



Life Sciences

Application Note

USD 2778

Filterability Testing and Virus Challenge of Pall® Minidisc Capsules with Pegasus™ SV4 Virus Removal Filter Membrane



Contents

| | |
|---|-----------|
| 1. Introduction..... | 3 |
| 1.1 Minidisc Capsule and Associated Equipment..... | 3 |
| 2. Filterability and Protein Transmission Trials..... | 5 |
| 2.1 How to Run a Filterability Trial | 5 |
| 2.2 Forward Prediction of Throughput Using V_{max} Analysis | 5 |
| 2.3 Typical Filterability Results | 7 |
| 2.4 Scale-Up | 8 |
| 2.5 Constant Flow Operation | 8 |
| 2.6 Protein Transmission | 8 |
| 2.7 Accounting for Minidisc Capsule Hold-Up Volumes | 8 |
| 2.8 Calculating Flux | 9 |
| 3. Virus Spike Challenge Testing | 10 |
| 3.1 How to Run a Virus Spike Challenge Test | 10 |
| 3.2 General Protocol Recommendations | 10 |
| 3.3 Typical Virus Spike Challenge Results | 11 |
| 4. Key Virus Filtration Factors..... | 12 |
| 4.1 Operating Differential Pressure | 12 |
| 4.2 Throughput / Processing Time..... | 13 |
| 4.3 Temperature and Viscosity | 13 |
| 4.4 pH and Ionic Strength | 13 |
| 4.5 Product Aggregation | 13 |
| 4.6 Protein Concentration | 14 |
| 4.7 Pre-filtration | 14 |
| 4.8 Summary of Recommended Design Space Using Pegasus SV4 Filters | 15 |
| 5. References..... | 16 |

1. Introduction

Of the available viral clearance (inactivation and removal) strategies, filtration is a robust technique that generally is not susceptible to minor changes in process conditions. Filtration's size exclusion mechanism complements any other inactivation or removal technique selected, as filtration targets a different property of a virus (physical dimensions). This allows for process designs based on orthogonal methods to achieve a high degree of virus safety. To date, virus filtration has demonstrated a high removal efficacy among virus clearance technologies, and has become a well-accepted orthogonal method for the clearance of contaminant and potential contaminant viruses.

Pegasus SV4 virus removal filters can provide a robust, economical solution for clearance of small non-enveloped viruses and large enveloped viruses, offering high throughput and providing constant, stable flow rates in both dilute and complex, concentrated biological fluids without dilution. These outstanding properties provide minimal flow decay over a wide range of process conditions and extended process times. This allows a much-improved degree of process control, as well as control of process costs, to provide maximum virus filtration efficiency and economy. This robust and efficient performance helps to qualify Pegasus SV4 virus removal filters as a 'platform technology'.

This application note provides assistance in filter qualification, validation and process development. It describes best practises for how to conduct Filterability Testing and Virus Spike Challenges.

To utilize the full performance of Pegasus SV4 virus filters, and to maximize process performance, Pall recommends working inside the design space recommended in this application guide, which is based on experience from a number of case studies carried out with a variety of biopharmaceutical manufacturers, and Pall internal application studies.

This guide is separated into three main sections:

Section 2 – Filterability and Protein Transmission Testing

- Filterability and protein transmission trials are often conducted initially to determine the flow, capacity and protein passage through a virus filter with a nutrient additive or product intermediate feed stream. The results show the potential throughput that a virus filter can provide during processing and can aid in projection of scale-up sizing and process cost modelling.

Section 3 – Virus Spike Challenge Testing

- Viral clearance validation is required to confirm that suitable virus retention can be achieved in process fluid under desired process conditions.

Section 4 – Key Virus Filtration Parameters

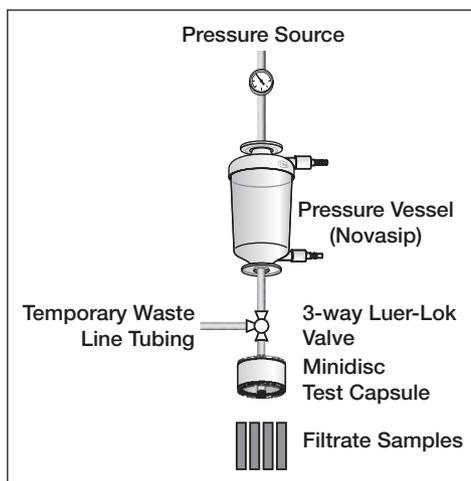
- A good understanding of the critical parameters involved in virus filtration and the typical Pegasus SV4 filter design space is required to ensure successful optimisation and robust virus validation.

1.1 Minidisc Capsule and Associated Equipment

The Minidisc capsule is a pre-assembled disposable filter capsule assembly incorporating Pall virus filter membranes. It is an effective and simple-to-use device for all small-scale virus filter testing requirements. This application note will guide the user in the best approaches for filterability, protein transmission testing and virus challenges of Pall membranes in the Minidisc capsule.

