Upstream Virus Safety: Protect Your Bioreactor By Media Filtration

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Background

Biopharmaceutical manufacturing processes involve a multilayered approach to microbial and virus testing to assure that the drug product is safe for human use. Screening raw materials, testing in-process intermediates and demonstrating the virus removal capabilities of the downstream process are critical to biosafety assurance. However, despite careful screening of raw materials, there remains a risk of introducing adventitious agents into bioreactors, which could impact manufacturing operations, cause significant business disruption and ultimately threaten drug supply to patients. Various technologies have been employed to minimize this risk. One of these, filtration, is a point of use operation that is easy to implement in the upstream process. This poster summarizes the performance of a filter specifically developed for virus removal from chemically defined cell culture media. The filter removes high levels of virus, mycoplasma and bacteria without impacting cell growth, antibody titer, or protein quality. The filter has robust performance over a broad range of conditions offering an effective, easy to implement solution for media treatment.

Viresolve® Barrier Filter for Upstream Processes

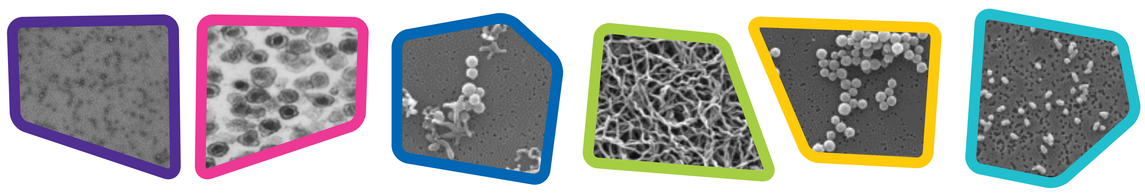
Until recently, virus risk reduction around upstream processes relied on careful sourcing of raw materials, screening cell banks for adventitious virus and careful control of facilities and workflow. Despite these precautions, bioreactor contaminations occurred resulting in significant disruption and cost for the companies involved. More recently, other options for virus reduction in upstream applications have been employed but these generally require costly investment and are often not suitable for all media components.

Filters specifically designed for processing chemically-defined cell culture media offer an alternative to capital-intensive methods using proven membrane technology to assure robust, broadly effective, size-based virus removal.

Technology	Pros	Cons
High Temperature Short Time (HTST)	Robust clearance Point-of-use Cost-effective at large scale	Conflicting clearance data Media compatibility Not cost-effective at small/mid scale
UV-C (254 nm)	Point-of-use	Virus dependent clearance Media compatibility Challenging at large scale
Irradiation (25-40 kGy)	Cost-effective	Virus dependent clearance Media compatibility Not point-of-use Best with small batches
Virus filtration with optimized upstream filters	Robust, sized based clearance Familiar format Compatible with most media Point-of-use	Not effective if media contains unusually large critical species

Excellent Retention of Broad Panel of Microorganisms

The Viresolve® Barrier filter can be integrated into single-use or stainless steel processes and can be used in place of a 0.1 µm or 0.2 µm filter; high retention (above the detection limit) has been demonstrated for large virus, bacteria and mycoplasma.



Minute Virus of Mice
Relevant contaminant
Target organism small virus
Typical LRV above 4
Worst case LRV ≥ 3

