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Sartocon® Hydrosart® TFF Cassettes for Downstream Processing of Lentiviruses

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Abstract

Lentiviral vectors are increasingly used in cell and gene therapies. To realize their clinical potential, it is critical that manufacturing processes are optimized for scalability and cost-effectiveness. This study explores the use of Hydrosart® membranes in Sartocon® TFF cassettes for the downstream processing of lentiviruses. We evaluated two cassette geometries, ECO and E-screen, in different pore sizes to determine optimal parameters for ultrafiltration and diafiltration. Our findings highlight the potential of these cassettes to enhance lentivirus purification, offering insights into achieving high titers and purity essential for clinical applications.

For further information, visit
sartorius.com/en/products/process-filtration/tangential-flow-filtration/tff-cassettes

Introduction

In recent years, lentivirus (LV) vectors emerged as potent tools for delivering genetic material into cells, and their use is now commonplace in academic laboratories and industry for both research and clinical gene therapy applications. As the LV-based therapeutics market grows, their applications are moving from localized to systemic disorders. As such, manufacturing processes must be optimized to ensure scalability, reproducibility, and cost-effectiveness. The requirements of LV processing vary with application, but high titers and purity are key critical quality attributes. One crucial aspect of LV production lies in the downstream purification steps, with ultrafiltration and diafiltration (UF | DF) playing central roles in achieving high product concentration (volume reduction) and buffer exchange in addition to the removal of low molecular weight impurities while retaining vector particles.

Sartorius offers two different cassette geometries for ultrafiltration and diafiltration operation, the ECO and E-screen formats. The ECO cassette is designed for tangential flow filtration (TFF)-based concentration and diafiltration of low viscosity solutions (<3cp), whereas the E-screen cassette is suited for highly viscous solutions (>3 cP and protein concentration >20%). Compared to the E-screen geometry, the ECO cassette features 26% more surface area per standard cassette width, making it possible to install more area in the cassette holder and decreasing feed pump power demand. Consequently, the demand for larger systems at scaled-up processing is reduced when operating with the ECO cassette format. Shear stress applied to the virus particles is also reduced during processing with this format. Furthermore, Hydrosart® high-performance UF | DF membranes have been optimized for biopharmaceutical process applications, which feature a broad pH and temperature range. These cellulose-based membranes are extremely hydrophilic, making them non-protein binding and virtually non-fouling.

In this study, two different cassette geometries of Sartocon Hydrosart® TFF (ECO and E-screen) were evaluated using harvested LV material. Each format was compared in two different pore sizes (100 and 300 kDa), and the optimal working parameters, such as flow rate and pressure set points, were established for each consumable. The performance of the different cassette configurations was compared with respect to product recovery (infective and particle titer), impurity removal (total protein and DNA), and speed (permeate flux).

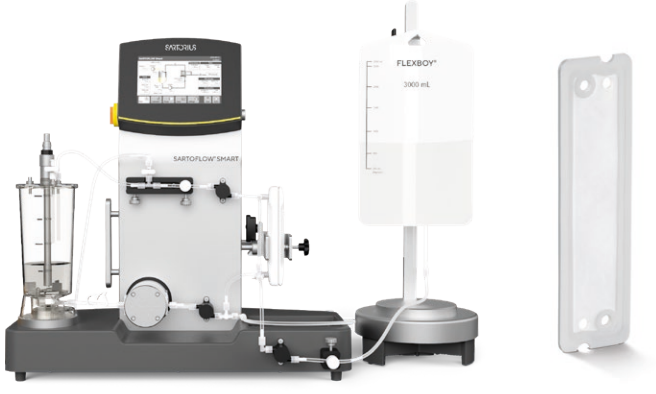
Materials and Methods

V-SVG lentivirus was produced in suspension in a 10 L Univessel® Glass bioreactor, controlled by a Biostat® B (Sartorius), through the transient transfection of HEK293 cells using PEIpro® (Sartorius). An endonuclease step was performed to digest nucleic acids for optimal results during the downstream processing. The harvest clarification was performed using a Sartopure® PP3 20 µm followed by a Sartopure® PP3 0.65 µm and Sartoclean® 2 0.8 µm (Sartorius) (all size 9 filters). Harvested LV was stored in aliquots, frozen at −80 °C, and used as feed for all the studies. The titer of the LV material was 1.1×10^8 TU/mL.

