# Viresolve® Barrier Filters
# performance guide


MilliporeSigma

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Preparation, Separation,
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# Introduction

Viresolve® Barrier filters are designed to remove viruses from cell culture media to reduce the risk of bioreactor contamination. This guide is a reference that provides summary information to assist in the evaluation of Viresolve® Barrier filters, as well as an overview of Viresolve® Barrier filter performance.

Viresolve® Barrier capsules are available in four production-scale sizes, from 0.05 $m^2$ to 1.0 $m^2$. A process development kit containing nine Micro filters (3.3 $cm^2$ each) is available for screening trials. These filters exhibit linear scalability from the Micro filter through the largest size production-scale filter. Consequently, most studies described in this guide use the Micro filters.

The results provided in this guide are applicable for the test media and conditions specified and do not predict performance with other media or experimental conditions. These results should not be considered as product claims or specifications. Users should execute their own studies to generate performance data that is representative of their process with Viresolve® Barrier filters.

Please contact Technical Service for more information on Viresolve® Barrier filters or visit

**EMDMillipore.com/ViresolveBarrier**

# overview of studies

## 1 Throughput Performance

Capacity of Viresolve® Barrier filters is compared to other commercially-available virus filters. Filter capacity was determined with several medium streams, including those containing hydrolysates.

The impact of higher concentrations of components that might cause filter fouling was evaluated. Process conditions that improve filterability of glucose streams using Viresolve® Barrier filters is discussed.

Viresolve® Barrier filter capacity is evaluated using both constant pressure and constant flow modes.

## 2 Microorganism Retention

Retention of viruses, mycoplasma and bacteria with Viresolve® Barrier filters is shown. Retention of minute virus of mice (MVM) was assessed under constant flow at filtration times up to four hours and under constant pressure for up to eight hours. The impact of different operating pressures on PhiX-174 retention using Viresolve® Barrier filters is summarized.

## 3 Maintaining Cell Culture Performance

Extensive characterization of media composition and cell culture performance was performed after Viresolve® Barrier filtration. Characterization included cell growth, antibody titer, and protein quality attributes.

## 4 Scalability

Scalability of flux and cell culture media capacity is demonstrated on Viresolve® Barrier filters from Micro filters (3.3 $cm^2$) to the largest production scale capsule filter (1.0 $m^2$). Virus retention is also shown to be consistent between the Micro filter and a 0.05 $m^2$ capsule filter.

# Throughput performance

Virus filters must achieve good capacity or volumetric throughput to be a cost-effective option for filtration of cell culture media. Viresolve® Barrier filters contain a single layer of virus-retentive asymmetric polyethersulfone (PES) membrane, leveraging the proven technology of the Viresolve® platform. This membrane was modified with a secondary chemistry to optimize these filters for high-flux virus filtration of cell culture media.

Viresolve® Barrier filters demonstrated good throughput performance across a variety of basal media and feed streams.

# Throughput Performance

## Methods

Micro filters were challenged at a constant pressure of 30 psi (2.1 bar). **Figure 1-1** shows the experimental setup. In cases where multiple filters were tested in parallel, a single pressurized feed vessel was used to challenge all filters.

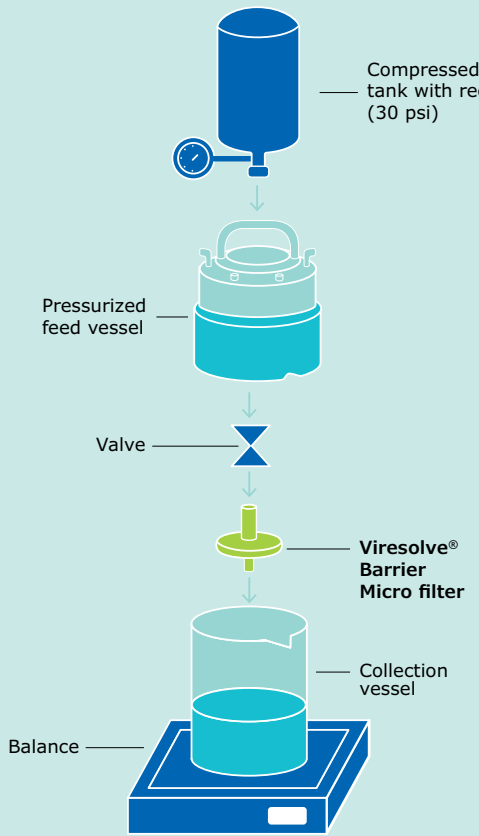



**Figure 1-1. Experimental setup for constant pressure testing**.

## Performance for a Range of Media

Viresolve Barrier® filters were challenged with a variety of chemically-defined media including both basal media and feeds.

## Results

The capacities of Viresolve® Barrier filters with several off-the-shelf media are presented in **Figure 1-2**. Average capacity of the basal medium is 1750 L/m² in 4 hours. Although performance is dependent on the medium composition, capacity of Viresolve® Barriers filters exceeded 1000 L/m² for the majority of the basal and feed media tested. This demonstrates that Viresolve® Barrier filters offer efficient filtration across a broad range of media.

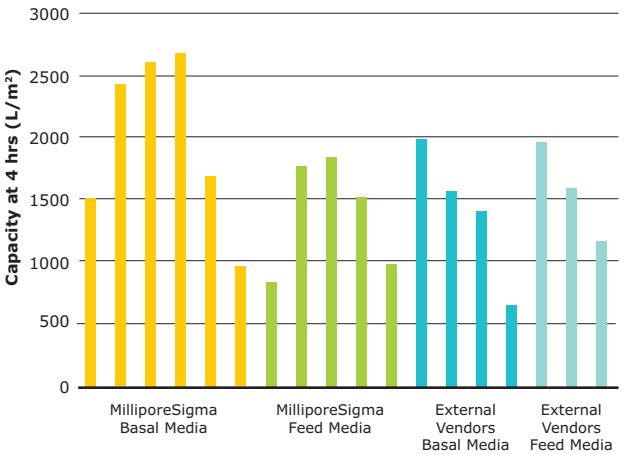



**Figure 1-2. Capacity of Viresolve® Barrier filters for a variety of media. Each bar represents performance of a different medium.**

## Comparison to Downstream Virus Filters

The throughput of a chemically-defined cell culture medium on Viresolve® Barrier filters was compared to throughput on commercially available virus filters designed for downstream processing.

## Results

**Figure 1-3** presents the throughput as a function of time for Viresolve® Barrier and downstream virus filters. Throughput of this cell culture medium on Viresolve® Barrier filters was notably higher than on downstream virus filters because the membrane in Viresolve® Barrier filters has been optimized for cell culture media processing.

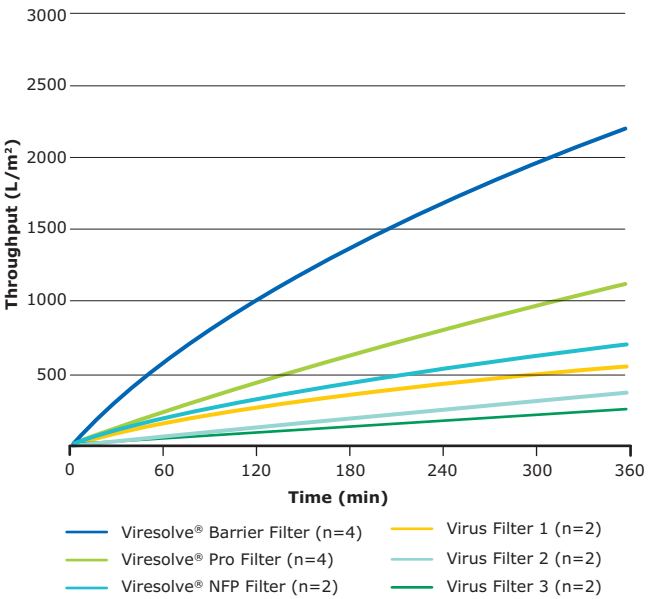



**Figure 1-3. Mean throughput of a chemically-defined medium using Viresolve® Barrier filter and various downstream virus filters**.

## Hydrolysate-containing Media

Viresolve® Barrier filters were challenged with customer-proprietary basal and feed media, including some that contain hydrolysates.

### Results

The capacity of Viresolve® Barrier filters for two different basal media and feeds was evaluated. Results are shown in **Figure 1-4**.

Throughput of this hydrolysate-containing basal medium was comparable to that of the chemically-defined basal medium. Viresolve® Barrier filters reached a capacity of approximately 1800 L/m² with hydrolysate-containing basal medium. Despite similar capacity for the two basal media, the hydrolysate containing-feed was more challenging to filter. This data demonstrates that it is possible to efficiently filter hydrolysate-containing media with Viresolve® Barrier filters. However, the identity and concentration of the hydrolysate can significantly impact performance.

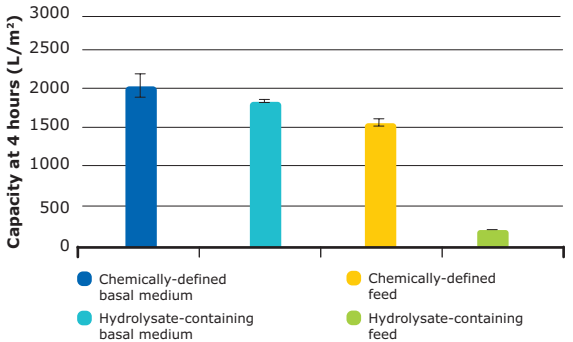



**Figure 1-4. Mean capacity of hydrolysate-containing media as compared to chemically-defined media, on Viresolve® Barrier filters (n=2 per category).**

## Processing Difficult-to-filter Components

In addition to the throughput studies using off-the-shelf media, the filters were also challenged with two commercially available basal media supplemented with higher concentrations of components that were identified as potentially high risk for filter fouling. Medium with a low level of a particular component was used as the baseline and was supplemented with higher levels of the component of interest to determine the impact on throughput. Components were selected based on characteristics that could impact filterability.

