Guidelines for selecting normal flow filters

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Introduction

Normal flow filters are used widely in biopharmaceutical operations to remove colloidal material, bacteria and viruses from growth media, buffers and process intermediates. A modern biopharmaceutical process typically contains 40 to 50 normal flow filtration operations from seed culture propagation to final vial filling. As shown in Figure 1, normal flow filtration accounts for approximately one-fourth of the total cost for downstream processing. Therefore, the choice of normal flow filter(s) has a potentially large impact on the total production cost for a biotherapeutic.

The variety of filter materials available to process development scientists is large—from depth media containing nominally-rated, micron-sized filtration-matrices to validated sterile filtration membranes containing sub-micron sized pores. The criteria for choosing an optimal filter is commonly application-specific and it is therefore important to understand these criteria when designing experiments, analyzing data, and comparing product attributes.

In general, normal flow filtration operations can be divided into three main categories:

- cell culture media sterilization
- buffer filtration
- product-stream filtration

Figure 2 shows a typical biopharmaceutical process and highlights where each of these filtration steps occurs.

Visit the MAb Production Scheme tool at:
www.amershammedia.com/MAb_final5/800x600_2.html

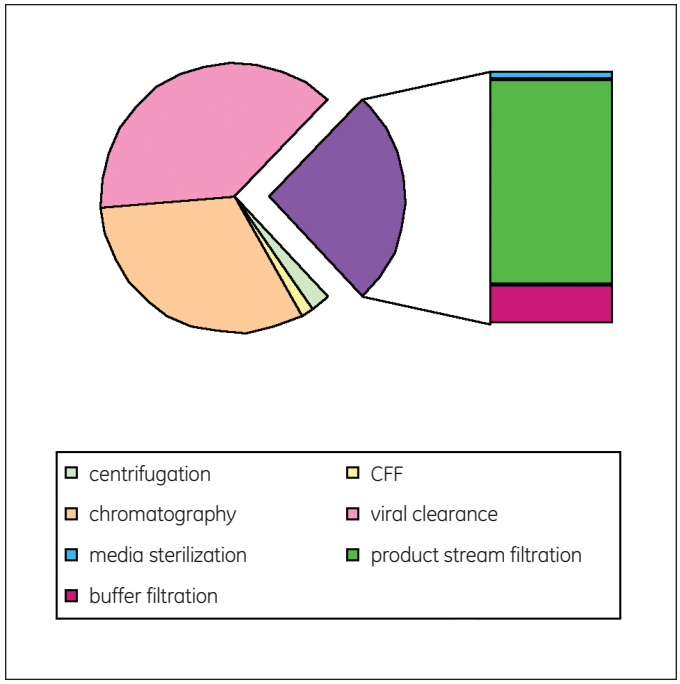

Fig 1. The relative cost of biopharmaceutical separations.

Cell culture media sterilization

One of the first unit operations in any biopharmaceutical operation is the preparation and sterilization of cell culture media. Cell culture media are nutrient-rich, buffered solutions containing amino acids, salts, vitamins and energy sources (e.g. glucose)—all of which are essential components for the culture of healthy cells. Over the past several decades, formulations have evolved from generic basal media supplemented with animal-derived sera, to more cell-line specific formulations which are serum-free, animal-derived component free and chemically-defined. The sterilization of these media is critical to successful cell growth and protein expression.

