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Application Note

Robust Removal of Minute Virus of Mice from Cell Culture Media with Viresolve® Barrier Filters

Parvovirus retention on Viresolve® Barrier filters for the protection of upstream processes

Summary

Viresolve® Barrier filters were evaluated for retention of Minute Virus of Mice (MVM) in a chemically defined cell culture medium over extended filtration times. Nominal and open lots of Viresolve® Barrier membrane were selected to represent a range of retention performance. Results demonstrated that Viresolve® Barrier filters containing open membrane provide an average of 3.8 logs of MVM retention. Viresolve® Barrier filters containing nominal membrane provide average retention of 5.1 logs.

Introduction

Bioreactors are at risk of contamination from adventitious agents from a number of sources, and in particular from the raw materials used in cell culture processes. A contamination event can interrupt manufacturing schedules and require costly investigations and cleaning remediation, resulting in lost revenue and potential disruptions in drug supply. Virus contamination incidents have led manufacturers to reexamine their risk assessments around virus prevention, and in many cases to evaluate implementation of virus reduction steps upstream of the bioreactor.

One approach to risk mitigation is to introduce virus filtration as a way to prevent adventitious virus ingress into cell culture processes[1]. Virus filters are easy to implement and use, and provide size-based removal of a broad range of both enveloped and non-enveloped viruses[2]. Viresolve® Barrier filters are specifically designed for high-flux virus filtration of chemically defined cell culture media, while providing high levels of virus retention. MVM is a small (18-24 nm), non-enveloped parvovirus that has been associated with several large-scale contamination events[3].

Objective

The objective of this study was to evaluate Viresolve® Barrier filters for MVM retention in a chemically defined cell culture medium.

Experimental Methods

MVM retention studies were performed with Viresolve® Barrier micro filters (3.3 cm^2) using four Viresolve® Barrier membrane lots: two lots of typical membrane (nominal) and two lots of membrane made near the low retention edge of the process window (open).

The filters were challenged at 30 psi constant pressure with CD OptiCHO™ cell culture medium spiked with MVM at a minimum titer of 2×10^6 $TCID_{50}$/mL. See Figure 1 for experimental setup.

Samples were collected from the filtrate pools at 2 and 4 hours of filtration. As media filtration is typically completed within 4 hours, this was selected as a conservative end point. For two of the lots, filtration was extended to 8 hours, to evaluate retention performance beyond the typical fed-batch media processing times. Titers were measured in each challenge solution and filtrate sample by cell-based $TCID_{50}$ assays using 324K indicator cells. Table 1 summarizes the throughput and number of filters tested.

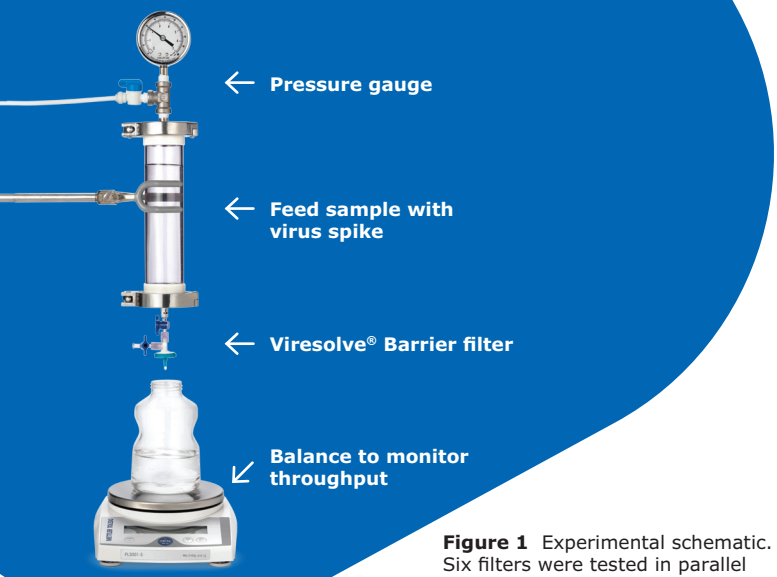


Figure 1 Experimental schematic. Six filters were tested in parallel from a single feed reservoir.