**Table 2-3. Components and characteristics potentially impacting filterability**

| Component | Rationale | Observed Impact |
|---|---|---|
| Insulin | Size (1-5 nm) | No |
| Dextran | Size (1-5 nm) | No |
| Fatty Acid Mix | Hydrophobicity | No |
| Hydrocortisone | Hydrophobicity | No |
| Poloxamer | Size or hydrophobicity | Yes |
| Iron Salts | Solubility | Maybe (solubility dependent) |

Figure 1-5 shows the capacity of Viresolve® Barrier filters for two basal media supplemented with the various potentially fouling components. Insulin and dextran did not impact capacity of the filters despite the relatively large size of these components. Similarly, the hydrophobic components, the fatty acid mix and hydrocortisone, did not impact filterability of basal media on Viresove® Barrier filters and no impact was observed when hydrophobic components, such as fatty acid, were combined with relatively large components, such as dextran.

Viresolve® Barrier filter chemistry is optimized for compatibility with poloxamer, but the capacity of the filter is still impacted to some extent by poloxamer. Basal media 1 and 2 contain 1.2 g/L and 2 g/L poloxamer respectively. However, when poloxamer concentration was increased to 5%, throughput reductions of 20% and 40% were observed in basal media 1 and 2 as compared to baseline media, **Figure 1-5.**

Solubility of metal salts, such as iron, can vary dependent on the salt, the formulation of the medium, and whether chelators are included. The baseline media in Figure 1-5 contain different iron salts, with a more soluble iron salt (iron salt 2) in basal medium 2. When basal medium 2 is supplemented with the same type and concentration of iron salt found in basal medium 1 (iron salt 1), filter throughput is reduced and is similar to basal medium 1 baseline. Addition of a chelator in an equimolar concentration to the iron salt mitigated the negative effect on throughput. When iron salt 2 was spiked to higher levels, the capacity did not decrease significantly from baseline, highlighting the dependence on salt type and medium formulation. Preparation conditions can impact the performance of these formulations; within a run, similar performance was observed between replicates. However, where multiple runs were performed, some run to run differences in throughput were observed as indicated by large standard deviations.

Overall, these results indicate the formulation of media can impact filterability, but the magnitude of the effect depends on media composition, type and concentration of components, and solubility of metal salts. Each cell culture medium should be evaluated for filterability on Viresolve® Barrier filters.

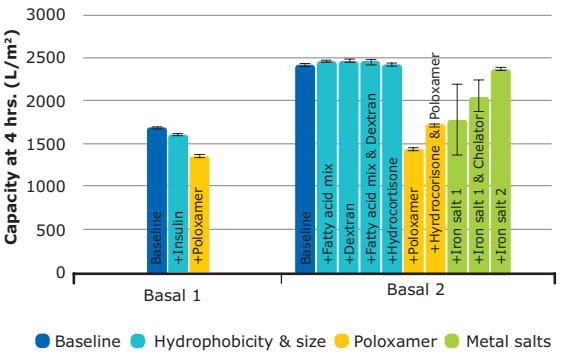



**Figure 1-5. Mean throughput of media supplemented with potentially fouling components on Viresolve® Barrier filters (n=3-6 per foulant).** Error bars indicate 1 standard deviation.

## Throughput Enhancement in Glucose Solutions

Glucose is an essential energy source for CHO cells, and is a common feed component. Although glucose is chemically defined, it poses a high risk for virus contamination since it is a rodent attractant; as such, virus filtration of glucose feeds is an application of particular interest. Typical glucose feeds, with 30 to 50% glucose, are viscous solutions, resulting in relatively low filtration flux. A study was performed to identify test conditions that improve filterability of glucose solutions on Viresolve® Barrier filters. Throughput was evaluated for glucose solutions ranging in concentration from 30% to 50% and at temperatures ranging from 21°C to 40°C.

### Results

**Figure 1-6** presents the mass of glucose filtered over 4 hours at different temperatures at a constant pressure of 30 psi (2.1 bar).

A higher mass throughput can be achieved by filtering the glucose at 30°C or 40°C compared to 20°C.

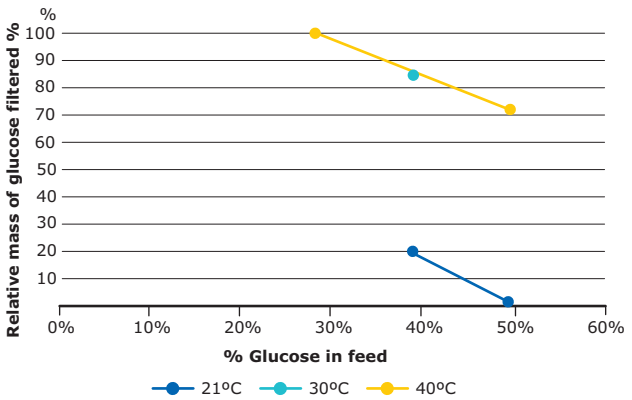



**Figure 1-6. Relative mass of glucose processed on Viresolve® Barrier filters in 4 hours, as a function of temperature and concentration. Data is normalized to mass of 30% glucose processed at 40ºC.**

Although smaller in magnitude than the temperature effect, reducing glucose concentration also improved the mass throughput at both high and low temperature.

Both elevated temperature and reduced concentration decrease the viscosity of the solution, resulting in increased efficiency of glucose filtration on Viresolve® Barrier filters.

## Performance in Constant Flow and Constant Pressure Modes

Depending on the specifics of a given process, media filtration may be performed under either constant pressure or constant flow. The objective of this study was to demonstrate that Viresolve® Barrier filters can be operated in either constant pressure or constant flow mode.

### Methods

Tests were executed using EX-CELL® Advanced CHO fed-batch basal medium. Three Viresolve® Barrier Micro filters were tested in each constant flow and constant pressure modes, with a single test for each operation mode.

For the constant flow test, filters were challenged at a flux of 291 LMH for 4 hours. Flux was set to maximize throughput while ensuring the pressure drop across Viresolve® Barrier filters did not exceed 20 psi (1.4 bar) at 4 hours. **Figure 1-7** shows the experimental setup.

For the constant pressure test, the filters were challenged at a constant pressure of 30 psi (2.1 bar) for 4 hours. A single pressurized feed vessel was used to challenge the filters **(Figure 1-1)**.

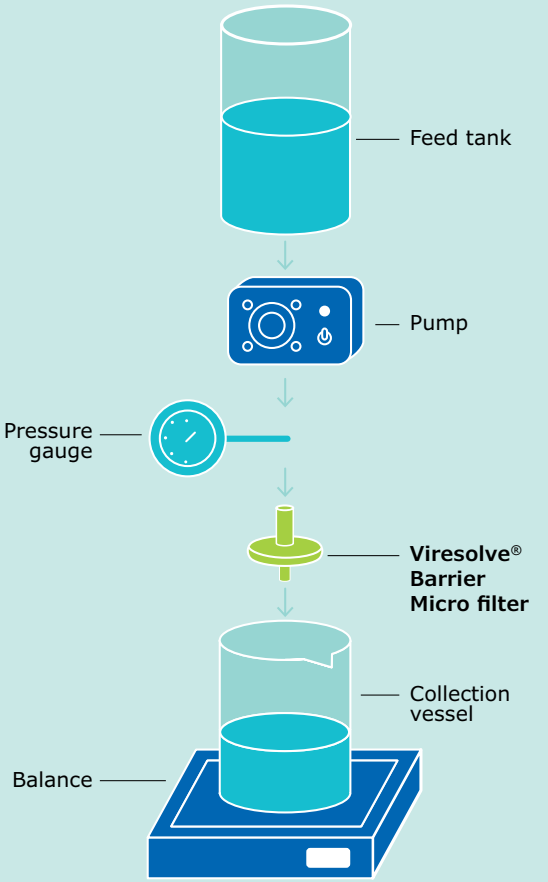



**Figure 1-7. Experimental setup for constant flow testing.**

## Results

Throughput of medium using Viresolve® Barrier Micro filters at constant pressure is shown in **Figure 1-8**. At 4 hours, the average media throughput at 30 psi (2.1 bar) constant pressure was 1685 L/m².

Throughputs of Viresolve® Barrier filters for the constant flow mode are shown in **Figure 1-9**. After 4 hours at the selected flux of 291 LMH, average medium throughput was 1163 L/m². Pressure drop across the filters in the constant flow mode was slightly under 20 psi (1.4 bar) at 4 hours. Because of the chosen test conditions, the average pressure across the filters was higher in the constant pressure testing, resulting in higher volumetric throughput. **Figure 1-10** shows the changes in permeability across the filters as a function of throughput under both constant pressure and constant flow conditions. Under these conditions, the constant pressure test, had a more rapid loss in permeability with increasing throughput than the constant flow test.

While Viresolve® Barrier filters can be operated in both constant pressure and constant flow modes, these results highlight the importance of understanding your process and system limitations and performing sizing studies with small-scale filters under the same operation mode and processing conditions that will be used for the full-scale process.

