Implementation of a Virus Barrier Media Filter into Fed-Batch Bioprocesses

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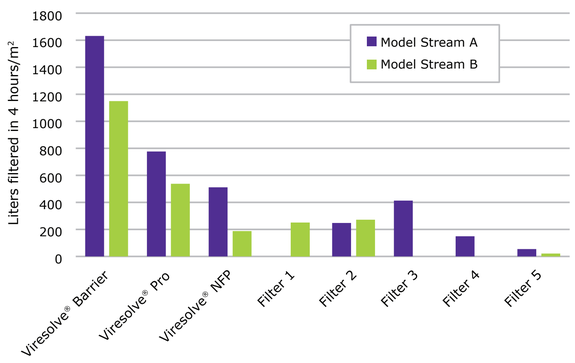
Introduction

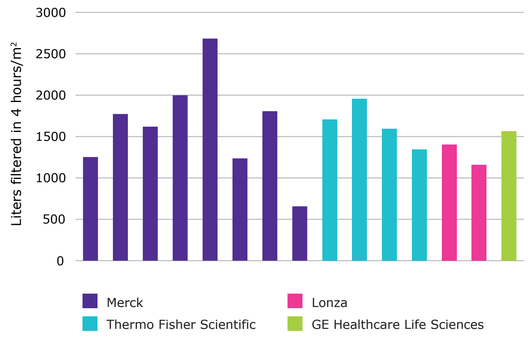
Upstream bioprocesses are at particular risk of contamination from adventitious agents. Typical 0.1 µm filters used at this step protect bioreactors from bacteria and mycoplasma, but offer no protection from viral contaminations. A new polyethersulfone (PES) virus barrier filter, Viresolve® Barrier filter, has demonstrated high levels of microorganism retention - full retention for bacteria and mycoplasma (>8.0 LRV - Log Reduction Value) and ~ 5 LRV for small viruses, such as parvoviruses. This filter is optimized for use with upstream components, and therefore exhibits higher flow and capacity than downstream virus filters. In this study, we evaluate the effects of implementing a Viresolve® Barrier filter into upstream processing.

Direct implementation of Viresolve® Barrier filter into bioreactor processes

Viresolve® Barrier filters are sterilizable and may be used in place of a 0.1 µm filter.



More than twice the volume of media can be filtered using Viresolve® Barrier filter in 4 hours as compared to existing downstream virus filtration technologies.

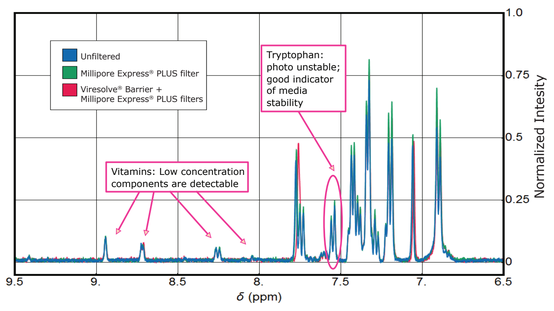


Viresolve® Barrier filter shows high capacity for efficient filtration of most commercially available chemically defined media.

Methods

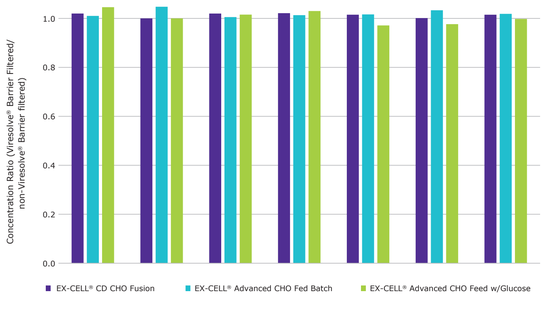
Given the small pore size of virus retentive filters, implementing virus filtration upstream of the bioreactor raises the question of whether critical cell culture media components might be removed. Therefore, it is important to evaluate the cell culture performance using filtered media to ensure that there has not been any negative impact to the process. EX-CELL® CHO media, Cellvento® CHO-200 medium and corresponding feeds were processed through Viresolve® Barrier filters, and media composition was compared to 0.2 µm Millipore Express® PLUS filtered controls. Fed batch cultures were performed in both shake flask and Mobius® 3L stirred tank bioreactors to verify that surfactants, such as poloxamer (which are essential for shear protection in stirred tank bioreactors and can be difficult to filter), had not been removed during filtration. Cell culture performance and protein quality were evaluated.

