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# Improving the Efficiency of Lentiviral Vector Concentration With Centrifugal Ultrafilters

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## Abstract

In this study, the performance of Vivaspin® Turbo 15 and Vivaspin® 20 centrifugal ultrafilters (Sartorius), as well as filters from two other manufacturers (referred to as products "A" and "B"), was evaluated for the concentration of lentiviral vector samples. Concentration performance was assessed based on filtration time, impurity removal, and infectious recovery. As a first step, the optimal process conditions for ultrafiltration of LVs with centrifugal ultrafilters were determined to be a centrifugal force of 500 *g* and a ≤10-fold volumetric concentration factor. Seven different centrifugal ultrafilters were tested under these process conditions. The best overall performance in terms of infectious LV recovery, impurity removal, and required centrifugation time was achieved with Vivaspin® Turbo 15 (100 kDa PES membrane). Our findings may be useful to ensure efficient ultrafiltration of other lentiviral vectors or similar large, enveloped viruses.

# Introduction

Lentiviral vectors are powerful tools for cell and gene therapy and biomedical research, providing an efficient means of delivering genetic material for stable integration into target cell genomes. With several approved products using LVs and many more in the pipeline undergoing clinical trials, the need for efficient LV production processes has never been more apparent. Challenges in LV bioprocessing occur largely due to the limited stability associated with their enveloped nature. Therefore, preservation of LV infectivity is a critical consideration [1]. Ultrafiltration (UF) plays a fundamental role in the downstream processing of lentiviral vectors. It is a separation technique that is used to concentrate and purify macromolecules and other particles, such as viruses. The process uses semipermeable membranes with nominal pore sizes that are intended to retain molecules above a certain molecular weight or diameter [2]. UF is widely used in academia, biopharma, and diagnostic industries, and is a key technique for many cell culture, structural biology, *in vitro* diagnostic, and environmental testing workflows.

Centrifugal ultrafilters – sometimes called centrifugal filters or spin filters - are well known laboratory consumables used for concentration and buffer exchange of organic and inorganic macromolecules. They are designed to operate in standard centrifuges. The selection of a centrifugal ultrafilter is based on the sample (feed) volume - typically in the range of a few microliters to several milliliters - and its properties, mainly the molecular weight or size of the target molecule. A feed is dispensed into the upper chamber of the filter and centrifugal force is applied. Under centrifugal force, the target molecule is simultaneously retained by the ultrafiltration membrane and concentrated in the upper chamber as the sample volume decreases due to solvent permeating the membrane. The concentration process with centrifugal ultrafilters can be stopped when the desired final (retentate) volume is reached and the retentate can be collected with a pipette, while the permeate is discarded. By diluting the retentate with another buffer and repeating the concentration process several times, a buffer exchange can be performed, e.g., for desalting or reformulation of the sample. This process is called diafiltration and is an efficient alternative to methods such as dialysis or gel filtration.

In this study, we first determined the ideal process conditions for the ultrafiltration of an LV sample using centrifugal ultrafilters. Under these optimized conditions, we compared the performance of different centrifugal ultrafilters, namely Vivaspin Turbo 15 and Vivaspin 20, with two different competitor products, "A" and "B". These ultrafilters included membranes of different materials and molecular weight cut-offs (MWCO). UF performance was evaluated based on process time, impurity removal, and infectious LV recovery.

# Methods & Results

A lentiviral vector was expressed by transient transfection in HEK293T/17SF suspension cells, using four plasmids. Cells were cultivated in a Univessel® 10 L bioreactor operated by a Biostat® B-DCU. Harvest and clarification of the LV-containing cell culture media were performed using Sartoclear Dynamics® Lab V, 500 mL, with 0.45 µm PES membrane and 5-8 g/L of diatomaceous earth. Centrifugal ultrafilters evaluated for UF of the clarified LV solution are listed together with their respective membrane material and molecular weight cut-off (MWCO) in Table 1.

