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Application Note

Cell Culture Media Performance using Viresolve® Barrier Filters

Characterization of chemically defined cell culture media processed with Viresolve® Barrier filters for bioreactor protection.

Summary

This study evaluates the performance of Viresolve® Barrier filters, designed to remove viruses from chemically defined cell culture media in a typical CHO fed-batch process. Extensive analytical characterization of cell culture media demonstrated no change in composition after Viresolve® Barrier filtration. Cell growth, antibody titer, and protein quality attributes were comparable after filtration with Viresolve® Barrier or sterilizing-grade filters.

Introduction

Bioreactors are at particular risk of contamination from adventitious agents introduced into the process from multiple sources including raw materials, equipment, and personnel. To ensure sterility, cell culture media and feeds are typically sterile filtered at the time of bioreactor fill. However, virus contamination incidents have led biotherapeutic manufacturers to consider an additional level of protection for their cell culture processes.

Given the small pore size of virus-retentive filters relative to other filters used upstream of the bioreactor, implementing a virus filter raises the question of whether critical cell culture media components might be removed by the filter. Therefore, it is important to evaluate cell culture performance and product quality attributes using virus-filtered media, to ensure that filtration does not impact the production process.

Objective

In this study, the composition and performance of cell culture media filtered with Viresolve® Barrier filters were compared to relevant controls.

Filter Properties

Viresolve® Barrier filters are comprised of asymmetric polyethersulfone (PES) membrane with a nominal pore size of 20 nm, designed to provide high flux and robust retention of adventitious agents. These filters have demonstrated typical log reduction values (LRVs) of at least 4 for minute virus of mice (MVM), quantitative retention of *Brevundimonas diminuta,* and high mycoplasma removal with typical LRV > 8 using *Acholeplasma laidlawii*.

Experimental Methods

EX-CELL® Advanced CHO basal medium and feeds were filtered through Viresolve® Barrier or Millipore Express® PLUS filters under constant pressure. Media composition pre- and post-filtration was characterized using nuclear magnetic resonance spectroscopy (^{1}H-NMR), high-performance liquid chromatography (HPLC), and inductively coupled plasma optical emission spectroscopy (ICP-OES). Cell culture performance and protein quality were assessed.

Table 1. Description of filters

Filter	Filtration Area (cm²)	Material	Catalog No.
Viresolve® Barrier	3.3	PES	**VBMSPDKNB9**
Millipore Express® PLUS (0.22 µm control)	40	PES	**SCGPU11RE**

Cell culture materials

- EX-CELL® Advanced CHO fed-batch basal medium
- EX-CELL® Advanced CHO feed 1
- 400 g/L glucose
- CHOZN® GS recombinant antibody-producing CHO cell line

Results

Media composition study

EX-CELL® Advanced CHO fed-batch basal medium and feeds were filtered through Viresolve® Barrier filters.

The basal medium was characterized pre- and post-filtration by ^{1}H-NMR at 400 MHz. The aromatic and aliphatic portions of the NMR spectrum are shown in Figures 1 and 2, respectively. There was no noticeable difference in the aromatic region of the spectra between Viresolve® Barrier filtered and unfiltered basal medium (Figure 1). The peaks corresponding to tryptophan, which is photo-unstable and sensitive to changes in the medium, were consistent. The aliphatic region of the spectra shown in Figure 2 provides information about additional components. Poloxamer, which has proven difficult to filter in some operations[1], showed no difference pre- and post Viresolve® Barrier filtration. The shift near 2 ppm in Figure 2, is from the histidine proton, which is sensitive to pH changes[2], and is not indicative of a change in composition of the cell culture medium.

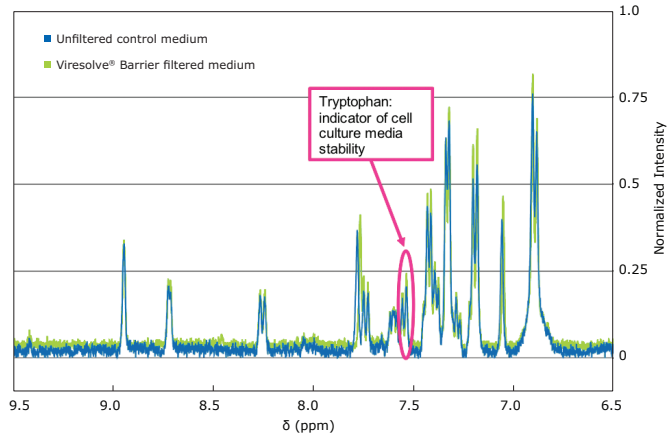

Figure 1

EX-CELL® Advanced CHO fed-batch medium ^{1}H-NMR aromatic region comparing Viresolve® Barrier filtered cell culture medium and unfiltered control.

