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Development of a Robust, Large-Scale Downstream Lentiviral Vector Process Using Tangential Flow Filtration

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Abstract

Lentiviral vectors (LVs) are crucial tools in cell and gene therapy, and the need to optimize their production processes is becoming increasingly important, especially as they gain traction in clinical applications. Ultrafiltration | diafiltration (UF | DF) processes are fundamental for concentrating viral particles, removing impurities, and exchanging buffers, critical steps in the production of high-quality LVs. Maintaining the biological activity of LVs is one of the fundamental obstacles in their purification. Therefore, several key factors must be considered during the tangential flow filtration (TFF) step. Membrane chemistry and pore size should be screened and optimized alongside the critical process parameters such as flow rate, transmembrane pressure, membrane throughput, and process time to ensure robust performance from the very early stages of process development.

This study details a process development strategy for establishing a high-performing TFF step to process LVs, emphasizing the importance of getting product and process insights during the early stages of UF | DF process development.
This approach aims to ensure that LVs production is aligned with product requirements, scalable, reproducible, cost-effective, and rapidly deployable. From small-scale screening experiments to a large-volume operation, the combination of different Hydrosart® TFF cassette configurations along with the Ambr® Crossflow, Sartoflow® Smart, and Sartoflow® Advanced systems offers a comprehensive solution to achieve these goals.

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Introduction

Lentiviral vectors (LVs) have become powerful and widely used tools in gene therapy and genetic research due to their unique ability to infect both dividing and nondividing cells and to integrate their genetic material into the host cell's genome. As their use expands, particularly in clinical applications, there is a growing need to optimize their manufacturing processes. Ensuring scalability, reproducibility, and cost-effectiveness is essential for making these therapies accessible and practical for widespread use.

Downstream purification is critical in LVs production, particularly in ensuring high titers, purity, and overall quality. Ultrafiltration and diafiltration (UF | DF) are key processes in achieving these goals and play an essential role in removing impurities while concentrating and exchanging buffers, making them indispensable in large-scale manufacturing. Sartorius offers flexible tangential flow filtration (TFF) solutions for process development and clinical trials, from laboratory environments up to commercial production batches.

Sartocon® Hydrosart® TFF cassettes are a powerful tool for virus purification in LVs production. With their high flux, low protein binding, and efficiency in virus concentration and impurity removal, these cassettes are critical for achieving high-quality, scalable, and cost-effective lentiviral vector production. Hydrosart® high-performance UF | DF membranes have been optimized for biopharmaceutical applications, well known for their extremely hydrophilic character and being less prone to fouling effects, allowing for extremely high fluxes.

The Ambr® Crossflow (5 – 100 mL) system is an advanced, high-throughput platform specifically designed for the development of UF | DF operations. This system is particularly useful in the early stages of process development, allowing researchers and manufacturers to rapidly screen several process and product parameters with a small amount of material requirements (can process up to 16 runs in parallel).

Within the product development- and bench-scale category, the Sartoflow® Smart (20 mL – 1 L) and Sartoflow® Advanced (200 mL – 10 L) systems are designed with scalability in mind, making them suitable for both small-scale development and large-scale commercial production. The modular design allows the systems to handle a variety of filtration cassettes and membrane configurations, making them highly adaptable to different bioprocessing needs.

This study outlines a process development strategy for establishing an efficient TFF step using Sartocon® Hydrosart® TFF cassettes for UF|DF in LVs production. The Ambr® Crossflow system and cassettes were initially used for high-throughput screening of different cassette pore sizes, providing early insights into infective LVs retention and product stability under high ranges of crossflow rates. The study was further optimized using Sartocon® Slice 200 cassettes in the Sartoflow® Smart bench system to establish a robust concentration and diafiltration unit operation of LVs at larger scales. The small-scale process was then scaled-up using the Sartocon® Slice (0.018 to 0.12 m^2) in the Sartoflow® Advanced system.

