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**Application Note**

# Pellicon® Capsules versus Hollow Fibers for Ultrafiltration/Diafiltration (UF/DF) in Viral Gene Therapy and Viral Vaccine Manufacturing

Tangential flow filtration (TFF) is an essential operation in the manufacturing of complex and life-saving biopharmaceuticals. Technologies used for TFF are hollow fiber modules and flat sheet filters, also known as cassettes. The hollow fiber design was developed by early pioneers in ultrafiltration (UF) technology for industrial applications (e.g., filtration of beverages) and low-pressure pharmaceutical applications (e.g., dialysis). The need to optimize the TFF process to produce therapeutics led to the development of the flat sheet TFF cassette.

Pellicon® cassettes were a result of such developments to provide improved processing speed, linear scalability, and high-pressure capability. Traditionally used for multi-use operation, for more than three decades, Pellicon® cassettes have provided robust and reproducible performance in bioprocessing applications. Growing populations, the emergence of new diseases, and recent advancement in novel modalities, such as viral gene therapies, are now driving the implementation of single-use manufacturing technologies to improve speed-to-market of critically needed therapies. The need to reduce process complexity and increase manufacturing flexibility while minimizing product and operational risks spurred the more recent development of Pellicon® Capsules.

Designed for efficient single-use UF/DF, the Pellicon® Capsule is a first-of-its-kind spiral-wound TFF filter engineered to provide linear scalability with comparable performance to our Pellicon® cassettes. This application note discusses methodology and experimental results in the performance evaluation of Pellicon® Capsules versus hollow fibers for UF/DF of viral vectors and describes the impact of either filter type on a TFF process.

## Study Background

TFF is typically used at two steps in the downstream purification process of viral gene therapies **(Figure 1)**. A first TFF operation (TFF1) is usually located after clarification and before chromatography. A final UF/DF step (TFF2) is performed post-chromatography to concentrate the purified viral vector and exchange into the formulation buffer. This study evaluates filter performance during the TFF1 step while using a virus model feed. A study with lentivirus for a TFF2 step is further highlighted.

