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Retention Characteristics of Virosart® Media When Filtering Chemically Defined Cell Culture Media

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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. Therefore, several 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 contamination 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.

Find out more: www.sartorius.com/virosart-media

Introduction

The Virosart® Media filter has been developed specifically for chemically defined cell culture media. The filter is an asymmetric polyethersulfone hollow fiber membrane with 20 nm nominal pore size rating that exhibits high capacity (1000 L/m² 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].

Virus validation studies are performed to show the effective removal of viruses including those that are known to contaminate processes. In these spiking studies the virus is added ("spiked") into the chemically defined media to evaluate the effective removal of viruses using a down-scale model of the production process[4-6]. The following equation is used to calculate the viral reduction and generate a log_{10} reduction value (LRV):

$$LRV = \log_{10} \frac{V_1 \cdot T_1}{V_2 \cdot T_2}$$

With:
V_1: Volume of starting material
T_1: Concentration of virus in the starting material
V_2: Volume of the material after the step
T_2: Concentration of virus after the step

This document reports on the retention characteristics of Virosart® Media when filtering chemically defined cell culture media. The following four studies were performed:

Study 1: MVM Retention

Study 2: Proof of Correlation of MVM and PP7 retention

Study 3: PP7 Retention in Different Cell Culture Media (CCM)

Study 4: Scalability of Retention

Study 1: MVM Retention

A number of companies have reported lost production lots due to contamination by small non-enveloped viruses such as MVM (Minute virus of mice) or vesivirus[1]. In this study the ability of the Virosart® Media filter to retain small non-enveloped viruses was tested. MVM as a relevant virus was used for the study. It is a single stranded, 18 – 26 nm, non-enveloped virus from the parvovirus family and is used as a worst-case, small non-enveloped virus model.

Materials and Methods
Spiking Studies were performed with representative scale-down models (figure 1). For this study Virosart® Media lab modules (5 cm², part number: 3V2--28-BVGML--V) from one production lot were used. Duplicate runs were performed with two different cell culture media EX-CELL® CD CHO-3* from Merck and ProCHO™5 from Sartorius at constant pressure of 2.0 bar | 30 psi. EX-CELL® CD CHO-3 is a chemically defined (non-animal origin, protein and peptide free) cell culture media containing polaxamer[7]. Surface active ingredients such as polaxamers, can have an impact on retention. Therefore, also ProCHO™5 from Sartorius containing 0.1% polaxamers was chosen to test the impact of polaxamers on retention. Finally, retention capabilities were determined using 20 mM KPI (potassium phosphate) buffer at pH 7.2. A buffer has been chosen as a worst-case set-up to exclude the possibility of interactions between media components, virus and the membrane.

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 then replaced by the cell culture media. 200 mL (400 L/m²) of the cell culture media was spiked with MVM at a 1% spike ratio. The spiked media was filtered at room temperature (20 – 22°C) at constant pressure of 2.0 bar | 30 psi. The filtration was stopped when the total volume of 200 mL was processed through the filter. Fractions of the filtrate were taken at 360 min for EX-CELL® CD CHO-3, KPI buffer and after 330 min for ProCHO™5. A hold control sample was taken and stored next to the process until the end of the virus filtration to determine the virus titer (hold) using the cell-line based $TCID_{50}$ assay.

Prior to the spiking study, a cytotoxicity and a virus interference assay was performed which showed no influence of media on cell growth and virus replication.

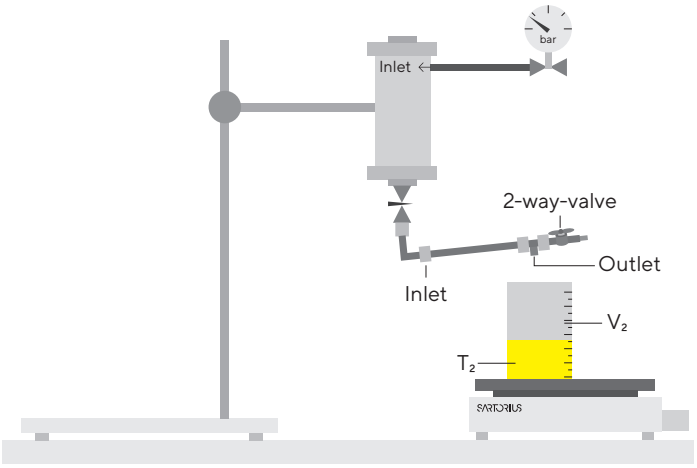

Figure 1: Experimental set-up for spiking study using Virosart® Media lab module 5 cm²

