

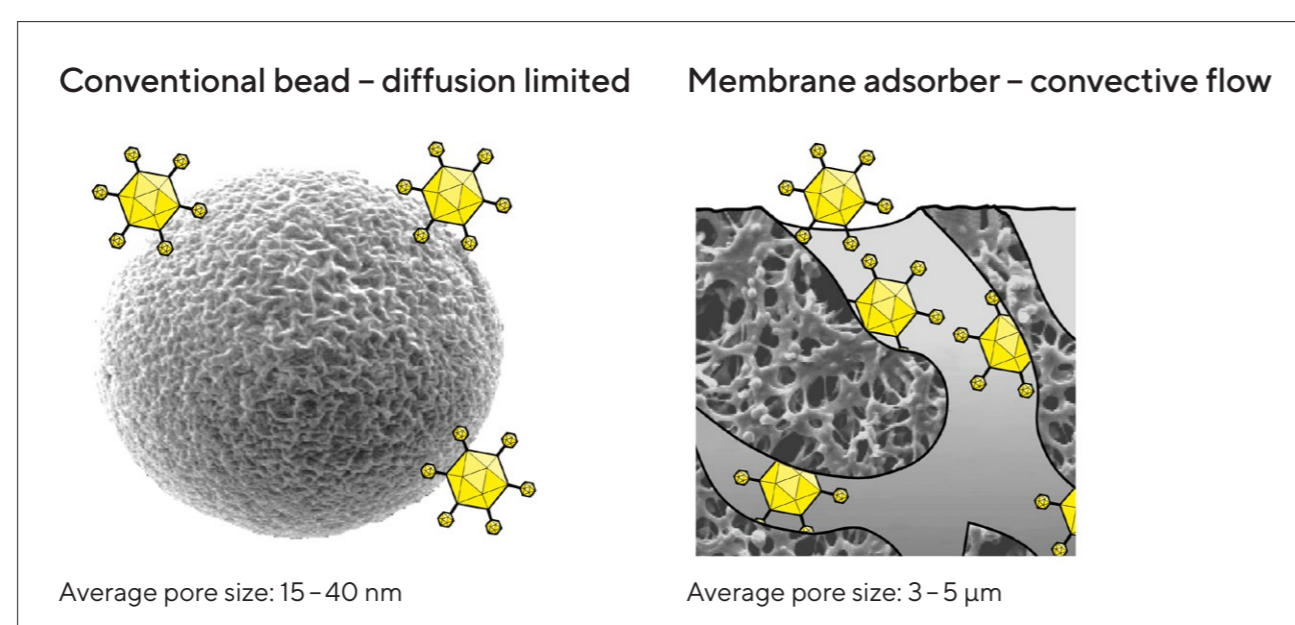
Robust Parvo Virus (MVM) Clearance on Sartobind® Membrane Adsorbers

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1. Introduction

The risk of virus contamination is a feature common to all biotechnology products derived from cell lines. It is a requirement for the downstream purification process to be validated to remove and/or inactivate potential viruses. The validated process must conform to an orthogonal approach which typically includes inactivation (low pH/solvents), Anion Exchange chromatography (AEX) and filtration. AEX chromatography is a powerful method to clear all types of virus based on charge.

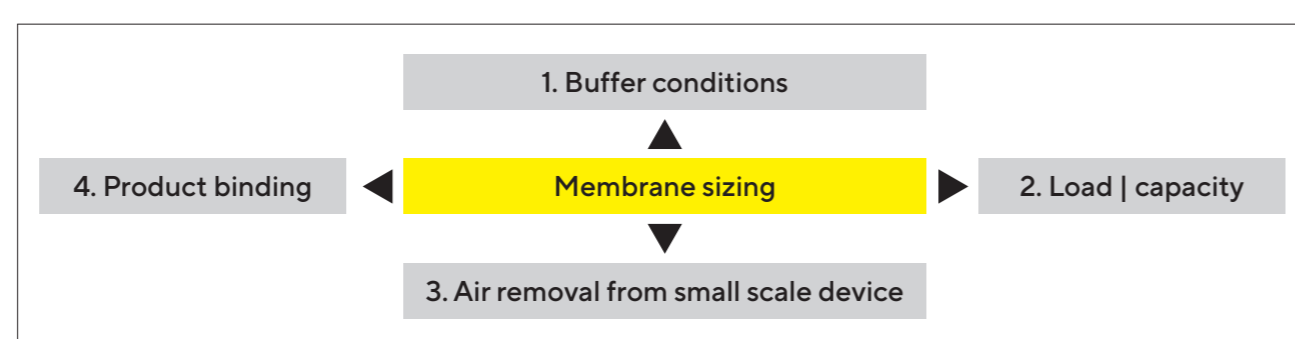
Compared to resins, membranes offer higher binding capacity for large molecules such as viruses, because the open pore structure allows the virus particles to easily enter the matrix and bind to the ligands. Membranes also show equivalent virus clearance capabilities, have 10–30 fold faster flow rates, use >75% less buffer and presents a flexible plug & play disposable solution which does not require cleaning validation. These characteristics make membrane adsorbers a perfect fit for flow through chromatography operations where virus clearance is expected.



