SARTORIUS

Simplifying Progress

Lab-Scale Tools for Advanced LV Downstream Processing

Jennifer J. Labisch*, Andreas Pickl

Sartorius Stedim Biotech GmbH, Lab Essentials Applications Development, Göttingen, Germany
* Corresponding author: Jennifer.Labisch@Sartorius.com

Introduction

Lentiviral vectors (LVs) are widely used as a gene delivery platform for the ex vivo generation of chimeric antigen receptor (CAR)-T cells in cancer immunotherapy. With the increasing demand for LV production, there is a growing need for materials and process strategies specifically tailored to these fragile, enveloped viral vectors. Established downstream processing (DSP) approaches for proteins, such as monoclonal antibodies (mAbs), cannot be directly applied due to the distinct physicochemical properties and size of LVs, necessitating careful process optimization. The shift to suspension-based LV production impacts DSP, particularly the harvest and clarification. Purification remains a bottleneck, as traditional anion exchange (AEX) and affinity chromatography often reduce viral infectivity. Therefore, chromatographic supports specifically designed for LVs are crucial to enhancing process efficiency. Additionally, while ultrafiltration techniques are commonly employed, they must be carefully optimized to prevent loss of viral activity.
This study presents a complete lab-scale DSP workflow for clarifying, purifying, concentrating LVs produced in suspension HEK cell culture.