Details of how to use Minidisc capsules are contained in the product's Instructions for Use (Pall publication USD 2877), which are provided in every product box. Care should be taken to follow these instructions closely to ensure successful operation. The following are some key specifications that are relevant to planning experiments and analysing data generated with the Minidisc capsule.

Figure 1
Laboratory equipment to use



Recommended Lab assembly (list below) Pegasus SV4 Minidisc capsules

Table 1
Recommended Equipment Details

| Part | Pall Part No. | Supplier |
|---|---|--------------|
| Novasip pressure vessel | C3EP1 | Pall |
| Sealkleen Pressure vessel (optional) | ZLK702G23LHKH4 | Pall |
| Adapter 1 in. TC/Male Stäubli connector | GFX0290 (two recommended for inlet and outlet) | Pall |
| TC clamp + silicone gasket (two each per part number) | SLK1TC23H4 | Pall |
| Female Stäubli fitting for inlet pressure tubing (6 mm O.D.) | GFX0236 | Pall |
| Tubing* | - | Lab Supplier |
| Luer lock valve and hosebarb adapters as required | - | Lab Supplier |
| Graduated cylinder | - | Lab Supplier |
| Pall Minidisc Capsules with Pegasus SV4 Virus Removal Filter Membrane | 10MCFSV4 (Pack of 3) | Pall |



Caution: All the upstream tubing and connectors used should be rated to at least 4.0 bar (58 psi).

Table 2
Key Specifications of the Minidisc Capsule

| | |
|---|---------------------|
| Effective Filtration Area (EFA) | 9.6 cm ² |
| Maximum Operating Pressure | 3.1 barg (45 psig)* |
| Maximum Recommended Differential Pressure | 3.1 bard (45 psid)* |

*Temporary pressures up to 3.4 barg (50 psig) are acceptable, but the target operating and differential pressures should not exceed 3.1 bar (45 psi) to allow for pressure fluctuations during testing. If tests at operating pressures exceeding 3.1 barg (45 psig) are required, a stainless steel disc holder and 47 mm membrane discs are available (Pall disc holder part number: FTK200; discs part number FTKSV404705 and FTKSV4047025 for a box of 5 or 25).

2. Filterability and Protein Transmission Trials

2.1 How to Run a Filterability Trial

For best results, a filterability trial should be conducted using a calibrated balance (accuracy ≤ 0.1 g) to collect and measure the filtrate mass over time. 1 g.mL^{-1} is a sufficiently accurate estimation of density for water and simple buffers, but the user should determine their product feed density if this is believed to be significantly different from water. The temperature should be kept at the same level as the full-scale process where possible, in order to give the correct product viscosity.

A buffer conditioning step can be used if desired, to reduce the risk of aggregate formation at the water-product interface of the pre-wetted membrane. A buffer flush of ≥ 3 mL will be sufficient to condition the membrane if water is drained from the upstream of the Minidisc assembly before processing the buffer. If buffer flux needs to be determined, measure the buffer flow rate over 10 minutes. Follow the procedures and instructions for water flow testing (in USD 2877), ensuring that at no point the Minidisc capsule runs dry.

We strongly recommend that filterability trials are run to full processing time for the most accurate estimation of performance. If time or product volume constraints are in place, then the longest processing time possible should be used and results forward-predicted using the V_{\max} model to estimate throughput.

2.2 Forward Prediction of Throughput Using V_{\max} Analysis

V_{\max} is the estimated value of the maximum capacity of a membrane^[1], i.e. the throughput that would be reached when the membrane is completely plugged, if time and feed quantity were not restricted and the membrane fouls in line with the standard pore constriction model.

V_{\max} is calculated from a plot of time over throughput (At/V) against time (t) and is the inverse of the gradient, as shown by the linear form of the standard blocking law equation below:

Equation 1

Constant Pressure V_{\max} Linear Equation

$$\frac{At}{V} = \frac{t}{V_{\max}} + \frac{1}{J_0}$$

A = filtration area (m^2), t = time (h), V = volume (L),
 V_{\max} = estimated maximum throughput capacity (L.m^{-2}), J_0 = initial flux ($\text{L.m}^{-2}.\text{h}^{-1}$).