2. How to size membrane adsorber for virus clearance

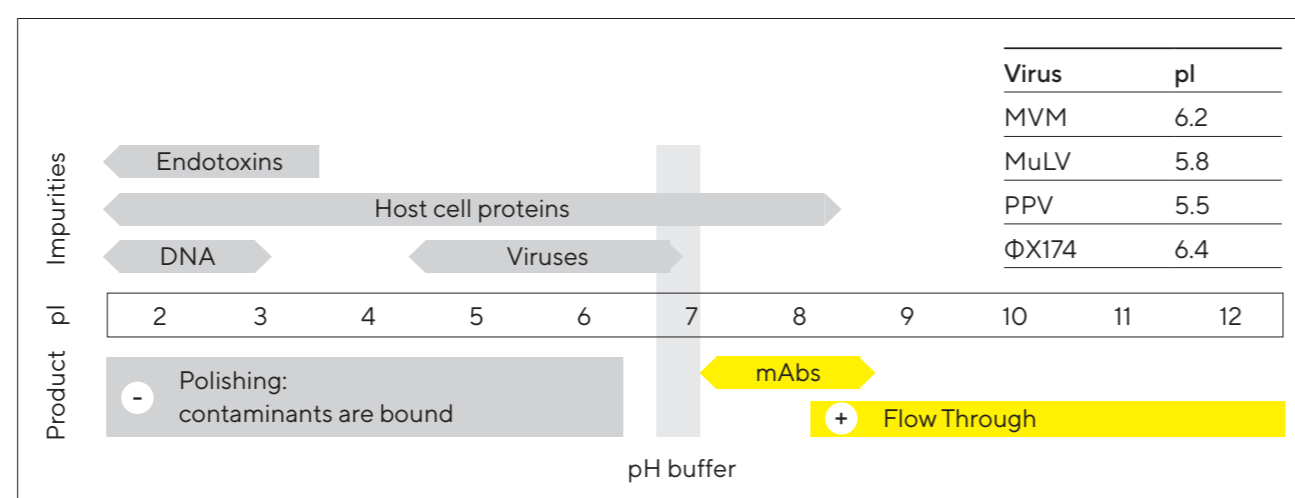
Anion Exchange chromatography is a very effective step for virus removal, and membrane chromatography is attractive because the high loading capacity results in very fast processing. However, since membranes are typically run at flow rates up to 30 times higher than conventional resins, there are specific considerations that should be taken into account when using membrane adsorbers.

There are 4 main considerations to take into account:



2.1 Buffer conditions

In order to achieve maximum virus clearance, it is important to choose conditions which prevent the product from binding to the membrane while facilitating the virus binding. Therefore, the pH of the buffer should be 0.5–1.0 unit higher than the isoelectric point (pI) of the virus to be removed and also 0.5–1.0 unit lower than the pI of the product.

