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Enhancing Lentiviral Vector Purification: Scalable Tangential Flow Filtration With Hollow Fibers

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Abstract

This application note presents a comprehensive analysis of Sartorius hollow fiber tangential flow filtration technology for the ultrafiltration | diafiltration of lentiviruses for gene therapy applications. The study evaluates the performance, scalability, and quality attributes of hollow fiber modules across different sizes and systems, focusing on virus recovery, infectivity, and impurity clearance. The results demonstrate that Sartorius hollow fiber technology offers a robust and scalable solution for lentiviral vector processing, ensuring high recovery rates and effective impurity removal while maintaining viral integrity.

Introduction

Lentiviral vectors (LVs) are pivotal in gene-modified cell therapies, such as CAR-T, due to their ability to efficiently deliver genetic material into cells. However, their production is challenging due to their shear-sensitive nature, particularly during tangential flow filtration (TFF) steps. Traditional purification methods, such as centrifugation and polyethylene glycol (PEG) precipitation, are not scalable and often compromise viral integrity.

During the early stages of LV purification post-harvest, TFF is commonly employed to optimize feed stream conditions for subsequent chromatography steps. This involves concentrating viral particles and adjusting buffer conditions to enhance chromatography efficiency. Additionally, TFF is used in late-stage downstream processing for the concentration and formulation of purified LVs. Hollow fiber (HF) modules used in TFF offer a gentle processing environment, minimizing shear stress and ensuring high recovery and purity of LVs, while maintaining their quality and efficacy.

This study evaluates the use of three HF module sizes for the concentration of a clarified LV harvest through ultrafiltration (UF), followed by a diafiltration (DF) step for buffer exchange. Scalability across HF module sizes was demonstrated on different TFF systems, and critical quality attributes (CQAs) were assessed to determine the impact on LV purity and infectivity.

Materials and Methods

Lentivirus Production & Clarification

HEK293T/17SF cells were inoculated into a 10 L Univessel® glass bioreactor (Sartorius) and cultivated at setpoints of 37 °C, 40% dissolved oxygen (DO), pH ≤ 7.25 and 202 rpm. After 2 days of batch culture, cells were transfected with plasmid DNA (pALD-Lenti system, Aldevron) using PEIpro® (Sartorius Polyplus). Six hours later, an enhancer was added, and the pH setpoint was decreased to ≤ 6.95. Three days post-transfection, 10 units/mL DENARASE® (c-LEcta) and 2 mM $MgCl_2$ were added to the culture broth and incubated for 1 hour at the above bioreactor control setpoints.

The nuclease-treated broth was subsequently filter-clarified using a filter train composed of three filters connected in series. The filter train consisted of Sartopure® PP3 20 µm, Sartopure® PP3 0.65 µm, and Sartopore® 2 0.45 µm filters. The first two stages, utilizing the Sartopure® PP3 filters, were designed for effective particle removal, ensuring the reduction of larger particulates from the LV material. The third stage, employing the Sartopore® 2 membrane filter, was implemented for bioburden reduction.

Filtration was performed at a flow rate of 30 mL/min (corresponding to 100 LMH for the first filter) and stopped upon reaching a pressure of 1 bar. Following clarification, the LV material was divided into approximately 500 mL aliquots and stored at –80 °C. The analytical results of the LV material post-clarification are detailed in Table 1.

Table 1: *Analytical Data of LV Material Post-Clarification*

Turbidity [NTU]	49.1
Infectious Titer [TU/mL]	7.3×10^7
Total Protein [µg/mL]	326
Total DNA [ng/mL]	934

TFF Consumables and Systems

Three sizes of 12" HF modules (750 kDa MWCO) with varying membrane areas were utilized in this study: the Pioneer, Discover, and Explorer modules (Table 2). All modules are single-use, gamma irradiated, and contain a mPES membrane. Before use, all HF modules were wetted with water using a Sartoflow® Smart at 650 mbar feed pressure and 350 mbar transmembrane pressure (TMP) for approximately 5 min. The wetted HF modules were integrity tested at 750 mbar feed pressure by a Sartocheck® 4 (4 min stabilization time, 2 min test time).

