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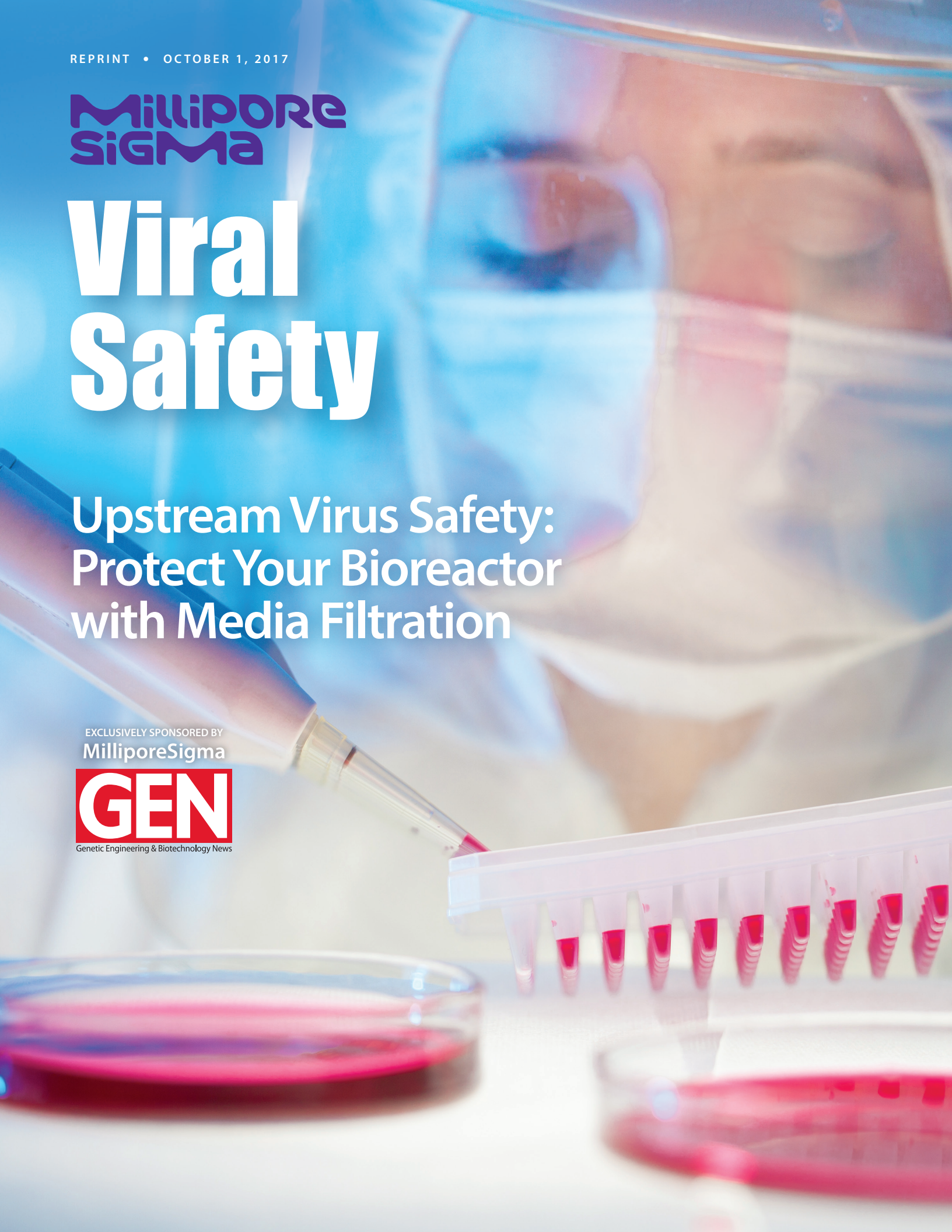
Viral Safety

Upstream Virus Safety: Protect Your Bioreactor with Media Filtration



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Upstream Virus Safety: Protect Your Bioreactor with Media Filtration

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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 may be used to minimize this risk. One of these, filtration, is a point-of-use operation that is easy to implement in the upstream process. This tutorial summarizes the performance of a filter specifically developed for virus removal from chemically defined cell culture media. The Viresolve® Barrier 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 and offers an effective, easy-to-implement solution for media treatment.

Risk reduction for viral contamination of upstream processes has traditionally relied on careful sourcing of raw materials, screening cell banks for adventitious virus, and control of facilities and workflow. Despite these precautions, bioreactor contaminations have 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 generally require costly investment and are often not suitable for all media components.

