® 4 instrument installed with Service Pack 4 met the accuracy and reproducibility targets. The accuracy is within 0.1 mL/min or 10% and the reproducibility is also well within 0.1 mL/

Integrity Testing

min (standard deviation) and 10% (coefficient of variance).

Table 1.

Test 1 Viresolve® Pro Device test

Test Type	Test Number	Device	Number of Devices	Initial State	Rewetting Between Tests
Diffusion with Variable Prepressurization (Viresolve® Pro Box Checked)	1	Modus 1.1	1	Dry	Yes
	2	Modus 1.2	1	Dry	Yes
	3	Modus 1.3	1	Dry	Yes
	4	Magnus 2.1	1	Dry	Yes
	5	Magnus 2.2	1	Dry	Yes
	6	Magnus 2.2	7	Dry	Yes

Table 2.

Results of the first Integritest® 4 instrument on the first set of Viresolve® Pro Devices*

Viresolve® Pro Device	Replicate No.	Viresolve® Pro Device Before Wetting	Manual Flow Rate at Room Temperature (mL/min)	Integritest® 4 System Flow Rate (mL/min)	Accuracy (Absolute or Relative)**	Reproducibility Standard Deviation or Coefficient of Variance)***
Modus 1.1	1	Dry	0.41	0.46	-0.05 mL/min	0.03 mL/min
	2	Wet	0.38	0.41	-0.03 mL/min	
	3	Wet	0.39	0.42	-0.03 mL/min	
	Average		**0.39**	**0.43**	**-0.04 mL/min**	
Modus 1.2	1	Dry	1.60	1.65	-3.1	0.9%
	2	Wet	1.61	1.66	-3.1	
	3	Wet	1.61	1.68	-4.3	
	Average		**1.61**	**1.66**	**-3.5**	
Modus 1.3	1	Dry	6.13	6.05	1.3%	1.3%
	2	Wet	5.98	5.89	1.5%	
	3	Wet	6.07	5.96	1.8%	
	Average		**6.06**	**5.97**	**1.5%**	
Magnus 2.1	1	Dry	17.8	17.0	4.5%	2.1%
	2	Wet	17.3	16.6	4.0%	
	3	Wet	16.8	16.3	3.0%	
	Average		**17.3**	**16.6**	**3.9%**	
Magnus 2.2	1	Dry	39.2	37.8	3.6%	1.6%
	2	Wet	38.4	37.2	3.1%	
	3	Wet	37.6	36.6	2.7%	
	Average		**38.4**	**37.2**	**3.1%**	
7 x Magnus 2.2	1	Dry	239.6	246.0	-2.7%	2.4%
	2	Wet	249.4	254.7	-2.1%	
	3	Wet	253.7	257.5	-1.5%	
	Average		**247.6**	**252.7**	**-2.1%**	

* If flow rate <1 mL/min, accuracy is absolute and reproducibility is standard deviation. If flow rate ≥1 mL/min, accuracy is relative and reproducibility is coefficient of variance.

**Absolute Accuracy = Manual Flow Rate – Integritest® 4 Flow Rate; Relative Accuracy = Absolute Accuracy/Manual Flow Rate

*** Coefficient of Variance = Integritest® 4 Flow Rate Standard Deviation/Average Integritest® 4 Flow Rate

Table 3.

Results of a second Integritest® 4 instrument on a second set of Viresolve® Pro Devices*

Viresolve® Pro Device	Replicate No.	Viresolve® Pro Device Before Wetting	Manual Flow Rate at Room Temperature (mL/min)	Integritest® 4 System Flow Rate (mL/min)	Accuracy (Absolute or Relative)**	Reproducibility Standard Deviation or Coefficient of Variance)***
Modus 1.1	1	Dry	0.35	0.41	-0.06 mL/min	0.02 mL/min
	2	Wet	0.34	0.38	-0.04 mL/min	
	3	Wet	0.34	0.40	-0.06 mL/min	
	Average		**0.34**	**0.40**	**-0.05 mL/min**	
Modus 1.2	1	Dry	1.41	1.45	-2.8%	2.2%
	2	Wet	1.37	1.41	-2.9%	
	3	Wet	1.37	1.39	-1.5%	
	Average		**1.38**	**1.42**	**-2.4%**	
Modus 1.3	1	Dry	4.67	4.68	-0.2%	1.0%
	2	Wet	4.59	4.64	-1.1%	
	3	Wet	4.77	4.73	-0.8%	
	Average		**4.68**	**4.68**	**-0.1%**	
Magnus 2.1	1	Dry	12.5	12.1	3.2%	2.0%
	2	Wet	11.9	11.7	1.7%	
	3	Wet	11.6	11.7	-0.9%	
	Average		**12.0**	**11.8**	**1.4%**	
Magnus 2.2	1	Dry	37.2	36.7	1.3%	1.1%
	2	Wet	37.0	35.9	3.0%	
	3	Wet	37.1	36.1	2.7%	
	Average		**37.1**	**36.2**	**2.3%**	
7 x Magnus 2.2	1	Dry	280.8	289.8	-3.2%	2.6%
	2	Wet	272.1	280.4	-3.1%	
	3	Wet	269.8	257.1	-2.0%	
	Average		**247.2**	**281.8**	**-2.7%**	

* If flow rate <1 mL/min, accuracy is absolute and reproducibility is standard deviation. If flow rate ≥1 mL/min, accuracy is relative and reproducibility is coefficient of variance.

**Absolute Accuracy = Manual Flow Rate - Integritest® 4 Flow Rate; Relative Accuracy = Absolute Accuracy/Manual Flow Rate

*** Coefficient of Variance = Integritest® 4 Flow Rate Standard Deviation/Average Integritest® 4 Flow Rate

Integrity Testing

Correlation Data with LRV

Defect Detection Sensitivity of the Air Diffusion and Binary Gas Integrity Tests

Overview

The purpose of integrity testing of virus filters is to detect the presence of defects that can compromise the retention capability of the filter. After device manufacturing, and as part of our multi-tier virus retention assurance strategy, every Viresolve® Pro Modus and Magnus Device undergoes both an air diffusion test and BGT. The air diffusion test can be used as a pre- and/or post-use test by the end user and can provide up to 4.5–5 LRV assurance. The BGT[1] is a proprietary test that provides up to 5.5–6 LRV assurance. Both BGT and the air diffusion test are key components of the multi-tier approach for assuring virus retention that has been implemented for Viresolve® Pro membrane and Devices[2].

Methods

Viresolve® Pro membranes were integrity tested using both the air-water diffusion test and BGT. Defects of controlled size, in the range 2–10 µm diameter, were introduced into 142 mm discs (effective filtration area of 127 cm^2) of the membrane. These defects were created by laser drilling in the center of the membrane discs. The membranes were tested in double layer format in 142 mm holders and the laser holes were drilled through both layers.

Prior to air diffusion or Binary Gas Testing, the membranes were first wetted by immersing the discs in RO water for about 5 minutes. The membranes were then placed into a 142 mm disc holder and the inlet of the holder was connected to a gas source. For the air diffusion test, pressurized air at 50 psig (3.45 Bar) was applied to the upstream side of the membrane. After draining out any excess water and about 5 minutes of equilibration time, the downstream air flow rate was measured using a mass flowmeter.

