Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Cell Culture Media Filtration
Filter Selection and Sizing

Introduction

As the global demand for biopharmaceuticals continues to rise, bioprocessing is evolving with a focus on maximizing productivity upstream through improvements in cell line development and cell culture media optimization. However, efficiently processing cell culture media can be challenging as many of these new media are highly concentrated and complex, with components that may lead to premature fouling of membrane filters.

Membrane filtration is a robust, easy-to-implement, time-tested solution for processing cell culture media that significantly reduces the risk of bioreactor contamination. Selecting a membrane filter for processing cell culture media is influenced by the desired level of microbial retention, compatibility of the filter materials with the fluid stream, and the filter capacity for your specific media.

The microbial retention properties of the filter may be the most critical element of filter selection. Risk assessments of your materials, process and manufacturing environment guide the level of microorganism retention required from your cell culture media filter. However, previous experience with the disruption resulting from contamination events may reduce your tolerance for contamination, resulting in a desire to minimize contamination risk, wherever possible.

While traditional sterilizing-grade filters are rated to have a membrane pore size of 0.2 µm or smaller, some adventitious agents such as mycoplasma, spirochetes and viruses can penetrate these filters, resulting in bioreactor contamination. Mycoplasma-retentive (0.1 µm) or virus-retentive (approximately 20 nm) filters are alternatives to sterilizing-grade filters and minimize the risk of contamination from smaller adventitious agents. However, while these smaller pore filter sizes reduce the risk of bioreactor contamination, the increased safety assurance comes with a tradeoff, of lower filtration flux and often lower filter capacity, Figure 1.

Filter capacity depends on the filtration membrane structure and the composition of the cell culture media; the latter vary widely in their constituents and formulations. In addition, media formulation impacts capacity: media products are sold both as pre-sterilized liquid solutions and dry powders, and hydration of powdered formulations must be controlled to achieve consistent, scalable filtration capacity. For any given cell culture medium, batch to batch variability may also affect filter capacity. Consequently, sizing calculations for filtration area requirements typically include safety factors to account for variability in cell culture media filtration throughput.

The purpose of this application note is to provide estimated filtration areas for different sterilizing-grade filters with a panel of media used for Chinese Hamster Ovary (CHO) cell culture processes.

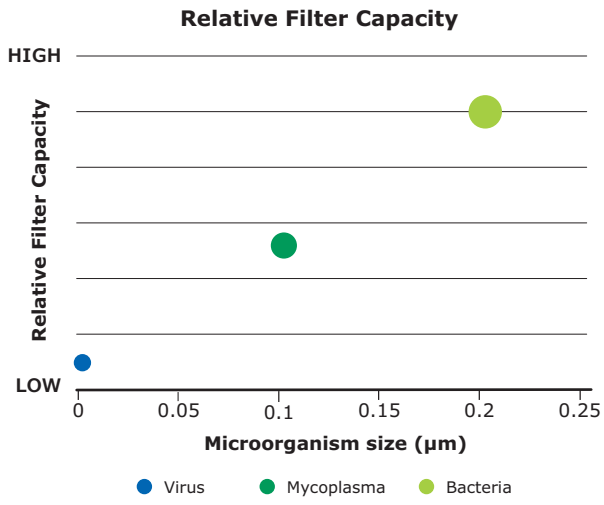

Figure 1. Relationship between retentive performance and filter capacity. Filters with smaller pore size have lower capacity than sterilizing grade filters. Conversly, filters designed to retain bacteria have higher capacity than virus-retentive filters. NOTE: Not to scale

Materials and Methods

The cell culture media used in this study are summarized in Table 1.

Table 1. Catalog media used in capacity testing

Name of Medium	Medium Type	Catalog No.
EX-CELL® Advanced CHO Fed-batch Medium	Chemically-defined medium*	24366C
EX-CELL® Advanced HD Perfusion Medium	Chemically-defined medium*	24370C
Cellvento® 4CHO COMP	Chemically-defined medium*	103795
EX-CELL® 302 Serum-free Medium	Serum-free medium	14324C
EX-CELL® Advanced CHO Feed 1 (with glucose)	Chemically-defined feed	24367C
EX-CELL® Advanced CHO Feed 1 (without glucose)	Chemically-defined feed	24368C
Cellvento® 4Feed COMP	Chemically-defined feed	103796

*Chemically defined media can contain proteins such as recombinant insulin or Long® R^3 growth factor

Filters used for capacity studies and their microbial retention characteristics are listed in Table 2.