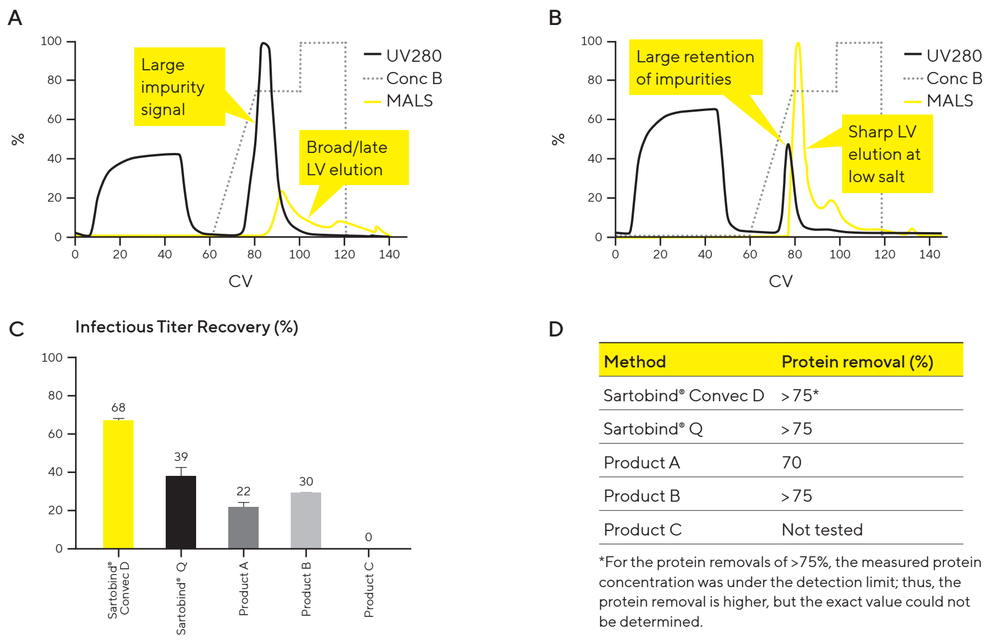
Methods

- **LV production:** LVs were produced by transient transfection of HEK293T/ 17SF suspension cells in a 2 L single use Univessel® bioreactor (Sartorius).
- **Harvest and clarification:** LVs were clarified using different vacuum-filters. Sartoclear Dynamics® Lab V50 (0.45 µm PES, Sartorius) with 5 g·L-1 (~50% wet cell weight) diatomaceous earth (DE) was used by adding the respective amount of DE to 50 mL of the cell culture broth and mixed, which was immediately filtered. For the other filtrations, the cell culture broth was centrifuged (800 *g*, 5 min), and the supernatant was clarified with Sartolab® RF50 (0.45 µm PES, Sartorius) or vacuum filtration units from other manufacturers (products A – C).
- **Purification:** LVs were purified using the novel Sartobind® Convec D membrane (Sartorius) in a MA15 device and the following buffers; Equilibration buffer: 20 mM Tris-HCl, wash buffer: 100 mM NaCl, 20 mM Tris-HCl, elution buffer (for step elution): 650 mM NaCl, 20 mM Tris-HCl, storage buffer: 50 mM HEPES, 20 mM $MgCl_2$, 5% sucrose (all buffers with pH7.2); flow rate: 6 $mLmin^{-1}$, equilibration 20 membrane volumes (MV), loading: according to LV titer in feed material and DBC10 of 2 x 10^{12} viral particles/MV, wash 20 MV and step elution 20 MV, elution fraction was diluted 1:5 in storage buffer. For gradient elution: 20 MV to 75% of elution buffer containing 1 M NaCl and 20 MV constant at 75%, followed by column wash at 100% salt buffer. Three purification kits from different suppliers (products A – C) were used as a comparison, buffers or buffer compositions were provided in these kits.
- **Concentration:** LVs can be concentrated before or after the chromatography step (or both) using Vivaspin® centrifugal ultrafilters or Vivaflow® TFF cassettes (Sartorius). Here we exemplarily show the ultrafiltration (UF) of LVs after the clarification (before the purification step) using centrifugal ultrafilters. Centrifugal ultrafilters were washed with PBS. 15 mL of clarified LV solution was loaded and UF was performed at different centrifugal forces (500, 1,000, and 2,000 *g*) and to various concentration factors to determine the ideal process conditions. Under the optimized conditions, the performance of centrifugal ultrafilters from different manufacturers were compared.
- **Analytical assays:** LV infectious titer - live-cell analysis system Incucyte S3 (Sartorius) + HEK293T cells were infected with LVs, expression of the transgene measured by real-time imaging; LV particle titer - p24 ELISA; Protein - Pierce Coomassie Bradford protein assay kit; dsDNA - Quant-iT Pico-Green dsDNA assay.

Results – Purification

After the clarification step, the LVs were purified using Sartobind® Convec D membrane adsorbers (Sartorius) and using purification kits from different suppliers (products A – C). Sartorius has developed the AEX membrane Sartobind® Convec D, a convective media with large membrane pores that enable convective mass transport, allowing large LV particles to reach binding sites.

Figure 2: *Purification Results*

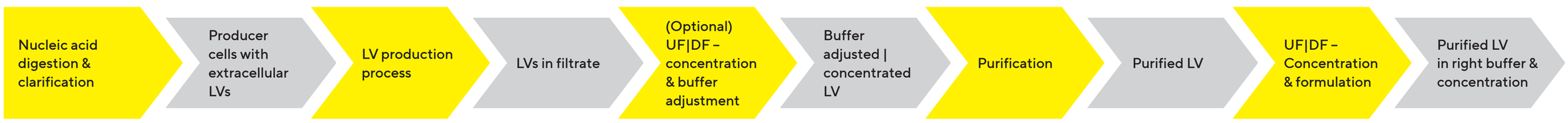


Method	Protein removal (%)
Sartobind® Convec D	>75*
Sartobind® Q	>75
Product A	70
Product B	>75
Product C	Not tested

*For the protein removals of >75%, the measured protein concentration was under the detection limit; thus, the protein removal is higher, but the exact value could not be determined.

Note: Chromatograms of (A) Sartobind® Q and (B) Sartobind® Convec D. LVs can be detected with MALS (multi angle light scattering), while impurities are detected by UV signal. (C) Infectious titer recovery of Sartobind® Convec D, Sartobind® Q and competitor LV purification kits (products A – C). (D) Protein removal of purification step.