Subsequently, the TFF experiments were conducted on Hydrosart® membranes in Sartocon® Slice 200 ultrafiltration cassettes (Sartorius; Figure 1) with both an "E" channel and "ECO" channel configuration in two different pore sizes (100 and 300 kDa). The process development was performed on a Sartoflow® Smart TFF System (Sartorius; Figure 2) using Sartocon® Slice 200 with 180 cm^2 membrane area.

The consumables tested were evaluated by controlling the process through a constant inlet pressure (constant pressure process method). A transmembrane pressure (TMP) scouting was performed for all cassettes to determine the optimal operating delta pressure (ΔP) and TMP conditions for UF | DF operation.

Figure 1: *Sartoflow® Smart TFF System (Left) and Sartocon® Slice 200 Cassette (Right)*



Note. The Sartoflow® Smart TFF System is presented in the configuration used for the study. The Sartocon® Slice 200 Cassette represents Sartorius' smallest scale-down device in the Sartocon® Cassette product family.

During the scouting, a permeate flow rate characterization study was carried out by adjusting the inlet (P1) and retentate pressures (P2) for several incremental delta (Δ) pressure values (ΔP = P1 – P2). For each module and ΔP, the TMP was ramped up until the corresponding permeate flow rate (flux) decreased. For both cases, the optimal TMP was selected as the inflection point of the permeate flow rate.

All experiments were conducted in duplicate using the same initial total loading volume of 0.5 L followed by a 10-fold concentration and 5 times diafiltration with a buffer composed of 5% sucrose, 20 mM $MgCl_2$, 50 mM HEPES, pH 7.5. After UF | DF, the system and cassettes were flushed twice with one hold-up volume each by recirculating 50 mL of diafiltration buffer for 5 min through the system. The flushes were then combined with the retentates.

Analytical testing included infective titer (TU), particle titer (p24 ELISA), total DNA (PicoGreen™) and total protein (Bradford) assays.

Results and Discussion

TMP Scouting

To assess the optimal operating TMP, the permeate flux was measured at various increasing TMP values and three different target Δ pressure (ΔP) values for each cassette type and pore size (Figures 2–5).

Sartocon® Slice 200 With Hydrosart® ECO Membranes

Figure 2: *Flux (Measured as a Function of TMP) at Three Different Delta Pressure Values (ΔP) for Hydrosart® ECO 100 kDa Membranes in Sartocon® Slice 200 Cassettes*

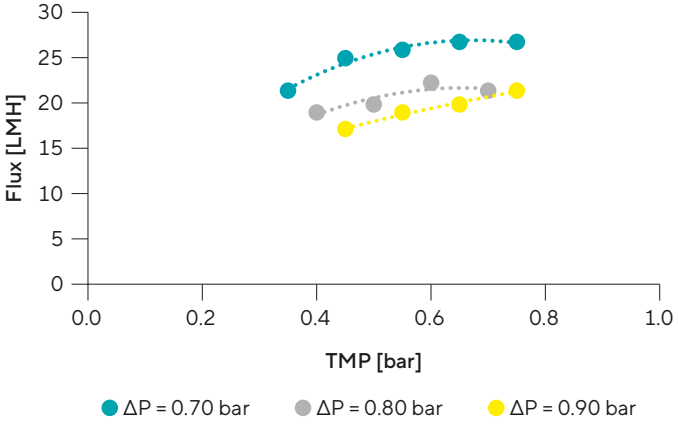
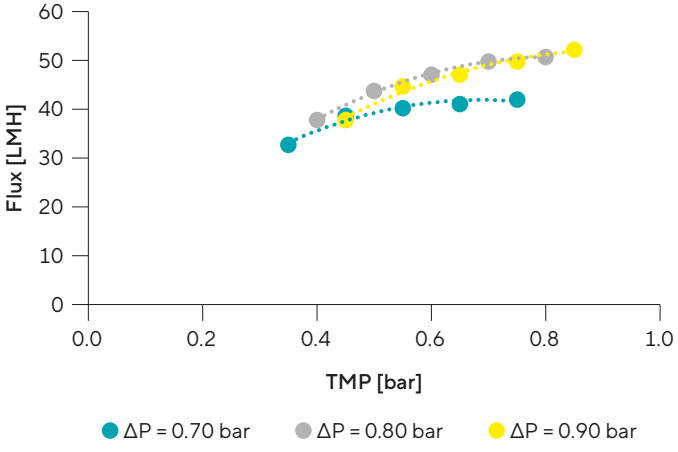


