SARTORIUS

Simplifying Progress

Virus Risk Mitigation in Viral Vector and Gene Therapy Cell Culture Media

Sherri Dolan[1], Alexander Schwartz[1]*, Kam Siu[1], Erin Gladstone[1], Noelle Ali[2], and Michael Lasse[2]

[1] Sartorius Stedim North America, Bohemia, New York, United States of America
[2] Sartorius Stedim Biotech, Göttingen, Germany
*Corresponding author: Alexander.Schwartz@sartorius.com

Introduction

Viral risk mitigation in biopharmaceutical production has historically relied upon a three-tiered integrated strategy for decades. First, the selection of raw material is evaluated to identify potential viral contaminants. If the risk for these contaminants cannot be fully eliminated, the second tier of relevant raw material and in-process testing must be implemented. Finally, as testing has been shown to allow some amount of false failing results, a validated downstream viral reduction strategy must also be demonstrated. This method of viral risk mitigation is commonly referred to as the viral risk mitigation tripod and has largely been credited with the significant patient safety seen in today's blood plasma-derived and recombinant therapeutics.

Like many risk strategies, the viral risk mitigation tripod is a dynamic process that does shift as process specifics change. While the reduction tier has become a central leg of blood plasma-derived and recombinant therapeutics, as novel therapies are developed with reduced or eliminated downstream processes the reliance on a downstream viral clearance validation will, in turn, be reduced or eliminated. Thus, the risk analysis for these new modalities shift towards raw material selection and testing to support the robust virus risk mitigation of the emerging viral vector and gene therapy markets.

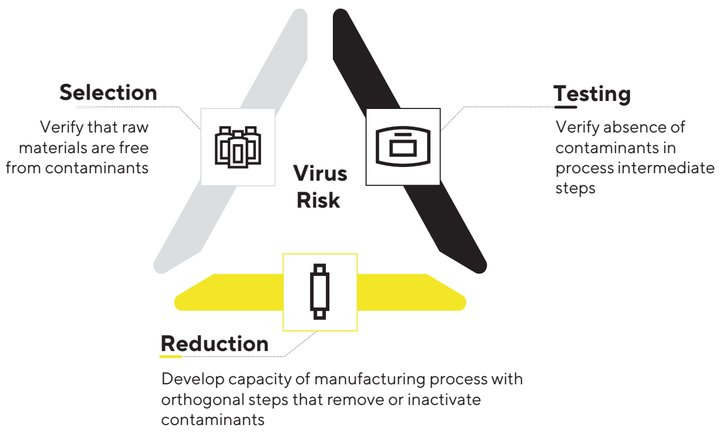

Figure 1: The viral risk mitigation tripod.

For these novel modalities, this shift in viral risk mitigation strategy can implement several different tactics that include, but are not limited to, the reduction or elimination of animal-derived media components – switching to chemically defined (CD) media, the implementation of virus clearance steps on media prior to use, and the use of closed aseptic and single-use processing. This poster evaluates the applications of these three methods in the commercialization of the viral vector and gene therapy processes and provides proven technologies to alleviate the pressure of the shifting viral safety tripod.

1. Virus Clearance Technologies for Media: Virosart® Media

Virosart® Media is specially designed for virus filtration of chemically defined cell culture media. This high-speed virus filter provides end-users with an economical solution suitable for upstream media virus filtration. High virus retention is validated for the Virosart® Media filter by logarithmic reduction values of ≥ 4 $\log_{10}$ for small non-enveloped viruses. Additionally, implementation into single-use processes is given by gamma irradiatable capsule designs. The Virosart® Media filter is comprised of a 20 nm hollow-fiber PES membrane that is specifically designed for upstream applications.

Performance of the Virosart® Media filter is independent of the use of powder or liquid media but can be strongly impacted by the media itself. While protein transmission for mAbs and large recombinant proteins is not possible for this filter, low concentration, and highly purified protein media supplements may be evaluated for filter effectiveness.

An efficient pre-filtration step, such as the Sartopore® 2 XLM (02/0.1 µm), could increase the capacity of the final virus filter. The optimum pre-filter to final filter ratio has to be identified during the development of the process step as this strongly depends on the specific media used.

Figure 2: The Virosart® Media filter hollow fiber design.

