Case Study: Virus Risk Mitigation in Cell Culture Media

Lab-scale to Pilot-scale – Virus Retentive Filtration and Cell Growth Studies

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1. Introduction

Ensuring virus safety is of utmost importance in the Biopharmaceutical industry. Mammalian cell culture processes present a unique challenge, as the handling and processing of these media allow for possible contamination events to occur. The cell culture bioreactor is a perfect environment for the proliferation of these contaminants and the introduction of a very low level contamination can quickly replicate into a major contamination. Past experience has shown that raw materials may be a high risk for introducing viral and bacterial contaminants. Bacterial contaminants can be easily removed by 0.1 or 0.2 µm sterilizing grade membranes, however small viruses (such as Vesivirus, MVM) are not removed by these filters. In addition, testing raw materials may not be adequate since low levels of virus contamination may go undetected, hence a mitigation strategy to treat all raw materials which enter the bioreactor for virus removal | inactivation is becoming more popular in the industry. Adventitious contamination events have occurred in the past and may have severe consequences, such as GMP facility contamination. Facility shutdown leading to drug shortages, financial losses and lost market share.

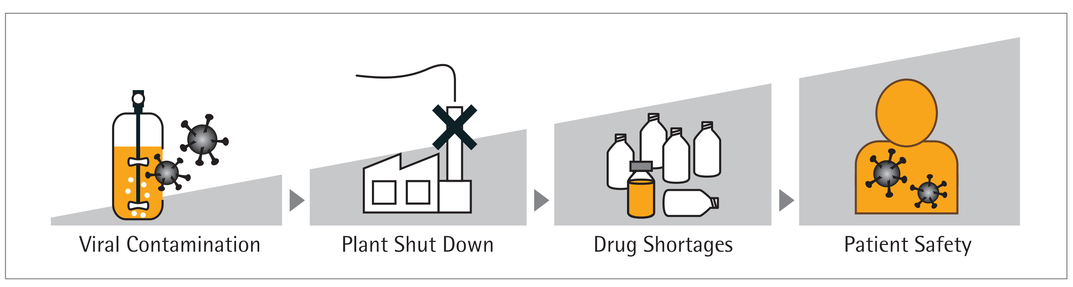

Fig 1: Upstream contaminations may lead to dire consequences such as GMP manufacturing plant shut down, drug shortages, and ultimately patient safety.

Current risk mitigation technologies such as HTST, UV-C, and gamma irradiation are useful, however not always easy to implement and are not cost effective. Size exclusion based filtration is the preferred technology for viral clearance, as it is robust and non-invasive. Current downstream virus retentive membranes do not possess the flux rate or economics when it comes to filtration of cell culture media. Therefore a novel membrane has been developed by Sartorius Stedim Biotech to mitigate contamination risks in the bioreactor from chemically defined media and raw materials.

The purpose of this study was to determine the capacity of Virosart® Media for Genentech's chemically defined media as well as to investigate the effect of filtration on subsequent cell growth performance.

In total, three studies were conducted:

Study 1: Small-scale (5 cm^2) filtration of chemically defined media with and without poloxamer

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Study 2: Pilot-scale (0.3 m^2) filtration of chemically defined media

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Study 3: Impact on cell culture growth of filtered media from pilot scale

2. New: Virosart® Media

The Virosart® Media filter mitigates virus contamination risks prior to the addition of nutrients plus other additives into the bioreactor system and has been developed for chemically defined cell culture media. The Virosart® Media filter is an asymmetric polyether sulfone hollow fiber membrane with 20 nm pore size rating that exhibits high capacity (1000 L/m^2 at 29 psi in 4 hour filtration time) for filtration of cell culture media while providing ≥ 4 LRV (log_{10} reduction value) for small non-enveloped viruses and ≥ 6 LRV for large enveloped viruses.

Fig. 2: Virosart® Media family: Process module (3 m^2 & 1 m^2), mid-scale module (0.3 m^2) and lab module (5 cm^2).

3. Small Scale Filtration: Study 1

This study was performed to determine the filter capacity for cell culture media at the lab-scale. Two runs were performed with the Virosart® Media module to compare the filter capacity of Genentech's chemically defined media (serum | protein free) with and without poloxamer. Poloxamer is a non-ionic surfactant which is used in cell culture media to protect cells from stressful shear conditions in bioreactors. The addition of poloxamer may help preserve high cell growth and viability which may be compromised without its use.

