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Application Note

Cell Culture Media Filtration

Selection and Sizing Filters for CHO Cell Culture Processes

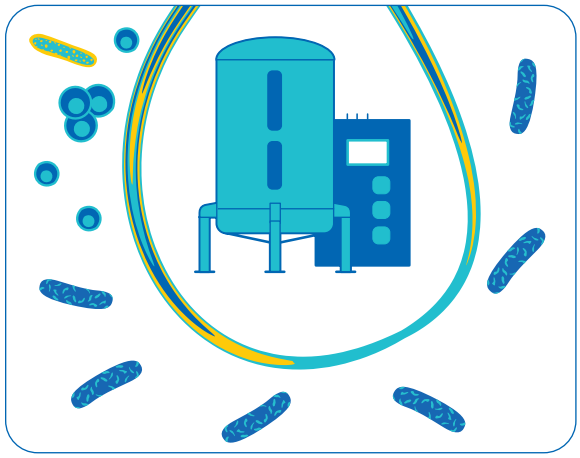
Introduction

As the global demand for biopharmaceuticals continues to rise, bioprocessing is increasingly focused 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 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, some adventitious agents such as mycoplasma, can penetrate these filters, resulting in bioreactor contamination. Mycoplasma-retentive (0.1 µm) filters are an alternative to sterilizing-grade filters and minimize the risk of contamination from smaller adventitious agents. However, while these smaller pore filters reduce the risk of bioreactor contamination, the increased safety assurance may come with lower flux and lower filter capacity.



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.

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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	Cat. 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 and mycoplasma 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
Millipore Express® SHR	0.1 µm	PES*, asymmetric	Mycoplasma & bacteria
Millipore Express® SHR with Prefilter	0.5/0.1 µm	PES*, asymmetric	Mycoplasma & bacteria

* Polyethersulfone (PES)

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). 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. 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.

