Biotech

Application Note

USD 3334

Validating Pegasus™ Prime Virus Membrane Filters: What Virus Spike Should I Use?

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Contents

1 General Recommendations

The purpose of virus filter validation is to evaluate the removal of viruses during an effective scaled-down simulation of the process, in order to demonstrate the removal of virus under the conditions seen at the process scale. Selection of a virus spike level must allow for quantification of virus removal, but not at the expense of adverse impact on the scaled down process. At the point of virus filtration, many unit operations have taken place which will significantly reduce any additional contamination that could potentially be caused by upstream viral contamination. Over-spiking during virus validation risks introducing contaminants that would normally not be present at that process position and will impact the filterability performance and reduce the accuracy of the scale-down model. The basis for successful spike selection is therefore:

- Use the purest spike available (ultracentrifugation essential)
- Use the most sensitive assay technique (large volume assays)
- Spike only what is needed to measure your target log reduction value (LRV)

2 Selecting a Spike

The preferred decision tree for spike selection is shown in Figure 1. This is based on information that can define the minimum required spike and checks to ensure that the filterability of the scale down mimic is not impacted by this spike. Care should be taken to ensure a reasonable safety margin and it is recommended to carry out all calculations with the experts from your chosen virus validation laboratory to assure your target LRV can be demonstrated.

An alternative approach is to use existing data and experience to select a reasonable spike level known not to cause fouling in typical testing, as detailed in Figure 2. This approach includes more risk to throughput, but can also be easier and quicker.

For a general guide as to what spike levels will have minimal impact on flow decay during a validation run, Figures 3 to 6 demonstrate the impact of 4 viruses commonly used in validation testing on a low fouling monoclonal antibody (mAb) solution:

- Minute virus of mice (MVM)
- Reovirus Type 3 (REO-3)
- Pseudorabies virus (PRV)
- Murine leukemia virus (MuLV)

The Figures 3 to 6 show, at a throughput of 1000 L/m^2, the percentage flow decay that a ‘total virus load’ causes (data dots), compared to the ‘standard’ flow decay of just the clean mAb ‘unspiked control’ depicted as a dashed horizontal line in each graph. Different total virus loads at 1000 L/m^2 throughput were achieved by varying the added virus spike standard stock quantity. We generally observe that the lowest total virus loads that we tested (between 10^{10} and 10^{11}) give filterability comparable to that of the unspiked control. Increasing total virus load, brings significant flow decay additional to that of the unspiked control, varying between the 4 different virus types and preparations.

All the spikes are standard preparations which have been ultracentrifuged (not gradient ultracentrifugation). This is a guide only and is specific for the given test mAb, solution conditions and virus testing laboratory spike preparation. Variation in these results due to different mAbs, buffers and spike preparations is expected and the level of fouling could be both lower and higher. Spikes of 1% and higher can potentially be used, as demonstrated, but are often undesirable due to increased fouling of the test virus filter. Some virus testing providers offer ultra-high purification spikes and these can be even lower fouling and offer more flexibility in

spike levels. When incorporating Pegasus Protect virus prefilters into your process, this can also significantly reduce spike related fouling for some viruses (see Figure 3).

Contact Pall if you require further assistance or information to help with virus clearance validation of Pegasus virus filters. Make sure to follow the general recommendations and work with your virus validation laboratory to ensure the spike does not prevent you from reaching the high throughputs possible with Pegasus Prime filters.