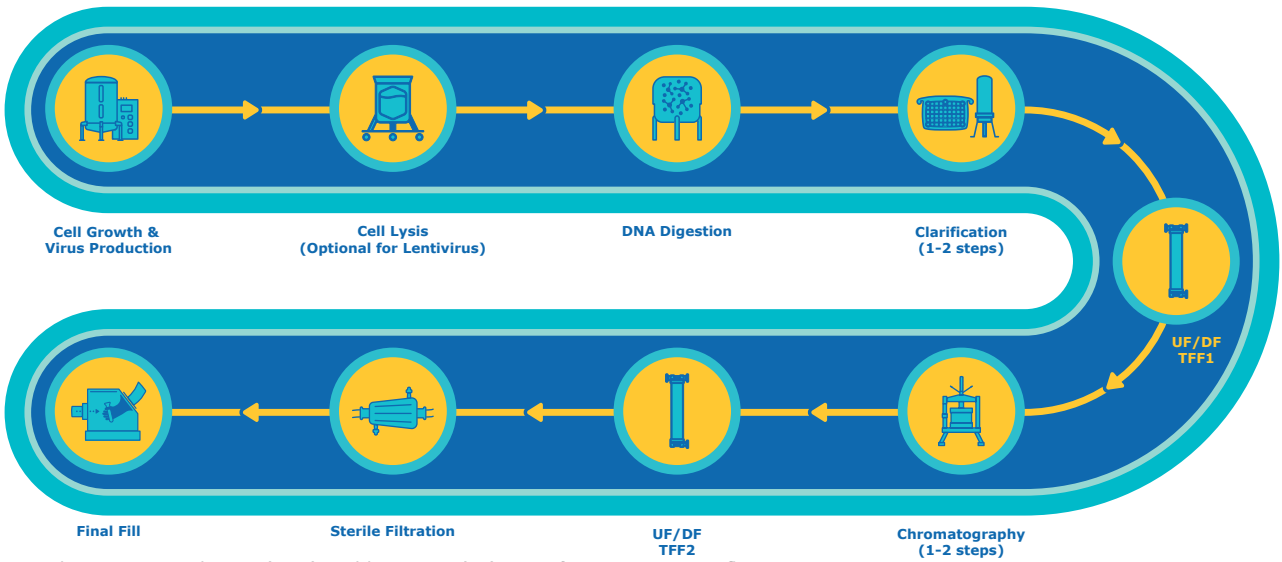


**Figure 1**. Adeno-associated virus (AAV) and lentivirus (LV) manufacturing process flow.

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# Materials

## Feed

Testing for the TFF1 step was performed using a virus model feed comprising a detergent-lysed, Benzonase® endonuclease-treated, depth filter-clarified, non-transduced HEK293 stream in phosphate-buffered saline (PBS) spiked with a bacteriophage of similar size to AAV2. Target titer was $1e^7$ phage/mL. Performance comparability of the model feed versus in-house AAV2 feed was previously described based on flux, yield, and impurity reduction[1].

## TFF Filters

Pellicon® Capsule and its scale-down filter, Pellicon® XL 50 cassette, were evaluated in this study as well as two hollow fiber modules, HF-A and HF-B, for performance comparison. Details for each TFF filter are listed in Table 1.

| Filter | Membrane Chemistry | Feed Channel | Fiber ID (mm)/L (cm) | Area ($m^2$) |
|---|---|---|---|---|
| Pellicon® Capsule | CRC | C screen | -- | 0.1 |
| Pellicon® XL 50 Cassette | CRC | C screen | -- | 0.005 |
| Hollow Fiber HF-A | mPES | Open | 0.5/60 | 0.029 |
| Hollow Fiber HF-B | mPES | Open | 0.5/20 | 0.0115 |

**Table 1**. Filters evaluated with 100 or 300 kDa NMWL.
NMWL = nominal molecular weight limit; CRC = composite regenerated cellulose; mPES = modified polyethersulfone

Pellicon® XL 50 cassette and hollow fibers were run with feed and permeate in co-flow mode (permeate port closed on feed end and open on retentate end) for all tests.

# Flux Evaluations

## Impact on Critical Flux

Initial comparison of filter performance evaluated the impact of crossflow rate on the critical flux for a permeate-controlled TFF system using a permeate pump. Tests were performed with 35 L/$m^2$ loading of virus model feed in total recycle mode. Pellicon® XL 50 cassette and hollow fiber HF-B with 100 kDa membrane were used for this study.

The results show how critical flux increases with crossflow rate for both filter formats **(Figure 2)**. However, the Pellicon® XL 50 cassette had at least three-times higher critical flux than the hollow fiber module at the same area-normalized crossflow rate, leading to higher productivity.

