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White Paper

Mastering Virus Filtration Clearance Studies

A Practical Guide for Study Success

Authors: Alexander Herrmann, Annie Leahy

Summary

This article will summarize best practices to achieve successful virus filtration (VF) viral clearance (VC) study targets with the Viresolve® Pro Device, a high flux virus filter that provides effective virus retention across a broad range of operating conditions. It will cover topics that impact filtration performance such as freeze/thaw effects, prefiltration, operating pressures, concentration effects, flux limitations, and will apply a quality by design (QbD) approach to study design. We'll also explore challenges such as virus spike prep purity and highlight conditions when alternative spiking approaches, such as inline spiking, should be considered.

Challenges of Virus Filter Clearance Studies

In the production of biopharmaceuticals such as monoclonal antibodies (mAbs), virus filtration is a dedicated virus reduction step critical to ensuring viral safety. During drug development, manufacturers are required to assess the level of viral clearance across the filter and other key virus removal steps to assess process clearance capabilities.

Viral clearance validation studies are generally performed at Contract Testing Organizations (CTOs) that have expertise in virological work. These studies involve challenging various unit operations, including the virus filter, with a panel of viruses of different sizes and physio-chemical characteristics using a representative scale down model[1].

While viral clearance studies are designed to replicate the product conditions and operating parameters of the manufacturing-scale filtration process, some artifacts arise that can impact the performance of the virus filter both in terms of filter capacity (volumetric throughput) and virus removal capacity. These artifacts can reduce throughput resulting in increased filtration area requirements and higher process costs. Over the course of two decades of Viresolve Pro® Device clearance evaluations, we have published best practice guides to minimize potential failure modes and ensure a successful filter clearance validation[2, 3].

Virus filters commonly operate in the normal flow orientation and remove viruses via size-based exclusion. Because the size of the monomeric mAb is close to that of the pores in the virus filter, high molecular weight mAb aggregates in the feed can foul the membrane, decreasing filter capacity[2].

To ensure optimal filter performance it is important to understand and mitigate the various root causes of protein aggregation in process fluid: high molecule concentration, long hold times, freezing and thawing, and agitation of the fluid.

Concentration Impact on Aggregation

Extensive virus filtration studies have shown that the VF performance of the Viresolve Pro® Device is independent of concentration for non-fouling feeds[4]. However, running virus filtration at or close to the upper concentration limit is one worst-case scenario because of the potential increase in levels of aggregates and altered monomers, the main drivers of filter plugging.

Changing elution pool collection conditions in chromatography steps or concentrating product via ultrafiltration can increase aggregate load and may reduce virus filter capacity. For this reason, process characterization should assess factors influencing concentration and compare with the expected variation in concentration from the preceding chromatography unit operation.

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Freeze/Thaw and Shipping Effects

Due to scheduling and logistical considerations for VC studies, many biomanufacturers freeze process fluid and ship it to the CTO for viral clearance evaluations. This freezing, while convenient, may increase aggregate formation in the process fluid which will reduce filterability when thawed. To understand a potential freeze/thaw effect, it is recommended to test the effects of age, freezing and thawing, and shipping on any fluid's filterability prior to a VC study.

Passing the thawed process fluid through a sterilizing grade microfilter or adsorptive prefilter prior to virus filtration can mitigate aggregation caused by the freeze/thaw cycle and restore the characteristic filterability[2]. **Figure 1** shows the impact freeze/thaw and the benefits of adsorptive prefiltration on process fluid. In this example, freeze/thawed process fluid (navy and turquoise) had greatly reduced volumetric throughput on the Viresolve® Pro Device as compared to fresh fluid (yellow). Restoration of the freeze-thawed fluid by prefiltration using Viresolve® Pro Shield prefilter (pink) improved throughput, to levels similar to fresh fluid.