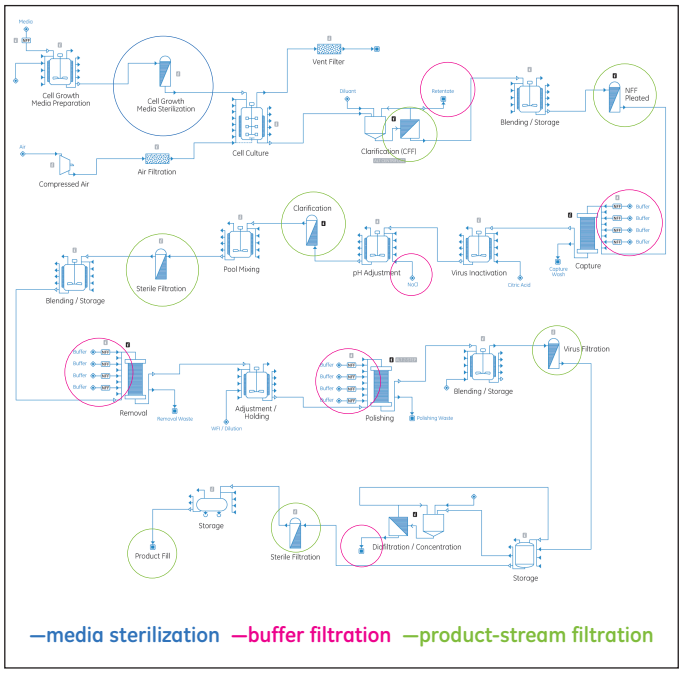

Fig 2. Normal flow filtration in biopharmaceutical production.

There are many characteristics one should look for when selecting a cell culture media filter. The following paragraphs describe some of the most important features.

Sterilizing-grade membranes

The term "sterilizing-grade filter" is defined in the FDA's document "Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice", which describes a sterilizing-grade filter as a filter that is "validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent." The validation of sterilizing-grade membranes is commonly performed using the procedure documented in ASTM F838-05, "Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration." In order to be labeled "sterilizing-grade", a filter must produce a sterile effluent when challenged with *Brevundimonas diminuta (B. diminuta)* at a minimum concentration of 10^7 colony forming units (CFU) per square centimeter (cm^2) of membrane area. Sterile filters are nearly always constructed of one or more sheets of polymeric membrane, either in pleated or flat-sheet form.

Low extractables

Media filters must not only retain contaminants, but they must also be chemically and biologically compatible with the cell culture media. This means that the filter must be constructed of components which are proven to be safe and that the materials which extract from the filter during normal operation have been quantified and characterized. Filter manufacturers will typically provide flushing recommendations for their particular filter products and also have standard specifications for many contaminants, including (but not limited to): total organic carbon (TOC), oxidizable substances, toxic compounds, particulates and fibers. Some manufacturers also offer application-specific testing of extractables.

Low nonspecific binding

Potential interactions between filters and culture media must be assessed carefully to ensure no inhibition of cell growth or protein expression. Cell cultures are highly sensitive to growth media composition; hence, the materials of construction for a sterilizing filter must be proven inert. Membranes employed in media sterilization operations should have low non-specific binding to ensure that key media ingredients are not removed during the filtration process.

Physical robustness

Physical robustness is important in all filtration applications but it is critical in media sterilization applications because of the stress which the filters undergo before, during and after use. A typical media filtration process consists of the following steps:

- filter installation and wet-out
- pre-use filter integrity test
- steam-in-place
- media filtration
- filter flush
- post-use filter integrity test
- clean-in-place
- filter disposal

Many of these steps result in physical, thermal and/or chemical stress on the membrane and other filter components. Nonetheless, every component must retain its functionality in order for the media sterilization operation to be considered successful.

High permeability

The term permeability refers to the flux rate achieved through a filter, normalized with respect to differential pressure. Permeability is typically reported with units of liters per square meter per hour per psi (LMH/psi). Permeability is important because most media sterilization processes have relatively short batch times (1 to 2 h) and filters having low permeability may drive the required filter area to be larger than the requirement based on filter capacity alone. (This results in a filter train which is underutilized with respect to capacity.) Media sterilization filters with high permeability result in a system which maximizes the filter throughput.

High capacity

The term capacity refers to the volume of feed which can be processed by a given membrane area before the membrane's resistance to flow becomes unacceptably high. Sterile media filters are expected to have high capacity (thousands of liters per square meter of membrane area). This is an important characteristic for media sterilization filters because of process economics, ease-of-use considerations and the minimization of non-specific binding. For sterilizing-grade filters which are designed for cell culture media sterilization, high capacity is achieved by the addition of an on-board prefilter layer—typically having a pore size rating of 0.4 to 0.8 microns.