**Table 1:** *List of centrifugal ultrafilters evaluated in our study, with their respective membrane material (RC: regenerated cellulose, PES: polyethersulfone) and molecular weight cut-off (MWCO).*

| Product | Membrane material | MWCO |
|---|---|---|
| Vivaspin Turbo 15 | RC | 100 kDa |
| | PES | 100 kDa |
| Vivaspin 20 | PES | 100 kDa |
| | | 300 kDa |
| | | 1,000 kDa |
| Product A | RC | 100 kDa |
| Product B | PES | 100 kDa |

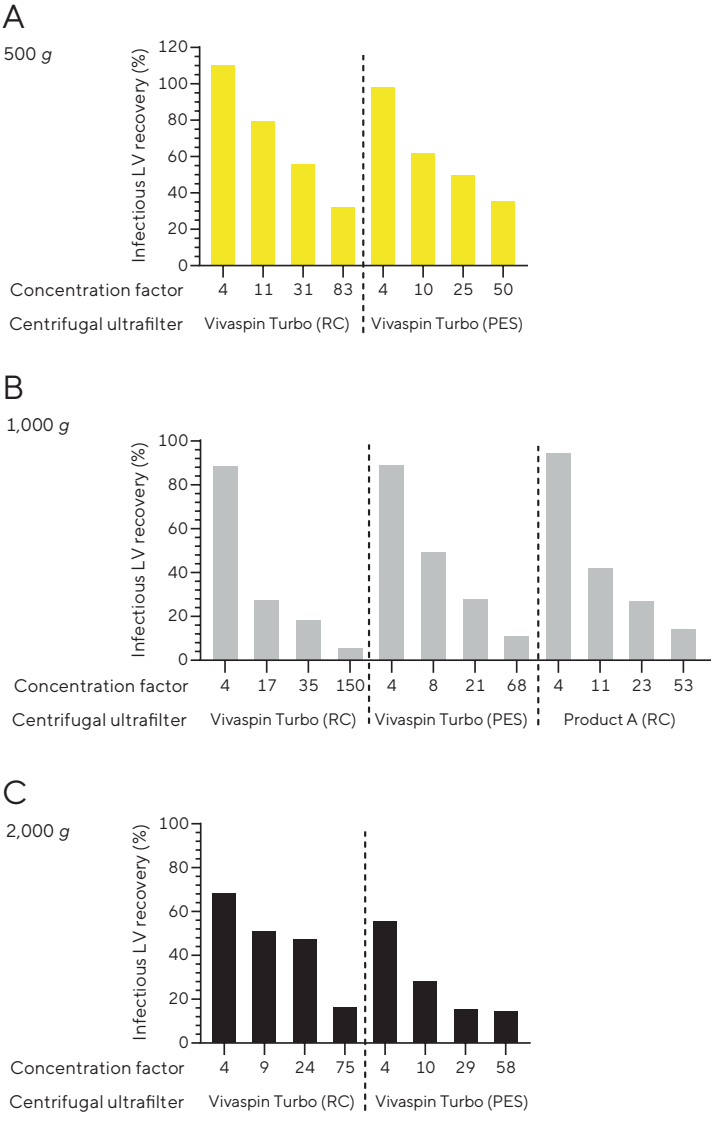
For all UF experiments, a Multifuge X1R centrifuge (Thermo Fisher Scientific) with swing-out rotor was used. Centrifugal ultrafilters were pre-washed once with phosphate-buffered saline (PBS). 15 mL of clarified LV solution was dispensed into each centrifugal ultrafilter and UF was performed at three different centrifugal forces: 500, 1,000, and 2,000 *g*. The concentration factor was varied in the experiments and is summarized in the results.

Analytical parameters to assess ultrafiltration performance were total dsDNA concentration (Quant-iT PicoGreen Assay, Thermo Fisher Scientific) and total protein concentration (Pierce Coomassie Bradford protein assay kit, Thermo Fisher Scientific), determined for both the feed and retentate samples. Furthermore, infectious particle titer was measured by transduction of adherent HEK293T cells with virus samples and measurement of transgene expression (green fluorescent protein) 60 h post-infection, using a real-time imaging approach with the Incucyte® S3 Live-Cell Analysis System.

# Results & Discussion

Our initial experiments sought to evaluate different process conditions for the concentration of LVs using commercially available centrifugal ultrafilters. LV samples were processed to four different volumetric concentration factors.
For comparison, the exact volumetric concentration factors are given on the x-axis of each chart shown in Figure 1A, since stopping the process at the same retentate volume was not possible for every design of ultrafilter tested, especially at higher concentration factors. Vivaspin Turbo 15 with RC and PES membranes (both 100 kDa) were centrifuged at 500, 1,000 or 2,000 *g*, respectively. In addition, Product A (100 kDa RC) was centrifuged at 1,000 *g* to verify the effect of different concentration factors on infectious recovery using an ultrafilter from another supplier.

