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Evaluation of Impact on Cell Growth using Chemically defined Cell Culture Media filtered through the Virosart® Media Filter

Application Note

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Abstract

The contamination of bioreactors with adventitious agents such as bacteria, mycoplasma, and viruses is a potential risk to patient safety. Viruses have been the cause of multiple bioreactor contamination events in recent years. A number of biopharmaceutical companies have reported production-scale bioreactor contamination events by small non-enveloped viruses such as minute virus of mice (MVM) or vesivirus.[1]

The consequences of such an event may be severe and result in GMP facility contaminations, along with drug shortages and financial losses (figure 1). Therefore, several large biopharmaceutical operations are evaluating risk mitigation strategies for the minimization of contaminations by adventitious agents. Classical sterilizing-grade filters and even 0.1 µm-rated filter membranes cannot prevent contaminations by small non-enveloped viruses.[2]

Size exclusion-based filtration is the preferred technology for virus clearance, as it is robust and non-invasive. The Virosart® Media filter mitigates virus contamination risks which may arise from the addition of nutrients and other additives into the bioreactor system. It has been developed specifically for chemically defined cell culture media. The Virosart® Media filter is an asymmetric polyethersulfone hollow fiber membrane with 20 nm nominal pore size rating that exhibits high capacity (1000 L/m^2 at 2 bar in 4 hour filtration time) for filtration of chemically defined cell culture media while providing ≥ 4 LRV ($\log_{10}$ reduction value) for small non-enveloped viruses and ≥ 6 LRV for large enveloped viruses.[3,4]

This study reports on the cell growth characteristics of chemically defined media that has been filtered with the Virosart® Media filter and shows no influence on growth media quality and therefore no impact on either cell metabolism or therapeutic protein production. Two cell culture cultivation runs were performed to compare media filtered with Virosart® Media with Sartopore® 2 XLM, a standard 0.1 µm filter. Before cultivation, a filtration study was performed to compare the filterability characteristics of five different media used during the cell culture. Filtration with the Virosart® Media was performed with and without pre-filtration with a Sartopore® 2 XLM.

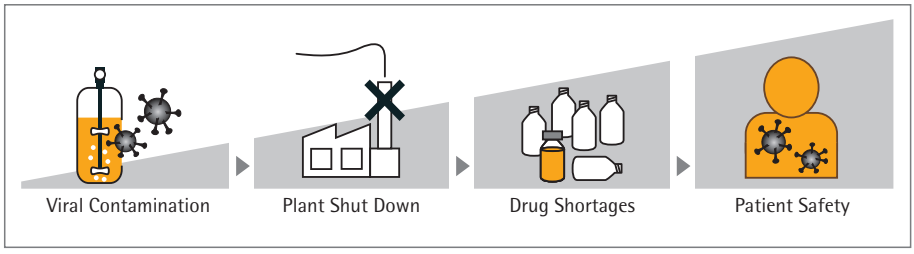

Fig. 1: Escalation cascade for a virus contamination event

The cell culture media used in this trial were developed by Sartorius Stedim. It is a chemically defined media solution comprising of five different media. Stock culture media (SM) is used for all seed culture steps. Production media (PM) is used as a basal media for the main culture. Feed media A (FMA) and feed media B (FMB) are added as a bolus feed on days 3 to 11 of the process.

FMA contains glucose and vitamins, amongst other components, whereas FMB contains amino acids and has a pH value of 11. The cell culture process requires glucose with a concentration of 400 g/L to be added from day 6 of the cultivation to maintain a minimum culture glucose concentration of 3 g/L.