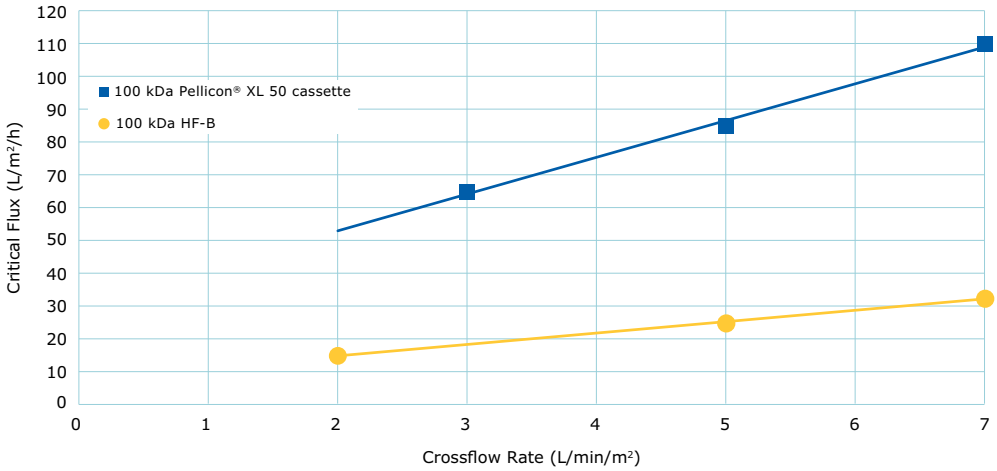



**Figure 2**. Critical flux vs Crossflow rate.

## Initial Permeate Flux Performance

For the next study, crossflow rate was fixed for each filter type and the test matrix expanded to evaluate permeate flux of the more open 300 kDa membrane, as well as performance testing during a typical transmembrane pressure (TMP)-controlled operation. The crossflow was set to give 5 liters/min/$m^2$ (LMM) for the Pellicon® Capsule and cassette. Hollow fiber HF-A was set at the same crossflow rate of 5 LMM, while hollow fiber HF-B was set at triple the crossflow rate, 15 LMM. Modeled shear rates were ~5100 $s^{-1}$ for the Pellicon® filters and ~6400 $s^{-1}$ for the hollow fiber modules.

Initial permeate flux data with virus model feed at 35 L/$m^2$ loading for TMP-controlled and permeate-controlled operations using the various filter types is reported as LMH (L/$m^2$/h) in **Figure 3**. For TMP control, initial flux is the flux at optimal TMP; and for permeate control, initial flux is 50% of the critical flux[1]. The results show Pellicon® Capsule had much higher flux than the hollow fiber modules for both TMP- and permeate-controlled operations using 100 and 300 kDa membranes. The hollow fiber modules had no more than 56% of the Pellicon® Capsule flux even at three times the crossflow rate. The data also demonstrates excellent scalability between the capsule and cassette within 15% flux difference in all cases.

