SARTORIUS

Application Note

December 2016

Keywords or phrases:
Virus, removal, clearance, pressure,
constantly high retention, stable performance

Impact of Pressure Release and Multiple Pressure Fluctuations on Virus Retention Performance of Virosart® HF Virus Retentive Filters

Sherri Dolan, Anika Manzke, Michael Lasse
Sartorius Stedim Biotech GmbH, August-Spindler-Str. 11, D-37079 Goettingen, Germany

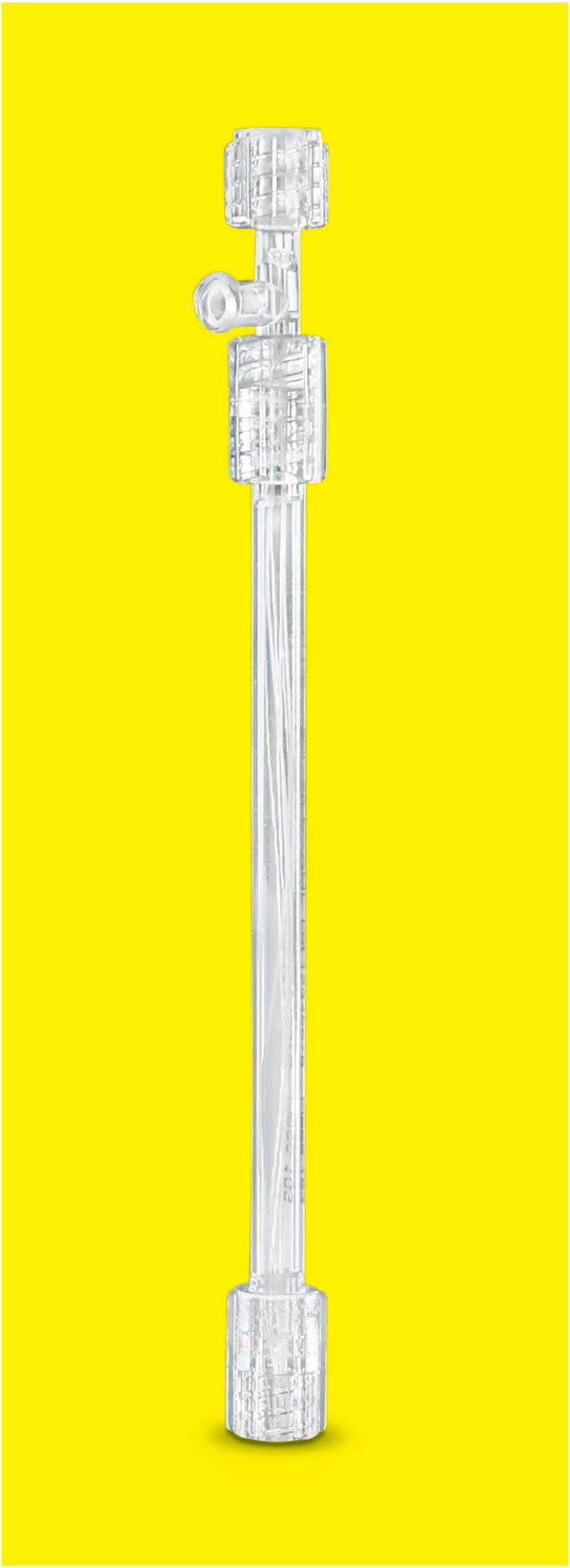
* Correspondence
Email: virusclearance@sartorius.com

Abstract

All biopharmaceutical products derived from human or animal origins must have a proven virus clearance concept to ensure the final patients' health before the product is released to commercial manufacturing or even clinical trials. The concept must cover known and unknown potential viral contaminants. Contaminating viruses can come from external sources or be present in cell lines. Endogenous retrovirus-like particles, for example, are present in most CHO cell lines. Regulators require that manufacturers perform a risk analysis and have a strategy to remove contaminating viruses during downstream processing.

The industry considers filtration to be a robust method of virus removal and hence it is a widely used method. Filtration relies on the principle of size-exclusion and can remove all types of viruses. Investigators have recently discovered that under specific conditions with some virus removal membranes, virus breakthrough can occur. Scientists believe that breakthrough is more likely to occur when the membrane is operated at high capacities, during flow-decay, under conditions of high or low process pressure or when the pressure is held and released. This study was conducted to determine the impact of pressure fluctuations on the retention of parvovirus using bacteriophage PP7 and the virus retentive filter Virosart® HF.

