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# AAV Studies Deploying a Closed, Irradiated, Single-use Flat Sheet TFF Device

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## Abstract

Closed systems provide risk mitigation for bioburden sensitive processes and create facility resource flexibility. Performance of a closed and irradiated tangential flow filtration (TFF) filtration device was compared to the analogous open and non-irradiated model for the concentration of adeno-associated virus (AAV) viral vector. Processing time, flux, trans-membrane pressure (TMP) and recovery were found to be equivalent, enabling process development to be rapidly executed in a single-use TFF cassette followed by facile bridging to a closed and single-use TFF device.

## Introduction

Viral vectors currently represent one of the most powerful in vivo gene therapy delivery systems with expanding clinical applications. This, in turn, drives production demand for viral vectors, such as adeno-associated virus, lentivirus, and adenovirus. Of the wide library of available vectors, AAV is the most frequently selected due to demonstrated safety, low immunogenicity and long-term transgene expression characteristics[1].

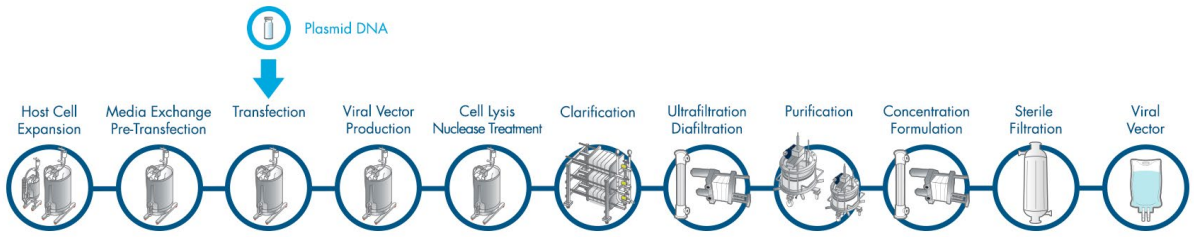


**Figure 1.** AAV production process containing two TFF steps

Downstream processing of AAV includes (Figure 1) the use of a series of purification technologies designed to produce a pure and concentrated final drug product. One of those technologies is TFF for ultrafiltration/diafiltration. Key execution metrics for ultrafiltration/diafiltration include time, yield, cost, facility compatibility and endotoxin level. Components within a generic TFF system typically include several reservoirs, a TFF cassette, 2 pumps and multiple flow meters. Integrating component into the system requires approximately 20 connections, each representing a point of contamination risk. Technologies frequently present potential process improvements with respect to time, cost, contamination, facility utilization and yield. With two instances of the TFF unit operation in the overall production, the benefits of TFF process improvements multiply.

The introduction of single-use TFF filters, such as the TangenX® SIUS® Cassette, reduced the typical TFF operation from 10 steps over the course of 12 hours to 3 steps completed with a dramatic

[1]Gene Therapy: A Paradigm Shift in Medicine, Pharma Intelligence, Informa UK Ltd., November 2018

savings in the unit operation time. Single-use TFF filters also can potentially reduce buffer consumption by 50%, saving both preparation time and reducing the cost of materials and waste disposal. The single-use SIUS® Cassette has now been engineered into a closed and irradiated system, the TangenX® SIUS® Gamma Device (Figure 2), which includes filter, manifold, clamps, tubing and AseptiQuik® connectors (Table 1). Set-up times and complexity are modestly reduced with the SIUS® Gamma Device, but most importantly, the nature of the closed system mitigates risk for the product, environment and operator in a fast-paced industry where safety and quality remain paramount.

**Figure 2.** TangenX® SIUS® Cassette and SIUS® Gamma Device

**Table 1.** TangenX® SIUS® Gamma Device engineers single-use process efficiencies into a closed and irradiated format

| | TangenX® SIUS® Cassette | TangenX® SIUS® Gamma Device |
|---|---|---|
| Single-use cassette | ✓ | ✓ |
| Pre-sanitized (0.2N NaOH) | ✓ | ✓ |
| L, E Screens | ✓ | ✓ |
| 0.1 - 2.5 $m^2$ surface area | ✓ | ✓ |
| Manifold | | ✓ |
| Clamps | | ✓ |
| Tubing stubs | | ✓ |
| Sterile connectors | | ✓ |
| Closed | | ✓ |
| Gamma-irradiation | | ✓ |

Construction and logistics of closed systems can be made challenging by proprietary connectors and vendor restrictions. Termination of the tubing with genderless AseptiQuik® connectors at each of the three ports enables the operator a multitude of connectivity options that integrate existing, smaller and more modular flow paths or tubing assemblies.

This case study describes optimization and scale-up of the SIUS® Gamma Device for concentration and diafiltration of clarified AAV vector samples derived from human hematopoietic stem cells (AAV-HSCs). AAV-HSCs represent a proprietary platform of adeno-associated viral vectors with unique properties developed by Homology Medicines, Inc. Delivery of AAV-HSCs occurs precisely and efficiently *in vivo* through either gene therapy or patient DNA repair processes of homologous recombination, a natural and nuclease-free gene editing mechanism. Usage in the lab environment and demonstration of equivalence of the irradiated SIUS® Gamma Device with the non-irradiated - SIUS® Cassette for concentration and diafiltration steps with AAV-HSCs was the primary goal.