Media	Description	Day Media added	Volume for 250 mL cultivation [mL]	NAO*	Protein-free	Peptide-free	Gln-containing	Poloxamer-containing	Hydrolysate-containing
Stock culture media (SM)	Basal media used for batch phase of main culture	1	15	Yes	Yes	Yes	Yes	Yes	No
Production media (PM)	Basal media used for batch phase of main culture	1	150	Yes	Yes	Yes	Yes	Yes	No
Feed media A (FMA)	Feed media used for fed-batch phase	3 –11	60	Yes	Yes	Yes	No	Yes	No
Feed media B (FMB)	Feed media used for fed-batch phase	3 –11	6	Yes	Yes	Yes	No	No	No
Glucose Feed 400 g/L	Feed media used for fed-batch phase	3 –11	6	Yes	Yes	Yes	No	No	No

Table 1: Five cell culture media used for 12 day fed-batch CHO cultivation

* Non-animal origin

I. Study 1: Filtration of Cell Culture Media

Material and Methods

The filterability characteristics of the five different cell culture media used for the CHO fed-batch cultivation were evaluated with the Virosart® Media. Filtration trials were performed on the Virosart® Media (VM), with and without Sartopore® 2 XLM as a pre-filter. Both trials on the Virosart® Media were performed in duplicate. Sartopore® 2 XLM is a 0.2 | 0.1 µm pleated polyethersulfone filter.

Virosart® Media lab modules (5 cm^2, part number: 3V2--28-BVGML--V) and Sartopore® 2 XLM SartoScale 25 0.1 µm (4.5 cm^2, part number: 5445358MV--LX--C) from different production lots were used in the trials.

The dehydrated media was reconstituted in deionized water according to manufacturer's instructions. Before each run, the filters were flushed for 15 minutes with deionized water at 2.0 bar | 30 psi using compressed air and the water flux recorded. The deionized water in the reservoir was exchanged to according cell culture media and the filtration at constant pressure of 2.0 bar | 30 psi was started at room temperature (20 – 22 °C | 68 – 71.5 °F). The filtration was stopped after 240 min or when the total volume was processed through the filter. The filtrate was collected and the weight recorded at specific time points in order to calculate the flow rate, flux and flux decay.

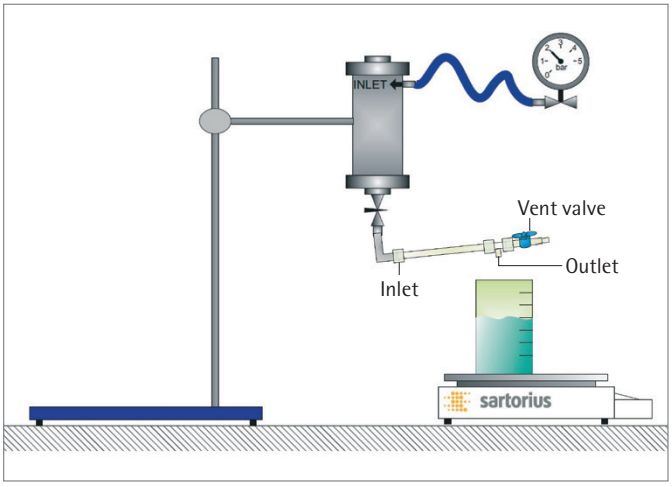

Fig. 2: Experimental set-up of small-scale filtration using Virosart® Media lab module 5 cm^2

Results and Discussion

The data presented in figure 5 and 6 show the capacity [L/m^2] vs. time [min] of the five different cell culture media being filtered for 240 min at 2.0 bar | 30 psi or when the total volume was processed over the filter such as was the case for FMB. The filtration of FMB has been extrapolated using V_{final} up to a filtration time of 240 min (shown in dotted lines) to allow for a comparison with the other media. The following formula describing V_{final} was applied:

$$\frac{t}{V} = \frac{1}{V_{final}} \cdot t + \frac{1}{Q_0}$$

with:

t	Time [min]
V	Capacity [L/m^2] filtered
V_{final}	Maximum capacity [L/m^2]
Q_0	Initial flux [LMH/bar]

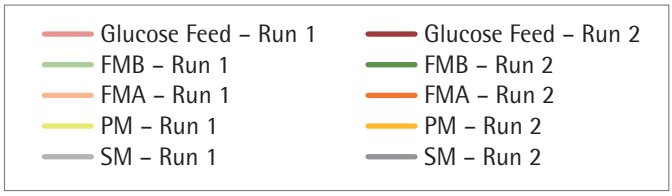

Fig. 3: Legend of 5 tested cell culture media in duplicate runs from fig. 4 – fig. 6