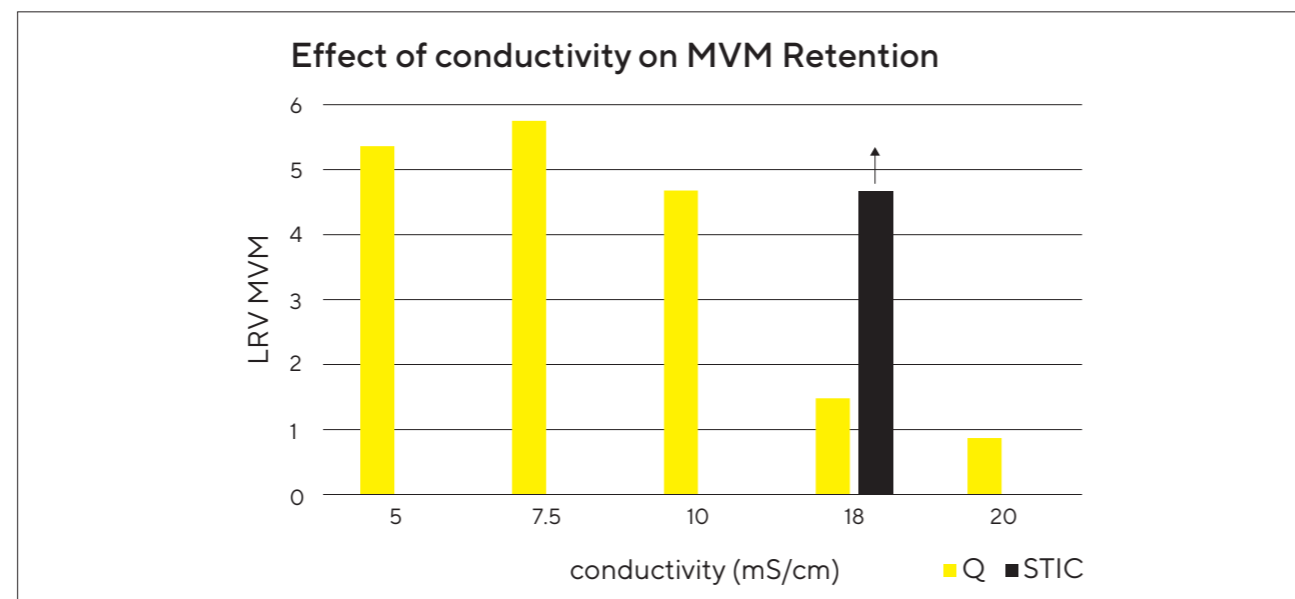


Optimal conditions:

Sartobind® Q: low conductivity (5–10 mS/cm) and neutral pH
Sartobind STIC®: no or low concentration of multivalent buffers, >10 mS/cm conductivity, neutral pH

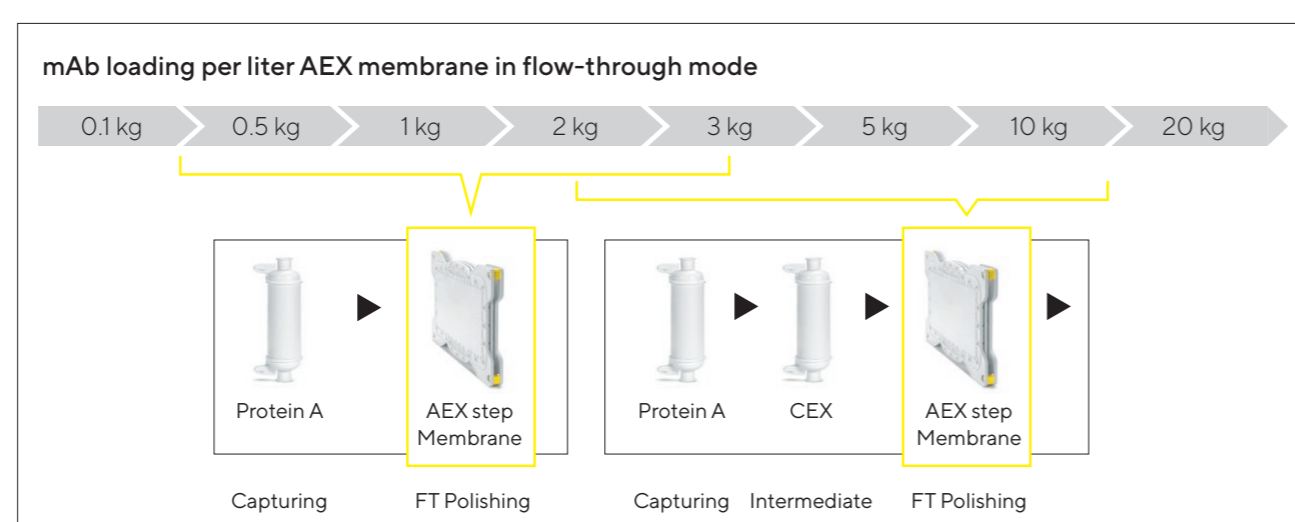
- Sartobind® pico (0.08 ml)
- Cellca mAb @ 5 g/L
- flow rate 10 MV/min
- load: 1 Kg/L on STIC
- 20 Kg/L on Q

