

Virus Risk Mitigation in Cell Culture Media

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A new virus-retentive membrane may be used to filter chemical-defined cell culture media for risk mitigation.



Image courtesy of Sartorius Stedim Biotech

The contamination of bioreactors with various infective agents such as bacteria, mycoplasma, and viruses is a potential risk to patient safety. Viruses have been the cause of multiple bioreactor contaminations in recent years (1). A number of biopharmaceutical companies have reported on production-scale bioreactor contaminations by small non-enveloped viruses such as minute virus of mice (MVM) or vesivirus (2). Many significant players within the industry participate in the Consortium of Adventitious Agent Testing (CAACB) to address this topic.

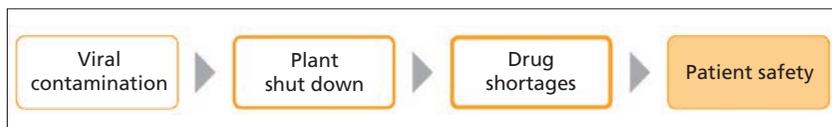
There are a range of methods available to manufacturers that reduce the risk of cell culture contaminations by bacteria or mycoplasma; however, the

contamination risk from viruses, specifically small non-enveloped viruses, is a much greater challenge to the biopharmaceutical industry even when using chemical defined media (1). Classical sterilizing-grade filters and even 0.1-micron membranes cannot prevent contaminations by small non-enveloped viruses.

Virus contamination is more difficult to detect than contamination caused by bacteria (3). Failure to detect viruses can result in the contamination of the entire downstream process and even the final drug product. A number of companies have reported lost production lots due to contaminations with MVM or vesivirus (2). In all these cases, the root-cause analysis showed that the most likely source was the contamination of



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Figure 1: Escalation cascade for a virus contamination event.

media components, such as salts, during storage at an early stage in the supply chain (4–6). Controls to prevent mice entering these warehouses have been inadequate.

The consequences of such a contamination event may be fatal for a patient.

Due to the high stability of these non-enveloped viruses, they can survive for long periods of time. Even one infectious particle is enough to contaminate a whole bioreactor. This one particle would find the perfect replication conditions in the bioreactor environment. It is almost impossible to identify a single particle within 1000 L of media before it enters the bioreactor.

IMPACT OF A CONTAMINATION

The consequences of such a contamination event may be fatal for a patient (see **Figure 1**). If a company identifies a contamination, the plant can be shut down and subjected to extensive cleaning efforts. This could lead to drug shortages and patients not receiving lifesaving drugs (7).

UPSTREAM VIRUS CLEARANCE SOLUTIONS

There is a demand for a new viral clearance concept for use in upstream processing. In the past, researchers have investigated tech-

nologies such as gamma irradiation, UV-C irradiation, or high temperature short time (HTST) for their ability to remove viruses from cell culture media. Bovine serum is gamma irradiated at external facilities that can handle large volumes (8). HTST is usually only economically feasible when preparing large volumes of media at high flow rates because of the large investment required to build customized systems (9). Unfortunately, not all media components are heat-stable and scalability of HTST system can be difficult. UV-C is limited by the flowrate that can be applied and hence has limited application when preparing large volumes of media. Furthermore, the data published on these methods show these technologies all have varying effectiveness in inactivating small non-enveloped viruses. Until now, filtration, which is a efficient technology that is especially effective for the small non-enveloped viruses, has not been widely adopted (10).

BOTTLENECKS OF DOWNSTREAM VIRUS FILTERS

To achieve the desired robust virus removal, some companies have implemented virus filters used in downstream processing for their media preparation to minimize the risk of viral contaminations. Companies can effectively use these filters for virus filtration of media produced for perfusion bioreactors because the flow rates are low due to long production times. The capacities for media itself are quite high, with classical downstream processing (DSP) filters

when filtering chemical defined media (up to 10.000 L/m² (11)). If the possibility exists to filter these volumes over multiple days instead of a few hours, classical DSP filters are a perfect solution and economically feasible as low filtration areas are sufficient. Unfortunately, during classical batch filtration, this is not the case. Due to the risk of bacterial contamination, the media must be filtered within a minimum of 24 hours and ideally during a working shift. To increase the overall speed of filtration, larger membrane areas are necessary, but this approach is expensive and makes this scenario unrealistic economically.

ECONOMICAL FEASIBLE SOLUTIONS

The industry needed to address the low flow rates and high cost of using virus filters designed for downstream processing application for cell culture media filtration for the virus filtration of media to be economically feasible. This was the basis for the first virus retentive filter membranes specifically developed for media filtration (10).