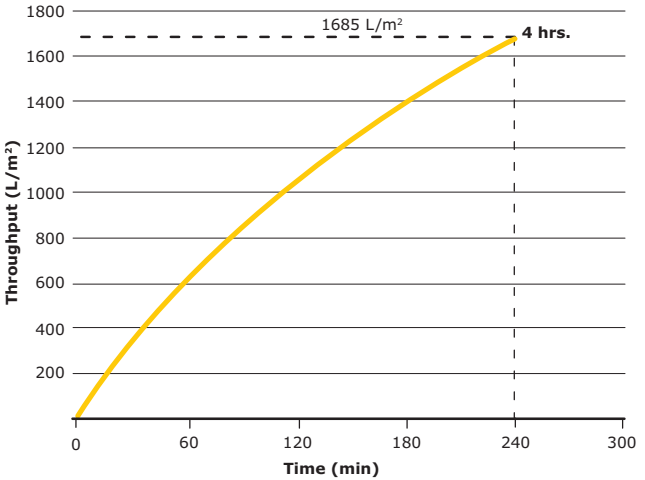



**Figure 1-8. Throughput of EX-CELL® Advanced CHO fed-batch medium on Viresolve® Barrier filters at 30 psi (2.1 bar) constant pressure**

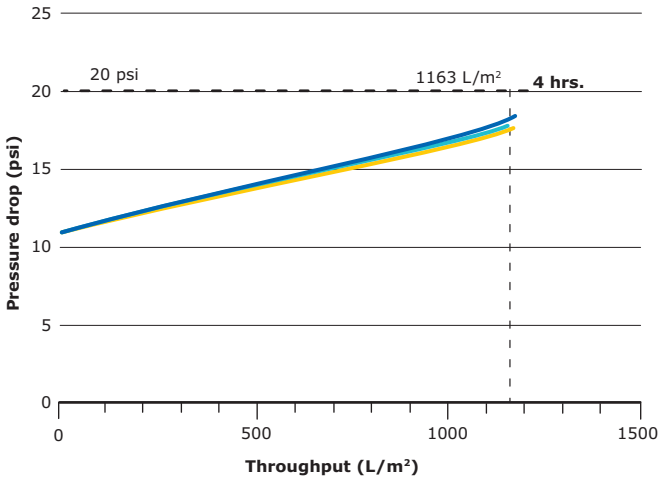



**Figure 1-9. Pressure drop across Viresolve® Barrier filters as a function of throughput for EX-CELL® Advanced CHO fed-batch medium at 291 LMH constant flow.**

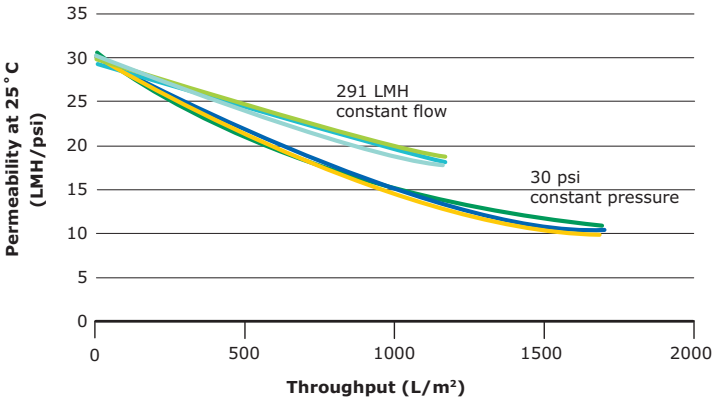
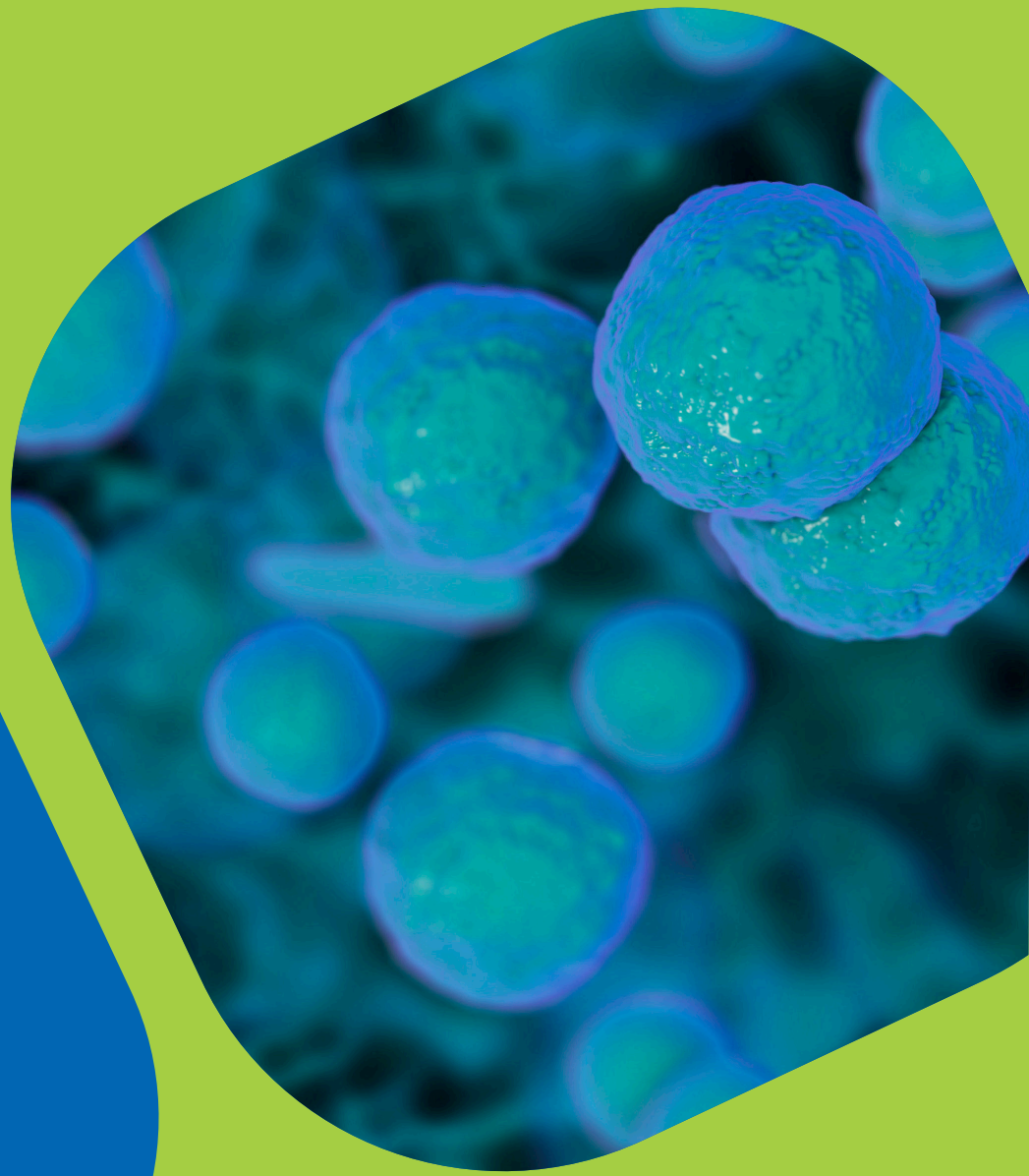



**Figure 1-10. Flow decay of EX-CELL® Advanced CHO fed-batch medium on Viresolve® Barrier filters at constant pressure and constant flow conditions.**

# Microorganism Retention

The objective of these studies was to demonstrate robust retention of a panel of microorganisms using Viresolve® Barrier filters. Filters used for cell culture media typically contain membrane with pore sizes of 0.2 µm (bacterial retention) or 0.1 µm (mycoplasma retention). However, some microorganisms are smaller than the retentive pores of these filters and may still present a risk of bioreactor contamination.

Retention studies were performed with Viresolve® Barrier filters and a panel of microorganisms. Sterilizing-grade performance was achieved for retention of bacteria; complete mycoplasma retention was demonstrated and a high level of virus retention was achieved.



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# Retention of Relevant Microorganisms

## Methods

Retention testing was performed at a constant pressure of 30 psi (2.1 bar) using a minimum of 3 replicates per test. **Tables 2-1 and 2-2** provide information on the microorganisms and experimental details for each of the challenge tests. For the mycoplasma and bacteria testing, the entire filtrate was processed through a recovery filter and recovered microorganisms were counted. For the virus testing, a sample of the filtrate pool was collected during the test and assayed for titer using cell based infectivity assays. Log reduction value (LRV) was determined by comparing microorganism load in the challenge solution with the load in the filtrate.

## Results

A summary of the bacteria and mycoplasma retention performance with Viresolve® Barrier filters is shown in **Figure 2-1**. Each bar represents the retention result of a single Viresolve® Barrier filter. Full retention (LRV ≥ 7.8) of bacteria and mycoplasma was observed.

A summary of virus retention performance with Viresolve® Barrier filters is shown in **Figure 2-2**. Each bar represents the retention result of a single Viresolve® Barrier filter; multiple lots were tested for MVM retention, as it is the target organism.