3 Example Calculations for Figure 1 Flowchart

Example 1
MuLV testing of a product with good prior knowledge

Minimum Target LRV	4.5	Chosen by the customer
LRV Safety Margin	0.5	Low risk due to existing data with this product
Dilution Factor (logs)	0	No cytoxicity / interference
Limit of Quantification (logs)	-1.2	Based on large volume assay of 50 mL and a 95% confidence interval [1]
Target Spike (logs)	3.8	Sum of above LRV values
Target Spike (virus titer/mL)	6.0×10^3	

Minimum Stock Titer	3.2×10^6	
Spike %	0.2%	Target spike / minimum stock titer

Target Throughput (L/m^2)	1000	
Expected Stock Titer (virus titer/mL)	1×10^7	For calculating likely fouling level
Total Virus Load (virus titer/m^2)	1.9×10^{10}	Target throughput × Expected stock titer × Spike % x 1000 mL/L

The example demonstrates viral clearance test design to high throughput with a more challenging virus such as MuLV. Figure 6 indicates that this level of load is relatively safe for filterability risk. A spiked filterability test would still be recommended, but in this scenario we are presuming prior knowledge with the product / virus combination and testing could go directly to validation.

Example 2
MVM evaluation of cytotoxic product with limited testing experience

Minimum Target LRV	5	Chosen by the customer
LRV Safety Margin	1	Based on risk assessment and high due to the limited experience of this product
Dilution Factor (logs)	0.5	1 in 3 dilution required to remove cytotoxic effect
Limit of Quantification (logs)	-1.2	Based on large volume assay of 50 mL (diluted from original filtrate sample as per above) and a 95% confidence interval [1]
Target Spike (logs)	5.3	Sum of above LRV values
Target Spike (virus titer/mL)	1.8×10^5	

Minimum Stock Titer	3.2×10^7	
Spike %	0.6%	Target spike / Minimum stock stock titer

Target Throughput (L/m^2)	750	
Expected Stock Titer (virus titer/mL)	1×10^8	For calculating likely fouling level
Total Virus Load (virus titer/m^2)	4.3×10^{11}	Target throughput × Expected stock titer × Spike % x 1000 mL/L

In this scenario, an increase in flux decay is likely because a high spike level is required. The high spike level is due to the high safety margin based on unknown risks of the virus stability and also the mild cytotoxic effects on the virus host cells requiring pre-dilution. Looking at this total virus load on Figure 3, this could potentially cause moderate issues with higher and non-representative flux decay in the scale down process simulation. A spiked filterability test to determine the effect of the spike on product filterability is highly recommended before running the test. Revisiting the volume of sample assayed, options to purify the spiked test sample, and even the target LRV or safety margin is also important to consider.

Figure 1
Preferred decision tree for virus challenge spike selection. Contact your chosen virus validation test laboratory for guidance on minimum spike calculations.