For the BGT, a 90/10 CO_2/C_2F_6 test gas, also at 50 psig (3.45 Bar) was introduced to the membrane, and a constant sweep gas rate was maintained through the vent port of the filter holder. Sweep and permeate gas flow rates were measured using separate mass flowmeters, and composition was measured using Fourier Transform Infrared Spectroscopy (FTIR). Measurements were recorded continuously until an essentially steady state permeate composition was achieved, typically within 15–20 minutes. This time was required to fully flush out the residual air from the volume downstream of the membrane, the sample lines leading to the FTIR, and the FTIR sample chamber.

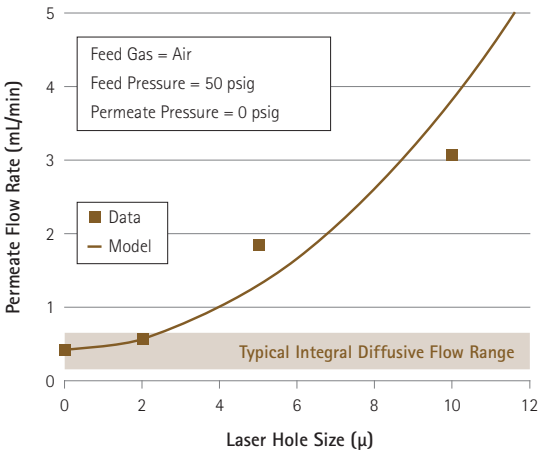
After air diffusion and Binary Gas Testing, the membrane devices were challenged with a solution consisting of the bacteriophage φX-174 spiked to 1 x 10^7 pfu/mL mixed with hIgG at 0.1 g/mL in 50 mM acetate buffer, pH 5.0. The solution was filtered through the membrane at 30 psig (2.1 Bar) until flux had declined by 75% compared to the clean buffer. Feed and permeate samples were collected and the infectious titer of φX-174 was determined using the plaque assay method.

Results

Figures 1 and 2 show air diffusion test and BGT results as functions of defect size. The solid lines are the model predictions for the air diffusion test and the BGT. The shaded regions in each graph show typical test value ranges for integral membranes. These regions represent background noise against which a signal for a defect must be compared. For the air diffusion test, the range of flow rates for an integral membrane may be relatively broad. Figure 1 shows that a 2 µm defect was not 'visible' to the air diffusion test because the additional flow rate due to the defect was not large enough to increase the total flow rate beyond the range typically measured for integral membranes. In contrast, the elevated C_2F_6 concentration in the permeate – about 1000 ppmv compared to less than 100 ppmv for an integral double layer membrane – of the BGT provided a clear signal for the same 2 µm defect. This result was an unambiguous demonstration of the superior defect detection sensitivity of the BGT. For defect sizes larger than 2 µm, the measured (and predicted) air flow rates and BGT values (i.e., C_2F_6 concentration in the permeate) were clearly higher than the integral range and therefore both tests provided a strong signal for a defect.

After integrity testing, the membranes were tested for virus retention as described earlier. The impact of the defects on virus retention is shown in Figure 3, where LRV_{75} as a function of defect size. The decrease in LRV_{75} due to defects was closely predicted by the model. LRV_{75} is plotted versus the air diffusion and BGT values in Figures 4 and 5. Here, we see that relationship between integrity test values and retention is closely predicted by the model calculations. This means that a maximum allowable integrity test value can be established for a desired level of LRV assurance based on a worst case assumption of a single defect. Multiple defects

of a smaller size that would generate the same integrity test values would have equal or less impact on LRV compared to a single larger defect, provided that the smaller defects are evacuated of the wetting liquid at the test pressure.



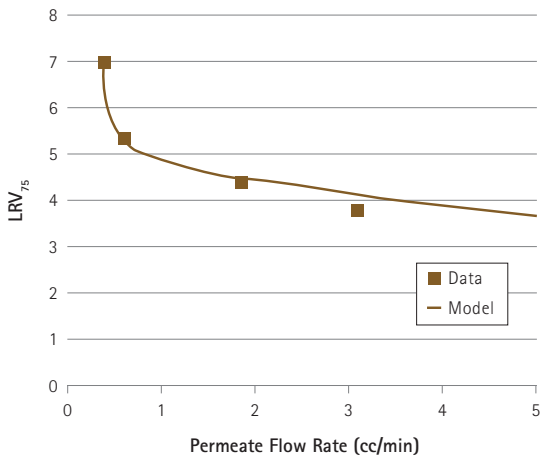
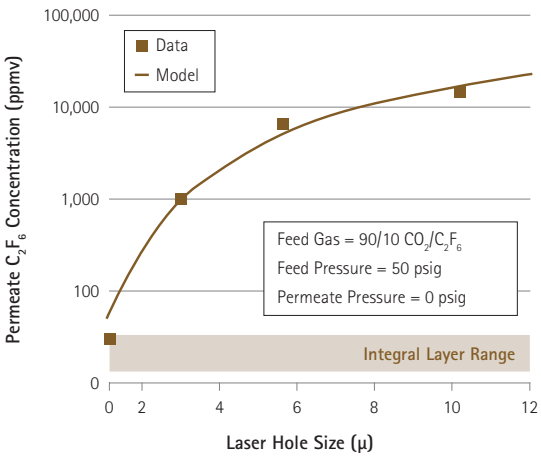


Figure 1.
Defect detection by the air diffusion test



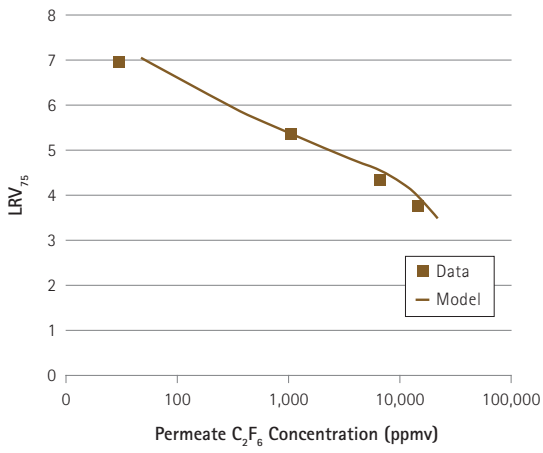


Figure 2.
Defect detection by the Binary Gas Test

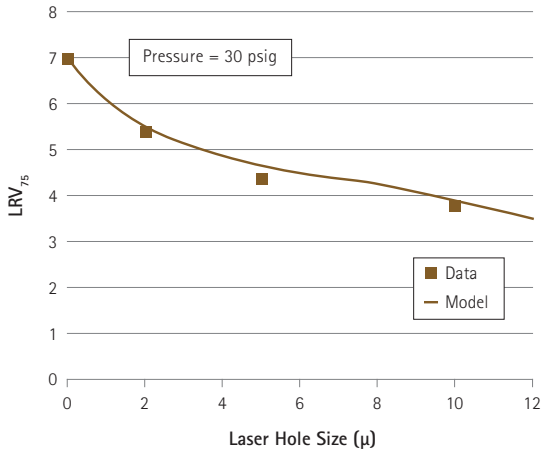


Figure 3.
Effect of defect size on LRV

Figure 4.
Relationship between LRV_{75} and air diffusion flow rate

Figure 5.
Relationship between LRV_{75} and Binary Gas Value

References

1. S. Giglia and M. Krishnan "High sensitivity binary gas integrity test for membrane filters" *Journal of Membrane Science*, Volume 323, Issue 1, 1 October 2008, pp. 60-66.2

2. "Multi-tier Approach to Assuring Virus Retention and Integrity of Viresolve® Pro Devices", Merck Millipore, Lit. No. AN1033EN00.

