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Robustness Study for Virus Retentive Filtration of Plasma Derivatives

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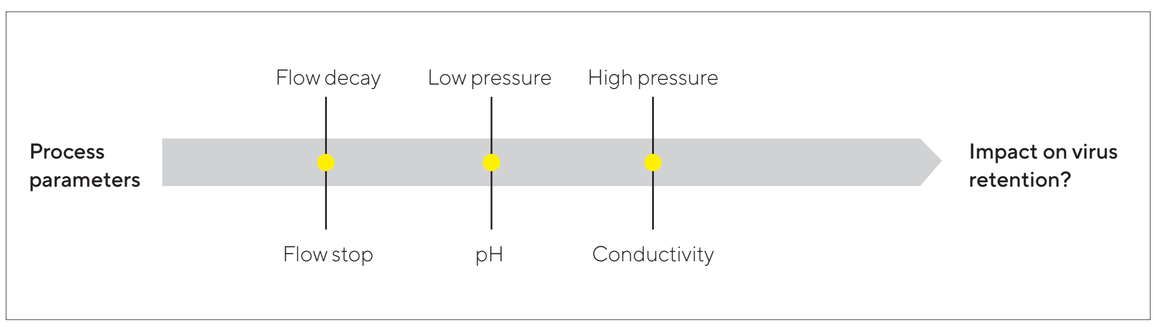
1. Introduction

Historically, plasma products such as IVIG, fibrinogen, and factor VIII are derived from human plasma, for which current regulatory guidelines such as the Annex 4 of the "WHO Technical Report, Series No. 924" and the "Guideline on plasma-derived medicinal products" from the EMA request at least two orthogonal steps for the inactivation and | or removal of viruses.

One method is virus filtration, which uses virus removal membranes having nominal pores sizes of 20 nm for effective size exclusion of both small, non-enveloped and large, enveloped viruses. Virus removal is usually demonstrated in spiking studies, where relevant viruses are deliberately added to the process stream ahead of the relevant unit operation.

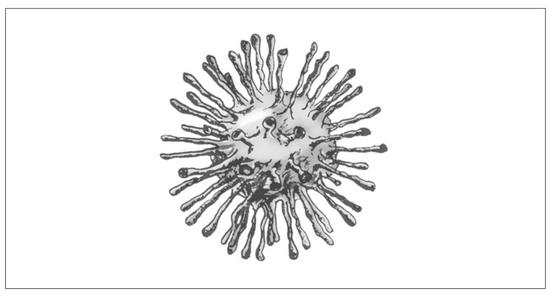
Besides normal spiking studies, the industry tends to perform additional robustness studies, proving to the authorities the effectivity of the virus removal step when deviating from the typical process parameters. However, investigations have recently discovered when operating at the edge of typical process parameters, with some virus removal membranes, virus breakthrough is more likely to occur.

This poster summarizes the results of different robustness studies looking at challenging process parameters potentially impacting virus retention.



2. Experimental details

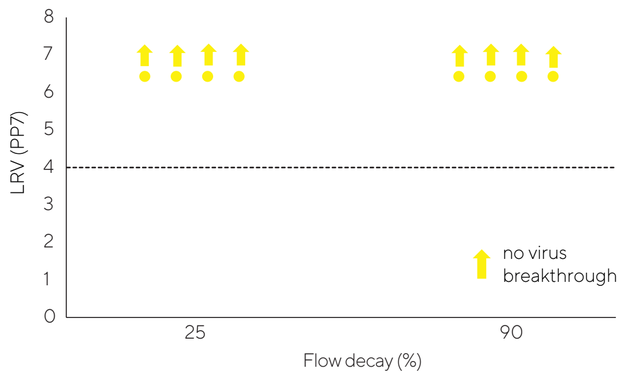
Model Virus
- Pseudomonas aeruginosa bacteriophage PP7
- Model virus for small viruses
- Size: ~ 25 nm; pI 4.5



Filter
- Commercially available virus filter (Virosart® HC)
- Down-scale device: 5.0 cm²

3. Flow decay

Two filtrate fractions were taken at 25 percent and 90 percent flow decay. The filtration was performed with 10 g/L IVIG in 20 mM KPI buffer, pH 7.2 at constant pressure of 2.0 bar with Virosart® HC lab modules (5.0 cm²). No virus breakthrough was detected under any conditions and replicates (N=4).