Filters specifically designed for upstream processing offer an alternative to capital-intensive methods, using proven membrane technology to assure robust, broadly effective, size-based virus removal *(Table)*.

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 sterilizing-grade filter; high retention (above the detection limit) has been demonstrated for large virus, mycoplasma, and bacteria *(Figure 1)*.

The graph *(Figure 2)* illustrates sustained high level of retention for minute virus of mice (MVM), a relevant small virus contaminant, during extended processing times for two different membranes. Membrane made near the limit of the manufacturing window shows MVM retention of approximately four logs sustained over 8 hours of processing. Typical nominal membrane shows over five logs of MVM retention over the same time period.

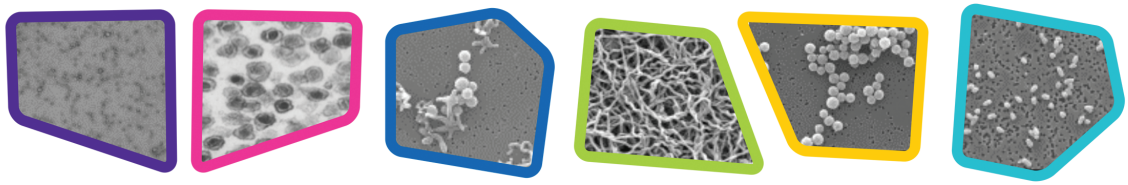
Virus retention across the Viresolve® Barrier Filter was evaluated at a range of processing conditions with different representative cell culture media. The filter is designed to retain a minimum of four logs Phi-

Comparison of Upstream Virus Prevention Technologies

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

Table.

Retention of a Broad Panel of Microorganisms with Viresolve® Barrier Filter



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

Figure 1.

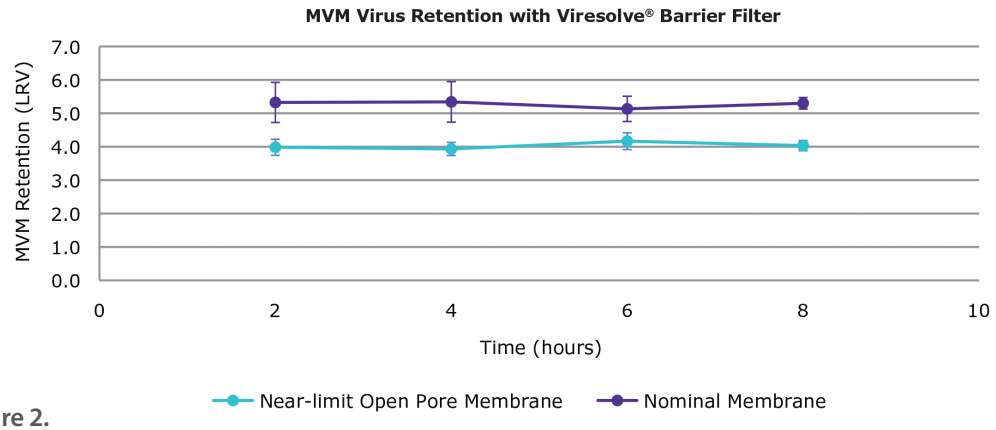


Figure 2.

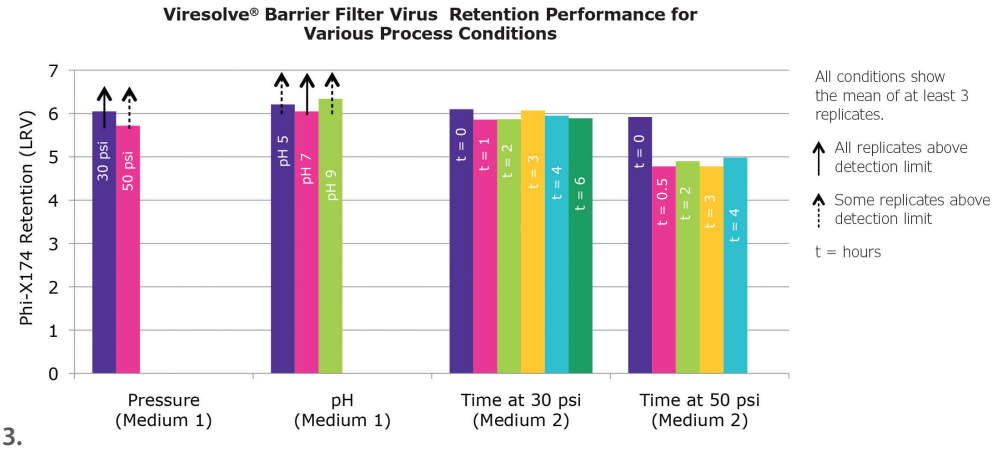


Figure 3.