**Figure 1:** *Infectious LV recovery after ultrafiltration at (A) 500 g, (B) 1,000 g, and (C) 2,000 g, using Vivaspin Turbo 15 (RC and PES), and Product A, all with a MWCO of 100 kDa. Volumetric concentration factors are indicated on the x-axes.*

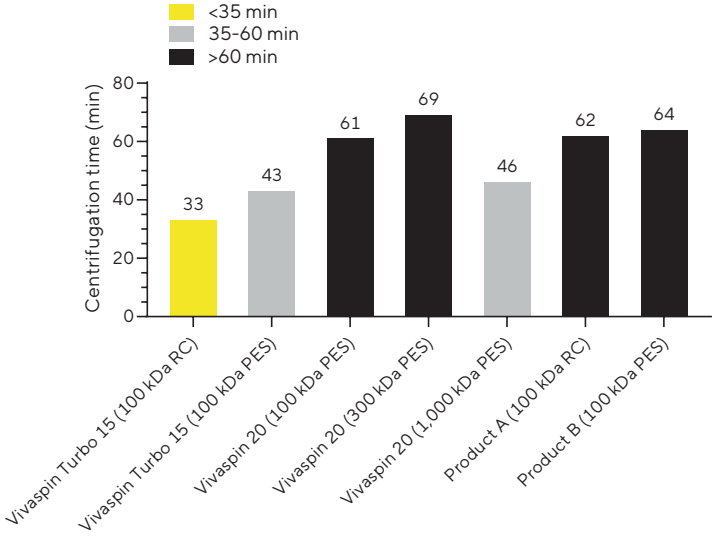



LV infectious titers after UF at different centrifugal forces showed that the highest recoveries were achieved at 500 *g* and that these decreased with increasing centrifugal force (Figure 1). Another interesting observation was that the infectious LV titer decreased with increasing concentration factor. These findings were consistent among all centrifugal ultrafilters that we tested. A possible explanation for the decreased titers is an increased propensity for LV aggregation at higher concentration factors. LV aggregates act as single infectious units in transduction assays, which may result in lower infectious titer measurements [3].
We concluded that the optimal process conditions for concentration of LVs with centrifugal ultrafilters were a centrifugal force of 500 *g* and a volumetric concentration factor ≤10-fold.

Under these conditions, infectious LV recovery was 100% and 60-80% at 4-fold and 11-fold concentration factors, respectively.

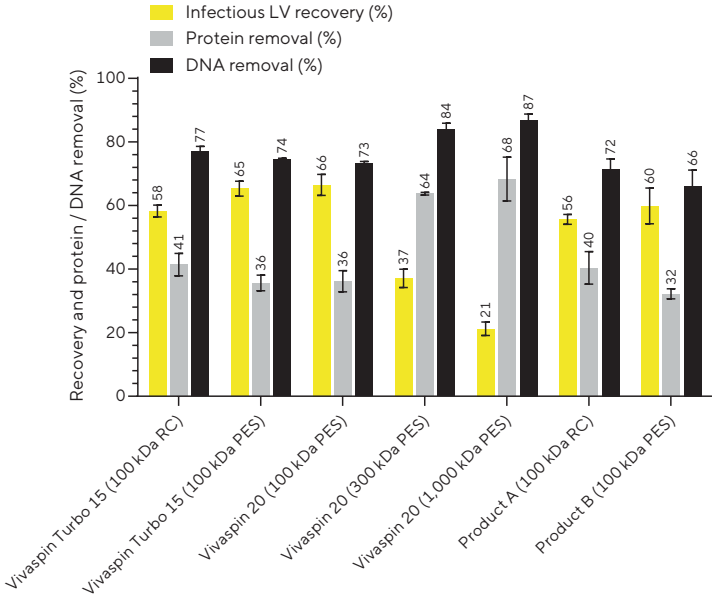
A comprehensive comparison of seven different centrifugal ultrafilters from three different suppliers was performed using our optimized process conditions (500 *g* until a concentration factor of 7.5-fold was reached). The ultrafilters tested were fitted with either regenerated cellulose (RC) or polyethersulfone (PES) membranes, with MWCOs of 100, 300, or 1,000 kDa. Figure 2 shows the centrifugation time required to concentrate our LV sample from 15 mL feeds.