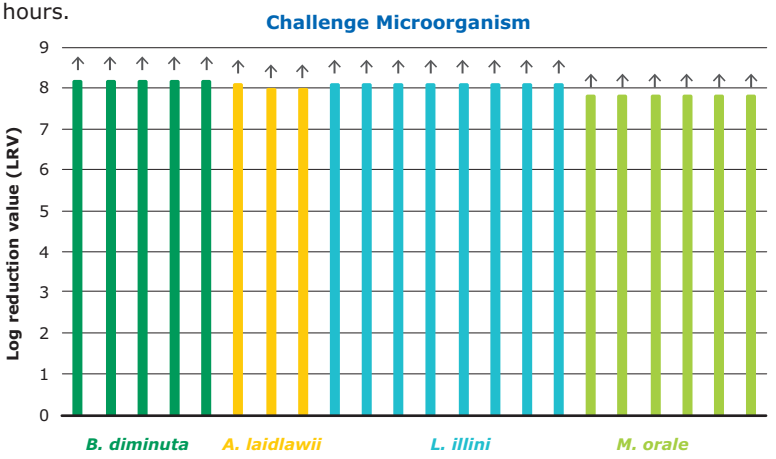
Full retention (LRV ≥ 6.1) was measured for x-MuLV at the test endpoint of 1 hour. For MVM, at least 4 logs retention was observed for all test filters with an average 5 logs retention at the test endpoint of 2 hours.

**Table 2-1. Microorganism panel used to test retention of Viresolve® Barrier filters.**

| | *Brevundimonas diminuta* | *Acholeplasma laidlawii* | *Leptonema illini* | *Mycoplasma orale* | Murine leukemia virus (x-MuLV) | Minute virus of mice (MVM) |
|---|---|---|---|---|---|---|
| **Description** | Rod-shaped bacteria | Mycoplasma | Spirochete bacteria | Small mycoplasma | Large enveloped virus | Small non-enveloped virus |
| **Significance** | ASTM® F838 test organism for sterilizing-grade filters | Standard model organism for 0.1 µm filters | Difficult to retain on 0.1 µm filters | Relevant contaminant; difficult to retain on 0.1 µm filters | Model large virus | Relevant contaminant; target organism |

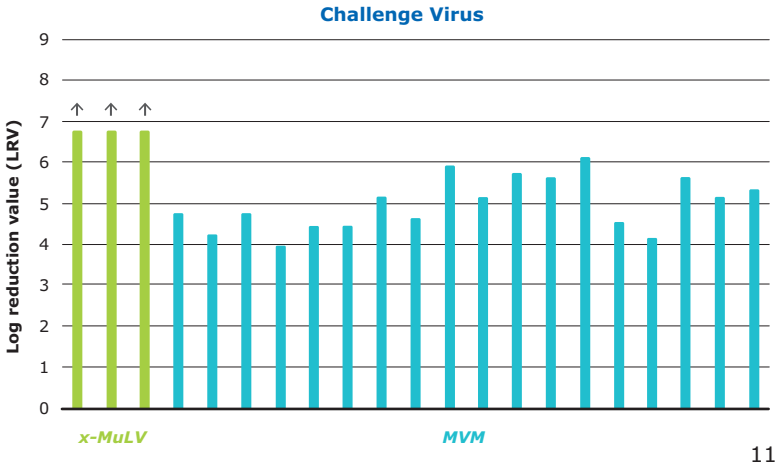
**Table 2-2. Retention testing details for Viresolve® Barrier filters. Micro filters were used for all retention testing at a constant feed pressure of 30 psi (2.1 bar).**

| | *Brevundimonas diminuta* | *Acholeplasma laidlawii* | *Leptonema illini* | *Mycoplasma orale* | Murine leukemia virus (x-MuLV) | Minute virus of mice (MVM) |
|---|---|---|---|---|---|---|
| **Medium** | 0.1% peptone | Phosphate buffer | 0.1% peptone | PPLO culture | Thermo Fisher Scientific CD OptiCHO™ medium | Thermo Fisher Scientific CD OptiCHO™ medium |
| **Challenge level** | $4.4 \times 10^7$ CFU/cm² | $3.4 \times 10^7$ CFU/cm² | $2.6 \times 10^8$ CFU/cm² | $2.0 \times 10^7$ CFU/cm² | $6.5 \times 10^5$ $TCID_{50}$/mL | $2.0 \times 10^6$ $TCID_{50}$/mL |




Note: Arrow indicates LRV was at assay limit

**Figure 2-1.**
**Challenge Microorganism**
**(left) Bacteria and mycoplasma retention using Viresolve® Barrier filters**




Note: Arrow indicates LRV was at assay limit

**Figure 2-2.**
**Challenge Virus (right)**
**Virus retention using Viresolve® Barrier filters**

## Retention of MVM at Extended Filtration Times

The objective of this study was to demonstrate robust MVM retention in cell culture media over extended filtration times with Viresolve® Barrier filters. Results demonstrated that Viresolve® Barrier filters containing nominal membrane provide average retention of 5.1 logs out to eight hours.

### Methods

Retention studies were performed with Viresolve® Barrier Micro filters, challenged at 30 psi (2.1 bar) constant pressure with CD OptiCHO™ cell culture medium spiked with MVM at a minimum titer of $2 \times 10^6$ $TCID_{50}$/mL. Filtration was performed to 8 hours. **Table 2-3** summarizes the capacity and number of filters tested.

### Results

Retention results out to the extended processing time of 8 hours are presented in **Figure 2-3**. MVM retention of at least five logs was consistent out to 8 hours at which point the filters were at 70-75% flow decay (data not shown).

These results demonstrate robust virus removal with no reduction in retention performance with virus loading, volume processed, or degree of filter plugging.

**Table 2-3. Average throughput and number of filters tested**

| Filtration Time (hours) | Average Throughput ($L/m^2$) |
|---|---|
| **2** | 1044<br>*n = 12* |
| **4** | 1720<br>*n = 12* |
| **8** | 2707<br>*n = 3* |

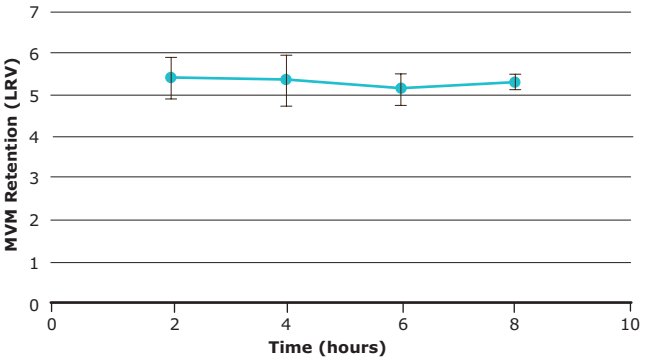



**Figure 2-3. MVM retention at an extended processing time of 8 hours.** Error bars indicate 1 standard deviation. (n=3)

## Retention of MVM in Constant Flow and Constant Pressure Modes

The objective of this study was to demonstrate robust retention of MVM with Viresolve® Barrier filters in both constant flow and constant pressure modes. Two commercial chemically-defined media were used to challenge the Viresolve® Barrier filters. For tests with each medium, high retention performance was achieved using both constant flow and constant pressure test modes.

### Methods

Tests were executed using EX-CELL® Advanced CHO fed-batch medium and CD OptiCHO™ medium. At least three Viresolve® Barrier Micro filters were tested in each constant flow and constant pressure test modes. The minimum MVM feed titer was $1 \times 10^6$ $TCID_{50}$/mL. Test equipment setup is shown for constant pressure and constant flow modes in **Figures 1-1 and 1-7**, respectively.

For the constant pressure test, the filters were challenged at 30 psi (2.1 bar) for 4 hours. A single pressurized feed vessel was used to challenge the filters in each test. For testing with CD OptiCHO™ medium, samples were collected from the filtrate pool at 2 hours and 4 hours. For testing with EX-CELL® Advanced CHO fed-batch medium, samples were collected from the filtrate pool at 30 minutes.

For the constant flow test, the filters were challenged with EX-CELL® Advanced CHO fed-batch medium at a constant flux of 250 LMH to maximize throughput while ensuring the pressure drop across the filters did not exceed 20 psi (1.4 bar) at 4 hours. Samples were collected from the filtrate pool at 1, 2, and 4 hours.

## Results

MVM retention for individual Viresolve® Barrier filters tested in CD OptiCHO™ medium under constant pressure conditions are presented in **Figure 2-4.**

After 4 hours of processing, filters were approximately 70% plugged with an average throughput of 1784 L/m$^2$. MVM retention was consistent at two and four hours with average LRV of 4.8 logs.

MVM LRV results for individual Viresolve® Barrier filters tested in EX-CELL® Advanced CHO fed-batch medium in constant flow mode are presented in **Figure 2-5.** No virus was detected in two of the filters at both 2 hours and 4 hours. Viral retention performance was consistent at the 1-hour, 2-hour and 4-hour time points with an average LRV of approximately 5.6 logs.

To compare performance under constant flow and constant pressure conditions, retention was evaluated when the filters were approximately 40% plugged. MVM retention results are shown in **Figure 2-6.**

Average LRVs at this common test endpoint were approximately 5.2 ± 0.6 logs and 5.6 logs under constant pressure and constant flow, respectively. Results indicate no difference in viral retention associated with constant pressure or flow operations.

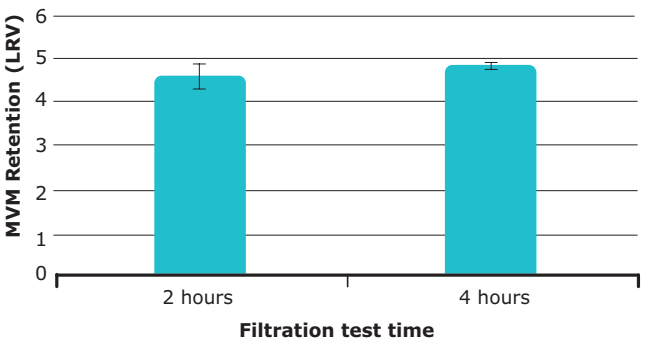



**Figure 2-4. Mean retention of MVM in CD OptiCHO™ medium under constant pressure conditions.** Error bars represent 1 standard deviation.