Materials and Methods

LV Production

V-SVG LVs were produced in suspension in a 10 L Univessel® Glass bioreactor, controlled by a Biostat® B control tower (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 Sartopore® PP3 20 µm, followed by a Sartopure® PP3 0.65 µm, and a Sartopore® 2 0.45 µm filter (Sartorius, all filters used were size 8). Harvested LVs were stored in aliquots, frozen at – 80 °C, and used as feed for all the studies. The titer of the LV material was 2.6×10^7 TU/mL.

TFF Process Screening Using the Ambr® Crossflow System

The Ambr® Crossflow system (Figure 1) comprises a high-throughput TFF processing unit that enables parallel operation with four independent crossflow channels per module, with up to four modules managed by one control station. A predefined recipe was run for each experiment. Two Ambr® CF Filters Hydrosart® with different pore sizes, 100 and 300 kDa, and an effective area of 10 cm^2, were evaluated under constant feed flow rates using the Ambr® Crossflow at 20, 40, and 60 mL/min (corresponding to 1,200, 2,400, and 3,600 LMH, respectively). A permeate flow rate characterization study was carried out to determine the optimal operating pump rate and TMP condition for every feed flow rate. For each module, the pump rate and TMP were ramped up until a decrease in the corresponding permeate flow rate was observed. The optimal TMP was selected as the inflection point of the permeate flow rate. All UF | DF trials were conducted in duplicate using the same initial total loading volume of 50 mL, followed by a 10-fold concentration and five-volume diafiltration (50 mM HEPES, 20 mM $MgCl_2$, 5% sucrose, pH 7.5).

By the end of the process, the system and cassettes were flushed twice with one hold-up volume each (2.5 mL) by recirculating diafiltration buffer for 5 min through the system. The flushes were combined with the retentates.

Transfer to the Sartoflow® Smart

The cassette configurations, Sartocon® Hydrosart® Slice 200 100 kDa and 300 kDa, with an effective filter area of 180 cm², were evaluated on the small-scale Sartoflow® Smart TFF system (Figure 1) by controlling the process through a constant inlet pressure (constant-pressure process method). TMP scouting was performed for all cassettes to determine the optimal operating delta pressure (ΔP) and TMP conditions for the UF | DF operation. During scouting, a permeate flow rate characterization study was carried out by adjusting the inlet (P1) and retentate pressures (P2) for several incremental delta pressure (ΔP) values (ΔP = P1 – P2). For each module and ΔP, the TMP was increased 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. The UF | DF trials were conducted in duplicate using a feed volume of 0.5 L (28 L/m² of membrane area), followed by a 10-fold concentration and five-volume diafiltration (50 mM HEPES, 20 mM $MgCl_2$, 5% sucrose, pH 7.5). By the end of the process, the system and cassettes were flushed twice with one hold-up volume each (50 mL) by recirculating diafiltration buffer for 5 min through the system. The flushes were then combined with the retentates.

Scale-Up Using the Sartoflow® Advanced

The scale-up experiment was conducted using the Sartocon® Hydrosart® Slice with an effective filter area of 0.12 m² on a larger-scale Sartoflow® Advanced TFF system (Figure 1). The optimal operating delta pressure (ΔP) and TMP conditions identified with the Sartoflow® Smart were used to process 3.25 L of feed volume, maintaining the same load density during scale-up (28 L/m² of membrane area). At the end of the process, the system and cassettes were flushed twice with one hold-up volume each (200 mL) by recirculating diafiltration buffer for 5 min through the system. The flushes were then combined with the retentate.

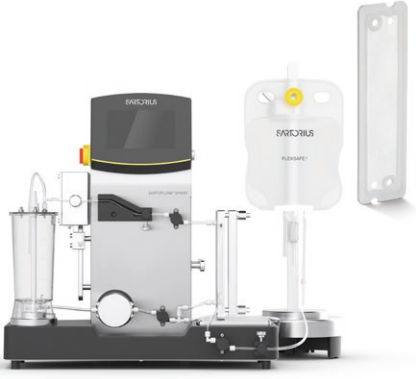
Analytical Methods

Analytical testing included infectious titer by transducing units assessment (TU) based on GFP expression (Incucyte® S3 Live-Cell Analysis System), total protein (Bradford), and residual DNA (PicoGreen) assays.