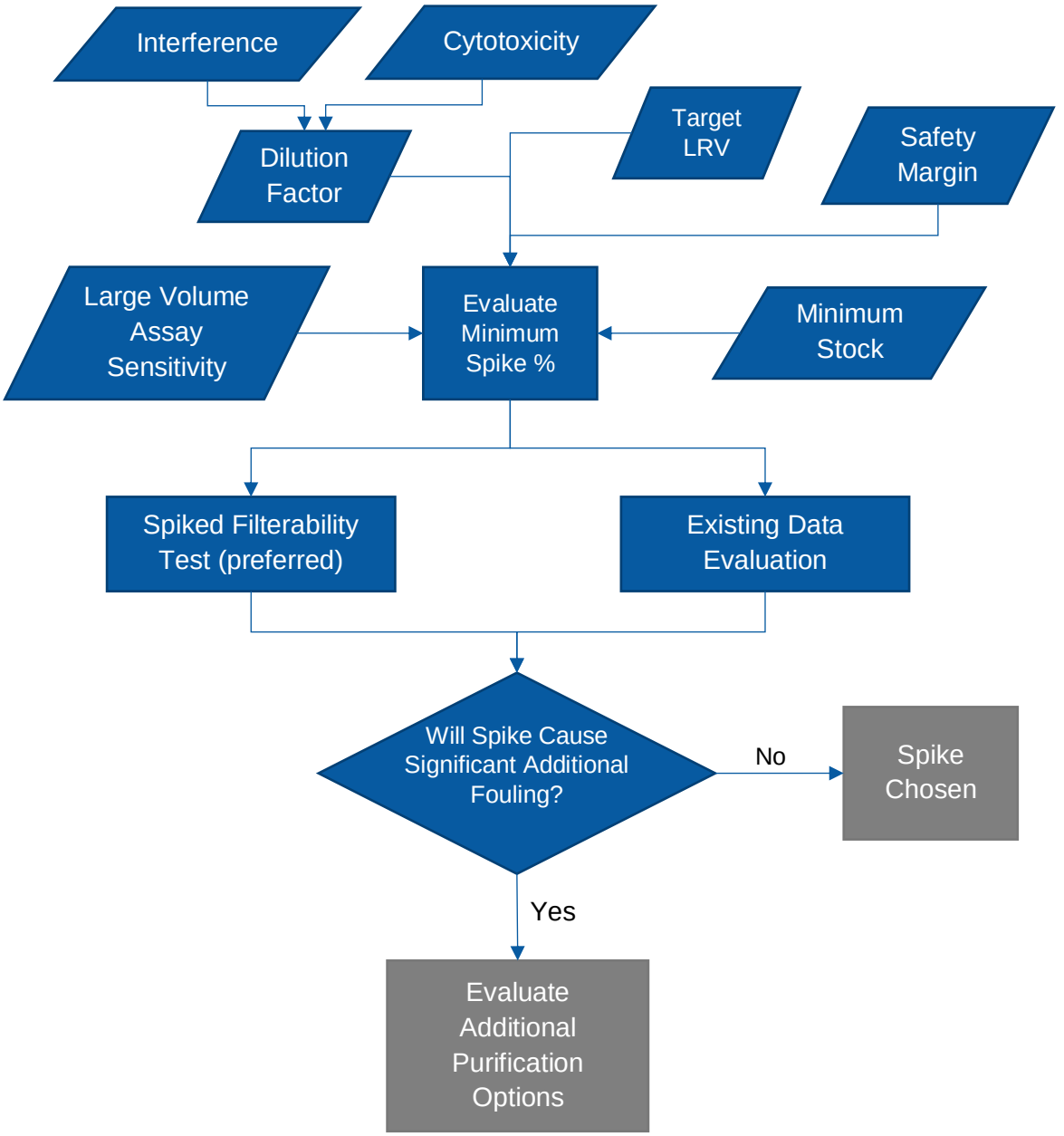
