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Evaluating the Robustness of Virus Clearance Under Challenging Filtration Conditions Using the Virosart® Media Filter

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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 contamination 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 viral 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 a LRV (log_{10} reduction value) of ≥ 4 log_{10} for small non-enveloped viruses and ≥ 6 log_{10} for large enveloped viruses[3,4].

Investigations have shown that some virus removal membranes used in downstream applications showed elevated small virus breakthrough under certain process conditions. In this study the impact of four different challenging process conditions on the Virosart® Media filter were tested:

Study 1: Impact of flow decay
Study 2: Impact of pressure release
Study 3: Impact of high and low operating pressure
Study 4: Impact of pressure pulsation

Study 1: Impact of Flow Decay

There are different criteria for ending a virus filtration that depend on the individual process validation and on process control. Reaching a validated flow decay limit is one criteria for terminating most downstream virus retentive filtration steps. In this study, the impact of flow decay was investigated on the virus retentive filter used in upstream applications. The filtration media was chosen to induce filter fouling accordingly.

Materials and Methods

Virosart® Media filter modules with an effective filtration area of 1.0 cm² (R&D devices) were tested for their PP7 retention capabilities (challenge level 1×10^8 pfu/mL) at increasing level of flow decay. Three fractions of filtrate were taken at approximately 15 mL, 116 mL (representing 75% flow decay) and after 140 mL of filtration (representing 80% flow decay). The filtration was performed in duplicate with Ex-Cell® CD CHO 325 cell culture media and at a constant pressure of 5.0 bar | 72.5 psi.

Results and Discussion

The LRV of PP7 was 5.6 log_{10} or greater irrespective of the extent of flow decay. Virus retention was independent of the level of blocking and was unaffected when the flow decay reached 75% and 80% of the original flow rate.

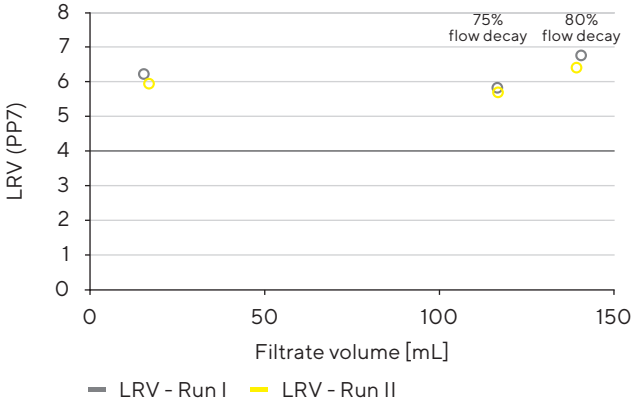

Figure 1: *LRV of PP7 Versus Filtrate Volume*

Note. The filtration media was chosen to induce filter fouling, separate filtrate fractions were collected at the beginning of the filtration and at 75% flow decay and 80% flow decay relative to the PP7 retention for Virosart® Media filter tested at 75 and 80% flow decay with Ex-Cell ® CD CHO 325 cell culture media.

Fraction	Filtration volume [mL]	Flow decay [%]	LRV – Run I	LRV – Run II
1	15	0	6.1	5.9
2	115	75	5.8	5.6
3	140	80	6.7	6.3

Table 1: *PP7 Retention at Different % Of Filter Fouling*

Study 2: Impact of Pressure Releases

During processing with virus retentive filters, the pressure across the membrane can be released regularly such as if the processing media is changed or in the event of a fire alarm. Studies have shown that virus breakthrough is more likely to occur when the pressure is released. The impact of pressure release on the virus retentive characteristics of Virosart® Media filter, therefore, has been investigated.

Material and Methods

Four lab modules from one production lot with nominal filtration areas of 1 cm² (R & D device) were tested for their PP7 retention capabilities. The filters were challenged at > 2.9×10^7 pfu/mL with Ex-Cell® CD CHO 325 cell culture media during which the pressure was released for five minutes. Fractions were taken after 30 minutes, 1 hour, 2 hours, 3 hours and 4 hours of filtration.

Results and Discussion

LRVs of about 5.0 $\log_{10}$ or greater were demonstrated in all fractions even after a mid-filtration pressure release of 5 minutes.

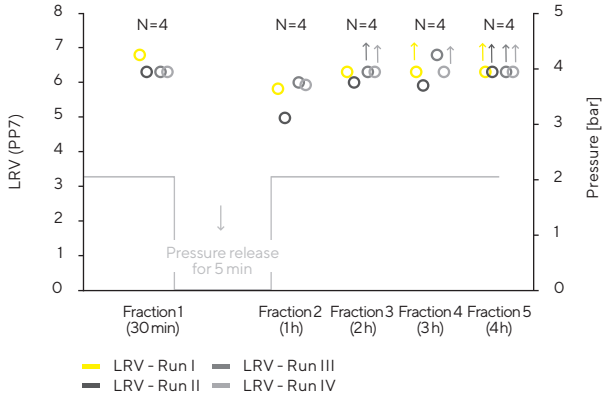

Figure 2: *PP7 LRV Retention for Virosart® Media Filter After Pressure Release of 5 Min With Absolute Retention Indicated by the Arrow*

Fraction	Filtration time	LRV – Run I	LRV – Run II	LRV – Run III	LRV – Run IV
1	30 min	6.6	6.2	6.2	6.2
5 min pressure release					
2	1 h	5.7	4.9	5.9	5.8
3	2 h	≥ 6.2	5.9	≥ 6.2	≥ 6.2
4	3 h	≥ 6.2	5.8	6.6	≥ 6.2
5	4 h	≥ 6.2	≥ 6.2	≥ 6.2	≥ 6.2

Table 2: *PP7 Retention During Pressure Release*

Study 3: Impact of High and Low Operating Pressure

Virosart® Media virus filters are validated at 2.0 bar | 30 psi operating pressure for PP7 retention to meet standard process requirements.[3] The filters may be used at pressures up to 5.0 bar | 72.5 psi operating pressure. In this study the effect of a low, normal and high operating pressure on PP7 retention was investigated.