The gradient should be determined from the linear portion of the graph only, as indicated in Figure 1. The initial flux should also be calculated from the same linear portion of the graph and is evaluated as the y-intercept of that linear data. This initial flux is not always as accurate as direct flux measurement, but will give a suitable forward prediction in many cases. Equation 1 can then be rearranged to estimate the throughput achieved at a given time:

Equation 2

Forward Prediction of Throughput at Constant Pressure

$$\frac{V}{A} = \frac{t}{\left(\frac{t}{V_{\max}} + \frac{1}{J_0}\right)}$$

Figure 2
Data Analysis for Forward Prediction

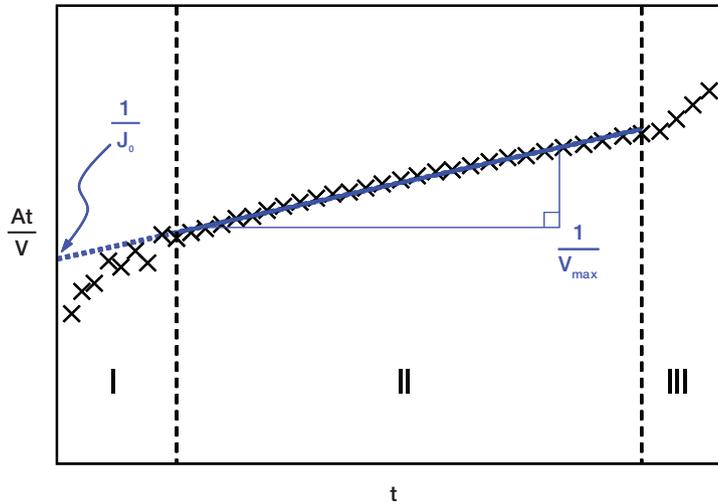


Figure 2 shows typical data collected during a filterability run, showing three phases of data collection.

- I – Start-up effects cause inaccurate and variable data due to the low flux decay relative to measurement accuracy and start time accuracy. The slope can be under or over-predicted and the time for this phase will vary between tests
- II – The linear portion of the graph used to determine V_{max} and J_0 for forward prediction
- III – End effects, only seen if the feed sample is filtered to completion and flow reduces to zero due to the feed running out

Caution should be taken when forward predicting. The following conditions should be adhered to, in order to minimize estimation errors:

- Forward predicted throughput no more than twice the measured throughput
- Forward predicted throughput < 90 % of the calculated V_{max}
- Coefficient of determination (R^2 value) > 0.95

The closer the raw data collection time is to the estimation time, the more accurate the estimated throughput will be. Fouling is more complex than a simple constriction mechanism, however, the model is the most appropriate of all the traditional membrane fouling mechanisms^[2] for small forward-predictions of limited data sets. Using V_{max} to forward-predict throughput relies on the assumption that the gradient measured from the At/V vs. t plot remains constant up to the estimation time. The V_{max} value itself should therefore be quoted as a maximum capacity with caution, since this definition is based on the assumption that the gradient remains constant until complete blockage. This is often a long and potentially very inaccurate extrapolation for high capacity membranes such as Pegasus SV4.

In general, V_{max} values for Pall virus removal filters are very high and exceed the throughput that can be reached in typical processing times. Most of the time membrane performance (batch area requirement) is either independent or weakly dependant on V_{max} and batch area requirement is governed by the processing time and membrane initial flux. For high V_{max} values, Pall does not recommend performance comparisons using V_{max} , whereas for cases when the membrane is plugged V_{max} can potentially be quoted with caution as highlighted above.

Contact Pall for more advanced fouling analyses if you believe that the model does not fit the raw data.

2.3 Typical Filterability Results

For filterability testing, proprietary monoclonal antibody solutions (MAbs) were used and a commercially available human gamma globulin (hlgG) solution was processed to represent plasma-derived product applications.