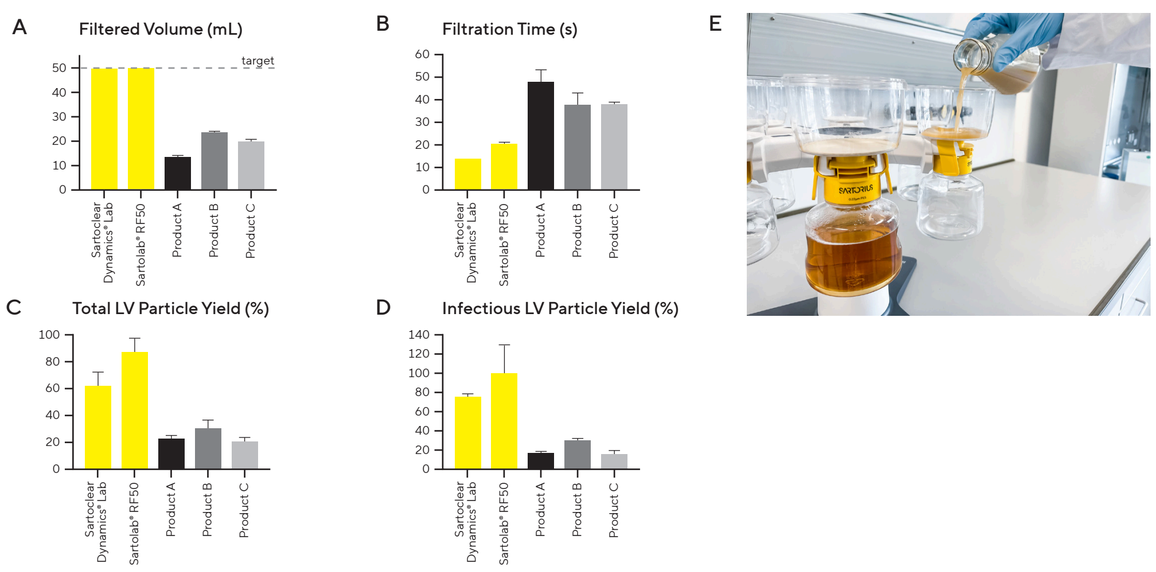
The Sartobind® Convec D membrane outperforms other products in terms of LV recovery. A sharp elution peak was achieved at a lower salt concentration compared to classical AEX matrices requiring 1 M NaCl for elution.
→ The Sartobind® Convec D membrane enables mild elution at lower salt concentrations, a sharp elution peak, resulting in high infectious LV recovery and a decreased binding of impurities.
→ Elution fractions can be further concentrated by ultrafiltration.



Results – Harvest and Clarification

Sartoclear Dynamics® Lab and Sartolab® RF50 were the only filters that could filter the target volume of 50 mL, while providing a fast filtration time. Filtration was especially fast using DE (Sartoclear Dynamics® Lab). Very high infectious LV yields (75 – 100%) were achieved using Sartolab® RF50 and Sartoclear Dynamics® Lab, while products A – C only resulted in 15 – 30% infectious LV yield. Incomplete filtration due to filter clogging with products A – C resulted in a lower overall yield (total number of recovered particles in filtrate). Harvest and clarification is very robust using Sartoclear Dynamics® Lab.

Figure 1: *Filtration Performance of Sartoclear Dynamics® Lab V50, Sartolab® RF50 and Products A, B, and C*



