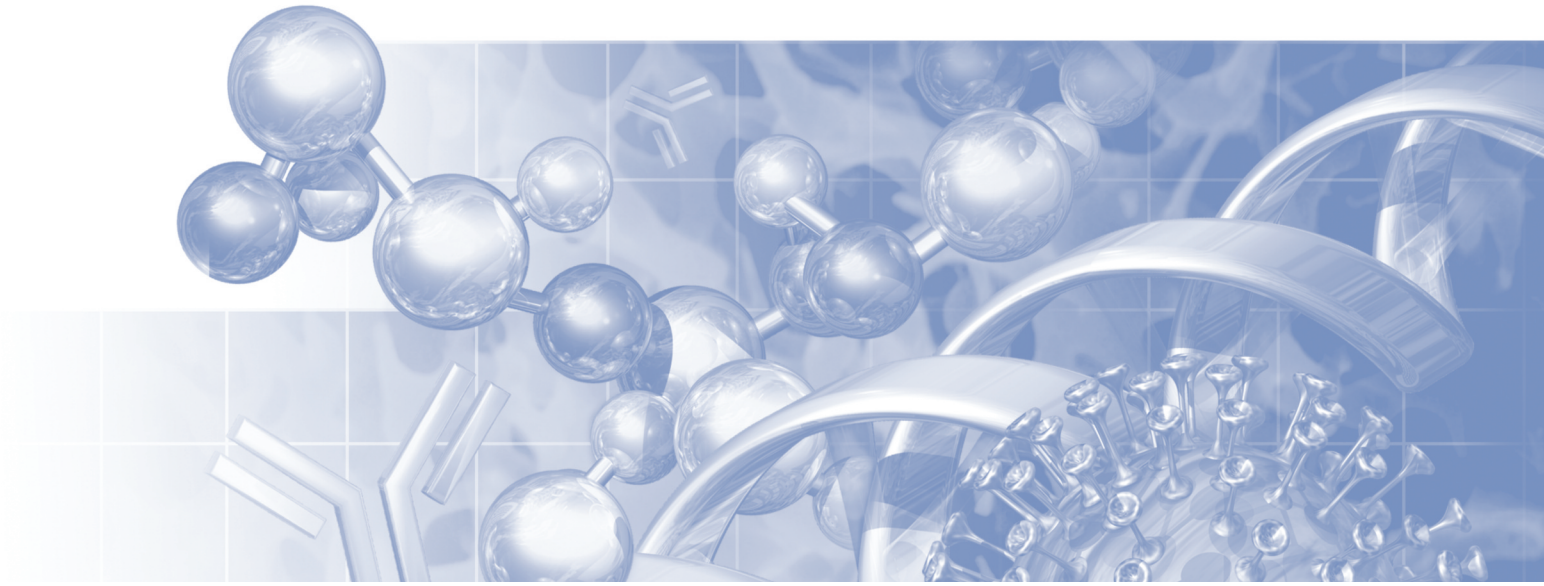
PALL Life Sciences

## Application Note

USD2849

# Design Space for Plasma Derivative Applications with Pegasus™ SV4 Virus Removal Filters



*Filtration. Separation. Solution.*SM

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## 1. Introduction

This Application Note adds plasma derivative application-specific information to Pall publications *USD 2778: Filterability Testing and Virus Challenge of Pall Minidisc Virus Removal Filter Capsules with Pegasus Grade SV4 Membrane and USD2846: Filterability Testing and Virus Challenge of Pegasus SV4 Virus Removal Membrane Filter Discs.*

Further recommendations are provided on how to get the best performance from Pegasus SV4 filters with reference to the particular challenges of solutions from plasma fractionation using plasma application data and design spaces.

## 2. Virus Spike Challenge Testing

The recommendations and details of virus spike testing in USD 2778 and USD 2846 are all applicable to plasma products, with the following amendments:

For initial virus spike challenges (or bacteriophage studies) the filtrate should be collected in at least two aliquots. The recommended maximum aliquot throughput for plasma-derived feeds is 50 $L.m^{-2}$ (56 mL for 47 mm discs in FTK200 disc holders, 50 mL for Minidisc capsules). 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.