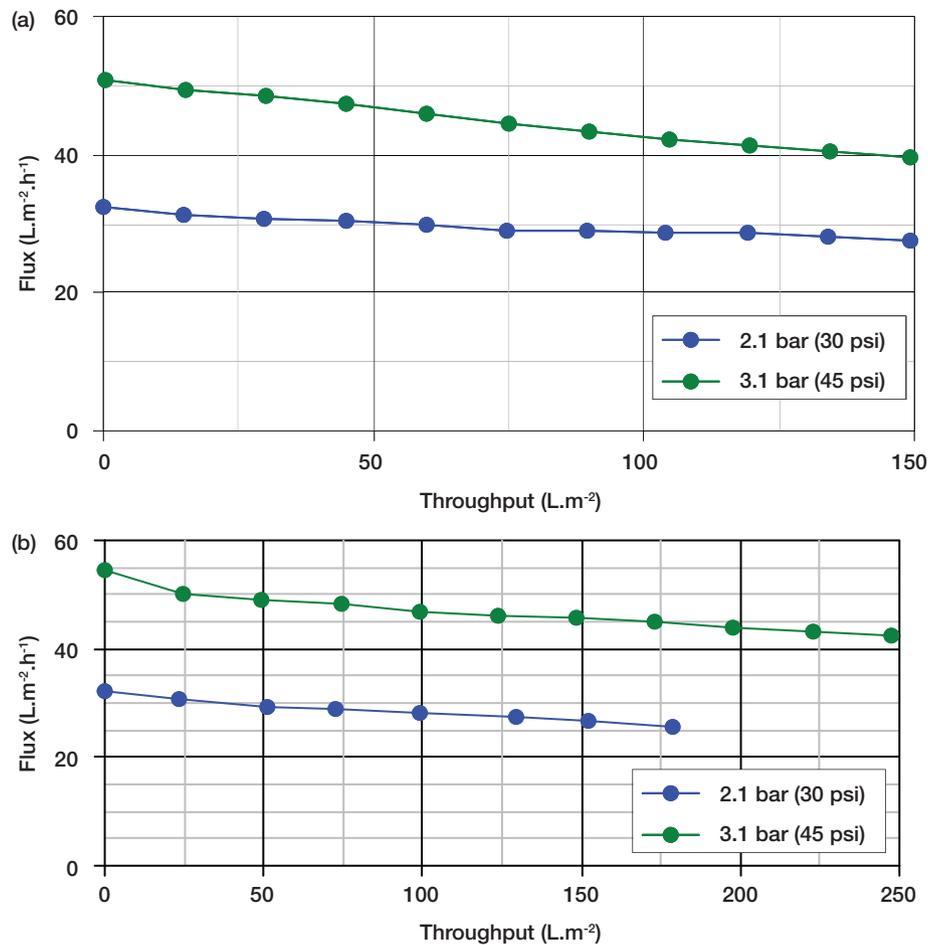
Figure 3(a) shows a typical flux profile for Pegasus SV4 membrane challenged with MAb solution. The fluxes remain constant throughout the experiment for both operating pressures. Increasing the operating pressure for Pegasus SV4 membranes to 3.1 bar (45.0 psi) yields higher flux without any negative impact on flux decay. Pall therefore recommends using an operating pressure of 3.1 bar (45.0 psi) to achieve the maximum flux performance. Results are typical for multiple MAb solutions tested, up to 25 g.L⁻¹.

Figure 3(b) demonstrates the robust nature of the Pegasus SV4 membrane tested in plasma-derived protein solutions, again showing that steady fluxes can be achieved at high operating pressures. The flux decay rates per unit mass are comparable to customer testing carried out with high purity IVIG solutions.

Due to complexities including process impurity levels, buffer conditions and donor profiles, results from plasma sources vary significantly from product to product and therefore direct comparisons must be made using your own specific feed solutions.

Figure 3

Increasing Performance at Higher Operating Pressure – Typical Flux Profiles for Pegasus Grade SV4 Membrane Filterability Tests at 2.1 bar (30 psi) or 3.1 bar (45 psi) with (a) 10 g.L⁻¹ (1 %) hlgG, (b) up to 25 g.L⁻¹ (2.5%) MAb



2.4 Scale-Up

Table 3 shows the relative effective filtration areas for scaling calculations.

Table 3

Pegasus SV4 Filter Scaling Calculations

| 47 mm disc (used with FTK200) Effective Filter Area (m ²) | Minidisc Capsule Effective Filter Area (m ²) | 10 in. Cartridges AB1USV4 Effective Filter Area (m ²) |
|--|---|--|
| 1.11×10^{-3} | 9.6×10^{-4} | 2.25 |

2.5 Constant Flow Operation

Pall recommends that small-scale filterability tests and virus validation studies are carried out at constant pressure. Difficulties in maintaining a constant flow and accurately measuring an increasing test pressure will typically generate more experimental noise than a constant pressure test and the results will be less reliable. Therefore constant pressure testing will always be preferable.

The flow decay for Pegasus SV4 membrane is slow and steady and therefore there is no difference seen between constant flow and constant pressure testing. Permeability (L.m⁻².h⁻¹ per unit pressure) decays relative to the product throughput and is independent of the pressure applied. Therefore using constant flow (or step-wise increases in the flow rate) to eliminate fouling due to high initial flow, as may be required in certain sterile filter applications, is not necessary for Pegasus SV4 virus filtration. What may appear to be a lower flux decline will actually be an equivalent permeability decline.

If required, the key to successful constant flow operation is a pump that is capable of supplying the required flow rates accurately up to the maximum test pressure, without pump slippage or pulsing of flow/pressure.

2.6 Protein Transmission

Protein transmission studies can be conducted concurrent with filterability testing by collecting samples of feed solution before and after filtration and subjecting them to protein assays.

Where the target protein is the major protein species, either a generic protein assay or a target protein-specific assay can be applied. Other assays may be employed on the filtrate to assess conformation, biological or enzymatic activity, as appropriate.

Typical protein transmission is >95 %, although the exact level will depend on the product concentration, quality, stability and process throughput. Care should be taken not to underestimate protein transmission from the Minidisc capsule by considering the hold-up volumes of the Minidisc capsule as described in Section 2.7.