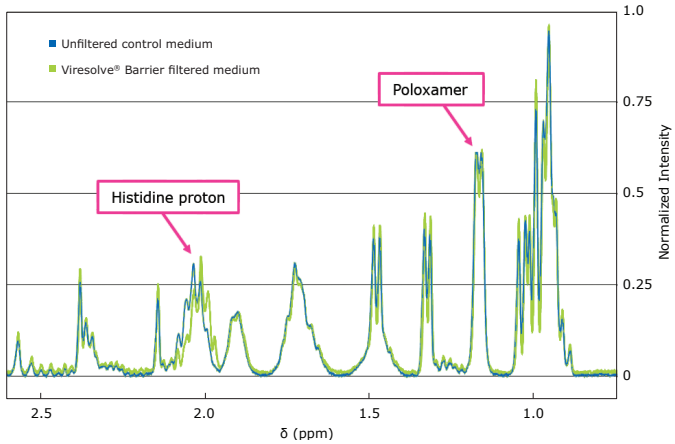

Figure 2

EX-CELL® Advanced CHO fed-batch medium ^{1}H-NMR aliphatic region comparing Viresolve® Barrier filtered cell culture medium and unfiltered control.

Cell culture media were evaluated using HPLC. Figure 3 shows the ratio of concentrations for individual media components before and after Viresolve® Barrier filtration. All detectable components in Viresolve® Barrier filtered EX-CELL® Advanced CHO fed-batch media were within 7% of the unfiltered control.

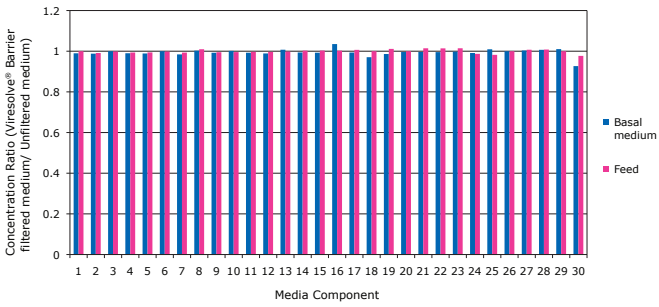

Figure 3

HPLC concentration ratio for Viresolve® Barrier filtered to unfiltered EX-CELL® Advanced CHO fed-batch cell culture media.

In addition to NMR and HPLC analyses, ICP-OES was used to detect trace metals in the media. Results demonstrated no change in metal concentrations after filtration (Figure 4). Note that some metals were below the detection limit of the assay.

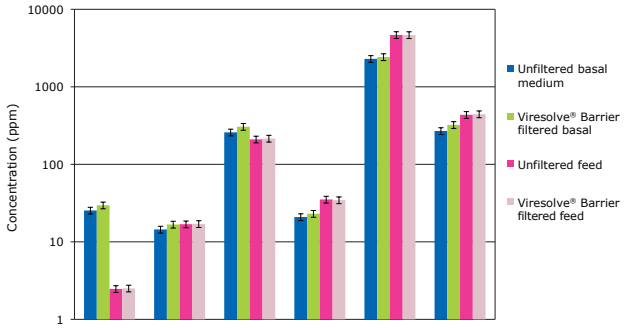

Figure 4

ICP-OES metal analysis of Viresolve® Barrier filtered and unfiltered EX-CELL® Advanced CHO fed-batch cell culture media.

Cell culture performance study

Viresolve® Barrier filtered EX-CELL® Advanced CHO fed-batch media were also used for monoclonal antibody (mAb) production with the CHOZN® GS recombinant cell line in Mobius® 3 L single-use bioreactors. Cultures were evaluated for cell growth, metabolites, titers, and protein quality.

The viable cell density indicated no differences in cell growth using Viresolve® Barrier filtered media compared to the controls, as seen in Figure 5. Similarly, no differences in viability, doubling time, population doubling level, peak IVC (integral viable cell density), pH, osmolarity, glucose, glutamate, or lactate concentration were observed. Protein quality analytics showed no changes in titer, glycan profile, charge variants or size distribution profile (monomer and high molecular weight).

Titers, measured by Protein A HPLC, showed there was no significant titer difference between a mAb produced with Viresolve® Barrier filtered cell culture media compared to the control (Figure 6).

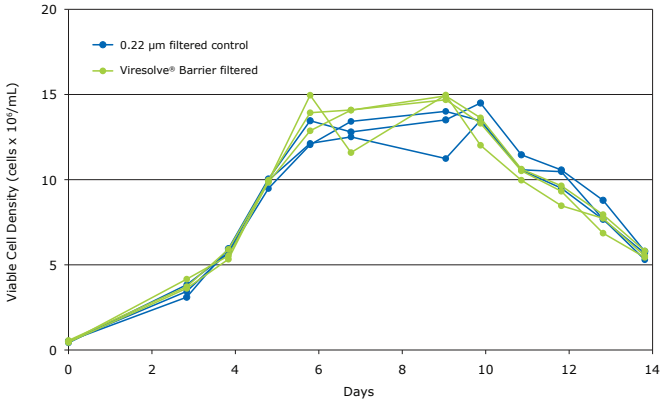

Figure 5

Viable cell density during fed batch cultivation for Viresolve® Barrier filtered and control filtered EX-CELL® Advanced CHO fed-batch medium and corresponding feeds.

