New Solutions for Virus Risk Mitigation in Cell Culture Media

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1. Introduction

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

The consequences of such a contamination event may be fatal for the patient, therefore, several large biopharmaceutical operations are evaluating risk mitigation strategies for the minimization of contaminations by adventitious agents. Classic sterilizing-grade filters (0.2 µm) and even 0.1 µm-rated filter membranes cannot prevent contaminations by small non-enveloped viruses.

In the past researchers have investigated technologies such as gamma irradiation or high temperature-short times (HTST). Size exclusion-based filtration is the preferred technology for viral clearance, as it is robust and non-invasive. In addition, virus retentive filters used in downstream processing have their bottlenecks when it comes to filtration of cell culture media due to the low flow rates. To increase the overall speed of filtration, larger membrane areas are necessary, but this approach is expensive and makes the scenario of DSP virus retentive filters used in USP unrealistic economically.

The newly developed Virosart® Media membrane mitigates virus contamination risks prior to the addition of nutrients and other additives into the bioreactor system and has been developed for chemically defined cell culture media.

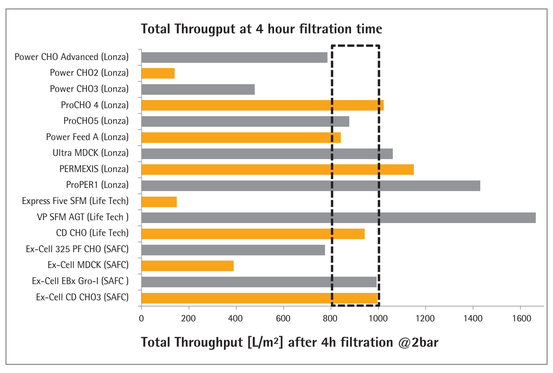
Technology	Robustness of Virus Retention	Scalability	Media Integrity	Flow rates	Large Volume	Process economics
Gamma irradiation γ	±	±	+	+		±
HTST 	±	-	±	+		±
DSP Virus filtration 	++	++	++	-		-

2. Filtration

Filtration throughput
The newly developed 20 nm Virosart® Media exhibits high total throughput of approximately 800 – 1000 L/m² within 4 hours filtration time for commercially available cell culture media. This makes it an economically feasible method for the batch preparation of media while reducing the risk of viral contamination.

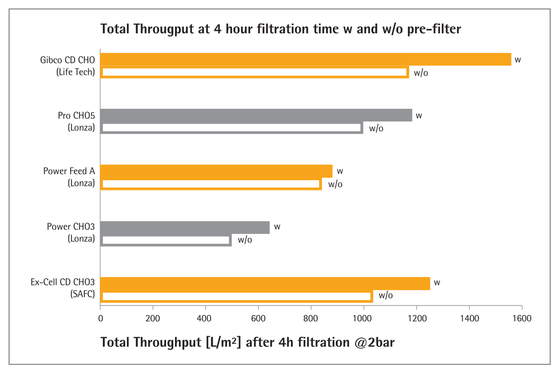
The data shows that volumes of media processed varied widely depending on the media. Some media tend to block the filter relatively quickly whereas other media did not appear to block the filter at all. Media supplements such as Pluronics® or soy hydrolysates are known to have an impact on the filtration performance and can reduce the flux rate drastically.

The throughput of Virosart® Media membrane was determined using 15 different media from three different suppliers. All runs were performed at constant pressure of 2.0 bar. The total throughput was measured after 4 hours of filtration.



Impact of 0.1 µm pre-filtration
The use of a 0.1 µm inline pre-filter is recommended. The total throughput is significantly increased by ≥ 20% for most of the tested media (4 of 5) by the use of an 0.1 µm inline pre-filter. The improvement is based on size-exclusion.

The filtration run was performed with 5 different cell culture media from 3 suppliers with and without the use of Sartopore® 2 XLM as an inline pre-filter. Sartopore® 2 XLM is a 0.2|0.1 µm pleated polyethersulfone filter. All runs were performed at constant pressure of 2.0 bar. The total throughput was measured after 4 hours of filtration. The pre-filter final filter ratio in this trial was 1:1 and further studies will be performed in order to optimize the pre-filter:final filter ratio.



3. Retention

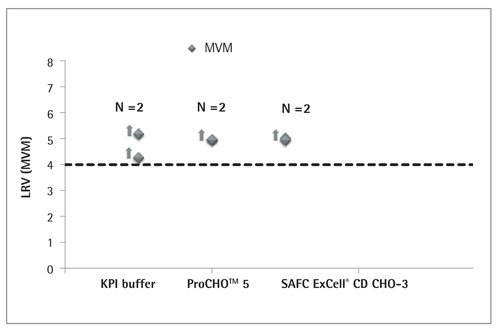
MVM Retention
Retention of small non-enveloped viruses e.g. MVM exceeds 4 LRV. MVM is a parvovirus of 18 – 26 nm diameter. Duplicate runs were performed at 2.0 bar constant pressure with 5 cm² lab modules for 3 different medias. A volume of 400 L/m² was filtered at a1% spike ratio. The analytical method ($TCID_{50}$) could not detect MVM in the filtrate.