Results and Discussion

Figure 2 shows MVM retention spiked in three cell culture media. The Virosart® Media removed MVM effectively to the limit of detection ($TCID_{50}$). A reduction of ≥ 4.22 in KPI buffer, ≥ 4.98 EX-CELL® CD CHO-3 and ≥ 4.98 ProCHO™5 was demonstrated in the filtrate fraction. Further details are shown in table 1.

The data indicate that surface active ingredients such as polaxamers have no impact on the retention characteristics of Virosart® Media as absolute retention was shown for both the EX-CELL® CD CHO-3 and for ProCHO™5 media both containing polaxamer. In addition, absolute retention is shown for Virosart® Media under the worst-case conditions of buffer filtration.

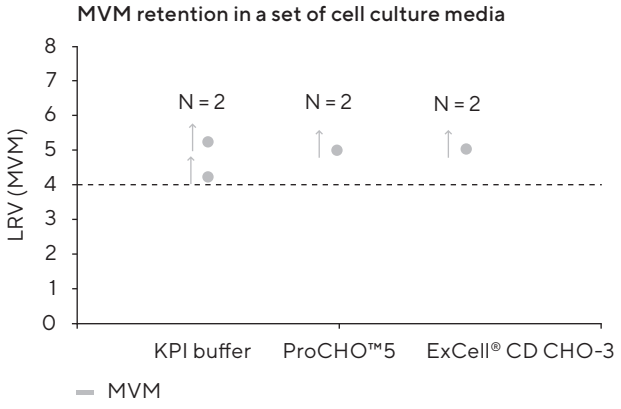

Figure 2: Pool of MVM retention in a set of cell culture media with absolute retention indicated by the arrow

Media	LRV – Run 1	LRV – Run 2
KPI buffer	≥ 5.22	≥ 4.22
ProCHO™5	≥ 4.98	≥ 4.98
EX-CELL® CD CHO-3	≥ 5.04	≥ 4.98

Table 1: MVM retention in a set of cell culture media

Study 2: Proof of Correlation of MVM and PP7

PP7 bacteriophage is an established model system that is often used to evaluate the removal capabilities of virus retentive filters. The FDA | PDA virus filter task force published a technical report (No. 41) that outlines the use of bacteriophage PP7 to characterize the retention characteristics of 20 nm virus retentive filters[8].

Materials and Methods

In this study, the correlation of MVM and PP7 retention for the upstream developed virus filter membrane was evaluated using representative scale-down models of the Virosart® Media (R&D devices of 1.0 cm^2) from a single membrane production lot. To investigate the correlation between MVM and PP7 retention, it is important that the membrane is in the same condition for both experiments, which are performed sequentially. For this reason, buffer was chosen instead of media not to cause any flux decay on the Virosart® Media.

PP7: *Pseudomonas aeruginosa* bacteriophage PP7 (ATCC 15692-B2) is a single stranded, 20–25 nm, non-enveloped, ssRNA bacteriophage from the Leviviridae family. Filtration was performed in constant pressure mode of 2.0 bar | 30 psi with 5 lab modules using PP7 spiked into 20 mM KPI Buffer pH 7.2. In total 25 mL were filtered with PP7 spiked at > 2.0×10^8 PFU/mL (Plaque Forming Units). No flux decay was observed. PP7 was harvested from suspensions of infected *Pseudomonas aeruginosa* by low speed centrifugation. The supernatants were pooled, filtered through a 0.2 µm filter, aliquoted and stored frozen until further use. Samples were incubated with bacteria to determine the bacteriophage PP7 titer. Subsequently, agar was added and the entire volume was spread on a petri dish. After an incubation period the plaques induced by bacteriophages were counted. All experimental samples were assayed for bacteriophage at three different dilutions. After an incubation period the number of plaques on a confluent layer of *Pseudomonas aeruginosa* were counted. Two dilutions with countable numbers of plaques were used for the calculation of the PP7 titer. Before each run, the filters were flushed for 15 minutes with deionized water at 2.0 bar | 30 psi using compressed air. *Pseudomonas aeruginosa* was grown as overnight culture. Before the shipment to the spiking house, the modules were inactivated using 0.1 M HCl.

