Concept of a Fully Single-Use Virus Filtration

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1. Introduction

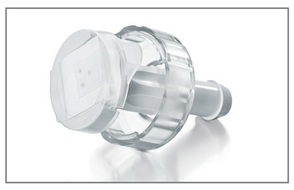
Downstream cell culture process schedules are often performed in an eight hour operation shift. Single-use process units enable a virus filtration step (i.e. preparation, operation and discarding) within one shift depending on processing volume. Further advantages of single-use in comparison to multi-use processes are: savings in cleaning validation, set-up time and buffer | WFI | NaOH and cleaning agents.

Virosart® HF and CPV are virus retentive filters especially developed for monoclonal Antibodies (mAb) and other recombinant protein applications. Virosart® Max is a pre-filter specially developed to maximize the virus filter performance.

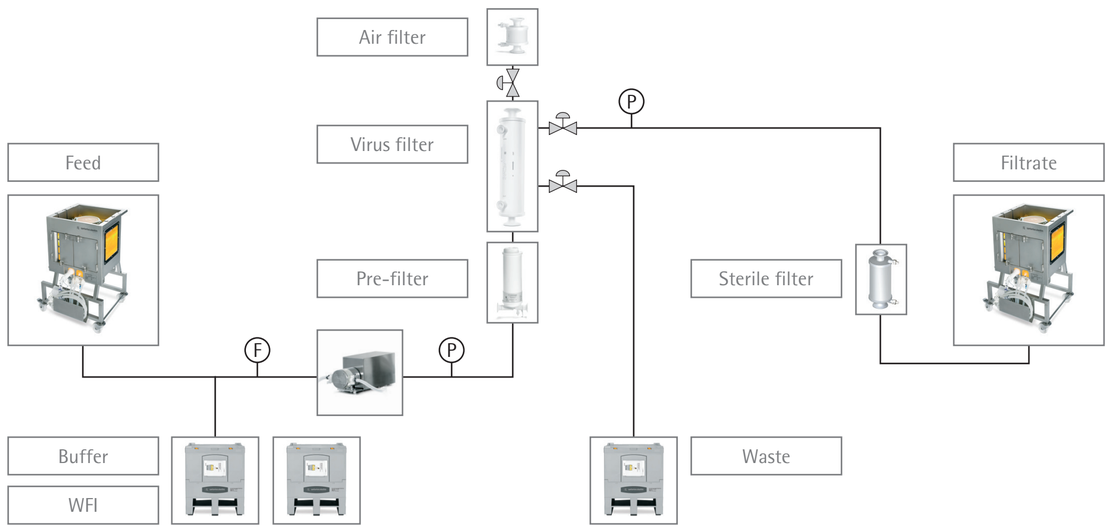
FlexAct® is a new system that enables a custom-configure flexible single-use solution for entire bio manufacturing steps. In this concept study, we outline a fully single-use virus filtration process unit including Virosart® HF, CPV and Max.

2. Sterility

- **Delivery:** The pre-assembled single-use equipment is sterilized, either by gamma irradiation or by autoclaving.
- **Installation:** In production the single-use equipment can be installed sterile through Opta® connections or non sterile via Tri-clamps.
- **State-of-the-art operation:** Virus filtrations are nowadays run under bioburden control. Therefore a sterile filter e.g. Sartopore® Platinum is placed as a standard between the virus retentive filter and the final bag.
- **Future operations:** In order to operate virus filtration in single-use systems in a fully sterile way, few challenges have to be overcome: Sterile pre-use integrity test (diffusion) but also smart handling of increased complexity of single-use equipment.



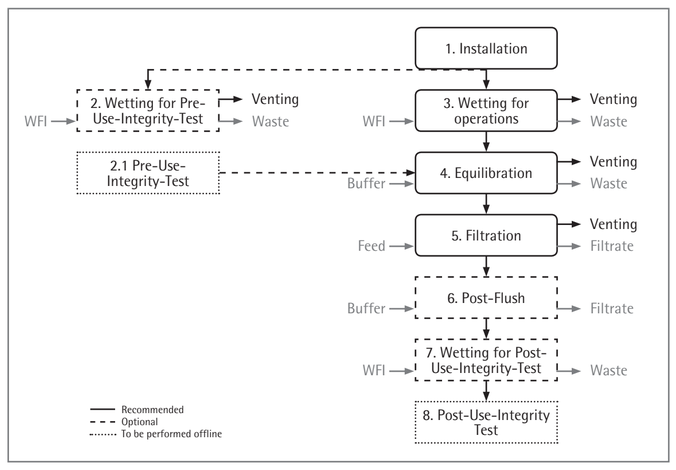
3. Set-up



4. Process Steps

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

- **Installation:** The single-use equipment has to be installed and all sensors have to be connected. The installation has to be easy, fast and operating errors during installation have to be minimized.
- **Wetting for pre-use integrity test (IT):** In order to perform an integrity test by diffusion the whole filter membrane has to be fully wetted with WFI.
- **Wetting for operation:** This guarantees, that the filter is particle free and completely wetted for the use of the whole filter capacity during the filtration.
- **Equilibration:** Prior to the filtration the filter and the equipment is equilibrated with the same buffer.
- **Filtration:** In this step the product is filtered over the virus filter. The filtration can be operated with constant pressure or constant flow.
- **Post flush:** A post flush with buffer is recommended to reach maximum product yield.
- **Flushing for post-use IT:** Prior to the post-use IT test the membrane has to be wetted with WFI.
- **Pre-use | Post-use IT:** The FlexAct® system should be able to wet the filter for pre-use IT and flush the filter for post-use IT. Afterwards the IT test is performed non sterile and with the Sartocheck® system outside of the FlexAct® system.



