Virosart® HF

High Speed Virus Filtration for mAbs and Recombinant Proteins

Application Guide

SARTORIUS

Keywords or phrases:
Virus filtration, virus clearance, mAb's, MVM, PP7

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Disclaimer

The evaluation of a virus filter is not confined to its capacity to retain viruses. Selection of a virus filter is influenced by numerous factors including, but not limited to, flow rate, total batch throughput, fluid compatibility, process fluid attributes, potential risk from virus passage, unit costs, validation costs and costs of ancillary equipment.

The ideal virus filter will retain all virus sizes and allow high protein transmission, while maintaining a high flow rate without significant virus breakthrough. The purpose of this document is to provide users of Virosart® HF with an overview of its product throughput and virus retention performance characteristics under different process and feed stream conditions. A variety of studies have been designed and performed that reflect typical feed stream conditions used from process development up to commercial manufacturing in the biopharmaceutical industry. In addition further experiments have been performed outside of the typical manufacturing processes to show the robustness of the Virosart® HF filter with respect to filter throughput and retention. The results presented in this guide provide an indication of filter performance under the specific conditions tested. Similar results are likely also under different operating conditions. However, this is not guaranteed and users are requested to confirm throughput and retention characteristics under their specific process and feed stream conditions. Our application specialists will gladly assist in your filtration trial work. If required please contact your local Sartorius representative to obtain further information on technical data, product integrity testing or general information concerning specific applications.

1. Introduction

1.1 Virus Filtration

The commercial manufacturing of therapeutic antibodies or recombinant proteins requires robust and reliable processes that are economical and deliver high yields, while generating a product that is effective and meets standards for human use. One factor posing a risk to the patient is the presence of viruses in the final product. A contamination of products derived from human or animal cells with viruses can have disastrous clinical consequences. Therefore, regulatory authorities stress the need to implement robust and orthogonal strategies for virus clearance in order to meet the requirements of a risk-based approach to virus clearance assessment.

Virus filtration has traditionally been accepted as a robust method for virus clearance. The Virosart® HF virus filter family incorporates an effective and robust virus clearance technology for small non-enveloped viruses and large enveloped viruses combining high flow rate, high virus retention and high product throughput.

The main application for Virosart® HF filters is the virus filtration of monoclonal antibodies (mAbs), antibody fragments (Fab) or small recombinant proteins (< 150 kD). Virosart® HF is used towards the end of the purification process of biopharmaceutical products for the virus filtration. At this stage, the purity of the biopharmaceutical product is at its highest and so fouling of the virus filter by contaminants (DNA, HCP, aggregates and lipoproteins) will be low.

1.2 Product Overview

1.2.1 Membrane

The Virosart® HF virus filter features an asymmetric polyethersulfone (PES) hollow-fiber membrane with a nominal pore size of 20 nm. This membrane is characterized by a funnel-like pore-size gradient to achieve robust retention of small viruses such as parvoviruses under challenging conditions without impeding the quantitative transmission of product molecules. Further, to reduce the adsorption of proteins, protein aggregates or other potentially fouling species, the membrane is surface modified with a hydrogel-forming, low binding polymer.

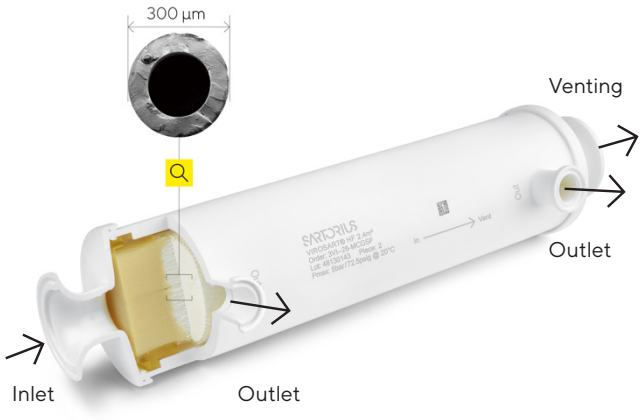

Figure 1: *Virosart® HF Device Set-up*

1.2.2 Filter Device

Scalable filters of different sizes were developed to provide appropriate size selection and flexibility from process development up to commercial processing.

Lab Modules
Nominal filtration area:
1.7 cm^2 & 5.0 cm^2

Typical filtration volume:
< 500 mL

To be used for:
- Scale-down work
- Flow & capacity studies
- Optimization of pre-filter to final-filter ratios
- GLP spiking studies

Mid-Scale Modules
Nominal filtration area:
200 cm^2 & 0.2 m^2

Typical filtration volume:
0.5 – 50 L

To be used for:
- Scale-up studies
- Clinical phases
- Small-scale production

Process Modules
Nominal filtration area:
0.8 m^2 & 2.4 m^2

Typical filtration volume:
> 50 L

To be used for:
- Large scale manufacturing

1.3 Product Release

Sartorius Stedim Biotech operates ISO 9001 and ISO 13485 certified Quality Management Systems to assure the consistent high quality of all membrane filters. All materials are selected carefully in accordance with current regulations, such as the FDA CFR's, cGMP's in-house guidelines and the specifications of our research and development department including the terms of delivery and acceptance of our purchasing department. Documentation begins with the inspection of the incoming raw materials including in-process materials, molded parts and sealing materials, etc. for manufacturing. Adherence to cGMP requirements (clean-room conditions, gowning and employee hygiene, etc.) which are monitored by documented in-process controls, ensures optimal quality control in standard operating procedures for production. In addition, all materials selected are highly unlikely to present a risk of TSE | BSE transmission. Our philosophy at Sartorius Stedim Biotech is to validate and test the membranes of our products extensively in order to assure the quality of all our filtration products. Below, an extract of membrane and final device release testing procedures for the Virosart® HF filter family is shown.

1.3.1 Membrane Release Testing

Extensive testing takes place during the whole membrane casting and release process. Membrane release testing includes, but is not limited to, the following tests:

Water and Buffer Flow Rate Test
Water | buffer permeability is an important parameter, as product throughput correlates to permeability. By testing the water and buffer permeability of the membrane, we can ensure reproducible, consistent and fast processing in the biopharmaceutical manufacturing process. Based on a defined sampling plan, the water and buffer flow rate at 2.0 bar | 30 psi operating pressure of each Virosart® HF membrane lot is tested. A 20 mM KPI buffer pH 7.2 is used as buffer.

Bacteriophage Challenge Testing
Retention of *Pseudomonas aeruginosa* bacteriophage PP7 is used as a release criteria of 20 nm retentive membranes. Bacteriophage PP7 is an accepted standard model virus for small viruses such as the parvovirus.(1) (2) Ideally suitable for the rapid release of membranes, bacteriophage PP7 is fast growing and can reach high titer. Bacteriophage challenge testing is performed using a buffer (low salt buffer, pH 7.2) and buffered IVIG (1 g/L in low salt buffer, pH 7.2) at an end point of 75% blocking. This low salt buffer at pH 7.2 has been chosen so as to exclude the possibility of interactions between protein components with the virus and the membrane. Filtering until an end point of 75% flow decay has been reached, represents testing that is commonly used during manufacturing and validation.

1.3.2 Product Release Testing

Two test methods are used for the release testing of devices and continuously assure the quality of Virosart® HF virus filters.

Water Flow Rate Test
Water permeability is an important parameter as protein throughput correlates to water flow rate. By testing the water flow rate of the final filter device, we can ensure reproducible, consistent and fast processing in the manufacturing process. The water flow rate of each individual Virosart® HF filter module is tested at 2.0 bar | 30 psi operating pressure.

Integrity Test (IT)
A water-based diffusion test is used to demonstrate the integrity of every final filter device. This non-destructive test ensures that only integral filters are released from production. Each individual Virosart® HF filter is tested for integrity (except non IT tested lab modules). The test pressure for diffusion testing is 4.5 bar | 65.25 psi and the testing and stabilization time is 5 minutes.

2. Throughput

2.1 Filtration Basics

2.1.1 Dead End Filtration

Modern virus filtration is a classical dead-end-filtration process. Pressure is applied on the feed stream side of the filter. The driving force for the filtration is the pressure difference across the membrane between feed and filtrate side.

2.1.2 Process Parameters

The course of a filtration can be described by different process parameters.

- **Differential Pressure:**
  DeltaP [Δp] is a process value that is defined as the difference between pressure on the feed [p_{Feed}] and filtrate side [$p_{Filtrate}$]. The differential pressure is usually controlled by the feed pressure as filtrate pressure is normally 0 bar | 0 psi.

  $\Delta p = [p_{Feed}] - [p_{Filtrate}] = [bar; psi]$

- **Flow Rate:**
  Flow rate [J] is a process value that is defined as the filtered volume [V] per time [t].

$$J = \frac{V}{t} = \left[\frac{L}{h}\ ;\ \frac{mL}{min}\right]$$

  Often, the flow rate [J] is normalized to the filter area [$A_{Membrane}$].

$$J_{Norm} = \frac{J}{A_{Membrane}} = \left[\frac{L}{m^2 h}\right]$$

- **Permeability:**
  The permeability [P] is a key process parameter that defines the filter performance and directly affects batch production time. The permeability is represented by the flow rate [J] over the membrane area [$A_{Membrane}$] during a given time period. The permeability normally decreases with increasing filtration time due to the fouling of the filter by larger particles such as aggregates.

$$P = \frac{J}{A_{Membrane} \times \Delta p} = \left[\frac{L}{m^2 h\ bar}\right]$$

- **Product Throughput:**
  Product throughput [V/A] describes the filtered volume [V] per membrane area [$A_{Membrane}$].

$$V/A = \frac{V}{A_{Membrane}} = \left[\frac{L}{m^2}\right]$$

- **Product Mass Throughput:**
  The mass throughput [m/A] defines the mass of product [m] per membrane area [$A_{Membrane}$].

$$m/A = \frac{m}{A_{Membrane}} = \left[\frac{kg}{m^2}\right]$$

- **Flow Decay:**
  The flow decay [J/J_{Buffer}] is defined as decay in product flow with respect to the initial buffer flow [J_{Buffer}] at a respective time point.

$$J/J_{Buffer} = \frac{J}{J_{Buffer}} = [\%]$$

2.1.3 Process Steps

Typically a virus filtration step includes following process steps:

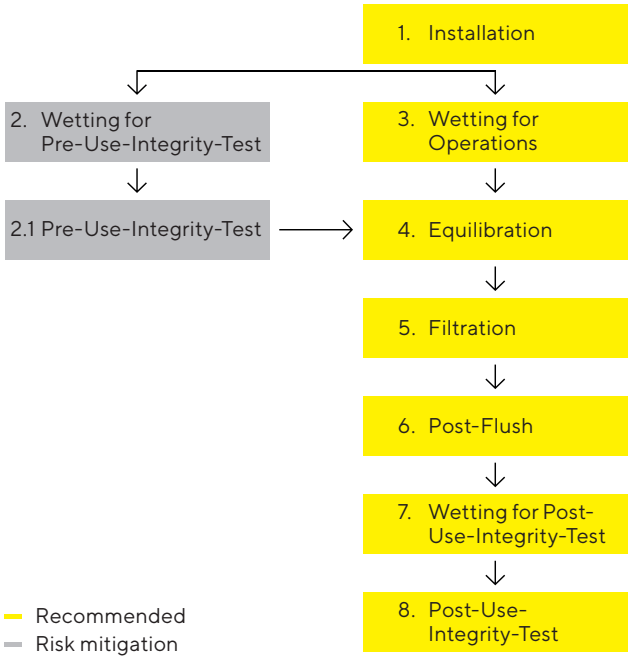

Figure 2: *Process Steps During Virus Filtration*

- **1. Installation:**
  The equipment and the filter need to be installed and all sensors have to be connected.

- **2. Wetting for Pre-use IT:**
  In order to perform a water based diffusion test, the entire filter membrane area needs to be fully wetted with water for injection (WFI).

- **2.1 Pre-use IT:**
  An IT Test is performed to prove the integrity of the filter. A pre-use IT test is not required from a regulatory perspective, however a pre-use IT is strongly advised for risk mitigation purpose.

- **3. Wetting for Operation:**
  This ensures that the filter is particle-free and completely wetted so that the entire filter area is used during filtration.

- **4. Equilibration:**
  Prior to the produt filtration, the filter and the equipment is equilibrated with buffer.

- **5. Filtration:**
  In this step the product is filtered through the virus filter. The filtration can be operated under constant pressure or constant flow conditions.

- **6. Post Flush:**
  A post-use buffer flush is recommended to achive maximum product yield.

- **7. Flushing for Post-use IT:**
  Prior to the post-use IT the membrane must be wetted with WFI.