Table 2. Filters for bacteria, mycoplasma, and/or virus removal from cell culture media and feeds

Filter	Membrane Pore Size	Composition & Symmetry	Organism Retention
Millipore Express® SHC	0.5/0.2 µm	PES*, asymmetric	Bacteria
Durapore® 0.22 µm	0.22 µm	PVDF**, symmetric	Bacteria
Millipore Express® SHR	0.1 µm	PES, asymmetric	Mycoplasma & bacteria
Millipore Express® SHR with Prefilter	0.5/0.1 µm	PES, asymmetric	Mycoplasma & bacteria
Durapore® 0.1 µm	0.1 µm	PVDF, symmetric	Mycoplasma & bacteria
Viresolve® Barrier	~20 nm	PES, asymmetric	Parvovirus, mycoplasma & bacteria

* Polyethersulfone (PES)
** Polyvinylidene fluoride (PVDF) membranes

Filtration Area Requirements

Estimates of filtration area requirements for the various filters were determined using the Vmax™ (Maximum Volume) method, which estimates minimum filtration area (A_{min}) required to meet process requirements[1]. Figure 2 shows a schematic diagram of typical Vmax™ test setup.

A series of small-scale filtration tests were performed under constant pressure of 10 psi (0.7 bar), except for tests with Viresolve® Barrier filters which performed at 30 psi (2.1 bar). Approximately 500 mL of each medium was processed through each filter. All tests were run in duplicate and filtration progress was tracked by changes in weight on a load cell connected to a data acquisition (DAQ) system.

Volumetric throughput versus time data was fitted to models of filter pore plugging[2,3] to determine minimum filtration area requirements for each filter with each cell culture medium. These values were used to establish recommended filter capsule sizes for meeting the processing requirements, with a given safety factor.

Results and Discussion

The first part of this study evaluated the throughput of a panel of cell culture media on different sterilizing-grade filters. The filters have different microbial retention characteristics determined by membrane pore size, and different capacities for retention of plugging components, driven by membrane symmetry and presence or absence of an integral prefilter. As cell culture media was processed through the membrane filters, flow through membrane pores was restricted, resulting in reduced flux and slower throughput over time.