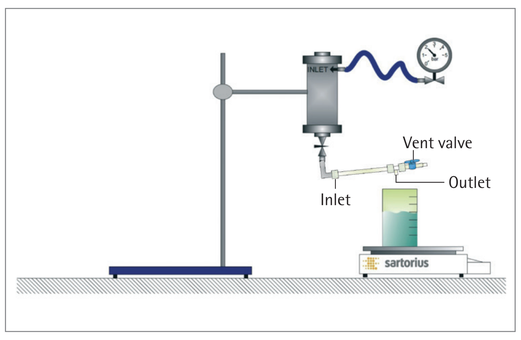

Fig. 3: Set-up of small scale filtration.

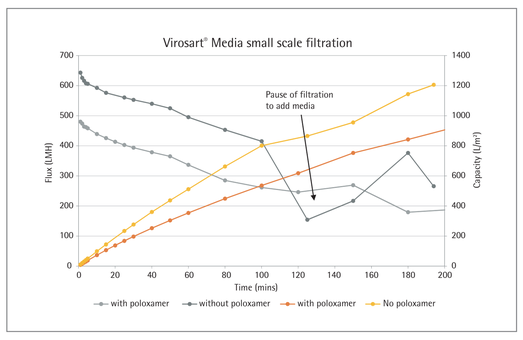

Fig. 4: Small-scale filtration of media with and without poloxamer, using the Virosart® Media lab module, comparing flux rate and capacity at constant pressure of 35 psi.

Flux (LMH) and capacity [L/m^2] data for chemical defined media with and without poloxamer are presented in figure 4. This figure shows a slight difference in capacity and flux when poloxamer is present. Lower flux and lower capacity are to be expected when poloxamer is present and is dependent on concentration of the substrate.

4. Mid-scale Filtration: Study 2

The lab-scale results were confirmed by processing 350 L of Genentech's media containing poloxamer through the Virosart® Media mid-scale module (0.3 m^2, part number: 3V2--28-GVGFL). The media was prefiltered using a Sartopore® 2 XLM (0.1 µm) pre-filter. The filtration was then performed and operated at a constant flux of 300 LMH using a Watson Marlow pump. The Virosart® Media filtered medium was then sterile filtered into a sterile bag and stored at 2 – 8 °C until used for the cell growth study (Study 3) as the media filtration was not performed under sterile condition. In commercial processing the sterile filter post Virosart® Media would not be used.

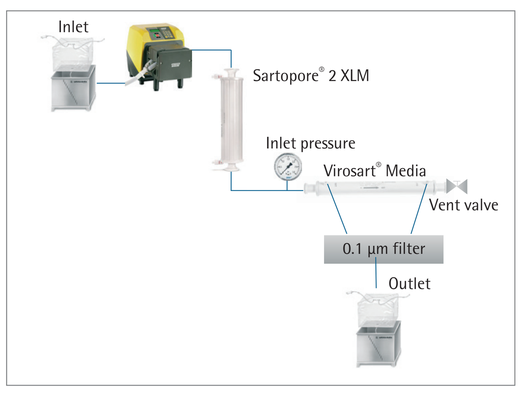

Fig. 5: Set-up of mid-scale filtration.

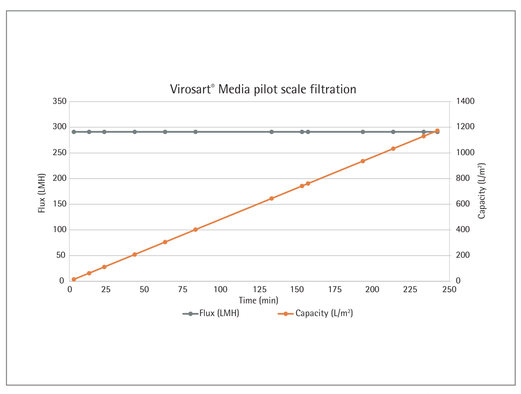

Fig. 6: The graphs shows the time vs. flux (grey) and capacity (orange) for 350 L batch filtration.

350 L of chemically defined media was filtered in 4 hours at constant flux (300 LMH). An overall capacity of 1174 L/m^2 was reached.