Integrity Test Methods and Virus Retention Assurance for Viresolve® Pro Magnus Devices

Overview

In the previous section, the defect detection sensitivity of the air diffusion test and BGT was evaluated using 142 mm discs containing controlled size defects (membranes drilled with laser holes). It was demonstrated that the air diffusion test could provide up to 4.5–5 LRV assurance and that the BGT was capable of providing up 5.5–6 LRV assurance. In this study, the air diffusion test and BGT were applied to a large population of Viresolve® Pro Magnus 2.1 and Magnus 2.2 Devices.

Methods

Twenty-three Viresolve® Pro Magnus 2.1 Devices and 10 Magnus 2.2 Devices were tested for this study. For each device size, at least three different device lots were included. All of the tests were run using an automated integrity test system that subjects each device to the following four tests:

1. Pressure hold

Dry devices were pressurized with air at 90 psig (1.5 times the rated forward operating pressure), with all ports closed. Pressure decay inside the device was monitored for 5 minutes to ensure that the housing was free of leaks.

2. Water permeability

The water permeability test consisted of wetting the devices by flushing with RO water at 30 psig for 15 minutes, and measuring the water flow rate.

3. Air diffusion

Pressurized air at 50 psig was introduced to the device inlet port. Water was allowed to drain out of the permeate outlet port and after water dripping stopped, the device was allowed to equilibrate for about 20 minutes at which point the downstream air flow rate was measured using a mass flowmeter.

4. Binary gas

The binary gas feed (a mixture of CO_2 and C_2F_6) at 50 psig was introduced to the device inlet port. The device is configured to allow a constant gas flow rate through the vent port and the permeate port of the filter holder. Vent and permeate gas flow rates were measured using separate mass flowmeters while the composition of the gas from the vent and from the permeate was measured using an FTIR (Fourier transform infrared spectroscopy). The device was equilibrated for 30 minutes, to fully flush out the residual air from the volume downstream of the membrane, the sample lines leading to the FTIR, and the FTIR sample chamber.

After air diffusion and BGT testing, the devices were challenged with a solution consisting of the bacteriophage φX-174 at approximately 1×10^7 pfu/mL in 50 mM acetate buffer, pH 5.0. This solution was filtered through the membrane at a quantity of 10 L/m^2 at 30 psig. Feed and permeate samples were collected and the infectious titer of φX-174 was determined using the plaque assay method.

Results

The relationship between virus retention and integrity value is shown in Figures 1 for air diffusion test and Figure 2 for BGT.[1] Each test provided the expected level of retention assurance, as indicated by data points and model lines in each figure.

The indicated specification for each integrity test was selected to ensure a robust manufacturing process capability (CpK ≥1.33) and a high level of retention assurance.

It should be noted that the air diffusion test and BGT do not indicate pore size distribution (other tests are available for this), but are designed only to detect defects. In Figures 1 and 2, the model calculation is based on a defect-free LRV of 7. For a membrane with lower intrinsic (defect-free) LRV, the level of LRV assurance provided by the tests would be limited to the intrinsic LRV of the membrane.

References

1. S. Giglia and M. Krishnan "High sensitivity binary gas integrity test for membrane filters" *Journal of Membrane Science*, Volume 323, Issue 1, 1 October 2008, Pages 60-66.

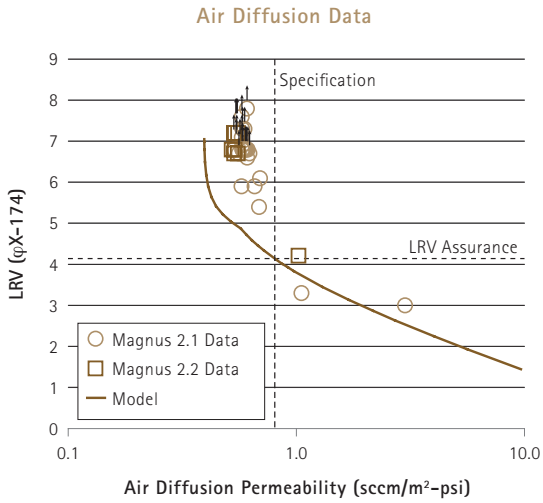


Figure 1.
LRV assurance provided by the air diffusion test

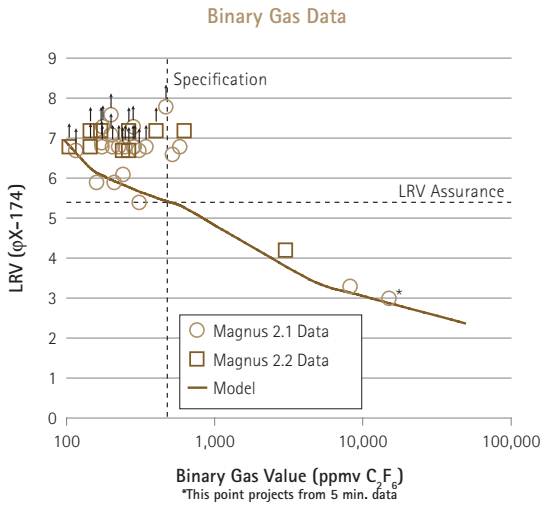


Figure 2.
LRV assurance provided by the Binary Gas Test

Best Practices

Wetting of Viresolve® Pro Devices Post Integrity Testing

Overview

Viresolve® Pro Devices are designed for easy wetting. However, re-wetting of devices after air diffusion integrity testing can be inhibited if residual air remains trapped within the membrane structure. The objective of this study was to determine the necessary conditions for successful re-wetting after air diffusion testing. It was demonstrated that re-wetting at 30 psig with 50 L/m^2 of water was sufficient to ensure proper wetting.

Methods

Four Viresolve® Pro Devices of three different sizes:

- Modus 1.3 SN08
- Magnus 2.1 SN02
- Magnus 2.1 SN03
- Magnus 2.2 SN02

were tested as part of this study. For each device, RO water was first fed to the device inlet at 5 psig until a continuous stream of water was observed exiting from the open vent valve. The vent valve was then closed and the wetting pressure was raised to 30 psig. The permeate flow rate was measured until it reached a steady state condition (typically within 5–15 minutes). Next, an air line was connected to the device inlet and pressure was increased to 50 psig and free water was drained from the outlet port. The device was then maintained at 50 psig for at least 20 minutes, as per the recommended air diffusion integrity test procedure for Viresolve® Pro Devices.

The entire procedure described above was repeated (after the device had been dried and a caustic [0.5 N NaOH] flush step was added).