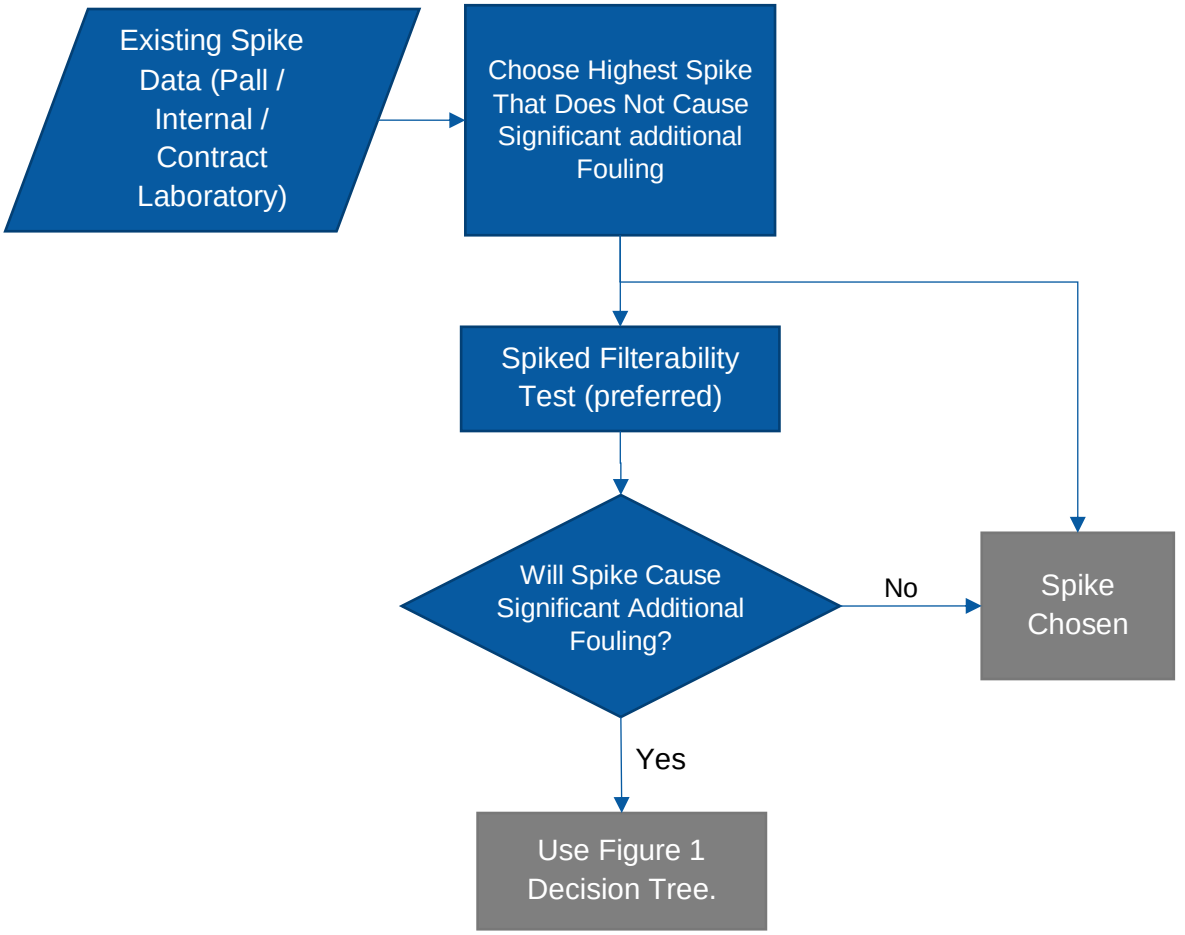