MVM: MVM a single stranded, 18–26 nm, non-enveloped virus from the parvovirus family has been used. The MVM study was performed using the same lab modules under the same experimental set-up and parameters as the PP7 study. 30 mL of 20 mM KPI buffer pH 7.2 were spiked at a 0.5% spike ratio. The filtrate was collected in a single fraction and analyzed for residual virus infectivity by the $TCID_{50}$ assay. Due to the aqueous composition of the test material, interference test with virus detection was not performed.

Results and Discussion

Figure 3 shows the correlation of MVM and PP7 bacteriophage clearance for Virosart® Media for 1 cm^2 lab modules in 20 mM KPI buffer pH 7.2. No flux decay was observed during the filtration of the PP7 solution to a total throughput of 250 L/m^2. Following the PP7 filtration, the MVM solution was filtered to a total throughput of 300 L/m^2 using the same modules. Virus clearance of at least 5 LRV were demonstrated for both MVM and PP7. Further details are shown in the table 2. In addition, the results confirm a correlation of MVM and PP7 retention for the newly developed virus filter membrane for virus risk mitigation upstream.

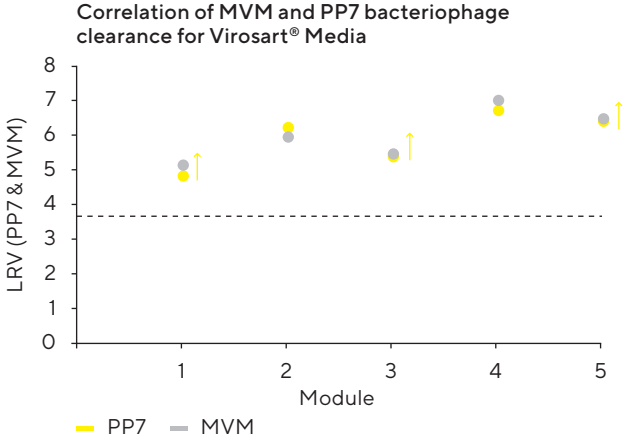

Figure 3: Correlation of MVM and PP7 bacteriophage clearance for Virosart® Media in KPI buffer. Absolute retention is indicated by the arrow.

Media	Module	LRV – PP7	LRV – MVM
KPI buffer	1	≥ 5.0	5.30
	2	6.5	6.15
	3	≥ 5.6	6.67
	4	7.0	7.45
	5	≥ 6.7	6.75

Table 2: MVM and PP7 bacteriophage clearance for Virosart® Media in KPI buffer

Study 3: PP7 Retention in Different CCM

Study 3 consisted of 3 different sub-studies:

- Study 3a: PP7 retention in a set of commercially available CCM
- Study 3b: Intra-membrane lot PP7 retention consistency
- Study 3c: Inter-membrane lot PP7 retention consistency

Materials and Methods

Pseudomonas aeruginosa bacteriophage PP7 (ATCC 15692-B2) was used as a well-established model organism to evaluate the virus removal capabilities of virus retentive filters[4-6]. In all three studies, the filtration was performed in constant pressure mode at 2.0 bar | 30 psi using PP7 spiked cell culture media. 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 was recorded. Cultivation, harvest and determination of the PP7 titer was performed as described in study 2.

Study 3a: PP7 Retention in a Set of Commercial Available CCM

Virosart® Media lab modules (5 cm^2, part number: 3V2--28-BVGML--V) from one production lot were tested for their ability to retain PP7 in five different cell culture media from three suppliers. Surface active ingredients such as poloxamers, can have an impact on retention. Therefore, ProCHO™ 5, ExCell® CD CHO-3 and Gibco™ CD CHO* were selected for this study to test the impact of surface active ingredients on the retention performance of Virosart® Media (table 4). All filtration runs were performed in duplicate at a constant pressure of 2.0 bar | 30 psi over 4 hours of filtration time. The filtration runs were performed for 4 hours and reached a capacity of 1000 L/m^2.