Murine leukemia virus
Model large virus
LRV >6.1

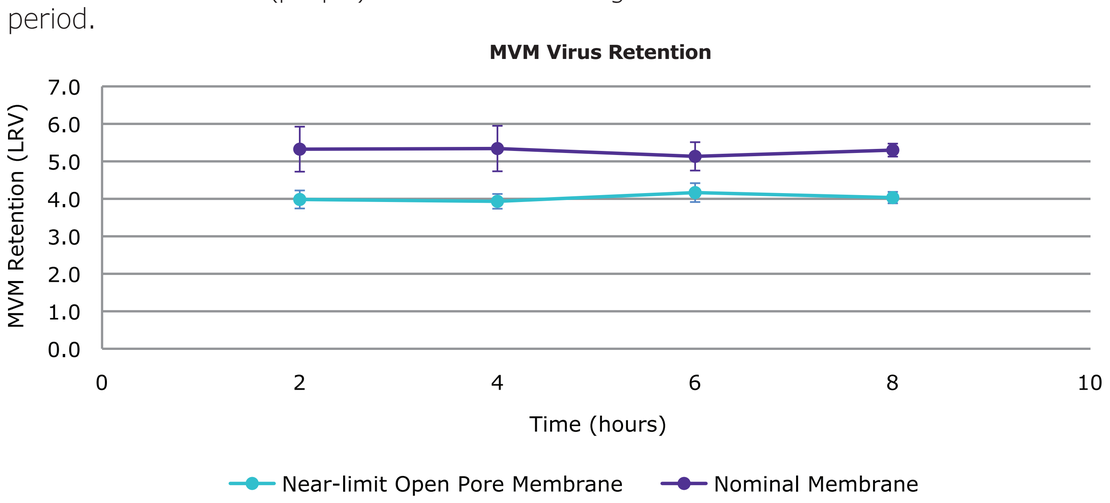
M. orale
Relevant contaminant
Can penetrate 0.1 µm filters
LRV >8

L. illini
Model spirochete bacteria
Can penetrate 0.1 µm filters
LRV >8

A. laidlawii
Standard mycoplasma model organism
Model organism for 0.1 µm filters
LRV >8

B. diminuta
Standard model bacteria
ASTM® F838-05 test organism
LRV >8

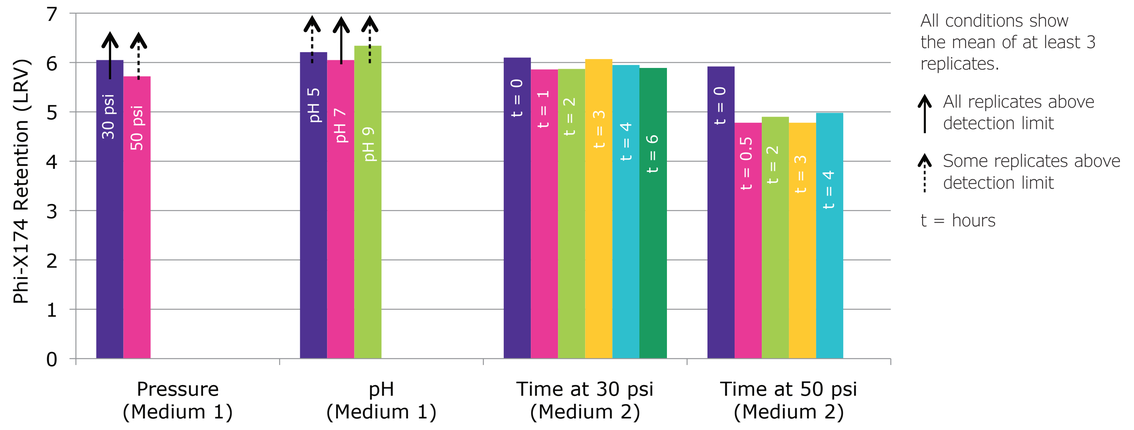
The graph below illustrates sustained high level of retention for MVM, a relevant small virus contaminant, during extended processing times for two different membranes. Membrane made near the limit of the manufacturing window (blue) shows MVM retention of approximately four logs sustained over 8 hours of processing. Typical, nominal membrane (purple) shows over five logs of MVM retention over the same time period.



Robust Virus Retention Across Multiple Conditions

Virus retention across the Viresolve® Barrier filter was evaluated at a range of processing conditions with different cell culture media. Bacteriophage Phi-X174 was used as a surrogate for MVM. The filter is designed to retain a minimum of four logs Phi-X174, even following prolonged processing. Virus retention remains high across a range of operating pressures and pH levels, even following extended processing times.

Retention performance for various process conditions

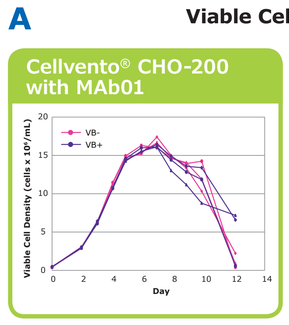


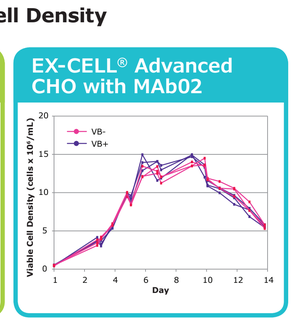
Cell Culture Media Composition and Cell Growth

Comprehensive analysis (mass spec or amino acid and soluble vitamin HPLC, NMR and ICP-OES) of two cell culture media before and after filtration through Viresolve® Barrier filter indicated no changes in media composition that could be attributed to filtration with the Viresolve® Barrier filter[1].