2.7 Accounting for Minidisc Capsule Hold-Up Volumes

During normal operation there will be a small volume of liquid within the Minidisc capsule that remains in the system when switching from one process fluid to the other. The total hold-up volume is 2.1 mL. The downstream hold-up volume is 0.7 mL.

Water or buffer will make up 2.1 mL of the initial filtrate collected from the filter. For virus challenges the effect on retention will be negligible, with an error of 0.01 in the LRV at the recommended aliquot volume of 100 mL. For any small grab sample taken directly from the capsule and not the pooled filtrate, the recovery will be accurate if taken after the first 2.1 mL of filtrate. Again the throughput at which these retentions and recoveries are quoted should be adjusted to account for hold-up volume, and this is detailed in Table 4.

For protein transmission the hold-up volumes will have a greater impact – particularly at lower volumes. Grab samples taken after the first 5 mL of filtrate will provide accurate assessments of the protein transmission, but for total pool measurements and full mass balance the total protein input must be based on a value that is. Alternatively an FTK200 disc holder and 47 mm disc can be used for more accurate transmission assessments.

Table 4
Throughputs at various filtrate volumes

| Volume Collected (mL) | Throughput (L/m ²) | Adjusted Throughput (L/m ²) to Account for Hold-Up Volumes (For Protein Transmission / Viral Retention) |
|-----------------------|--------------------------------|---|
| 1.0 | 1.0 | 0.0 |
| 2.1 | 2.2 | 0.0 |
| 10.0 | 10.4 | 8.2 |
| 25.0 | 26.0 | 23.9 |
| 50.0 | 52.1 | 49.9 |
| 100.0 | 104.2 | 102.0 |
| 150.0 | 156.3 | 154.1 |
| 200.0 | 208.3 | 206.1 |
| 300.0 | 312.5 | 310.3 |
| x | x | (x – 2.1) |
| | <u>0.96</u> | <u>0.96</u> |

2.8 Calculating Flux

When aliquots are taken, the flux (L.m⁻².h⁻¹) for that aliquot is simply the total unadjusted throughput (L.m⁻²) divided by the time (h). If continuous data is collected using a balance then the flux can be charted throughout the experiment. Many different options for calculating flux from continuous data exist with varying complexity. One solution Pall recommends is that the flux at a given data point should be calculated by the slope of the throughput and time data up to five (5) minutes either side of the data point. Calculating the instantaneous flux between every time point collected can lead to significant variation in the calculated flux due to the discrete nature of the filtrate drops, especially when collecting data over small time intervals.

Contact Pall if you require further advice on flux and throughput analysis or any other aspect of Minidisc operation.

3. Virus Spike Challenge Testing

The validation of virus filtration processes requires special attention, as both the filter manufacturer and end user serve vital roles. The filter manufacturer has responsibility for ensuring that each filter will perform to the same specification. The filter user must demonstrate that the selected filter satisfies the needs of their process.

Important factors to consider in designing product-specific viral filter retention validation studies:

Proper design of the product-specific validation study is critical to ensure success of the study. Usually the retention study follows a scaled down version of the full-scale process. Some of the important factors that need to be addressed in the study design are:

- i) The choice of spike viruses (models)
- ii) Target reduction factor
- iii) Spike virus titer
- iv) Test feedstock comparable to process feedstock with respect to, e.g., concentration, temperature, chemistry, etc
- v) Equivalence of scale-down filter to process scale filter
- vi) Same volume to filter area ratio for the test and process filter
- vii) Inclusion of proper study controls
- viii) Inclusion of measures aimed at removing virus aggregates potentially present in the spiked challenge solution (e.g. spike prefiltration)

3.1 How to Run a Virus Spike Challenge Test

Contact your local Pall representative for detailed instructions of the steps required for successful operation of virus spike challenges, specifically the pre and post-use testing required (see also Pall publication USD2848: Instructions For Use – Filterability/Viral Challenge Test Procedure For Pegasus SV4 Virus Removal Membrane Filter Discs).

As with filterability studies, it is strongly recommended to run virus spike challenges in constant pressure operation. Difficulties in maintaining a constant flow and accurately measuring the test pressure will typically generate more experimental noise and variability compared to a constant pressure tests and the results may not be representative of the large scale process filter. Therefore constant pressure testing will always be preferable, even where a pump is to be used at process scale, with the pressure limits set by the expected full scale pump performance. A constant pressure will also always be required to run post-use installation checks to verify the correct operation of the challenge and release the samples for viral assay.

3.2 General Protocol Recommendations

It is best practice to minimize the amount of non-viral contaminants added to the product in spike studies to keep maximum equivalency between viral validation and production-scale feedstreams. Therefore excessive spiking, which also increases virus preparation-derived contaminants, is not ideal. Pall recommends that virus spikes should be designed on the basis of required input titer rather than a particular spike percentage. Our recommended approach is to use a spike level that achieves a 10^6 pfu.mL⁻¹ input titer (or another appropriate target titre based on your requirements).