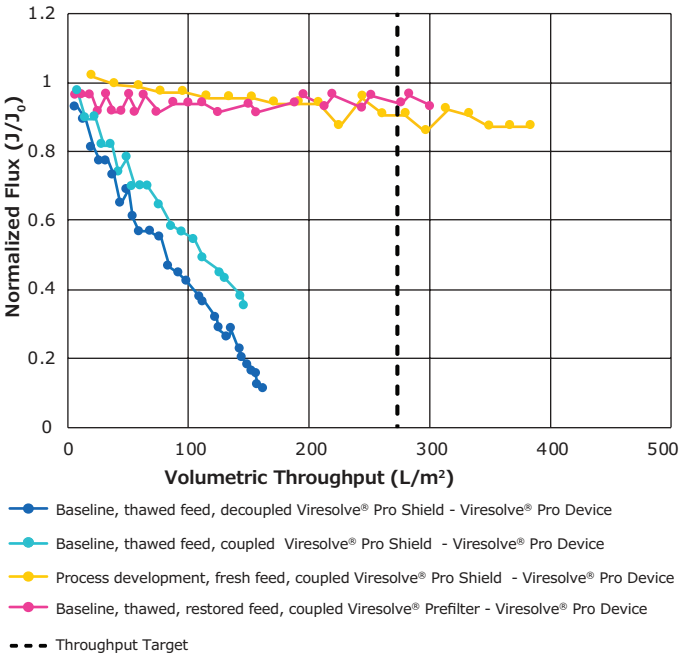

Figure 1. Fresh vs. frozen material and coupled vs decoupled adsorptive prefilter.

Microfiltration

Microfilters are commonly used during viral clearance studies. Following thaw of frozen load material, a 0.2 µm filter is typically used to remove high molecular weight protein aggregates.

Microfilters are also used to filter virus-spiked process fluids as a control for process-intermediate mediated viral aggregation. Microfiltration can be used to assess if there are viral aggregates larger than the pore size of the control filter or if the virus is interacting with the product to form larger particles. Occasionally, non-specific interactions between a particular virus and a mAb results in significant aggregation. In these cases, switching to a similarly sized different virus may mitigate aggregation. The level of virus-virus or virus-fluid aggregation is determined by sampling and assaying the spiked feed before and after microfiltration. If there is no change in viral titer across the microfilter, the spiked feed is assumed to have few aggregates. Monodispersed virus represents the highest level of challenge for a virus filter.

The pore size of the microfilter depends on the size of the virus being spiked. For smaller parvoviruses like Minute Virus of Mice (MVM) and Porcine Parvovirus (PPV), a 0.1 µm filter is typically used. For other viruses, like Xenotropic Murine Leukemia Virus (XMuLV) or Reovirus (Reo-3), a 0.2 µm filter is typically used. For the larger viruses, like Pseudorabies Virus (PRV), a 0.45 µm filter is preferred to avoid retaining any virus on the microfilter.

For vacuum microfiltration of spiked process fluid, it is recommended to use a low-level vacuum source to prevent overly strong flow that could cause foaming. The filter unit should be placed at a 45° angle to allow filtrate to flow down the side wall of the collection container minimizing the likelihood of aggregate formation from air-liquid interface or splashing.

For process intermediates known to aggregate easily, the vacuum microfiltration should be replaced with gentler pressurized microfiltration. Under some situations, the post-spike microfiltration step can be omitted and the virus-spiked process fluid applied directly to the virus filter. Determination of virus aggregation may be accomplished by sampling and filtering a small aliquot of the virus-spiked fluid separately through a syringe control microfilter (**Figure 2**). This avoids potential aggregation that could be introduced by microfiltration of the entire load while enabling assessment of viral aggregation.

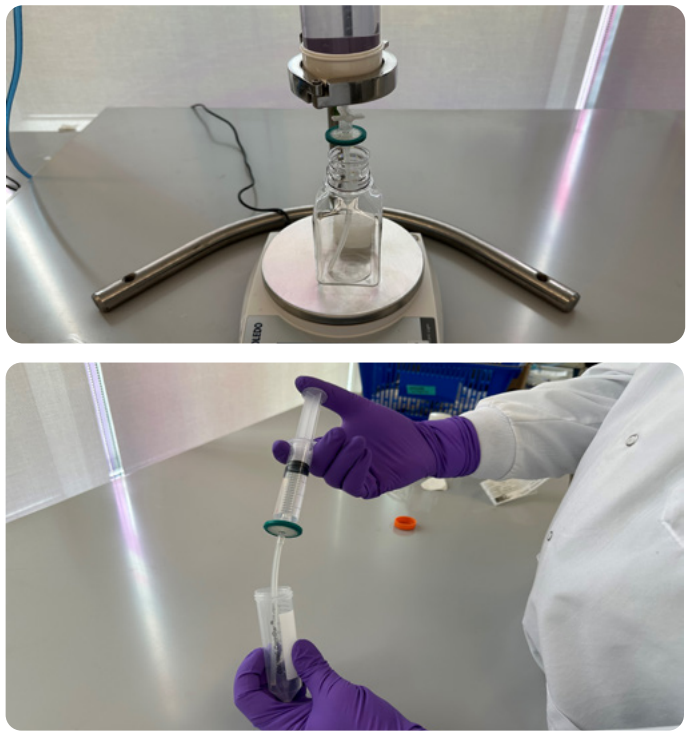

Figure 2. Gentler, pressure-driven microfiltration (top); Syringe virus control microfiltration check (bottom).