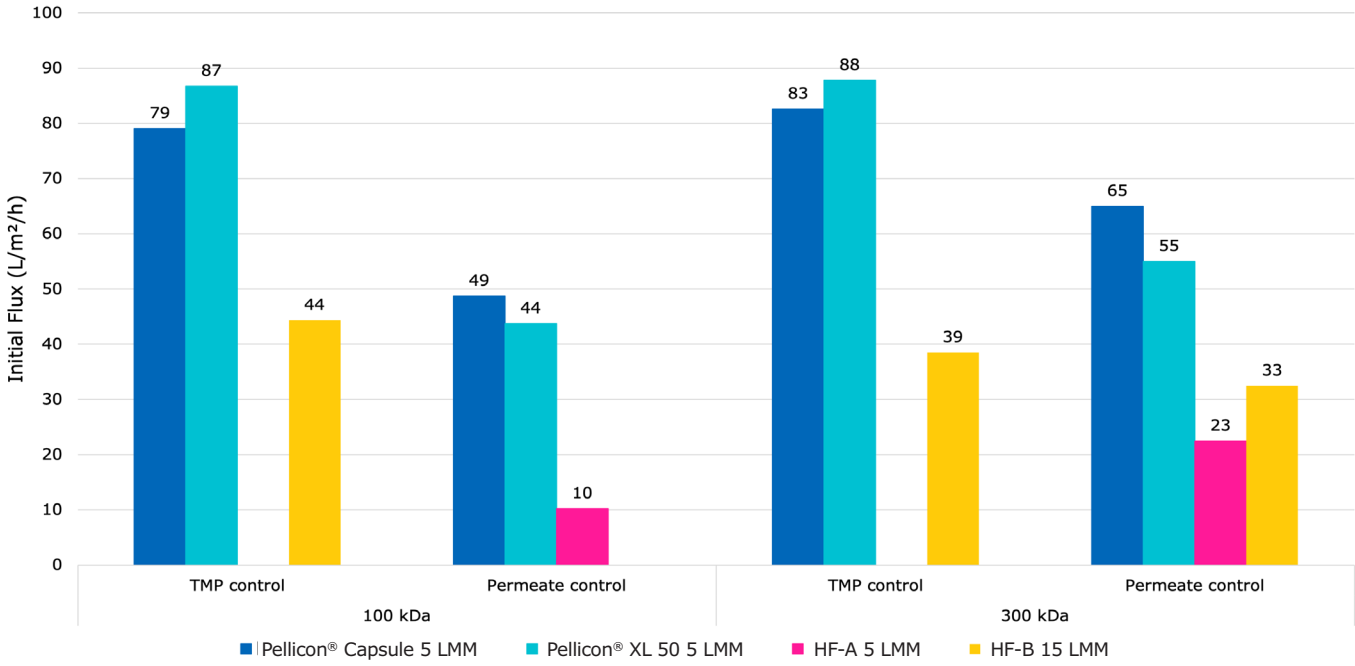



**Figure 3**. Initial operating flux performance comparing filter types and operating systems for 100 and 300 kDa NMWL. For TMP control, initial flux is the flux at optimal TMP. For permeate control, initial flux is 50% of the critical flux. Pellicon® XL 50 cassette: two cassettes in parallel for 100 $cm^2$.

## Process Simulation

### Permeate Control Case Study with Virus Model Feed

A simulation was run to evaluate TFF performance during a UF1/DF/UF2 process with 35 L/m$^2$ of virus model feed using Pellicon® Capsule and the hollow fiber modules. The process goal was to concentrate four-fold (4×) in batch mode, diafilter with five diavolumes (5 DV) of HEPES buffer at constant volume, and then concentrate two-and-a-half-fold (2.5×) in batch mode for an overall volumetric concentration factor (VCF) of 10×. A permeate-controlled operation was used for the process simulation with 300 kDa membrane.

Crossflow rates were set to 5 LMM for Pellicon® Capsule and hollow fiber HF-A; HF-B was set to 15 LMM. Retentate pressure was set initially at ~5 psi. Permeate flux was controlled to 50% of the critical flux for the first concentration step and reduced to 25% for the subsequent diafiltration and final concentration steps. Feed and permeate samples were collected throughout the process. Performance was evaluated based on flux, processing time, Benzonase® endonuclease removal, and virus yield.

Flux and TMP profiles during the TFF process simulation are shown in **Figure 4**, where flux is fixed and TMP can rise. Pellicon® Capsule and hollow fiber HF-A achieved the 10× concentration target. However, hollow fiber HF-B could not reach 10× concentration due to the larger system hold-up volume required to attain a 3× crossflow rate of 15 LMM compared to the capsule and HF-A.

