An Improved Method for Virus Filter Qualification and Implementation

Using Flow Decay to Determine Processing Endpoint

Paul Genest, Jennifer Campbell, Susan Crump, Mark Cabatingan, and Fudu Miao

Virus clearance is critically important to biopharmaceutical manufacturers. One key strategy enjoined in regulatory guidelines is to assess the ability of downstream unit operations to provide such clearance. In addition, guidelines also recommend using specific steps, where necessary, to achieve robust virus clearance that augments what is achieved across a manufacturing process. In recombinant protein manufacturing, low pH, solvent detergent, chromatography, and virus filtration are common viral-reduction unit operations. Virus filtration is considered a robust clearance step because the removal mechanism is based on size, so it is less sensitive than are other steps to solutions or operating conditions.

Current practices for conducting virus filter validation studies use a scale-down of "anticipated" manufacturing scale conditions, with equivalent hydraulic conditions (differential pressure or flux), volumetric throughput (L/m^2), feed concentration, and so on. The throughput (L/m^2) at which virus reduction is demonstrated in a qualification study becomes the maximum the manufacturer can run in its process. However, qualification based on volumetric throughput may limit manufacturers to processing within protein concentrations tested in qualification. Furthermore, addition of virus stock solutions during spiking significantly limits volumetric throughput obtainable across a virus filter.

The alternative method we present here uses flow decay as a more pertinent measure for determining endpoints during filter qualification and manufacturing, particularly for some parvovirus filters. Using a flow-decay endpoint may provide a higher level of virus-retention assurance. Furthermore, qualifying virus removal based on flow decay may also increase manufacturing flexibility.

Product Focus: Recombinant proteins

Process Focus: Downstream processing

Who Should Read: Process development and manufacturing, filter manufacturers, QA/QC

Keywords: Virus filters/filtration, validation, flux, LRV, monoclonal antibodies, throughput, scale-down studies

Level: Intermediate

The Theory Behind Virus Filtration

An effective and economical virus filtration step must balance the need for high levels of small virus clearance — generally >4 log retention values (LRV) — with high protein recovery (>98%) and maximum flow rate and capacity. Given the small size difference between typical therapeutic proteins (4–10 nm) and small-virus particles (~20 nm), this is a challenging application.

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All small-virus filters have an inherent pore size distribution. Within small-virus filters, pores are either retentive or nonretentive to small viruses.

Figure 1 shows a simplified model membrane with retentive (10 nm) and nonretentive (60 nm) pores. It is assumed that a 28 nm virus is challenged at 1 × 10^7 pfu/mL (pfu = plaque-forming units). Table 1 shows that LRV is dependent on the percent flow through nonretentive pores (**1**). When nonretentive pores are present at a density of 1 in 1,000, they represent only 3.48% of the flow area but correspond to 58% of the total flow. This results in an LRV of 0.25. Membranes that achieve approximately 4-log virus reduction, based on this simplified model, will have only one nonretentive pore in 10,000,000 pores, and flow through those nonretentive pores will be 0.01% of the total flow (99.99% of the flow will be through retentive pores).

That simplified model also suggests that if small retentive pores were to become plugged more rapidly than larger nonretentive pores, more flow would be diverted through the nonretentive pores, and the LRV would decline. Previous studies (**2–4**) have shown a strong relationship between LRV decline and filter flow decay for Viresolve NFP (normal flow parvovirus) filters. Flow decay is defined as $(1\text{-}Q/Qi)$, where Qi = buffer permeability. Therefore, monitoring filter flow decay, both during virus filter validation studies and during manufacturing, is useful for tracking LRV decline behavior and ensuring expected virus reduction.

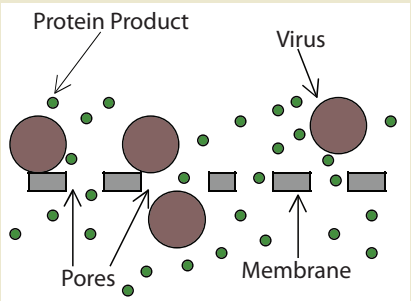
The following data corroborate the direct relationship between LRV decline and flow decay for a Centocor monoclonal antibody (MAb) molecule. The data also indicate virtually no relationship between LRV and throughput across the concentration range expected in the manufacturing process. Monitoring flow decay during virus filtration is also shown as the optimal approach to ensuring expected virus reduction.