Survey of media sterilization filters

GE Healthcare performed a study to evaluate the most common filters which are designed for media sterilization. In this study, five commercially-available cell culture media were prepared from dry powder per the manufacturer instructions. The tested media are shown below:

- DIFCO Miller LB Broth for *E. coli*
- DIFCO YPD Broth for yeast
- Invitrogen™ RPMI-1640 basal media supplemented with Sigma™ Type IV soy peptone
- Hyclone SFM4CHO Utility
- SAFC Biosciences EX-CELL 302

Each of the media was tested on a panel of sterilizing-grade membranes from Pall, Millipore, Sartorius and GE Healthcare. Solutions were filtered at a constant pressure of 10 psid and the volume filtered as a function of time was recorded until the flow rate had decayed by at least 50% or until the solution was exhausted. Based on the test results, estimations of the required number of equivalent 10 inch filter cartridges were made for a 12 000 L batch of media filtered in 2 h. Results are presented in Figure 3.

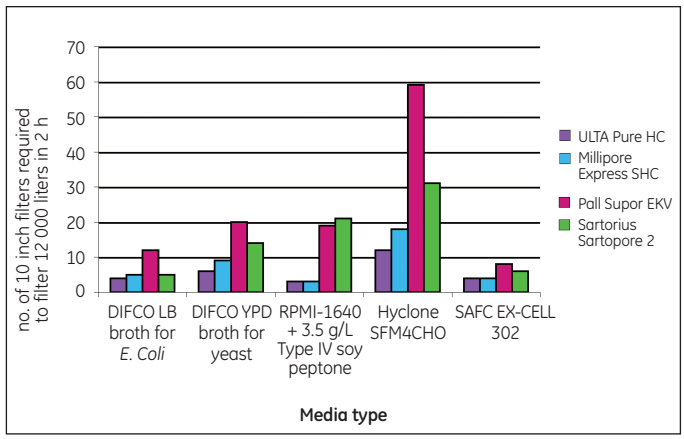

Fig 3. Comparative performance of ULTA HC to competitive sterile filters for media filtration (smaller bars represent better performance).

Filter recommendations

ULTA™ Pure HC consistently outperforms competitive filters for cell culture media operations. It combines the benefits of high-capacity and high-permeability with excellent physical, chemical and thermal robustness. ULTA Pure HC filters are preflushed at the point of manufacture resulting in a filter which requires no pre-flushing to remove extractable components. The ULTA Pure HC membrane and filter device are backed by an extensive validation package which includes:

- 100% integrity testing of every device
- testing on a lot sampled basis to confirm: (i). bacterial endotoxin levels < 0.25 EU/mL; (ii). water bubble point
- extensive chemical compatibility information
- 10-fold 130ºC autoclave lifetime
- 30-fold 130ºC steam-in-place lifetime

Buffer filtration

Buffer solutions are used widely in nearly every step of biopharmaceutical production processes. In fact, buffer filtration is the most commonly performed filtration operation in any biopharmaceutical process. During operation of the bioreactor, buffers are used in order to control pH and osmolality of the cell culture media. At cell harvest, buffers are used to precondition filters and to assist in product recovery operations. Chromatography steps employ numerous buffers for such operations as column conditioning, column elution and column regeneration. Once a biopharmaceutical is ready for formulation, buffers become a key ingredient in the bulk drug substance. Finally, buffers are used throughout the process for clean-in-place operations.

Biological and particle contaminants present in buffers can have a large impact on process efficiencies and final product quality. Therefore, normal flow filtration is one of the first steps (after dissolution) in any buffer preparation process. Buffer filtration is key to protection of chromatography columns and ultrafiltration operations and to production of an endotoxin-free final product.

The following paragraphs describe the key characteristics of a buffer filter.