Figure 2
Decision tree for selecting a virus challenge spike based on prior data.



The decision tree in Figure 1 is preferable as it minimizes the risk of spike related fouling affecting the scale-down model.

Figure 3

Effect of different spike levels of ultracentrifuged MVM on the filterability performance of a low fouling mAb solution (1.5 g/L mAb, 75 mM Tris, pH 7.5, 6 mS/cm) at various spike concentrations. Data points are 0.1%, 0.3%, 3% spikes from a stock titer of 7.7 log_{10} virus titer/mL, tested to 1000 L/m^2. Standard ultracentrifuged spike from one specific laboratory, does not necessarily represent batch and vendor variation.

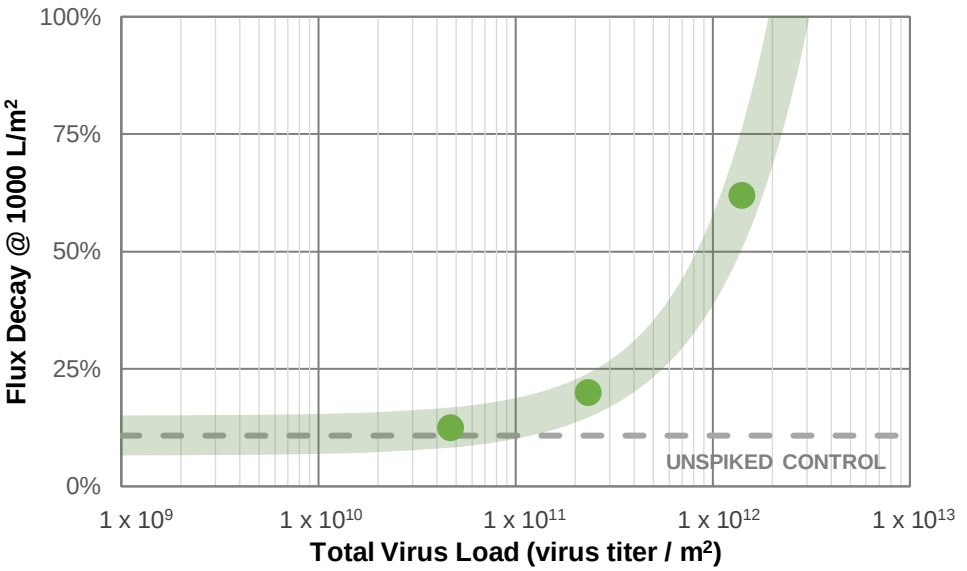


Figure 4

Effect of different spike levels of ultracentrifuged REO-3 on the filterability performance of a low fouling mAb solution (1.5 g/L mAb, 75 mM Tris, pH 7.5, 6 mS/cm). Data points are 0.05%, 0.3%, and 3% spikes from a stock titer of 8.0 log_{10} virus titer/mL, tested to 1000 L/m^2. Standard ultracentrifuged spike from one specific laboratory, does not necessarily represent batch and vendor variation.

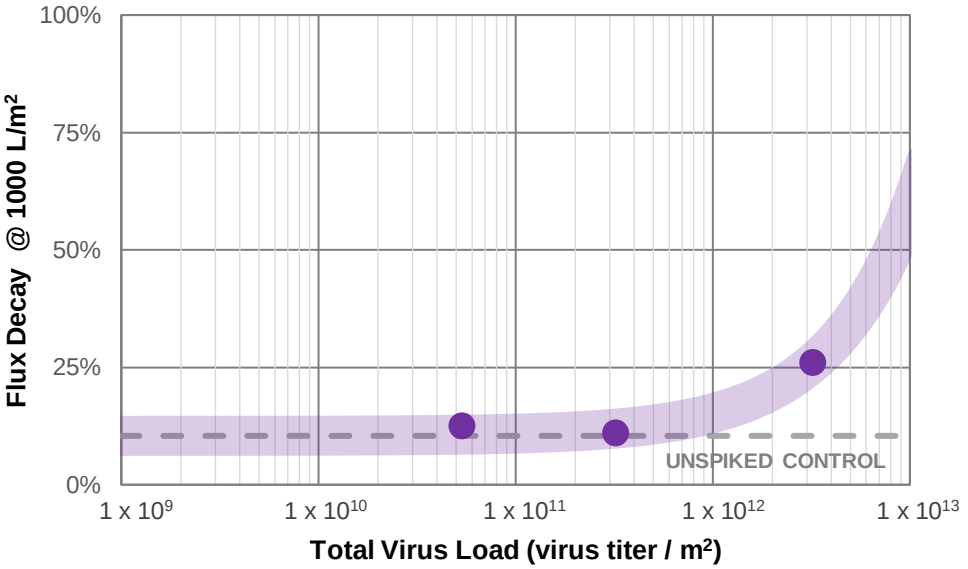


Figure 5

Effect of different spike levels of ultracentrifuged PRV on the filterability performance of a low fouling mAb solution (1.5 g/L mAb, 75 mM Tris, pH 7.5, 6 mS/cm). Data points are 0.05%, 0.3%, and 3% spikes from a stock titer of 8.1 log_{10} virus titer/mL, tested to 1000 L/m^2. Standard ultracentrifuged spike from one specific laboratory, does not necessarily represent batch and vendor variation.