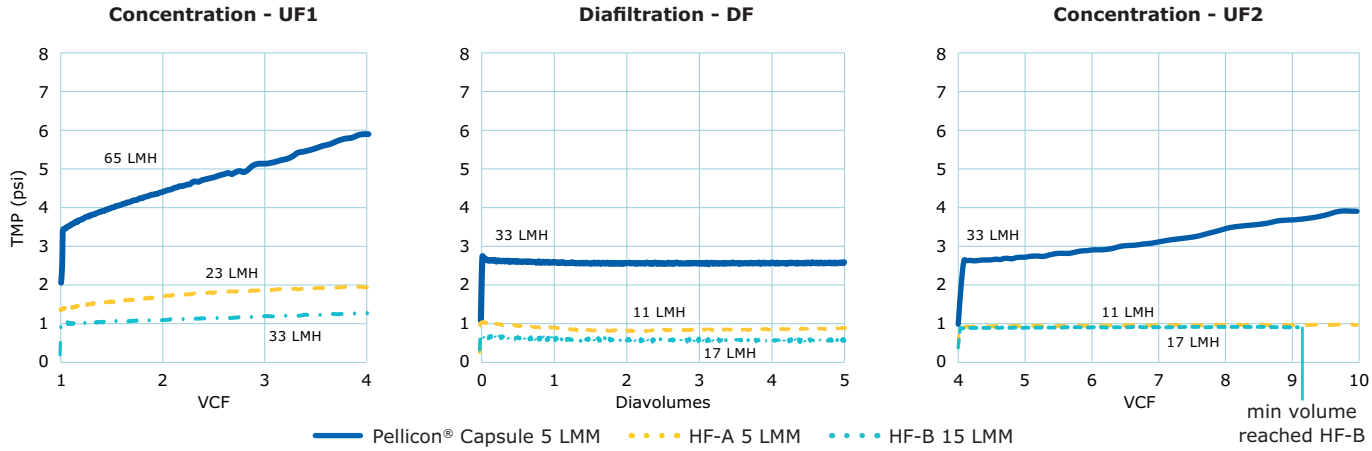



**Figure 4**. Flux and TMP during process simulation for a permeate-controlled UF1/DF/UF2 process with 300 kDa membrane. Flux values above represent the nominal flux rounded to the nearest whole number. Actual flux values during processing were ±10%.

Although a crossflow rate of 5 LMM allowed for reduced system hold-up volume and achievable VCF target of 10× for HF-A, a tradeoff of lower flux resulted in 3.2× longer processing times for the hollow fiber compared to Pellicon® Capsule **(Table 2)**. Even at three-times the normalized crossflow rate, hollow fiber HF-B still took longer than capsule with 60% more run time. The longer required run time for hollow fibers is consistent with their lower starting flux. Benzonase® endonuclease clearance was 96% or more for all filters, and virus yield was comparable at 98% or greater.

| Filter | Run Time | Run Time Normalized to Capsule |
|---|---|---|
| Pellicon® Capsule, 5 LMM | 112 min | 1.0 |
| HF-A, 5 LMM | 354 min | 3.2 |
| HF-B, 15 LMM | 181 min | 1.6 |

**Table 2**. Run time for a permeate-controlled UF1/DF/UF2 process simulation with 300 kDa membrane.

## Sizing Scenarios and Process Impact Analysis

The results of the 300 kDa permeate-controlled process simulation were applied to build two theoretical sizing scenarios using Pellicon® Capsule and the hollow fibers:

*Scenario I* assumes the same loading is used for all filters. This scenario compares run times and maximum possible VCF for a 100 L batch at 35 L/m² loading **(Table 3)**.

This scenario shows that when using the same loading (35 L/m²), the Pellicon® Capsule runs 2.9× faster than hollow fiber HF-A at the same normalized crossflow rate. The capsule is still 1.7× faster even when hollow fiber HF-B uses triple the capsule crossflow rate. Although HF-B was able to save some time compared to HF-A, the higher crossflow rate reduces the hollow fiber system's maximum possible VCF by a factor of ~4×.

**Scenario I: 100 L batch, 35 L/m², 300 kDa, permeate control**

| Parameter | Pellicon® Capsule 5 LMM | HF-A 5 LMM | HF-B 15 LMM |
|---|---|---|---|
| Area (m²) | 2.9 | 2.9 | 2.9 |
| Run time (hr) | 1.9 | 5.5 | 3.3 |
| Run time normalized to capsule | 1.0 | 2.9 | 1.7 |
| Max VCF | 33 | 33 | 8.3 |
| Feed flow (L/min) | 15.8 | 14.8 | 43.8 |
| Mobius® TFF system | TF2S | TF2S | Flow rate too high |

**Table 3.** Modeling of a 100 L scale scenario for processing a TFF1 batch at 35 L/m² loading based on flux data from the 300 kDa simulation study.

**Note:** Calculated area is before applying a safety factor. Maximum VCF is theoretically based on the system minimum recirculation volume, which depends on crossflow rate and resultant piping. The Mobius® TFF system provides permeate control with automated valve.

*Scenario II* restricts the time required to complete the TFF process. This scenario compares filtration area required and maximum possible VCF for processing a 1000 L batch in 4 hours, which requires variable loading **(Table 4)**.