Conclusion:
1. Increasing conductivity has a negative effect on MVM clearance with Q membrane
2. STIC® membrane: retention good at higher conductivity



2.2 Membrane loading

Careful consideration must be taken when determining the loading capacity for membrane adsorbers for maximum virus clearance. When used as a second purification step, typical capacities range from 0.1–3 kg protein/L membrane in flow through mode. The actual capacity is dependent on the impurity level. When used as a third purification step, typical capacities range from 2 kg/L up to 20 kg/L.



To accurately determine loading capacity for maximum virus clearance, a virus spiking study must be performed.

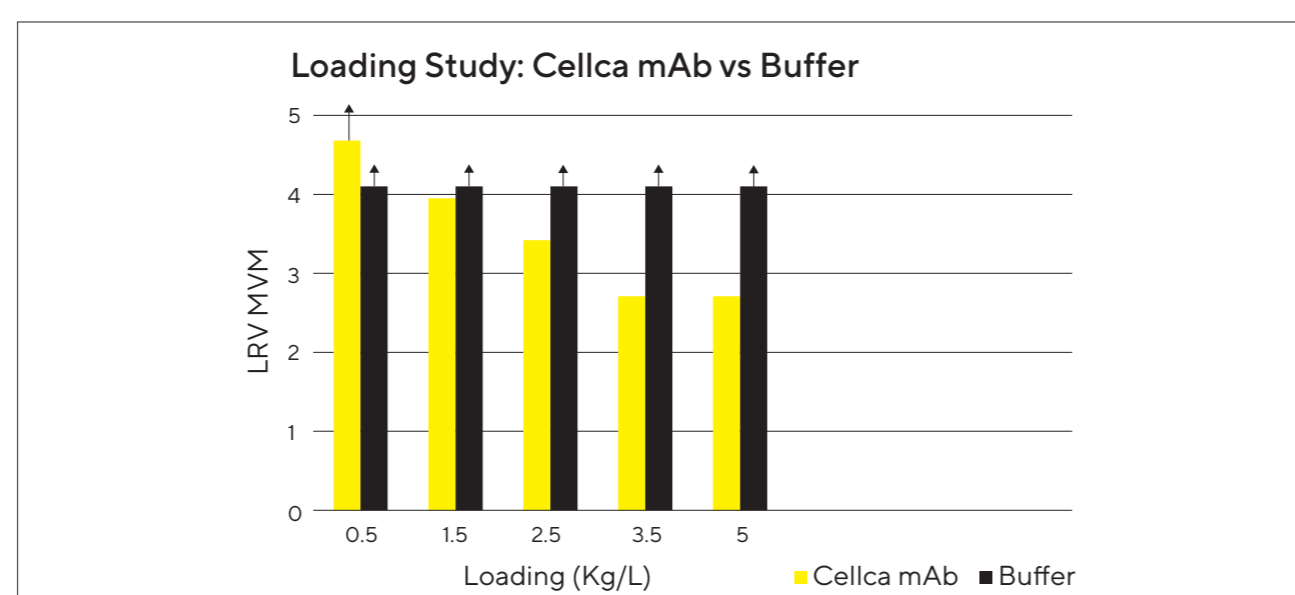
Sartobind STIC® pico, buffer alone:

- 20 mM Tris, 150 mM NaCl, pH 7.5 (18 mS/cm conductivity)
- flow rate 20 MV/min
- pool: >5.89 LRV