Media composition was not affected



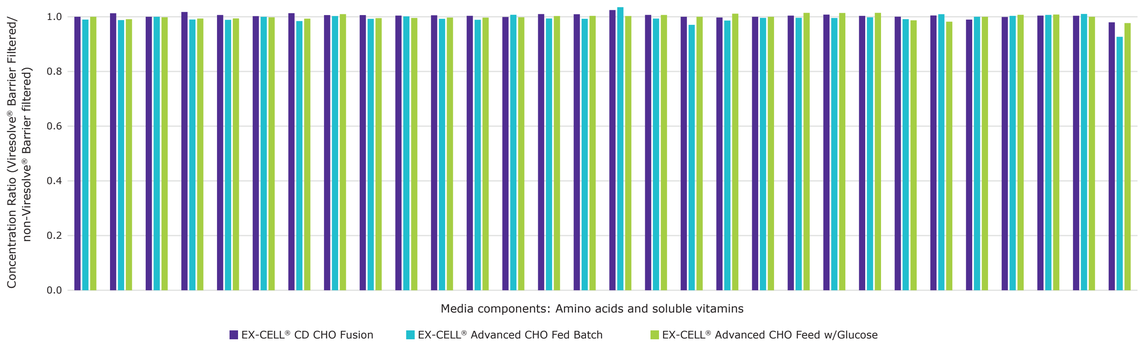
Nuclear Magnetic Resonance Fingerprinting – Aromatic Region

NMR fingerprinting of EX-CELL® Advanced CHO Fed-batch medium shows no change before and after filtration. NMR shows that the component levels have not changed and no new components have been introduced. This same trend was observed in the aliphatic region – of particular note the Pluronic® F68 peak was unchanged (data not shown).



Inductively Coupled Plasma/Optical Emission Spectrometry

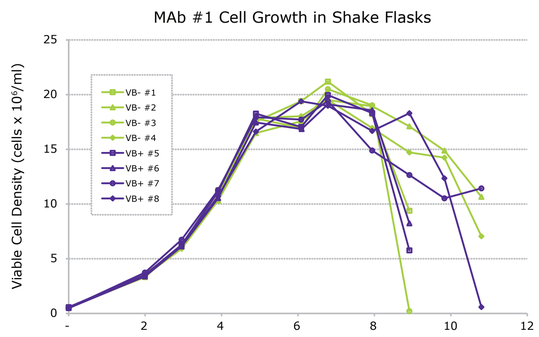
The graph above shows the concentration ratio of metals in the EX-CELL® CHO media and feed following Viresolve® Barrier filtration compared to Millipore Express® PLUS filtration as measured by ICP-OES. No significant differences were observed in metal concentrations.

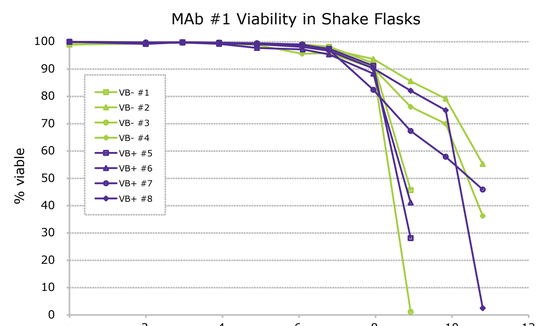


High Performance Liquid Chromatography

Amino acid analysis conducted by ion-exchange chromatography post column Ninhydrin method and water-soluble vitamin analysis by reversed-phase C-18 chromatography. The graph above shows no significant difference in the media concentration of amino acids and soluble vitamins following Viresolve® Barrier filtration compared to Millipore Express® PLUS filtration for EX-CELL® CHO media and feed.

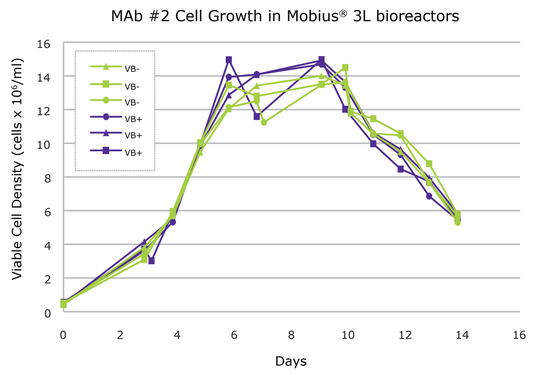
No change in cell culture performance

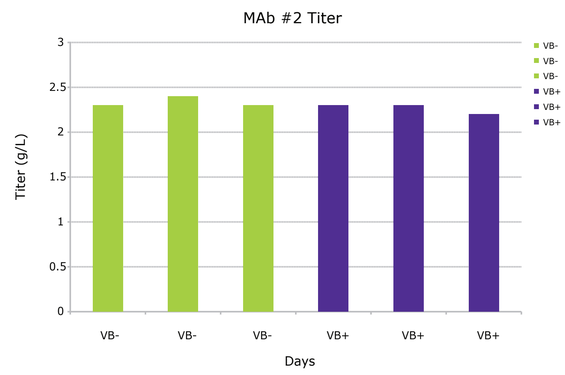




Cell performance in shake flasks

Two recombinant CHO cell lines were cultured in 125 mL shakers in fed batch culture. MAb #1 utilized Cellvento® CHO-200 medium and feeds and mAb #2 (data not shown) utilized EX-CELL® CHO media and feeds. No change in cell growth was observed when media and feeds were processed with (VB+) or without (VB-) Viresolve® Barrier filters. Cell viability was also unaffected. Osmolarity, pH, glucose, glutamate, lactate, or NH_3 levels were within limits (as measured by BioProfile® FLEX biotech analyzer). Titer, as measured by POROS® Protein A HPLC, was also consistent.