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



Introduction

Virus filtration with Virosart® virus retentive filters is an integral part of the orthogonal virus clearance technology platform of Sartorius Stedim Biotech. This orthogonal technology platform features virus clearance by filtration (size exclusion), inactivation and adsorption. The Virosart® product range includes four different virus retentive membranes, in order to provide the best solution for every application.

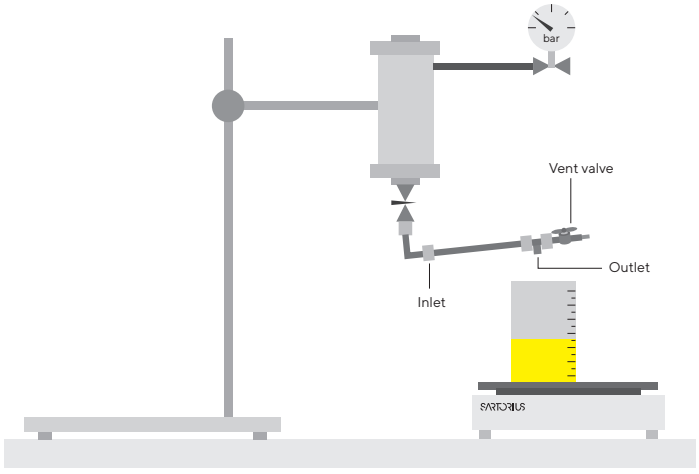
Objective

The objective of this study is to determine the impact of pressure fluctuation on the retention of parvovirus using bacteriophage PP7, an established model system for parvoviruses. Parvoviruses are renowned for being small viruses that are typically difficult to remove.

The newly developed Virosart® HF virus retentive filter membrane, from Sartorius, was tested. Virosart® HF is a 20 nm single layer hydrophilic surface modified PES membrane within a hollow fiber format, which provides high flow rates and superior capacity. The high packing density combines extremely low hold up and flushing volumes with low footprint requirements. It is the only gamma irradiatable virus retentive filter on the market and is designed for fully single-use manufacturing. The delivery of the filter elements in a sterile condition allows ease of use and rapid installation.

Materials

- Compressed nitrogen together with the Sartorius constant pressure test system with stainless steel sample reservoir
- Virosart® HF 1.7 cm^2 lab module
- Sartorius Balances
- Virosart® Max Minisart®, 5 cm^2
- Buffer: 50 mM Tris, 10 mM NaCl, pH 8.1
- Product: mAb; c = 5 g/L



Picture 1: *Set-up of Virosart® HF Lab Modules*

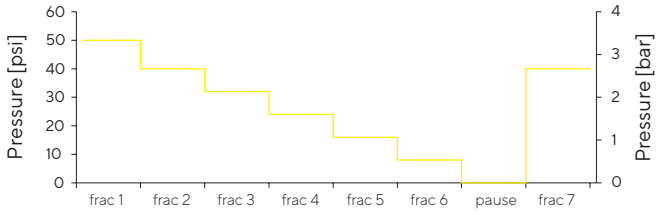
Method

A virus spike of PP7 with infectivity titer >7 log_{10}/mL was added to a monoclonal antibody solution. The mAb solution, c = 5 g/L, had been pre-filtered offline using a 5 cm^2 Virosart® Max, a 0.1 µm adsorptive pre-filter for Virosart® virus retentive filters, prior to being spiked.
A pressure control device for monitoring and varying the trans-membrane pressure during the filtration experiments was connected to the pressure vessel containing the spiked feed solution.

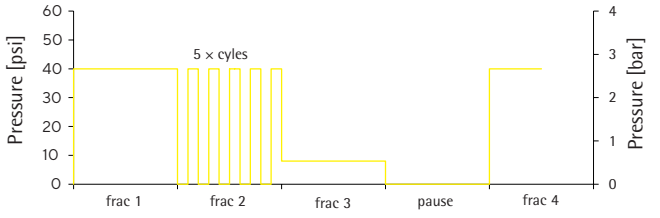
Afterwards the solution was loaded on the final virus retentive Virosart® HF up to a capacity of 240 L/m^2.
The filtrate volume was collected in a container and measured automatically using a balance connected to data recording software.