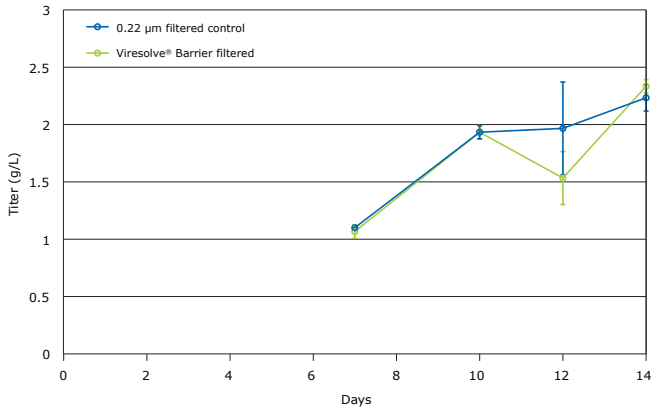

Figure 6

Titer of CHOZN® GS produced mAb with Viresolve® Barrier filtered media compared to the control.

Glycan analysis of the purified mAb was performed using 2-aminobenzamide fluorescent labeling and ultra performance liquid chromatography (UPLC). Consistent glycan profiles were observed for mAbs produced with Viresolve® Barrier filtered and control filtered media (Figure 7).

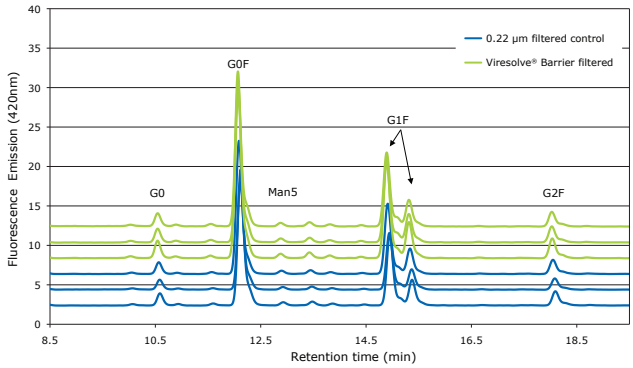

Figure 7

Glycan profile of CHOZN® GS produced mAb using Viresolve® Barrier filtered cell culture media compared to the control.

Charge variants were analyzed by weak cation-exchange chromatography (WCX). Results of the WCX showed no difference in the concentrations of acidic, neutral, or basic variants when using Viresolve® Barrier filtered media (data not shown).

Size exclusion chromatography (SEC) was used to determine the levels of mAb aggregates. SEC analysis showed a high concentration of monomer purified from all cultures (Table 2). Small amounts of high molecular weight (HMW) species were detected, and no fragments were observed. Based on test results, there is no difference in protein aggregate profile for antibodies produced with Viresolve® Barrier filtered and control filtered media.

Table 2. SEC analysis of CHOZN® GS produced mAb using Viresolve® Barrier filtered cell culture media compared to the control

Aggregate Profile (Peak %, UV @ 280 nm)		
	Monomer	**HMW**
0.22 µm filtered control	**98.7 ± 0.02**	**1.3 ± 0.02**
Viresolve® Barrier filtered	**98.8 ± 0.04**	**1.2 ± 0.04**

Conclusion

The results of this study demonstrate that cell culture media compositions were unaffected by Viresolve® Barrier filtration. Cultures in 3 L stirred tank bioreactors showed no difference in cell growth or protein titer compared to the control. In addition, mAbs produced using Viresolve® Barrier filtered media showed no difference in aggregate concentrations, glycan profiles, or charge variants compared to the control.

Viresolve® Barrier filters can be used in upstream processes to reduce the risk of contamination, contributing to an overall viral risk mitigation strategy.

References

1. C. Schulz, J. H. Vogel, K. Scharfenberg, Influence of Pluronic F-68 on the Ultrafiltration of Cell Culture Supernatants, in Animal Cell Technology, 1st ed., M. J. Carrondo, B. Griffiths, J. L. Moreira, Ed. Netherlands: Springer, 1997, pp. 373-378.
2. O. K. Baryshnikova, T. C. Williams, B. D. Sykes, Internal pH indicators for biomolecular NMR, Journal of Biomolecular NMR, 41 (2008), pp. 5–7.

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