Two benchtop TFF systems were used in this study and operated in parallel (Figure 2). The Ambr® Crossflow system was used for the Pioneer and Discover HF modules and operated under a clean bench. The Sartoflow® Smart system was used for the Explorer HF module and operated in a closed manner within an uncontrolled environment. Both systems were sanitized to reduce contamination risks during processing and sampling for analytical measurements. Sanitization was conducted by circulating 1.0 M NaOH through the system for 30 min at room temperature. Neutralization was performed by rinsing with sterile buffer (20 mM HEPES, pH 7.4) until a pH of 7 was reached.

Table 2: *HF Modules Used in the Study*

Hollow Fiber Material Number	**MWCO [kDa]**	**Fiber Lumen [mm]**	**Fiber Length [inch]**	**Fibers**	**Fibers Membrane Area [cm²]**	**Recommended Batch Volume [mL]**	**Feed | Retentate Connector**	**Permeate Connector**
Pioneer 12" SU75010PIO12L1	750	1	12	2	17.3	3 – 80	Luer-Lock	Luer-Lock
Discover 12" SU75010DIS12LL	750	1	12	6	52	10 – 250	Luer-Lock	Luer-Lock
Explorer 12" SU75010EXP12S0	750	1	12	18	155	150 – 750	½" Tri-Clamp	3/16" Hose Barb

Figure 1: *Hollow Fiber Modules and TFF Systems*

Pioneer Module

Explorer Module

Discover Module

Ambr® Crossflow

Sartoflow® Smart

UF | DF Process Parameters

The clarified LV material was subjected to a 10-fold concentration using UF, followed by DF with five diavolumes under TFF conditions as outlined in Table 3. The diafiltration buffer consisted of 20 mM HEPES pH 7.0, 75 mM NaCl, and 2.5% (w/v) sucrose. All experiments were performed in duplicate.

Table 3: *TFF Process Settings*

Parameter	Unit	Pioneer 12"	Discover 12"	Explorer 12"
TFF System		Ambr® Crossflow	Ambr® Crossflow	Sartoflow® Smart
Feed Flux	LMH	817	816	821
Amount of Fiber	#	2	6	18
Membrane Area	cm^2	17.3	52	155
Fiber Diameter	mm	1	1	1
Shear Rate	s^{-1}	2,000	2,000	2,000
TMP	mbar	500	500	500
Feed Flow	mL/min	23.6	70.7	212.1
Feed Volume	mL	69.2	208	620
Load Density	L/m^2	40	40	40
UF End Volume	mL	6.9	20.8	62.0
DF Buffer Volume	mL	34.5	104	310

Analytics

Samples were taken from the LV feed material (post-clarification) and from the retentate of each HF module at the end of the TFF process. Samples were analyzed for virus particle titer, infectivity, and host cell impurity clearance. Appropriate sample dilutions were determined in pretests, and samples were measured in triplicate.

The total protein content post-clarification was measured using the Pierce™ Coomassie (Bradford) Protein Assay Kit (Thermo Fisher Scientific). The virus particle titer was assessed using the QuickTiter™ Lentivirus Titer Kit (Lentivirus Associated HIV p24, Cell Biolabs Inc). For quantifying the infectious virus titer, the Incucyte® S3 Live-Cell Analysis System (Sartorius) was employed. Adherent HEK293 T cells (ACC 635, DSMZ) were infected with serially diluted LV samples, and GFP expression was measured. Host cell protein (HCP) levels were determined using the HEK293 HCP ELISA Kit (Cygnus Technologies). Total DNA was measured with the Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo Fisher Scientific). Virus recovery, as well as HCP and DNA clearance after UF | DF, were calculated relative to the feed material.