Results and Discussion

Figure 4 shows PP7 retention at 2.0 bar | 30 psi constant pressure in a set of commercial cell culture media over a 4 hour filtration time. PP7 retention exceeded 4 LRV for all 5 tested cell culture media. The data indicate that surface-active ingredients such as poloxamers have no impact on the retention characteristics of Virosart® Media.

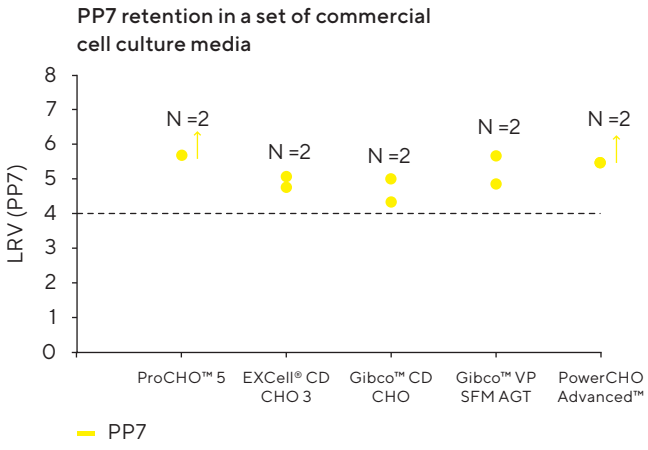

Figure 4: PP7 retention in a set of commercial cell culture media. Absolute retention is indicated by the arrow

Media	LRV – Run 1	LRV – Run 2
ProCHO™ 5	≥ 5.7	≥ 5.7
ExCell® CD CHO-3	4.8	5.1
Gibco™ CD CHO	5.0	4.4
Gibco™ VP SFM AGT™	5.7	4.9
PowerCHO Advanced™	≥ 5.5	≥ 5.5

Table 3: PP7 retention in a set of commercial cell culture media

Media	Pro-CHO™ 5	ExCell® CD CHO-3	Gibco™ CD CHO	Gibco™ VP SFM AGT™	Power CHO Advanced™
Supplier	Sartorius	Merck	Life Tech	Life Tech	Sartorius
Cat.No	WPW-045D	C1490-1L	10743029	12559027	12-929Q
NAO*	Yes	Yes	Yes	Yes	Yes
Protein free	Yes	N/A	Yes	No	Yes
Peptide free	No	No	Yes	Yes	N/A
CD**	No	Yes	Yes	N/A	Yes
L-gluta-mine	No	No	No	No	N/A
Polox-amer	0.1%	0.2%	Yes	0%	N/A

Table 4: Set of cell culture media used for PP7 retention studies[7-9]
* NAO: Non Animal Origin ** CD: Chemically defined

Study 3b: Intra-Membrane Lot PP7 Retention Consistency

The intra-membrane lot consistency for Virosart® Media lab modules (5 cm², part number: 3V2--28-BVGML--V) was tested from one production lot using two different chemically defined cell culture media ProCHO™ 5 and ExCell® CD CHO-3. 12 to 15 lab modules were used for each cell culture media. The filtration was performed in constant pressure mode of 2.0 bar | 30 psi. In total, two fractions were taken: First fraction was taken at 0–3 hours and second fraction taken at 3–4 hours. The study is representative of a four-hour filtration batch of 1000 L cell culture media with 1 m² process modules.

Results and Discussion

Figure 5 and 6 prove intra-membrane lot PP7 retention consistency for both ProCHO™ 5 and ExCell® CD CHO-3 cell culture media. PP7 retention exceeds an LRV of 4 over the 4 hour filtration time for all tested modules in both cell culture media.