- **8. Post-use IT:**
  An IT test is performed to prove the integrity of the virus filter. A post-use IT is mandatory from a regulatory perspective for the release of a batch.

2.1.4 Process Control

- **Set up**
  Figure 3 describes a virus filtration set-up for commercial processing. Typically three solutions are needed (WFI, buffer, feed), as well as 2 types of sensors (flow, pressure), a peristaltic pump and valves.

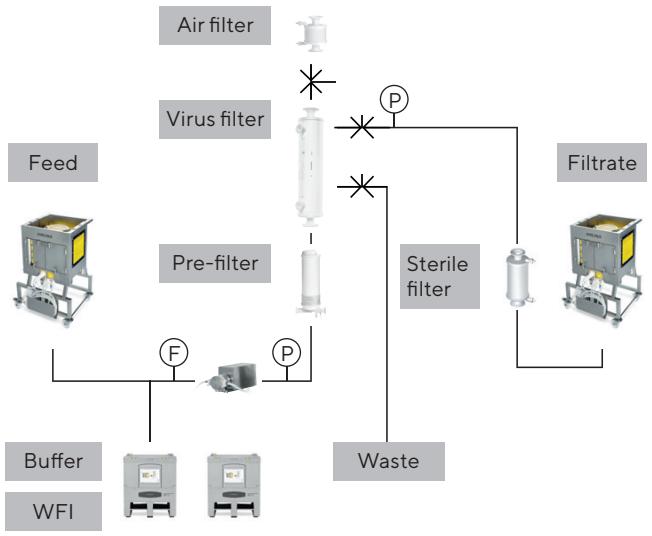

Figure 3: *Set-up for a Typical Virus Filtration Unit Operation*

- **Mode of Filtration**
  There are two ways to operate a virus filter:

  - 1. Constant pressure: The filtration is operated at a constant pressure of 2.0 to 3.0 bar|30.0 to 43.5 psi. The mode of operation for most virus filtration steps is constant pressure. The pressure may be generated by either a compressed air source or pump.

  - 2. Constant flow rate: The filtration is operated with a constant flow rate. The flow is normally controlled at a Δp of 1.0 bar|14.5 psi at the beginning of the filtration. After reaching a certain pressure increase during the filtration, the flow rate is lowered to maintain the pressure.

- Filtration Stop Criteria
  Processes are typically stopped after the total batch has been processed through the virus filter. During spiking studies typically the max. total batch throughput (L/m² or g/m²) is validated.

2.2 Filtration of mAbs

The Virosart® HF filter is specially developed for the filtration of mAbs and recombinant proteins. The virus filtration unit operation is typically located towards the end of the purification process. At this stage, the purity of the biopharmaceutical product is at its highest and virus filter fouling due to contaminants (DNA, HCP, aggregates and lipoproteins) is low. However, filterability can highly depend on the individual product molecule (size, pI, hydrophobicity) and its impurity profile (aggregates, fragments, HCPs, DNA).

Factors such as buffer composition, pH value, conductivity, operating pressure are known to potentially impact product throughput and productivity. In this study, the filtration performance for a variety of different representative mAbs is summarized to illustrate the range of typical product throughputs.

Materials and Methods
Virosart® HF lab modules (1.7 cm² or 5.0 cm²) were challenged with different mAb feed streams. In total, 10 different antibody solutions ranging in concentrations from 3 to 30 g/L were filtered at neutral pH value. The filtrations were performed at constant pressure of 2.0 bar|30 psi using a 0.1 µm PES pre-filter (Sartopore® 2 XLM 4.5 cm²). In order to compare all filtration runs, the total product mass throughput was compared after 3 hours of filtration time. If the filtration was stopped before 3 hours of processing, the filtration was extrapolated using V_{Max} up to a filtration time of 3 hours. This allowed a comparison to be made between the feed streams. The following formula describing V_{Max} was applied:

$$\frac{t}{V/A} = \frac{1}{V/A_{Max}} \times t + \frac{1}{J_0}$$

with:

t	Time [h]
V/A	Product throughput [L/m²] filtered
V/A_{Max}	Maximum product throughput [L/m²]
J_0	Initial flow rate [L/m²h]

Results and Discussion

High product mass throughput is demonstrated for Virosart® HF. However, the product mass throughput depends strongly on the individual molecule feed stream. Throughput after 3 hours of filtration ranges from 1 up to 12 kg/m² (figure 4). In all runs shown, the individual target volume was met.

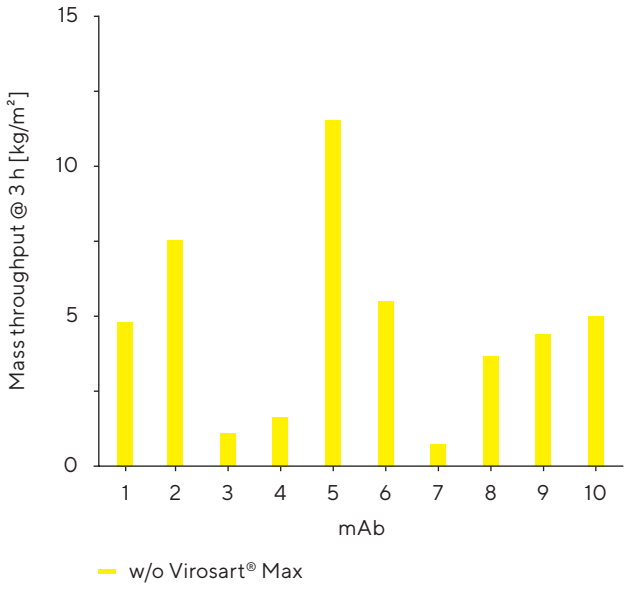

Figure 4: *Product Mass Throughput for Different Antibody Feed Streams After 3 Hours of Filtration at 2.0 bar | 30 psi Operating Pressure Using Virosart® HF*

2.3 Consistent Filter Throughput

Biopharmaceutical manufacturers expect consistent and reproducible throughputs from virus filters. During the development of the Virosart® HF and during routine production Sartorius Stedim Biotech ensures exceptional quality standards are maintained by implementing and conducting comprehensive release tests as described in chapter 1.3.

Materials and Methods

In the following experiment, consistent throughput was confirmed by evaluating the performance of an antibody feed stream (mAb, IVIG) as well as buffer for inter- and intra-lot consistency of Virosart® HF virus filters. All filtration runs were performed at constant pressure of 2.0 bar | 30 psi.

To show consistent mAb throughput, Virosart® HF lab modules (1.7 cm²) from three different filter lots were challenged with a representative mAb solution of 5 g/L in 20 mM Tris HCL, pH 7 with 150 mM NaCl. The runs have been performed in duplicate, as shown in figure 5.

In addition, 96 filters from 8 different lots of filter modules were tested to prove inter- and intra-lot filter consistency for buffer permeability (figure 6). Virosart® HF lab modules with a filtration area of 5.0 cm² were used for the runs. To facilitate comparison, buffer permeability results were normalized to a temperature of 25 °C | 77 °F. 20 mM KPi at pH 7.2 was used as a buffer.

During membrane release testing, bacteriophage PP7 retention is measured at 75% blocking. Also the time when 75% blocking is reached is documented. As the bacteriophage PP7 spike is not blocking the Virosart® HF membrane, these data can also be taken to compare consistent protein throughput of Virosart® HF. 42 lab modules from 8 different lots were challenged with highly blocking IVIG solution at a concentration of 1 g/L in 20 mM KPI buffer pH 7.2. The IVIG throughput was normalized to reference modules based on inhomogeneous IVIG feed stream from different batches (figure 7).

Results and Discussion

Inter- and intra- lot consistency of Virosart® HF performance is confirmed.

Consistent mAb throughput is shown for both membrane lots of Virosart® HF lab modules as shown in figure 5. Throughput varied minimally by 5% from 413 to 463 L/m² demonstrating consistent inter- and intra-membrane lot performance. The data shown in figure 6 and figure 7 are representative for Virosart® HF product release testing. Consistent buffer permeability and protein throughput is shown for the Virosart® HF lab modules with minimal variation (less than ± 10%) within the given specifications.

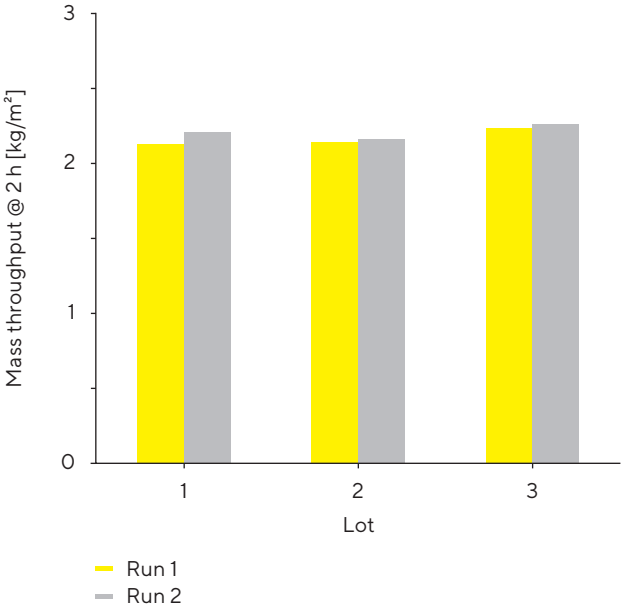

Figure 5: *Product Throughput for 3 Different Lots of Virosart® HF Filter Tested in Duplicate Runs*

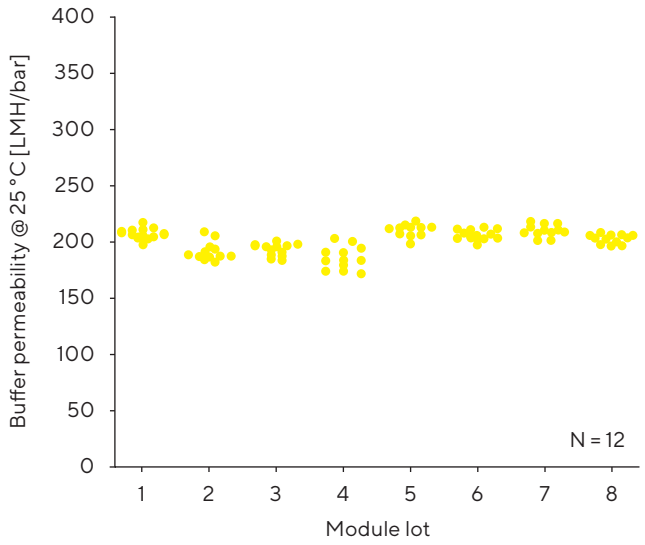

Figure 6: *Buffer Permeability for Virosart® HF Lab Modules Shown for 96 Filters of 8 Different Lots*

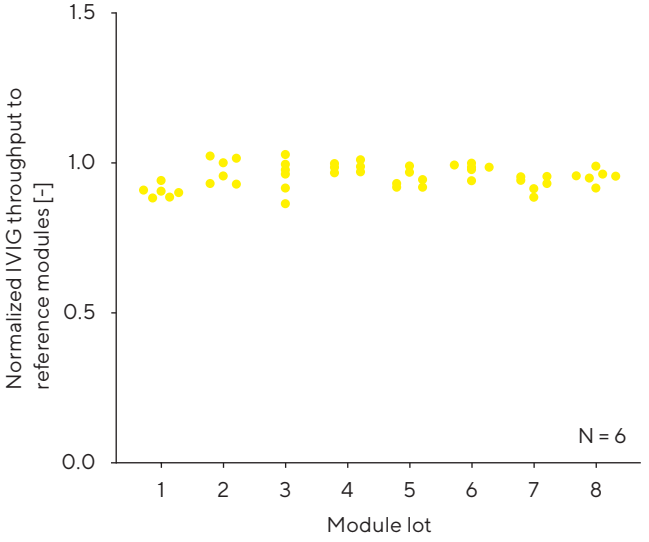

Figure 7: *Protein Throughput for 42 Modules of 8 Different Module Lots of Virosart® HF Using IVIG. Protein Throughput Has Been Normalized to Reference Modules*

2.4 Protein Transmission

Short processing times and high product yields significantly contribute to process economics. A post-use flush is commonly performed after the filtration process in order to increase the product yield. High yields also result from the high selectivity of the virus filter to retain viruses and to allow product to pass through the filter. Protein transmission close to 100% is expected for commercially available virus filters. In the experiment conducted, the initial protein transmission was determined.