METHODS

Retention performance

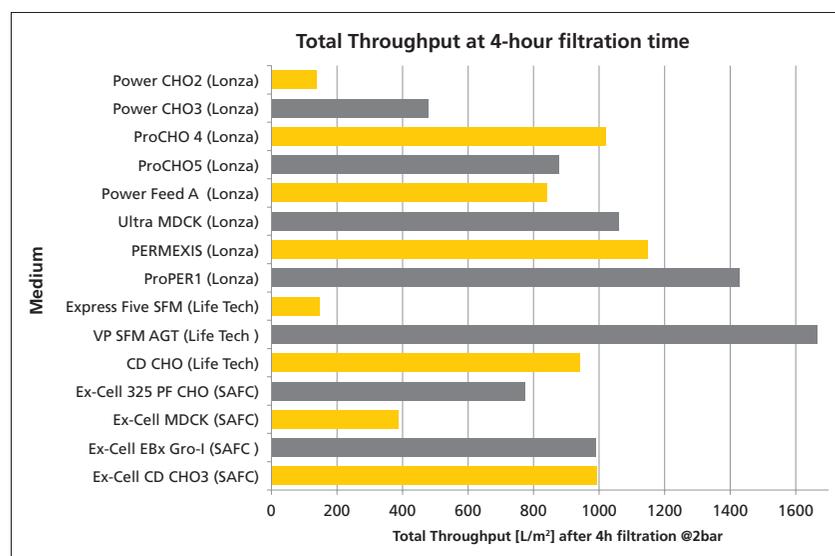
Each lab module with a filtration area of 5.0 cm² was challenged with virus. As a worst-case model for small non-enveloped viruses, MVM, a single stranded, 12-26 nm, non-enveloped, ssDNA virus from the parvovirus family has been used. Duplicate runs were performed at 2.0 bar constant pressure with 5.0 cm² lab modules for 3 different media. A volume of 400 L/m² was filtered at a 1% spike ratio.

Membrane throughput testing

The throughput of the membrane was determined using 15 different media from three different suppliers. All trails were performed at

Table 1: Retentive capacities of the new virus retentive media filter, lab modules (5 cm²).

Organism	Strain	Description	Medium	LRV Lab Module 1	LRV Lab Module 2
Minute virus of mice	ATCC VR-1346	Worst-case model virus for small non-enveloped, ssDNA viruses from the parvovirus family	KPI buffer	≥ 5.22	≥ 4.22
			ProCHO™ 5	≥ 4.98	≥ 4.98
			SAFC EXCell® CD CHO-3	≥ 5.04	≥ 4.98

Figure 2: Throughput overview within four-hour filtration time with 15 cell culture media (CCM) at constant pressure 2.0 bar; virus media filter, lab modules.

constant pressure of 2.0 bar. The total throughput was measured after four hours of filtration.

RESULTS

Retention performance

Table 1 provides a summary of the retention data from testing performed with the new membrane. The membrane provides virus retention performance that is equivalent to those filters designed for use in downstream processing applications. Retention of the small non-enveloped virus MVM exceeded four logs. The analytical methods could not detect MVM in the filtrate. It is reasonable to expect that the membrane will retain agents larger than these small viruses that could potentially contaminate cell cultures.

Membrane throughput with different chemically defined cell culture media

The throughput of the membrane was determined using 15 different media from three different suppliers. **Figure 2** shows that the volumes of media processed within four hours at 2-bar pressure varied widely depending on the media formulation. The Lonza Power CHO2, for example, blocked the membrane relatively quickly; however, the Lonza ProPER1 media did not appear to block the membrane at all.

For some of the commercial cell culture media, the use of an inline 0.1-micron filter increased the membrane throughput significantly (7). This was only successful with some of the media formulations and not with others. Protective agents, such

For some of the commercial cell culture media, the use of an inline 0.1-micron filter increased the membrane throughput significantly.

as pluronics, reduce flux rates drastically (10). Reducing the pluronic concentration or filtering it before adding it to the media can increase filter throughput significantly. The industry should perform additional research to understand fully the influence that different media components can have on the blockage of media filters. It could use this research to design media with improved filterability characteristics without affecting its performance during cell culture.

Nevertheless, the research performed shows that the new membrane developed typically filters approximately 1000 L/m² within four hours of commonly available cell culture media. This makes it an economically feasible method for the batch preparation of media

while reducing the risk of viral contaminations of cell cultures.

CONCLUSION

In this article, the authors have reported on a new virus-retentive membrane that upstream process engineers can use to filter chemical defined cell culture media for risk mitigation. The authors have demonstrated that that this method is able to provide a greater than four-log reduction in small non-enveloped viruses. The high throughput of the membrane when used to filter a range of different chemical defined media enables biopharmaceutical companies to use the membrane in this upstream application without having a detrimental impact on the process economics. This membrane has the potential to be a crucial component of a risk-based approach to minimizing virus contamination events allowing the robust production of biopharmaceuticals.

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