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. This is especially important in plasma applications where heterogeneous and immunological or complex and sensitive proteins have a higher risk of adverse interactions with the non-viral spike contaminants. Pall recommends that virus spikes should be designed on the basis of required input titer rather than a particular spike percentage. Pall's recommended approach is to use a spike level that achieves a $10^6$ $pfu.mL^{-1}$ input titer (or another appropriate target titer based on your requirements).

## 3. Case Study: IVIG Filtration Design Space

In order to demonstrate how important buffer conditions can be to process performance in the specific example of intravenous immunoglobulin (IVIG) filtration, a study examining various points within a pH, NaCl concentration and IVIG concentration design space was carried out.

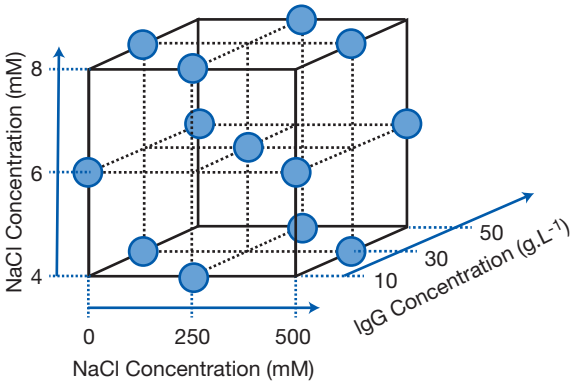
Depending on specific capabilities, purification strategies and donor profiles, each IVIG solution will be different and should be assessed individually. The trends demonstrated here show that there can be dramatic variation of the physical properties of different IVIG intermediates and that any generic performance values could either over or under estimate performance in a particular product.

### 3.1 Materials and Methods

A human gamma globulin with similar fouling levels to those seen in testing with customer IVIG intermediate feedstreams was frozen at a protein concentration of 50 $g.L^{-1}$ and -20 °C. The thawed IVIG solution was adjusted to the correct conditions using addition of NaCl, pH adjustment using NaOH / HCl and dilution. All solutions contained a maltose stabilizer to IgG ratio of 2:1 (w/w). A Box-Behnken design for three factors was used as detailed in Figure 1. Each combination in the design was tested using Pall Minidisc capsules with Pegasus SV4 Virus Removal Filter Membrane in triplicate at a constant pressure of 3.1 bar (45 psi) and a standard lab temperature of 22 ± 2 °C. The centre-point was replicated three times, each with another set of triplicate tests.

**Figure 1**

*Box-Behnken design for IVIG protein concentration, salt concentration and solution pH*




The experimental design employed allows the major trends across a wide design space to be observed. In addition it allows the important extreme combinations of buffer conditions to be tested without the need for operating tests outside of these extremes.