Materials and Methods
A model feed stream of buffered IVIG at a concentration of 1 g/L in 20 mM KPI buffer, pH 7.2 was filtered using the Virosart® HF lab modules (5.0 cm^2). In order to determine the initial protein transmission in the first experiment, 5 mL of IVIG was filtered in duplicate runs. During the filtration two fractions were collected. The 1st fraction (2 mL) was discarded due to potential dilution resulting from the flushing prior to the filtration.

The protein concentration of the 2nd fraction (3 mL) was determined using a photometer at a wavelength of 280 nm (figure 8). The filtration was performed at 2.0 bar | 30 psi operating pressure.

Results and Discussion
Protein transmission is high throughout the entire IVIG filtration process. Figure 8 shows that the initial protein transmission in both runs exceeded 99%.

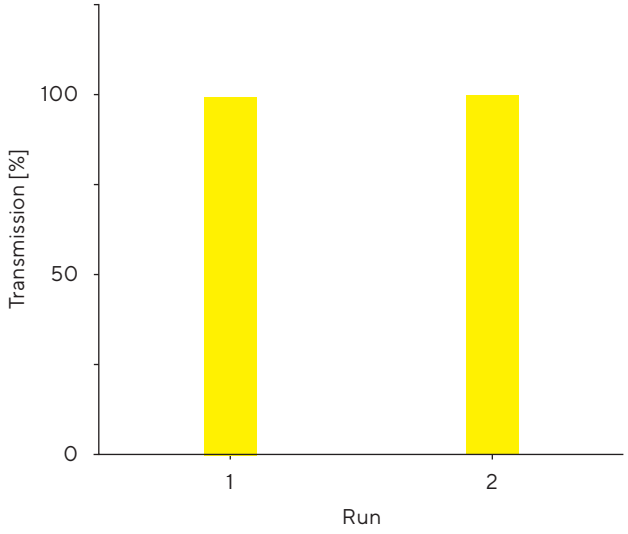

Figure 8: *Initial Protein Transmission for Virosart® HF Using IVIG*

2.5 Scalability

Virosart® HF filter modules are available in 5 different sizes. These range from lab modules (1.7 and 5.0 cm^2) used for scale-down work and GLP virus validation studies, mid-scale modules for pilot runs (200 cm^2 and 0.2 m^2) to process modules (0.8 and 2.4 m^2) used for commercial processing. From a process development perspective, scalability is a key factor to successfully transfer processes to large scale.

Materials and Methods
A set of experiments has been performed to demonstrate the scalability of flow rate and protein throughput across the size range of Virosart® HF filters. All runs were performed at 2.0 bar | 30 psi operating pressure.

Water flow rates for Virosart® HF filter family (device sizes of 1.7 cm^2, 5.0 cm^2, 200 cm^2, 0.2 cm^2, 0.8 m^2 and 2.4 m^2) have been tested using 3 different lots of filter modules with 10 filters from each lot. To facilitate the comparison of different runs, water flow rates were normalized to a temperature of 25 °C | 77 °F (figure 9).

Three different 5.0 cm^2 Virosart® HF lab modules and one 0.8 m^2 process module were challenged with a 2 g/L buffered IVIG model solution at 2.0 bar | 30 psi constant pressure until 95% flow decay was achieved. The pre-treatment of lab and process modules was identical (figure 10).

Results and Discussion
Scalability of water flow rates and protein throughput is demonstrated through the whole filter family.

Figure 9 shows the water flow rates for different filter sizes. These are within specification and are consistent across the entire range.

Figure 10 shows that results from down-scale modules can be scaled-up based on 2 to 3 down-scale experiments. An average of the filtered protein mass per filtration area vs. time filtration data for the three laboratory modules was taken and compared to the corresponding data for the process module.

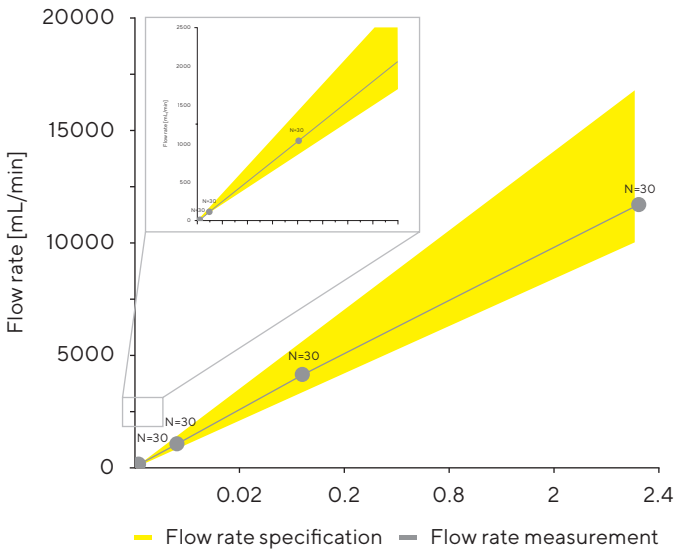

Figure 9: *Average of Water Flow Rates at 2.0 bar | 30 psi Through the Entire Product Family of Virosart® HF*

2.6 Post-Flushing Volume

A post-use flush is often performed during the virus filtration step in commercial production in order to recover protein and increase yield. The Virosart® HF filter family was developed to have low hold up volumes and thereby reduce product dilution as well as reduced buffer consumption and costs. The goal of this experiment was to determine the flushing volume required to reach a 99% recovery of the target protein.

Materials and Methods

A Virosart® HF process module with 0.8 m² surface area was used to determine the flushing volume required to reach 99% product recovery. Buffered IVIG solution with a concentration of 2 g/L was processed through the filter at 2.0 bar | 30 psi until a 40% flow decay was reached. A slightly higher concentration was used in this experiment to reach faster the level of flow decay. The feed stream was then changed from the IVIG solution to a buffer. An in-line UV-detector in the filtrate stream was used to determine the protein concentration.

Results and Discussion

Buffer savings are possible with Virosart® HF due to minimal post flushing volumes. Figure 11 shows that the flushing volume required in order to reach 99% product recovery is 3 L/m².

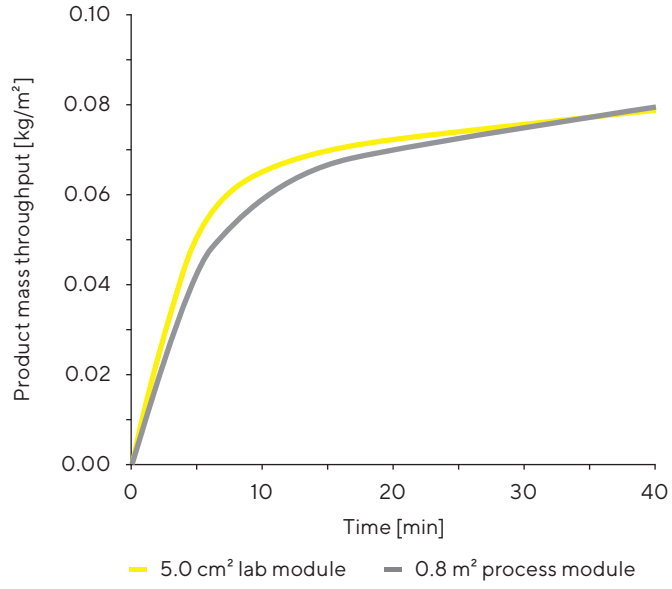

Figure 10: *Product Mass Throughput of the Virosart® HF Lab (5.0 cm²) And Process Module (0.8 m²)*

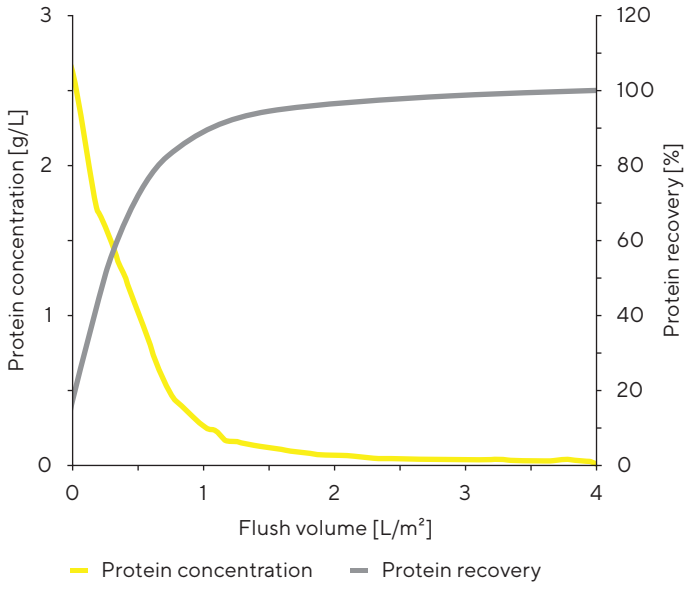

Figure 11: *Post-flushing Volume for Virosart® HF*

2.7 Hold-up Volume

It is important to know filter wet and hold-up volumes in order to optimize production processes. The Virosart® HF modules are designed and developed to have minimal wet and hold-up volumes.

Materials and Methods

The wet volume and the hold-up volume was determined for every Virosart® HF module in the product family.

- **1. Wet Volume:**
  Defined as volume of water required to completely fill the module (upstream and downstream side of the membrane). In order to determine the wet volume the difference in weight between empty and filled capsules is determined for each module (figure 12, top).

- **2. Hold-up Volume:**
  Defined as the volume left in the module once emptied with pressurized air at 4.5 bar | 65.2 psi inlet pressure. Here, only the downstream side of the filter contains liquid as indicated in figure 12, bottom. After emptying, the module was weighed to determine the hold-up volume.

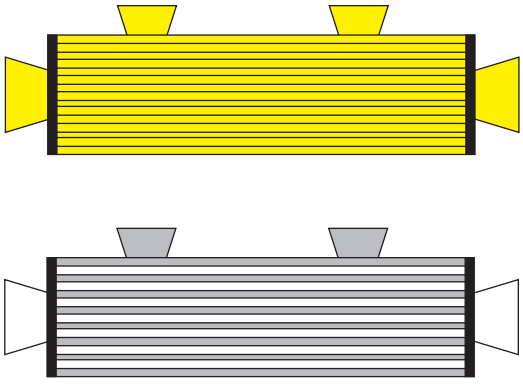

Figure 12: *Wet Volume (Top) And Hold-up Volume (Bottom) Of the Virosart HF®*

Results and Discussion

Minimal need of hold and flushing volumes for the entire family of Virosart® HF. The results are presented in table 1 for each size.

	Area	Wet volume [mL]	Hold-up volume [mL]
Lab module	5.0 cm²	2	2
Mid-scale module	200 cm²	50	3
	0.2 m²	100	20
Process module	0.8 m²	650	80
	2.4 m²	575	170

Table 1: *Wet and Hold-up Volume for Virosart® HF Filter Family*

3. Process Optimization

3.1 Fouling Behavior of Virus Filters

Virus filtration performance is often a function of feed stream properties such as buffer composition, pH, conductivity, process pressure, impurity and aggregate levels, protein concentration and protein characteristics. These can lead to different fouling behaviors being observed during filtration operations.

The main reason for flow decay in virus filtration is fouling caused by fouling species that are present in the feed stream such as process impurities and protein aggregates. Aggregates may have a size similar to the pores of the virus filter and become retained by the membrane.

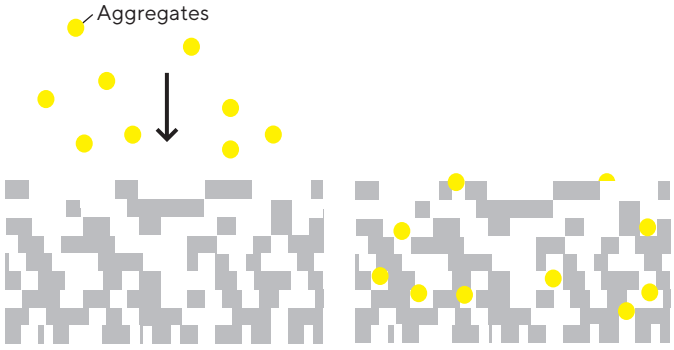

Figure 13: *Virus Filter Fouling Due to Aggregates Beeing Present in the Feedstream*

Solutions for Virus Filter Fouling

- Adsorptive pre-filtration (chapter 3.2) to remove process impurities and aggregates.
- Application of virus filtration unit operation further downstream, if possible, where impurities and aggregate levels are lower.

A change of feed stream conditions can also have a positive impact on the fouling behavior:

- Dilution (chapter 3.3)
- Conductivity (chapter 3.4)
- pH (chapter 3.4)
- Buffer

3.2 Adsorptive Pre-Filtration With Virosart® Max

Virosart® Max is a pre-filter which is designed to significantly increase the performance of downstream virus filters. The Virosart® Max pre-filter primarily acts via its adsorptive capacity, together with size exclusion mechanisms, to retain typical virus filter foulants.