Results

TMP Optimization

The TMP optimization was conducted with the Pioneer 12" testing module within the Ambr® Crossflow system, using clarified LV material as the feed. The shear rate was maintained at 2,000/s, and various TMPs were tested: 250, 500, 750, and 1,000 mbar. To measure stable permeate flux, a flux stabilization time of 30 min was applied for each TMP point.

Figure 2: *TMP Optimization Curve: Relationship Between TMP and Flux Using Pioneer 12" With Clarified LV Material*

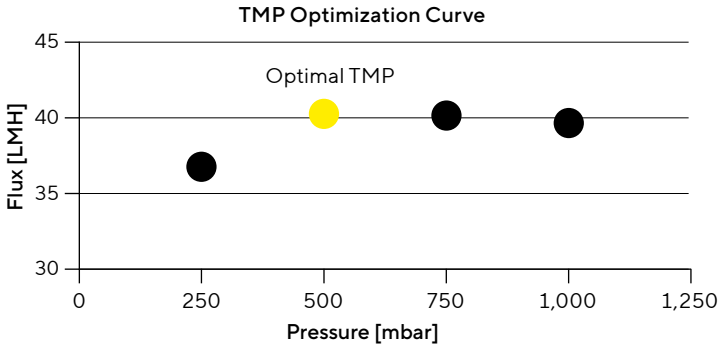
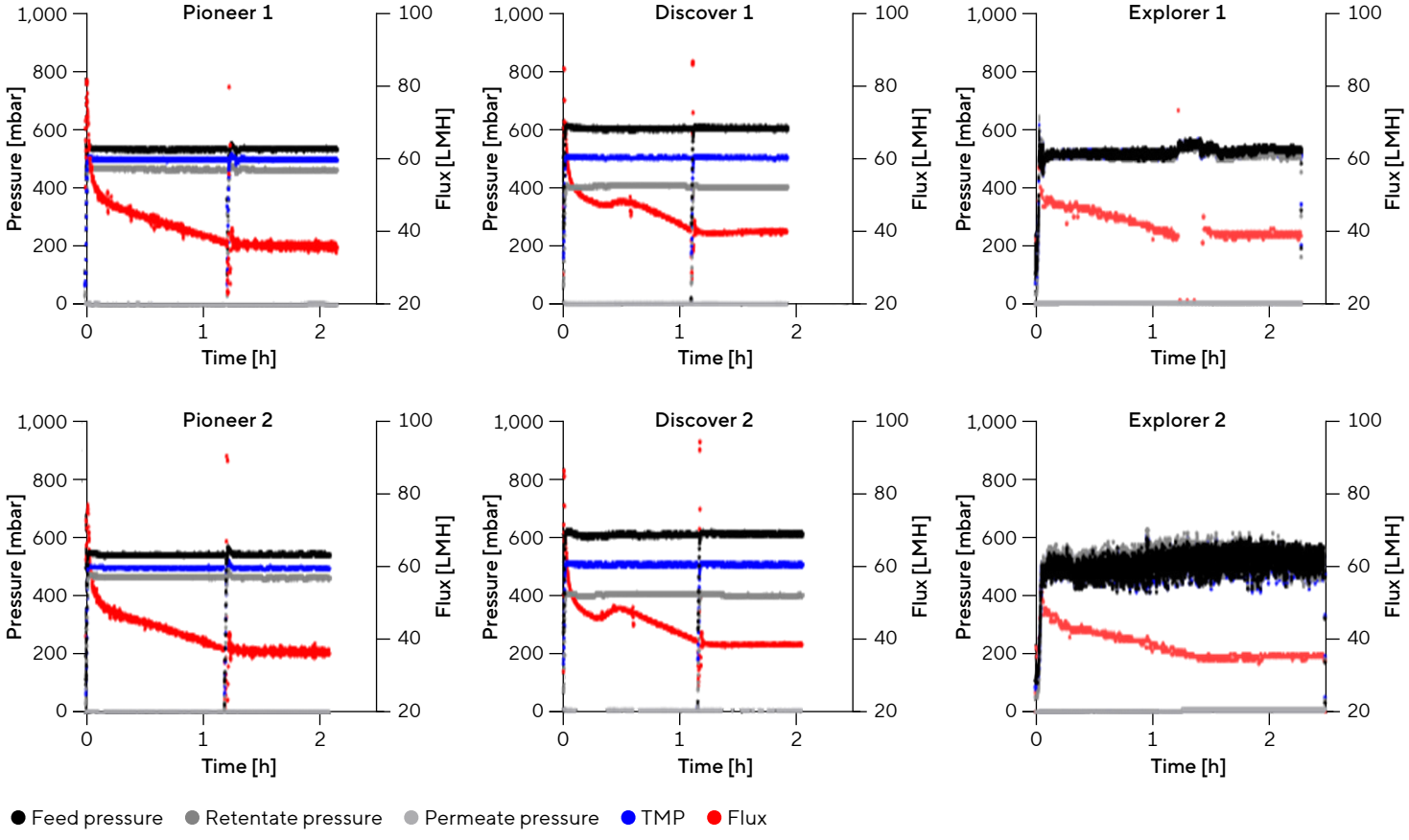