Validated 0.2 micron membranes

Buffer filtration is commonly done with 0.2 µm membrane filters to reduce bioburden or to achieve sterility of the buffer and to remove particulate contaminants. The choice between a sterilizing-grade and a bioburden-reduction filter[1] is often dependent on the final use of the buffer. For example, sterility is a requirement for buffers used as additions to the bioreactor in order to prevent contamination of the cell broth, while bioburden reduction may be sufficient for buffers used in chromatography operations, which are often not aseptic processes. Bioburden reduction filters are generally less expensive (per unit membrane area) and require less filtration area for a given batch size (thereby improving their economics even further). In either case, it is important for regulatory purposes that the membrane is validated for retention of bacteria and that the retention can be correlated to an in-process integrity test.

Chemical compatibility

Buffer filters must have broad chemical compatibility, since buffers used in biopharmaceutical production span a wide range of pH levels (1-14), and must withstand exposure to alcohols and (occasionally) other organic chemicals.

Physical robustness

Filters used in buffer preparation must withstand the rigors of steam-in-place and/or autoclaving. They must also be validated for multiple sterilization cycles since buffer preparation areas may be designed to re-use filters. Buffer filters should remain integral within a wide range of operating conditions in order to avoid filter failures which can lead to batch reprocessing, lost product and/or costly regulatory investigations.

High permeability

Buffer filtration is a high-volume, short-time operation. Since buffers are generally fluids with low particle loading, they do not tend to plug membrane filters. Therefore, permeability (rather than capacity) becomes the key determining characteristic in the size of the filtration system. The use of high permeability membranes can result in significant reductions in the amount of filter area needed for buffer preparation. Small filtration footprints are desirable because they are not only cheaper in terms of consumables, but they also require smaller capital investments and reduce the risk of filter integrity failures.

Survey of filters for buffer filtration

GE Healthcare performed a study to evaluate the most common filters which are used for buffer filtration. In this study, eight common buffers spanning a range of concentration, pH and organic content were prepared and tested on a panel of buffer filtration membranes from Pall, Millipore, Sartorius and GE Healthcare. The tested buffers are shown below:

- 1 M hydrochloric acid (pH = 1)
- 1 M sodium hydroxide (pH = 13)
- 50 mM Tris-buffered saline (pH = 8)
- 10 mM phosphate-buffered saline (pH = 7)
- 50 mM phosphate-citrate buffer (pH = 5)
- 0.1 M acetic acid
- 10% ethanol
- 6 M urea

Solutions were filtered at a constant pressure of 10 psid and the volume filtered as a function of time was recorded until the solution was exhausted. Based on the test results, membrane permeability for each filter tested was calculated and estimations of the required number of equivalent 10 inch filter cartridges were made for a 12 000 L batch of buffer filtered in 1 h. Results are presented in Figure 4.

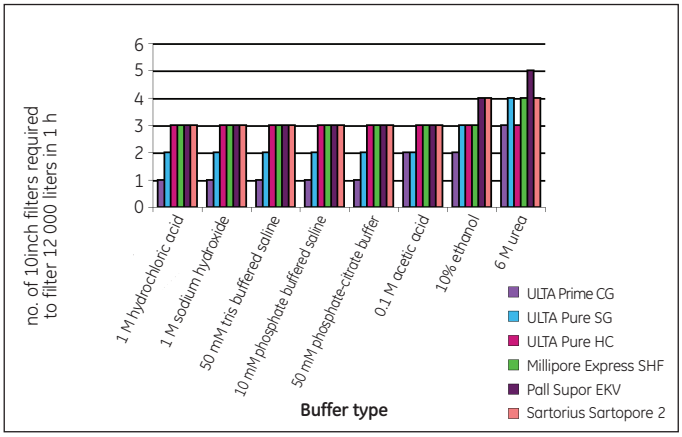

Fig 4. Comparative performance of ULTA filters to competitive sterile filters for buffer filtration (smaller bars represent better performance).

Filter recommendations

ULTA Pure SG (for buffer sterilization) and ULTA Prime CG (for bioburden reduction) consistently outperform competitive membranes and ULTA Pure HC provides competitive filter sizing with the added assurance of an onboard prefilter layer. All three filter grades are constructed using polyethersulfone 0.2 micron membrane which is physically robust and chemically resilient, so they perform reliably regardless of the buffer being prepared. Additionally, all three employ a final membrane which is validated for bacterial retention using the ASTM F838-05 methodology[2]. (LRV > 7 for ULTA Pure SG and HC and LRV > 5 for ULTA Prime CG).