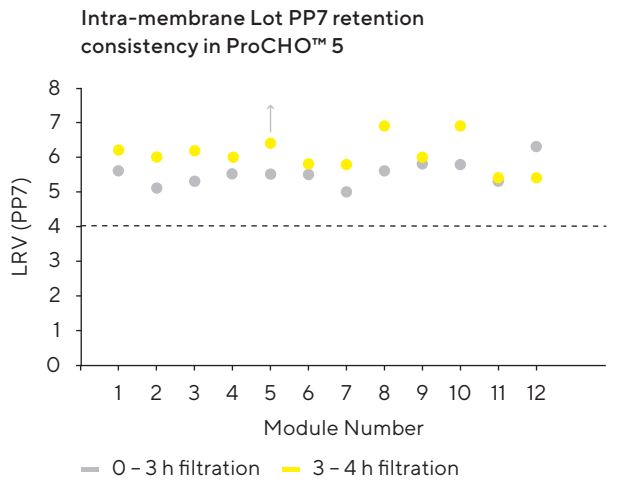

Figure 5: Intra-membrane lot PP7 retention consistency in ProCHO™ 5 cell culture media

Fraction	LRV 0–3 h	LRV 3–4 h
1	5.6	6.2
2	5.1	6.0
3	5.3	6.2
4	5.5	6.0
5	5.5	≥ 6.4
6	5.5	5.8
7	5.0	5.8
8	5.6	6.9
9	5.8	6.0
10	5.8	6.9
11	5.3	5.4
12	6.3	5.4

Table 5: Intra-membrane lot PP7 retention consistency in ProCHO™ 5 cell culture media

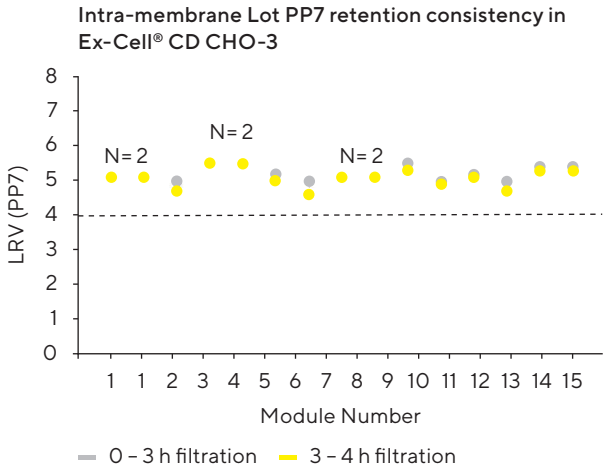

Figure 6: Intra-membrane lot PP7 retention consistency in ExCell® CD CHO-3 cell culture media

Fraction	LRV 0–3 h	LRV 3–4 h
1	5.1	5.1
2	5.1	5.1
3	5.0	4.7
4	5.5	5.5
5	5.5	5.5
6	5.2	5.0
7	5.0	4.6
8	5.1	5.1
9	5.1	5.1
10	5.5	5.3
11	5.0	4.9
12	5.2	5.1
13	5.0	4.7
14	5.4	5.3
15	5.4	5.3

Table 6: Intra-membrane lot PP7 retention consistency in ExCell® CD CHO-3 cell culture media

Study 3c: Inter-Membrane Lot PP7 Retention Consistency

The lot-to-lot membrane consistency of Virosart® Media lab modules (5 cm², part number: 3V2--28-BVGML--V) was evaluated. Three different membrane lots (5 modules each) were tested for their ability to retain PP7 spiked into ProCHO™ 5 cell culture media. The filtration was performed in constant pressure mode at 2.0 bar | 30 psi. Two fractions were taken, the first after 1 hour and the second after 2 hours.

Results and Discussion

Figure 7 provides PP7 retention data from three different lots of Virosart® Media modules (5 modules each) at 2.0 bar | 30 psi in ProCHO™ 5 cell culture media. Robust inter-lot PP7 retention consistency has been demonstrated and PP7 LRVs exceed 5.

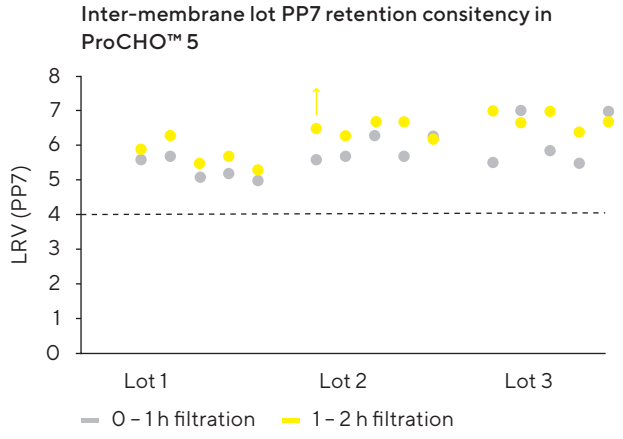

Figure 7: Inter-lot PP7 retention consistency in ProCHO™ 5 cell culture media. Absolute retention is indicated by the arrow.