Sartobind STIC® pico: 5 g/L Cellca mAb

- 20 mM Tris, 150 mM NaCl, pH 7.5 (18 mS/cm)
- flow rate: 20 MV/min
- pool up to 1 Kg/L: >4.67
- pool up to 5 Kg/L: 3.15 LRV

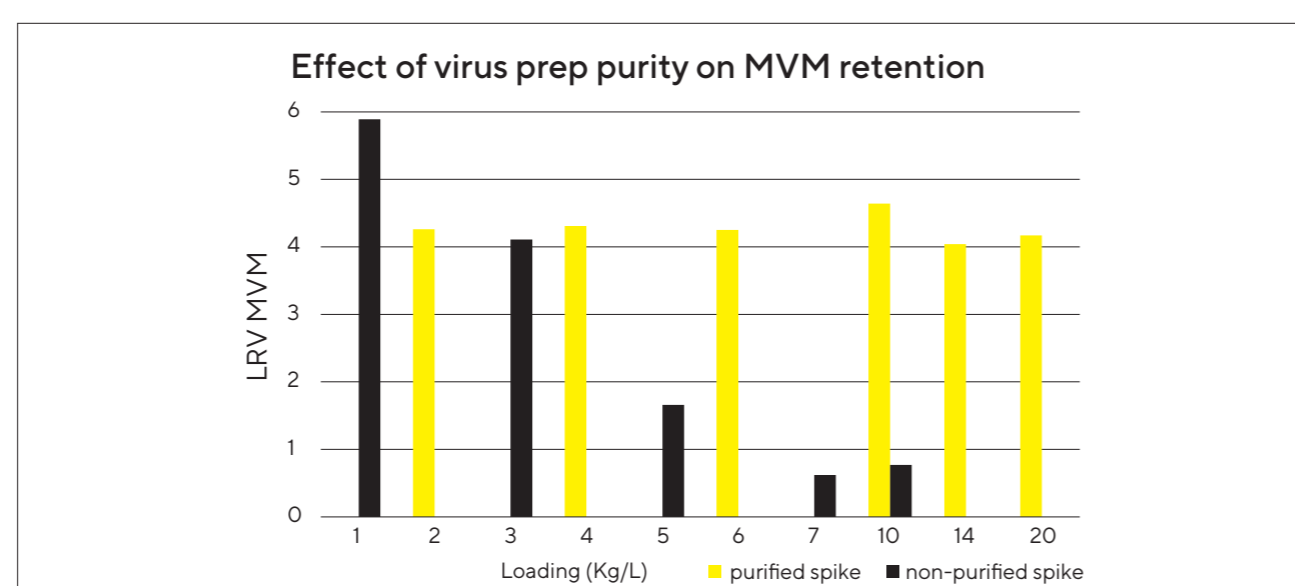
Conclusion:
protein is competitively binding to membrane



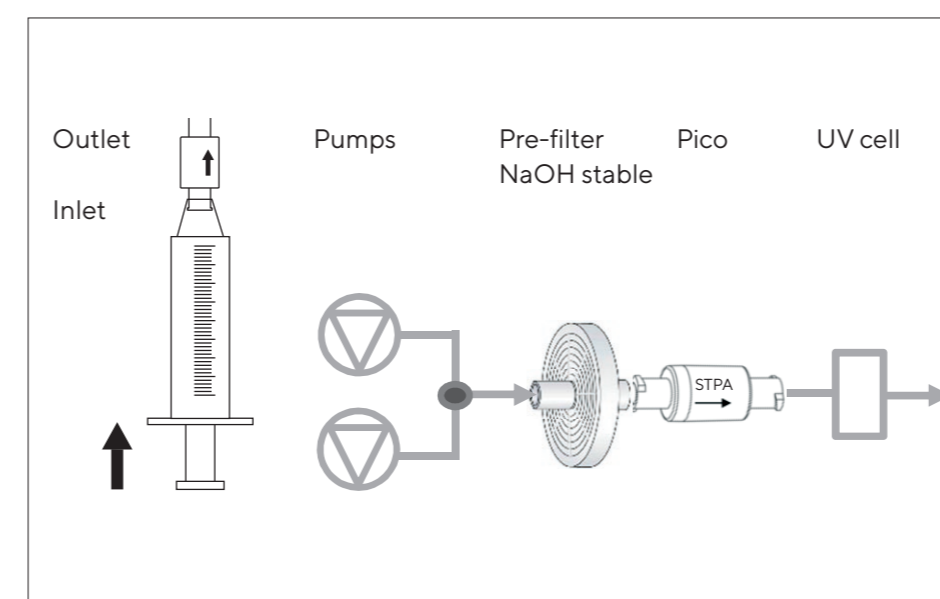
Sartobind® Q pico (0.08 ml)