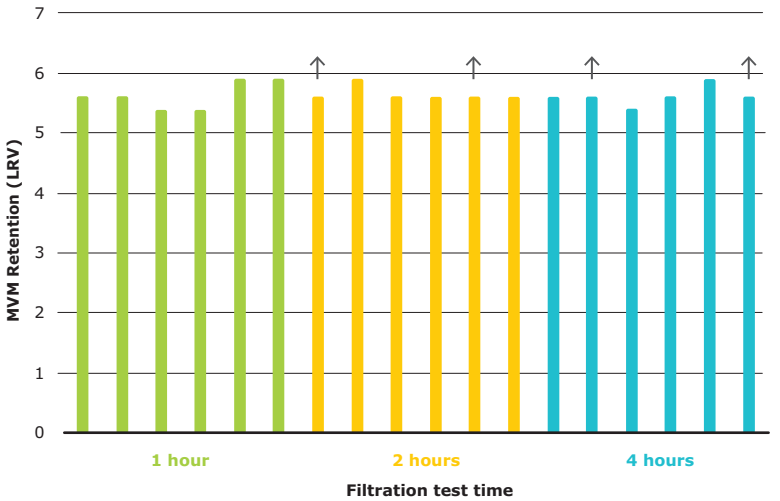



**Figure 2-5. Retention of MVM in EX-CELL® Advanced CHO fed-batch medium at constant flow conditions.** At both two and four hours hours, two filters exhibited retention at the assay limit.

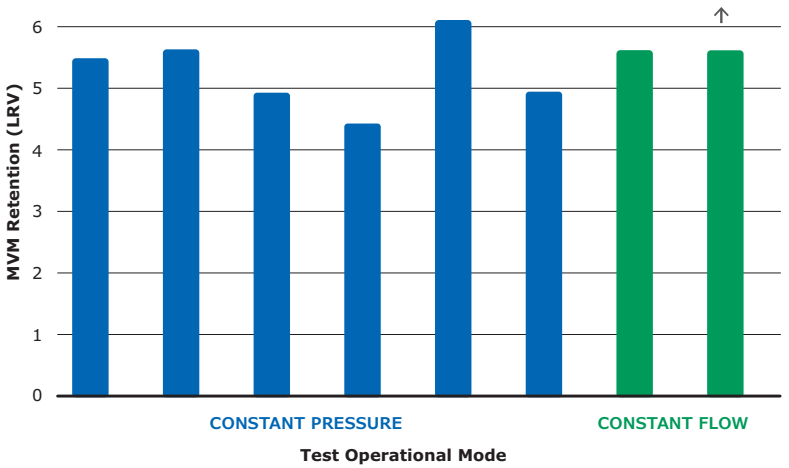



**Figure 2-6. Retention of MVM in EX-CELL® Advanced CHO fed-batch medium run under constant pressure and constant flow modes, with filter plugged 37-40%.**

## Retention of PhiX-174 at Different Operating Pressures

The objective of this study was to evaluate the performance of Viresolve® Barrier filters at constant operating pressures of 10 and 30 psi. The feed stream was EX-CELL® Advanced CHO fed-batch medium with PhiX-174, a model for parvovirus because of its size (approximately 27 nm). The results demonstrated consistent, robust retention at both 10 and 30 psi operating pressure.

### Methods

Six Viresolve® Barrier Micro filters were wet with water and water flux was measured at 30 psi (2.1 bar) pressure. Water flux was then measured in three of these filters at 10 psi (0.7 bar) and then those three filters were challenged with the feed solution at 10 psi (0.7 bar). The three remaining filters were challenged with the feed solution at 30 psi (2.1 bar). The feed solution consisted of EX-CELL® Advanced CHO medium spiked with PhiX-174 at a titer of approximately $2 \times 10^7$ pfu/mL. For all tests, samples were collected directly from the filter outlet at 4 hours of filtration.

### Results

Virus retention results at 4 hours of filtration are shown in **Figure 2-7**. Virus retention of at least 6.0 logs was observed in all devices at operating pressures of both 10 and 30 psi. These results indicate robust virus retention under a range of operating pressures.

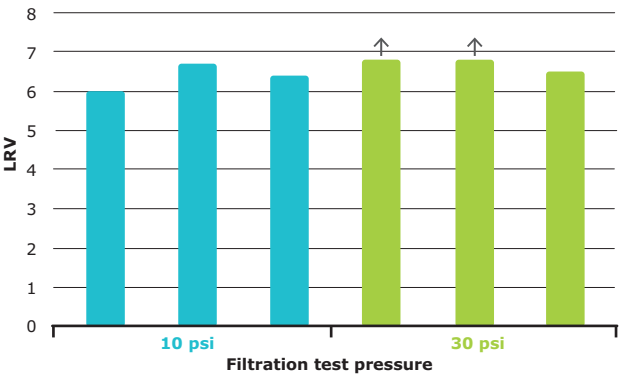



**Figure 2-7. PhiX-174 retention values from individual Viresolve® Barrier devices run at 10 and 30 psi**

# Maintaining cell culture performance

This study evaluates cell culture performance in a CHO fed-batch process following media filtration with Viresolve® Barrier filters. Extensive analytical characterization of cell culture media demonstrated no change in composition after Viresolve® Barrier filtration. Cell growth, antibody titer, and protein quality attributes were comparable using media filtered with Viresolve® Barrier and sterilizing-grade filters.

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## Media Composition Study

### Methods

EX-CELL® Advanced CHO basal medium and feeds were filtered through Viresolve® Barrier or control 0.22 µm (Millipore Express® PLUS) filters under constant pressure. Media composition pre- and post-filtration was characterized using nuclear magnetic resonance spectroscopy (¹H-NMR), high-performance liquid chromatography (HPLC) and inductively coupled plasma optical emission spectroscopy (ICP-OES). Cell culture performance and protein quality attributes were assessed.

**Cell culture materials included the following:**

- EX-CELL® Advanced CHO fed-batch basal medium
- EX-CELL® Advanced CHO feed 1
- 400 g/L glucose feed
- CHOZN® GS recombinant antibody-producing CHO cell line

1 C. Schulz, J. H. Vogel, K. Scharfenberg, *Influence of Pluronic F-68 on the Ultrafiltration of Cell Culture Supernatants, in Animal Cell Technology, 1st ed.*, M. J. Carrondo, B. Griffiths, J. L. Moreira, Ed. Netherlands: Springer, 1997, pp. 373-378.

2 O. K. Baryshnikova, T. C. Williams, B. D. Sykes, *Internal pH indicators for biomolecular NMR, Journal of Biomolecular NMR*, 41 (2008), pp. 5–7.

### Results

EX-CELL® Advanced CHO fed-batch basal medium and the two feeds were filtered through Viresolve® Barrier filters. The basal medium was characterized pre- and post-filtration by ¹H-NMR at 400 MHz. The aromatic and aliphatic portions of the NMR spectrum are shown in **Figures 3-1 and 3-2**, respectively. There was no noticeable difference in the aromatic region of the spectrum between Viresolve® Barrier filtered and unfiltered basal medium (**Figure 3-1**). The peaks corresponding to tryptophan, which is photo-unstable and sensitive to changes in the medium, were consistent before and after filtration.

The aliphatic region of the spectrum shown in **Figure 3-2** provides information about additional components. Poloxamer, which has proven difficult to filter in some operations[1], showed no difference pre- and post Viresolve® Barrier filtration. The shift near 2 ppm in **Figure 3-2**, is from the histidine proton, which is sensitive to pH changes[2], and is not indicative of a change in composition of the cell culture medium.

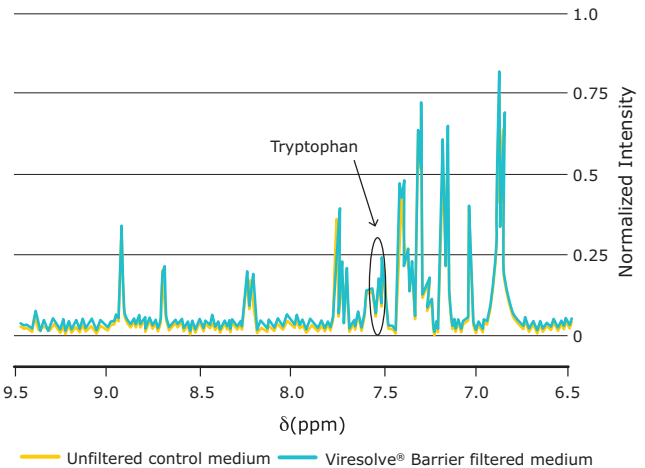


**Figure 3-1. EX-CELL® Advanced CHO fed-batch medium ¹H-NMR aromatic region comparing Viresolve® Barrier filtered cell culture medium and unfiltered control.**

Cell culture media components were evaluated using HPLC. **Figure 3-3** shows the ratio of the concentrations for individual media components before and after Viresolve® Barrier filtration, for both the EX-CELL® Advanced CHO basal medium and feed. All detectable components in Viresolve® Barrier filtered EX-CELL® Advanced CHO fed-batch basal medium and feed were within 7% of the unfiltered control.