Figure 3: *Flux (Measured as a Function of TMP) at Three Different Delta Pressure Values (ΔP) for Hydrosart® ECO 300 kDa Membranes in Sartocon® Slice 200 Cassettes*



The optimal TMP range was selected as the range approaching the pressure-independent zone of the process, where further increases in pressure do not linearly increase permeate flux. The selected TMP range was 0.50–0.60 bar (at ΔP = 0.70) and 0.50–0.60 bar (at ΔP = 0.80) for the 100 kDa and 300 kDa ECO cassettes, respectively (Figures 2–3).

Sartocon® Slice 200 With Hydrosart® E-Screen Membranes

Figure 4: *Flux (Measured as a Function of TMP) at Three Different Delta Pressure Values (ΔP) for Hydrosart® E-Screen 100 kDa Membranes in Sartocon® Slice 200 Cassettes*

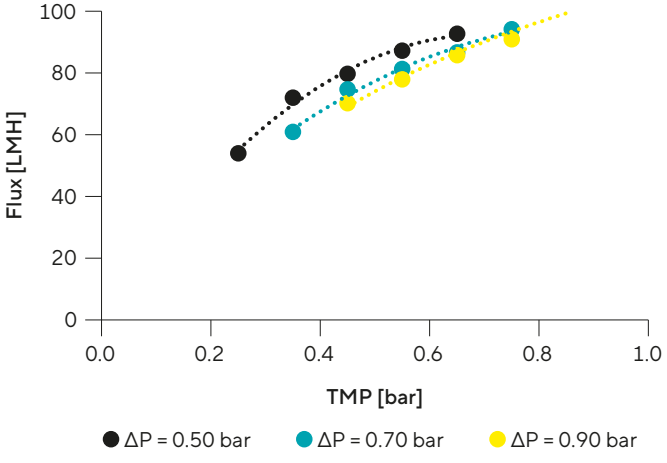
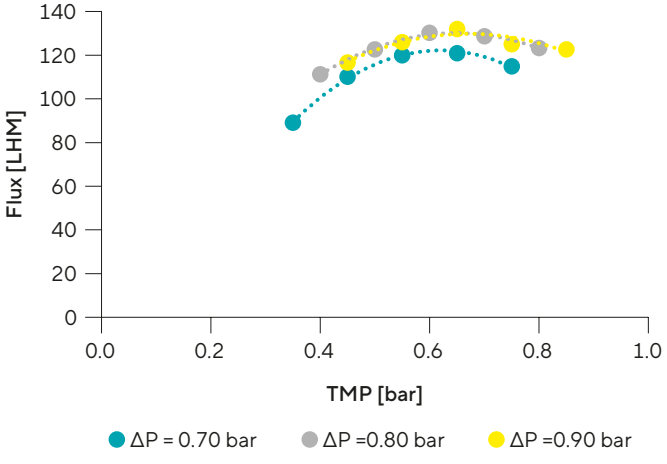


Figure 5: *Flux (Measured as a Function of TMP) at Three Different Delta Pressure Values (ΔP) for Hydrosart® E-Screen 300 kDa Membranes in Sartocon® Slice 200 Cassettes*



In the same way, the selected TMP range was 0.50–0.60 bar (at ΔP = 0.50) and 0.50–0.60 bar (at ΔP = 0.80) for the 100 kDa and 300 kDa E-screen cassettes, respectively (Figures 4–5).

Performance of Sartocon® Cassettes for Concentration and Diafiltration of LV

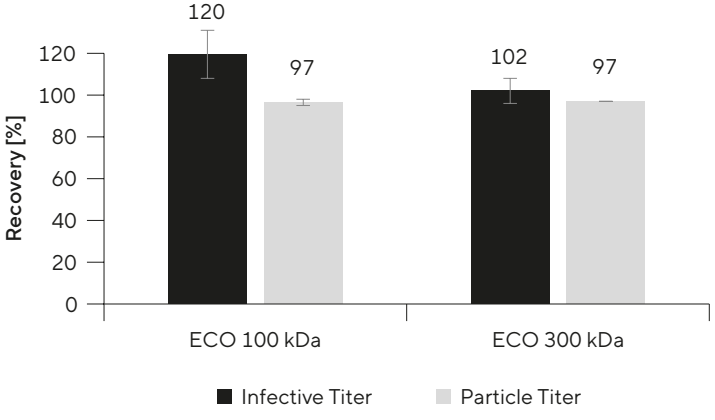
With the identified optimal setpoint for the process parameters TMP and ΔP for each ultrafiltration cassette type, the cassettes were further evaluated with regards to their performance for concentration and diafiltration of harvested LV material (Tables 1–2, Figures 6–9).