5. Cell Growth Studies: Study 3

A study was performed to compare the cell growth profiles using filtrate produced by Virosart® Media filter (Study 2) verses a standard 0.1 µm filter. Filtrate from these filters was used to culture the cells across 8 passages using shake flasks. A standard CHO cell line was used to inoculate the duplicate shake flasks. The viable cell concentration (VCC) and cell viability were recorded during each cultivation.

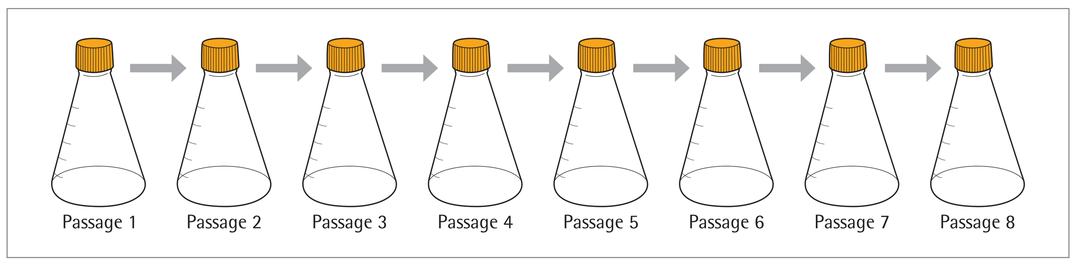

Fig. 7: Set-up of cell growth study.

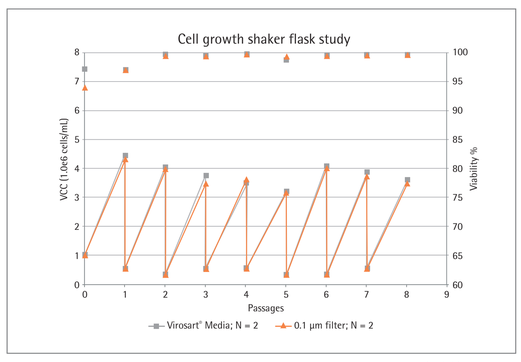

Fig. 8: Cell growth study comparing viable cell concentration (VCC) and viability with Virosart® Media filtered media with the standard 0.1 µm filtered media.

There was no difference in cell viability observed between media filtered with the Virosart® Media filter and media filtered with standard 0.1 µm filter. The viability was > 95 % for both filters.

6. Economic Analysis

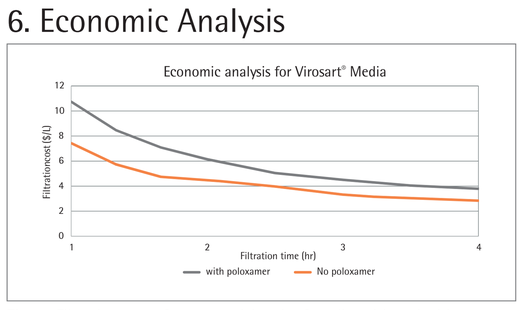

Fig. 9: Filtration cost of 350 – 400 L batch of media, with and without poloxamer calculated based on the small-scale filtration performed. This graph shows that a longer processing time will reduce costs.

The cost effective filtration of cell culture media is feasible with Virosart® Media and mitigates the risk of viral contaminations of cell cultures. In this study filter costs are in the range of $ 3 – 4/L media filtered to process a batch size of 350 – 400 L (calculated based on the small-scale filtration performed). For most of the other types of media, filtration costs are in the range of $1 – 3/L.

7. Summary and Conclusion

The results presented here demonstrate that the Virosart® Media filter had high capacities and good process economics for the media being tested. The presence of poloxamer had a negative effect on the capacity of the Virosart® Media filter. However, approximately 1000 L/m^2 of chemically defined media containing poloxamer could still be filtered with a Virosart® Media in little less than 4 hours for both the lab scale and mid-scale modules.

Growth studies comparing virus filtered media and standard filtered media (0.1 µm) in shake flasks showed no differences in growth or viability. Although the Virosart Media® membrane shows no impact on cell growth and cell metabolism in the system studied, we recommend users perform cell growth evaluations under their specific culture conditions.

In this study, filter costs are in the range of $3 – 4/L, media and may be lower for media which do not contain poloxamer or where longer process times are used.