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Comprehensive Virus Clearance Evaluation of the Sartobind® Q Membrane Using Four Historical Biologics

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Introduction

Virus Clearance is critical to the development of a successful clinical program. The potential sources for virus contamination include master cell banks (endogenous virus) as well as Adventitious viruses. As guided by the Regulatory agencies, live virus spiking studies are performed to quantify the reduction factor of virus which is expressed in LRV (log reduction value) or LRF (log reduction factor). The LRV is a quantitation of the reduction value of the virus present in the starting product versus the amount of virus present in the processed product (filtrate). Typical model viruses used for early phase studies include MMV (murine minute virus) and xMuLV (Xenotropic Murine Leukemia Virus). MMV is a small (18 – 24 nm), ssDNA, non-enveloped parvo virus.
This virus is considered to be 'worst case' for anion exchange chromatography (AEX), due to its small size and high pI value, therefore was the chosen virus for this study.

Platform purification processes are commonly used within the industry, with its benefit being a decrease of workload for process development. A typical platform process is represented below where three orthogonal steps may be claimed for enveloped virus (inactivation, charge-based, and size-based) and two orthogonal steps may be claimed for virus clearance for non-enveloped virus (charge-based and size-based):

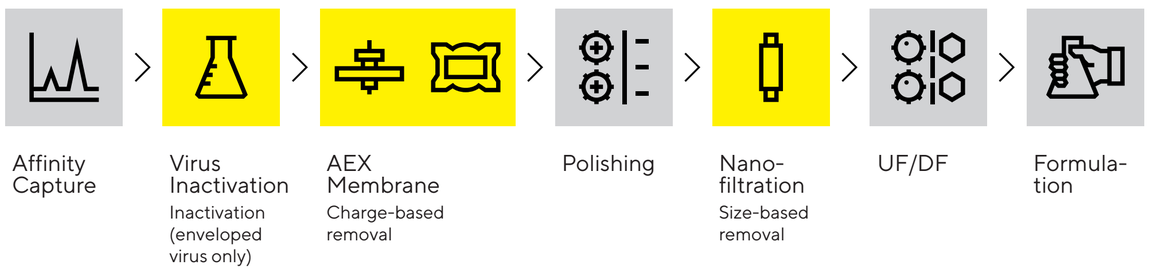

Figure 1 *Virus Clearance with a Platform Purification Process*

1. Anion Exchange Membrane Adsorbers

Sartobind® Q membrane adsorber is designed to remove charged contaminants from therapeutic proteins at accelerated flow rates by AEX. The high throughput is a direct result of negligible mass transfer effects and is made possible by the >3 µm macroporous membrane with 4 mm bed height. Sartobind® Q is used as the workhorse to remove negatively charged contaminants: DNA, host cell proteins, viruses and endotoxins.
Attractive properties of membrane adsorbers vs. traditional AEX columns.

Efficient
- Higher throughput (g/h) for trace impurity removal

Economical
- Saves capital
- No hardware investment and maintenance
- No column packing, testing, regeneration
- No re-use validation
- Less unspecific binding – higher yield
- Less labor
- Buffer consumption may be decreased 95%

Easy to use
- Disposable
- Simple and fast set up
- Handling like a filter capsule

Sartobind® Q Pico
The Pico (0.08 mL) is useful for challenging the membrane to high loading capacity (>5 g/mL)

Sartobind® Q Nano
The Nano is often used when validating lower loading capacities

Figure 2 *Two Small Scale Devices Are Available for Virus Clearance Validation Studies and the Performance of These Two Devices Were Tested in This Study.*

2. Study Design

The study was performed to assess the potential virus removal capacity of a Sartobind® Q membrane adsorber in the purification process of four monoclonal molecules (mAb, DVD, Bispecific 1 and Bispecific 2) using MMV.
The study was divided into two parts.

Part 1: Scalability study
The purpose is to compare the performance and interchangeability of the 1 mL Nano with the 0.08 mL Pico device (for Bispecific 1 only) for virus validation studies. For this study, both Nano and Pico devices were loaded up to 5 kg material/L membrane.

Part 2: Loading study
The purpose is to determine the maximum loading capacity of each test molecule while maintaining >4 LRV of virus retention. For this study, the membrane was loaded up to 20 kg material/L membrane using an 0.08 mL Pico device. Five fractions were taken during the loading and analyze for the presence of MMV.