Figure 2 highlights the flux performance across the tested TMP values. For a standard UF | DF process, the optimum TMP to run a process is at the inflection point of the curve, where near-maximum flux is achieved without applying excessive pressure. This point is crucial as it prevents exceedingly high protein wall concentrations (polarization) or membrane fouling. The curve shows a peak flux at 500 mbar, confirming its suitability as the optimal pressure setting for the process. Consequently, a TMP of 500 mbar was selected for all subsequent UF | DF runs.

UF | DF Processing of Clarified LV

To evaluate the scalability and reproducibility of HF modules across different TFF systems during the UF | DF of clarified LVs, we assessed critical process parameters (CPPs) and CQAs. CPPs encompass the scalability of pressure profiles, flux, and process duration, while the CQAs evaluate the consistency and effectiveness of virus recovery, infectivity, and impurity removal.

Figure 3: *Pressure and Flux Profiles of Different HF Modules With Clarified LVs*



Note. Two modules of each HF size were assessed for permeate flux, along with feed, retentate, permeate, and TMP pressures during the UF | DF process. Modules marked '1' were run in parallel, with the same approach applied to those marked '2'.

Scalability of Pressure and Flux Profiles

The scalability of process parameters was evaluated by comparing pressure profiles—including feed, retentate, permeate pressures, and TMP—across three module scales and permeate fluxes: Pioneer, Discover, and Explorer.

Across all modules, pressure parameters remained within a 20% variation range, supporting the scalability of both HF modules and the TFF systems. The Discover modules exhibited a slightly elevated feed pressure, consistent with their higher flow rate, despite using the same flow kit design as the Pioneer module on the Ambr® Crossflow system.
The pressure difference of approximately 50 mbar between Discover and Pioneer is minimal and can be attributed to Discover's higher fiber density within the same housing. Pressure profiles for the Explorer module, run on the Sartoflow® Smart system, closely aligned with those of both Pioneer and Discover, further validating their scalability. Minor fluctuations observed in Explorer 2 were attributed to pump variability inherent to the Sartoflow® Smart system (Figure 3).

Despite differences in module sizes, reproducible flux measurements were achieved in all trials, underscoring the scalability across HF modules. The Explorer module exhibited slightly lower overall flux values; however, its initial flux showed reduced variability, which is expected with increased fiber surface area. This indicates enhanced process robustness at larger scales (Table 4).