Sartocon® Slice 200 With Hydrosart® ECO Membranes

Table 1: *Parameters of LV UF | DF Runs Performed Using Hydrosart® ECO 100 and 300 kDa Membranes on the Sartocon® Slice 200*

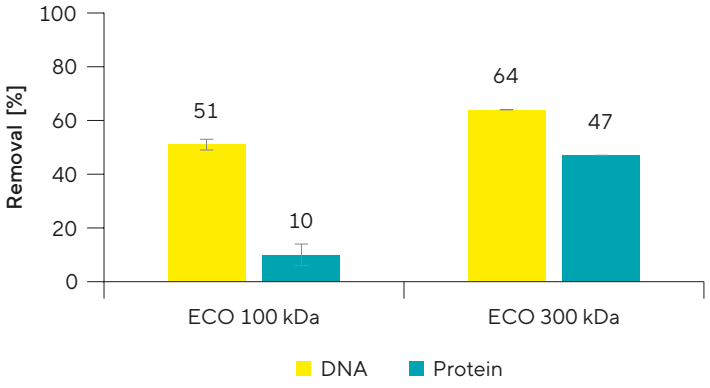
ECO	100 kDa	300 kDa
Inlet pressure (bar; constant)	0.90	0.95
Pump rate (variable | automatic; %)	7–13	6–15
Δ pressure (ΔP; bar; constant)	0.70	0.80
TMP (bar)	0.50–0.60	0.50–0.60
Average flux (UF | DF; LMH)	49 | 27	55 | 24
Total run time (UF | DF; min)	55	57

Figure 6: *Infective and Particle Titer Recovery of LV UF | DF Performed With Hydrosart® ECO 100 and 300 kDa Membranes on the Sartocon® Slice 200*



Note. Values are % mean +/- stdev; n = 2.

Figure 7: *DNA and Protein Removal (% mean +/- stdev; n = 2) From an LV UF | DF Performed With Hydrosart® ECO 100 and 300 kDa Membranes on the Sartocon® Slice 200*



Note. Values are % mean +/- stdev; n = 2.

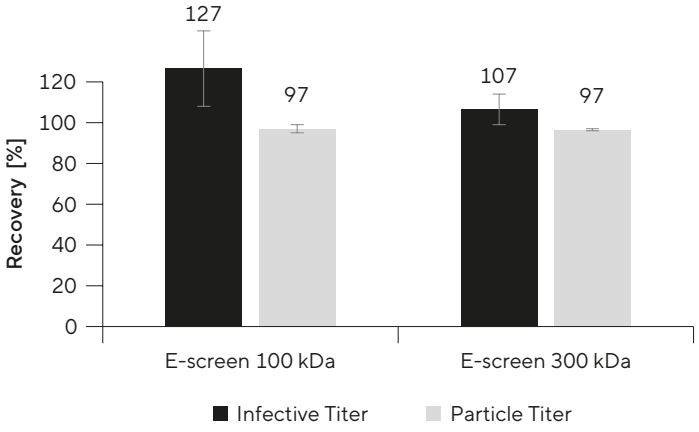
The TMP was kept in the same range for both pore sizes of the ECO cassette configuration, even though a higher ΔP was selected for the 300 kDa (0.80 bar) compared to the 100 kDa (0.70 bar) pore sizes. Given the selected parameters, the flux profiles and, therefore, the processing times, were very similar for both cases (Table 1). Both particle and infective titer were very similar with both pore sizes (~100%; Figure 6). Contaminant removal efficiencies increased with the larger pore size for DNA (51 and 64%) and protein (10 and 47%) for 100 and 300 kDa, respectively (Figure 7).