Handling

Some molecules are prone to aggregation when exposed to increased agitation, splashing, or air-liquid interfaces. Gentler handling techniques help ensure successful outcomes by avoiding potential aggregation. The preferred method of vessel-to-vessel transfer is gently and slowly pouring the process fluid down the inner wall of the pressure vessel at a ~ 45° angle. Gentler methods of transfer, such as gravity siphoning using tubing, can be used for sensitive molecules. In situations where the fluid is sensitive to handling, steps that can introduce air-liquid interfaces, such as vacuum-filtration, should be avoided or substituted with the pressure-driven or syringe filter methods.

Adsorptive Prefilters

Many virus filtration processes incorporate adsorptive prefiltration prior to the virus filter to remove aggregates and fouling species in the process fluid and maximize virus filter throughput capacity. While prefilters can be highly beneficial to virus filter performance and mitigate the impact of process variability, they can also complicate viral clearance studies. Because most prefilters either don't have a claim for virus retention or would retain a virus through charge or other mechanisms, it is frequently not feasible or desirable to process virus-spiked process fluid through a coupled or inline prefilter and virus filter.

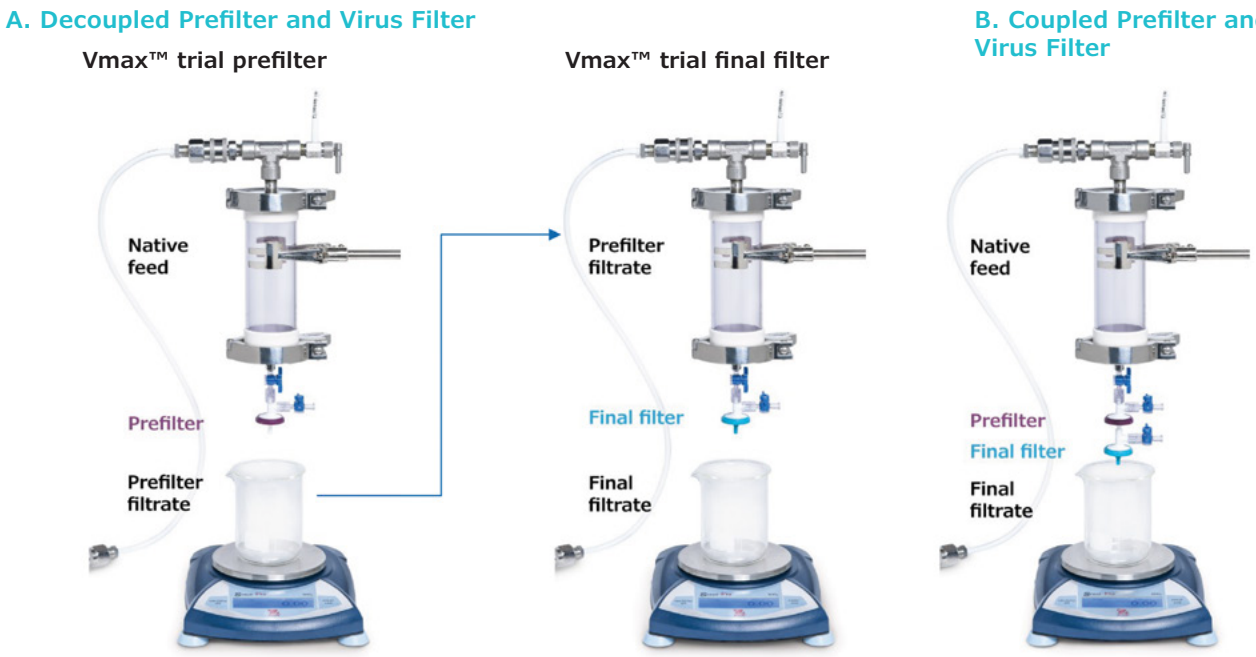

Figure 3. Decoupled (left, center) vs. coupled (right) prefilter and virus filter

One solution is to decouple the prefilter and virus filter. The process fluid is processed through the prefilter, collected, spiked with virus, sampled, then loaded onto the virus filter to assess clearance (See **Figure 3**). This process solves the problem of spiking across the prefilter but requires careful handling of the spiked load material to avoid potential filterability impacts[2].

Several important steps can help ensure successful prefiltration. Before beginning, affix a dip tube to the outlet of the prefilter and feeds into the bottom of the collection vessel. This mitigates any splashing or air-liquid interfacing that could occur. Because prefilters rely on adsorption, residence time on the prefilter media is very important, otherwise the surface chemistry of the prefilter won't have enough contact time with the load material to adsorb foulants. The residence time is set by the flowrate through the decoupled adsorptive prefilter, which should match the flowrate of the virus filter at its operating pressure; the tighter pores of the virus filter are the rate limiting step for throughput, so a coupled adsorptive prefilter can only flow as fast as the virus filter.