In addition to NMR and HPLC analyses, ICP-OES was used to detect trace metals in the media. Results demonstrated no change in metal concentrations after filtration for all metals present at concentrations above the detection limit of the assay **(Figure 3-4).**

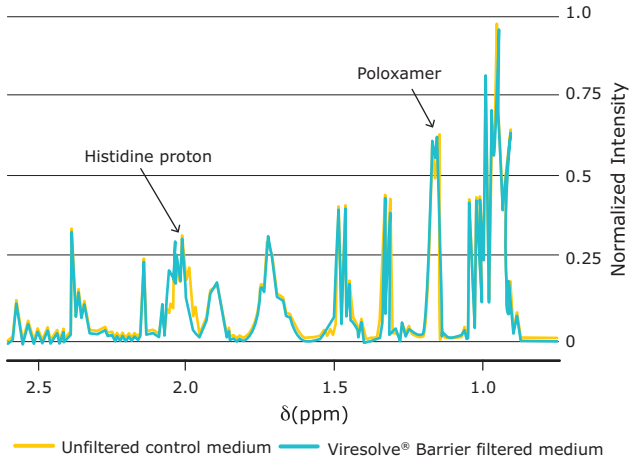


**Figure 3-2. EX-CELL® Advanced CHO fed-batch medium ¹H-NMR aliphatic region comparing Viresolve® Barrier filtered cell culture medium and unfiltered control.**

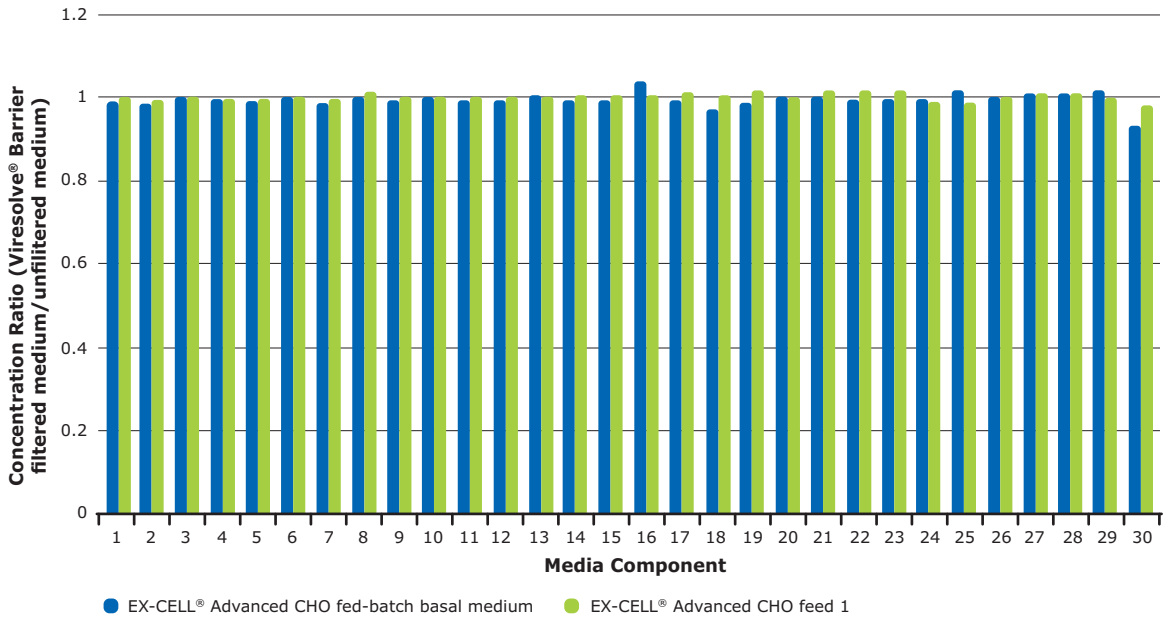


**Figure 3-3. HPLC concentration ratio for Viresolve® Barrier filtered to unfiltered EX-CELL® Advanced CHO fed-batch cell culture media.**

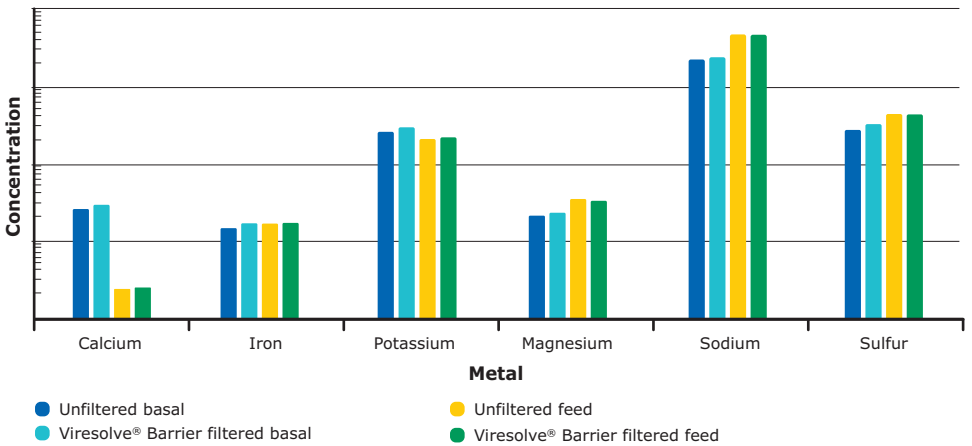


**Figure 3-4. ICP-OES metal analysis of Viresolve® Barrier filtered and unfiltered EX-CELL® Advanced CHO fed-batch cell culture media.**

## Cell Culture Performance Study

Viresolve® Barrier filtered EX-CELL® Advanced CHO fed-batch media were also used for monoclonal antibody (mAb) production with the CHOZN® GS recombinant cell line in Mobius® single-use bioreactors. Bioreactors were monitored for a number of performance and quality attributes.

The viable cell density indicated no differences in cell growth using Viresolve® Barrier filtered media compared to the controls, as seen in **Figure 3-5**.

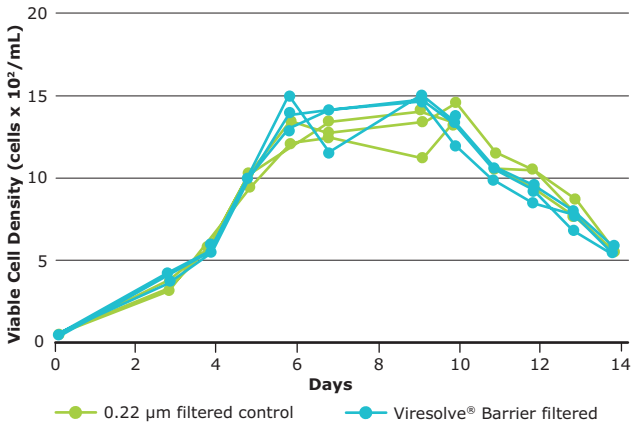


**Figure 3-5. Viable cell density during fed-batch cultivation for Viresolve® Barrier filtered and control filtered EX-CELL® Advanced CHO fed-batch medium and corresponding feeds, using Mobius® 3 L single-use bioreactor.**

Similarly, no differences in cell viability, doubling time, population doubling level, peak IVC (integral viable cell density), pH, osmolarity, glucose, glutamate, or lactate concentration were observed (data not shown).

In **Figure 3-6**, titers at day 14, measured by Protein A HPLC, showed no significant differences between a mAb produced with Viresolve® Barrier filtered cell culture media (VB+) and the control (VB-) in both 3L and 50L Mobius® single-use bioreactors

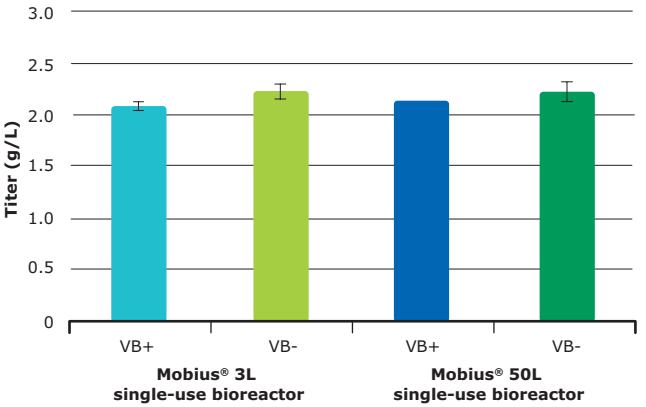


**Figure 3-6. Day 14 titer of CHOZN® GS-produced mAb with Viresolve® Barrier filtered and control filtered EX-CELL® Advanced CHO fed-batch medium and corresponding feeds, using Mobius® 3 L and 50 L single-use bioreactors.**

## Protein Quality Study

Glycan analysis of the purified mAb was performed using 2-aminobenzamide fluorescent labeling and ultra performance liquid chromatography (UPLC). Consistent glycan profiles were observed for mAbs produced with Viresolve® Barrier filtered and control filtered media **(Figure 3-7)**.

Charge variants were analyzed by weak cation-exchange chromatography (WCX). Results of the WCX showed no difference in the concentrations of acidic, neutral, or basic variants when using Viresolve® Barrier filtered media vs control filtered media (data not shown).

Size exclusion chromatography (SEC) was used to determine the levels of mAb aggregates. SEC analysis showed a high concentration of monomer purified from all cultures. Small amounts of high molecular weight (HMW) species were detected, and no fragments were observed. No difference in protein aggregate profile was identified for antibodies produced with Viresolve® Barrier filtered and control filtered media, as shown in **Table 3-1**.

The results of this study demonstrate that cell culture performance and protein quality attributes are not impacted by filtration of cell culture media through Viresolve® Barrier filters.