2. Study Design for Evaluation of CD Media Virus Filtration

Two Chemically Defined (CD) media were selected for this evaluation and are outlined in the materials section below. Media were selected to cover a range of cell culture types that have historically been used in the viral vector and viral vaccine space. Prior to final virus filtration, media was adjusted from 2–8 °C storage to room temperature overnight. Media was then processed through Sartopore® 2 XLM (02/0.1 µm) Sartoscale disposable units (17.3 cm²) at 0.1 bar (1–2 psi) in a constant pressure system. Virosart® Media Lab Modules (5.0 cm²) were then wetted with sterile water for at least 20 minutes at 2 bar (29 psi) prior to use. Pre-filtered material was then processed over Virosart® Media filters at 2 bar (29 psi) in a constant pressure test system. Filtrate mass throughput was collected using data logging software for at least four hours. See figure 3 for a diagram of the experimental setup.

Media Selection

4Cell® MDCK CD Medium is a chemically defined, serum-free, protein-free, animal component-free, hydrolysate-free medium designed for the growth and infection of Madin-Darby Canine Kidney (MDCK) cells in suspension conditions, either for research use or for further manufacturing purposes.

The 4Cell® BHK-21 CD Medium is complete medium, ready-to-use, chemically defined, serum-free, animal component-free, antibiotics-free, and hydrolysate-free; designed to reduce costly product purification steps of BHK to support high-density growth and maintenance of BHK-21 suspension cell lines used for viral vaccine production.

Media Selection

- Virosart® Media Lab Modules (5.0 cm²); PN 3V2-28-BVGML-V
- Sartopore® 2 XLM (17.3 cm²) Sartoscale; PN 5445358MS--FF--M
- 4Cell® MDCK CD Media; PN CFV3FA2003
- 4Cell® BHK-21 CD Media; PN CFV3FA0002

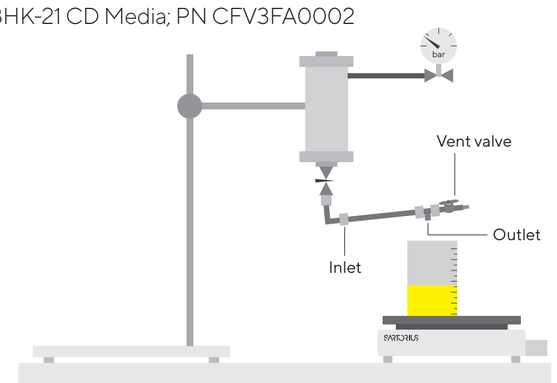

Figure 3: Constant pressure test system for hollow fiber membrane filters.

3. Results of CD Media Virus Filtration

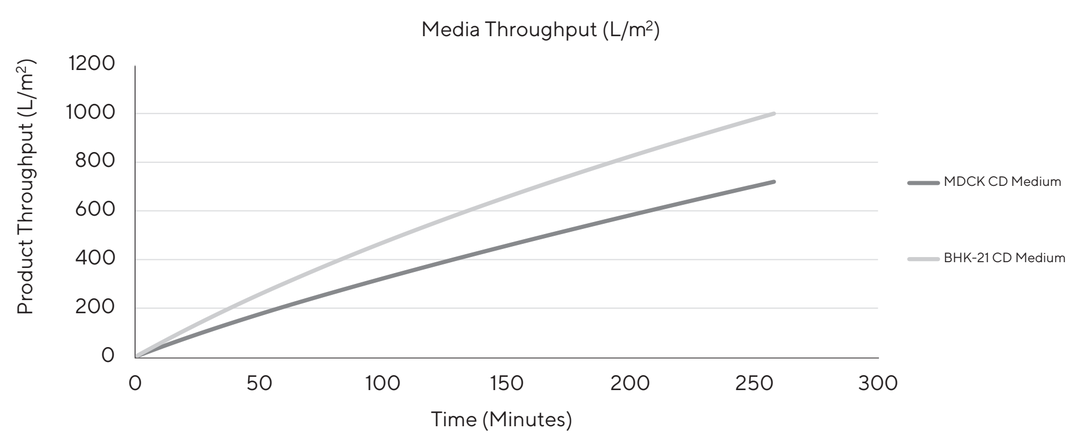

Figure 4: Graph of media throughput over time for evaluated chemically defined medias.