Figure 4 shows cell culture media filtration data performed with the Virosart® Media without the Sartopore® 2 XLM as a pre-filter. In all trials feed media B, production media and seed culture media showed very good filter capacities and tended to block the filter relatively slowly (figure 4). FMB showed the highest filtration capacity such that a total volume of 500 mL could be filtered in 120 min. Both production media and seed culture media approached their total capacity in 240 min filtration time of 1000 L/m^2 and indicating some blocking around 60 % at 240 min total filtration time. Both production media and seed culture media do contain poloxamers and L-glutamine. The presence is known to have an impact on the filterability.[5] Extrapolated feed media B reaches over 2000 L/m^2 in 240 min.

Glucose feed and high concentrated media which tended to block the filter more rapidly (figure 4). Both media give an overall capacity of 500 L/m^2 in 240 min filtration time. The glucose feed may contain residues of high molecular weight carbohydrates or molasses resulting in the filter blockage of up to 70 %.

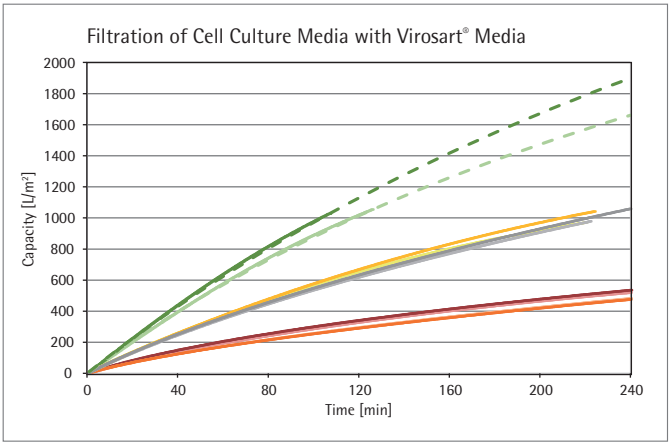

Fig. 4: Filtration of five different cell culture media with Viorsart® Media at constant pressure of 2.0 bar | 30 psi for 240 min filtration

Data determining the impact of Sartopore® 2 XLM as a pre-filter for all five different cell culture media is presented in figure 5. Sartopore® 2 XLM minimally increased the filtration performance for glucose feed, FMA, FMB, production media and seed culture media.

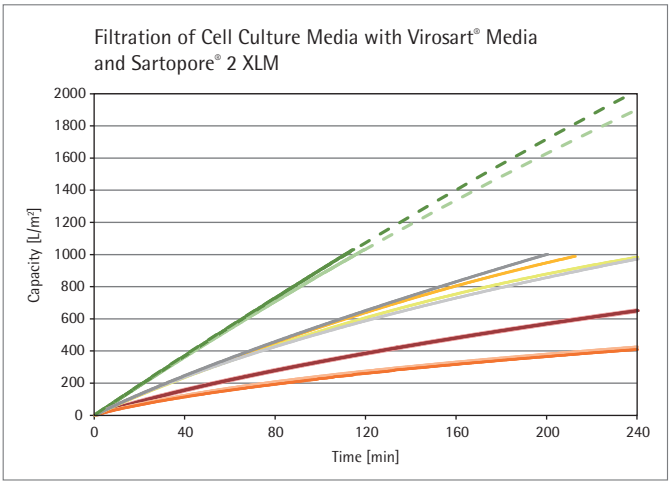

Fig. 6: Filtration of five different cell culture media with Virosart® Media and Sartopore® 2 XLM as pre-filter at constant pressure of 2.0 bar | 30 psi for 240 min filtration

Figure 6 provides the cumulated capacity after 240 min filtration time with (w) and without (w/o) the use of Sartopore® 2 XLM as a pre-filter. The data shows a capacity of 2000 L/m^2 for FMB, 1000 L/m^2 for process media and seed culture media and around 500 L/m^2 for FMA and glucose feed with concentration of 400 g/L.