Material and Methods

The runs have been performed in house at Sartorius Stedim Biotech bacteriophage lab to verify the retention capabilities at a low (0.1 bar | 1.5 psi), normal operating pressure (1.0 bar | 14.5 psi) and a high (5.0 bar | 72.5 psi) operating pressure. The PP7 retention capabilities of four lab modules from one production lot and with an effective filtration area of 1.0 cm^2 (R & D device) were tested at each operating pressure. The virus stock solution was suspended in Ex-Cell® CD CHO 325 cell culture media at a PP7 challenge level of 7.6 > 10^7 pfu/mL. A sample of filtrate was taken after 5 mL (0.1 bar | 1.5 psi) and after 50 mL (1.0 bar | 14.5 psi and 5.0 bar | 72.5 psi) of filtration.

Results and Discussion

No impact of high and low operating pressures on the virus retention capabilities could be seen under the conditions tested. LRVs of approximately 5.5 log_{10} at low and high pressures has been demonstrated.

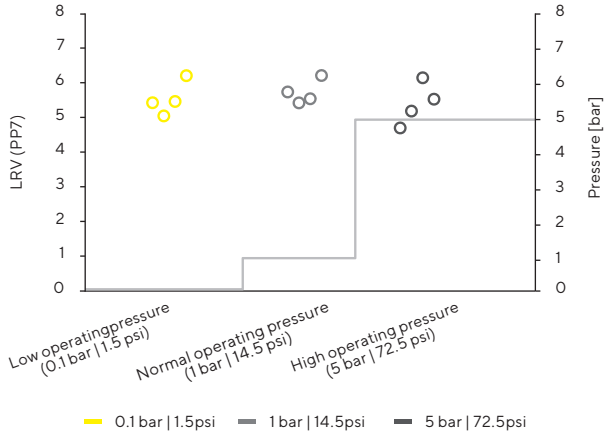

Figure 3: *No Impact of Different Operating Pressures on PP7 Retention*

Filtration	Filtration volume [mL]	LRV – Run I	LRV – Run II	LRV – Run III	LRV – Run IV
0.1 bar | 1.5 psi	5	5.5	5.1	5.5	6.3
1 bar | 14.5 psi	50	5.8	5.5	5.6	6.3
5 bar | 72.5 psi	50	4.8	5.2	6.2	5.6

Table 3: *PP7 Retention at High and Low Operating Pressure*

Study 4: Impact of Pressure Pulsation

Peristaltic pumps may be used to drive the filtration of cell culture media and they can cause pressure pulses. The degree of pressure pulses depends on the pump itself. There are low pulsation pumps available such as Quattroflow pump and pumps with more pulsations such as Watson Marlow pumps. The effect of these pressure pulses on the retention capability of Virosart® Media filter was studied.

Material and Methods

Two gamma-irradiated lab modules (5.0 cm^2) from a single production lot were tested for their PP7 retention capabilities (challenge level 5.3 × 10^7 pfu/mL) with Ex-Cell® CD CHO 325 cell culture media. A Watson Marlow 520 peristaltic pump was used with pulsation of 0.3 bar | 4.4 psi (analog manometer) at average operating pressure of 1.4 – 1.5 bar | 20.3 – 21.8 psi as a worst-case scenario. Fractions were taken after 15, 50, 100 and 150 mL of filtration.

Results and Discussion

No impact of pulsation on retention was observed. All LRVs were 5.3 log_{10} or higher.

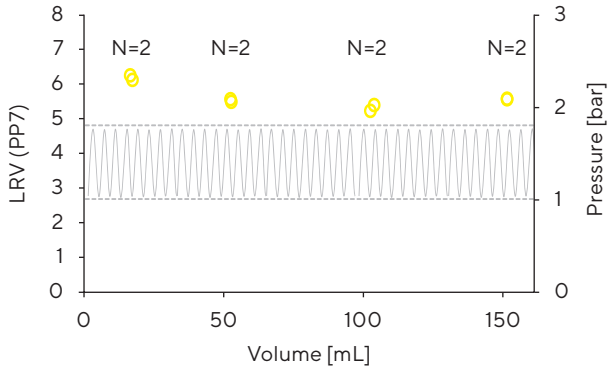

Figure 4: *No Effect of Pressure Pulsation on PP7 Retention With High LRVs of 5.3 Log_{10} and Higher Tested Under Worst Case Conditions With a Peristaltic Pump*

Fraction	Filtration volume [mL]	LRV – Run I	LRV – Run II
1	15	6.2	6.3
2	50	5.5	5.6
3	100	5.4	5.3
4	150	5.6	5.6

Table 4: *PP7 Retention During Pressure Pulsation With Peristaltic Pump*

Summary and Conclusion

The results presented demonstrate that the Virosart® Media filter is the filter of choice for upstream applications where high capacities and low processing costs are required. The filter delivers LRVs that exceed 4 log_{10}, even under challenging processing conditions such as where flow decay has occurred, when pressure is released, at high and low operating pressure and during pressure pulsations.

Although the Virosart® Media filter showed reliable retention even under challenging conditions, we recommend users to perform virus retention studies with their media and under their specific process conditions.

References

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