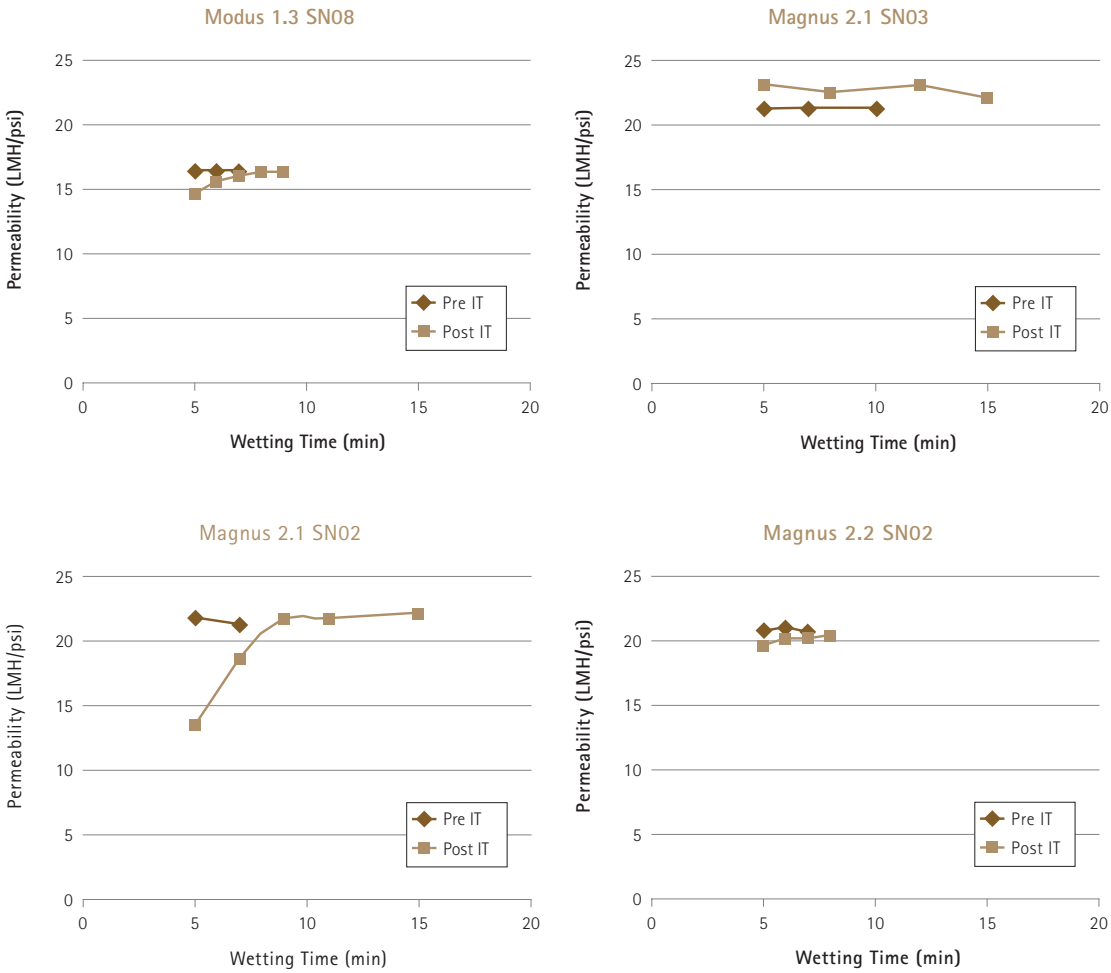


Figure 1.
Water wetting of Viresolve® Pro Modus and Magnus Devices pre- and post- air diffusion integrity testing

Results

Permeability as a function of time for each of the initially screened devices is shown in Figure 1. For all but one of the devices, water permeability reached a steady state value within about 5 minutes (~50 L/m^2) both before and after air diffusion testing. The Magnus 2.1 SN02 did exhibit slower wetting post air diffusion testing, resulting in the need for more extensive testing.

Figure 2 shows permeability versus time volume for each of three wetting steps for Viresolve® Pro Magnus 2.1 Device SN02. Wetting with water after diffusion testing was slower than before diffusion testing, but as can be seen from the right hand panel of Figure 2, steady-state permeability was below the recommended flush volume of 50 L/m^2. Caustic permeability also reached steady-state in about 5 minutes. Thus, re-wetting with water after air diffusion testing can be used to restore permeability before the caustic flush step.

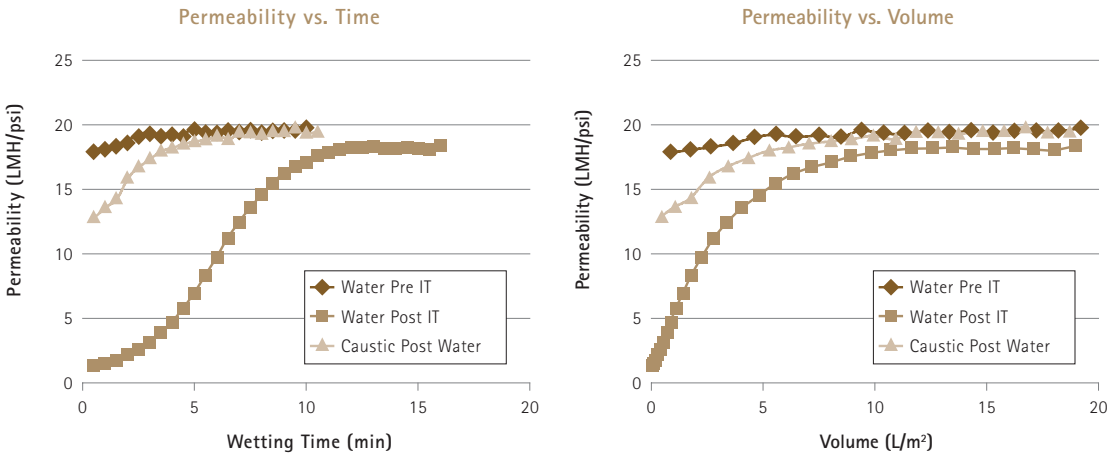


Figure 2.
Water wetting and caustic flushing of a Viresolve® Pro Magnus 2.1 Device (SN02)

Integrity Testing

Recommended Integrity Testing Procedure of Viresolve® Pro Device with Integritest® 4 System and Competitive Models

Overview

The membrane morphology and multilayer construction of Viresolve® Pro Device requires different integrity testing methods compared to microporous filters such as Durapore® membrane filters. To address this issue, a software update for the Integritest® 4 system was released in February 2009 to include a pre-pressurization step in the diffusion test. The competitive systems referenced in this study do not include this feature.

The objectives of this study were to:

- Establish best practices for automatic integrity testing the Viresolve® Pro Devices on the Integritest® 4 system, as well as on the competitive systems
- Determine which automatic test method best correlates to the downstream manual measurement
- Determine which automatic test method is applicable for end users that integrity test Viresolve® Pro Devices on competitive testers

We performed this testing to guide end users in the proper practice for integrity testing Viresolve® Pro Device using automated integrity testers other than the Integritest® 4 system.

Methods

All testing was performed at our facilities using a Viresolve® Pro Magnus 2.1 Device as the test filter. This filter was tested on the Integritest® 4 system with Service Pack 4, and on competitive systems X and Y. A total of 54 runs were executed under four main test conditions that the end user would potentially encounter as test programs:

1. A short stabilization (time varied per unit and was kept at this pre-programmed default time)
2. A long stabilization (set to 30 minutes)
3. Pre-pressurization with short stabilization
4. Pre-pressurization with long stabilization

The pre-pressurization involves pressurizing the filter to 55 psi for 15 minutes, 0 psi for 3 minutes, and then back to 55 psi for 10 minutes.

The device was wetted with water at 30 psi for 15 minutes prior to each run. Diffusion test pressure was 50 psi. The first 45 runs (Phase 1) were triplicate runs of each test condition above, followed by a repeat of the short stabilization time.