Table 4: *Average Flux Values Across Different Experiments in UF and DF Steps*

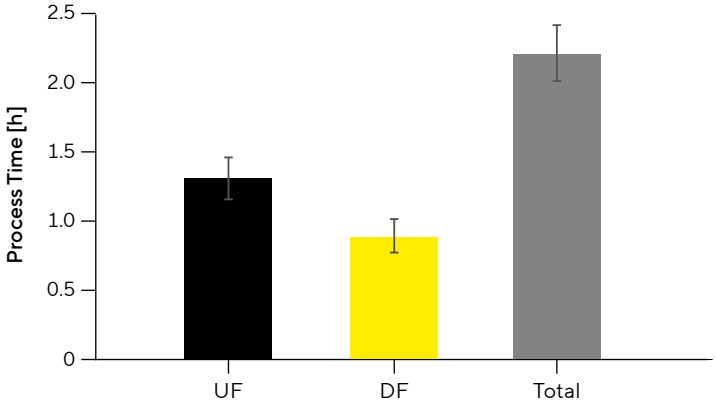
HF Experiment	UF Start	UF End	DF End
Pioneer 1	31 ± 8	21 ± 1	19.5
Pioneer 2	32 ± 8	23 ± 1	20.8
Discover 1	34 ± 6	25 ± 5	24.5
Discover 2	32 ± 6	23 ± 5	22.5
Explorer 1	30 ± 5	24 ± 1	23.2
Explorer 2	26 ± 5	19 ± 1	19.0

Note. UF start flux was measured 15 min post UF initiation to ensure process stabilization.

Low Process Time Variation

The total process time across all modules and trials ranged from approximately 2.0 to 2.5 hours, with the Pioneer and Discover modules completing slightly faster than the Explorer (data not shown). This difference is likely attributable to the automated features of the Ambr® Crossflow system, which minimize delays at the initiation of each UF | DF step. On average, the 10-fold concentration step required approximately 1.3 hours, while the 5-volume diafiltration step took around 0.9 hours to complete (Figure 4).

Figure 4: *Average UF, DF, and Total Process Times Across HF Modules*



Impurity Clearance and Infectivity

The study evaluated the clearance of HCPs and total DNA, as well as the recovery of infectious titers across the HF modules. Total amounts of HCP, DNA, and infectious titer were determined by analyzing both feed and retentate volumes.

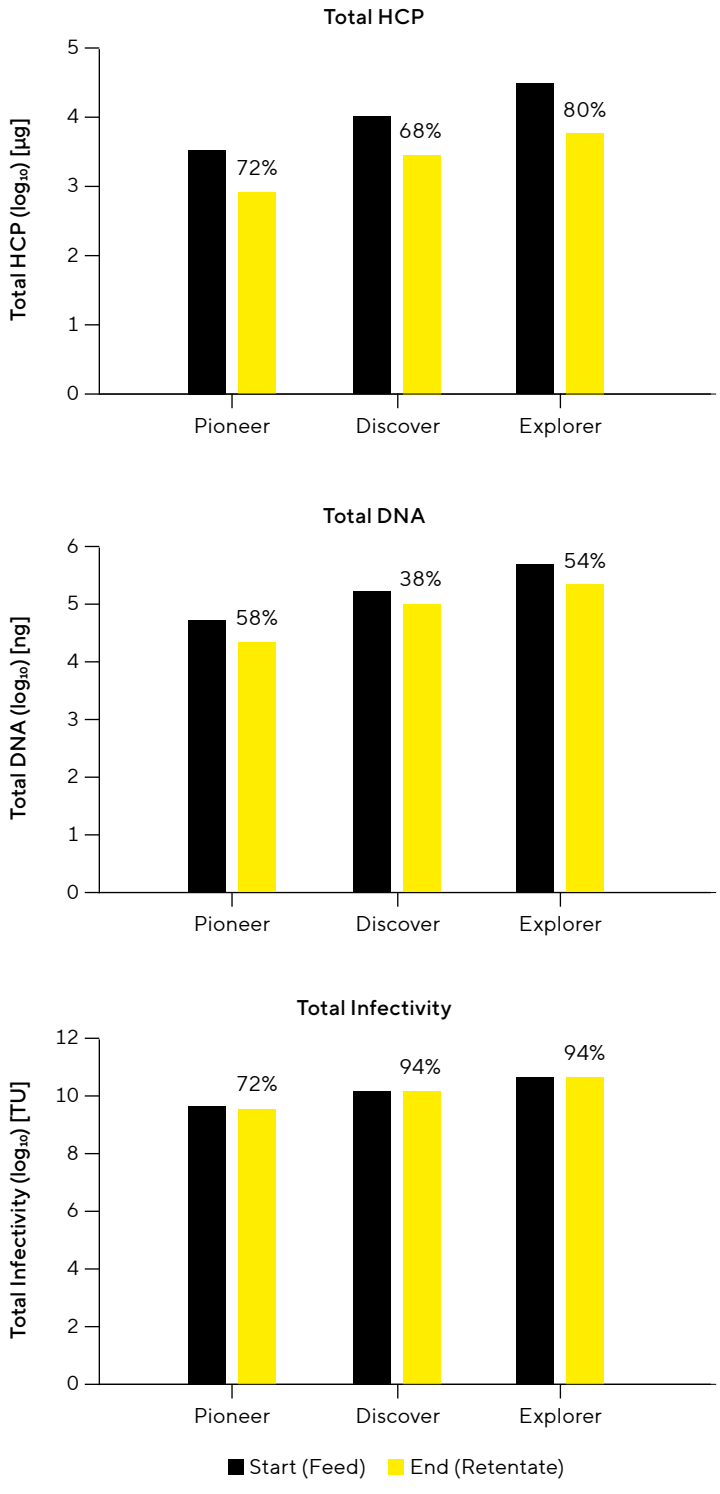
HCP clearance was most effective in the Explorer module, achieving an average reduction of 80%, compared to 68 – 72% observed in the smaller Pioneer and Discover modules. Across all modules, the average HCP reduction was 73%, indicating efficient removal. DNA clearance averaged 50% across modules, with both Pioneer and Explorer showing slightly more robust removal than the Discover module.