X174, which is used as a surrogate organism for MVM. Virus retention remains high across a range of operating pressures and pH levels, even after extended processing times *(Figure 3)*.

Virus filters designed for downstream applications are inefficient for processing cell culture media. *(Figure 4A)*. The Viresolve® Barrier Filter leverages the proven technology of the Viresolve® platform with asymmetric polyethersulfone (PES) membrane technology and a novel secondary chemistry formulated for optimal processing of chemically defined media. This unique filter provides good volumetric throughput across a range of "off the shelf" and proprietary media *(Figure 4B)*.

Comprehensive analysis (mass spec or amino acid and soluble vitamin HPLC, NMR, and ICP-OES) of two cell culture media and their respective feeds 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.

Cell culture was performed using filtered media 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 *(Figure 5A)* or antibody titers *(Figure 5B)* 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).

Summary

Risk-based analysis of bioprocess manufacturing processes highlights weaknesses in design and, therefore, 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 final layer of protection before 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 high level of virus removal across a range of conditions, increasing confidence that microorganisms will not be introduced to the bioreactor. ■

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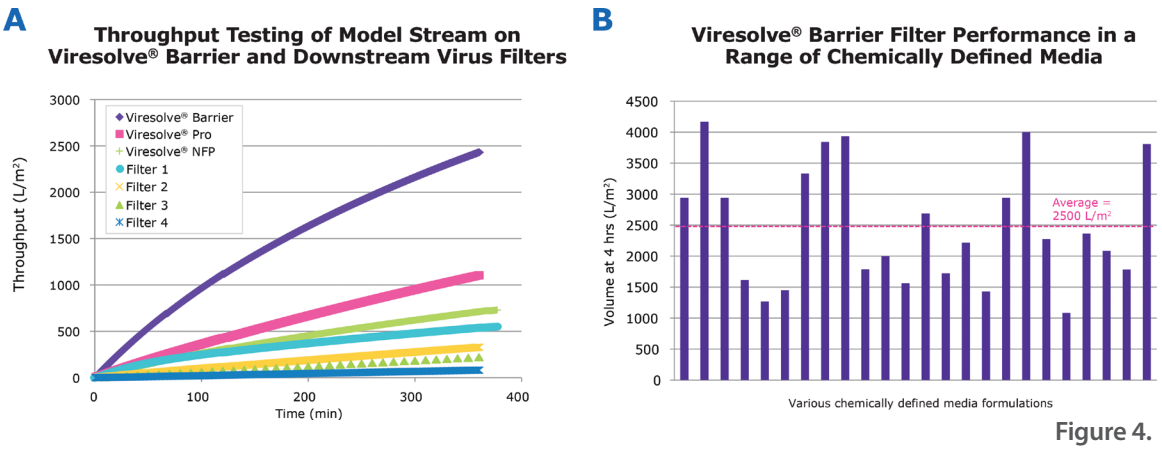
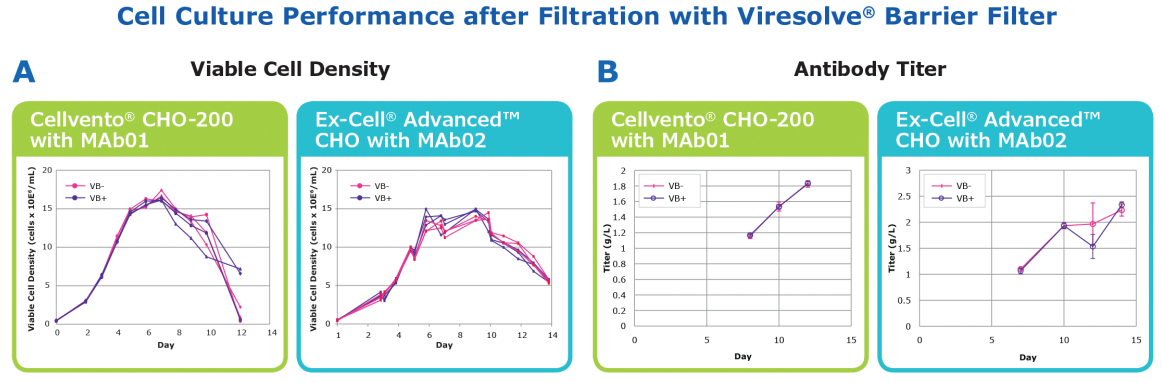


Figure 4.



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

Figure 5.