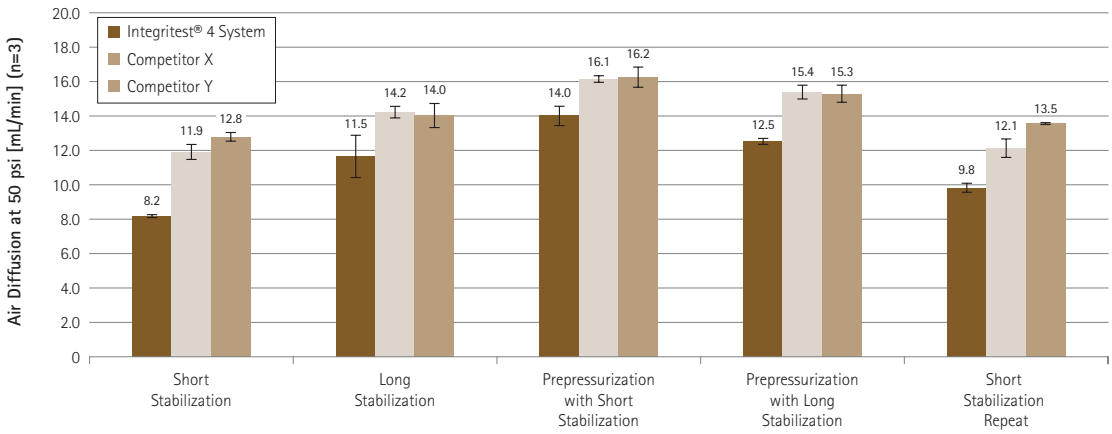


Figure 1.

Viresolve® Pro Magnus 2.1 integrity testing with automatic upstream testers, diffusion specification less than 20 mL/min at 50 psi

The final nine runs (Phase 2) were performed using:

1. A long stabilization (30 minutes)

2. Pre-pressurization with short stabilization

3. Increase air pressure to 3.4 bar(d) (50 psid)

A simultaneous manual measurement was taken using a graduated cylinder and bucket of water.

Results

Figures 1 and 2 show graphical representation of the diffusion values obtained in both phases of the study.

Based on the graph below, short stabilization (1) should not be used on any tester to integrity test Viresolve® Pro Devices. Using only short stabilization time, the measured diffusion value will not be valid relative to the diffusion specification determined by Merck Millipore.

The other test methods yield more accurate measurements with respect to the diffusion specification.

The goal of the Phase 2 testing was to run the remaining viable test methods and compare the automatic measurement to a manual measurement taken downstream.

The Integritest® 4 system was designed to accurately measure device integrity based on the manual test method.

Our acceptance criteria for the Integritest® 4 system states that the automatic value must be within 0.1 mL/min for flow rates less than 1 mL/min and +/- 10% for flow rates greater than 1 mL/min of the manual value.

For the Integritest® 4 system, the optimal procedure is the pre-pressurization step with short stabilization. This procedure produces results that are within the acceptance criteria.

End users that have automated integrity testers manufactured by other vendors can use any of these three procedures: long stabilization (2), pre-pressurization with short stabilization (3) and pre-pressurization with long stabilization (4). All yield diffusion values that are in agreement with the downstream manual measurement and our acceptance criteria. The end user should choose one of these conditions based on their capabilities to validate the procedure on the automated competitive tester.

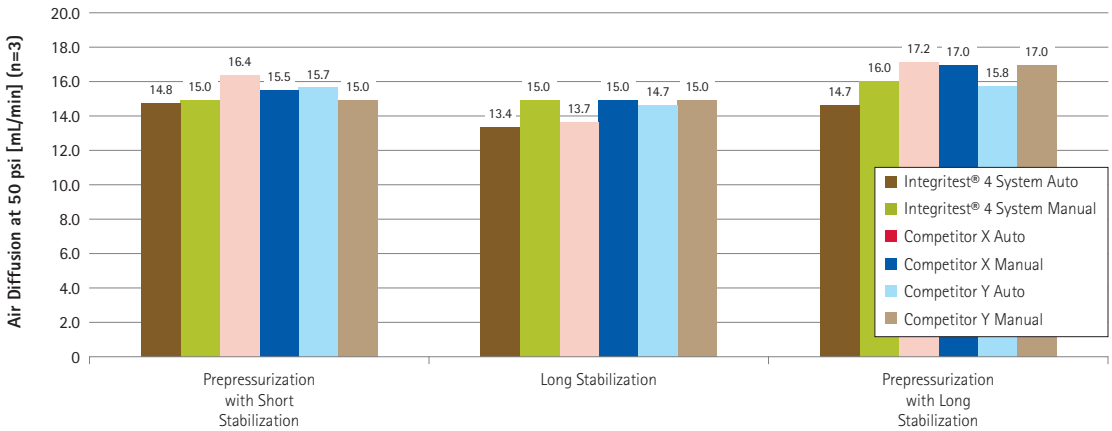


Figure 2.

Viresolve® Pro Magnus 2.1 Device integrity testing with automatic upstream testers and downstream manual test

Scalability

Overview

Viresolve® Pro Devices are available for the processing of different size batches, corresponding to different area of membrane provided in different types of devices. Since process development and validation work is typically performed on smaller devices than the devices used during production, an undersized process-scale device may result in incomplete batch processing. It is essential that the performance of Viresolve® Pro Devices scale in a predictable and consistent manner. Additionally, excessively oversized systems do not use the available filtration area effectively and will cause the cost of this process step to be unnecessarily high. Viral clearance validation is typically performed on small scale devices, thus, it is essential that viral clearance of larger-scale devices (i.e. Modus and Magnus Devices) is equivalent to viral clearance of small-scale devices. For these reasons, the Viresolve® Pro Solution was designed so that it scales predictably and linearly between all sizes and formats. This section provides data that demonstrate the scalability of permeability, capacity, and virus retention in the Viresolve® Pro Solution. Data are also provided demonstrating integrity testing and product recovery for larger-scale Viresolve® Pro Devices.

Table 1 details the different size Viresolve® Pro Devices available and the effective filtration area for each device. Details for how these areas were determined can be found in the Viresolve® Pro Solution Validation Guide. The smallest devices available (Micro Devices) are intended for Process Development (PD) and Validation work. The Modus and Magnus Devices are used for increasingly larger pilot-scale and process-scale batches.

Table 1.
Viresolve® Pro Devices and effective filtration area

Device Type	Effective Filtration Area (m^2)
Micro Device Validation and PD Kits	0.00031
Modus 1.1 Device	0.0172
Modus 1.2 Device	0.07
Modus 1.3 Device	0.22
Modus 2.1 Device	0.51
Modus 2.1 Device	1.53

Scaling Strategy

Viresolve® Pro Micro Devices in our Process Development Kit can be used for process development and optimization in order to minimize the volume of fluid required. Appropriate operating conditions can be determined and optimized, including many of the parameters discussed in the Process Optimization section of this guide. Validation of viral clearance may be performed using Viresolve® Pro Micro Devices from our Validation Kit. These devices undergo the same binary gas integrity test at the end of manufacturing that the larger scale devices undergo. In order to scale-up performance of Viresolve® Pro Devices, performance characteristics such as flow rate (for constant flux operation) and capacity should be normalized by the effective filtration area. The flow rate (at constant flux) or capacity of a different size device can be calculated by multiplying the effective filtration area of the device of interest. For larger-scale devices, LRV can be expected to be equivalent to validated reduction values obtained using smaller-scale devices.