By removing these virus filter foulants, process robustness can be increased significantly. The species that typically are responsible for fouling virus filters are product aggregates and smaller hydrophobic species. Using the patented technology of Virosart® Max (DE 10 2011 105 525 B4), aggregates and hydrophobic species are bound very efficiently through hydrophobic interactions of the polyamide membrane material, independent of process conditions such as pH and conductivity.

Since the Virosart® Max removes most of the hydrophobic species by adsorption, the surface area of pre-filter needed is important to determine. Two different studies have been performed to test the impact of adsorptive pre-filtration and to optimize the ratio of pre-filter to final-filter area.

3.2.1 Use of Adsorptive Pre-Filters

In this study, the effect of Virosart® Max on the overall product throughput using Virosart® HF was tested.

Materials and Methods

The overall product throughput of Virosart® HF lab modules was tested with and without in-line adsorptive pre-filtration with Virosart® Max. Different mAb solutions ranging in concentration from 3 g/L up to 30 g/L were filtered with and without the use of Virosart® Max. All filtration runs were run at 2.0 bar | 30 psi constant pressure. The filtration runs were extrapolated up to 3 hours filtration time using V_{Max}.

Results and Discussion

By the use of Virosart® Max as an adsorptive inline pre-filter (figure 14), mass throughput may be increased significantly for most of the feed streams tested. The overall product throughput can be improved by 10% up to 50% specifically for feed streams containing a high amount of foulants such as aggregates. The use of an adsorptive inline pre-filter before the virus filter is recommended to guarantee process robustness and to improve process economics.

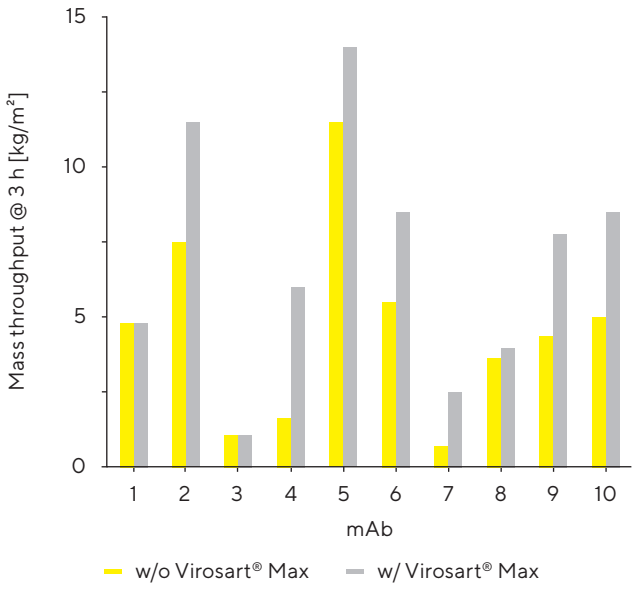

Figure 14: *Impact of Inline Pre-filtration on the Overall Throughput of Virosart® HF*

3.2.2 Optimization: Pre-filter to Final-filter Ratio

Further improvement of process economics can be achieved by optimizing the ratio of adsorptive pre-filter to final-filter area. Since the Virosart® Max removes most of the hydrophobic species by adsorption, the surface area of pre-filter needed is important to determine. By increasing adsorptive pre-filter area, more foulants that tend to block the virus filter, e.g. aggregates can be removed.

Materials and Methods

In this study, different pre-filter to final filter ratios were tested in order to achieve optimal virus filter throughput for two specific feed streams (IVIG, mAb). Filtration was carried out at a constant pressure of 2.0 bar | 30 psi. In the first experiment a high fouling IVIG feed stream was filtered with different adsorptive pre-filter to final-filter ratios of 0:1, 1:1 and 4:1 (figure 15). In the second experiment the mAb feed stream was filtered with a 0:1, 3:1 and 6:1 pre-filter to final-filter ratios (figure 16).

Results and Discussion

The throughput of Virosart® HF increases with increased adsorptive pre-filtration area of Virosart® Max. As shown in figure 15, using the virus filter alone resulted in a continuously decreasing flow rate as the virus filter is challenged with a constant concentration of foulants. By increasing the adsorptive pre-filtration area, the overall product throughput over the virus filter can be extended significantly. Here specifically the adsorptive capacity increases thus extending the loading phase. During the loading phase the capacity of the adsorptive pre-filter is not fully used and no foulants can breakthrough. During the further course of filtration, flow decay sets in as a consequence of overloading the pre-filter.

Product throughput can be increased significantly by increasing the adsorptive pre-filter area as shown in figure 15 and figure 16 for the mAb feed stream used. The capacity can be doubled by using a pre-filter to final-filter ratio of 3:1 with an overall capacity of 1.5 kg/m² after 3 hours of filtration when compared to the case without adsorptive pre-filter. The highest product throughput of 2.0 kg/m² can be reached by using a 6:1 pre-filter to final-filter ratio.

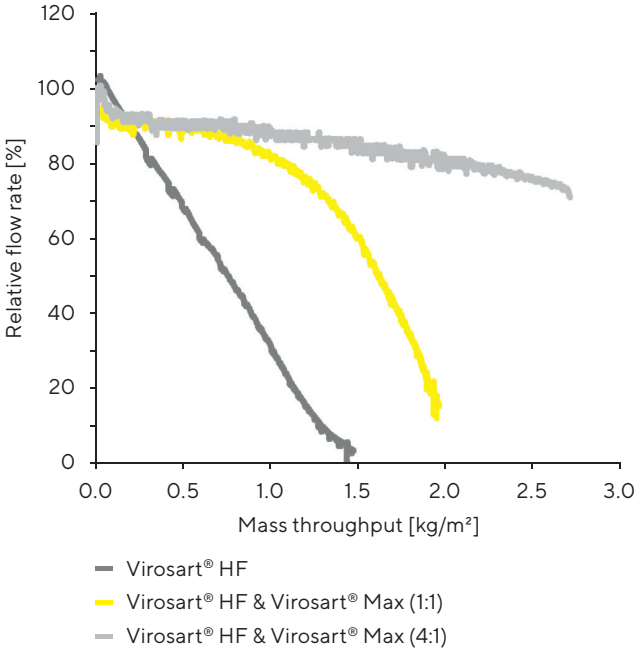

Figure 15: *Virus Filter Throughput Can Be Increased by Increasing the Adsorptive Pre-filtration Area*

3.3 Dilution

There are feed streams that can be challenging for virus filters to process even with the use of an adsorptive pre-filter. In this experiment the impact of feed stream dilution was investigated to improve product throughput over the virus filter. Dilution can influence molecule folding as well as protein-protein interaction and can therefore have a positive impact on product throughput performance of the virus filter.

Materials and Methods

To start, a mAb feed stream was filtered at target concentration. Afterwards the feed was diluted in two steps, each time by factor 1.5. Filtration has been extrapolated using V_{Max} up to a filtration time of 3 hours to allow comparison. All filtration runs were performed at 2.0 bar | 30 psi constant pressure.

Results and Discussion

Dilution of feed stream can increase product throughput significantly. As seen in figure 17, the overall product throughput over the virus filter can be improved by dilution for challenging feed streams. The product throughput can be increased from 2 kg/m² up to approx. 6.5 kg/m² after 3 hours of filtration by applying a two-step dilution.

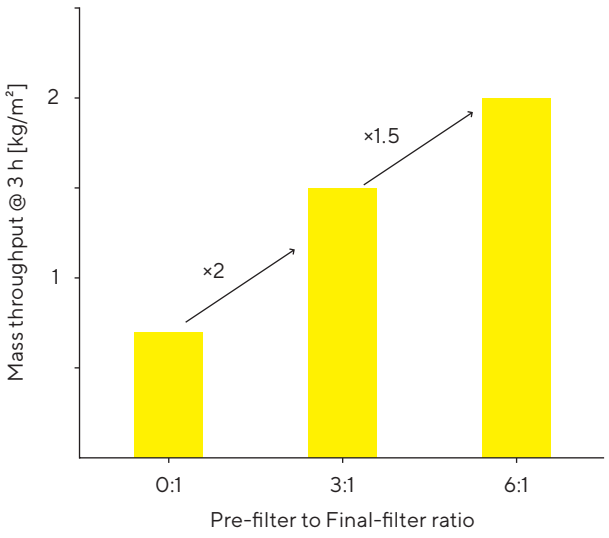

Figure 16: *Optimization of Adsorptive Pre-filter Area Can Improve Overall Virus-Filter Throughput*

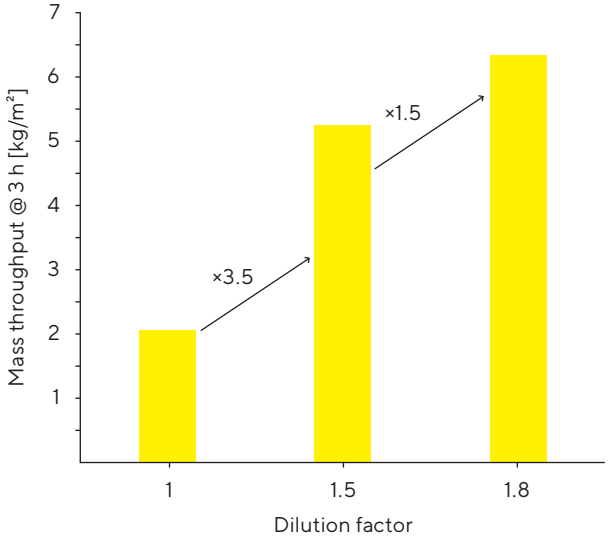

Figure 17: *Dilution of Feed Stream Impacting Product Throughput Over the Virus Filter*

3.4 pH and Conductivity

Adapting the pH and conductivity of the feed stream can influence molecule folding as well as protein-protein interaction and can therefore have an impact on product throughput performance during virus filtration.

Materials and Methods
The effect of pH-shift and conductivity shift on the product throughput of Virosart® HF lab modules was tested. pH value and conductivity of a challenging-to-filter mAb feed stream were adjusted. The pH was varied around neutral pH (7, 8, 8.5) and different conductivities (100, 200, 300 mM) were chosen (figure 18).

Results and Discussion
Adapting pH and conductivity can positively impact virus filter throughput.

The optimal feed stream conditions for the mAb appears to be at neutral pH and a conductivity of 100 mM showing 6 times the product throughput in comparison to the other conditions tested as shown in figure 18.

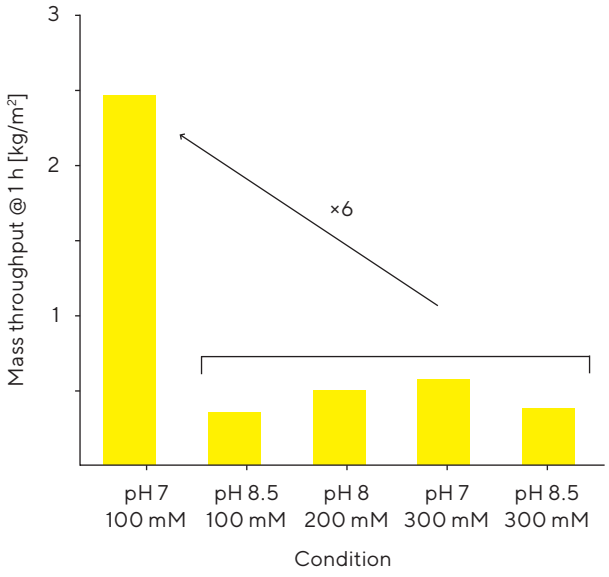

Figure 18: *Variation of pH and Conductivity Impacting The Virus Filter Throughput*

3.5 Decision Tree for Virus Filter Throughput Optimization

The figure 19 summarizes the chapter optimization based on a decision tree for virus filter optimization.