Table 1: Showing the log retention value's (LRV) dependence on percent flow through nonretentive pores

Large Pore Density	Large Pore (% Total Area)	% Flow Through Large Pores	LRV*
1 in 10^3	3.48	58	0.25
1 in 10^4	0.36	11	0.94
1 in 10^5	3.6×10^{-2}	1.3	1.8
1 in 10^6	3.6×10^{-3}	0.1	3
1 in 10^7	3.6×10^{-4}	0.01	4
1 in 10^8	3.6×10^{-5}	0.001	5
1 in 10^9	3.6×10^{-6}	0.0001	6

* LRV = log (volume of feed challenged × concentration of virus in the feed/volume of the permeate × concentration of the virus in the permeate)

Figure 1: A simplified model membrane with retentive (10 nm) and nonretentive (60 nm) pores



Materials and Methods

Three MAb concentrations were tested (4, 6, and 9 g/L) on three NFP filter lots in triplicate. The filters had 0.00035 m^2 of filter area. They were wetted with water and their NWP (normal water permeability) measured. Then they were equilibrated with buffer. The buffer permeability was measured and then challenged with MAb containing 1×10^7 ϕX-174 viruses/mL.

NWP, buffer permeability, and MAb/ϕX-174 filtration were carried out using constant pressure at 30 psi. Preparation of ϕX-174 and virus assay methods are previously described (**4**). Cumulative weight (volume) was measured and recorded with time. Grab samples (instantaneous from permeate, not from pooled permeate) were collected at 0–20% flow decay, and at 50%, 75%, and 90% flow decay (grab LRV = log [concentration of virus in the feed/concentration of virus in the permeate]). Permeate pool samples were collected at 75% flow decay. The samples collected were used to establish the instantaneous and final pool LRVs. Instantaneous LRVs were plotted as a function of volumetric throughput (L/m^2), mass loading (g/m^2), and percent flow decay. Figure 2 shows a schematic of the experimental set up used to generate the data.

Results

Figure 3 shows flow rate as a function of throughput (L/m^2) for the three product concentrations tested in triplicate. Tests were performed on three different filter lots (one lot shown here). All filter lots show similar flow decay behavior. Filter flow starts high at 200–300 $L/m^2/h$ and decays during processing. The rate of flow decay increases as the MAb concentration increases. If virus removal were qualified based on volumetric throughput, then a different throughput endpoint would need to be defined for each concentration expected in manufacturing. That increases the validation burden by requiring qualification studies at varying drug product concentrations.

Figure 4 shows instantaneous LRV as a function of throughput for the three MAb concentrations tested in triplicate. Tests were performed on three different filter lots (one lot shown here). All filter lots show similar LRV decline behavior. LRV starts at 5.5–6.5 and declines during processing, presumably as small virally retentive pores plug during processing. The rate of LRV decline increases as the MAb concentration increases. Consistent with previous observations (**4**), the data show no relationship between LRV and throughput across the protein concentrations tested. For

Figure 2: Schematic of the experimental set up used to generate the data

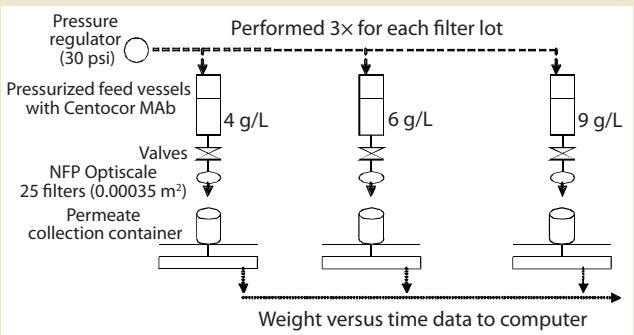
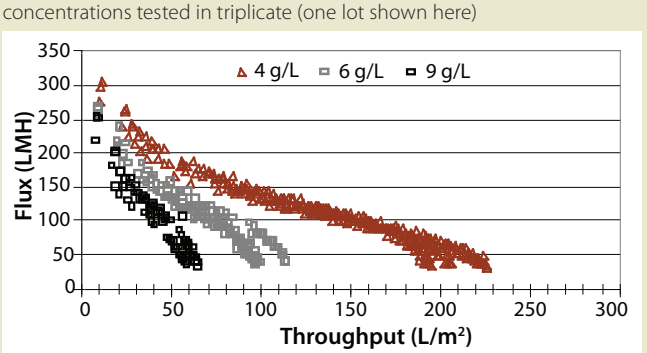


Figure 3: Flow rate as a function of throughput (L/m^2) for three product concentrations tested in triplicate (one lot shown here)



example, at a throughput of ~60 L/m^2, the instantaneous LRV ranges from 3 to 6.

