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Implementation of Virus Filtration Unit Operations and Evaluation of Virus Clearance Under Challenging Process Conditions

Sherri Dolan[1], Abhijit Shirke[2], Alexander Schwartz[1*], Dharmesh Kanani[2], Michael Zaykaner[2], Ruby Gao[1] and Michael Lasse[3]

[1]Sartorius Stedim North America, Bohemia, New York, United States of America; [2]Teva Pharmaceuticals, West Chester, PA, United States of America ; [3]Sartorius Stedim Biotech GmbH, Göttingen, Germany
* Corresponding authors: Alexander.Schwartz@sartorius.com & Abhijit.Shirke@tevapharm.com

1. Introduction

As a part of the downstream virus clearance requirement, multiple orthogonal unit operations should be evaluated. In contemporary virus clearance submissions, it is common to evaluate a capture column (Protein A), a polishing column (AEX, CEX, or HIC), low pH inactivation, and Virus Retentive Filtration (VRF). Since VRF is considered to function from a defined size-exclusion based principle, VRF has been thought to perform independently from the physicochemical properties of virus, making VRF a robust tool for virus removal for viruses greater than 20 nm.

There are still some cases that are a potential for viral breakthrough during viral clearance validation studies. These challenging process conditions include low pressure filtration[1], pause in filtration[1], over challenging of VRF membrane[2], significant flow decay[3], and low-pH high-conductivity effects[1]. As these effects have been observed on virus filters commercially available, it is advisable that these challenging process conditions be considered during validation study design and execution.

Sartorius Stedim offers a wide variety of 20 nm VRFs. These include the Virosart® HF, Virosart® CPV, Virosart® HC, and Virosart® Media along with a pre-filter specifically designed to work in conjunction with VRFs, the Virosart® Max. The Virosart® HF was specifically designed and now implements specific quality release tests to mitigate the effects described above. Here, in collaboration with Teva Pharmaceuticals, the Sartorius Stedim Virus Clearance Technology Team looked to create a case study evaluating the Virosart® Max and Virosart® HF in real-world worst-case conditions for process performance, virus clearance capability, and finally ease of scale up processing.

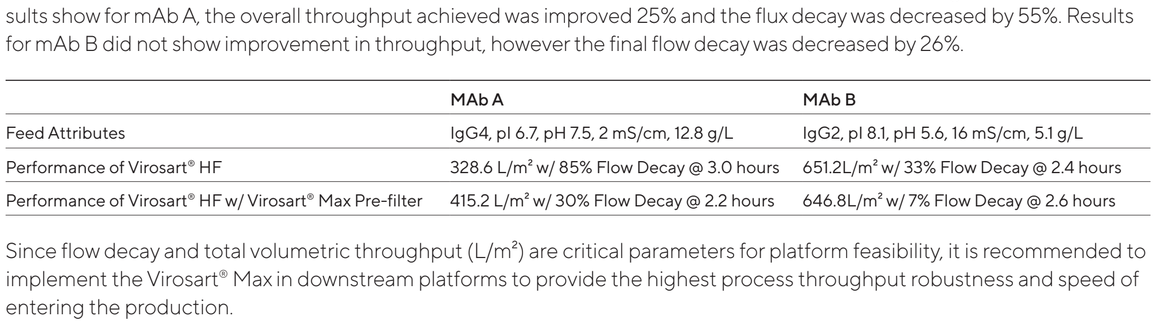

Figure 1: Tripod for risk mitigation.