Product-stream filtration

Biopharmaceutical products are filtered numerous times in the course of their manufacture to control bioburden, remove precipitates and separate solid contaminants (e.g, fines from chromatography resins or diatomaceous earth flushed from depth filters). In most cases, sterilizing-grade filters are used for product stream filtration, although growing concerns about cost-of-goods is resulting in increased use of bioburden reduction filters for these steps.

The following paragraphs describe the key characteristics one should look for when selecting a product-stream filter.

Validated 0.2 micron membranes

As is the case with buffer filtration, the choice between a sterilizing-grade and a bioburden-reduction filter is often dependent on the unit operation to which the filtration is coupled. For example, sterilizing grade membranes are required for filtration of bulk drug substance and usually desired for filtration steps performed prior to any product hold. However, bioburden reduction membranes may be sufficient and more economical for many intermediate filtration steps (e.g. prior to a chromatography column) and therefore their use can result in significant cost savings. As mentioned previously, regardless of the choice of filter, it is important to choose a membrane which is validated for retention of bacteria and that the retention can be correlated to an in-process integrity test.

Physical robustness

Product-stream filtration is the highest value normal flow filtration operation in any biopharmaceutical process. Filter failures which occur during product-stream filtration require time-consuming and costly investigations and may result in lost product. As a result, normal flow filters used for product-stream filtration must be constructed to withstand a broad range of operating conditions with respect to temperature, pressure and pH. Furthermore, product-stream filters should be 100% tested by the manufacturer and should include instructions for integrity testing at the point-of-use.

High capacity

Product-streams are some of the most challenging filtration steps and filter performance can vary widely due to the strong effects of filter-fluid interactions. Therefore, it is important to select a filter which is optimized to provide high capacity, regardless of the protein-type, concentration and/or formulation buffer. Furthermore, many product-stream filtrations are coupled to relatively low flow unit operations (e.g, centrifugation, cell harvest or chromatography column loading), thereby making differences in membrane permeability less important when determining the required filtration area.

Low extractables

Product-streams contain a drug substance which will eventually be administered to a human patient. Therefore, product stream filters must be constructed of components which are proven to be biologically safe. Biological safety is demonstrated by the performance of the USP<88> Class VI Plastics Test for Biological Reactivity and the burden of obtaining this information rests with the filter manufacturer who should include test results as part of a validation package. In addition, vendors should be able to provide information regarding filter effluent quality in terms of total organic carbon (TOC), buffering capacity, non-volatile residue (NVR), bacterial endotoxins and particle or fiber shedding.

Low protein binding

Membranes and other materials used to construct filter cartridges and capsules should not bind proteins or preservatives which are in the fluid as this may lead to product loss or decreased shelf-life. Figure 5 shows the amount of several proteins bound by microporous membranes cast from several common polymers. Most modern membranes are cast from PES or PVDF to ensure minimal product loss.

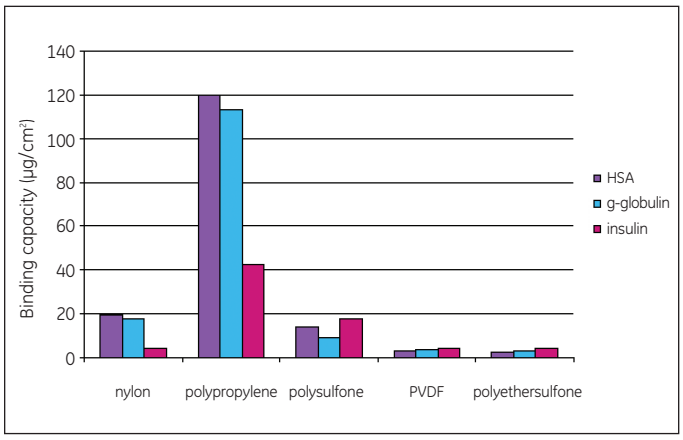

Fig 5. Protein binding on various membrane materials.