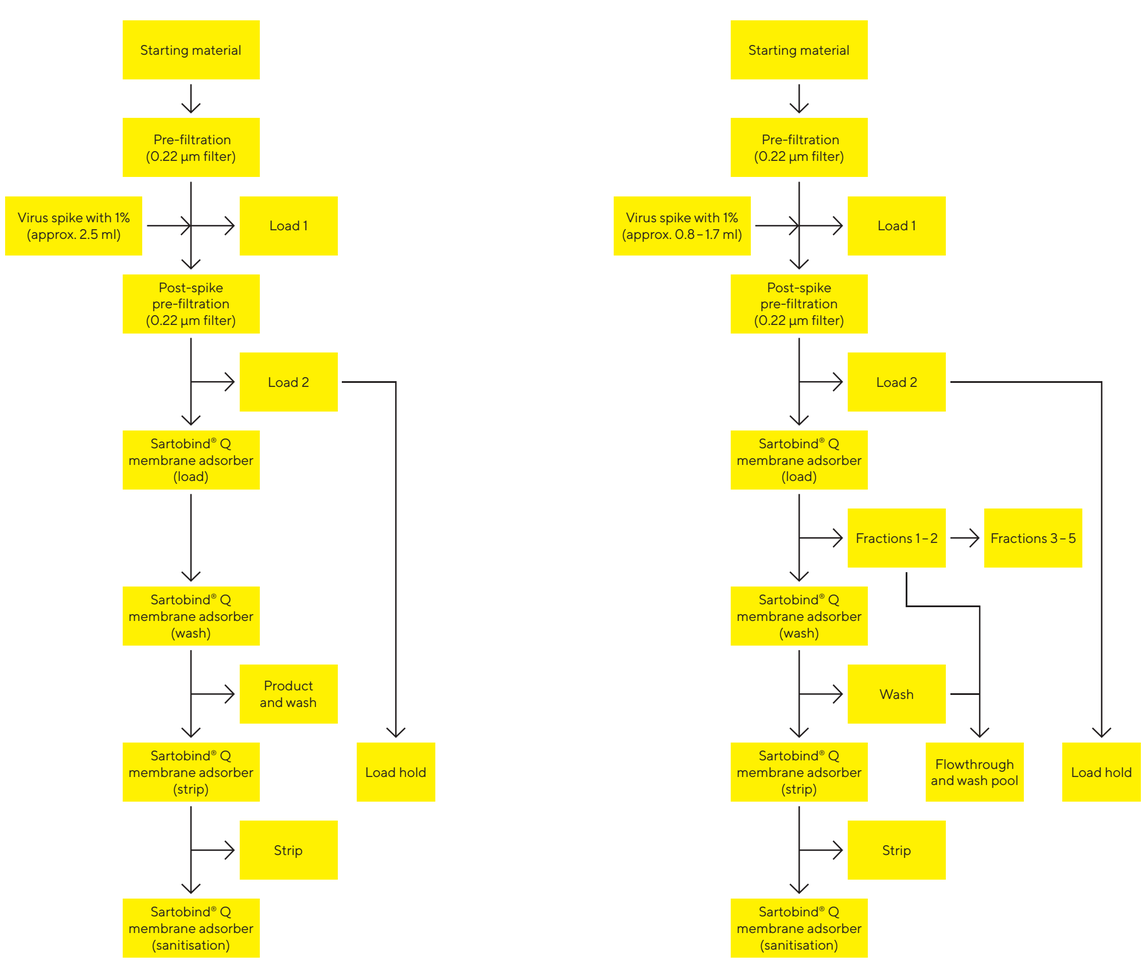

Figure 3 *Study Design Overview*

Loading study - Fractions were collected as follows:

Fraction	1	2	3	4	5
g_{mAb}/ml_{Q}	0 – 2	2 – 5	5 – 10	10 – 15	15 – 20

3. Data and Results

Test Material Characteristics

Molecule	pH	pI	Conductivity (ms/cm)	Concentration (mg/ml)	Hcp (ppm)	Dna (ppm)	% HMW
Bispecific 1	7.1	6.9	2.87	20.45	0.6	<0.15	1.9
mAb	7.2	8.9	3.58	19.58	3.1	<0.15	2.2
DVD	7.0	9.0	5.18	3.4	1	<0.88	2.9
Bispecific 2	7.1	6.8	3.08	11.6	3.4	<0.26	32.6

Table 1 *Overview of the Four Different Molecules (Product Types) Chosen for This Study to Cover a Diverse Set of Characteristics. All test materials were sourced from their respective GMP campaigns as AEX loads (sampled post depth filtration). These test materials are identical to the materials used for the respective GLP virus clearance studies using competitor membrane (data shown in Figure 6) with the exception of DVD.*

Part 1: Scalability Study

These results suggest the Nano and Pico devices are comparable with regards to MMV clearance and either device may be used for virus clearance studies. The Pico device is recommended when loading to high capacities or when product amount is limited.

Test material: Bispecific 1, loading up to 5 kg mAb/L membrane adsorber.

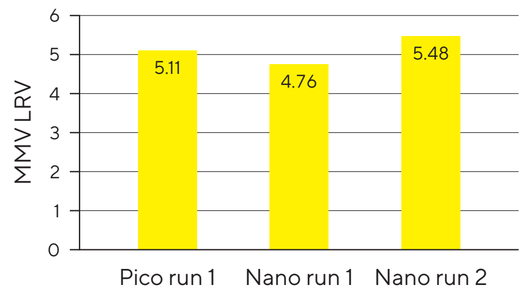

Figure 4 *Sartobind® Q Scalability Study*

Part 2: Loading Study

A loading study using Sartobind® Q membrane was performed to determine the maximum amount of test material which can be loaded onto the membrane adsorber (in the flow through mode) before the virus removal capacity is reduced. Determining an accurate loading capacity can be critical to achieving an economical large-scale process. Underloading the membrane will result in excessive costs, while overloading the membrane will result is lower than expected virus and impurity clearance. The ultimate goal is to determine the amount of test material which can be loaded on the membrane before the membrane becomes overloaded (by impurities, etc) and virus breaks through into the filtrate.