Figure 5 shows instantaneous LRV as a function of mass loading (g/m^2) for the three MAb concentrations tested in triplicate. Tests were performed on three different filter lots (one lot shown here). All filter lots show similar LRV decline behavior. LRV starts at 5.5–6.5 and declines during processing. The rate of LRV decline, with respect to mass loading, depends less on concentration than on volumetric loading. That suggests filter plugging is not related to volume, but to the amount of protein applied to the filter. Thus the validation endpoint could be defined by mass loading when testing multiple concentrations (the range expected from the upstream unit operation). This is advantageous when chromatographic performance may produce variable elution concentrations. However, if a shift occurs in the aggregate content of a feed stream, increased plugging could result at an equivalent mass loading, resulting in increased viral passage.

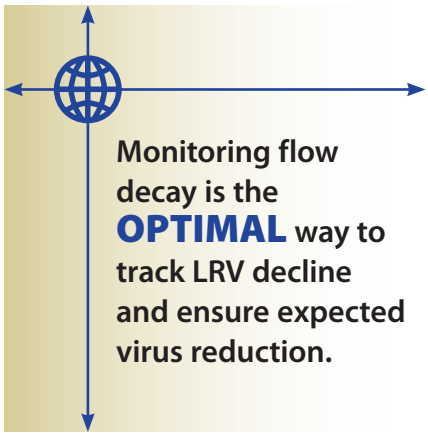


Figure 6 shows instantaneous LRV as a function of flow decay for the three MAb concentrations tested in triplicate. Tests were performed on three different filter lots (one lot shown here). Filter LRV starts at 5.5–6.5 and declines during processing. The rate of LRV decline is independent of MAb concentration. LRV depends only on the degree of flow decay or plugging. At 75% flow decay the LRV is 4–5, regardless of throughput or concentration.

Figure 7 shows pool LRVs at 75% flow decay for one membrane lot (4, 6, and 9 g/L, respectively) tested in triplicate. Two other membrane lots were also tested. All pool LRV values at 75% flow decay, for all filter lots, MAb concentrations, and all repeat runs were >4 logs.

Discussion

Flow decay is believed to correlate to LRV more strongly than volumetric or mass loading. We believe that this is due to the following effect: Virus filters plug and flow decays because drug product aggregates (dimer, trimer, and so on) plug small retentive pores more rapidly than larger nonretentive pores. Such plugging leads to increased flow through nonretentive pores, and the LRV declines.

The amount of aggregation may also depend on the concentration of the drug product. Disproportionately more plugging material (aggregate) may be in a 9 g/L feed than in a 4 g/L feed, which may explain why the 9 g/L feed shows slightly more LRV decline than the 4 g/L feed during mass loading. The degree of that effect is likely to be drug-product specific. Assuming that feed characteristics do not drastically change, fluctuations in drug product concentration and quality (amount of

Figure 4: Instantaneous LRV as a function of throughput for three MAb concentrations tested in triplicate (one lot shown here)

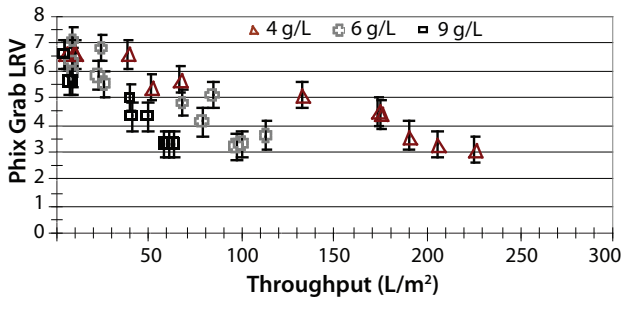


Figure 5: Instantaneous LRV as a function of mass loading (g/m^2) for three MAb concentrations tested in triplicate (one lot shown here)