Figure 1: *TFF Cassettes and Systems Used at Each Stage of the Study*

Ambr® Crossflow and Ambr® CF Filters Hydrosart® (Screening)

Sartocon® Hydrosart® Slice 200 and Sartoflow® Smart (Small Scale)



Sartocon® Hydrosart® Slice and Sartoflow® Advanced (Scale Up)

Results and Discussion

Screening Experiments Using the Ambr® Crossflow

The Ambr® Crossflow was used to screen TFF conditions using the two Ambr® CF Filters Hydrosart® configurations with different molecular weight cut-offs (MWCOs) of 100 and 300 kDa (10 cm^2) for LVs processing.

TMP Optimization

Optimizing TFF process conditions should include determining the combination of TMP and crossflow rate that yields the highest flux while minimizing the formation of a gel layer. Through TMP scouting, the optimal TMP was first defined under different constant feed flow rates (1,200, 2,400, and 3,600 LMH) for both cassette configurations (Figures 2 and 3).

Figure 2: *TMP Optimization Curves for 100 kDa Ambr® CF Filters Hydrosart® Under Different Constant Feed Flow Rates (1,200, 2,400, and 3,600 LMH)*

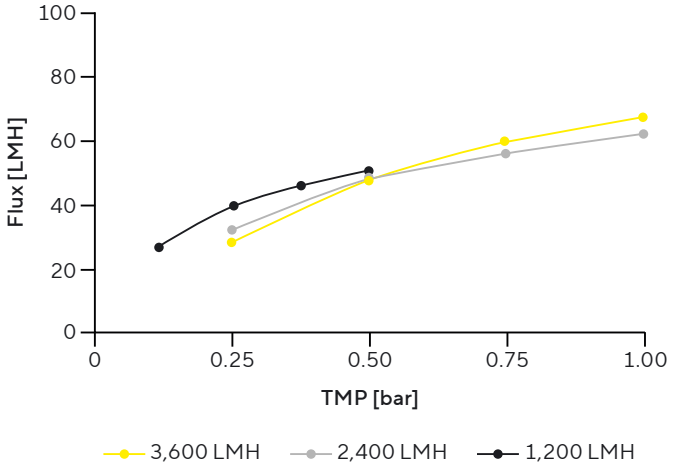
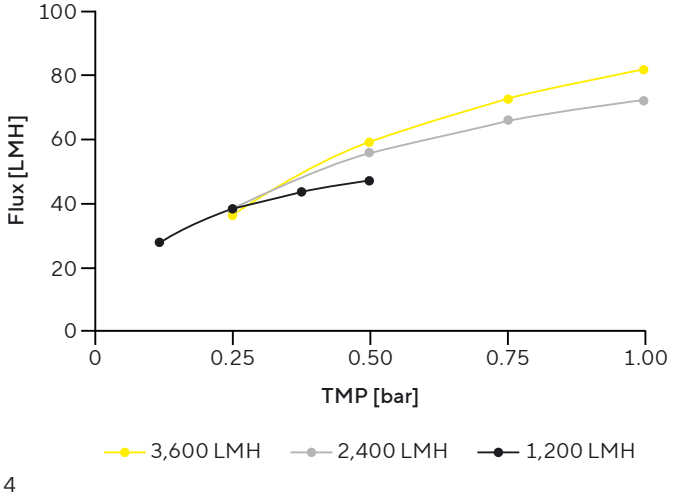


Figure 3: *TMP Optimization Curves for 300 kDa Ambr® CF Filters Hydrosart® Under Different Constant Feed Flow Rates (1,200, 2,400, and 3,600 LMH)*



The optimal TMP was selected as the range approaching the pressure-independent zone of the process, where further increases in TMP do not linearly increase permeate flux. The TMP ranges were identified for every feed flow rate and for each ultrafiltration cassette configuration (Table 1).