- Cellca mAb @ 10 g/L
- 20 mM Tris, 25 mM NaCl, pH 7.2 buffer (<5 mS/cm)
- flow rate 20 MV/min

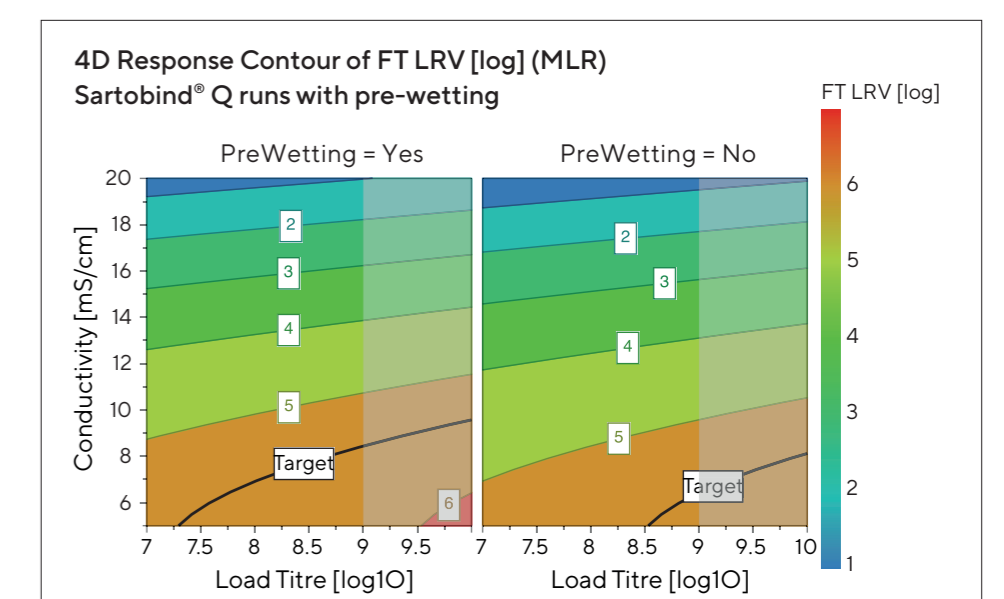
Conclusion:
1% purified spike
pool: 4.74 LRV (Ivp)
1% non-purified virus prep
pool: 1.32 LRV



2.3 Membrane wetting of small scale device



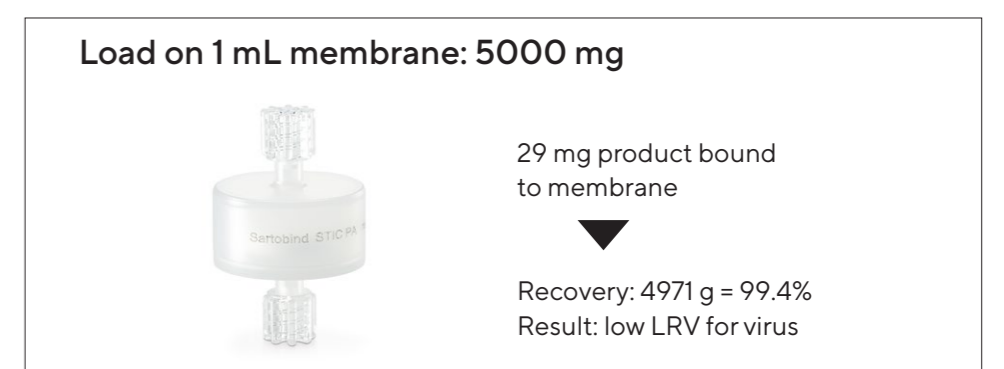
It is very important to completely remove all air from the small scale devices. Failure to do so may result in low and/or variable LRV (especially with hydrophobic proteins). It is also recommended to place a prefilter in line before the small scale Sartobind® membrane. Please follow vendor recommendations for wetting and proper use of device.