Three studies were performed as follows:

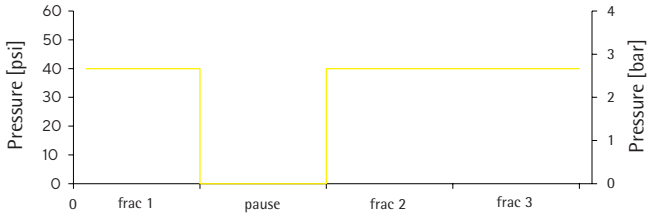
1. Multi-step pressure reduction



2. Rapid pressure changes, low pressure and pressure hold



3. Pressure release study

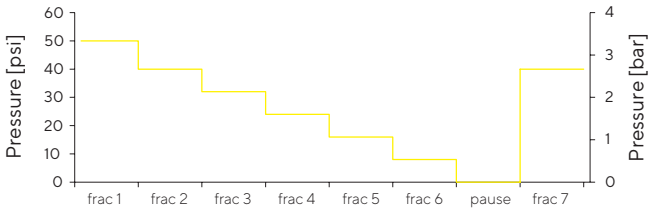


Results

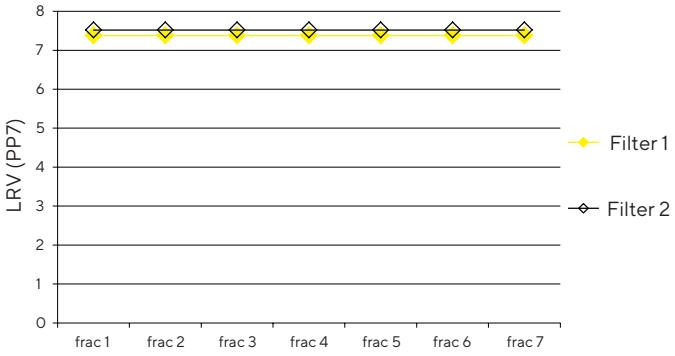
Study 1: Multi-step pressure reduction
Duplicate filtration runs were performed with two 1.7 cm², Virosart® HF lab modules. Flow was started by pressurizing the reservoirs to 50 psi | 3.44 bar and an initial 6 ml fraction taken.

6 ml fractions were collected at each of the following pressure steps (40 psi | 2.75 bar, 32 psi | 2.2 bar, 24 psi | 1.65 bar, 16 psi | 1.1 bar and 8 psi | 0.55 bar). The filtration was then paused for 10 minutes and the pressure returned to 40 psi | 2.75 bar before the final fraction was collected. Graph 1 shows the pressure profile during the experiment. Graph 2 shows the corresponding LRV profile of the fractions.

	Pressure (psi)	LRV (PP7) Virosart® HF Filter 1	LRV (PP7) Virosart® HF Filter 2
Load		7.38	7.52
Fraction 1	50	≥ 7.38	≥ 7.52
Fraction 2	40	≥ 7.38	≥ 7.52
Fraction 3	32	≥ 7.38	≥ 7.52
Fraction 4	24	≥ 7.38	≥ 7.52
Fraction 5	16	≥ 7.38	≥ 7.52
Fraction 6	8	≥ 7.38	≥ 7.52
10 minute pause	0		
Fraction 7	40	≥ 7.38	≥ 7.52



Graph 1: *Pressure Course During Multi-Step Pressure Reduction*



Graph 2: *Multi-Step Pressure Reduction Onto Virosart® Showing Constant Independent Retention Performance.*

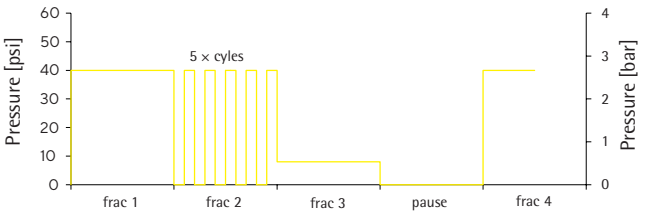
Summary of Study 1:
No fractions of the filtrate showed any virus breakthrough allowing the conclusion that multi-step pressure reduction had no effect on the retention of Virosart® HF lab modules.

Study 2: Rapid pressure change, low pressure and pressure release

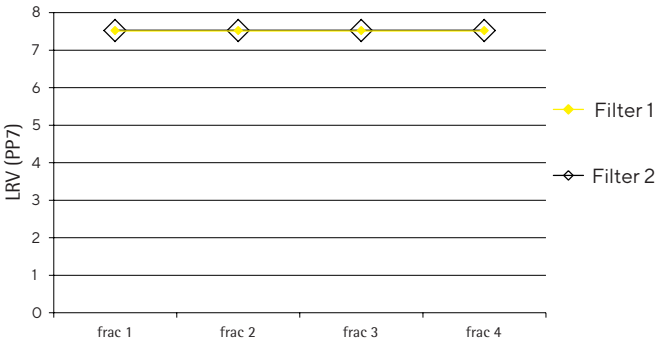
Duplicate filtration runs were performed with two 1.7 cm^2, Virosart® HF lab modules. The reservoirs were pressurized to 40 psi | 2.75 bar and the first fraction (10 ml) was taken. While the second 10 ml fraction was collected the pressure was rapidly switched off and on 5 times (fraction 2). Afterwards the filtration was allowed to proceed at a low pressure of only 8 psi | 0.55 bar during which the third 10 ml fraction was collected.