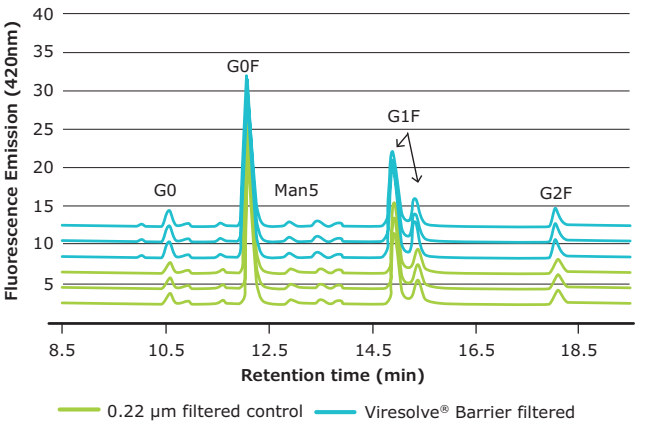


**Figure 3-7. Glycan profile of CHOZN® GS-produced mAb with Viresolve® Barrier filtered and control filtered EX-CELL® Advanced CHO fed-batch medium and corresponding feeds, using Mobius® 3 L single-use bioreactor.**

**Table 3-1. SEC analysis of CHOZN® GS-produced mAb with Viresolve® Barrier filtered and control filtered EX-CELL® Advanced CHO fed-batch medium and corresponding feeds, using Mobius® 3 L single-use bioreactor.**

| Aggregate Profile (Peak %, UV @ 280 nm) | | |
|---|---|---|
| | Monomer | HMW |
| **0.22 µm filtered control** | 98.7 ± 0.02 | 1.3 ± 0.02 |
| **Viresolve® Barrier filtered** | 98.8 ± 0.04 | 1.2 ± 0.04 |

# scalability

Process development work is typically performed on smaller filters than those used during production. Use of these smaller filters enables optimization of filter performance under the intended processing conditions, while minimizing cost, materials and time. The Micro filters are intended for process development work, while capsule filters are suited for filtration of larger volumes typical of pilot-scale and production-scale batches. Viresolve® Barrier filters scale linearly all device sizes.

4

## Performance from Bench Scale to Production Scale Filters

Permeability and capacity are expected to remain constant across filter sizes as they are normalized per unit area. Retention of virus is expected to be consistent across the filter sizes. The objective of this study was to evaluate permeability, capacity and virus retention across the various sizes of Viresolve® Barrier filters.

## Permeability Performance

### Materials and Methods

**Table 4-1** describes the available sizes of Viresolve® Barrier filters. The Micro filters are intended for process development work, while capsule filters are suited for filtration of larger volumes typical of pilot-scale and production-scale batches.

Water flow was measured under both constant pressure and constant flow. For the capsules, pressure at the device inlets and outlets were recorded for accurate pressure drop measurements. Filtrate temperature was also monitored so that variations in viscosity could be accounted for in scalability calculations.

**Table 4-1. Viresolve® Barrier filter sizes.**

| Viresolve® Barrier | Effective Filtration Area ($m^2$) |
|---|---|
| **Micro filter** | 0.00033 |
| **Capsule filter** | 0.05 |
| **Capsule filter** | 0.15 |
| **Capsule filter** | 0.5 |
| **Capsule filter** | 1.0 |

### Results

The average water flow rate for each filter size is shown in **Figure 4-1**. Flow rates were normalized to 30 psi (2.1 bar), as testing was performed using both constant pressure and constant flow conditions.

**Figure 4-1** confirms a linear relationship between flow rate and filtration area. The dotted line indicates the predicted flow rate of the various Viresolve® Barrier device sizes based on permeability of the Micro devices. The close agreement between observed and predicted flow rates demonstrates the permeability is scalable.

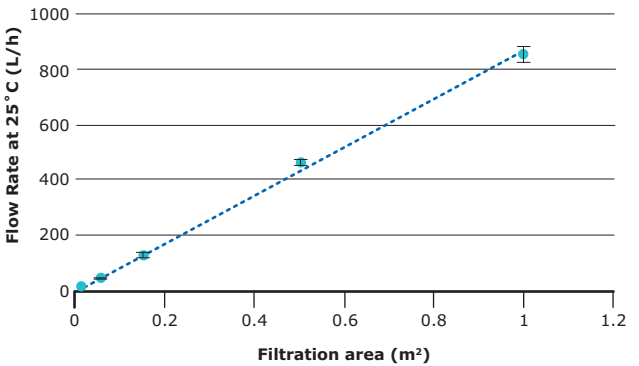


**Figure 4-1. Linear scalability of flow rate for range of Viresolve® Barrier filters, from Micro filter to 1.0 $m^2$ capsule, at 30 psi (2.1 bar).** Error bars represent 1 standard deviation, and in some cases are small enough that they are masked by the data point itself.

## Capacity Performance

### Materials and Methods

Scalability of capacity for filters of different areas was evaluated under constant pressure and flow conditions. For each mode of operation, Micro filters were tested in parallel with the production scale filters. Experimental set ups are shown in **Figures 4-2 and 4-3**.

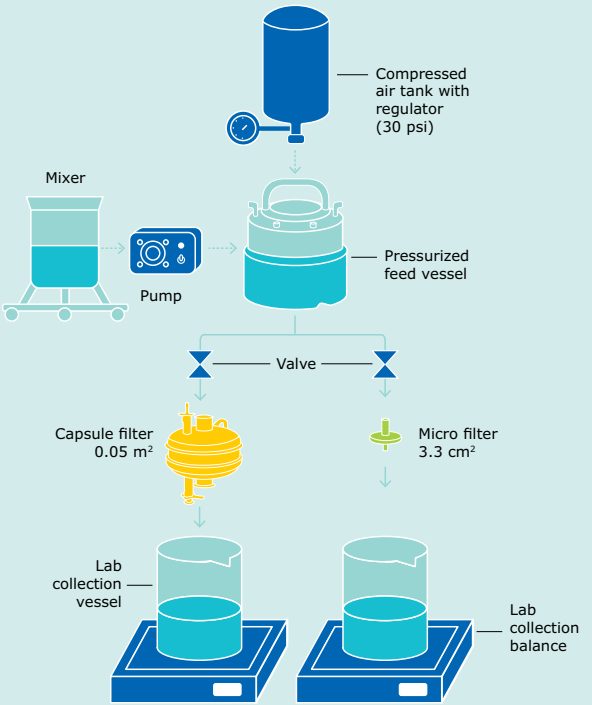


**Figure 4-2. Experimental setup for constant pressure test.**

A commercially-available cell culture medium that has demonstrated low throughput on Viresolve® Barrier filters was selected for scalability assessments. This medium was chosen to represent a highly fouling stream, which may have different scaling performance than a low fouling stream, shown in the previous section.

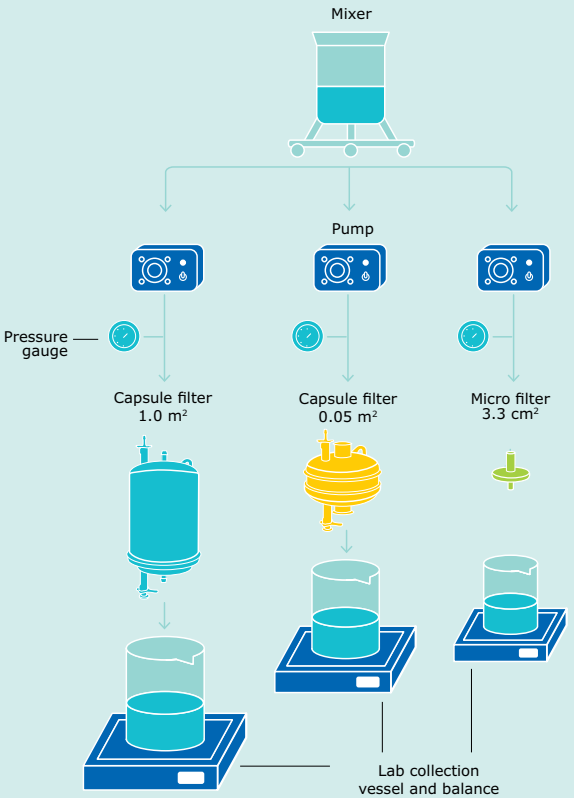


**Figure 4-3. Experimental setup for constant flow test.**

Mobius® Power MIX single-use mixing systems were used to prepare the cell culture medium, in batches ranging from 100 to 800 L. During the constant flow test, the medium was transferred directly from the mixer through the Viresolve® Barrier filters, using a peristaltic pump. For the constant pressure test, the prepared medium was pumped from the mixer to the pressure feed vessel. **Table 4-3** lists the test conditions and process endpoints.

For the constant flow tests, the flow rate was 300 LMH and the filtration endpoint was 30 psi. Capacity was also measured at an intermediate point of 20 psi. The number and size of filters tested for the two constant flow runs are shown in **Table 4-4**.

For testing in constant pressure mode, a feed pressure of 30 psi (2.1 bar) was selected. The 2-hour filtration endpoint was selected as Viresolve® Barrier filters reached approximately 90% flow decay by this time. A single run was performed in constant pressure operation.

Pressures at the inlet and outlet of each device were recorded, as well as filtrate temperature. These parameters were used to ensure that the correct pressure drops and viscosities were used to normalize the fluid flow rates. The filters for the constant pressure run are listed in **Table 4-5.**

**Table 4-3. Capacity test conditions.**

| Viresolve® Barrier | Operating mode | Process endpoint |
|---|---|---|
| **Constant flow** | 300 LMH | 30 psi (2.1 bar) |
| **Constant pressure** | 30 psi (2.1 bar) | 2 hours (V90) |

## Results

The scalability factor is defined as the ratio of the capacity for a given filter to the average capacity of the Micro filters at a specified process endpoint. The average capacity scalability factor in constant flow mode for both runs is shown in **Figure 4-4**. Scalability is shown at the process endpoint of 30 psi (2.1 bar) and an intermediate point when pressure reached 20 psi (1.4 bar).