This scenario shows that to obtain the same process time (4 hours), Pellicon® Capsule only requires 35% of the filtration area and 37% of the feed flow of the hollow fiber module when the filters operate at the same crossflow rate—i.e., compared to the HF-A case. If the crossflow rate of the hollow fiber is tripled to increase its flux (HF-B case), the capsule requires only 58% of the hollow fiber filtration area and 21% of its feed flow. It should also be noted that the higher feed flow of the hollow fiber system reduces the maximum possible VCF by a factor of ~4×.

**Scenario II: 1000 L batch, 4-hour process, 300 kDa, permeate control**

| Parameter | Pellicon® Capsule 5 LMM | HF-A 5 LMM | HF-B 15 LMM |
|---|---|---|---|
| Loading (L/m²) | 73.2 | 25.4 | 42.3 |
| Area (m²) | 13.7 | 39.4 | 23.7 |
| Area normalized to capsule | 1.0 | 2.9 | 1.7 |
| Max VCF | 40.0 | 22.2 | 11.1 |
| Feed flow (L/min) | 75.7 | 204.6 | 362.4 |
| Mobius® TFF system | TFF 80 | Flow rate too high | Flow rate too high |

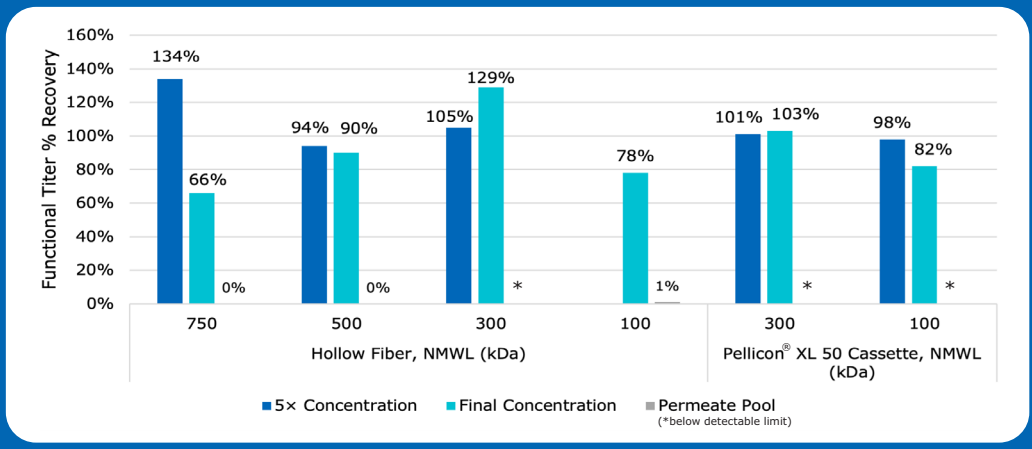
**Table 4.** Modeling of a 1000 L scale scenario for processing a TFF1 batch in 4 hours based on flux data from the 300 kDa simulation study.

**Note:** Calculated area is before applying a safety factor. Maximum VCF is theoretically based on the system minimum recirculation volume, which depends on crossflow rate and resultant piping. The Mobius® TFF system provides permeate control with automated valve.