A safety factor is recommended for sizing virus filters allowing for larger effective filtration area than the optimal value determined during process development. Among other factors, this safety factor can be rationally determined by accounting for process variability, data extrapolation and scale differences. An approach for rationally defining safety factors for filter sizing has been suggested by Lutz[1].

Materials and Methods

Viresolve® Pro PV Micro, Modus 1.1, Modus 1.3, and Magnus 2.1 Devices were built specifically for this study; all devices contained the same lots of membrane. Devices of each type were tested for permeability, capacity, and LRV in parallel for each run so that variability in the feed preparation or operating conditions would affect devices at each scale, equally. Some devices were treated with a caustic sanitization procedure while others were not sanitized.

Table 2 outlines the number of each type of device tested under each condition. Figure 1 shows schematically the set-up used to test the devices.

The procedure for devices treated for caustic sanitization is detailed in Table 3, while Table 4 details the procedure for devices tested without caustic sanitization.

Devices that were caustic-treated were first flushed with water, then integrity tested, then flushed with water again, and then flushed with 0.5 N NaOH for 10 minutes at 15 psi. After this flush, the 0.5 N NaOH solution was held in the devices for 16 hours without flow. For devices caustic-treated or devices not caustic-treated, Modus and Magnus Devices were integrity tested both before and after processing using an Integritest® 4 automated integrity tester with Service Pack 4.

Micro Devices were not integrity tested. All devices were flushed with water and buffer, and then challenged with a solution containing 24 g/L BSA in FA buffer with at least 106 pfu/mL of φX-174. Each device was challenged until the flow decayed by 75% of the value obtained during the buffer flush and the device capacity (V_{75}) was reached. At this point, grab and pool samples of the filtrate were collected and assayed for φX-174. A product recovery buffer flush was performed on Modus and Magnus Devices, followed by a water flush prior to the final integrity test.

The pressure and filtrate mass were monitored as a function of time and the temperature of each fluid was recorded for each step. Pressure measurements were made at the inlet to each device format to account for differences due to elevation and fluid flow through the feed manifold.

Product recovery was calculated for Magnus and Modus Devices using the following equation:

$$\% \text{ recovery} = \frac{V_{filtrate} \times C_{filtrate}}{V_{challenge} \times C_{challenge}}$$

Device Type	Number of Devices with Caustic Pretreatment	Number of Devices without Caustic Pretreatment
Micro Device	15	45
Modus 1.1 Device	3	3
Modus 1.3 Device	3	3
Magnus 2.1 Device	3	3

Table 2.
Quantity of devices tested for Viresolve® Pro Device scalability

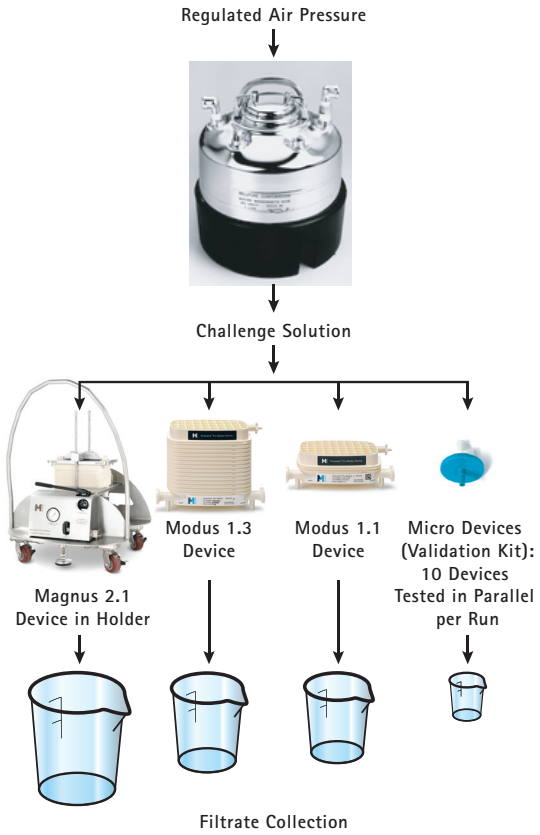


Figure 2.
Schematic overview of scalability testing

Scalability

Table 3.
Testing procedure for devices which were caustic-treated

Sequence	Step	Fluid	Pressure (psig)	Duration (min)
1	Wetting	DI water	30	15
2	Integrity testing	Air	Automated	Automated
3	Wetting	DI water	30	15
4	Caustic flush	0.5 N NaOH	15	10
5	Caustic hold	0.5 N NaOH	0	960
6	Water flush	DI water	30	15
7	Buffer flush	Buffer	30	15
8	Protein challenge	BSA	30	~40 (V_{75})
9	Product recovery	Buffer	30	~10 (10 L/m^2)
10	Water flush	DI water	30	~10 (10 L/m^2)
11	Integrity testing	Air	Automated	Automated

Table 4.
Testing procedure for devices which were not caustic-treated

Sequence	Step	Fluid	Pressure (psig)	Duration (min)
1	Wetting	DI water	30	15
2	Integrity testing	Air	Automated	Automated
3	Water flush	DI water	30	15
4	Buffer flush	Buffer	30	15
5	Protein challenge	BSA	30	~40 (V_{75})
6	Product recovery	Buffer	30	~10 (10 L/m^2)
7	Water flush	DI water	30	~10 (10 L/m^2)
8	Integrity testing	Air	Automated	Automated

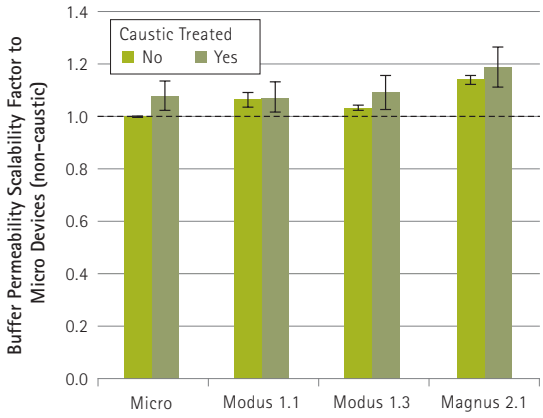


Figure 2.
Scalability factor for buffer permeability

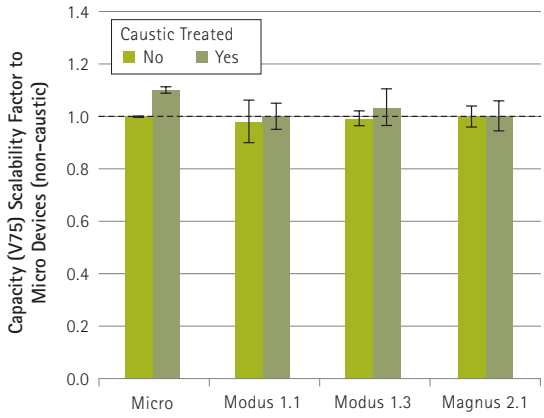


Figure 3.
Scalability factor for capacity

Results

The average buffer permeability scalability factor for each device format is shown in Figure 2. The scalability factor was defined as the ratio of the permeability for a given device to the permeability for Micro Devices (without caustic pretreatment). The error bars represent one standard deviation of the scalability factor determined for each run. Modus Devices scaled within 10% of the permeability of Micro Devices, while Magnus Devices scaled within 20%. These results demonstrate linear scalability and indicate that Viresolve® Pro Device users will experience similar processing times regardless of the scale.