Correlation of MVM & PP7
The correlation of MVM and PP7 retention for the newly developed virus filter membrane is confirmed. PP7 is a bacteriophage that is commonly used as a model virus for 20 nm virus retentive filters. The runs were performed at constant pressure of 2.0 bar with 1cm² R&D lab devices in 20 mM KPI buffer, pH 7.2. No flux decay was observed during 250 L/m² of PP7 filtration. Afterwards 300 L/m² with MVM was filtered over the same modules.

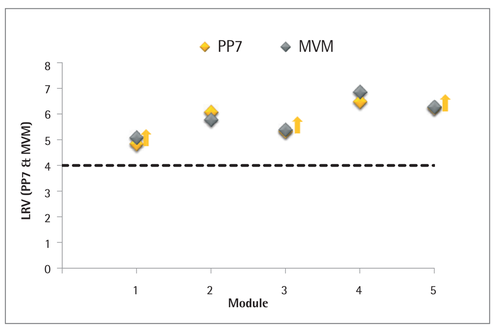
PP7 Retention in different CCM
PP7 retention is larger than 4 LRV for a set of commercial cell culture media. The filtration was performed in duplicate runs at 2 bar constant pressure over 4 hour filtration time with Virosart® Media lab modules. The test is representative for 4 hour filtration batch of 1000 L with 1 m² process modules.

Scalability of Retention
Scalability of LRV is given for the whole product family of Virosart® Media from lab (5 cm²) to mid-scale (0.3 m²) up to process scale (1 m²). 15 devices of each module sizes were tested on their PP7 retention capability in 20 mM KPI buffer, pH 7.2 at 2 bar constant pressure.

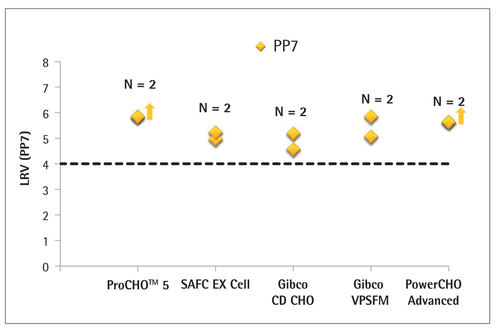
MVM retention in a set of cell culture media



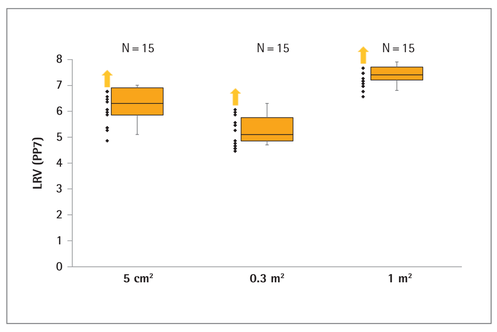
Correlation of MVM an PP7 bacteriophage clearance for Virosart® Media



PP7 retention in a set of commercial cell culture media



Scalability of PP7 retention

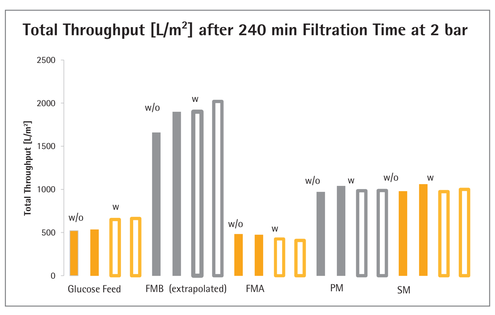


4. Cell Growth Study

Two fed-batch cultivation runs were performed to compare cell growth characteristics of chemically defined media. One cultivation used media which has been filtered through Virosart® Media, the reference cultivation was using media filtered with Sartopore® 2 XLM as a standard 0.1 µm filter.

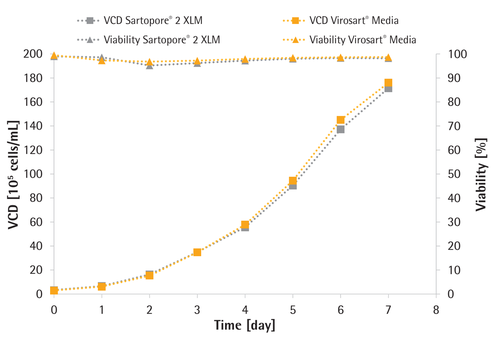
Filtration of Cellca Media
Before cultivation, a filtration study was performed in duplicate runs to compare the filterability characteristics of five different media used during the cell culture. Filtration with the Virosart® Media was performed with (w) and without (w/o) pre-filtration with a Sartopore® 2 XLM with high level of reproducibility of throughput of 500 to 2000 L/m².