2. Background and Process Development

For many large biopharmaceutical companies, a platform manufacturing process is desired for ease in development so that speed to clinic is achieved. In many cases, that means finding process technologies that can handle diverse feed materials. For virus removal filtration (VRF), critical attributes for a platform technology include the following:

- Ability to process diverse feed streams while achieving high virus retention
- Process economics in achieving consistently high total feed throughput

These critical attributes can be affected by:

- Feed material concentration
- pH
- Conductivity
- Product aggregation

Some feed streams may contain process impurities that impact the performance of the virus filter. In these situations, the implementation of a prefiltration step to remove these impurities may improve the performance of the virus filter from 30 -100%. The Virosart® Max is a triple-layer, 0.1 µm, polyamide pre-filter specifically designed for use in VRF applications with recombinant therapeutics. Due to its polyamide chemistry, the Virosart® Max filter uses the mechanisms of both size-exclusion and adsorption to provide consistently clean VRF feed materials without negatively impacting product yield or quality. In the table below, we show how prefiltration with Virosart® Max can improve the overall throughput while decreasing flux decay. The results show for mAb A, the overall throughput achieved was improved 25% and the flux decay was decreased by 55%. Results for mAb B did not show improvement in throughput, however the final flow decay was decreased by 26%.

	MAb A	MAb B
Feed Attributes	IgG4, pI 6.7, pH 7.5, 2 mS/cm, 12.8 g/L	IgG2, pI 8.1, pH 5.6, 16 mS/cm, 5.1 g/L
Performance of Virosart® HF	328.6 L/m² w/ 85% Flow Decay @ 3.0 hours	651.2L/m² w/ 33% Flow Decay @ 2.4 hours
Performance of Virosart® HF w/ Virosart® Max Pre-filter	415.2 L/m² w/ 30% Flow Decay @ 2.2 hours	646.8L/m² w/ 7% Flow Decay @ 2.6 hours

Since flow decay and total volumetric throughput (L/m²) are critical parameters for platform feasibility, it is recommended to implement the Virosart® Max in downstream platforms to provide the highest process throughput robustness and speed of entering the production.

3. Virus Clearance Study Design

A small-scale model was used to demonstrate robust MVM virus clearance under low-pH and high-conductivity conditions. Several factors were evaluated such as throughput, pH, conductivity, virus prep purity, and flow decay. The run identification and process parameters are listed below.

Table 1: Additional Feed Characteristics: pI ~8, Concentration 11 - 12 g/L, and Conductivity 12 - 16 mS/cm

Run ID	Molecule	pH	Inlet Pressure	Process Pause	MVM Virus Purity*
1	MAb B	5.6	1 bar	30 minute	Ultracentrifuge Grade
2		5.6	2 bar		Ultracentrifuge Grade
3		5.6			True Spike™
4		7.0			Ultracentrifuge Grade
5		7.0			Ultracentrifuge Grade
6	MAb C	5.2			Ultracentrifuge Grade

*The authors would like to acknowledge Charles River Laboratories and Tareq Jaber for their contributions to study design and testing support.

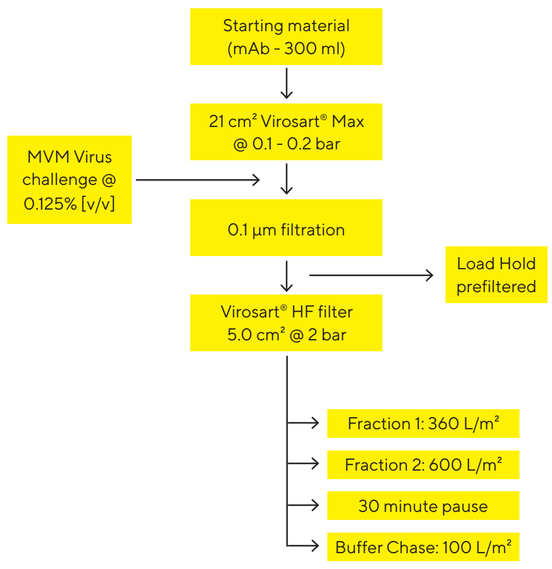

Figure 2: Viral Clearance Run Process Process Flow Diagram

Figure 2 depicts the run diagram used throughout the study. All fractions were provided to the testing lab for analysis and a simulated pool was prepared for run cumulative Logarithmic Reduction Value (LRV) results.