Table 1: *Optimal Operating TMP Ranges Identified Between Membrane- and Gel-Controlled Layer Regions for Each Feed Flow Rate Using 100 kDa and 300 kDa Ambr® CF Filters Hydrosart®*

Flow Rate [LMH]	TMP [bar]	
	100 kDa	300 kDa
1,200	0.25–0.35	0.25–0.35
2,400	0.65–0.75	0.70–0.80
3,600	0.75–0.85	0.85–0.95

The TMP value was selected from the middle point of the identified TMP optimal range for every condition and consumable configuration (Table 1). The cassette formats used during the screening study differ structurally from those employed at larger scales, particularly in terms of channel length and the presence and design of turbulence promoters. The lower inherent turbulence in the Ambr® CF Filters Hydrosart® leads to significantly different flow dynamics, resulting in operating parameters, such as crossflow velocity and TMP, that differ substantially from those in larger-scale systems during this study. Lower feed flow rates were also tested; however, under these conditions, the achievable TMPs were very low with abnormally low fluxes (data not shown). For these reasons, this section focused on a range of conditions that reflect acceptable process durations and TMP values more representative of standard UF|DF operations and focus on the outcome of infective viral retention within the pore size feed flow rates ranges tested.

UF | DF

The screening UF|DF experiments were performed using the optimal setpoints for process parameters (TMP and feed flow rate) for each Ambr® CF Filters Hydrosart®. These trials aimed to have insights on processing times, confirm infective LVs retention by the selected pore sizes and check on viral stability under high shear rates ranges (Table 2, Figure 4 and 5).

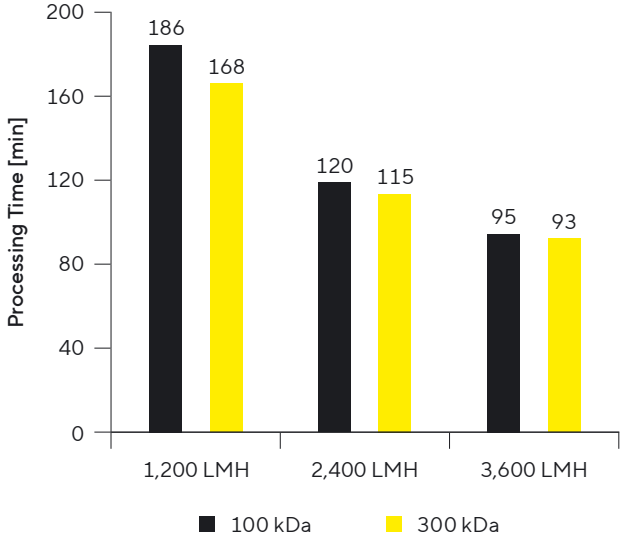
Table 2: *Average Flux [LMH] During UF | DF Steps Using 100 and 300 kDa Ambr® CF Filters Hydrosart® at Different Feed Flow Rates (With the Respective Average Inlet Pressure P1 [bar]) and Selected TMP*

Ambr® CF Filter Hydrosart® 100 kDa			
Feed Flow Rate [LMH]	1,200	2,400	3,600
TMP [bar]	0.30	0.70	0.80
Average Flux (UF) [LMH]	44	64	77
Average Flux (DF) [LMH]	13	20	27

Ambr® CF Filter Hydrosart® 300 kDa			
Feed Flow Rate [LMH]	1,200	2,400	3,600
TMP [bar]	0.30	0.75	0.90
Average Flux (UF) [LMH]	46	64	78
Average Flux (DF) [LMH]	15	21	28