Media	Description	Day Media added	Media components
Stock culture media (SM)	Basal media used for batch phase of main culture	1	NAO, protein & peptide free, Pluronic®
Production media (PM)	Basal media used for batch phase of main culture	1	NAO, protein & peptide free, Pluronic®
Feed media A (FMA)	Bolus feed used for fed-batch phase	3 – 11	NAO, protein & peptide free, Pluronic®, glucose, vitamins
Feed media B (FMB)	Bolus feed used for fed-batch phase	3 – 11	NAO, protein & peptide free, amino acids
Glucose Feed 400 g/L	Feed media used for fed-batch phase	3 – 11	NAO, protein & peptide free

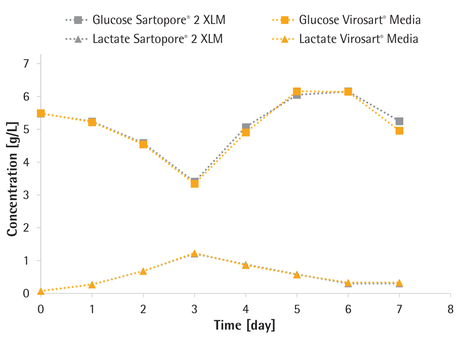


Fed-batch Culture of CHO Cells
Viable cell density (VCD), viability and metabolic profiles are comparable between the CHO fed-batch process (Cellca 2) in the ambr® 250 modular system. The 12-day main culture comprises of a 3-day batch phase and a 9-day fed-batch phase. Since the main influence of media alteration is visible in the growth phase of the process, the focus of this study was the first 7 days of the process. Both cultures reached a VCD of approx. 17 × 10^6 cells/mL after 7 days. Viability was in a range of 95 – 99 %. The glucose and lactate profiles 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. Overall, there is no influence on growth media quality and therefore no impact on either cell metabolism or therapeutic protein production of media filtered through Sartopore® 2 XLM or Virosart® Media.

Viable Cell Density and Viability during Cell Cultivation

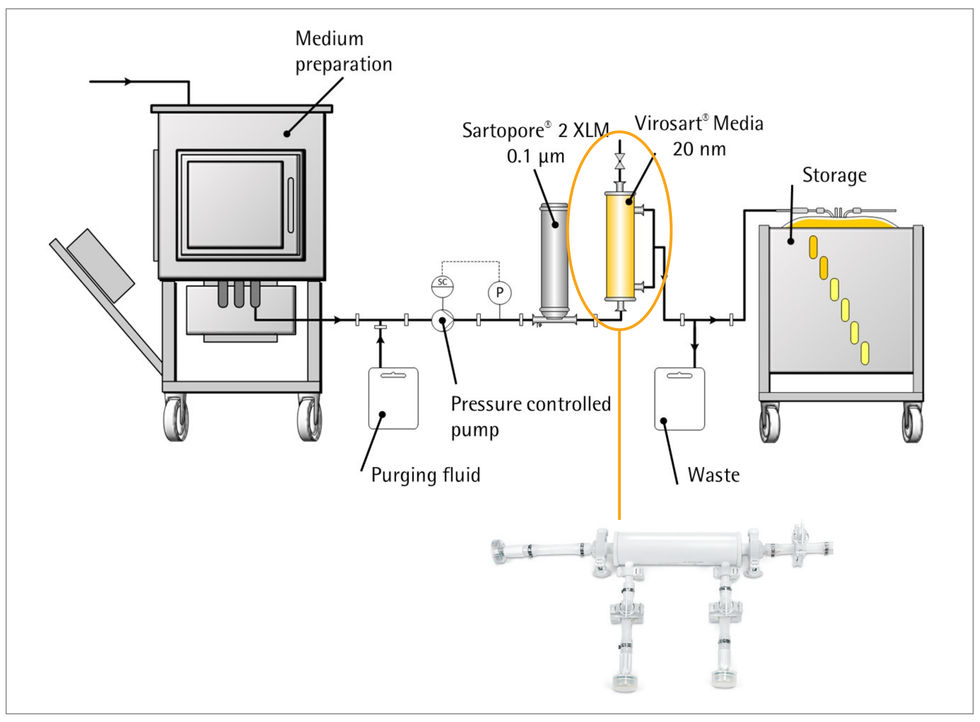


Glucose and Lactate Concentration during Cell Cultivation



5. Process Integration

Virosart® Media is specially developed for the filtration of chemically defined cell culture media for scales up to 5000 L. The filter comes gamma sterilized and will also be available in a gamma sterilized filter transfer set. Below the implementation of Virosart® Media for a ≤ 2000 L single-use process is shown. High operating pressure up to 3 bar is possible due to the reinforced pressure stable tubes as well as the metal clamps.



6. Summary

- Virus filtration is the technique of choice for risk mitigation in cell culture media.
- Superior filtration capacity can be reached with approx. 1000 L/m² in 4 hours filtration time at 2 bar for chemical defined cell culture media.
- Highest virus safety is given with ≥ 4 LRV for small-non enveloped viruses and ≥ 6 LRV for large enveloped viruses.
- No impact on cell growth and cell metabolism.
- The high packaging density of the filter elements combines extremely low hold-up volume with low footprint requirements.
- Easy implementation into single-use processes is given by gamma irradiatable capsule design.