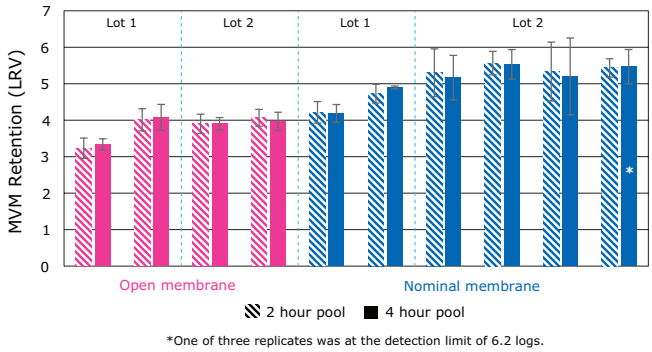
Table 1. Average throughput (L/m²) and number of filters tested per membrane lot.

Filtration time	Open Membrane		Nominal Membrane	
(hours)	Lot 1	Lot 2	Lot 1	Lot 2
2	1157 *n* = 6	1116 *n* = 6	1109 *n* = 5	1044 *n* = 12
4	1865 *n* = 6	1857 *n* = 6	1787 *n* = 5	1720 *n* = 12
8	Not tested	2986 *n* = 3	Not tested	2707 *n* = 3

Results

A summary of MVM retention with Viresolve® Barrier filters at 2 and 4 hours is presented in Figure 2. The bars indicate mean log reduction values (LRVs) of different membrane rolls, and the error bars indicate the LRV range. All individual LRVs, including those from open membrane lots, met the target of at least 3.0 logs. Approximately 3 logs of MVM retention should be sufficient to remove low levels of virus not detected in routine raw material screenings. Open membrane lots provided an average of 3.8 logs MVM retention at the 4-hour time point; nominal membrane lots, which are typical Viresolve® Barrier filters, provided an average of 5.1 logs retention at the same time point.

Retention results out to the extended processing time of 8 hours are presented in Figure 3. For both nominal and open membrane lots, MVM retention was consistent from 2 to 8 hours of processing, despite 70-75% flow decay after 8 hours of processing. These results demonstrate robust virus removal with no reduction in retention performance with virus loading, volume processed, or degree of plugging.



*One of three replicates was at the detection limit of 6.2 logs.

Figure 2 MVM retention of open and nominal membrane at 2 and 4 hours. Multiple lots were tested; each bar represents the mean LRV of a different membrane roll.

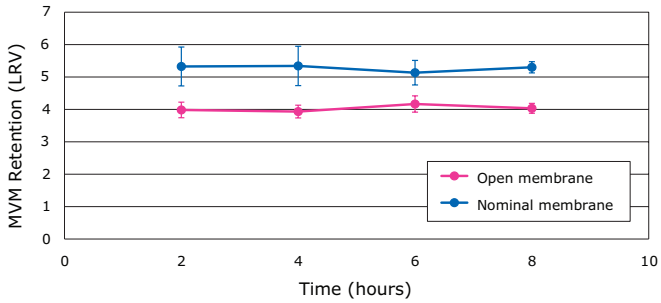


Figure 3 One lot each of open and nominal membrane were used to evaluate MVM retention to an extended processing time of 8 hours.

Conclusion

This study demonstrated high levels of MVM retention performance for multiple lots of Viresolve® Barrier filters over 8 hours of processing. Open membrane provided 3.8 logs of MVM retention in a chemically defined medium. Nominal membrane, present in typical Viresolve® Barrier filters, provided an average of 5.1 logs retention. Testing of multiple lots of both open and nominal membrane over a range of typical processing times has demonstrated that Viresolve® Barrier filters offer a robust solution for virus removal from chemically defined cell culture media. Implementation of Viresolve® Barrier filters reduces the risk of introducing viral contamination into a cell culture process, providing a high level of virus safety assurance.

References

1. Liu, S. et. al. Development and Qualification of a Novel Virus Removal Filter for Cell Culture Applications. Biotechnol. Prog. **2000**, 16:425-434.
2. Gefroh, E.; Dehghani, H.; McClure, M.; Connell-Crowley, L.; Vedantham, G. Use of MMV as a Single Worst-Case Model Virus in Viral Filter Validation Studies. PDA J. Pharm. Sci. Technol. **2014**, 68:297-311.
3. Garnick, R. L. Experience with Viral Contamination in Cell Culture. Dev. Biol. Stand. **1996**, 88:49-56.

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