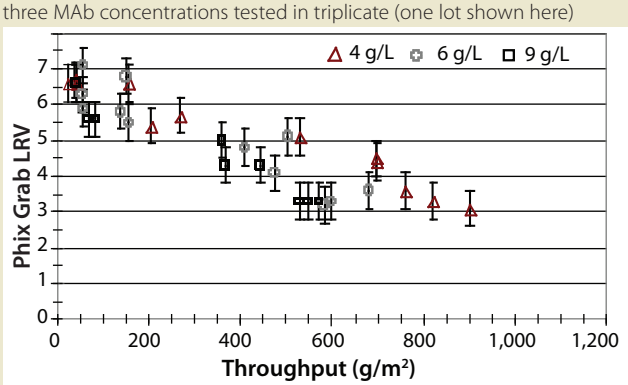


Figure 6: Instantaneous LRV as a function of flow decay for three MAb concentrations tested in triplicate (one lot shown here)

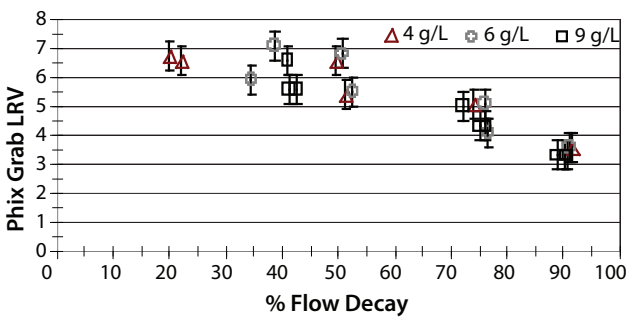
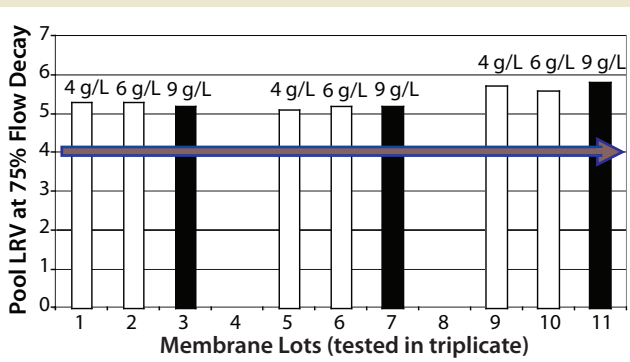


Figure 7: Pool LRVs at 75% flow decay for one membrane lot (4, 6, and 9 g/L, respectively) tested in triplicate



aggregate) do not appear to affect LRV when flow decay is specified.

No correlation is found between LRV and volumetric throughput (L/m^2) across MAb concentrations tested. A better correlation exists between LRV and mass loading (g/m^2). A validation endpoint defined by mass loading offers the advantage of being applicable to a range of drug product concentrations.

A stronger correlation exists between LRV decline and flow decay. Monitoring flow decay is the optimal way to track LRV decline and ensure expected virus reduction. Validation studies conducted with flow decay monitoring produce the required data to safely support full-scale manufacturing processing at a range of product concentrations. With maximum flow decay limits in place, the LRV of a full-scale process can be better predicted.

References

1 Levy R, Phillips M, Lutz H. Filtration and the Removal of Viruses from Biopharmaceuticals. *Filtration in the Biopharmaceutical Industry.* Meltzer T, Jornitz M, Eds. Marcel Dekker Inc.: New York, NY, 1998.

2 Brorson K. Virus Filter Validation and Performance. Presented at *Recovery of Biological Products XII*, Litchfield, AZ, 2–7 April 2006; American Chemical Society, Division of Biochemical Technology; www.acs.org.

3 Parkkinen J, at al. (Finnish Red Cross Blood Service). A Modified Caprylic Acid Method for Manufacturing Immunoglobulin G from Human Plasma with High Yield and Efficient Virus Clearance. *Vox Sang.* 90(2) 2006: 97–104.

4 Bolton G, et al. Normal-Flow Virus Filtration: Detection and Assessment of the Endpoint in Bio-Processing. *Biotechnol. Appl. Biochem.* 42(Part 2) 2005: 133–142.

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