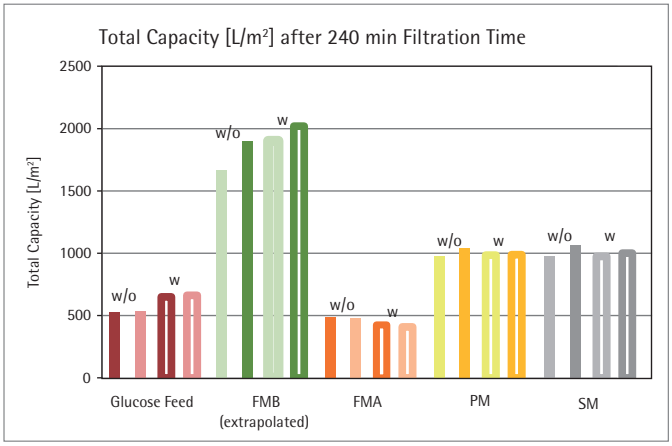

Fig. 6: Total capacity after 240 min filtration time at constant pressure of 2 bar | 30 psi in duplicate runs with (w) and without (w/o) pre-filter

II. Study 2: Fed-Batch Culture of CHO Cells

Material and Methods

A CHO fed-batch process was used to evaluate the growth of cells in Virosart® Media filtered media. The process comprises five seed culture steps. To eliminate early growth influence, the media used in the seed train was either filtered with the Virosart® Media filter or the sterile filter. Two of the media filtration conditions (Virosart® Media and Sartopore® 2 XLM) were repeated to prepare sterile media for the cell culture processes.

The 12-day main culture comprises of a 3-day batch phase and a 9-day fed-batch phase. The culture is inoculated with 0.3×10^6 cells/mL and the peak viable cell density (VCD) is typically reached at day 8 with $19 - 27 \times 10^6$ cells/mL with a viability of 99 %. Since the main influence of media alteration is visible in the growth phase of the process, the focus of this study was the first 7 days of the process.

The bolus feeding from day 3 comprises FMA, FMB and a highly concentrated glucose solution (400 g/L). The amount of FMA and FMB added is constant throughout the complete fed-batch phase. Typically, additional glucose is needed on day six to maintain a glucose concentration of 3 g/L in the cell broth. The feeding process is automated using pumps. The harvest is performed during the dying phase during which the VCD should be above 15×10^6 cells/mL with a viability of more than 70 %.

The process was performed in an ambr® 250 modular 2-vessel system. The ambr® 250 modular is an innovative high performance benchtop bioreactor system for parallel fermentation or cell culture. It consists of a fully single-use bioreactor integrated to sensors (e.g. pH, dissolved oxgen) with a working volume range of 100 to 250 ml, five reagent bottles, off-gas measurement and one peristaltic pump per bioreactor (figure 7). The system is modular and expandable from 2 to 8 bioreactors.

For the cultivation, the filtration was repeated under sterile conditions. Volumes according to table 1 were taken from the pool of cell culture media filtered with Virosart® Media and with Sartopore® 2 XLM.

Fig. 7: ambr® 250 modular

Results and Discussion

The evaluation of cell growth using chemically defined media that had been filtered with Virosart® Media and Sartopore® 2 XLM is shown in figure 8 and 9. The viable cell density (VCD) and viability trends of both cultures were comparable and no influence of Virosart® Media could be detected (figure 8). Both cultures reached a VCD of approx. 17×10^6 cells/mL after 7 days. Viability was in a range of 95 – 99 %.