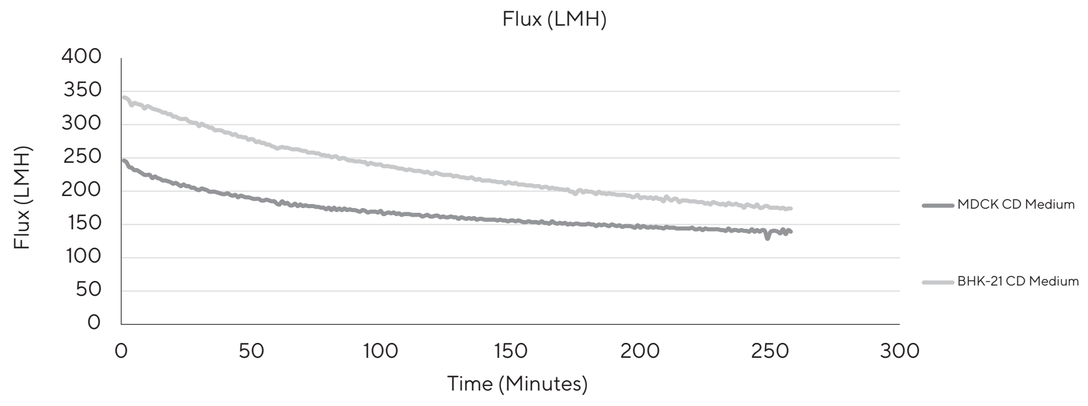

Figure 5: Graph of filter flowrate (flux) over time for evaluated chemically defined media.

Run ID	Media	Total Throughput (L/m²)	Total Time (hrs)	Capacity in 4hrs (L/m²)
1	4Cell® MDCK CD Media	719.96	4.4	678.18
2	4Cell® BHK-21 CD Media	999.40	4.3	946.58

Figure 6: Table of filter performance for respective chemically defined media.

4. Media Virus Filtration Scale Up

One of the benefits of using a hollow fiber membrane is its inherent linear scalability based on membrane area. Further benefits of using the Virosart® Media filter include its ability to be delivered gamma sterilized, along with its integration into sterile single-use filter transfer sets. The Sartopore® 2 XLM is also gamma stable and can similarly be made into sterile single-use filter transfer sets. To further aid in single-use processing, both the Virosart® Media filter and the Sartopore® 2 XLM are available in the Maxicaps MR® format. With the Maxicaps MR®, one can implement several large-scale filters in a pre-constructed and pre-sterilized manner, in-parallel on a single-use cart. The advantage of such a system in GMP single-use settings allows with near-instant set-up and takedown using aseptic connections to maintain a sterile boundary without the need for additional filter holders. With the use of closed aseptic systems as described above, a true viral barrier can be maintained for processing media into cell culture or bioreactors. Given the capacities listed for four-hour processes earlier, this indicates a single 6 unit Maxicaps MR® Virosart® Media can process up to 4069L of 4Cell® MDCK CD Media and 5679L of 4Cell® BHK-21 CD Media.

# of Units per Module	3 Unit	6 Unit
Sartopore 2® XLM	7.2 m²	14.4 m²
Virosart® Media	3.0 m²	6.0 m²

(A) (B)

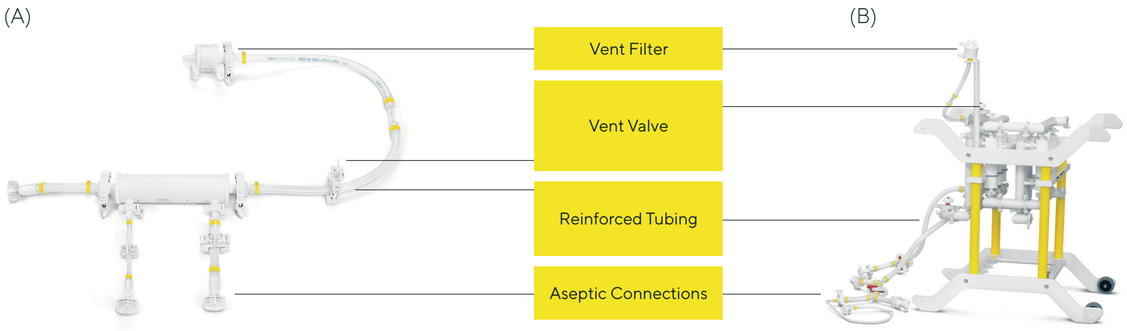

Figure 7: (A) Sterile single-use filter transfer sets for the Virosart® Media. (B) Maxicaps MR® format for Virosart® Media.

5. Discussion and Conclusion

The data presented shows that the Virosart® Media filter is capable of filtering CD media for a diverse set of cell types within the viral vector and viral vaccine space. As would be done with all media filters, cell growth studies should be conducted to assure media effectiveness post filtration. Furthermore, additional media can be evaluated to broaden the scope of this application to cell lines such as HEK293 and Vero.

The scaleup simulation details the potential for commercial filtration with these specific media at the largest of scales. Of note, the application of CD media filtration to commercial viral vector or viral vaccine processes may not require the same process size that has become a hallmark of recombinant protein manufacturing. This makes the proposed processes potentially commercially viable as a true virus barrier technology to commercial cell culture processes. Efforts should be made in the commercialization activities of future viral vector and viral vaccine processes to evaluate technologies for efficient screening of cell culture media and raw materials as a better business practice and as tools to ensure virus risk mitigation.