4. Filtration and Virus Clearance Results

All runs were successfully completed and achieved targeted volumetric loading of greater than 600 L/m² with varying amounts of flow decay depending on product and process conditions.

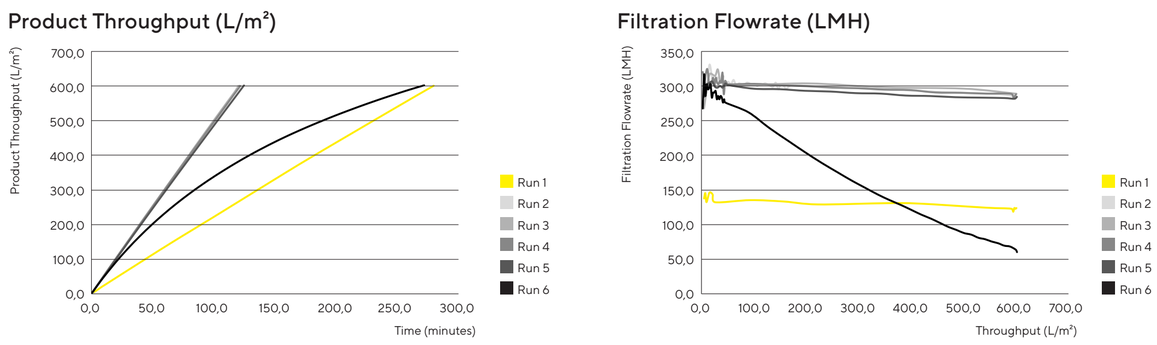

Figure 3: Filtration graphs documenting the volumetric throughput and flow decay compared to the normalized buffer flow rate.

All tested fractions and simulated pool samples demonstrated complete virus clearance, i.e. no MVM infectivity detected. All LRVs reported are above 5.2.

- The following LRVs defined for the final fractions are referred to the chase and simulated pool
- Results are derived from non-interfering final dilutions

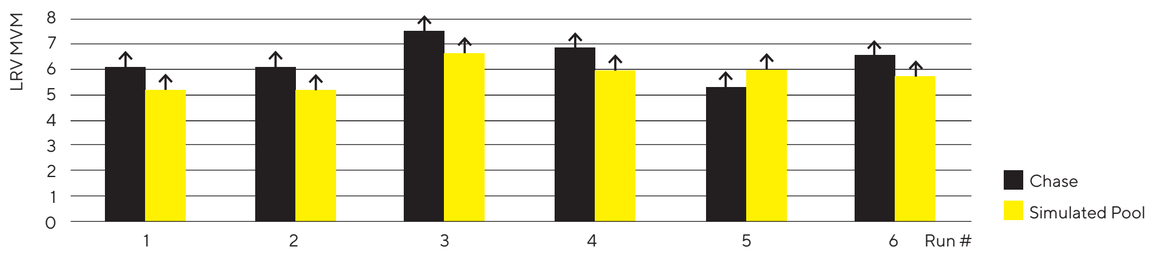

Figure 4: LRV results documenting complete and robust viral clearance among all tested fractions and simulated pool samples.

5. Virus Clearance Discussion

All runs were able to achieve the targeted load of 600 L/m². As the sample set demonstrates a broad range of filtration performance that includes higher flow decay and low-pressure conditions, the runs together give a great example of challenging process conditions.

As previously mentioned, the Virosart® HF filters are specifically designed and tested to avoid the impact of challenging conditions on the retention during virus filtration processses. The quality release of the hollow fiber membrane used in Virosart® HF includes PP7 bacteriophage retention under adverse conditions. The above virus clearance results confirm that the quality metrics are a success and result in a final product that provides robust virus retention under challenging process conditions. Furthermore, the design space demonstrates lack of impact from any of the challenging process conditions which include low pressure filtration, process pause evaluation, feed streams with low-pH and high conductivity buffer conditions, and high flow decay models.