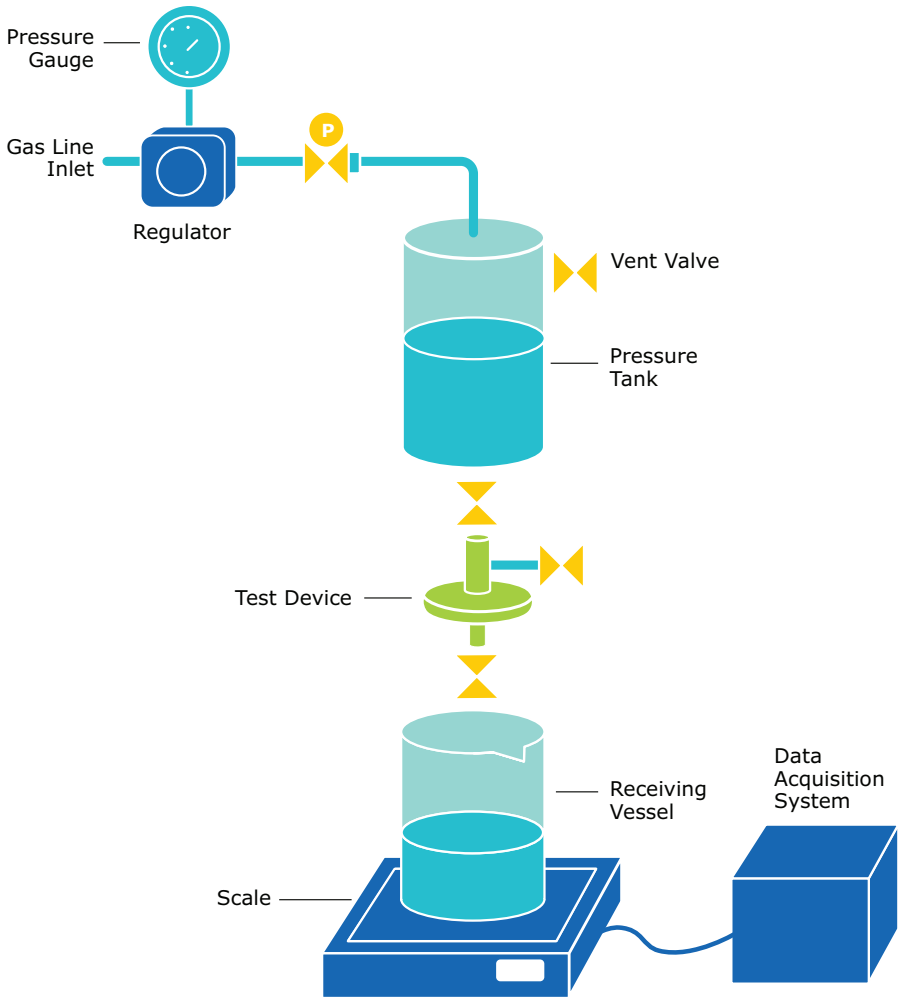

Figure 2. Setup for Vmax™ constant pressure filtration studies

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

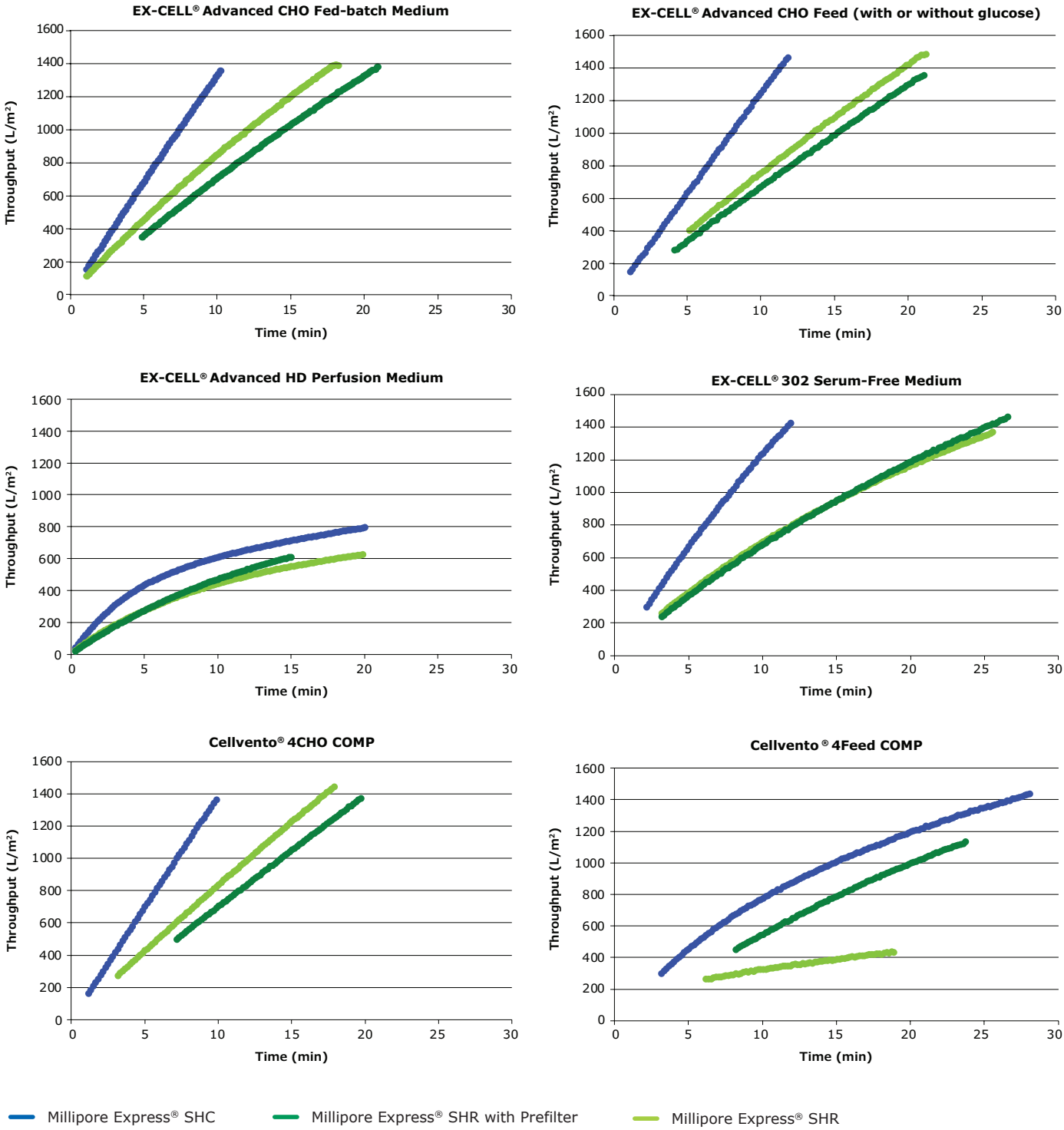

Figure 3. Throughput of catalogue media on filters with different microbial retention characteristics.
The medium is listed in the title of graphs **A-F**.

The various filters tested exhibit differences in permeability due to membrane structure, surface chemistry or presence of an onboard membrane prefilter. Millipore Express® SHC and Millipore Express® SHR with Prefilter filters have onboard 0.5 µm PES prefilters which remove larger particles, and protect the sterilizing-grade membrane, enabling higher throughput and filter capacity.

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. For biopharmaceutical manufacturers with low tolerance for bioreactor contamination, these filters offer an effective risk mitigation solution.

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 (Amin) 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.

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
Millipore Express® SHR (0.1 µm)	0.32	0.06
Millipore Express® SHR with Prefilter (0.5/0.1 µm)	0.32	0.09

Table 4 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 4. Filter sizing recommendations by medium.

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® XLT20
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® XLT20
EX-CELL® Advanced HD Perfusion Medium							
Millipore Express® SHC	10 (0.7)	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XLT20	Opticap® XLT30 (HA*)	2 x Opticap® XLT20 (HA*)
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XLT20	Opticap® XLT30 (HA*)	2 x Opticap® XLT20 (HA*)
EX-CELL® 302 Serum-Free Medium							
Millipore Express® SHC	10 (0.7)	Opticap® XL300	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XLT20
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL3	Opticap® XL10	Opticap® XLT20	Opticap® XLT30
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
Cellvento® 4Feed COMP							
Millipore Express® SHC	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XLT20	Opticap® XLT30
Millipore Express® SHR with Prefilter	10 (0.7)	Opticap® XL600	Opticap® XL3	Opticap® XL5	Opticap® XL10	Opticap® XLT20	Opticap® XLT30

*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.

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.
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4. Lutz H. Rationally Defined Safety Factors for Filter Sizing. *J. Membrane Sci.* 341(1–2) 2009: 268–278.

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