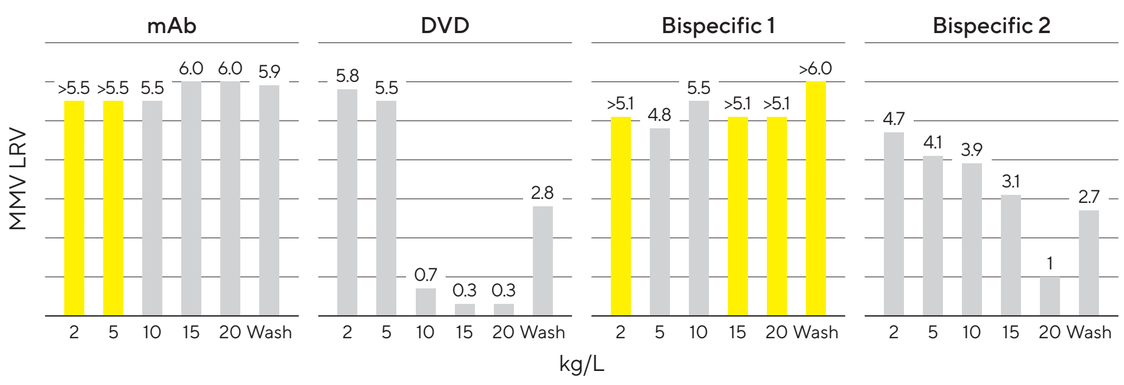

Figure 5 *Fractioned Membrane Loading Capacity (kg/L)*
Solid yellow bars indicate complete removal – no virus detected in sample

This study was performed using the Sartobind® Q Pico (0.08 mL) device at a flow rate of 10 MV/min. The membrane was loaded with test material up to 20 kg/L and 5 filtrate fractions, plus one wash fraction, were taken and analyzed for the presence of virus.

Molecule	Loading Capacity (kg/L)	MMV LRV
Bispecific 1	20	5.1
mAb	20	5.5
DVD	5	5.6
Bispecific 2	5 | 15	4.4 | 4.0

Table 2 *The results of the loading study show the mAb along with the Bispecific 1 can be loaded up to 20 kg test material/L membrane without any breakthrough of MMV. DVD and Bispecific 2 showed MMV virus breakthrough after loading 5 kg/L, however virus retention at 5 kg/L loading is >4 LRV.*

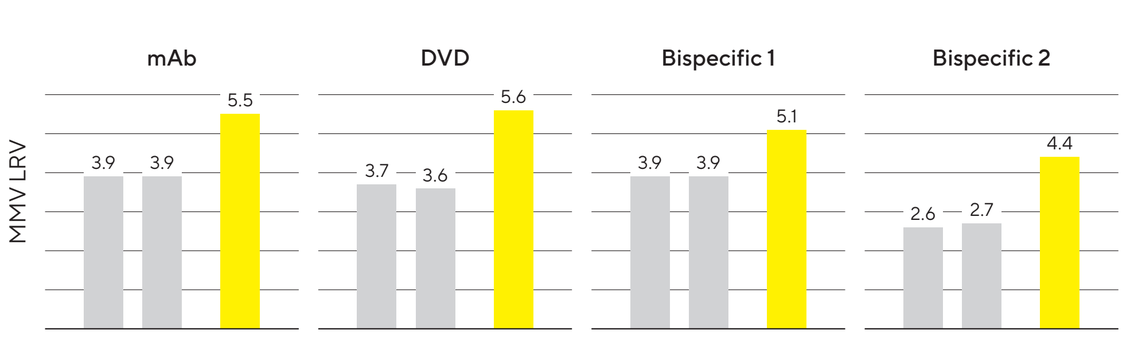

Figure 6 *MMV Clearance Comparison of Sartobind® Q versus Competitor Q Membrane*

Comparison of Sartobind® Q MMV retention with competitor membrane. Sartobind® Q outperformed the competitor for all four molecules at higher loading capacities. For all runs, the load virus titers were comparable.

4. Conclusions and Future Directions

- Sartobind® Q Nano and Pico devices showed similar results which increased confidence in using the Pico device, which in turn, enables decreased material requirements as well as the ability to perform virus validation studies at high loading capacities.
- Two out of four molecules showed high virus retention at maximum loading capacities. The other two molecules showed high virus retention at lower capacity; however, the determined load capacity was higher than the capacity used for competitor membrane.
- The maximum loading capacity will be impacted by the presence of negatively charged molecules (such as impurities/aggregates) that bind to the membrane in addition to virus.
- Sartobind® Q membrane showed higher virus retention at similar load conditions when compared to the competitor membrane.
- Next step: Complete cost analysis of using membrane adsorbers at high load capacity in late-stage manufacturing with chromatography resin.