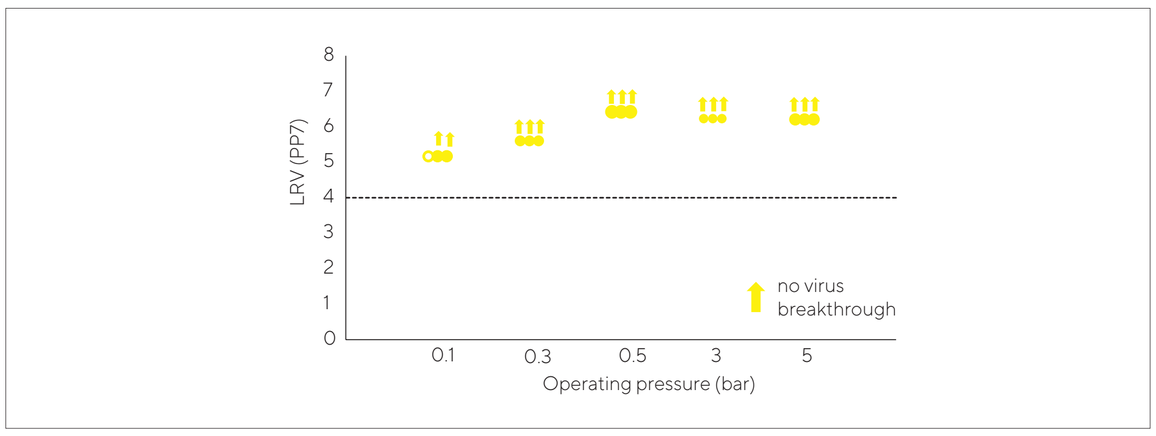


PP7 retention is independent of the level of flow decay, with absolute retention of > 6 log_{10} (without virus breakthrough).

4. Pressure

4.1 Low pressure | high pressure

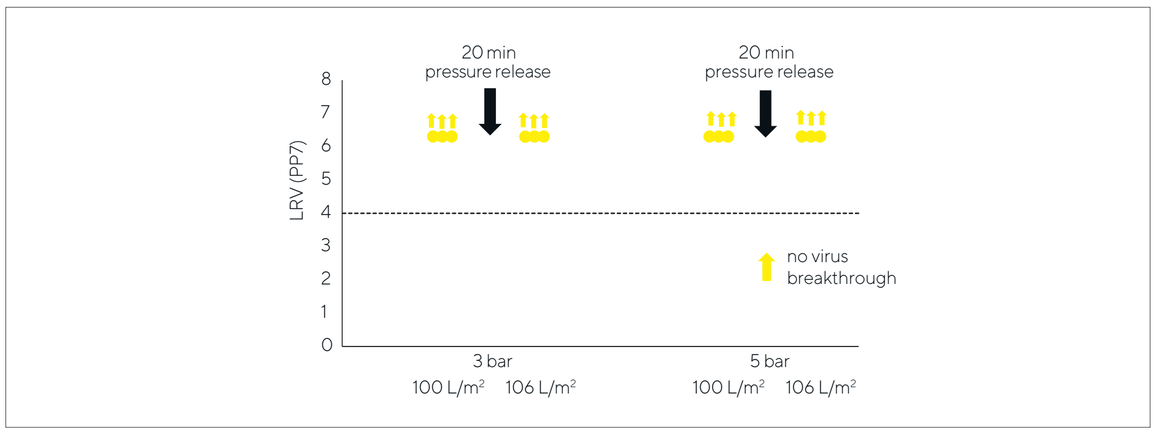
Virus retention at low (0.1 bar | 0.3 bar | 0.5 bar) and high (3.0 bar | 5.0 bar) operating pressures were tested with Virosart® HC. PP7 retention is shown in 20 mM KPI buffer, pH 7.2. A pooled fraction was taken after 100 L/m² of filtration at a PP7 challenge level between 4.0×10^6 – 6.0×10^7 pfu/mL.