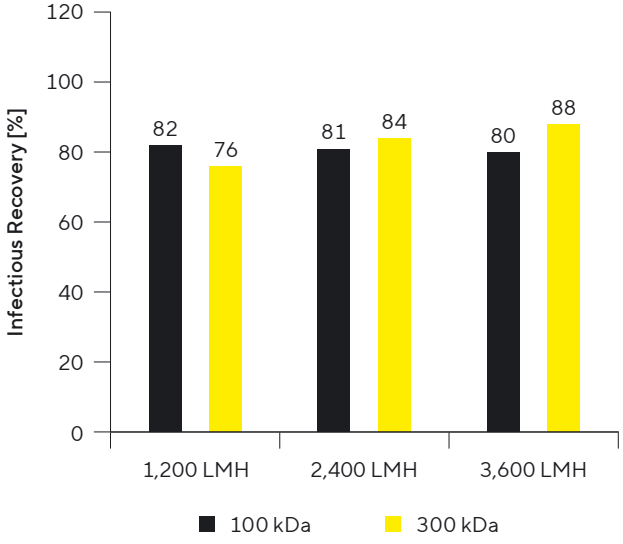
Note. Values show mean ± stdev; n = 2

Figure 4: *Processing Time [min] for UF | DF Using Ambr® CF Filters Hydrosart® 100 kDa and 300 kDa at Different Feed Flow Rates*



Note. Values show % mean; n = 2

Figure 5: *Infectious LV Particle Recovery [%] After UF | DF Using Ambr® CF Filters Hydrosart® 100 kDa and 300 kDa at Different Feed Flow Rates*



Note. Values show % mean; n = 2

The results showed that higher feed flow rates, leading to higher crossflow rates, reduced processing time, with a two-fold reduction for the 100 kDa membrane and a 1.8-fold reduction for the 300 kDa membrane (Figure 4). Although increased flow rates could potentially affect LVs infectivity due to higher shear forces, the infective recovery remained stable (80-85% recovery with ≤5% variation) across the tested flow rates and pore sizes (Figure 5). In this regard, the Ambr® CF Filters Hydrosart® enabled for high shear conditions exposure without detectable loss in LVs infectivity, providing valuable insights into process optimization and potential threshold effects for scale-up.

Transfer to the Sartoflow® Smart

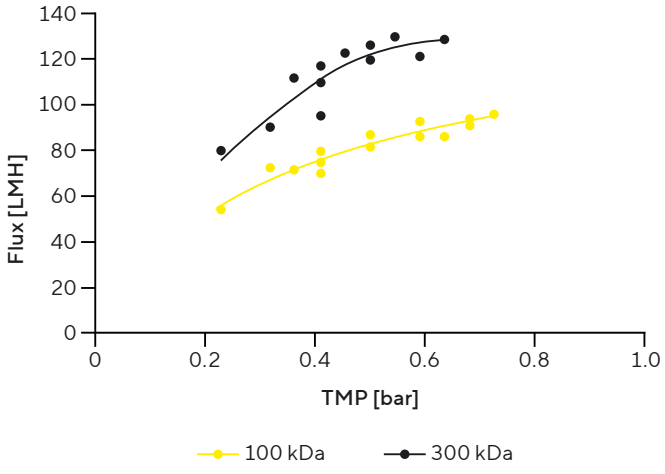
Differences in the cassette structures play an important role when scaling a TFF process, particularly factors such as channel path length, the presence of screens | spacers, and the number of stacked membrane layers. For this reason, the Ambr® CF Filters Hydrosart® were used exclusively for screening, as they are structurally different from the cassettes used in the following studies.

With this understanding, the process was then transferred to the Sartoflow® Smart, using the Sartocon® Hydrosart® Slice 200, and further optimized.

TMP Optimization

A TMP optimization study was performed A TMP optimization study was performed using two Sartocon® Hydrosart® Slice 200 cassettes with 100 and 300 kDa MWCOs (180 cm^2), across different inlet pressures (P1): 0.5 – 1.30 bar for the 100 kDa cassette and 0.5 – 1 bar for the 300 kDa cassette, with the tested TMP combinations within defined ranges (Figure 6).

Figure 6: *TMP Optimization Curves for Sartocon® Hydrosart® Slice 200 at Different Inlet Pressures (P1) and TMP Combinations Within Defined Ranges*



Note. Inlet pressures ranged from 0 – 5 – 1.3 bar for 100 kDa cassettes and 0.5 – 1.0 bar for 300 kDa cassettes.