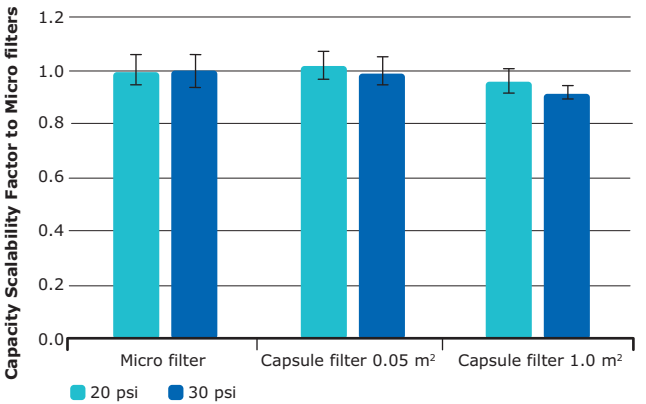


**Figure 4-4. Scalability factor for capacity at 300 LMH constant flow operating condition when pressure reached 20 psi (1.4 bar) and test endpoint of 30 psi (2.1 bar).**

**Table 4-4. Number of filters tested for each capacity test run in constant flow mode.**

| Filter Type | Effective Filtration Area ($m^2$) | Run 1 | Run 2 |
|---|---|---|---|
| **Micro filter** | 0.00033 | n = 4 | n = 4 |
| **Capsule filter** | 0.05 | n = 4 | n = 2 |
| **Capsule filter** | 1.0 | – | n = 2 |

Both production-scale filter sizes scale within 8% of the Micro filters. All filters, irrespective of scale, reached ~68% flow decay at the end of the test when pressure reached 30 psi.

Permeability decreases with throughput when Viresolve® Barrier filters are challenged with this highly-fouling media. Similar fouling behaviors were observed for all filter sizes. An example of the permeability profiles for different sized filters is shown in **Figure 4-5**.

The average capacity scalability factor for the 0.05 $m^2$ capsule filters in constant pressure mode is shown in **Figure 4-6**. The 0.05 $m^2$ capsule filters scale within 5% of the Micro filters. All filters reached approximately 90% flow decay by the 2-hour test endpoint.

In summary, both capacity and permeability scale linearly on Viresolve® Barrier filters in both constant flow and constant pressure modes of operation. This has been demonstrated using both water and a fouling, chemically-defined medium. Performance of the production-scale Viresolve® Barrier capsule filters can be predicted by the Micro filters.

Note that, although Viresolve® Barrier filters do exhibit linear scalability, a safety factor is still recommended to account for typical process variations.

## Retention Performance

### Materials and Methods

Retention performance was assessed under constant pressure conditions using Micro and capsule filters containing the same lot of membrane. **Figure 4-7** shows the experimental setup.

**Table 4-5. Number of filters tested for each capacity test run in constant pressure mode.**

| Filter Type | Effective Filtration Area ($m^2$) | Number of filters |
|---|---|---|
| **Micro filter** | 0.00033 | n = 4 |
| **Capsule filter** | 0.05 | n = 8 |

In a single run, filters were challenged with CD OptiCHO™ medium containing at least $10^6$ pfu/mL of PhiX-174 at a constant pressure of 30 psi (2.1 bar). After 30 minutes of filtration time, a filtrate sample was collected from each device and assayed for PhiX-174 titer.

### Results

Retention results are shown in **Figure 4-8**. The difference in average LRV between Micro and capsule filters is 0.1 logs. These results demonstrate that production-scale filters provide the same level of virus retention as Micro filters.

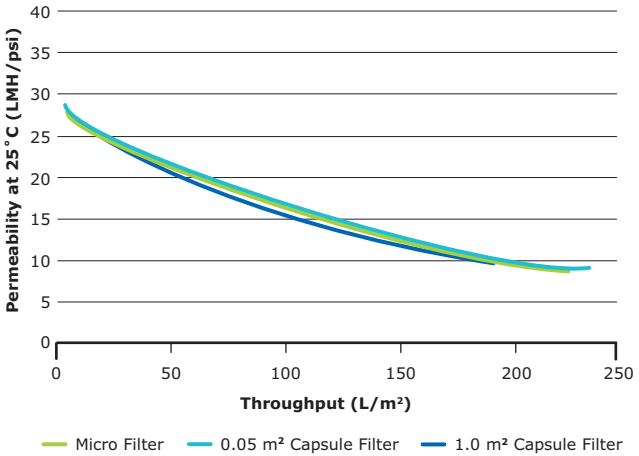


**Figure 4-5. Flow decay of filters during capacity test in constant flow mode.**

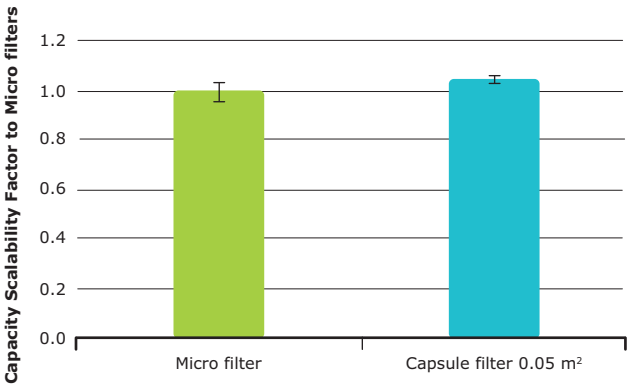


**Figure 4-6. Capacity scalability factor in constant pressure mode.** The error bars represent full range of data.

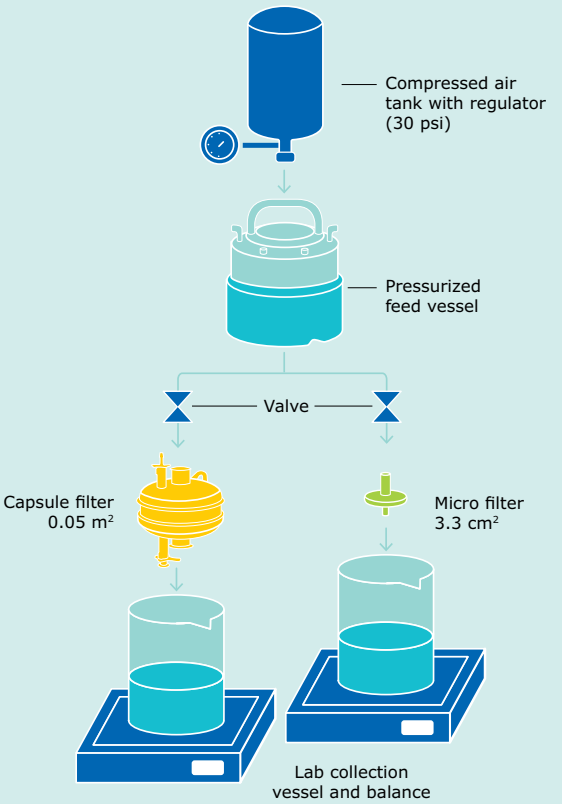


**Figure 4-7. Experimental setup for retention tests under constant pressure using Viresolve® Barrier filters.**

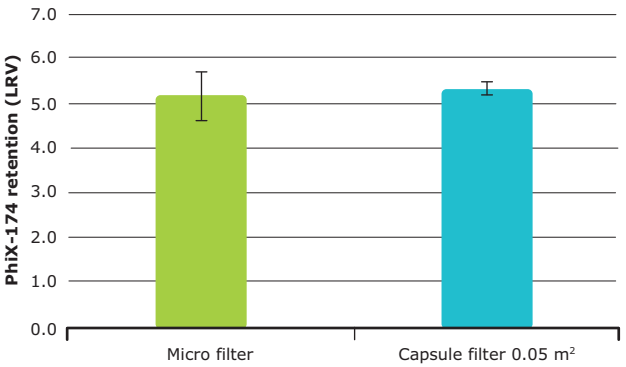


**Figure 4-8. Mean retention performance of Viresolve® Barrier Micro and capsule filters.** The error bars represent the full range of data.

# summary of studies

## 1 Throughput Performance

Virus filters must achieve good capacity or volumetric throughput to be a cost-effective option for filtration of cell culture media. Viresolve® Barrier filters contain a single layer of virus-retentive asymmetric PES membrane, leveraging the proven technology of the Viresolve® platform. This membrane was modified with a secondary chemistry to optimize the filters for high flux virus filtration of cell culture media.

Viresolve® Barrier filters provide good throughput performance across a range of basal media and feed streams.

## 2 Microorganism Retention

Retention studies were performed using Viresolve® Barrier filters and a panel of microorganisms representing a range of potential cell culture contaminants.

Sterilizing-grade performance was achieved for retention of bacteria; complete mycoplasma retention was demonstrated and a high level of virus retention was achieved.

## 3 Maintaining Cell Culture Performance

Media filtration should not affect cell growth and protein production of the cell culture process. Extensive analytical characterization of cell culture media demonstrated no change in composition after Viresolve® Barrier filtration.

Cell growth, antibody titer and protein quality attributes were comparable in media filtered with Viresolve® Barrier and sterilizing-grade filters.

## 4 Scalability

Viresolve® Barrier Micro filters are intended for process development work, while capsule filters are suited for pilot and production-scale batches. Capacity and throughput scaled linearly across the range of Viresolve® Barrier filters under constant flow and pressure operations. Consistent virus retention was observed from bench to production-scale devices.

## For additional information

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please visit **EMDMillipore.com/contactPS**

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