Sartocon® Slice 200 With Hydrosart® E-Screen Membranes

Table 2: *Parameters of LV UF | DF Runs Performed Using Hydrosart® E-Screen 100 and 300 kDa Membranes on the Sartocon® Slice 200*

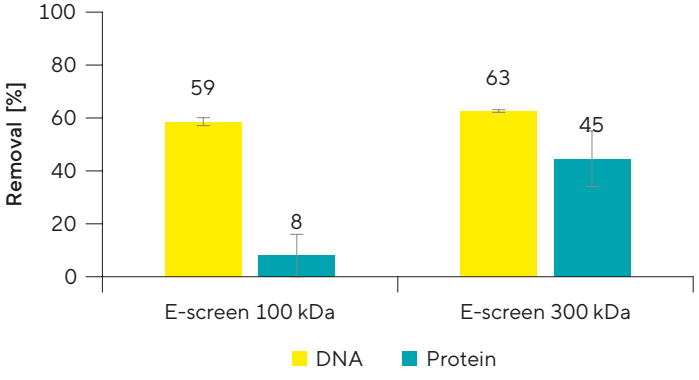
E-Screen	100 kDa	300 kDa
Inlet pressure (bar; constant)	0.80	0.95
Pump rate (variable | automatic; %)	16 – 22	22 – 28
Δ pressure (ΔP; bar; constant)	0.50	0.80
TMP (bar)	0.50 – 0.60	0.50 – 0.60
Average flux (UF | DF; LMH)	87 | 44	108 | 57
Total run time (UF | DF; min)	30	23

Figure 8: *Infective and Particle Titer Recovery of LV UF | DF Performed With Hydrosart® E-Screen 100 and 300 kDa Membranes on the Sartocon® Slice 200*



Note. Values are % mean +/- stdev; n = 2.

Figure 9: *DNA and Protein Removal (% mean +/- stdev; n = 2) From an LV UF | DF Performed With Hydrosart® E-Screen 100 and 300 kDa Membranes on the Sartocon® Slice 200*



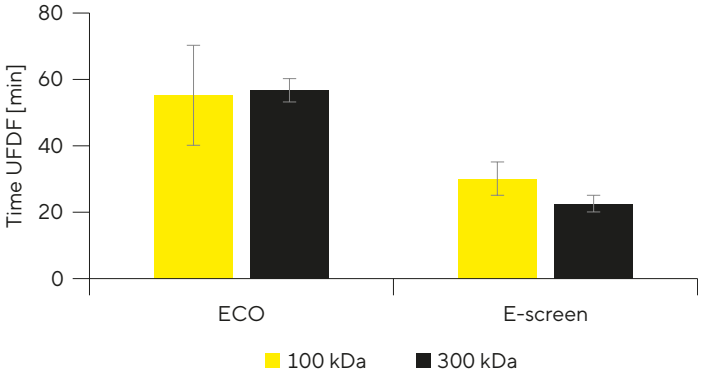
Note. Values are % mean +/- stdev; n = 2.

For the E-screen configuration cassettes, the TMP values were kept in the same range for both pore sizes, with a higher difference of selected ΔP values between the 300 kDa (0.80 bar) compared to the 100 kDa (0.50 bar) pore sizes. This considerable difference is also reflected in pump rate differences (automatically adjusted) during the trials, with 20 – 30% lower values for the 100 kDa compared with the 300 kDa cassette. The average fluxes were considerably higher using 300 kDa, resulting in a lower processing time than 100 kDa (Table 2).

Both particle and infective titer were very similar with both pore sizes (~100%; Figure 8). contaminant removal efficiencies, those were similar for both pore sizes for DNA (59 and 63%) and increased with the larger pore size for protein (8 and 45%) for 100 kDa and 300 kDa, respectively (Figure 9).