Lot	Fraction	LRV 1	LRV 2	LRV 3	LRV 4	LRV 5
Lot 1	0 – 1 h	5.6	5.7	5.1	5.2	5.0
	1 – 2 h	5.9	6.3	5.5	5.7	5.3
Lot 2	0 – 1 h	5.6	5.7	6.3	5.7	6.3
	1 – 2 h	≥ 6.5	6.3	6.7	6.7	6.2
Lot 3	0 – 1 h	5.5	7.0	5.9	5.5	7.0
	1 – 2 h	7.0	6.7	7.0	6.4	6.7

Table 7: Inter-lot PP7 retention consistency in ProCHO™ 5 cell culture media

Study 4: Scalability of Retention

Virosart® Media lab modules are typically used for R&D and scale-down work. Virus validation studies must also be performed on a representative down-scale model.[4-6] It is therefore necessary to ensure robust and scalable retention across the entire range of modules including at the large-scale modules.

Materials and Methods

The scalability of PP7 retention was studied for the whole product family of Virosart® Media from lab (5 cm²) to mid-scale (0.3 m²) up to process scale (1 m²). *Pseudomonas aeruginosa* bacteriophage PP7 (ATCC 15692-B2) is the ideal organism for the study because it is an established model system to evaluate the removal capabilities of virus retentive filters. In addition, it can be grown to high quantities that are necessary especially for the larger filtration areas.

The filtration processes were performed at a constant pressure of 2.0 bar | 30 psi. PP7 was spiked into 20 mM KPI buffer pH 7.2. All devices had been gamma irradiated prior to use. 15 modules of each filter size were tested from 3 different production lots. For the lab modules, 25 mL KPI buffer was filtered with PP7 spiked at > 1.0×10^{10} PFU/mL. 10 L of KPI buffer spiked at > 3.0×10^{8} PFU/mL was filtered through the mid-scale and process scale devices. Cultivation, harvest and determination of the PP7 titer was performed as described in study 2.

Results and Discussion

Figure 8 shows the PP7 retention capability of 15 devices of each module size. The scalability of PP7 retention has been demonstrated across the whole product family of Virosart® Media filters from lab (5 cm²) to mid-scale (0.3 m²) up to process scale (1 m²).

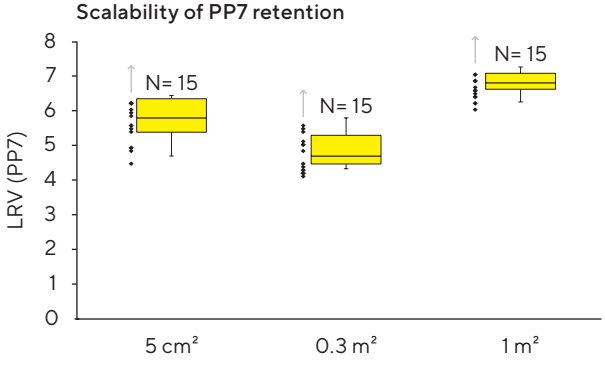

Figure 8: Scalability of PP7 retention over the whole product family of Virosart® Media. Absolute retention is indicated by the arrow.

Summary and Conclusion

The results presented demonstrate that the Virosart® Media is the filter of choice for upstream applications where high capacities and process economics are required. Retention of small non-enveloped viruses e.g. MVM exceeded 4 LRV for all three cell culture media tested. The analytical method ($TCID_{50}$) could not detect MVM in any filtrate. In addition, a correlation between MVM and PP7 retention was confirmed for the newly developed virus filter membrane. PP7 retention exceeded an LRV of 4 when spiked into a set of commercial available cell culture media. Robust intra-membrane lot and inter-membrane lot PP7 retention consistency has been shown for the cell culture media tested. Additionally the scalability of retention was confirmed across the whole product family of Virosart® Media. Although the Virosart® Media membrane showed reliable retention, we recommend users to perform virus retention studies with their media and under their specific process conditions.

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(9) Link: https://www.thermofisher.com

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