The optimal TMP range was selected as the range approaching the pressure-independent zone of the process, where further increases in pressure no longer result in a linear increase in permeate flux. For both cassette types, this region corresponded to inlet pressure range regions already identified in the previous screening phase (Table 1) using the Ambr® Crossflow system and Ambr® CF Filters Hydrosart® (Figures 2 and 3 , marked in yellow). The optimization process on the Ambr® Crossflow system provided valuable insights regarding the appropriate pressure ranges for transferring the process to the larger system. The TMP was maintained within the same range for both Sartocon® Hydrosart® Slice 200 cassette configurations, although slightly higher ΔP values were observed for the 300 kDa cassette (0.50 – 0.90 bar) compared to the 100 kDa (0.70 – 1.0 bar) cassette, within the same TMP range. Operating within these ranges, the Sartocon® Hydrosart® Slice 300 kDa achieved considerably higher fluxes compared with the 100 kDa cassette, showing a trend consistent with that previously observed on the Ambr® Crossflow system, though to a lesser extent (Table 2).

The optimal TMP range identified was 0.50 – 0.60 bar (at ΔP = 0.70) for the 100 kDa Sartocon® Hydrosart® Slice 200, and 0.50 – 0.60 bar (at ΔP = 0.80) for the 300 kDa Sartocon® Hydrosart® Slice 200.

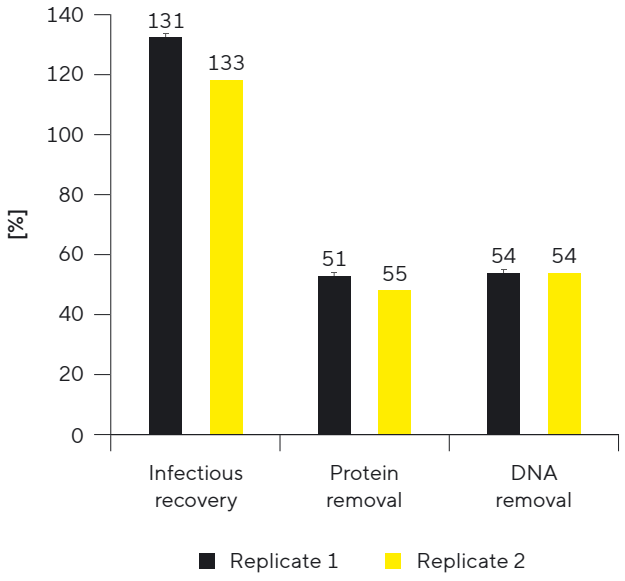
UF | DF

The cassettes were further evaluated with regard to their ability to retain infectious particles during the concentration and diafiltration of harvested LVs material. Given the higher fluxes observed within the same range and its previously observed ability to retain LVs particles, the 300 kDa Sartocon® Hydrosart® Slice 200 was selected for this study, using the identified optimal setpoints for the process parameters TMP and ΔP (Table 3; Figure 7).

Table 3: *Parameters of LV UF | DF Runs Performed Using 300 kDa Sartocon® Hydrosart® Slice 200 Cassettes on the Sartoflow® Smart (Small Scale)*

300 kDa Sartocon Slice 200	Replicate 1	Replicate 2
Inlet pressure (fixed) [bar]	0.95	0.95
Δ pressure (ΔP) [bar]	0.80	0.80
TMP [bar]	0.55	0.55
Average flux (UF) [LMH]	122	120
Average flux (DF) [min]	47	45
Processing time (UF | DF) [min]	31	32

Figure 7: *Infectious LV Particle Recovery [%], Protein Removal [%], and DNA removal [%] after UF | DF Using Sartocon Hydrosart® Slice 200 300 kDa and Sartoflow® Smart System (Small Scale).*