The filtration was then paused for 10 minutes and the pressure returned to 40 psi | 2.75 bar before the final fraction was collected. Graph 3 shows the pressure profile during the experiment. Graph 4 shows the corresponding LRV profile of the fractions.

	Pressure (psi)	LRV (PP7) Virosart® HF Filter 1	LRV (PP7) Virosart® HF Filter 2
Load		7.52	7.52
Fraction 1	40	≥ 7.52	≥ 7.52
Fraction 2	0 – 40 cycle 5 ×	≥ 7.52	≥ 7.52
Fraction 3	8	≥ 7.52	≥ 7.52
10 minute pause	0		
Fraction 4	40	≥ 7.52	≥ 7.52



Graph 3: *Pressure Course During Rapid Pressure Change, Low Pressure and Pressure Release*



Graph 4: *Rapid Pressure Change, Low Pressure and Pressure Release Onto Virosart® HF Showing Complete Retention Performance*

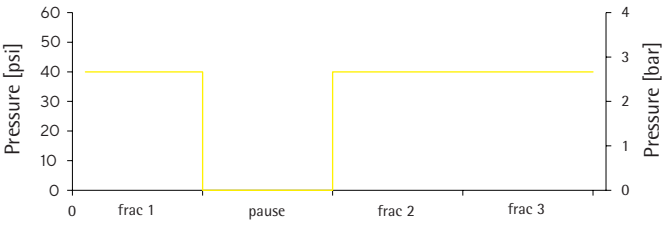
Summary of Study 2:

No fractions of the filtrate showed any virus breakthrough allowing the conclusion that the rapid pressure changes had no effect on the retention of Virosart® HF lab modules.

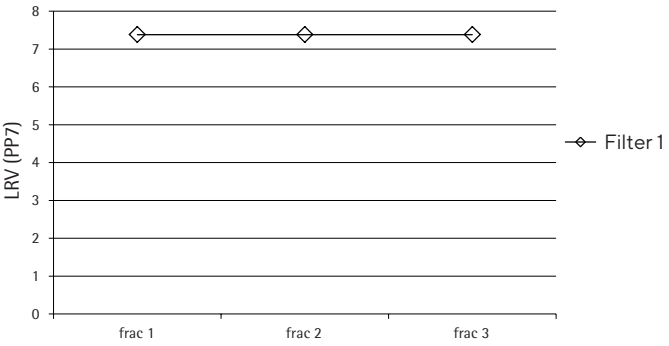
Study 3: Pressure release study

A single filtration run was performed with a 1.7 cm², Virosart® HF lab modules. Flow was started by pressurizing the reservoir to 40 psi | 2.75 bar. Upon reaching 50% of the total loading (fraction1: 120 L/m²) the system was de-pressurized and held at 0 psi | 0 bar for 10 minutes. Following the de-pressurized hold, the system was re-pressurized to 40 psi | 2.75 bar and the remaining material collected as fraction 2 (total loading capacity 240 L/m²). Finally the system was de-pressurized, wash buffer added to the reservoir, and the wash fraction was collected. Graph 5 shows the pressure profile during the experiment. Graph 6 shows the corresponding LRV profile of the fractions.

	Pressure (psi)	LRV (PP7) Virosart® HF Filter 1
Load		7.38
Fraction 1: (Load 0 – 120 L/m²)	40	≥ 7.38
10 minute pause	0	
Fraction 2: (Load 120 – 240 L/m²)	40	≥ 7.38
Fraction 3: Buffer flush	40	≥ 7.38



Graph 5: *Pressure Course During Pressure Release Study*



Graph 6: *Pressure Release Onto Virosart® HF Showing Complete Retention Performance*

Summary of Study 3:

No fractions of the filtrate showed any virus breakthrough allowing the conclusion that the performed pressure release during virus filtration had no effect on the retention of Virosart® HF lab modules.

Conclusions

This application note summarizes three filtration studies investigating the effect of pressure variations on the retention of PP7 bacteriophage by Virosart® HF lab modules.

The virus retention of the Virosart® HF lab modules was unaffected by pressure changes in any of the studies.

It can be concluded that the Virosart® HF lab modules, provide robust retention under the conditions studied in the experiments described in this application note.

Although this membrane offers high virus retention under a wide range of processing conditions, users are recommended to evaluate the impact of pressure excursions onto membrane performance under their specific process conditions.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

Pubilcation No.: SPK4112-e | Order No.: 85037-552-82 | Status: 07 | 06 | 2022