2.4 Product binding

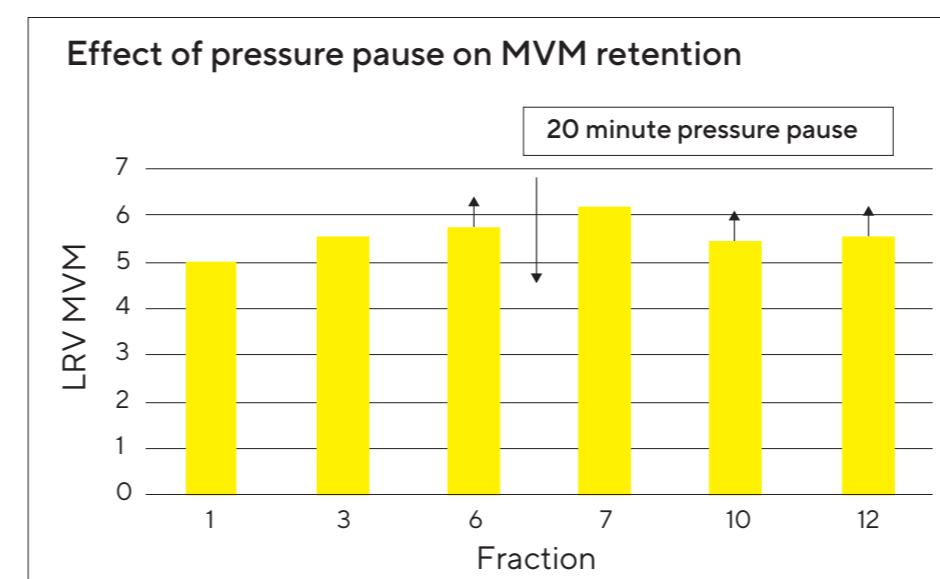
In order to achieve high LRV product binding must be minimized. Simply measuring product recovery is not sufficient to detect product binding on the membrane. Since the protein (BSA) binding capacity of the membrane is 29 mg/mL membrane, it is not possible to detect such a small loss in recovery when loading large amounts of product in flow through mode. In order to determine whether product is binding to the membrane, a dynamic binding capacity (DBC) study should be performed.

Non-recommended conditions include very low conductivity and running at a pH close to (+/- 0.5 unit) or above the pI of the product.



3. Robustness of MVM clearance

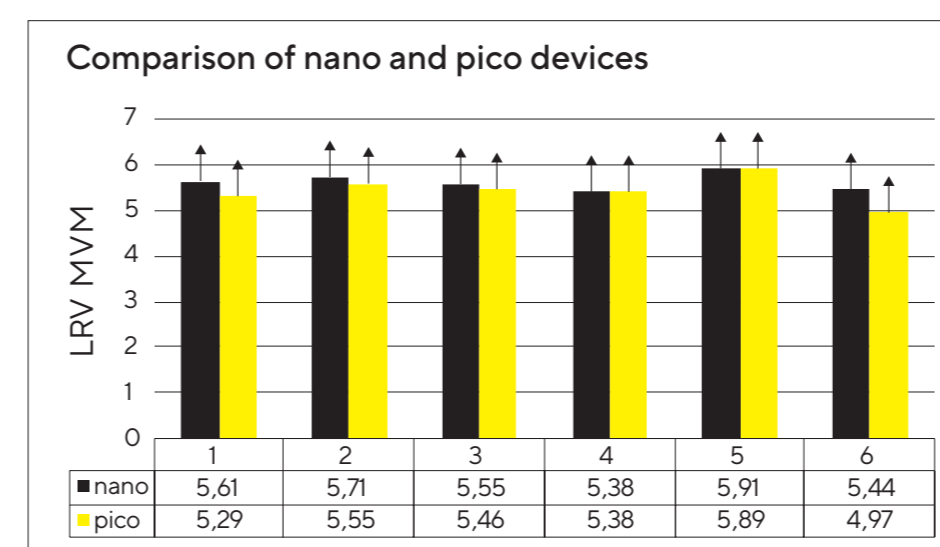
3.1 Process pause



Pressure pause during processing did not effect MVM virus retention.