6. Virus Filtration Scale Up

One of the benefits of using a hollow fiber membrane is its inherent linear scalability based on membrane area. Further benefits of using the Virosart® HF filter include its ability to be delivered gamma sterilized, which allows for the filter to be made into sterile single use filter transfersets. The Virosart® Max is autoclavable and can similarly be made into sterile single use filter transfersets. To further aid in single use processing, both the Virosart® HF and the Virosart® Max can now be made into the Maxicaps® MR format allowing for several large-scale filter capsules to be implemented in a pre-constructed and pre-sterilized in-parallel single use cart. The Maxicaps® MR allows for ease of set-up in GMP single use settings with near instant setup and takedown using aseptic connections to maintaining a sterile boundary and no additional filter holders are needed. Given the large scale of the Maxicaps® MR Virosart® HF 6-unit, processing capacity is now up to 8,600 L given a 600L/m² capacity using a single connection device.

Surface Area per Module	2 Unit	3 Unit	4 Unit	5 Unit	6 Unit	9 Unit
Virosart® Max	Not available	6.3 m²	Not available	Not available	12.6 m²	18.9 m²
Virosart® HF	5.6 m²	7.2 m²	9.6 m²	12.0 m²	14.4 m²	Not available

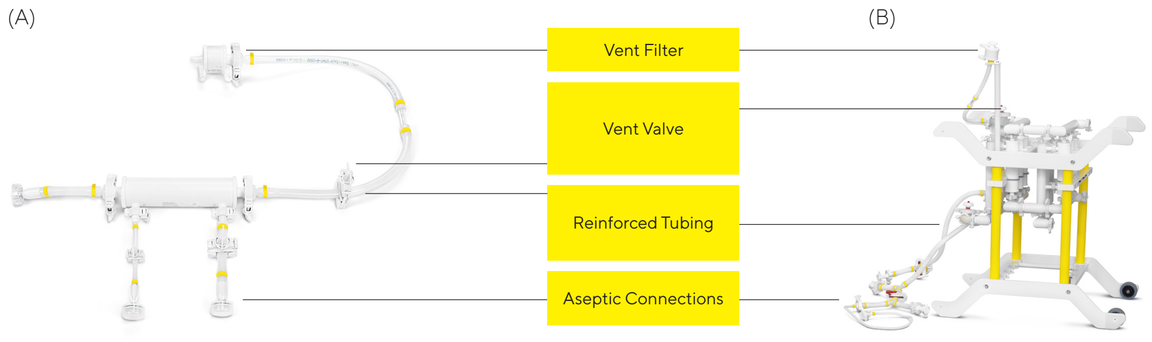

Figure 5: (A) Sterile Single-Use Filter Transfer Sets for the Virosart® HF. (B) Maxicaps® MR format for Virosart® HF filters.

7. Conclusion

The data presented demonstrates that Virosart® HF filters can provide complete virus clearance with significant virus retention under challenging process conditions that include low pressure filtration, process pause evaluation, feed streams with low-pH and high-conductivity buffer conditions, and high flow decay models. The advanced quality release testing that is conducted on each lot for Virosart® HF significantly contributes to the overall robustness regarding process performance and virus retention. Quality release testing for Virosart® HF includes flow rate and bacteriophage retention testing under challenging conditions. Furthermore, due to the gamma stability of the Virosart® HF membrane, sterile transfersets and Maxicaps® MR formats are now available which now provide up to 14.4 m² of filter area and 8,600 L of processing capability.

References: [1]Dishari et.al., 2015; [2]Chen et.al., 2014; [3]Bolton et al., 2005.

Authors: Alex Schwartz, Sherri Dolan, Rubi Gao and Michael Lasse (employees of Sartorius) & Abhijit Shirke, Dharmesh Kanani and Michael Zaykaner (employees of Teva)