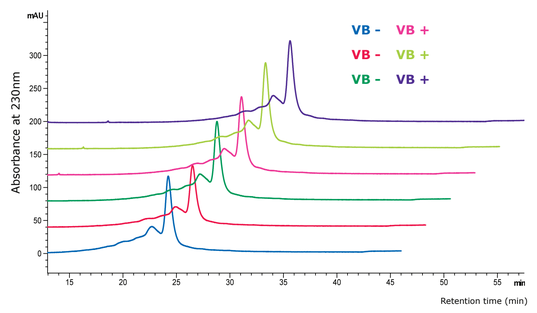


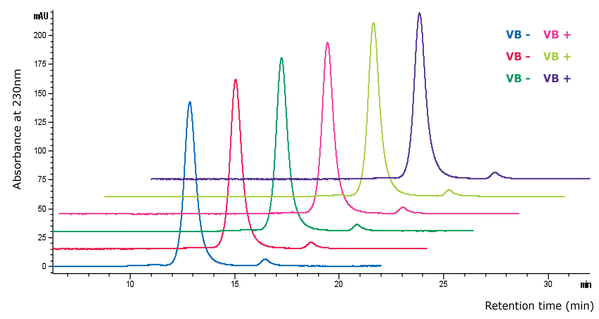


Cell performance in 3L bioreactors

Recombinant CHO cell mAb #2 was expanded for fed batch production in 3L bioreactors utilizing EX-CELL® CHO media and feeds. As in the small-scale shake flasks, no change in cell growth was observed when media and feeds were processed with (VB+) or without (VB-) Viresolve® Barrier filters. Titer, as measured by POROS® Protein A HPLC, was also consistent.

Product quality of recombinant antibody was unaffected



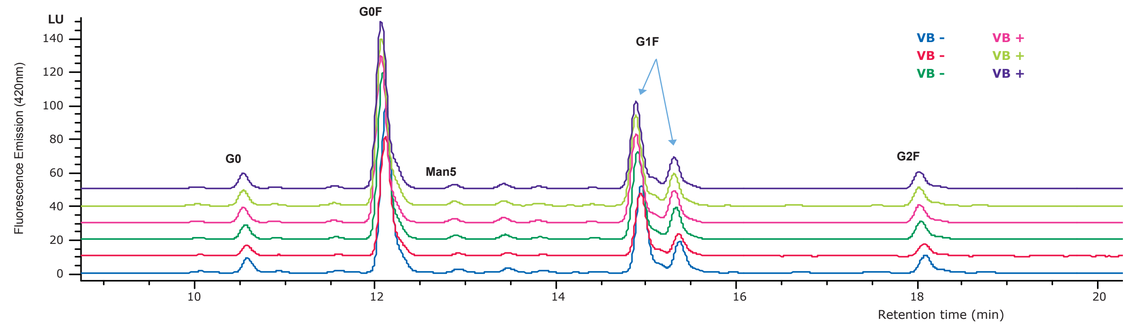


Charge heterogeneity

Weak cation-exchange chromatography was employed to analyze the Eshmuno® A purified antibody mAb#2 from bioreactor culture. No notable differences were seen in the amount of acidic, neutral or basic peaks between antibody purified from either culture system with (VB+) or without (VB-) Viresolve® Barrier filtration.

Aggregate Profile

The size exclusion chromatography profile for mAb#2 showed very high amounts of monomer for antibody purified from cultures, which was unaffected by filtration with (VB+) or without (VB-) Viresolve® Barrier filters. Consistently, very small amounts of a high molecular weight species were noted and no fragments were observed.



Glycan Analysis

Glycan analysis of the purified mAb#2 was performed using 2-AB fluorescent labeling and NP-UPLC. Consistent glycan patterns for both culture systems were observed for the antibody produced from media and feeds with (VB+) or without (VB-) Viresolve® Barrier filtration.

Summary

The risk of virus contamination of the bioreactor remains a concern for biotherapeutic manufacturers, as there is no universal technology that provides a reliable, cost effective solution for virus removal of all components of cell culture media. This study evaluated the Viresolve® Barrier filter, which provides an efficient and easy way to protect bioprocesses from adventitious virus contamination.

Study results demonstrated that media and feed compositions were unaffected by filtration through the Viresolve® Barrier filter. Cultures, both in shake flasks and scaled up to stirred tank bioreactors, showed no differences in cell growth or titer. In addition, the secreted antibodies showed no differences in the glycosylation pattern, amount of aggregates or charge variants. Preliminary work (not shown) in Mobius® 50 L bioreactors shows similar results.

Filtration of media and feeds with Viresolve® Barrier filter exhibits efficient filtration performance, high virus retention and minimal cell culture impact, and offers a viable option to improve the overall virus risk mitigation strategy for the manufacture of biotherapeutics.