The mean flux of the UF | DF steps was very similar in both replicates, resulting in a total process time of 30 minutes with a variance of 1 minute between replicates (~3%). Infectious titer recoveries were very similar across both replicate runs. In both cases, the TFF cassette configurations successfully retained infectious particles with the established protocol (~ 100%), with no viral particles detected in the permeate. Contaminant removal efficiencies were also evaluated as a critical factor in TFF design, as they directly impact performance profiles. Higher contaminant content can contribute to fouling (trapped proteins and DNA), which increases membrane resistance over filtration time and directly affects membrane productivity. Both replicate trials demonstrated a consistently high efficiency in clearing proteins and DNA, with average removal rates of 53 and 54%, respectively, and low or no variance between trials.

Overall, excellent reproducibility was observed in both process parameter profiles and the quality of the final LVs product.

Scale Up Using the Sartoflow® Advanced System

UF | DF

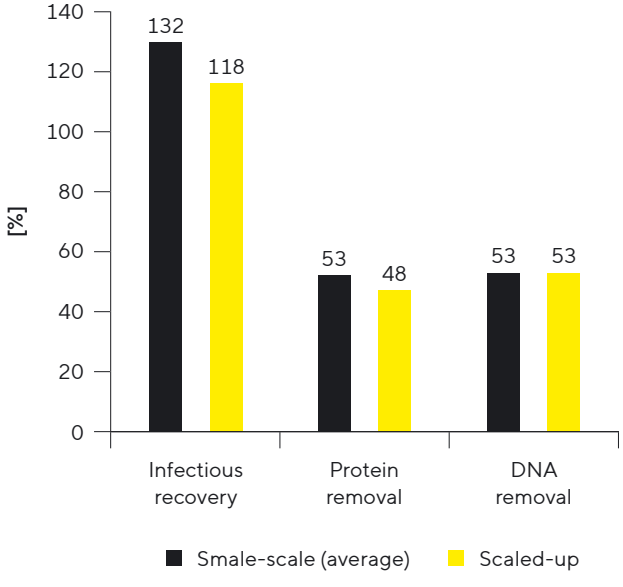
The ability to scale up the established process was assessed using the Sartocon® Hydrosart® Slice in the Sartoflow® Advanced system. The loading density (L/m² of cassette area) was maintained, and process parameters (such as TMP and ΔP) were scaled to match the conditions in the small-scale Sartocon® Hydrosart® Slice 200 cassette runs (Table 3), as this cassette format represents the smallest scale-down device in the Sartocon® Cassette product family.

Table 4: *Parameters of LV UF | DF Runs Performed Using 300 kDa Sartocon® Hydrosart® Slice Membranes on the Sartoflow® Advanced (Scaled-Up) Compared to Small-Scale Experiments*

	Small Scale	Scaled Up
Inlet pressure (fixed) [bar]	0.95	0.95
Δ pressure (ΔP) [bar]	0.80	0.80
TMP [bar]	0.55	0.55
Average flux (UF) [LMH]	121	129
Average flux (DF) [min]	46	48
Processing time (UF | DF) [min]	31	24

Note. Values show mean; n = 2

Figure 8: *Infectious LV Recovery [%], Protein Removal, and DNA Removal [%] After UF | DF Using 300 kDa Hydrosart® Sartocon® Slice Cassette and a Sartoflow® Advanced System (Scaled-Up) Compared to the Small-Scale Experiments*



Note. Values show mean; n = 2

Conclusion

The average flux of the UF | DF on the Sartoflow® Smart and the scaled-up trial on Sartoflow® Advanced was very similar. A similar viral recovery was obtained in the small-scale experiment compared to the larger-scale run (132 vs. 118%). However, this variance remains in the range of the quantification assay. The contaminant removal results were largely unchanged during scale-up, with protein removal of 53% vs. 48%, and DNA removal of 54% vs 54% for the small-scale and larger-scale, respectively (Figure 8).

Overall, processing time, virus recoveries, and contaminant removal levels obtained at small scale were consistent with the results from the scaled-up run, suggesting linear and predictable scaling from the Sartocon® Slice 200 cassette format to the Sartocon® Slice format.