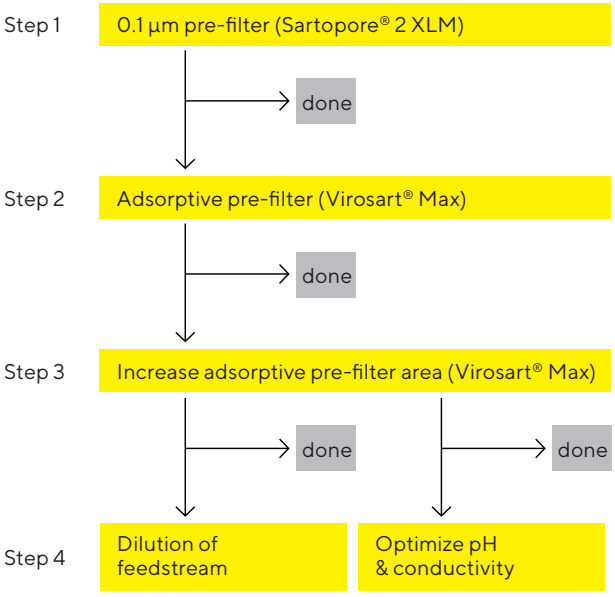

Figure 19: *Decision Tree for Virus Filter Throughput Optimization*

4. Virus Clearance

4.1 Introduction

4.1.1 Definition of LRV

Retention during virus clearance unit operations is calculated as a $\log_{10}$ reduction value (LRV), which is the logarithmic ratio of the virus titer in the feed [C_{Feed}] to the virus titer in the filtrate [$C_{Filtrate}$]:

$$LRV = \log_{10} \frac{C_{Feed}}{C_{Filtrate}}$$

Typical reduction values given by filter manufactures for virus filters are:

- ≥ 4 LRV for small non-envleoped viruses
- ≥ 6 LRV for large envleoped viruses

4.1.2 Model Virus

Validation studies with viruses, so called "spiking studies", are generally performed in contract laboratories that have the capabilities to prepare, realize and document spiking studies based on scale-down models from customer-specific manufacturing processes. An initial study is normally required prior to the manufacture of clinical Phase I material and is aimed validating effective and adequate clearance of viruses. A subsequent and more comprehensive study is then conducted prior to clinical phase III to provide evidence of the effective and adequate clearance of relevant and known viruses along with the removal of a range of novel and unpredictable viruses.

They are differentiated as follows: (3)

- Relevant virus: The identified virus, or of the same species as the virus that is known, or likely to contaminate the cell substrate or any other reagents or materials used in the production process.
- Specific model viruses: Viruses that are physically and chemically similar to relevant viruses like same genus or family.
- Non-specific model viruses: Viruses that represent wide range of physicochemical properties in order to test the ability of the system to eliminate virus in general.

Virus	Description	Enveloped	Genome Type	Size [nm]
PPV	Model virus for small non-enveloped virus	No	ssDNA	18 – 24
MVM	Model virus for small non-enveloped virus	No	ssDNA	18 – 26
MuLV	Model virus for large enveloped viruses	Yes	ssRNA	80 – 110
PP7	Proven bacteriophage model for parvoviruses (9)	–	ssRNA	26

Table 2: *Viruses and Bacteriophages Used for Following Studies (2) (8)*

Further details regarding model viruses can be found in the virus information guide (10). Data showing no virus breakthrough are indicated in the figures by filled circles and data with virus breakthrough are illustrated by empty circles respectively.

4.2 Virus Retention

4.2.1 MuLV Retention

Murine Leukemia Virus (MuLV) is a standard model virus used to validate the clearance of large enveloped viruses in products derived from cell lines. (5) Typically the virus is used in spiking studies performed prior to entrance into phase I and phase III clinical trials.

Materials and Methods
MuLV retention results were summarized from different individual experiments. The runs were performed at different contract testing organizations with different feed steams and experimental set-ups. All studies were performed with duplicate runs at an operating pressure of 2.0 bar | 30 psi. Therefore specific conditions such as product concentration, buffer composition product throughput, fractions taken, MuLV spike level etc. are process specific (figure 20).

Results and Discussion
High retention of MuLV with no virus breakthrough detected for all runs of Virosart® HF.

MuLV results from different experiments were collected in figure 20. Filtration runs were performed at different contract testing organizations with different feed steams most at neutral pH with typical operating pressure of 2.0 bar | 30 psi. In all trials, no virus breakthrough was detected. The distribution between the resulting LRVs originates from different virus stock titer used and is therefore caused by the different input titer in the feed material within the experiments.

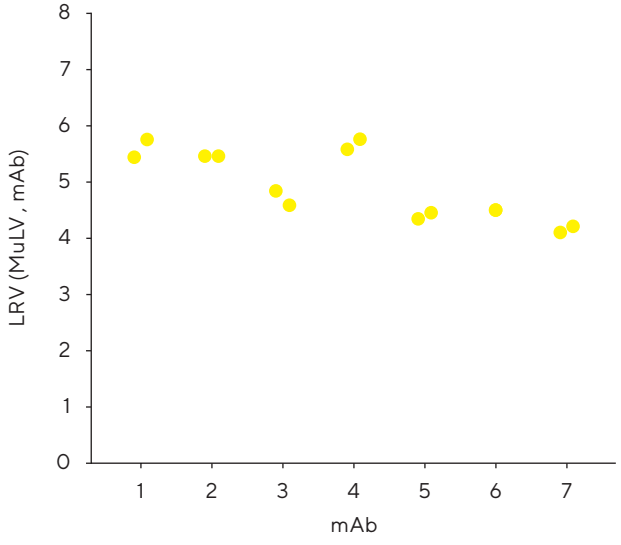

Figure 20: *Summary of MuLV rRetention From Different Studies. LRV Distribution Is Resulting From the Different Feed Virus Titer*

4.2.2 PPV Retention

Porcine Parvo Virus (PPV) is a standard model virus used to evaluate the clearance of small non-enveloped viruses in products derived from cell lines. (5)

Materials and Methods
The Virosart® HF was tested with a feed stream comprising PPV spiked IVIG solution at a concentration of 1 g/L in 20 mM KPI buffer, pH 7.2. Two spiked runs were performed with different virus titer in the feed with the first containing 5×10^5 pfu/mL and the second containing 5×10^6 pfu/mL. The LRV results present fractions taken up to 25% and up to 70% flow decay. The log reduction value was also determined in the post-wash fraction and the overall filtrate and wash pool. The studies were performed by an external contract laboratory.

Results and Discussion
LRVs of 6 and greater are shown in figure 21 for all fractions taken for both runs. The LRV for PPV was also high in the post-wash fraction. The filter achieved high and reproducible LRVs for PPV regardless of the extent of fouling.

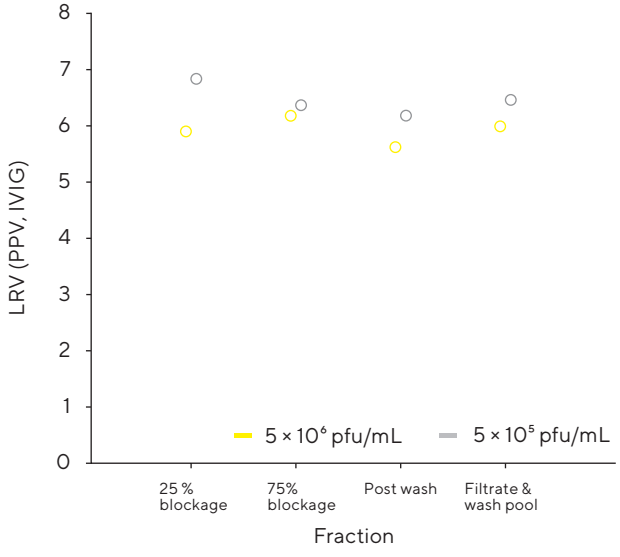

Figure 21: *Retention of PPV at 25% And 70% Blockage as Well as Post Wash*

4.2.3 MVM Retention

Minute Virus of Mice (MVM) is a standard model virus used to evaluate the clearance of small-non enveloped viruses in products derived from cell lines. (5)

Materials and Methods
Different studies were performed with Virosart® HF lab modules showing MVM retention in duplicate runs.

The runs were performed in different contract testing organizations with different feed steams and experimental set-ups. Therefore, specific conditions such as product concentration, buffer composition, product throughput, fractions taken, MVM spike level etc. are process specific. All runs were performed at constant operating pressure of 2.0 bar | 30 psi.

Results and Discussion
Virosart® HF meets the retention requirements of a high-performance parvovirus-retentive filter with minimal lot-to-lot variability. Figure 22 shows that the filter achieved robust LRVs of greater 5 even under challenging conditions. In most trials absolute retention was observed. The distribution results from the spike titers used in the experiments, as well as from the experimental conditions.

4.3 Robust Retention

4.3.1 Retention in Different Feed Streams

Feed streams to be processed by virus filtration are highly individual and can differ significantly from process to process. Feed stream can vary in parameters such as the molecule of interest (size, pI, hydrophobicity), impurity profile (aggregates, fragments, HCPs, DNA) and the formulation (buffer type, pH, conductivity, additives). Therefore, Virosart® HF was tested with respect to retention capabilities of PP7 in different model feed streams: Buffer, IVIG, and three mAb solutions.

Materials and Methods
Virosart® HF lab modules were challenged with PP7 spiked model feed solutions (challenge level > 5×10^7 pfu/mL). In total, five different feed solutions were tested at neutral pH. Retention data in buffer (20 mM KPi buffer, pH 7.2) and IVIG (1 g/L IVIG, 20 mM KPI buffer, pH 7.2). For mAb containing feed streams, mAb concentrations ranged from 4 – 12 g/L. All filtrations were performed at 2.0 bar | 30 psi standard operating pressure in duplicate runs.

Results and Discussion
Virosart® HF shows robust and high PP7 clearance for all tested model solutions with LRVs of above 6.

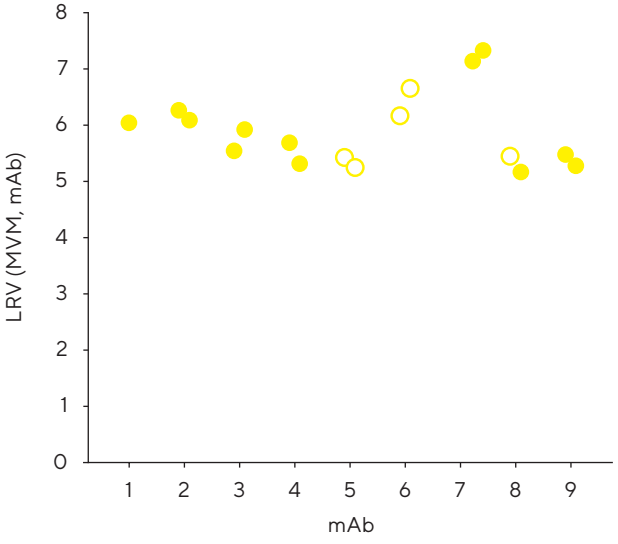

Figure 22: *Summary of MVM Retention From 10 Different Studies With Absolute Retention for Most Results*

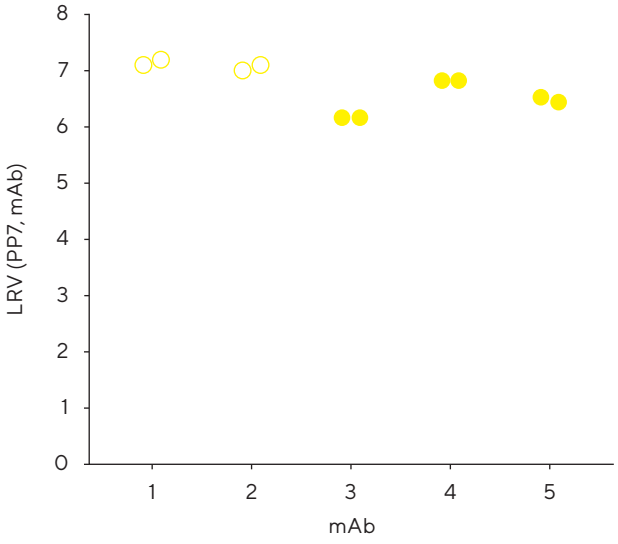

Figure 23: *Robust PP7 Retention in 5 Tested Model Feed Streams*

4.3.2 Virus Filter Intra- and Inter-Lot Retention Consistency

Understanding the intra- as well as inter-lot consistency of virus filters such as Virosart® HF with respect to virus retention is of especially high importance during validation studies as well as during clinical and commercial processing.

Materials and Methods

Product release data from randomly chosen virus filter membrane lots of Virosart® HF (5.0 cm^2) were collected. The filtrations were performed in constant pressure mode at 2.0 bar | 30 psi.

In the first experiment 48 Virosart® HF modules from eight virus filter lots were used for determination of PP7 retention in buffer (20 mM KPi, pH 7.2) using ≥ 10^8 pfu/mL in the feed (figure 24). Under conditions more challenging and representative for biopharmaceutical processes, the other 36 modules were used to determine PP7 retention in buffered IVIG (1 g/L IVIG, 20 mM, pH 7.2) at a flow decay of 75% using ≥ 10^8 pfu/mL in the feed (figure 25).

Results and Discussion

Robust intra- and inter-lot PP7 retention consistency was demonstrated for Virosart® HF. Retention exceeded 6 LRVs using buffer as well as under 75% flow decay.