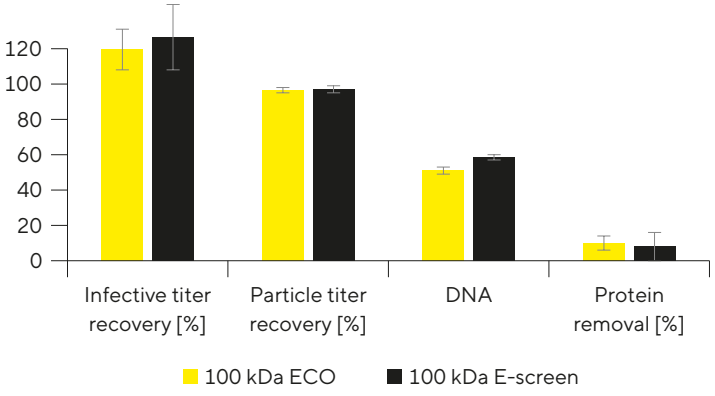
Hydrosart® ECO Versus E-Screen

Figure 10: *Processing Times for UF | DF of LV Performed With Hydrosart® ECO and E-Screen (100 and 300 kDa) Membranes on Sartocon® Slice 200 Cassettes*



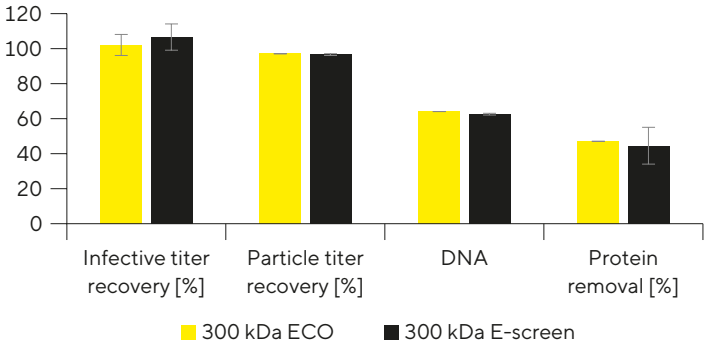
Note. Min +/- stdev; n = 2.

Figure 11: *Infective and Particle Titer Recovery & dsDNA and Protein Removal for UF | DF of LV Performed with Hydrosart® ECO and E-Screen (100 kDa) Membranes on Sartocon® Slice 200 Cassettes*



Note. Min +/- stdev; n = 2.

Figure 12: *Infective and Particle Titer Recovery & dsDNA and Protein Removal for UF | DF of LV Performed with Hydrosart® ECO and E-Screen (300 kDa) Membranes on Sartocon® Slice 200 Cassettes*



Note. Min +/- stdev; n = 2.

The pump rate (automatically adjusted by the system) was 2–4 times lower for the ECO compared to the E-screen cassette format and this difference was more significant for the bigger pore size. E-screen achieved higher fluxes (and therefore lower processing times) at a cost of higher pump rates. Again, this difference was more significant for the 300 kDa (60% faster) compared with the 100 kDa (36% faster; Figure 10).

Particle and infective titer recoveries were very similar for both cassette screen and pore size configurations (~100%; Figure 11). Both cassette configurations retained infectious particles, and the pore size did not impact this outcome. In all experimental runs, no virus particles were identified in the permeate.

In addition, a higher clearance of proteins was observed with the 300 kDa pore size for both cassette configurations. Similarly, DNA clearance increased with the larger pore size for the ECO format and was kept relatively constant for the E-screen format (Figure 12).

These differences in contaminant removal efficiencies are a very important factor when designing a TFF trial, as they directly affect profiles. A higher contaminant content has a major impact on the occurrence of fouling (trapped proteins and DNA) which can cause membrane resistance to increase with filtration time, directly reflected in the productivity of the membrane.

Conclusions

In this study, Hydrosart® 100 and 300 kDa TFF membranes in two different cassette formats were evaluated for their performance in the UF | DF of LV. For every cassette configuration, the identified optimal processing parameters resulted in high LV recoveries, both in particle and infective titer (95 – 100%), while reducing contaminating DNA (51 – 63%) and, to a lesser extent, protein (10 – 47%). The DNA and protein removal at this step is important to consider, as a typical LV process only includes one chromatography step, so the high performance of TFF is crucial. Contaminant removal was shown to be dependent on the pore size selected but independent of the cassette format.

Compared to the E-screen cassette format, the ECO cassette required 2 – 4x less pump power. Higher pumping rates for the E-screen cassette generally resulted in higher permeate flux and shorter process times, most evident for the bigger pore size. Due to the fragility of the LV, shorter process time is always preferred; however, the shear stress generated could impact the quality of the product lowering the infectivity titer. The ECO cassettes still allowed LV concentration in a reasonable time and with gentler processing conditions, as well as reduced pump capacity, which is important when operating at larger scales. Overall, excellent reproducibility of the LV UF | DF process was demonstrated, ensuring reliable process performance.

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