This study outlines a process development approach for creating a robust TFF step for UF | DF processing LVs using Sartorius solutions. We demonstrated the value of obtaining product and process insights early in development to enable the creation of a scalable, reproducible, and cost-effective manufacturing process that meets the product and market demands.

The Ambr® Crossflow system was used in the early stages of lentiviral vector TFF process development, enabling for an initial assessment of the infectivity of lentivirus particles across different pore sizes and recirculation rates, looking for prioritizing infective lentivirus particles retention. Its advanced multi-parallel processing capabilities enabled a fast and simple evaluation of the impact of different pore sizes and process parameters on processing times and retention of infectious LVs particles. Only small volumes of material were required for these screening experiments, avoiding the depletion of expensive and often limited product.

The insights from the screening trials were transferred and further optimized using the Sartoflow® Smart System and the Sartocon® Hydrosart® Slice 200 cassette format, which represents the smallest scalable device of the Sartocon® product family. The optimized process parameters resulted in impressive infectious LVs particle recovery while achieving significant removal of proteins and DNA. Both replicate runs showed highly comparable performance in terms of TFF process parameters and final product quality, demonstrating robust reproducibility.

This study also demonstrated the efficient scalability from small-scale to larger-scale applications, with membrane surface areas ranging from 0.018 to 0.12 m^2, using the Sartocon® Hydrosart® Slice with the Sartoflow® Advanced. Furthermore, this study underscores the suitability and competitiveness of Sartocon® Hydrosart® TFF cassettes for UF | DF operations in LVs production processes, with exceptional LVs recoveries (~100%), contaminant clearance (50 – 60%), high reproducibility across runs, and scalability from small-scale to larger-scale applications.

In conclusion, the TFF process development strategy using Sartorius solutions enabled rapid selection of reliable ultrafiltration consumables and screening of process conditions, while providing early insights into potential product impact concerns, which contributed to the rapid establishment of a high-performing LVs TFF step.

References

1. Sartorius. (2021). Hydrosart® ultrafiltration cassettes [Datasheet]. Sartorius. https://shop.sartorius.com/shop/medias/Hydrosart-Ultrafiltration-Cassettes-Protein-purification-concentration-and-diafiltration.pdf?context=bWFzdGVyf-GRvY3VtZW50c3wyODI1NjR8YXBwbGljYXRpb24vc-GRmfGRvY3VtZW50cy9oNjMvaGI0LzkwNzYxMzQyOD-EyNDYucGRmfGMxMzFhOTcyMTBiNDYxZDJjZGQ-wNThmNDdmNmJjN2FkYWMxODQyNjJjYWFiZmJkY-mI5OTMzMDZiOGUyY2Y0OGQ

2. Sartorius. (2025). Ambr® Crossflow system [Datasheet]. Sartorius. https://www.sartorius.com/download/297806/data-ambr-crossflow-spc2084-e-pdf-data.pdf

3. Sartorius. (2024). Sartoflow® Smart TFF system [Datasheet]. Sartorius. https://www.sartorius.com/download/1150102/sartoflow-smart-datasheet-en-b-2803216-sartorius-pdf-data.pdf

4. Sartorius. (2021). Sartoflow® Advanced TFF system [Datasheet]. Sartorius. https://www.sartorius.com/download/1242510/sartoflow-advanced-datasheet-en-b-2803170-sartorius-pdf-data.pdf

5. Cardoso, S., Engelhard, J., & Tappe, A. (2024). Sartocon® Hydrosart® TFF cassettes for downstream processing of lentiviruses [Application note]. Sartorius. https://www.sartorius.com/download/1672534/sartocon-hydrosart-tff-cassettes-application-note-en-b-pdf-data.pdf

6. Moreira, A. S., Cavaco, D. G., Faria, T. Q., Alves, P. M., Carrondo, M. J. T., & Peixoto, C. (2020). Advances in lentivirus purification. Biotechnology Journal, 16(1). https://doi.org/10.1002/biot.202000019

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