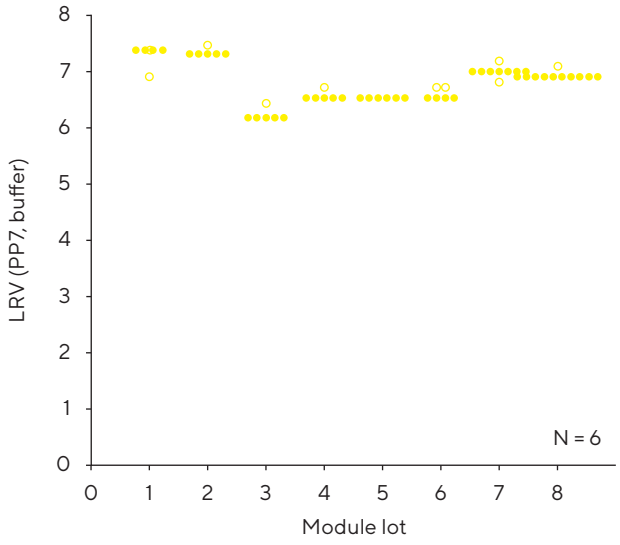

Figure 24: *Robust and Consistent PP7 Retention for Virosart® HF Lab Modules (5.0 cm^2) Confirmed for 8 Module Lots With 6 Filters Each Tested in Buffer*

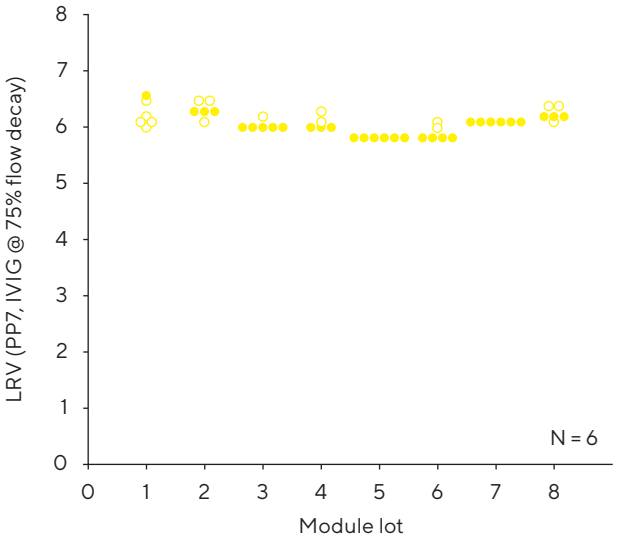

Figure 25: *Robust and Consistent PP7 Retention for Virosart® HF Lab Modules (5.0 cm^2) Confirmed for 8 Module Lots With 6 Filters Each Tested at 75% Blocking Using IVIG.*

4.3.3 Scalability of Retention

Virosart® HF lab modules are typically used for R&D, process scale-down work and virus validation studies. However, in commercial processing larger devices with larger filtration areas are used. It is, therefore, necessary to ensure robust and scalable retention across the entire size range of Virosart® HF filter family.

Materials and Methods

The scalability of PP7 retention was studied for the entire product family of Virosart® HF from lab (1.7 & 5.0 cm^2) through mid-scale (200 cm^2 & 0.2 m^2) to process scale (0.8 & 2.4 m^2). All filtrations were performed at a constant pressure of 2.0 bar | 30 psi. All modules were spiked with PP7 at a minimum concentration of 1.0×10^8 PFU/mL. PP7 was spiked into 20 mM KPI buffer pH 7.2 (figure 26) or into 1 g/L IVIG in KPI buffer (figure 27).

Results and Discussion

The scalability of PP7 retention has been demonstrated across the entire product family of Virosart® HF filters from lab (1.7 cm^2) up to process scale (2.4 m^2).

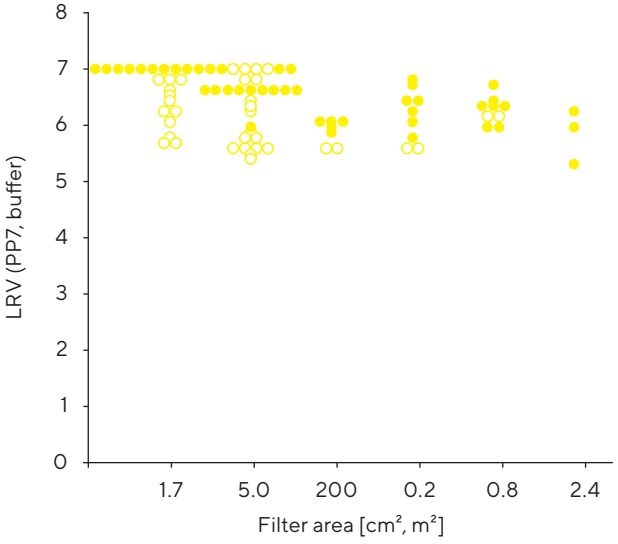

Figure 26: *PP7 Retention for the Whole Module Family of Virosart® HF in Buffer*

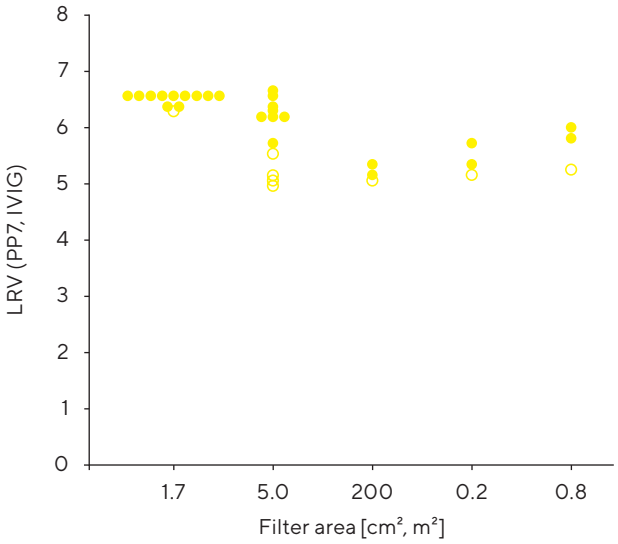

Figure 27: *PP7 Retention for the Whole Module Family of Virosart® HF in IVIG*

4.4 Retention Under Challenging Conditions

Recent investigations have shown that virus breakthrough can occur under specific conditions with certain virus removal membranes. Breakthrough is more likely to occur when the membrane is operated at high product throughput, at elevated flow-decay, under conditions of high | low process pressure, when the pressure is held and released, during pressure pulsation or at low pH or high salt concentrations. The following sections show results from virus retention experiments performed under all of these challenging conditions.

4.4.1 Flow Decay

Different virus filters show increased level of virus breakthrough at increasing level of flow decay of 75% or higher.

Materials and Methods
22 lab modules (1.7 cm^2) from different production lots were tested for their PP7 retention capabilities (challenge level > 10^7 pfu/mL) at increasing level of flow decay. Three fractions were taken, namely at 25%, 75% and 90% flow decay. The filtration was performed with IVIG (1 g/L, 20 mM, pH 7.2) at a constant pressure of 2.0 bar|30 psi.

Results and Discussion
PP7 retention was independent of the level of flow decay for Virosart® HF. LRVs of 5.9 and higher were achieved. No virus breakthrough could be detected under the conditions tested for most of the fractions taken.

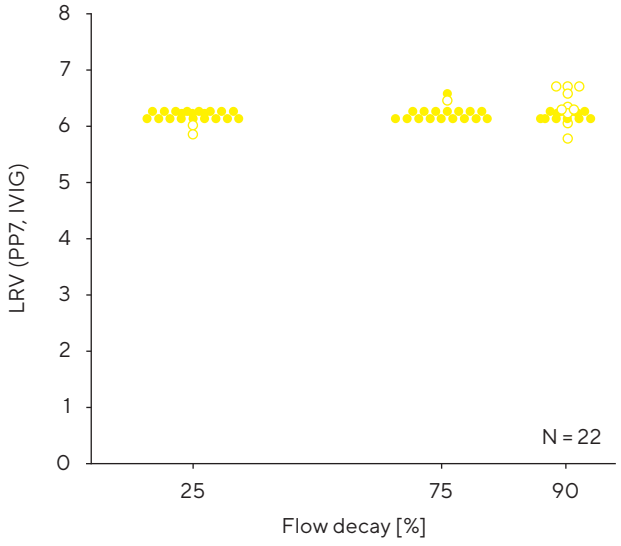

Figure 28: *PP7 Retention Tested at Different Levels of Flow Decay for Virosart® HF*

4.4.2 Different Operating Pressures

Virosart® HF virus filters are typically validated at 2.0 bar|30 psi operating pressure to meet standard processing requirements. The filters are released for operation up to 5.0 bar|72.5 psi operating pressure. Experiments have been performed to show that operating at low or high operating pressures does not affect the retention characteristics of Virosart® HF. The experiments have been performed with buffer only and with IVIG at 75% flow decay.

Materials and Methods
Retention capabilities were determined at low (0.1 bar|1.45 psi), normal operating pressure (1.0 bar|14.5 psi) and high (5.0 bar|72.52 psi) operating pressures (figure 29). 12 lab modules (5.0 cm^2) were tested for retention of the PP7 bacteriophage. PP7 (challenge level > 10^7 pfu/mL) was spiked in 20 mM KPI buffer, pH 7.2. A pooled fraction was taken after 5.0 mL (0.1 bar|1.45 psi) and after 50 mL (1.0 bar|14.50 psi and 5.0 bar|72.52 psi) of filtration.

Experiments were performed at 3 different operating pressures of 1.0 bar|14.5 psi, 3.0 bar|43.5 psi, 4.0 bar|58 psi (figure 30). 20 lab modules (5.0 cm^2) from one production lot were tested for retention with a buffered IVIG (1g/L) solution spiked with PP7 (challenge level > 10^7 pfu/mL). A pooled fraction was taken after 75% flow decay.

Results and Discussion
Under the condition tested, no significant impact of the different operating pressures on virus retention capabilities was observed in buffer and at 75% flow decay. LRVs of approximately 6 for low, moderate and high pressures were demonstrated, as shown in figure 29. Robust LRVs of 5 or greater were demonstrated at 75% flow decay (figure 30).

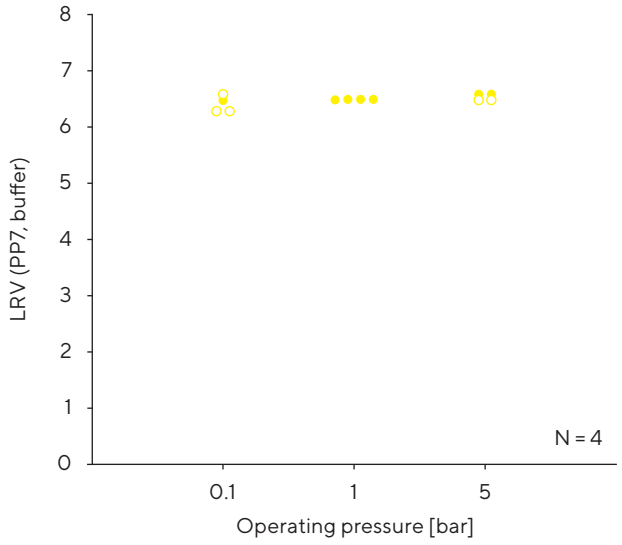

Figure 29: *Different Operating Pressures of 0.1 bar, 1.0 bar and 5.0 bar Tested on PP7 Retention for Virosart® HF*

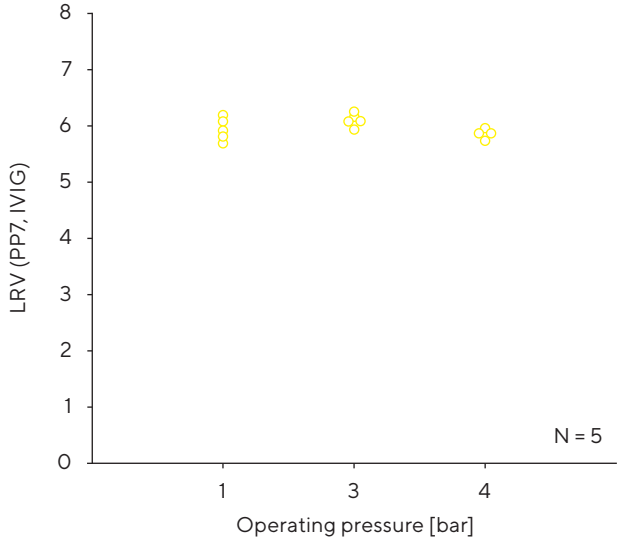

Figure 30: *Different Operating Pressures on PP7 Retention at 75% Blocking Tested for Virosart® HF*

4.4.3 Pressure Release

During commercial processing pressure release occurs on a regular basis e.g. if the processing media is changed. Data has shown that virus breakthrough is more likely to occur with some virus filters when the pressure is released. For this reason, a pressure release cycle is often included in spiking studies to show the retentive capabilities of the virus filter under this processing condition.