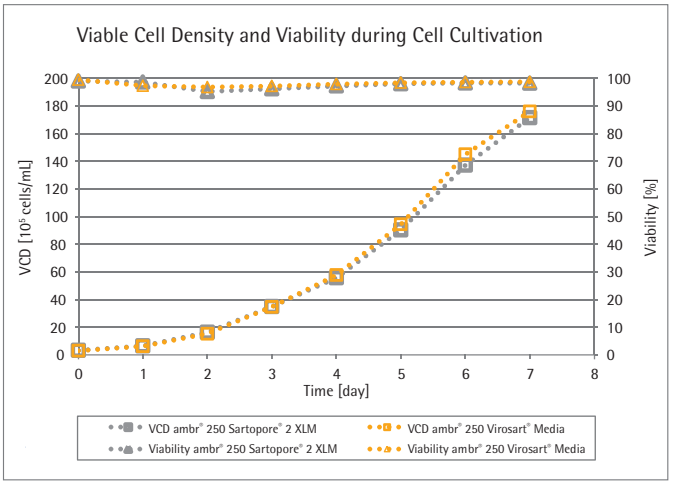

Fig. 8: Viable cell density and viability during CHO fed-batch cultivation in an ambr® 250 modular bioreactor system using media filtered with a Virosart® Media and a standard Sartopore® 2 XLM for comparison

The glucose and lactate profiles is shown in figure 9 are comparable irrespective of the filters used. The lactate profile peaks at a value of 1.2 g/L on day 3 while the glucose peaks at a value of 6.1 – 6.2 g/L on day 5 and 7.

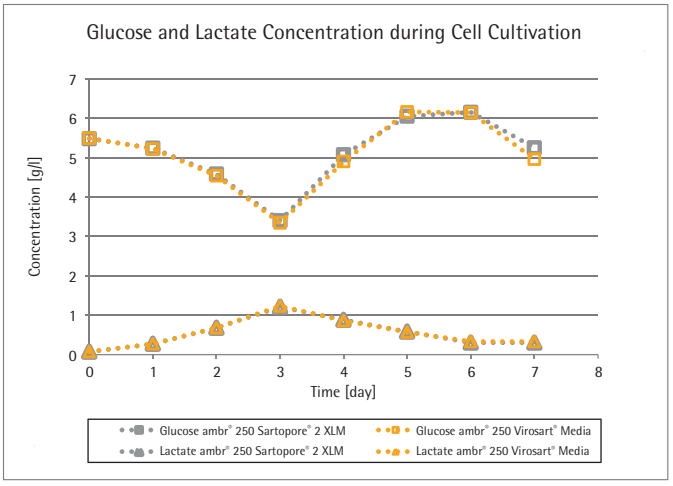

Fig. 9: Glucose and lactate concentration during CHO fed-batch cultivation in an ambr® 250 modular bioreactor system using media filtered with a Virosart® Media and a standard Sartopore® 2 XLM for comparison

III. Summary and Conclusion

The data demonstrates that the Virosart® Media is the filter of choice for upstream applications where high capacities and process economics are desired. Overall, the filtration of the five different cell culture media with and without Sartopore® 2 XLM as pre-filter show a high level of reproducibility of capacity. Over a filtration time of 240 min, capacities of 500 to 2000 L/m^2 were observed depending on the cell culture media being filtered.

Viable cell density, viability and metabolic profiles are comparable between the CHO fed-batch process in the ambr® 250 modular system with filtered media from Sartopore® 2 XLM and Virosart® Media. There was no difference in the performance of media filtered through a Sartopore® 2 XLM or a Virosart® Media.

Our studies demonstrate no impact on cell growth and cell metabolism in the system studied. We recommend, however, users perform cell growth evaluations under their specific conditions.

IV. References

[1] A. Kerr and R. Nims, Adventitious viruses detected in biopharmaceutical biopharmaceutical bulk harvest samples over a 10 Year Period. PDA J Pharm Sci Technol, 2010. 64(5): p. 481-5.

[2] A. Manzke and B. Kleindienst, Virus Risk Mitigation in Cell Culture Media, BioPharm International, October 2016

[3] Application note: Retention Characteristics of Virosart® Media when filtering Chemically Defined Cell Culture Media, Publication No.: SPK4116-e, Order No.: 85037-560-92

[4] Application note: Evaluation the Filterability of Chemically Defined Cell Culture Media with the Virosart® Media Filter, Publication No.: SPK4115-e, Order No.: 85037-560-91

[5] Application note: Influence of Cell Culture Media Components on the Filtration Characteristics of Virosart® Media, Publication No.: SPK4118-e, Order No.: 85037-560-94

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