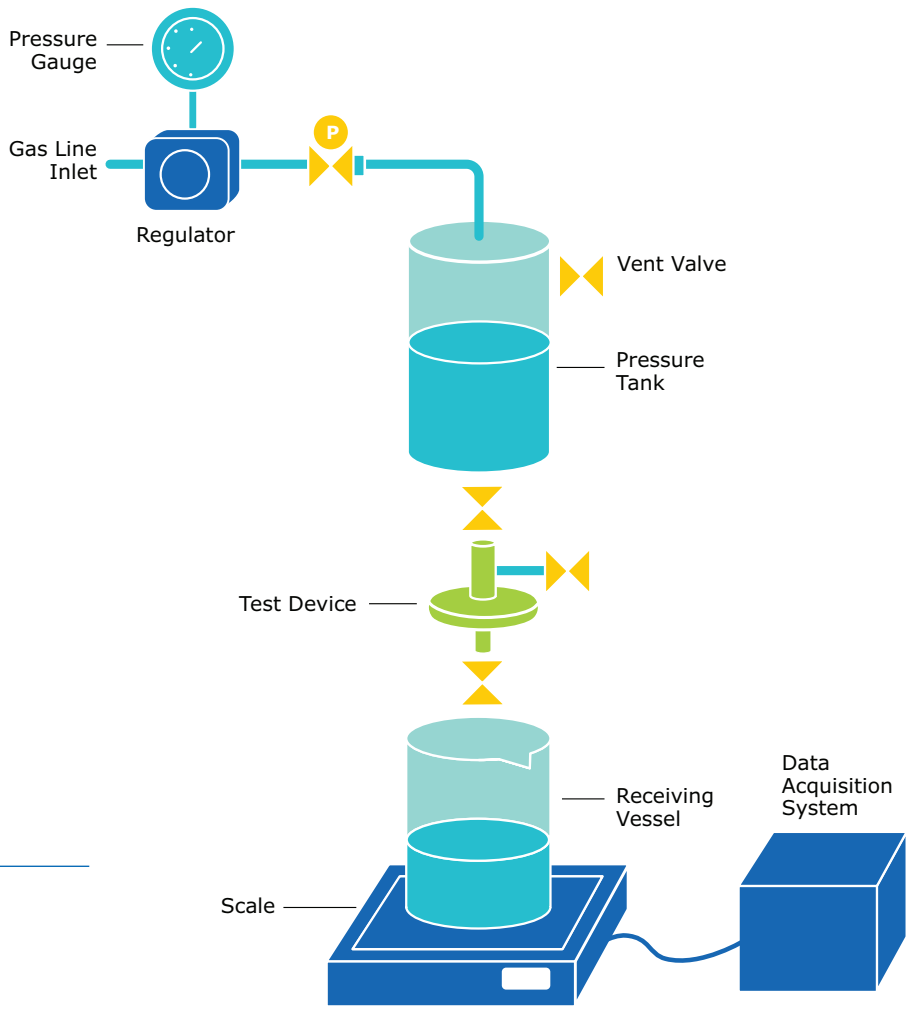

Figure 2.
Setup for Vmax™ constant pressure filtration studies

Figures 3A through 3F show throughput for the panel of media on the different filters.