- Sartobind® pico (0.08 mL)
- 200 mL of 5 g/L Cellca 2 mAb (load 12.5 kg/L)
- 20 mM Tris, 25 mM NaCl, pH 7.2
- Flow rate: 10 MV/min
- Fraction 6: immediately prior to flow stop
- Flow stop for 20 min
- Fraction 7: immediately after flow stop

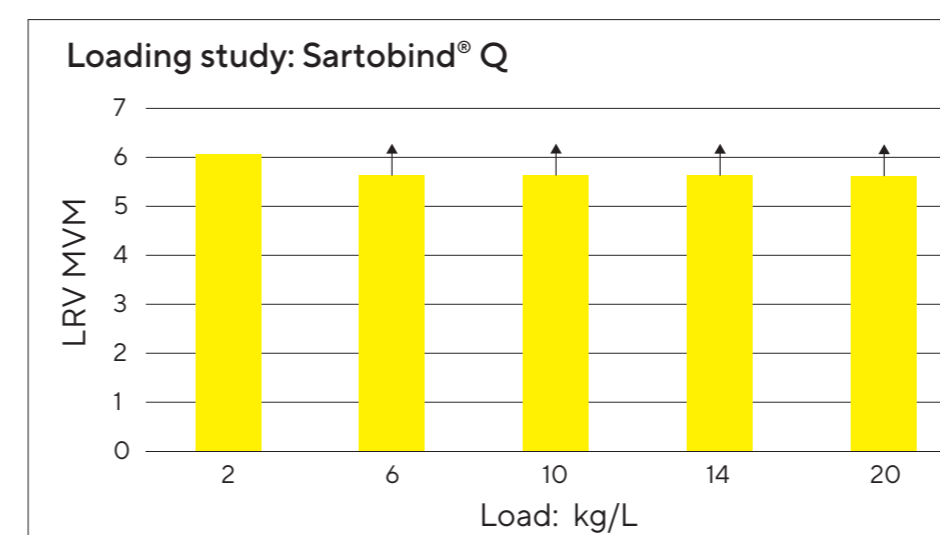
3.2 Consistency



Either Sartobind® Q Nano (1 mL) or Pico (0.08 mL) may be used for virus spiking validation. Retention of MVM is consistent as shown in the graph

- Run 1–4: 20 mM Tris, 25 mM NaCl, pH 7.2 buffer
- Run 5–6: 5 g/L Cellca 2 mAb (load 5 kg/L)
- Conditions same as 3.1

3.3 High product loading



Complete MVM clearance with increasing load onto Sartobind® Q.

- Sartobind® pico (0.08 mL)
- 320 mL of 5 g/L Cellca 2 mAb (load 20 kg/L)
- 20 mM Tris, 25 mM NaCl, pH 7.2
- Loading flow rate: 20 MV/min
- pool: 5.77 LRV

4. Considerations

For optimal virus removal, certain considerations need to be taken into account with regards to the equipment used as well as process conditions.

Equipment set-up

- Do not reuse small scale device
- Wet filter and remove air as stated in the user's manual
- Clean with NaOH to remove glycerol and bioburden
- Pre-filter sample with 0.2 or 0.1 µm filter
- Place NaOH stable pre-filter (0.2 or 0.45 µm) in line
- Measure pH and conductivity of eluate to confirm equilibration
- Use dedicated sample pump
- Do not load product through mixer
- Use flow restrictor when using pico

Process considerations

- Choose appropriate buffer conditions
- Determine loading capacity by performing virus spiking study
- Ensure product is not binding to membrane
- Characterize process robustness with respect to product loading, pH & conductivity

5. Conclusion

Robust parvo virus removal can be achieved using Sartobind® membrane adsorbers. Membrane adsorbers, like Sartobind® can be loaded to high capacities at high flow rates, and offer consistent performance in an easy to use disposable format. For optimal virus removal, certain considerations need to be taken into account: buffer conditions, product, air removal from filter and product binding. Acknowledgement: Cella 2 mAb provided by the BioProcessing (Corporate Research) team of Sartorius Stedim Biotech GmbH, Goettingen