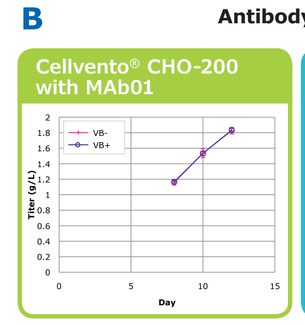
Cell culture using filtered media was performed in shake flasks (Cellvento® CHO-200 medium with MAb01) or Mobius® 3L Bioreactors (EX-CELL® Advanced CHO medium with MAb02). No significant changes in viable cell densities (A) or antibody titers (B) were observed and analysis of antibody charge heterogeneity, aggregate profile, and glycan profile indicated no changes as a result of the cell culture media filtration (not shown).

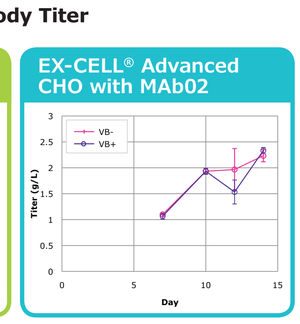
A **Viable Cell Density**





B **Antibody Titer**



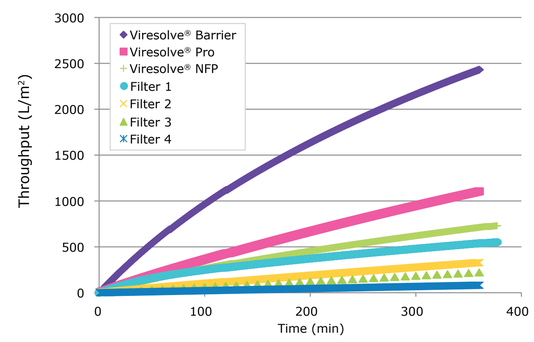


VB+ Viresolve® Barrier filter **VB-** Sterilizing-grade filter (control)

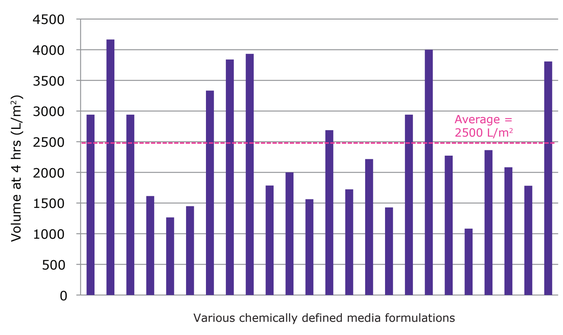
Cell Culture Media Filtration

Virus filters designed for downstream applications are inefficient for processing cell culture media (C). The Viresolve® Barrier filter leverages the proven technology of the Viresolve® platform with asymmetric polyethersulfone membrane technology and a novel secondary chemistry formulated for optimal processing of chemically defined media. This unique filter provides good volumetric throughput for a range of "off the shelf" and proprietary chemically defined media (D).

C **Throughput Testing of Model Stream on Viresolve® Barrier and Downstream Virus Filters**



D **Performance in a Range of Chemically Defined Media**



Summary

Risk-based analysis of bioprocess manufacturing processes highlights weaknesses in design and offers opportunities for improving specific elements that can impact virus safety. The Viresolve® Barrier filter is specifically designed to reduce risk early in production by adding a layer of protection to the bioreactor, enhancing existing materials sourcing, selection and facility control processes. The filter is easy to use, does not impact cell culture processes, and provides a level of virus removal across a range of conditions to provide confidence that microorganisms will not be introduced to cell culture processes.

[1] Cunningham, K, Carbrello C, et al. (2016) Nanofiltration as an Effective Means to Prevent Virus Contamination of Cell Culture Processes. Poster submission BPI 2016.

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