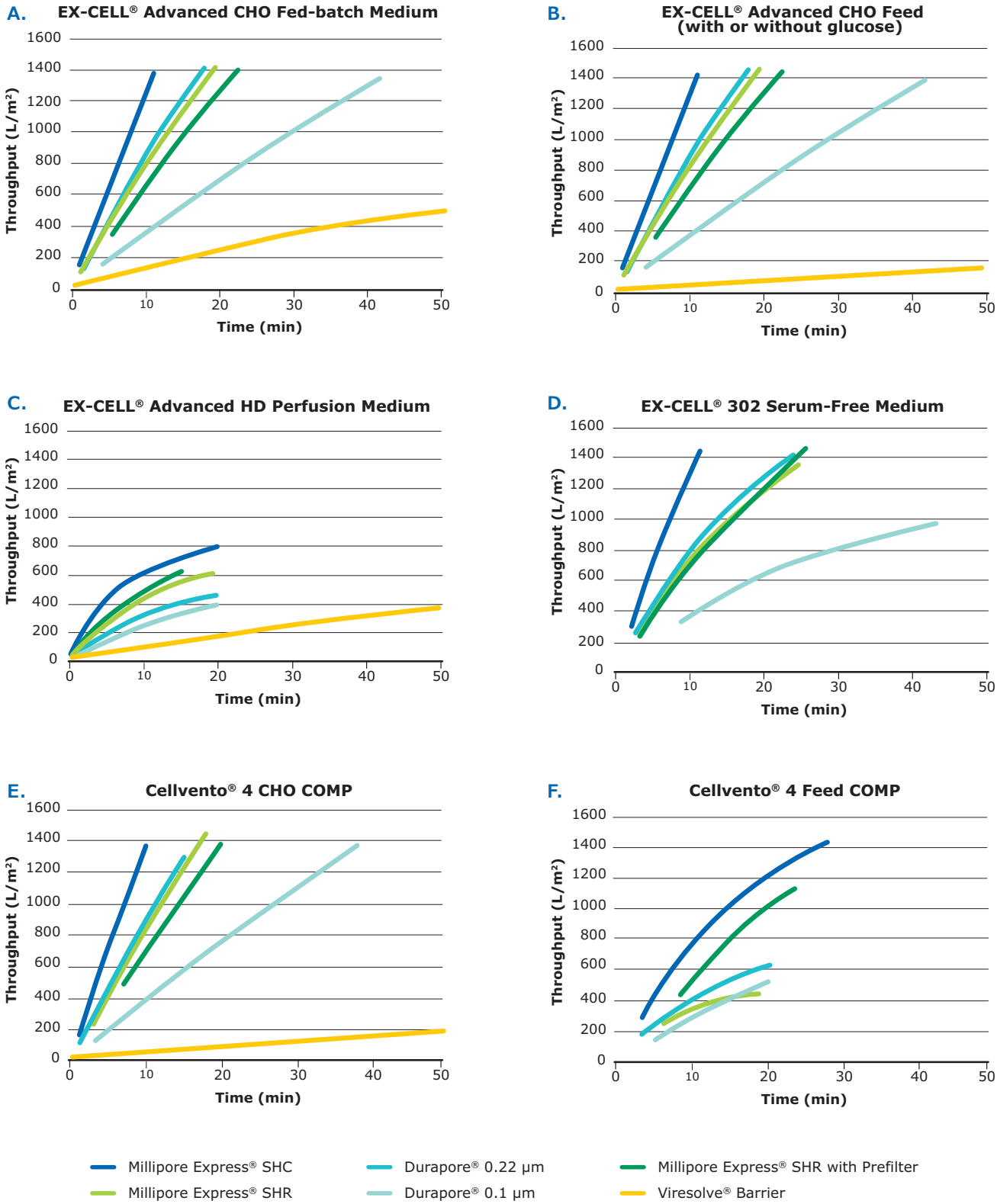

Figure 3. Throughput of catalogue media on filters with different microbial retention characteristics. The medium is listed in the title of graphs A-F.

Due to differences in membrane structure, layering and surface chemistry, different filters exhibit different permeabilities. The asymmetric membrane structure of Millipore Express® filters leads to higher initial flux than Durapore® filters of the same rated pore size. Millipore Express® SHC and Millipore Express® SHR with Prefilter filters have an onboard 0.5 µm PES prefilter layer which removes larger particles, protecting the sterilizing-grade membrane and enabling higher throughput and smaller filter area than filters containing Durapore® membrane.

Collectively, these results indicate that Millipore Express® SHC filters achieved the highest throughput of all sterilizing-grade filters for all cell culture media and feeds tested. For filters designed to reduce mycoplasma and remove bacteria, Millipore Express® SHR with Prefilter filters achieved the highest filtration throughput with most of the cell culture media. While the throughput of all cell culture media tested with Viresolve® Barrier filters was lower with sterilizing-grade filters, it is the only filter that provides virus removal capabilities in addition to complete mycoplasma and bacteria removal. For biopharmaceutical manufacturers with low tolerance for bioreactor contamination, these filters offer a solution for maximum risk reduction.

The throughput data was processed using the models to estimate filtration area, and sample results for one medium/feed combination is shown in Table 3. Note that a safety factor is not included in A_{min} calculations shown. For this non-plugging medium, the prefilter in Millipore Express® SHR with Prefilter filter did not improve throughput, and the additional resistance of the prefilter layer may increase filtration area requirements.

Before implementing any filter, it is recommended to perform studies to assess cell growth productivity in filtered media. Such studies complement Vmax™ studies and are an important element of filter selection.

Table 3. Minimum filtration area requirement (A_{min}) for EX-CELL® Advanced CHO Fed-batch Medium and Feed with Glucose; safety factors not included. Sizing tests for all filters were run at 10 psi, except Viresolve® Barrier filter which was run at 30 psi.

Filter	A_{min} for 1000 L basal medium (m^2)	A_{min} for 200 L feed (m^2)
Millipore Express® SHC (0.5/0.2 µm)	0.16	0.03
Durapore® 0.22 µm	0.27	0.06
Millipore Express® SHR (0.1 µm)	0.32	0.06
Millipore Express® SHR with Prefilter (0.5/0.1 µm)	0.32	0.09
Durapore® 0.1 µm	0.55	0.10
Viresolve® Barrier	2.16	0.34

Table 4 (following page) lists the suggested filter sizes for processing different volumes of the various cell culture media in typical processing times. These filter sizing recommendations include a minimum safety factor of 1.5[4]. For non-plugging media, the size of the filter outlet should be considered to ensure it does not affect flow.

Table 1. Filter sizing recommendations by medium. Viresolve® Barrier filters were not tested with all media.