Survey of filters for product-stream filtration

GE Healthcare performed a study to evaluate the most common filters which are used for product-stream filtration. In this study, two protein-containing feedstreams (1% bovine serum albumin and a monoclonal antibody purified on Protein-A chromatography and subjected to low pH viral inactivation) were used to challenge a panel of membrane filters from Pall, Millipore, Sartorius and GE Healthcare. The specifics of the feedstreams are shown in Table 1.

Feedstream	Concentration	pH	Buffering Solution	Previous Treatment
Bovine serum albumin	1% w/v	7.0	Phosphate-buffered saline	None
Monoclonal IgG	10.5 g/L	7.0	20 mM sodium citrate buffer[3]	Held for 40 min. at pH = 3.8

Table 1

Solutions were filtered at a constant pressure of 10 psid and the volume filtered as a function of time was recorded until the flow rate had decayed by at least 50% or until the solution was exhausted. Based on the test results, estimations of the number of equivalent 10 inch filter cartridges were made for a 2 000 L batch of product filtered in 1 h. Results are presented in Figure 6.

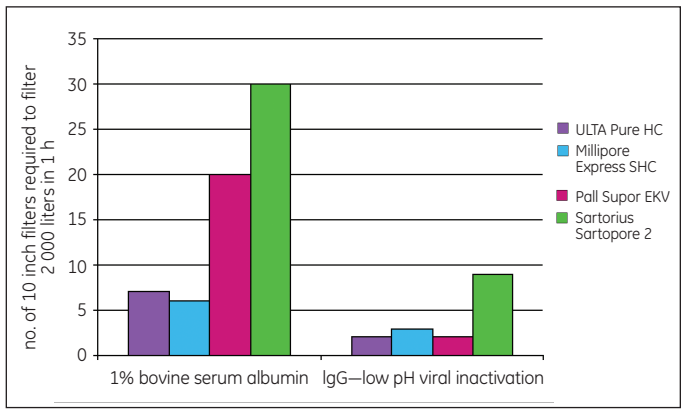

Fig 6. Comparative performance of ULTA Pure HC to competitive filters for product-stream filtration (smaller bars represent better performance).

In addition, experiments were run to compare the performance of ULTA Pure HC and ULTA Prime CG. As shown in Figure 7, ULTA Prime CG provides equal or better filter capacity which, when coupled with a lower per-filter cost, can translate to significant cost savings for applications where sterile-effluent is not required.

Filter recommendations

ULTA Pure HC and ULTA Prime CG exhibit consistently high capacities over a wide range of feed streams. Both are constructed of materials which are low in extractables and which exhibit low protein binding, thereby ensuring high-purity, high-yield filtrate.

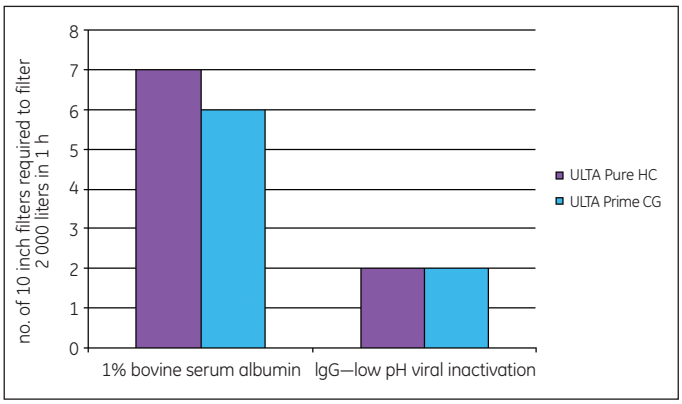

Fig 7. Comparative performance of ULTA Pure HC and ULTA Prime CG for product-stream filtration (smaller bars represent better performance).

Conclusions

ULTA Pure SG, ULTA Pure HC and ULTA Prime CG capsules and cartridges have been specifically optimized to provide consistently high performance with complex biological solutions by combining high capacity and fast flow rates. The inherent low protein binding properties of the ULTA membranes minimize product loss due to adsorption. The filters have low extractable levels and broad chemical compatibility. The membrane is inherently hydrophilic and the filters can be easily and repeatedly integrity tested.