If the extra handling that comes with decoupling reduces filterability that isn't mitigated by the gentlest handling practices, or if it is suspected that some interaction between the process load material and the virus is causing fouling, in-line injection of virus may be a viable solution.

In-line injection is the process of spiking virus into the process fluid between a coupled prefilter and virus filter. This utilizes syringe pumps, narrow high-pressure tubing, in-line mixers, and a different method of spiking buffer rather than spiking the process fluid directly[5].

Process Development

The considerations above should all be investigated during process development and before the clearance studies at the CTO. Process development (PD) studies should test a process intermediate under a range of conditions (concentration, pH, conductivity, freeze/thaw) as well as different operating conditions (temperature, pressure, prefilters, decoupling, etc.) to fully characterize filtration behavior. These data can be used to define the process parameters critical for viral clearance and in setting worst-case limits for testing in a specific viral clearance step.

While the Viresolve Pro® Device has undergone extensive characterization and is known to provide robust virus retention across a wide range of feed and process conditions, protein aggregates in the process fluid can impact the filterability of the fluid and the throughput targets of the virus filter[4]. Before clearance evaluations, it is important to assess multiple conditions and handling techniques during PD to understand how they impact filterability.

Such characterization will be useful knowledge that can be applied to future molecules using the same manufacturing template. The application of previously generated viral clearance data to a specific product should be supported by demonstration of similarity of the processes across manufacture of different products involved, similarity of the product intermediates, and an assurance that product-specific attributes do not affect virus reduction[1].

Importance of the Baseline Run in the Viral Clearance Study

At the start of any clearance study before proceeding with virus spiked runs, performing an unspiked "baseline" run helps assess VF performance of the process fluid for comparison to process development data. This baseline run involves running the process intermediate for testing through the virus filter with no virus. This run should simulate each step in the virus spiking process, including mixing performed after the virus spike and vacuum microfiltration. Data from this run should uncover and help address any issues with the feed or viral clearance study procedures.

If the filter's performance with the process intermediate in the baseline run matches previous data (PD and/or manufacturing scale), then the baseline run has demonstrated a qualified, scaled-down model, verifying that no artifacts (feed concentration, hold time, freezing and thawing, shipping, or decoupled adsorptive prefiltration) are affecting the process. If this baseline run differs from the expected behavior, then steps need to be taken to mitigate the impacts of these artifacts on virus filter performance before executing the clearance runs with virus.

Addition of Virus Spike

Several factors should be considered when choosing the level of virus spike that achieves both target log reduction value (LRV) and target throughput. Common virus spike percentages range from 0.1 % to 1.0 %. At typical virus stock titers, those percentages usually provide enough virus load to demonstrate a target LRV of >4–6.

Although higher spike levels may seem more desirable at the outset, there are a couple reasons not to exceed a 1% virus spike in virus filter clearance studies. ICH recommends that a virus spike "should be added to the product in a small volume so as not to dilute or change the characteristics of the product. Diluted test-protein sample is no longer identical to the product obtained at commercial scale"[1]. Another important aspect to consider is the virus purity: impurities in virus spike preparations can cause fouling of the virus filter, decreasing the filter throughput. Because of this, it is important to confer with CTO personnel regarding the titer and purity of the virus prep prior to spiking.

Cytotoxicity/viral interference of the virus assay should also be assessed by the CTO prior to spiking runs. It could impact the LRV if the dilution required to mitigate potential cytotoxicity, interference, etc. is such that significant assay sensitivity is lost. If a particular virus is interacting non-specifically with a mAb and a large dilution is required, switching to another virus might be the best solution.

If there are concerns that even a low level of virus spike will negatively impact filterability, it may be valuable to perform a scoping or mock-spiked run in addition to an unspiked baseline, to confirm hydraulic performance[3].

Conclusion

The VF VC study uses a scale-down model to demonstrate the virus removal capacity of the filter and, as part of a comprehensive clearance study, can demonstrate the viral safety of the medicinal product. Many factors in the VC study can directly and negatively impact the performance of the filter, limiting throughput. This article has highlighted areas to be characterized and understood during process development prior to the clearance validation study to ensure that potential artifacts do not negatively impact the scale-down model. The greatest value of solving these issues in advance is that the baseline run can be executed and virus spiking runs can be performed as planned, without extra troubleshooting. With this advance knowledge, and high-quality virus spikes, viral clearance studies can proceed smoothly to a successful endpoint, where the target LRV and optimized filter throughput are achieved.

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MS_WP14401EN Ver. 1.0 70722 07/2026