Sterile filter

Birte Kleindienst, Anika Manzke (Marketing Virus Clearance)

Concept of a Fully Single-Use Virus Filtration

Robust retention in virus filtration is faced with challenges: of operation via peristaltic pump, operating pressure and process interruptions.

Pressure and Flow are essential parameters to control and monitor the virus filtration.

Virosart® Max is a pre-filter specially developed to maximize the virus filter performance.

Virosart® HF and CPV are virus retentive filters especially developed for monoclonal Antibodies (mAb) and other recombinant protein applications.

Delivery: The pre-assembled single-use equipment is sterilized, either by gamma radiation, UV light or autoclaving.

Installation: In production the single-use equipment can be installed sterile through Teflon® connection or non-sterile via Femtoseal®. Purification is planned as intended between the syringe filter and the final bag.

Future operations: In order to operate virus filtration in single-use systems a fully sterile way few challenges have to be overcome: sterile pre-use integrity test (puncture) but also smart handling of increased complexity of single-use equipment.

The pre-use integrity test (puncture) has to be executed, the syringe filter membrane has to be fully worked with a needle.

Wetting for operation: The filter and the equipment is equilibrated with the same buffer.

The single-use equipment has to be installed and all required process steps, besides the IT, are automated with the FlexAct® system.

Virosart® HF, CPV and Max.

Independent of operating pressure or virus retention (PFP) of four Virosart® HF 5 cm² lab modules tested in high (5 bar), medium (1 bar) and low (0.1 bar) operation pressure 20 mM KPi buffer, pH 7.2 LRV (PP7) in buffer

<table>
<thead>
<tr>
<th>Pressure Level</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRV (PP7)</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The system should be operated in the following order of process steps:

1. Installation: The single-use equipment has to be installed sterile through Teflon® connection or non-sterile via Femtoseal®.
2. Wetting for Pre-use IT:
3. Flow control of process
4. Filtration:
5. Equilibration:
6. Post-use IT:

Constant pressure or constant flow.

Use-of-the-whole-filter capacity during filtration.

Validated flow decay is reached

Maximale delta p is reached

Validated flow decay is reached

All required process steps, besides the IT, are automated with the FlexAct® system.

Virosart® HF, CPV and Max.

Independent of process interruptions (pressure release) on virus retention (PP7) of four Virosart® HF 5 cm² lab modules in 20 mM KPi buffer, pH 7.2. Pressure was released once after 15 mL for 5 minutes. Fractions were collected after 15 mL, 20 mL, 75 mL and 75 mL.

<table>
<thead>
<tr>
<th>Pressure Release</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRV (PP7)</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Independent of operating pressure a virus filtration step (i.e. preparation, Downstream cell culture process schedules depending on processing volume. Further

Fractions Run 1 Run 2
<table>
<thead>
<tr>
<th>Pressure</th>
<th>15 mL</th>
<th>25 mL</th>
<th>35 mL</th>
<th>45 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>Run 1</td>
<td>Run 2</td>
<td>Run 1</td>
<td>Run 2</td>
</tr>
<tr>
<td>15 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>25 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>35 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>45 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
</tbody>
</table>

All required process steps, besides the IT, are automated with the FlexAct® system. Process control and stop criteria can be chosen individually in the software of the FlexAct® system.

FlexAct® 2.0 VR

The functional test was performed including all process steps described in chapter 4. Constant pressure mode was chosen as process control and pressure as well as Flow were recorded using the single-use sensors shown above.

Virosart® HF and a 1.1 cm² filter were chosen for the same study. As a model feed solution 5 g/L BSA in 0.1 M glycine buffer, pH 5.5 was used. Also glycine buffer was taken as an equilibration and post-flush buffer.

The functional test was performed including all process steps described in chapter 4. Constant pressure mode was chosen as process control and pressure as well as Flow were recorded using the single-use sensors shown above.

Virosart® HF and a 1.1 cm² filter were chosen for the same study. As a model feed solution 5 g/L BSA in 0.1 M glycine buffer, pH 5.5 was used. Also glycine buffer was taken as an equilibration and post-flush buffer.

The system should be operated in the following order of process steps:

1. Installation: The single-use equipment has to be installed sterile through Teflon® connection or non-sterile via Femtoseal®.
2. Wetting for Pre-use IT:
3. Flow control of process
4. Filtration:
5. Equilibration:
6. Post-use IT:

Constant pressure or constant flow.

Use-of-the-whole-filter capacity during filtration.

Validated flow decay is reached

Maximale delta p is reached

Validated flow decay is reached

All required process steps, besides the IT, are automated with the FlexAct® system.

Virosart® HF, CPV and Max.

Independent of process interruptions (pressure release) on virus retention (PP7) of four Virosart® HF 5 cm² lab modules in 20 mM KPi buffer, pH 7.2. Pressure was released once after 15 mL for 5 minutes. Fractions were collected after 15 mL, 20 mL, 75 mL and 75 mL.

<table>
<thead>
<tr>
<th>Pressure Release</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRV (PP7)</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Independent of operating pressure a virus filtration step (i.e. preparation, Downstream cell culture process schedules depending on processing volume. Further

Fractions Run 1 Run 2
<table>
<thead>
<tr>
<th>Pressure</th>
<th>15 mL</th>
<th>25 mL</th>
<th>35 mL</th>
<th>45 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>Run 1</td>
<td>Run 2</td>
<td>Run 1</td>
<td>Run 2</td>
</tr>
<tr>
<td>15 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>25 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>35 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>45 mL</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
<td>a,b</td>
</tr>
</tbody>
</table>

All required process steps, besides the IT, are automated with the FlexAct® system. Process control and stop criteria can be chosen individually in the software of the FlexAct® system.