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

1. Introduction

The risk of virus contamination is a feature common to all biotechnology products derived from cells lines. It is a requirement for the downstream purification processes 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), Solvent/ detergent, AEX chromatography is a powerful method to clean a types of virus based on charge.

Compared to toxins, membranes offer higher binding capacity for large molecules such as viruses, because the open pore structure allows the virus to be captured easily enter the matrix and not to the ligands. Membranes also show equivalent virus clearance capabilities, having 30-300 faster flow rates, use 75% less buffer and presents clearances is expected.

Membranes also show equivalent virus clearance performances, allowing the virus particles to easily enter the matrix and bind to the ligands.

2. How to size membrane adsorber for virus clearance

Anion Exchange chromatography is a very effective step for viruses, 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.

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, 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 pH of the product.

Optimal conditions:

- Sartobind® Q: low conductivity (5–10 mS/cm) and neutral pH
- Sartobind® STIC: neutral medium concentration of multivalent buffers, >75% less buffer and presents clearances is expected.

2.2 Membrane loading

Careful consideration must be taken when determining the loading capacity for membrane adsorbers for maximum virus clearance. When using a second purification step, typical capacities range from 0.1–1.4 g protein, membranes flow through mode. The actual capacity is dependent on the multiplicity level and can be used as a high throughput stage, typical capacities range from 2g/L up to 20g/L.

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

3. Robustness of MVM clearance

3.1 Process parameters

Pressure pause during processing did not affect MVM virus retention.

Effect of pressure pause on MVM retention

<table>
<thead>
<tr>
<th>Fraction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Load Titre</td>
<td>0.00</td>
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<tr>
<td>Recovery</td>
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</table>

3.2 Consistency

Comparison of nano and pico devices

<table>
<thead>
<tr>
<th>Device</th>
<th>PreWetting</th>
<th>Flow Through Flow Rate</th>
<th>LRV MVM</th>
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<tbody>
<tr>
<td>Nano</td>
<td>Yes</td>
<td>20 mL/min</td>
<td>5.8</td>
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<tr>
<td>Pico</td>
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3.3 High product loading

Complete MVM clearance with increasing load onto Sartobind® Q.

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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 use small scale device
- Use filter and demin water as stated in the user’s manual
- Clean with NaOH to remove glycerol and surfactants
- Use the same sample with 0.5-2.0 µm bio
- Place NaOH stable pre-filter (2.0 or 2.0 µm)
- Measure pH and conductivity of buffer to confirm equilibration
- Use cleaned sample pump
- Do not load product through filter
- Use flow restrictor when using nanos

Process considerations

- Choose appropriate buffer conditions
- Determine loading capacity by performing virus spiking study
- Ensure no product is binding to membrane
- Characterize robustness with respect to product loading, pH, and 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: Cellca 2 mAb provided by the BioProcessing (Corporate Research) team of Sartorius Stedim Biotech GmbH, Göttingen.

Conclusion:

Sartobind STIC® pico, buffer alone:

- 20 mM Tris, 25 mM NaCl, pH 7.2 buffer
- Flow rate 20 mL/min
- pool ≤ 8.9 mS/cm

Sartobind® STIC pico, 5 g/L Cellca mAb:

- 20 mM Tris, 25 mM NaCl, pH 7.2 buffer
- Flow rate 20 mL/min
- pool up to 1 g/L
- ≤ 4 g/L
- pool up to 5 g/L 3.15 mS/cm

Conclusion:

protein is completely binding to membrane

Sartobind® Q: 0.08 mL

Cellca mAb 0.5 g/L

20 mM Tris, 20 mM NaCl, pH 7.2 buffer (flow rate 20 mL/min)

Conclusion:

% of pool capacity

pool ≤ 4 g/L/min

100% product recovery

pool ≤ 3.15 mS/cm

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<tr>
<td>0</td>
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Effective of virus prep purity on MVM retention

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2.3 Membrane wetting of small scale device

It’s 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 prefiltet in front of 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 is not possible to detect such a small loss in recovery when loading large amounts of protein 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 pH of the product.

Acknowledgement: Cella 2 mAb provided by the BioProcessing (Corporate Research) team of Sartorius Stedim Biotech GmbH, Göttingen.