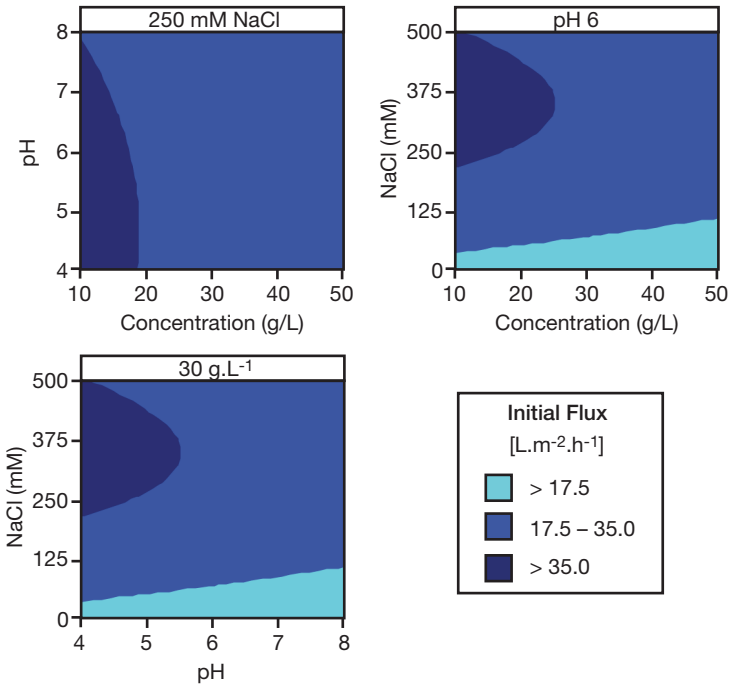
## 3.2 Results and Discussion

Reversible self-association occurs in standard IVIG solutions between IgG molecules to cause transient dimer formation mediated by electrostatic FAb interactions[1-3]. This does not occur in the majority of other therapeutic protein molecules, even monoclonal antibodies (however it has been demonstrated in isolated cases[2]). This is because of the variety of IgG combinations in pooled plasma sources where antibodies from different donors form idiotype-antigen binding combinations which increase in occurrence with a higher number of donors[3]. Reversible self-association increases the viscosity of the feed[1,3] and is shielded by increasing salt content[1-3] – hence the strong impact of salt concentration on initial flux seen in Figure 2. There is also a weak interaction of salt content with pH, indicating that the self-association effect is stronger at higher pH, probably due to the reduction in charge repulsion as there will be a mix of positive and negative net charges at pH 8, which is in the isoelectric point (pI) range of IgG[4].

The overall increases in viscosity due to concentration have less impact on Pegasus SV4 filter membrane flux compared to salt content, as demonstrated in Figure 2. In comparison the influence of pH is negligible. This confirms that the Pegasus SV4 membrane is capable of processing proteins with high concentrations and that large reductions in initial fluxes are mediated by physical changes in the viscosity of the IVIG due to solution conditions.

**Figure 2**

*Effect of IVIG protein concentration, pH and NaCl concentration on Pegasus SV4 filter membrane initial flux at 3.1 bar (45 psi) constant pressure*

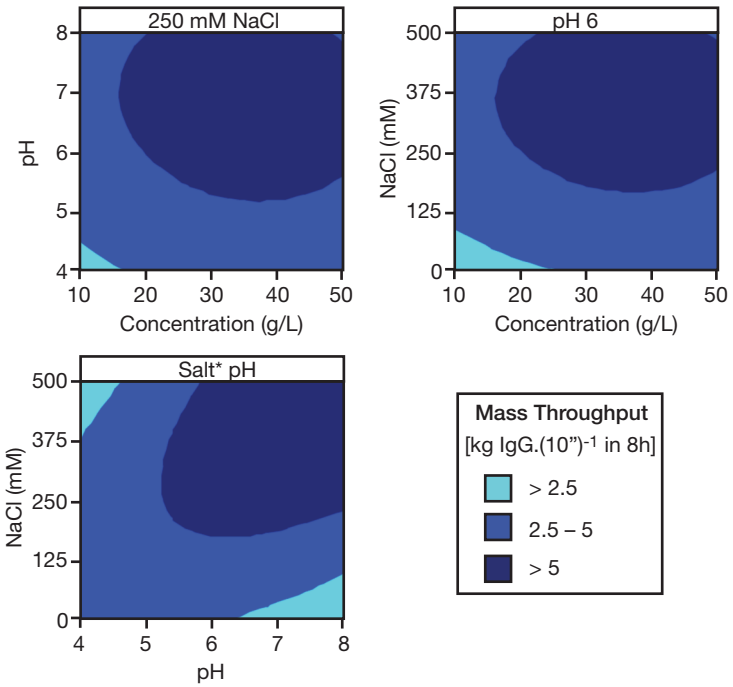



The impact of IVIG protein concentration, pH and NaCl concentration on the overall performance of Pegasus SV4 filter membrane, as defined by the total mass of IgG capable of being processed by one 10-inch pleated cartridge (2.25 $m^2$ effective filtration area, projected from filter disc performance), is shown in Figure 3. This combines the physical changes in viscosity across the design space with the difference in product stability and the ability of Pegasus SV4 filters to process these variable challenges. The end product is a wide design space where close to optimum performance for the Pegasus SV4 filter is replicated across a wide range of conditions. The data points showing lower filter performance results are all in positions where there are indications of protein instability (medium to high pH at low NaCl concentration, medium to high NaCl concentration at low pH – see next paragraph). The optimum IVIG concentration in this case is in the range of 30 - 50 $g.L^{-1}$, indicating that higher concentrations provide optimum performance (5 – 6 kg/10 inch/8 hours) without the need to fine adjust the concentration of the process intermediate.