High spike percentages (>1 %) can be necessary, for example due to low stock titres. In these specific cases the robustness to fouling of Pegasus SV4 allows the use of these spike percentages without additional flux decay impacting on the throughput that can be validated. The spike level should always be minimised to maintain equivalency and control any additional contaminants, but spikes of up to 5 % can generate acceptable throughputs where required. Discuss this further with your virus test laboratory for additional options or contact your local Pall representative for assistance.

Filterability and protein transmission trial results should guide the target throughput for virus filtration. For initial virus spike challenges (or bacteriophage studies) the filtrate should be collected in at least two aliquots. The recommended maximum aliquot volume is 100 mL (104 L.m⁻²). Once retention data has been established then aliquot volumes can be increased based on assessment of the data with respect to target retention. Contact your local Pall representative for more detailed discussion of aliquot plans.

Collection of aliquots should be in individual graduated sterile containers. The time taken to collect each aliquot should be recorded in order to calculate the flux.

If a product recovery buffer flush sample is required we recommend priming the upstream volume with buffer and flushing through 3 mL of buffer, or another appropriate amount as determined by protein transmission studies.

Virus clearance is measured by the log titer reduction (LTR) or log reduction value (LRV), which is the base-10 logarithm of the ratio of the total virus input and total virus measured in all filtrate aliquots. For an individual aliquot or grab sample this simplifies to the ratio of feed concentration (C_{feed}) to filtrate concentration ($C_{filtrate}$).

Equation 3

Log Titer Reduction (LTR) or Log Reduction Value (LRV)

$$LTR, LRV = \log_{10} \left(\frac{C_{feed}}{C_{filtrate}} \right)$$

3.3 Typical Virus Spike Challenge Results

Table 5

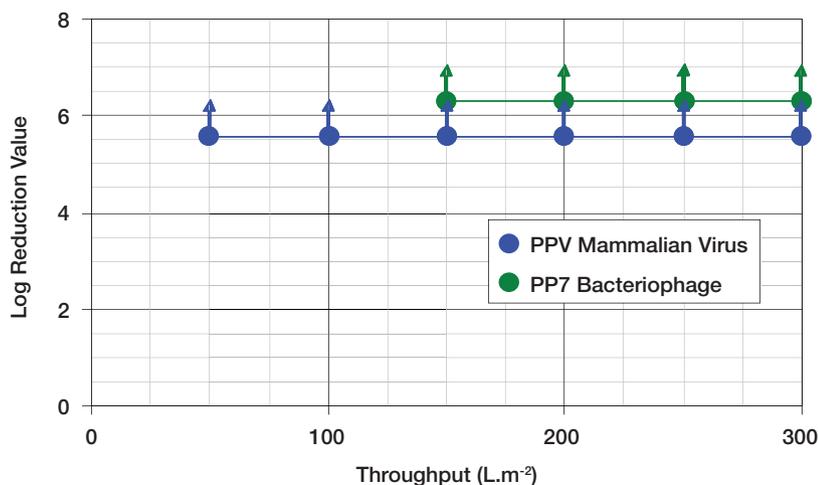
Example virus spike challenge results for Pegasus SV4 virus filters

| Carrier Fluid | Virus Spike Reduction |
|--|------------------------------|
| Plasma derivative against hemophilia | > 4 log PPV |
| Mammalian cell cultured derived protein | > 4.8 log PPV |
| Post cation exchange monoclonal antibody | > 5.5 log PP7 |

Table 5 shows details of the typical virus removal performance that can be expected from Pegasus SV4 membrane in a variety of protein feed solutions. Figure 4 shows typical performance of Pegasus SV4 membrane in a 1 g.L⁻¹ BSA solution as per the PDA guidelines^[3] for bacteriophage model parvovirus (PP7) and porcine parvovirus (PPV). Live virus testing was carried out at an independent virus validation test laboratory. Filtration was carried out at 3.1 bar (45.0 psi) to achieve the maximum flux performance and demonstrate that Pegasus SV4 membrane is capable of robust viral clearance under these conditions.

Figure 4

Live virus (PPV, $n=4$) and model virus (PP7, $n=9$) retention performance of Pegasus Grade SV4 virus filter membrane in 1 g.L^{-1} BSA at 3.1 bar (45 psi). Live virus testing was carried out at an independent virus validation test laboratory



4. Key Virus Filtration Factors

There are several product and process parameters that may affect microbial, including virus, retention by filtration.

| Product Parameters | Process Parameters |
|------------------------|--|
| Protein concentration | Batch size |
| Amount of aggregates | Temperature |
| Amount of contaminants | Process (filtration) time |
| pH | Pressure differential or flow rate |
| Viscosity | Throughput (volume to filter area ratio) |
| Ionic strength | Pre-filtration |

Details of these parameters are outlined in PDA Technical Report 41-08^[4], 'Virus Filtration'.