**Figure 2:** *Time to concentrate LVs 7.5x at 500 g using different centrifugal ultrafilters. Process times are categorized to <35 min (yellow), 35-60 min (grey), or >60 min (black). The LV sample was concentrated from an initial (feed) volume of 15 mL to a final (retentate) volume of approximately 2 mL.*



The fastest time to concentrate of 35 minutes was observed when using Vivaspin Turbo 15 (100 kDa RC). This was followed by the Vivaspin Turbo 15 (100 kDa PES) and Vivaspin 20 (1,000 kDa PES) with a total process time of around 45 minutes. The four remaining units required longer centrifugations, resulting in process times >60 minutes. The infectious LV recovery, protein removal, and DNA removal for all seven centrifugal ultrafilters are summarized in Figure 3.

**Figure 3:** *Infectious LV recovery (yellow), protein removal (grey), and DNA removal (black) after 7.5x concentration of LVs at 500 g using different centrifugal ultrafilters. The LV sample was concentrated from 15 mL to approximately 2 mL.*




The highest infectious LV recoveries of around 65% were obtained when using Vivaspin Turbo 15 and Vivaspin 20 (both with 100 kDa PES membranes). The second highest LV recoveries of 60% were observed in the retentate from Product B (100 kDa RC). Vivaspin 20 with 300 and 1,000 kDa PES membranes showed the lowest LV recoveries of ≤37%, indicating that higher MWCOs are not well-suited for concentration of LVs. Due to their smaller size relative to the nominal pore sizes of these membranes - especially those with a 1,000 kDa MWCO – a proportion of the LVs may have been retained inside the membrane pores, experienced increased shear stresses leading to degradation, and/or passed through the membrane.

The highest rates of impurity removal (≥84% DNA and ≥64% protein) were observed with Vivaspin 20 (300 kDa and 1,000 kDa PES), most likely due to the larger MWCO allowing a higher proportion of impurities to permeate the membranes. However, due to the low LV recovery, these ultrafilters would not be considered suitable for concentrating LV samples. Among the centrifugal ultrafilters with 100 kDa MWCO membranes, the highest rate of DNA removal was ≥72% when using Vivaspin Turbo 15 (PES and RC), Vivaspin 20 (PES), and Product A (RC). Similarly, more proteins (around 40%) were removed when using Vivaspin Turbo 15 and Product A (both RC), followed by a slightly lower removal rate (36%) with Vivaspin Turbo 15 and Vivaspin 20 (both PES). Table 2 provides an overview of all our results, indicating the best performance in yellow.

**Table 2:** *Summary of recovery, impurity removal and process time data, used to assess the performance of different centrifugal ultrafilters when concentrating an LV sample 7.5x at 500 g. Each value is color-coded from best (yellow) to worst (white).*

| Device | Infectious recovery (%) | Protein removal (%) | DNA removal (%) | Process time (min) |
|---|---|---|---|---|
| Vivaspin Turbo (100 kDa RC) | 58.26 | 41.39 | 76.9 | 33 |
| Vivaspin Turbo (100 kDa PES) | 65.3 | 35.63 | 74.41 | 43 |
| Vivaspin 20 (100 kDa PES) | 66.49 | 36.2 | 73.29 | 61 |
| Vivaspin 20 (300 kDa PES) | 37.07 | 63.75 | 83.89 | 69 |
| Vivaspin 20 (1,000 kDa PES) | 21.23 | 68.34 | 86.89 | 46 |
| Product A (100 kDa RC) | 55.64 | 40.39 | 71.57 | 62 |
| Product B (100 kDa PES) | 59.9 | 32.25 | 66.15 | 64 |

# Conclusion

We have found that the optimal process conditions for concentrating LV samples with centrifugal ultrafilters are a relatively low centrifugal force of 500 *g* and a volumetric concentration factor ≤10-fold. Vivaspin 20 with 300 and 1,000 kDa PES were shown to be unsuitable for this application due to low LV recoveries. Taking a holistic view of the performance of the tested 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 (DNA and protein) removal. However, when also taking process time into account, the use of Vivaspin Turbo 15 is preferred, as the time to concentrate was 30-48% less compared to the other three ultrafilters we tested with 100 kDa MWCO membranes. The test procedure and results presented here will serve as a useful guide for identifying and implementing optimal concentration conditions for lentiviral vectors and other large, enveloped viruses that may behave similarly.

# References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# Abbreviations

| | |
|---|---|
| (ds)DNA | (Double-stranded) deoxyribonucleic acid |
| HEK | Human embryonal kidney cells |
| LV | Lentiviral vector |
| MWCO | Molecular weight cut-off |
| PBS | Phosphate-buffered saline |
| PES | Polyethersulfone |
| RC | Regenerated cellulose |
| UF | Ultrafiltration |

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