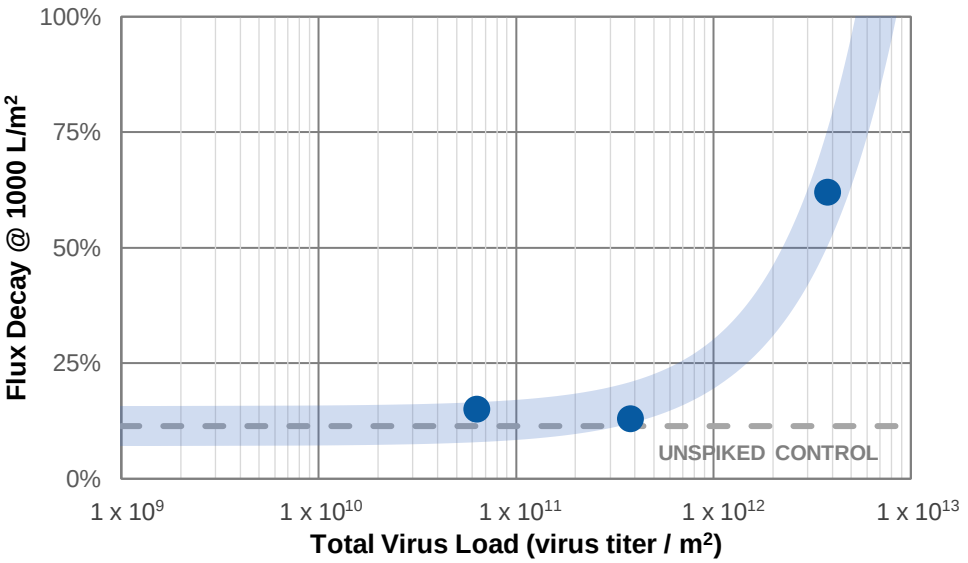
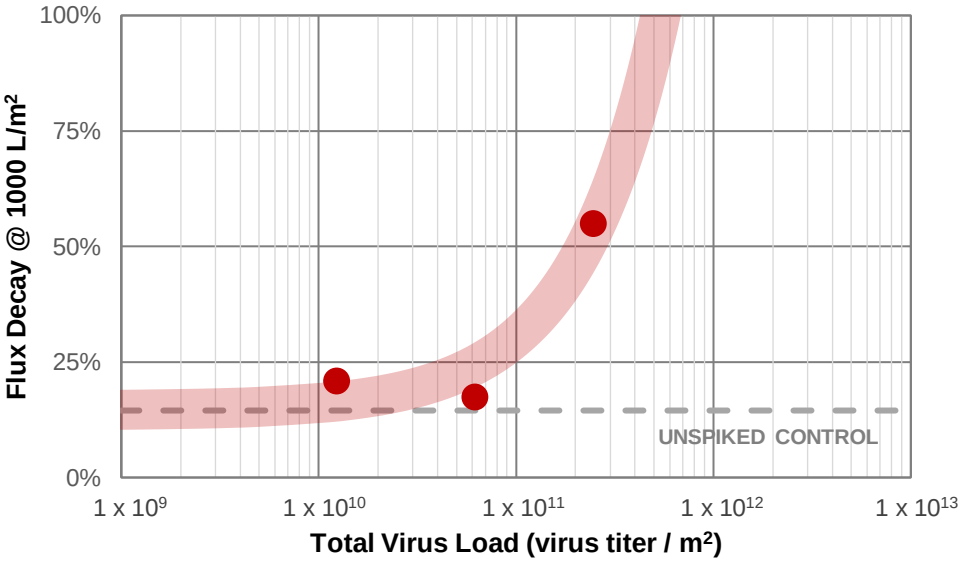


Figure 6

Effect of different spike levels of ultracentrifuged MuLV on the filterability performance of a low fouling mAb solution (1.5 g/L mAb, 75mM Tris, pH 7.5, 6 mS/cm). Data points are 0.1%, 0.5%, and 2% spikes from a stock titer of 7.1 log_{10} virus titer/mL, tested to 1000 L/m^2. Standard ultracentrifuged spike from one specific laboratory, does not necessarily represent batch and vendor variation.



References:

[1] ICH Q5A, Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, Appendix 3.

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