General guidance for all filters are addressed in detail in PDA Technical Report 26-08^[5], 'Sterilizing Filtration of Liquids' and much of this guidance can be applied to virus filters.

These parameters should be considered when running filterability optimization studies and designing viral clearance validation tests for virus filters. Specific recommendations for Pegasus SV4 virus filters are detailed in the following sections:

4.1 Operating Differential Pressure

As shown in Figure 3, Section 2.3, increasing operating differential pressure increases the flux of Pegasus SV4 membrane and this is maintained across the course of the test. Testing with polyclonal human IgG solutions having different fouling levels at a variety of different pressures has demonstrated no significant change in the level of fouling of the Pegasus SV4 filter membrane (as measured by V_{max}) from 2.1 barg (30 psid) to 3.1 barg (45 psid) operating differential pressure.

Typical bacteriophage clearance by Pegasus SV4 filter membrane in a 1 g.L^{-1} BSA solution (as per the PDA recommendations^[3]) is >4 logs and consistent from 2.1 barg (30 psid) to 3.1 barg (45 psid) operating differential pressures. This demonstrates that with Pegasus SV4 filter membrane, the optimum filterability performance seen at higher pressures does not impact retention performance.

For operating pressures above 3.1 barg (45 psig) the FTK200 stainless steel disc holder and 47 mm Pegasus SV4 discs are available.

Optimal operating differential pressure for Pegasus SV4 Minidisc capsule

3.1 barg (45 psid)

4.2 Throughput / Processing Time

Under many process conditions, other virus filters characterized by high initial flow rates will display rapid decay in flow and become less economical over time compared to a fouling resistant constant flow filter such as the Pegasus SV4 filter. Hence, the most economical approach for virus filtration is to allow for longer processing times using a fouling resistant filter and therefore achieve higher throughputs with a minimized cost per batch.

Viral filtration validation testing must be run to at least the expected maximum process throughput (volume to filter area ratio). This will correspond to the expected maximum process time. Filterability studies should also be run to the maximum throughput, although initial scouting studies can use smaller volumes and forward predict performance. This is important due to factors related to process throughput and time such as product stability over the processing time and changes in performance at the higher loading levels present during extended processing.

4.3 Temperature and Viscosity

Higher processing temperatures can reduce product viscosities and thereby increase filtration flux. Lower temperatures tend to increase viscosities and reduce filtration flux rates.

4.4 pH and Ionic Strength

Ionic strength and pH may have effects on processing parameters like filtration flux rates and total throughput, but also can affect properties of the spiked viruses in the carrier fluid. Therefore, careful control of pH is required during all virus filter testing.

No specific recommendations are given for pH and ionic strength when using Pegasus SV4 filter membrane, as optimal conditions may vary for different products. Other buffer components, e.g. stabilizers, excipients, etc., can also impact the overall filterability performance. In general, extremes of pH (<4, >8) and high ionic strength (>1 M) should be avoided unless there is evidence of product stability at these conditions.

4.5 Product Aggregation

Product aggregation can be caused by a variety of factors such as extremes of temperature, ionic strength and pH (also at $\text{pH} \approx \text{pI}$). Not only percentage aggregate content, but also aggregate size distribution may impact virus filter performance. Process steps including virus inactivation and freeze thawing can also introduce aggregation. Some products may also aggregate over time due to intrinsic instability.

An important benefit of Pegasus SV4 filter membrane is its high resistance to fouling for a range of aggregates, which results in outstanding throughput capacity in both dilute and complex, concentrated biological fluids. This enables maximum virus filtration economy and efficiency. General precautions present during product development to maintain product stability are typically sufficient to ensure that the Pegasus SV4 filter is capable of processing the aggregate burden in product feedstreams with low flux decay. Where significant flux decay is seen, Pall recommends the use of pre-filtration to improve the overall process performance (see Section 4.7 for details).

4.6 Protein Concentration

Pall Pegasus SV4 filters have proven to achieve stable flows over a wide range of process conditions, including different protein concentrations, because of robustness of flux and resistance to fouling.

For all protein solutions, an optimum concentration will exist where a given mass can be processed with the minimum amount of filter area. It will be a balance between three effects:

1. Reduced flow at higher concentrations due to increased viscosity
3. A decrease in capacity at higher concentrations
3. Reduction in process volume at higher concentrations

As well as minimizing costs, operating at or close to this optimum is also preferable since variations in batch concentration will have lower impact on performance. This is especially true for a robust, fouling-resistant virus filter such as the Pegasus SV4 filter, which typically has a relatively wide and flat optimum design space.