This study demonstrates a strong interaction specific to IVIG. This displays the importance of understanding any design space which forms the backbone of quality by design (QbD) approaches. At low pH, increasing salt concentration shields the electrostatic repulsion that helps to limit aggregation and leads to a lack in stability and increase in fouling. At high pH we see the exact opposite effect because of the mixed net charges on the IgG (pH inside the pI range). Increasing salt concentration shields the electrostatic attraction that generates instability. These two opposite corners of the design space should be avoided.

**Figure 3**

*Effect of IVIG protein concentration, pH and NaCl concentration on Pegasus SV4 mass throughput at 3.1 bar (45 psi) constant pressure*




The data in Figure 3 points towards non optimal performance at low pH and low NaCl concentration – however this is affected by the low viscosity at this extreme of low conductivity and at higher processing times the comparison is more favourable. Also the non pH-adjusted IVIG source (pH = 4.8) generated stronger results, indicating that the stability starts to decline as pH drops from this point and more complex effects may be present at smaller scales in addition to the overall design space trends. At final formulation, commercially available IVIG products typically have low or zero salt content and mid-range pH values close to pH 5[5]. Similar buffer conditions to these can be expected in many process intermediates and several strong filterability results with IVIG intermediate solutions have been generated in this area of design space at extended processing times.

This case study highlights some specific interactions and broad effects over a wider design space than proper application of response surface design methodology would generally recommend. True design spaces for IVIG processing will be more constrained by specific process options being considered and implementation of these approaches to a smaller design space is required for optimization. The important factors for consideration in the following section should not be tested at these process extremes and instead the focus should be on variations around the process target to confirm the robustness of Pegasus SV4 filters.

## 4. Key Virus Filtration Factors

There are several product and process parameters that may affect microbial, including virus, retentive filter performance.

| 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 | Pre-filtration |
| Ionic strength | Throughput (Volume to Filter Area Ratio) |

Details of these parameters are outlined in PDA Technical Report 41-08[6], 'Virus Filtration'. General guidance for all filters are addressed in detail in the PDA Technical Report 26-08[7] '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

The benefits of higher pressures described in the original application notes are valid for all feeds, since 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 maximum throughput capacity) from 2.1 bard (30 psid) to 3.1 bard (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[5]) is >4 logs and consistent from 2.1 bard (30 psid) to 3.1 bard (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 bar (45 psi) the Pall FTK200 stainless steel disc holder and 47 mm Pegasus SV4 virus removal membrane filter discs should be used.

| | |
|---|---|
| Optimal operating differential pressure for Pegasus SV4 filter membrane | 3.1 bard (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.

In various plasma products, there are wide ranges of process conditions where flow decay due to membrane fouling is low for Pegasus SV4 filter membrane. Even with high fouling fluids, the higher viscosities involved can often reduce the rate of flow decay with respect to process time. This can result in low overall filtration costs (less filter area) calculated for the complete full-scale process time.

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. Less stable plasma proteins can often lead to the need for extreme temperatures to maintain optimal stability. Note should be taken that operating filtration at 4 °C or 37 °C will lead to > 30% changes in water flux alone due to viscosity variation compared to a reference point of 20 °C[8]. Many protein solutions will show similar percentage viscosity variations due to their aqueous base, but the exact performance may be product-specific, especially with highly viscous solutions.

### 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 buffering 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. A specific example of the impact of buffer conditions is detailed in the IVIG case study (Section 3).

### 4.5 Product Aggregation

Product aggregation can be caused by a variety of factors such as shear, extremes of temperature, ionic strength and pH (also at pH ≈ 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

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. This throughput will be a balance between three effects:

1. Reduced flow at higher concentrations due to increased viscosity.

2. 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.

Optimum protein concentrations for Pegasus SV4 filters are typically > 30 g.L-1. In general, it is not necessary or recommended to dilute feeds or select a process position for virus filtration with a low 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 $g.L^{-1}$.