Materials and Methods

Five lab modules (5.0 cm^2) from one production lot were tested for their PP7 retention capabilities. PP7 (challenge level > 1×10^7 pfu/mL) were spiked in 20 mM KPI buffer, pH 7.2. A 5-minute pressure release was performed and four pool fractions were taken. These fractions were taken after 30, 40, 140 and 150 L/m^2 of filtration. The filtration was performed at 2.0 bar | 30 psi operating pressure.

Results and Discussion

High LRVs of above 5.5 are shown in figure 31 for all fractions, even after pressure release. Further studies were performed with 5 g/L mAb model solution, which confirmed these results. (6)

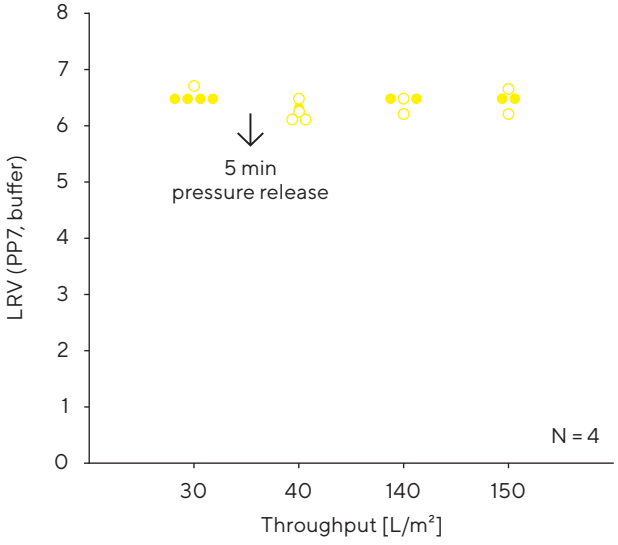

Figure 31: *Impact of Pressure Release on PP7 Retention of Virosart® HF*

4.4.4 Pressure Pulsation

Compressed air is typically used as a pressure source to run a virus filter in stainless steel, but pumps can also be used. In single-use manufacturing peristaltic pumps are commonly used when performing virus filtrations with single-use assemblies in order to operate at low back pressure. Peristaltic pumps can create pressure pulsation which could potentially impact retention performance of the virus filter. Therefore, the retention performance of Virosart® HF filters was assessed using a peristaltic pump as the fluid delivering force.

Materials and Methods

Two lab modules (5.0 cm^2) from one production lot were tested for their ability to retain PP7 at a challenge level of 1×10^7 pfu/mL in 20 mM KPI buffer, pH 7.2. A Watson Marlow 520 peristaltic pump was used with pulsation of ± 0.3 bar | ± 4.4 psi at medium operating pressure of 1.4 – 1.5 bar | 20.3 – 21.8 psi. This was deemed a worst-case scenario for pump pulsation. Fractions were taken after 30, 100, 200 and 300 L/m^2 of filtration.

Results and Discussion

No impact of pressure pulsation on the retention for Virosart® HF observed. No virus breakthrough was detected and overall LRVs were above 6.5. However, we recommend pumps with lower pulsation (e.g. 4 piston diaphragm pump) in commercial processing due to the shear sensitivity of most proteins.

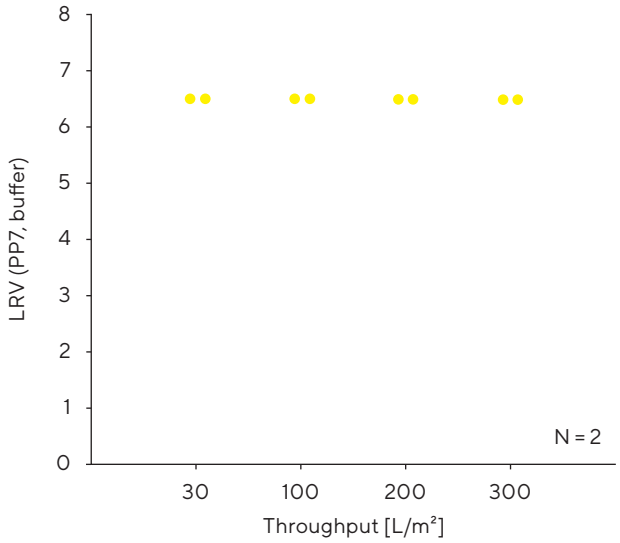

Figure 32: *The Impact of Pressure Pulsation Tested on PP7 Retention*

4.4.5 High Virus Load

Some virus retentive filters on the market show the tendency for increased virus breakthrough with increasing virus load on the virus filter. In this experiment the impact of PP7 virus load on the retention of Virosart® HF was evaluated.

Materials and Methods

Six Virosart® HF lab modules (1.7 cm^2) from one lot were used in these experiments. The filters were challenged with 20 mM KPi buffer, pH 7.2 spiked with PP7 at a high challenge level of 2.5×10^8 Pfu/mL. In total, four pool fractions were taken after 60, 350, 900 and 1000 L/m^2 of filtration. The fractions taken are representative for different overall virus loads 1.5×10^{13}, 9.4×10^{13}, 2.4×10^{14}, 2.6×10^{14} pfu/m^2. The filtration runs were performed at a constant pressure of 2.0 bar | 30 psi.

Results and Discussion

Robust virus retention at increased virus load observed with Virosart® HF. No impact of virus load on PP7 retention of Virosart® HF was observed under the conditons tested even with a high PP7 load of 10^{14} pfu/m^2 as shown in figure 33.

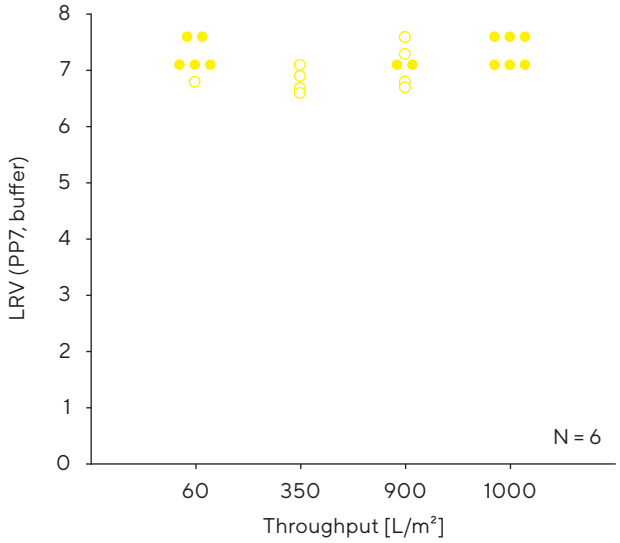

Figure 33: *Impact of High Load on PP7 Retention Investigated*

4.4.6 Low pH & High Salt Concentration

The feed streams processed during the virus retentive step typically have neutral pH values and low salt concentration. Feed streams at low pH values and high salt concentrations are rare. However, some virus filters have shown increased virus breakthrough under these conditions. Analogous to the high virus load experiments at neutral pH (see section 4.4.5), the impact of low pH and high salt concentration was evaluated at similarly challenging high virus loads.

Materials and Methods

Four Virosart® HF lab modules (1.7 cm^2) were tested for their retention capabilities under two different conditions. The filters were challenged with acetate buffer at pH 4.3 with a high salt concentration (200 mM). PP7 bacteriophage was spiked at a challenge level of 1×10^8 pfu/mL. Different fractions were taken as the virus loading level increased from 1.6×10^{13} to 4.7×10^{14} pfu/m^2. These are representative for throughputs of 60, 350, 950 and 1200 L/m^2. The filtration runs were performed at a constant pressure of 2.0 bar | 30 psi.

Results and Discussion

No impact of worst-case conditions on retention was observed. The retention at low pH and high salt concentration, high product throughput and high PP7 challenge level was above 6 LRV.

4.4.7 Detergents

Some feed streams may contain surface active ingredients such as detergents. In this study, the effects of detergents on the retention capabilities of Virosart® HF were evaluated using two representative solutions containing Tween 80 or SDS.

Materials and Methods

Virosart® HF lab modules (1.7 cm^2) from one production lot were tested for their ability to retain PP7 at a challenge level of greater than 1×10^8 pfu/mL in two model solutions containing detergents: 0.1% Tween 80 in 20 mM KPI pH 7.2 and 0.1% SDS in 20 mM TRIS pH 10.2. Four fractions were taken, which included a pressure release of 20 minutes. Each solution was tested with four modules each. The filtration runs were performed at 2.0 bar | 30 psi operating pressure.

Results and Discussion

No impact of detergents on the retention of Virosart® HF could be detected. LRVs of more than 7 were observed even though the two model feeds contained either Tween 80 or SDS.

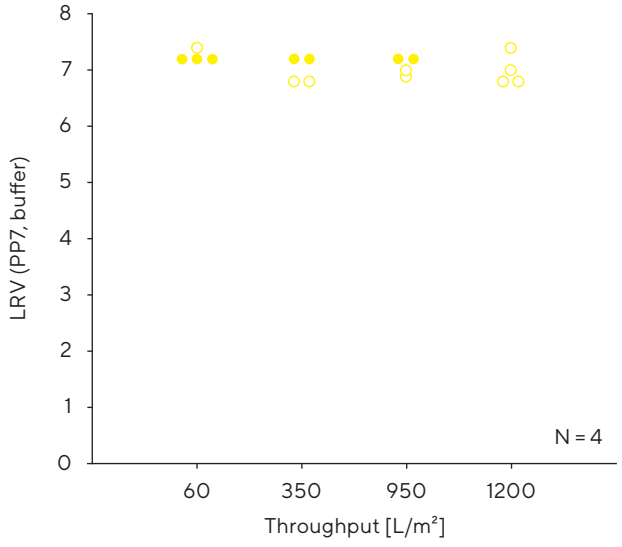

Figure 34: *Retention at Low pH and High Salt Concentration During High Challenge Load*

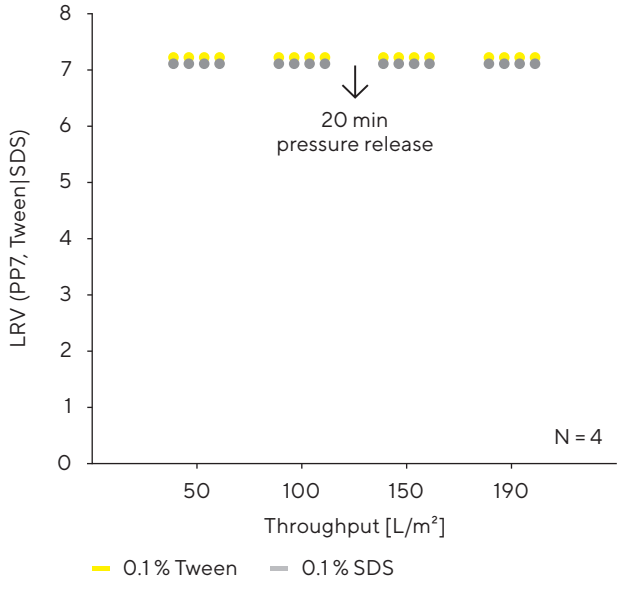

Figure 35: *Impact of Surface Active Ingredients Such as SDS and Tween on PP7 Retention of Virosart® HF*

4.5 Simulation of Transportation Conditions

Testing was performed to simulate the transportation of filter modules from the location of their production to their location of use.

4.5.1 Drop-Test

A drop-test is part of the shipping validation performed in accordance to guideline ASTM 7386. During this test the final shipping box is dropped in a strictly defined way from a specified height (1 m | 3.3 ft).

Materials and Methods
12 gamma irradiated lab modules (5.0 cm^2) from one production lot were tested for their ability to retain PP7 at a challenge level of 2.1×10^8 Pfu/mL in 20 mM KPI buffer, pH 7.2 after they have been dropped from 1 m | 3.3 ft height. The filtration process was perfromed at a constant pressure of 2.0 bar | 30 psi. A pool fraction was taken after 100 L/m^2 of filtration volume.