Optimum protein concentrations for Pegasus SV4 Minidisc capsules are typically $> 30 \text{ g.L}^{-1}$ and performance is stable around these optima. Therefore, typical variations in process concentrations are not likely to impact on Pegasus SV4 performance but should still be considered in robustness studies. In general, it is not necessary or recommended to dilute or select a process position for virus filtration with a lower concentration. However, for certain extremely high fouling feeds or products with unusual viscosity trends, this may be necessary and is more likely to be beneficial where undiluted concentrations exceed 50 g.L^{-1} . Where concentrations are low ($<20 \text{ g.L}^{-1}$) Pegasus SV4 performance will still be strong, however where there are process positioning options it is likely that performance will be improved by operating at higher concentrations.

Optimal Protein Concentration for Pegasus SV4 Minidisc capsules

20 – 50 g.L^{-1}

Higher concentrations are possible but performance may be better at lower concentration process intermediates in this range or after dilution.

4.7 Pre-filtration

Pre-filtration requirements will vary from feed to feed based on the presence of various sizes of aggregates or contaminants. Pegasus SV4 filters are capable of performing without any pre-filtration beyond upstream sterilizing filtration (0.2 or $0.1 \mu\text{m}$ membrane) that may be already built into the purification process. This has been demonstrated in many MAb and plasma protein tests and highlights the robustness to flow decay of the Pegasus SV4 membrane. Where an existing sterilizing grade pre-filter is not in place, we recommend Pall Fluorodyne® II DJL filters ($0.1 \mu\text{m}$ rated). Where required, there are several other Pall sterilising grade filter options (see Table 5).

Each process and its associated contaminant profile are different and where high levels of particulate contaminants are present, protection of Pegasus SV4 filters by membrane pre-filtration may be required. If flow decays are substantially faster than the typical performance seen in Figure 3 (Section 2.3), an additional pre-filter should be considered. Where flux decay is $<20 \%$ the process is unlikely to benefit from an additional pre-filter. Where flux decay is $>50 \%$ a pre-filter is likely to make the process more economical.

A range of pre-filter options capable of removing finer aggregates are available as listed below and should be discussed with your Pall representative for recommendations.

Table 6*Pre-filter Recommendations*

| Market | Process Fluid | First Pre-filter | Second Pre-filter (optional*) | Final Filter |
|----------------|---|--|--|--------------------------------|
| General Market | Typical combination for many fluids | Fluorodyne II DJL Fluorodyne EX EDT | Not required | Pegasus SV4 Ultipor VF DV20 |
| Biotech Market | Low fouling fluids such as monoclonal antibodies after purification by chromatography | Supor® ECV Supor EKV Supor EBV Ultipor® N66 NF | Not required | Pegasus SV4 Ultipor VF DV20 |
| Plasma Market | Typical combination for plasma Fractionation | Supor ECV Supor EKV Supor EBV Ultipor N66 NF Fluorodyne EX EDF | Ultipor VF DVD Pegasus ULV6 Ultipor VF UDV50 | Pegasus SV4 Ultipor VF DV20 |

*for fine particles or aggregates < 0.1 µm a second prefilter might make the process more economical.

4.8 Summary of Recommended Design Space Using Pegasus SV4 Filters

Please note that the following design space specifications are a guideline for optimal performance of Pegasus SV4 filters. Prior knowledge and understanding of the particular feed to be tested should also be applied and filterability studies are recommended before virus spiking to confirm performance and reproducibility.

Table 7*Design space recommendations when using Pegasus SV4 filters*

| | |
|-------------------------------------|--|
| Differential pressure | 3.1 bar (45 psi) |
| pH and Ionic Strength | <ul style="list-style-type: none"> Highly product specific optima General recommended ranges: <ul style="list-style-type: none"> pH 4 – 8 <1 M ionic strength More extreme values can be tested if the product remains stable under those conditions. |
| Protein concentration | <ul style="list-style-type: none"> Optimum performance at higher concentrations (20 – 50 g.L⁻¹) > 50 g.L⁻¹ may require further studies for high fouling and highly viscous solutions as a lower concentration may provide improved performance |
| Pre-filtration | <ul style="list-style-type: none"> 0.2 µm sterilizing grade filter as a minimum Flux decay < 20 % no additional pre-filter required Flux decay 20-50 % additional pre-filter recommended Flux decay > 50 % additional pre-filter required (see Table 5 for pre-filter options) |
| Spike titer | 10 ⁶ pfu.mL ⁻¹ |
| Spike concentration | Minimum required to generate target spike titer <ul style="list-style-type: none"> ≤ 1 % = minimal additional flux decay 1-5 % = acceptable additional flux decay if necessary |
| Virus challenge aliquot throughputs | <ul style="list-style-type: none"> Minimum 2 aliquots ≤ 111 mL (100 L.m⁻²) per aliquot for initial tests |

5. References

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