The average capacity scalability factor for each device format is show in Figure 3. As with Figure 2, all values are normalized to the capacity obtained on Micro Devices that had not undergone caustic pretreatment. Each device type scales within 10% of the Micro Devices. These results demonstrate that the Viresolve® Pro Solution Device scale linearly with respect to capacity, resulting in a similar throughput per unit area regardless of the device size and format.

Furthermore, the devices tested demonstrated similar fouling behavior at each scale. Figure 4 displays the fouling profiles for one of the test runs, expressed as

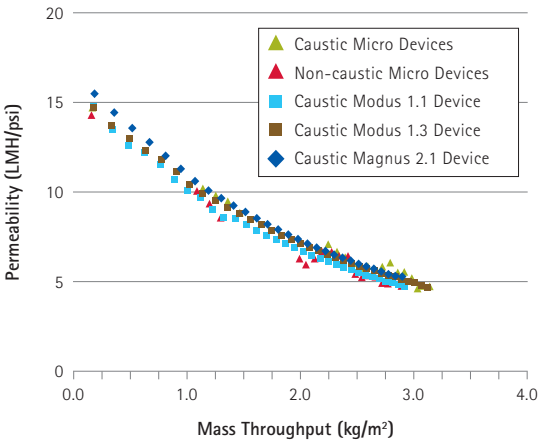


Figure 4.
Fouling behavior for Viresolve® Pro Devices

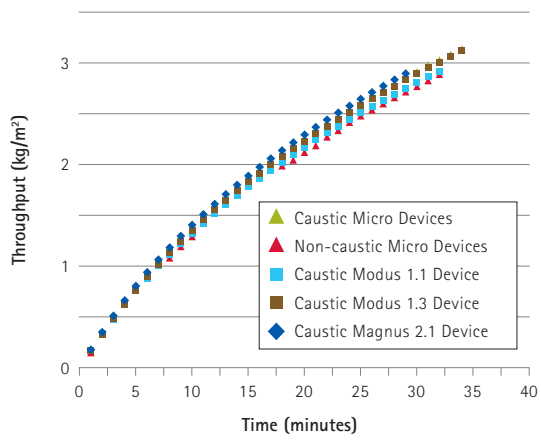


Figure 5.
Mass throughput scalability for Viresolve® Pro Devices

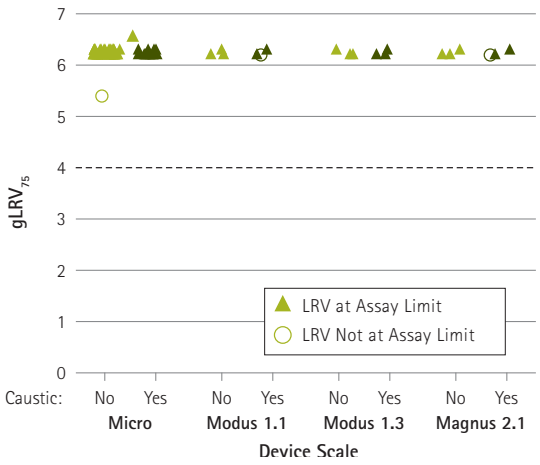


Figure 6.
Grab LRV data collected at 75% flow decay

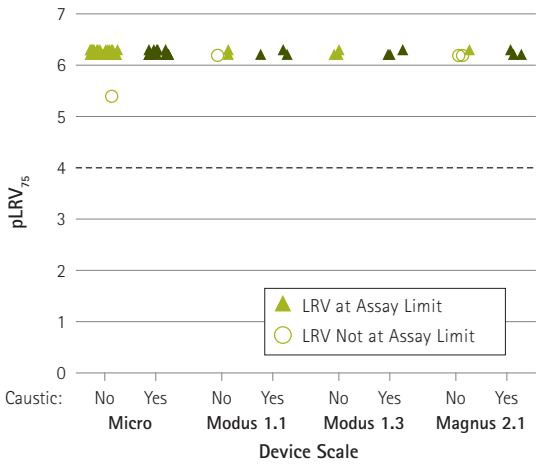


Figure 7.
Pool LRV data collected at 75% flow decay

Scalability

Figure 8.
Area-normalized air diffusion rates for Modus and Magnus Devices

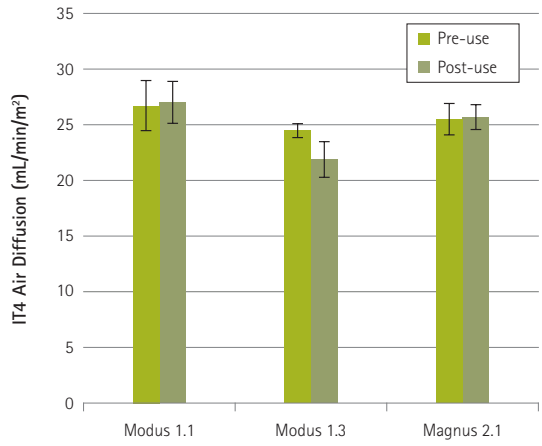
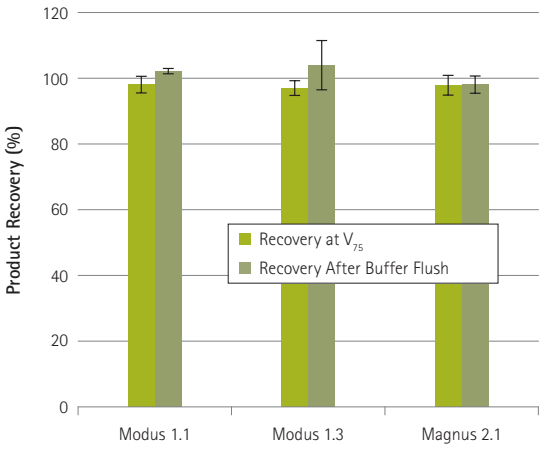


Figure 9.
Protein recovery in Modus and Magnus Device filtrate pools



the permeability of each device as a function of mass throughput. For Micro Devices, the performance of multiple devices within a run were combined, with gaps in the data representing time intervals during which grab samples were collected to monitor LRV. The agreement in these data indicates that the same underlying phenomena govern filter performance at each scale, and that capacity will scale linearly between device formats for any filtration endpoint, whether defined by throughput or flux. This feature is reinforced in Figure 5, which shows the mass throughput as a function of time. For a given processing time, the throughput per unit area is nearly indistinguishable between the different device scales.

Retention data corresponding grab and pool samples collected at 75% flow decay are displayed in Figures 6 and 7, respectively. All LRV data were greater than 4 logs; most or all of the data for each device format is at or near the limit of the assay. The consistently high LRV across all devices demonstrates that devices in the Modus and Magnus formats can obtain LRV values comparable to Micro Devices.

In order to provide assurance of the integrity of each Modus and Magus Device, the Integritest® 4 automated integrity tester was used both before and after each test. Each device passed with an air diffusion value below the specification before and after processing. The results from these tests were normalized by the membrane area and are summarized in Figure 8.

The protein recovery for Modus and Magnus Devices is summarized in Figure 9. At each scale, on average greater than 95% of the protein was recovered in the filtrate pool at V_{75}. The amount of protein recovered increased when the devices were flushed with 10 L/m^2 of buffer.