Filter Recommendations	Operating Pressure, psi (bar)	Batch Volume (Filtration Time) 50 L 0.5 hour	100 L 0.5 hour	200 L 1 hour	500 L 1 hour	1000 L 2 hour	2000 L 2 hour
EX-CELL® Advanced CHO Fed-batch Medium							
Millipore Express® SHC	10 (0.7)	Opticap® XL300	Opticap® XL600	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL3	Opticap® XL10	Opticap® XL10	Opticap® XL20
Viresolve® Barrier	30 (2.1)	0.50 m^2 capsule	1.0 m^2 capsule	1.0 m^2 capsule	2 × 1.0 m^2 capsule	2 × 1.0 m^2 capsule	4 × 1.0 m^2 capsule
EX-CELL® Advanced CHO Feed, with or without glucose							
Millipore Express® SHC	10 (0.7)	Opticap® XL300	Opticap® XL600	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL20
Viresolve® Barrier	30 (2.1)	0.50 m^2 capsule	0.50 m^2 capsule	1.0 m^2 capsule	2 × 1.0 m^2 capsule	2 × 1.0 m^2 capsule	4 × 1.0 m^2 capsule
EX-CELL® Advanced HD Perfusion Medium							
Millipore Express® SHC	10 (0.7)	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL20	Opticap® XL30 (HA*)	2 x Opticap® XL20 (HA)
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL20	Opticap® XL30 (HA)	2 x Opticap® XL20 (HA)
Viresolve® Barrier	30 (2.1)	0.50 m^2 capsule	1.0 m^2 capsule	1.0 m^2 capsule	2 × 1.0 m^2 capsule	4 × 1.0 m^2 capsule	5 × 1.0 m^2 capsule
EX-CELL® 302 Serum-Free Medium							
Millipore Express® SHC	10 (0.7)	Opticap® XL300	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL20
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL3	Opticap® XL10	Opticap® XL20	Opticap® XL30
Cellvento® 4CHO COMP							
Millipore Express® SHC	10 (0.7)	Opticap® XL300	Opticap® XL600	Opticap® XL600	Opticap® XL3	Opticap® XL3	Opticap® XL3
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL10
Viresolve® Barrier	30 (2.1)	0.50 m^2 capsule	0.50 m^2 capsule	0.50 m^2 capsule	2 × 1.0 m^2 capsule	2 × 1.0 m^2 capsule	3 × 1.0 m^2 capsule
Cellvento® 4Feed COMP							
Millipore Express® SHC	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL20	Opticap® XL30
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XL20	Opticap® XL30

*HA denotes high area filters

Conclusions

These results provide a benchmark for filter sizing and are specific to the conditions listed; it is recommended that you perform similar studies under your own processing conditions. Upon request we can provide customized sizing recommendations for different operating pressures or flow conditions, processing times, and batch volumes. For applications where Durapore® PVDF filters are preferred, sizing information for Durapore® 0.22 µm and 0.1 µm filters is available upon request.

Proper filter selection for your cell culture medium and feeds includes three considerations:

- Risk assessment of your retention needs
- Throughput testing to determine filtration area requirements and filter sizing at desired process conditions
- Cell culture studies with filtered media to confirm acceptable cell culture performance

As a leader in cell culture media and sterile filtration, we seek to simplify filter selection and provide you with the optimum filtration solutions tailored to your needs to help reach your process goals.

References

1. Vmax™ Constant Pressure Test for Sizing Aseptic Filters [BR3860EN].
2. Bolton, Glen R., Austin W. Boesch, and Matthew J. Lazzara. "The effects of flow rate on membrane capacity: development and application of adsorptive membrane fouling models." *Journal of Membrane Science* 279.1-2 (2006): 625-634.
3. Giglia, Sal, and Greg Straeffer. "Combined mechanism fouling model and method for optimization of series microfiltration performance." *Journal of membrane science* 417 (2012): 144-153.
4. Lutz H. Rationally Defined Safety Factors for Filter Sizing. *J. Membrane Sci.* 341(1–2) 2009: 268–278.

For additional information
please visit **MerckMillipore.com**

To place an order or receive technical assistance
please visit **MerckMillipore.com/contactPS**

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

Millipore®
Preparation, Separation, Filtration & Monitoring Solutions

Lit. No. MK_AN5144EN Ver. 1.0
4/2020