However, due to significant variability in the analytical assay for DNA quantification, a reliable comparison across modules could not be established. Additionally, it is important to note that total DNA measurement captures both host cell and viral DNA (Figure 5).

While the removal of HCP and DNA impurities is beneficial, the primary focus remains on preserving the integrity of the LVs during TFF processing, as demonstrated by infectious particle recovery. Throughout the UF | DF process, the infectious titer remained stable, with no notable loss, ensuring the retention of infective lentivirus across all modules. Recovery rates for the infectious titer varied between 72% and 94%, with the Discover and Explorer modules achieving the highest recovery at 94%, suggesting that modules with larger membrane areas deliver more robust performance (Figure 5).

In summary, the UF | DF process across all HF modules was completed in approximately 2.2 hours, with an average flux of 28 LMH. The consistent process time and average flux indicate a well-optimized process that balances speed and quality. Viral recovery was consistent, with no significant loss in infectivity. Impurity clearance was effective, achieving around 70% HCP and 50% DNA removal. These results confirm the performance and scalability of the process, with consistent pressure profiles, flux, and product quality across different module sizes. The findings obtained with a challenging LV feed stream containing high impurity levels are likely transferable to other intermediate TFF steps in downstream LV processing.

Figure 5: *Impurity Clearance and Infectious Titer Recovery Across all HF Modules*



Conclusion

The study highlights the effectiveness of TFF using HF modules in purifying LVs. The HF technology enhances recovery yields by concentrating and purifying LVs while minimizing shear stress, crucial for maintaining viral integrity. The devices demonstrated scalability and reproducibility, with consistent performance across different module sizes and TFF systems. The Explorer module showed superior impurity clearance, contributing to the safety and efficacy of the product. The HF technology can be integrated into various stages of the LV downstream processing workflow. Based on the demonstrated scalability, the process can be adapted to varying production scales, from research to clinical manufacturing. These attributes make it an ideal choice for the production of LVs in gene therapy applications, addressing the challenges associated with traditional purification methods. Integrating Sartorius HF technology into LV production workflows can significantly enhance the quality and yield of therapeutic products.

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