Results and Discussion
No effect of the drop test on PP7 retention was observed. LRVs of greater than 5.5 were demonstrated for all filters tested (figure 36). However, it is not recommended to drop the boxes. (6)

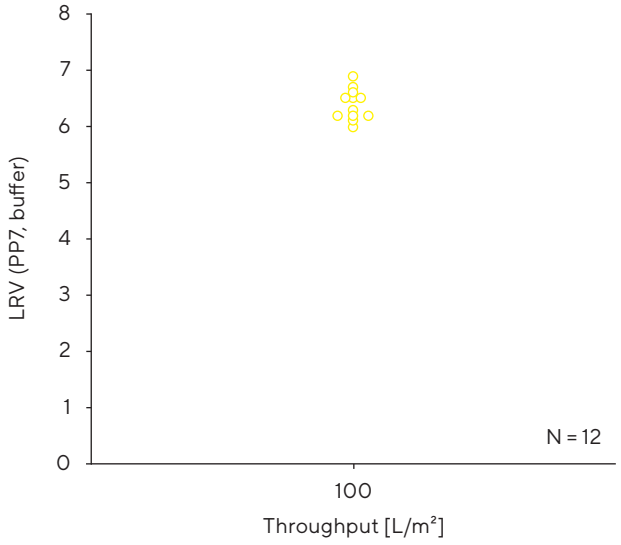

Figure 36: *Effect of Drop-Test on PP7 Retention Studied With 5.0 cm^2 Lab Modules*

4.5.2 Thermal Stress

The effect of temperatures lower than 0 °C | 32°F, that may occur during the transportation and storage of Virosart® HF filters, was evaluated with respect to retention performance

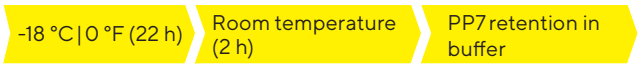

Figure 37: Simulation of thermal stress during transportation

Materials and Methods
Four lab modules (5.0 cm^2) from one production lot in double protective plastic bags were stored at –18 °C | –0.4 °F for 22 hours followed by storage at room temperature for two hours. This process was repeated for 3 cycles (figure 38). PP7 retention (challenge level > 10^8 Pfu/mL) was then determined in 20 mM KPI buffer at pH 7.2. The filtration was performed at 2.0 bar | 30 psi constant pressure. A pool fraction was collected after 100 L/m^2 of filtration.

Results and Discussion
No effect of thermal stress conditions on PP7 retention was observed. Robust LRV of above 6 is shown. However, note that the shelf life is validated under the storage conditions described in the validation guide (6).

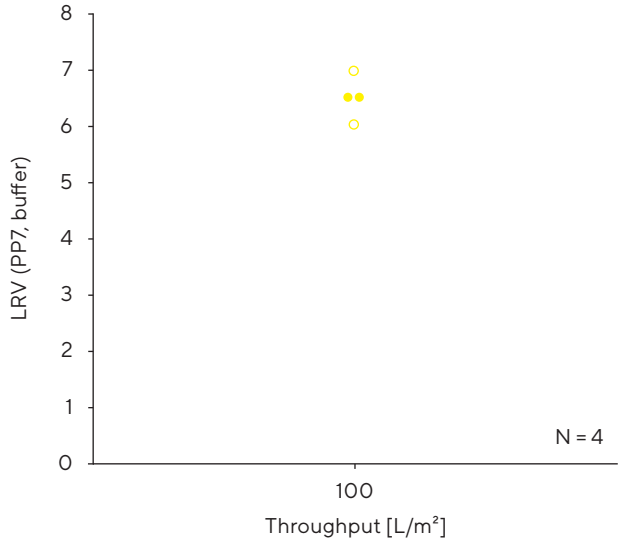

Figure 38: *Effect of Thermal Stress Transport Condition on PP7 Retention Investigated*

4.5.3 Mechanical Stress

Plastics show an increased brittleness at low temperatures which represents the worst case condition during mechanical stress tests. Therefore, the effect of mechanical stress conditions during transport and storage on the retention performance of Virosart® HF filters was evaluated.

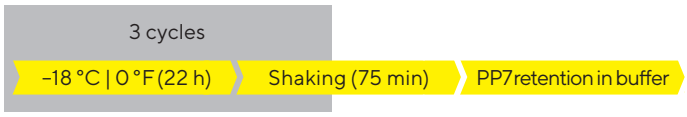

Figure 39: Simulation of mechanical stress transport condition

Materials and Methods
Four lab modules (5.0 cm²) from one production lot were stored at – 18 °C | 0 °F before shaking on a laboratory shaker for 75 minutes (without packaging). PP7 retention was assessed at a challenge level > 10^8 Pfu/mL. The PP7 virus was solublized in 20 mM KPI buffer at pH 7.2. The filtration was performed at a constant pressure of 2.0 bar | 30 psi . A pool fraction was taken after 100 L/m².

Results and Discusion
No effect of mechanical stress condition on PP7 retention was observed. Robust LRV of above 5.9 is shown. However, note that the shelf life is validated under the storage conditions described in the validation guide (6).

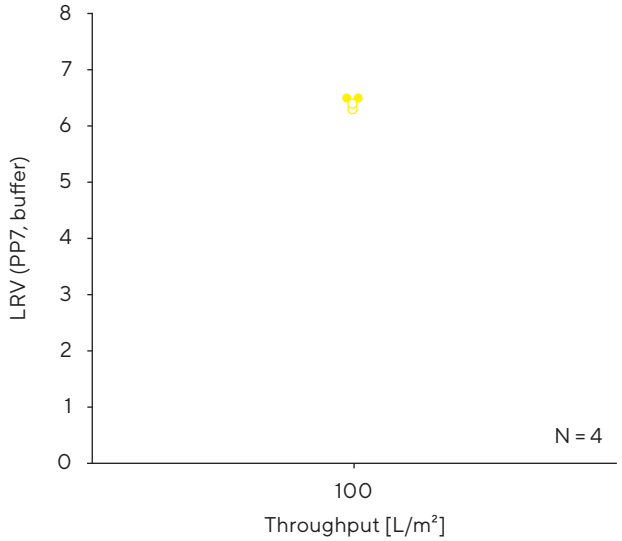

Figure 40: *No Effect of Mechanical Stress Transport Condition on PP7 Retention*

5. Integrity Test

5.1 Definition

Virus retentive filters such as Virosart® HF are integrity tested based on a diffusion test. A constant test pressure is applied on the upstream side of the wetted membrane (figure 41). A pressure drop is created on the upstream side as the air molecules are diffused through the membrane towards the downstream side of the filter. The Sartocheck® filter integrity tester is measuring the pressure drop on the upstream side. Integrity test is performed at one defined test pressure of 4.5 bar | 65 psi with the test criterion of a max. diffusion rate. The IT has passed when the measured diffusion rate does not exceed the limit value.

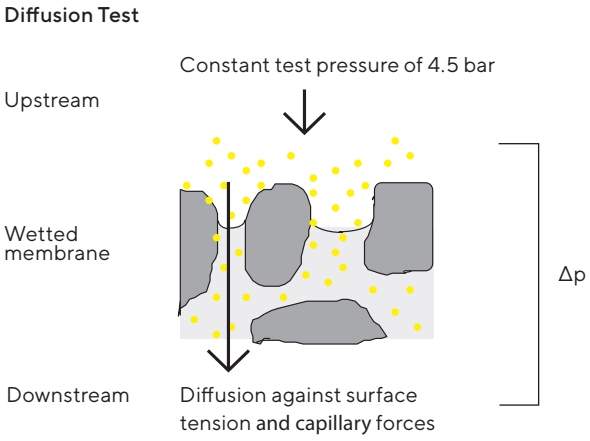

Figure 41: *Schematic Drawing of the Integrity Test Based on Diffusion. Applying Test Pressure to Upstream Side Results in Gradient From Upstream Side to Downstream Side, Thus a Net Flux in the Direction of Filtration. This Situation Is Relevant for Integer Devices, Where the Bubble Point of the Membrane Has Not Been Reached by the Test Pressure.*

5.2 Reasons

An appropriate measurement method is required using non-destructive techniques that facilitate a statement with respect to filter integrity. In addition, integrity testing of virus retentive filters is necessary to

- fulfill regulatory requirements
- confirm integrity of the filter element prior to and after use
- detect system leaks in the filtration system
- prevent contamination events

The FDA "Guidelines on Sterile Drug Products Produced by Aseptic Processing", state: "After a filtration process is properly validated for a given product, process and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. Normally, integrity testing of the filter is performed after the filter unit is assembled and sterilized prior to use. More importantly, however, such testing should be conducted after the filter is used in order to detect any filter leaks or perforations that may have occurred during filtration." (11)

Further a correlation between bacteriophage PP7 retention of Virosart® HF filters and a non-destructive integrity test has to be established in order to ensure filter reliability prior to using the filter.

5.3 Correlation of IT and PP7 Retention

A correlation is established between destructive bacteriophage PP7 retention and non-destructive integrity test for Virosart® HF. Therefore a positive and a negative correlation was performed during product validation to confirm the integrity test value.

- Positive correlation: IT passed and retention passed
- Negative correlation: IT not passed and retention not passed (performed with limiting filter samples)

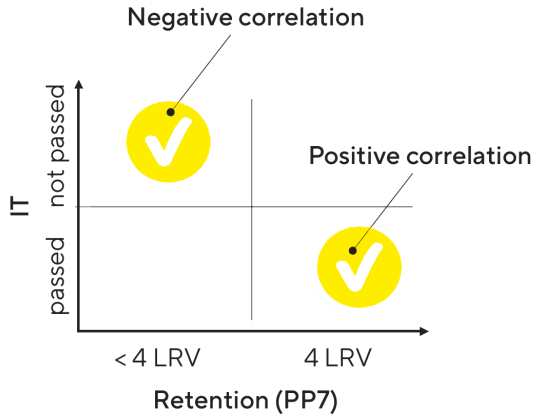

Figure 42: *Correlation of Integrity Test and PP7 Retention for Virosart® HF by Positive and Negative Confirmation of Correlation*

Materials and Methods
Virosart® HF process modules (0.8 m^2) from different production lots were tested for their retention capability of the bacteriophage PP7. The negative correlation was performed with limiting filter samples.

Figure 43: Test set-up for correlation of PP7 and IT

As described in figure 43 the filters were wetted in the flow through mode for > 20 minutes at an inlet pressure of 2.5 bar | 36 psi against an outlet pressure of 2.0 bar | 30 psi. After wetting, the modules were tested for integrity by a diffusion test at 4.5 bar | 65 psi. Next, the elements were then challenged with a minimum of 10^7 PP7/mL in 20 mM potassium phosphate buffer (KPi), pH 7.2 at 2.0 bar | 30 psi. Fractions for LRV were collected after 10 liters of filtration. Finally, an integrity test based on diffusion was performed post PP7 retention test at 4.5 bar | 65 psi.

Results
Positive (grey) and negative correlation (yellow) confirm IT-limit of 20 mL/ min for Virosart® HF process modules (0.8 m²). The diffusion data presented in figure 44 was recorded post-use.

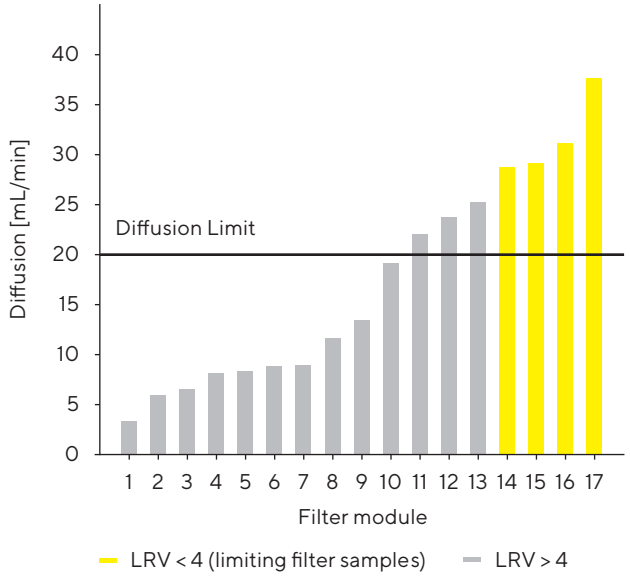

Figure 44: *Correlation of PP7 Retention and Diffusion Value for Virosart® HF Process Modules (0.8 m²)*

6. Abbreviations

DNA	Deoxyribonucleic acid
HCP	Host Cell Protein
IVIG	Intravenous immunoglobulin
IgG	Immunogamma Globulin
IT	Integrity tested
KPI	Potassium Phosphate
LRV	Log reduction value
mAb	Monoclonal antibody
MuLV	Murine Leukemia Virus
MVM	Minute Virus of Mice
Pfu	Plaque forming units
PES	Polyethersulfone
SDS	Sodium dodecyl sulfate
WFI	Water for injection

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