Regardless of scale or operation, GE Healthcare has an ULTA filter with proven performance in every application, including:

- Media sterilization—Filters incorporating high capacity/high flow membrane formats minimize filtration system sizes while meeting full validation and integrity test requirements.
- Buffer filtration—Sterilizing-grade and bioburden reduction filters providing high permeability and wide chemical compatibility.
- Product stream filtration—Bioburden reduction filters reduce or eliminate bioburden and particulates immediately before chromatography columns and UF/DF operations. Sterilizing-grade filters provide the highest level of assurance prior to product hold steps and during bulk drug substance formulation and final vial filling.

ULTA Pure SG, ULTA Pure HC and ULTA Prime filters from GE Healthcare offer solutions for a comprehensive range of normal flow filtration applications which increase process efficiency from early phase product development through to full-scale biopharmaceutical production.

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1. Bioburden reduction filters are not defined by an industry standard. The term "bioburden reduction" is a designation used to describe a class of filters which provide a high level of microorganism retention (i.e., LRV 4-6), but do not yield a sterile effluent under the high bacterial load called for in the ASTM F838-05 test method. Manufacturer claims on bioburden reduction filters vary from "typical" retention data to full validation of a minimum LRV. In practice, most bioburden reduction filters are of a 0.45 or 0.2 µm rating and may yield a sterile fluid in common usage, where bacterial loads are much lower than those used in the ASTM challenge. Nevertheless, process-specific claims of fluid sterilization through the use of a bioburden reduction filter are generally not appropriate.

2. ULTA Pure SG and ULTA Pure HC are validated for a LRV > 7 of *B. diminuta* and ULTA Prime CG is validated for a LRV > 5.

3. 20 mM sodium citrate was used as the elution buffer during the Protein-A purification and sodium phosphate buffer was used to adjust the pH to 3.8 and to neutralize the feed stream.

Quick guide to selection of normal flow filters

Product attribute	Media sterilization	Buffer filtration	Product-stream filtration
Sterilizing-grade membranes	Required. Media must be free of all viable organisms before inoculation of the bioreactor.	Application specific. Depends on use of the buffer. BBR[1] filters may be substituted for cost reduction purposes and/or performance advantages.	Application specific. Depends on the process process step. BBR filters may be substituted for cost reduction purposes and/or performance advantages.
Low extractables	Required to ensure proper growth of cells.	Required to protect product quality.	Required to protect product quality.
Low non-specific binding	Required to prevent loss of trace nutrients critical to growth of cells.	Not generally applicable to buffer filtration. (Buffers do not generally contain proteins.)	Required to ensure high product yield.
Physical robustness	Critical to ensure process robustness.	Required as filters may undergo numerous SIP and IT operations.	Required to ensure product quality.
High permeability	Required due to short batch processing times.	Required due to short batch processing times.	Not generally applicable to product-stream filtration where flow rates are relatively low.
High capacity	Required due to process economics and ease-of-use considerations and to minimize non-specific binding.	Important, but generally true of all membrane filters. (Buffers do not contain high concentrations of plugging agents.)	Critical. Product-streams are often the most challenging filtration steps.
Chemical compatibility	Not generally applicable since cell culture media is aqueous based and at neutral pH.[2]	Required. Buffers span a wide range of pH levels (1-14) and may include alcohols and (occasionally) other organic chemicals.	Not generally applicable since biotherapeutics are aqueous based and not typically held at extreme pH.[2]
GE Healthcare recommendation	ULTA Pure HC	ULTA Pure SG (sterilizing-grade) ULTA Prime CG (BBR)	ULTA Pure HC (sterilizing-grade) ULTA Prime CG (BBR)

1 BBR = bioburden reduction
2 Caustic-based clean-in-place (CIP) solutions are one exception. PES membranes are designed to be compatible with caustic solutions.

For contact information for your local office, please visit: www.gelifesciences.com/contact

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