In the IVIG case study presented here (Section 3), optimum performance was seen in the 30-40 g.L-1 range. The broad design space optimum shown confirms that batch to batch variations in concentration have minimal impact on the overall performance of Pegasus SV4 filter membrane and that even where optimum concentrations are sub 50 $g.L^{-1}$, there is often little benefit from dilution.

Where concentrations are low (<20 $g.L^{-1}$), Pegasus SV4 filter performance will still be strong, however, where there are process positioning options, it is likely that performance will be improved by conducting virus filtration at the higher concentration position.

| | |
|---|---|
| Recommended Protein Concentration for Pegasus SV4 Filters | 20 to 50 $g.L^{-1}$ |

*Higher concentrations are possible but performance may be better at process intermediates within 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 µm membrane) that may be already built into the purification process. This has been demonstrated in many plasma protein tests and highlights the robustness to flow decay of the Pegasus SV4 filter membrane. Where required, there are several Pall sterilizing grade filter options (see Table 1).

Each process and its associated contaminant profile are different and where high levels of contaminants are present, further protection of Pegasus Grade SV4 filters may be required. If flow decays are high, 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.

Common plasma fractionation product contaminants that are not removed by 0.2 or 0.1 µm sterilising grade filters can be reduced by a sub-0.1 µm filter such as the Ultipor® VF DVD virus prefilter. Also, large virus (e.g. retrovirus) grade virus filters such as Pegasus LV6 or Ultipor VF DV50 filters can provide even further protection as aggregates with similar sizes to retroviruses can be present, particularly in some IVIG intermediates.

Where pre-filtration is not capable of limiting flux decay to below 50%, further options should be discussed with your local Pall representative. For example, alternative process positioning or buffer conditions to maximize stability and minimize aggregation may yield significant improvements in virus filtration process performance.

**Table 1**

*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<br>Fluorodyne EX EDT | Not required | Pegasus SV4<br>Ultipor VF DV20 |
| Biotech Market | Low fouling fluids such as monoclonal antibodies after purification by chromatography | Supor® ECV<br>Supor EKV<br>Supor EBV<br>Ultipor N66 NF | Not required | Pegasus SV4<br>Ultipor VF DV20 |
| Plasma Market | Typical combination for plasma Fractionation | Supor ECV<br>Supor EKV<br>Supor EBV<br>Ultipor N66 NF<br>Fluorodyne EX EDF | Ultipor VF DVD<br>Pegasus ULV6<br>Ultipor VF UDV50 | Pegasus SV4<br>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 in plasma-derived products. 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 2**

*Plasma Process Design Space Recommendations when using Pegasus SV4 Filters*

| | |
|---|---|
| **Differential pressure** | 3.1 bar (45 psi) |
| **pH and Ionic Strength** | • Highly product specific optima<br>• General recommended ranges:<br>■ pH 4 - 8<br>■ < 1 M ionic strength<br>• More extreme values can be tested if the product remains stable under those conditions |
| **Protein Concentration** | • Optimum performance at higher concentrations (20 - 50 $g.L^{-1}$).<br>• Concentrations > 50 $g.L^{-1}$ will require further studies for high fouling and highly viscous solutions as a lower concentration may provide improved performance. |
| **Pre-Filtration** | • 0.2 µm sterilizing grade filter as a minimum<br>• Flux decay < 20% no additional pre-filter required<br>• Flux decay 20 - 50% additional pre-filter recommended<br>• Flux decay > 50% additional pre-filter required<br><br>(see Table 1, Section 4.7 for pre-filter options) |
| **Spike Titer** | • $10^6$ $pfu.mL^{-1}$ |
| **Spike Concentration** | Minimum required to generate target spike titer<br>• ≤ 1% = minimal additional flux decay<br>• 1 - 5% = acceptable additional flux decay if necessary |
| **Virus Challenge Aliquot Throughputs** | • Minimum 2 aliquots<br>• ≤ 50 $L.m^{-2}$ per aliquot (56 mL for 47mm discs, 50 mL for Minidisc capsules) for initial tests |

## 5. References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