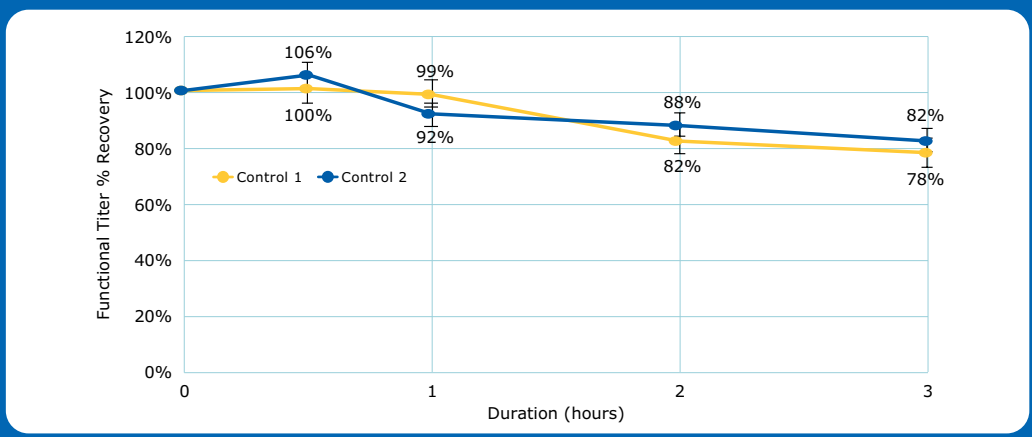
## Lentivirus Spotlight

Pellicon® XL 50 cassettes and hollow fibers were evaluated during an optimization study of the TFF2 step for lentiviral vector purification. Control samples were held at room temperature for the duration of the experiment to quantify temperature degradation of lentivirus. Virus yield was quantified for both filter formats and various membrane cutoffs following the TFF2 step. Yield calculations were normalized to their respective benchtop control samples. Lentivirus yield was comparable at the manufacturers' stated NMWL of 300 kDa for the cassette and 300 or 500 kDa for the hollow fiber (A). Evaluation of the benchtop control samples of lentivirus feed taken over a 3-hour period found recovery to decrease with time (B). Given that lentivirus yield is sensitive to process time, processing with speed can be critical for optimal yield, hence, operating with higher flux is highly desirable.

**(A)**




**(B)**




Based on this study, optimum parameters for operation were developed for the cassette and hollow fiber and are detailed below. The cassette gave 30% higher flux while staying below a conservatively low pressure limit. Due to linear scalability, similar results can be expected when scaling up to Pellicon® Capsule.

| Parameter | Filter Format and Developed Operating Conditions | |
|---|---|---|
| TFF Filter | Pellicon® XL 50 Cassette | Hollow Fiber |
| NMWL | 300 kDa | 300 or 500 kDa |
| Construction | C screen, Ultracel® membrane | 0.5 mm lumen, 20 cm long, mPES membrane |
| Process | Permeate-controlled operation (constant flux and feed flow) | |
| Shear, 4000 $s^{-1}$ | ~4.1 LMM | ~9.4 LMM |
| Operating flux | 53 LMH* | 40 LMH |
| Concentration | 5x | |
| Diafiltration | 5 DV | |

*Flux was set conservatively low for the cassette since it is based on flux recorded at a self-imposed pressure limit of 12 psi during the critical flux excursion, before the critical flux was reached.

## Conclusion

Hollow fiber modules give lower flux at a given shear; therefore, higher membrane area or processing time is required compared to Pellicon® Capsules. Crossflow rates of hollow fibers are generally increased to get a higher production rate, but flux is still lower than Pellicon® Capsules and the resulting system may be limited in achieving high concentration targets.

In contrast, Pellicon® Capsules achieve higher and more stable flux than hollow fibers, resulting in more efficient TFF systems while giving comparable virus yield. The resulting system is also smaller, which enables higher concentration targets.

Since Pellicon® Capsule is a pre-sterilized, plug 'n play, holderless device—a similar form to hollow fibers, it can more easily replace hollow fiber modules in existing single-use TFF systems, while gaining process benefits such as faster processing time or reduced membrane area required and minimal system working volume for an overall more productive TFF operation. Our technical experts are ready to guide your conversion efforts, from process development to implementation, to help you achieve your goals faster.

### Summary of Benefits

**Robustness & Reliability:** Each sheet of membrane is supported by the device itself and self-balancing pressures, providing a higher degree of construction reliability.

**Efficiency:** Lower crossflow rates result in smaller pumps and systems, reducing overall overhead costs as well as minimum working volumes to achieve higher concentrations.

**Linear Scalability:** The normalized crossflow rate is independent of capsule size, providing fast and reliable scaling from benchtop to commercialization, including scalability to our Pellicon® cassettes.

**Optimum Recovery:** Void-free Ultracel® composite membrane provides low fouling and low binding for excellent product retention and recovery.

### References

1. Technical Brief, Evaluation of TFF Operating Control Strategies and Scalability for Viral Vector Process Development. TB11669EN.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

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