No impact of low | high operating pressures on the virus retention capabilities with robust LRVs (log reduction value) of approximately 6 log_{10}.

4.2 Flow stop

Virus retention after a 20-minute flow stop at high operating pressures of 3.0 and 5.0 bar was tested for Virosart® HC. The filters were challenged with PP7-spiked KPI buffer; 20 mM, pH 7.2. 2 fractions were taken as indicated after 100 L/m² mL and after 106 L/m² of filtration.

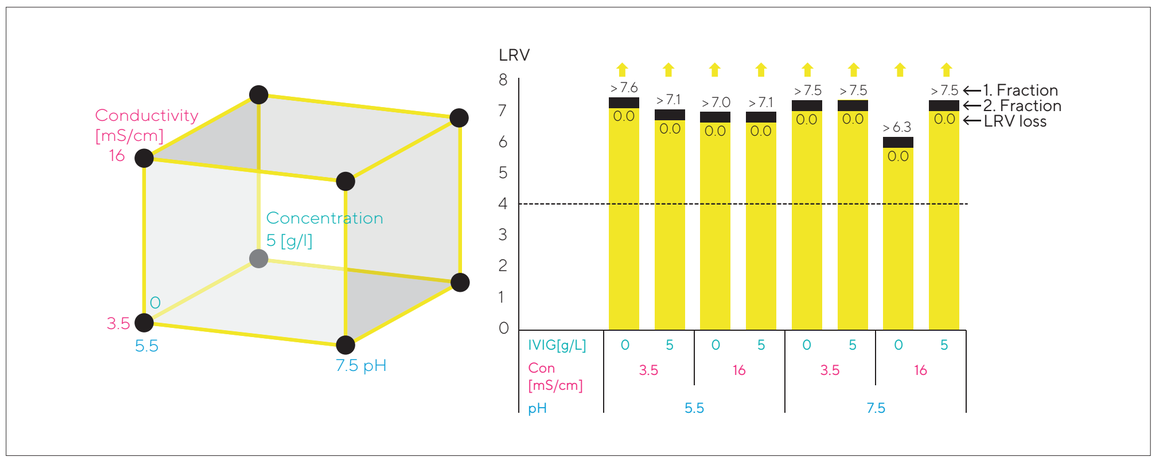


Virus retention of > 6.3 log_{10} are shown for all fractions, even after 20-minute flow stop.

5. Case Study: Impact of Solution Composition

A full factorial DOE (2^3) has been performed with Virosart® HC in order to characterize the impact of solution conditions on virus removal capability. Conductivity, pH, and IVIG concentrations have been varied. The filters were challenged with PP7 at a spike level of 10^7 pfu/mL.

All filtration runs were performed at 2.0 bar operating pressure. Overall two fractions were taken: The first one after 50 L/m², then a 15-minute flow stop was performed, then the second fraction was taken after 100 L/m².



PP7 retention is independent from the condition tested for Virosart® HC. Pressure release, protein concentration, as well as conductivity have no impact on retention characteristics. The retention values for both factions are shown, as well as the LRV loss. There was no virus breakthrough detected in any filtration runs.

6. Summary

This poster summarizes the results of a robustness study with Virosart® HC looking at various process parameters known to potentially impact virus retention.
- Flow decay up to 90 percent shows no impact on retention
- Stable and high LRV during pressure release, high and low operating pressure
- Absolute retention of PP7 (above 6 LRV) with various pHs, conductivities, and protein concentration