5. Sensors

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

a) Pressure sensor

2 pressure sensors one before and one after the final virus filter:

- Pressure control of process
- Operating | maximal allowed pressure
- DeltaP control
- Back pressure of membrane

b) Flow sensor

1 flow sensor before the pump:

- Flow control of process
- Flow decay

6. Process Control

a) Process control

There are 2 ways how a virus filtration is operated.

- **Constant pressure:** The filtration is operated with constant pressure control at operating pressure of 2 – 3 bar.
- **Constant flow:** The filtration is operated with a constant flux. At the beginning of the filtration the flow is controlled via delta p at 1 bar.

b) Stop criteria of filtration

There are different stop criteria for a virus filtration which depend on the process validation of the customer and on the process control:

- Validated total throughput has been passed over the virus filter
- Maximum delta p is reached
- Validated flow decay is reached

7. Retention under Process Conditions

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

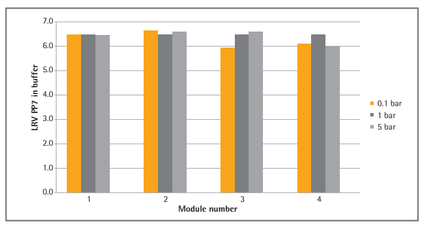
a) LRV independence from operation via peristaltic pump

Virus retention with PP7 as a model virus was performed with Virosart® HF lab modules using a peristaltic pump (Watson-Marlow 520) under worst case conditions in 20 mM KPI buffer, pH 7.2 at an operating pressure of 1.5 bar ± 0.3 bar. Virus filtration in the FlexAct® system will be performed with the Quattroflow-1200 pump due to its lower shear forces.

Fractions	Run 1	Run 2
15 mL	≥ 6.5	≥ 6.5
35 mL	≥ 6.5	≥ 6.5
50 mL	≥ 6.5	≥ 6.5
60 mL	≥ 6.5	≥ 6.5

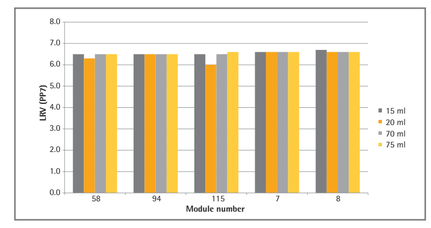
b) LRV independence from operating pressure

Independence of operating pressure on virurs retention (PP7) of four Virosart® HF 5 cm² lab modules tested on high (5 bar), medium (1 bar) and low (0.1 bar) operating pressure in 20 mM KPI buffer, pH 7.2.



c) LRV independence from process interruptions

Independence of process interruptions (pressure release) on virus retention (PP7) of five 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, 70 ml and 75 ml.

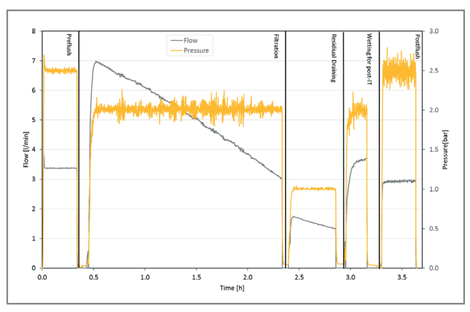


Pressure release after 15 ml for 5 min

8. Case study with FlexAct® 2.0 VR

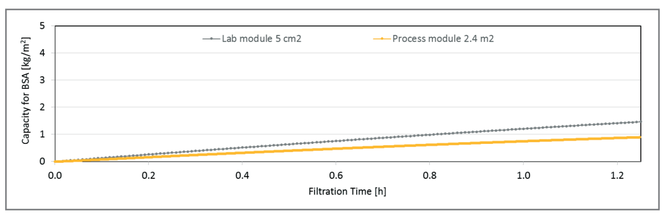
a) Functional test with Virosart® HF

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® Max 2.1 m² as an in-line pre-filter, Virosart® HF 2.4 m² as the virus filter and a 0.1 µm sterile filer were chosen for the case 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.



b) Scale-up study of Virosart® HF

In addition, the scalability from Virosart® HF 5 cm² lab module up to the 2.4 m² process module was confirmed using 5 g/L BSA in 0.1 M glycine buffer. Time for filtration of 3 Kg BSA was 5 hours by reaching around 80% total blocking.



9. Conclusion

- FlexAct® is an ideal system to realize a fully single-use virus filtration with Virosart® HF, CPV and Max.
- Due to its modular set-up the system provides high flexibility to integrate different sizing of pre-filters and virus retentive filters.
- All required process steps, besides the IT, are automated with the FlexAct® system except the integrity test. Process control and stop criteria can be chosen individually in the software of the FlexAct® system.