References

1. H. Lutz. "Rationally defined safety factors for filter sizing". *Journal of Membrane Science*, 341 (2009) 268-278.

Case Study

Processing with Viresolve® Pro Devices

Overview

In this study, the feasibility and performance of an entire Viresolve® Pro Device process using an inline Viresolve® Prefilter will be demonstrated. The process included installation, flush, pre-use integrity testing, sanitization, buffer conditioning, protein processing, recovery flush, and post-use integrity. This study used the 0.017 m^2 size Viresolve® Pro Modus Device with an antibody protein at 20 mg/mL concentration.

Methods

A Viresolve® Pro Modus 1.1 Device was flushed with 100 L/m^2 of purified water (PW) at 30 psid. An automated tester was used to run the pre-use 50 psig air-water diffusion integrity test with a spec of ≤0.71 mL/min. After venting, a 50 L/m^2 PW flush at 30 psid was used to remove additional air and restore filter permeability. Sanitization involved a 10 L/m^2 flush of 0.5 N NaOH and at 15 psi to saturate the module. After a 45 min hold, a 50 L/m^2 PW flush at 30 psid was used to remove the NaOH. Filtrate pH and conductivity was measured to verify the flush.

A Viresolve® Prefilter was flushed with 100 L/m^2 of PW at 600 LMH and installed in front of the Viresolve® Pro Device and flushed with 50 L/m^2 (of Viresolve® Pro area) of an acetate buffer at 30 psid. A 0.2 µm filtered 20 g/L MAb feedstock was then processed at 500 L/m^2 (10 Kg/m^2) through both at an inlet pressure of 30 psig. After processing, a 50 L/m^2 (of Viresolve® Pro area) buffer flush at 15 psig was used to recover additional product. During this flush, filtrate samples were taken to assess recovery.

The prefilter was then removed and the Viresolve® Pro Device flushed with 50 L/m^2 of PW. An automated tester was used to run the post-use 50 psig integrity test. An extra 50 L/m^2 PW flush at 30 psid was used to check permeability recovery. This final flush is not part of normal operation.

A feed manifold was used that allowed switching between fluids without the introduction of air. Modules were vented between steps. Purified water was introduced into the manifold from a purification system with standard line pressure. Acetate buffer, 0.5 N NaOH sanitant and protein were introduced from a sealed stainless pressure can using compressed air. Filtrate volumes were recorded using a data acquisition system.

Results

- All integrity tests passed with a pre-use value of 0.33 and a post-use value of 0.41 mL/min
- The 50 L/m^2 PW flush at 30 psid reduced the NaOH conductivity from 92,000 to 19.0 µ S/cm, comparable to the PWI conductivity of 12.2 µ S/cm

As shown in Figure 1, the initial normalized water permeability (NWP) is 15.5–16.0 LMH/psid, in-line with the expected 10–25 LMH/psi range. Integrity testing drops the permeability to 1-2 LMH/psid due to the introduction of air. The 50 L/m^2 PW flush at 30 psid consistently removes trapped air, restoring the initial permeability. Protein processing permeabilities were 8–10 LMH/psid, roughly half the water permeability. The recovery step partially restores NWP and the PW flush before post-use integrity testing restored NWP fully

As shown in Figure 2, the post-processing buffer flush filtrate (A^{280}) values show the product recovery from the module. The area under this curve was integrated as a measure of protein mass. The % mass recovered was calculated as the ratio of the area under the curve to the left of the L/m^2 value, divided by the total area under the curve. Assuming no significant adsorption or dead volume, this data suggests that the recommended 50 L/m^2 recovery flush will recover 99% of the product out of the module. This data also supports the adequacy of the pre-processing 50 L/m^2 conditioning buffer flush.

Figure 1.
Effects of each process step

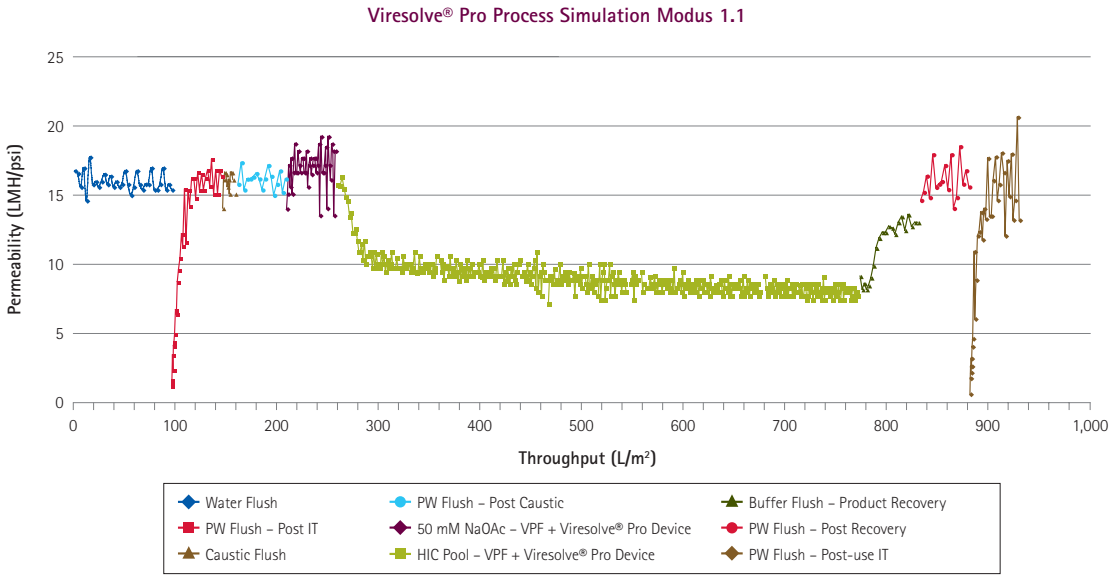
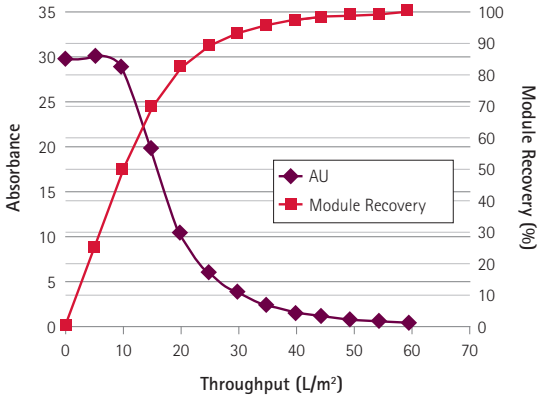


Figure 8.
Protein recovery



Appendix

Nomenclature

CHO: Chinese Hamster Ovary

gLRV: an instantaneous LRV at a specified sample point, i.e., "grab sample"

J/Jbuffer: normalized flux – the instantaneous flux divided by the flux as measured with buffer

LRV: Log Reduction Value

MAb: Monoclonal Antibody

OD_{280}: Optical Density at a wavelength of 280 nm

pLRV: a cumulative LRV at a specified sample point, i.e., "pool sample"

PP7–LRF4: a rating defined by the Parenteral Drug Association (PDA) for PP7 Bacteriophage defining a minimum log reduction factor of four

$TCID_{50}$: tissue culture infectious dose 50 assay

V_{50}: the volumetric throughput at which the flow rate is reduced to 50% of the flow rate measured with buffer

V_{75}: the volumetric throughput at which the flow rate is reduced to 75% of the flow rate measured with buffer

V_{90}: the volumetric throughput at which the flow rate is reduced to 90% of the flow rate measured with buffer

Please note: All of the φX-174 and MMV was supplied by our R&D group unless otherwise noted in the chapter.

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