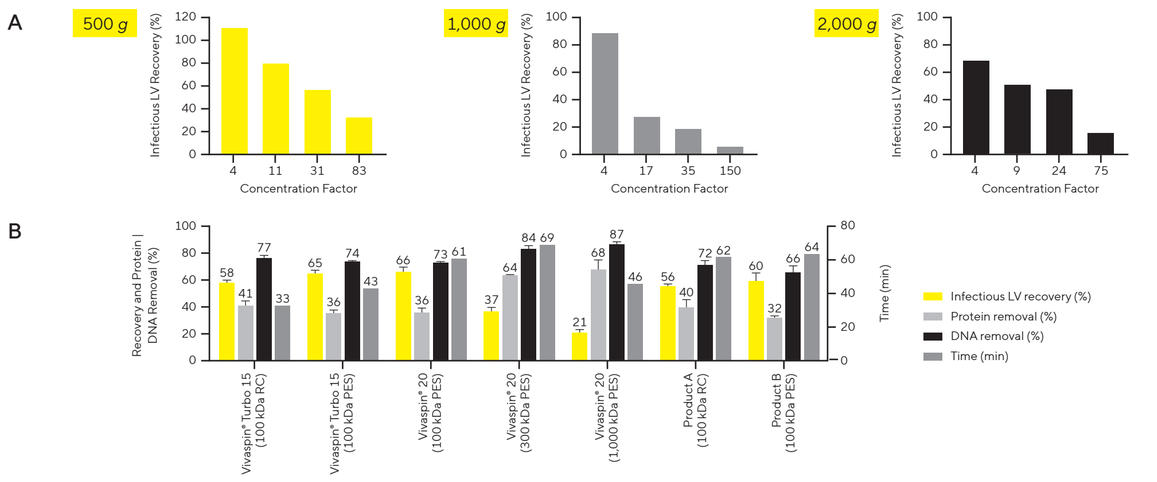
Note: Volume filtered until filter clogging occurred (max. volume 50 mL); B: Filtration time (until filter clogging); C: Total LV particle yield; D: Infectious LV particle yield; and E: experimental setup. Mean ± standard deviation, N=3

→ Harvest and clarification is very robust using Sartoclear Dynamics® Lab, this product is especially suited for LVs produced in HEK suspension culture as no additional centrifugation step is required.
→ Sartolab® RF50 is especially useful for LVs produced with adherent cell culture as cell-free supernatant can be directly clarified.

Results – Ultrafiltration

LV infectious titers after UF were highest at 500 *g*, decreasing with higher centrifugal force. Another interesting observation was that the infectious LV titer decreased with increasing concentration factor, likely due to LV aggregation. Aggregates act as single infectious units in transduction assays, reducing measured titers. A comprehensive comparison of seven centrifugal ultrafilters from three suppliers was conducted under optimized conditions (500 *g*, 7.5-fold concentration).

Figure 3: *Concentration of LVs by Centrifugal Ultrafilters*



Note: (A) Infectious LV recovery at different centrifugal forces and varying concentration factors. (B) Infectious LV recovery, impurity removal and time to concentrate LVs 7.5x at 500 *g* using different centrifugal ultrafilters. Mean ± standard deviation, N=3.

→ MWCOs of 300 and 1,000 kDa PES were shown to be unsuitable for this application due to low LV recoveries.
→ Of the centrifugal ultrafilters with 100 kDa MWCO membranes, Vivaspin® Turbo 15 (RC or PES), Vivaspin® 20 (100 kDa PES), and Product A (RC) showed the highest rates of infectious LV recovery and impurity removal.
→ Considering process time, Vivaspin® Turbo 15 is preferred, reducing concentration time by 30 – 48% compared to other tested ultrafilters with 100 kDa MWCO.

Conclusion

- Sartoclear Dynamics® Lab and Sartolab® RF50 enable efficient LV harvest and clarification, achieving 75 – 100% infectious LV yields. Sartoclear Dynamics® Lab suits HEK suspension cultures by eliminating centrifugation, while Sartolab® RF50 is ideal for adherent cultures.
- Sartobind® Convec D ensures superior LV purification, achieving high yields (68%) while enabling efficient protein removal and mild elution at lower salt concentrations, and a sharp elution peak, making it optimal for LV purification.
- Vivaspin® Turbo 15 (100 kDa) offers the highest LV recoveries (58 – 65%) among tested ultrafilters, with efficient impurity removal and a 30 – 48% reduction in process time.

Literature | published data:
- Guide for needed DE amount: **https://sar.to/jarlo**
- Application Note (harvest and clarification): **https://sar.to/kxejc**
- Paper (harvest and clarification): **https://doi.org/10.1016/j.jbiotec.2020.12.004**
- Paper (ultrafiltration): **https://doi.org/10.1002/elsc.202400057**
- Paper (infectious titer real-time imaging): **https://doi.org/10.1371/journal.pone.0254739**