## Materials and methods

TangenX® SIUS® Cassette and SIUS® Gamma Device installations were performed according to manufacturer instructions. After installation of SIUS® Cassettes, system tubing, filter plate, insert and connections were sanitized with 0.2 N NaOH. SIUS® Cassettes arrive pre-sanitized and packaged with 0.2 N NaOH and therefore do not require pre-sanitization, but the assembled tubing, insert and flow path still required sanitization. The SIUS® Gamma Device was simply installed into a stainless-steel holder, connected to a pre-sanitized flow path with the AseptiQuik® connectors and flushed with buffer.

While Repligen performs integrity tests on all individual cassettes prior to shipment, a recommended pre-use integrity test was performed for confirmation of cassette status immediately prior to use for all runs. The integrity test entails a simple, qualitative pressure-hold step using the peristaltic pump to pressurize the upstream side of the cassette to 7 - 8 psi. With the pump stopped and the feed/retentate tubing clamped shut, the pressure was monitored for 1 minute to confirm the ability of the system to maintain pressure.

## Process development membrane and configuration screening

Prior to optimization and scale-up, screening experiments determined the most appropriate molecular weight cut-off (MWCO) HyStream membrane for clarified AAV-HSC concentration. A Repligen KrosFlo® KR2i TFF System was used to concentrate 1 L 20X - 0.05 L with a 0.01 $m^2$ sized cassette. Retain samples were collected from the product pool at 1, 5, 10, 15 and 20X concentration factors for further analysis.

Experiments with 0.01 $m^2$ 300 kD L-screen (Part Number XP300LP1L) and open channel (Part Number XP300LP1J) were also performed but found to be less appropriate for the process.

## Optimization and scale-up

Optimization studies were performed using the TangenX® LHV System, 100 mL of clarified AAV-HSC feed stream at a titer of approximately 109 vg/mL in combination and a 0.01 $m^2$, 100 kD L-Screen HyStream SIUS® Cassette. Flux excursions were performed at crossflow fluxes of 3, 5 and 7 LPM/$m^2$.

Conformational experiments with a 0.01 $m^2$, 100 kD L-Screen Cassette (P/N XP100LP1L) were run at the optimized operating conditions, a constant transmembrane pressure (TMP) of 15 psi with no imposed permeate flux control and crossflow flux of 7 LPM/$m^2$.

10 X scale-up step and diafiltration were performed using a KrosFlo® KR2i TFF System, 10 L of clarified AAV-HSC product at a titer of approximately 109 vg/mL and a 0.1 $m^2$, 100 kD L-Screen HyStream Cassette (P/N XP100L01L) 20 X concentration. The final 0.5 L was diafiltered with constant volume against 10 diavolumes.

## Scale-up scenarios

Based on data from the 10 L process, operating parameters for 10X concentration at 50 L and 200 L scales were developed (Table 2). A processing time of approximately 2 hours was set as a requirement and therefore time was entered as a constant during calculations. During the 10 L experiment, normal flow filterability of the feed material was evaluated at multiple points during and at the conclusion of diafiltration (data not shown). Diafiltration did not improve the 0.2 μm filterability of the concentrated AAV-HSC product. Because diafiltration at the 10 L scale did not demonstrate process improvement, scaleup scenarios were limited to concentration only.

**Table 2.** Scale-up scenarios

| | Batch volumes (L) | | |
|---|---|---|---|
| | 10 | 50 | 200 |
| Concentration factor | 20 | 10 | 10 |
| Diafiltration volumes | 10 | 0 | 0 |
| Average flux (LMH) | 50 | 65 | 65 |
| Time required (hr) | 2.5 | 2 | 2 |
| Permeate volume concentration (L) | 9.5 | 45 | 100 |
| Permeate volume diafiltration (L) | 5 | 0 | 0 |
| Required area ($m^2$) | 0.1 | 0.35 | 1.4 |
| Load $L/m^2$ | 86.2 | 144.4 | 144.4 |

10 X concentration of 50 L with no diafiltration in 2 hours was determined to require four 0.1 $m^2$ 100 kD L-Screen SIUS® Cassettes, resulting in a loading of 125 $L/m^2$. Analogously, 10 X concentration of 200 L with no diafiltration was determined to require a single 1.5 $m^2$ SIUS® Cassette with a loading of 133 $L/m^2$.

Equivalent performance of the non-irradiated and gamma irradiated TFF products was demonstrated with a side-by-side split batch of clarified AAV-HSC harvest using a HyStream membrane, 100 kD 0.5 $m^2$ cassette (P/N XP100G05L) and a HyStream, 100 kD 0.5 $m^2$ SIUS® Gamma Devices (HyStream membrane, 100 kD, 0.5 $m^2$) (PN:XP100L05L).

Yield equivalence between the SIUS® Cassette and SIUS® Gamma Device based processes was measured using orthogonal analytical methods. Digital droplet PCR (ddPCR) was used to quantitate viral genomic targets. ELISA was used to quantitate a viral capsid using a protein target.

## Results

### Membrane and configuration screening

The HyStream 100 kD L-Screen cassette flux averaged 49.5 LMH (Liter/$m^2$/hour) during a deviation free run. The HyStream 300 kD L-Screen cassette averaged 50 LMH flux but exhibited signs of fouling toward the end of the run. The open (J) channel HyStream 300 kD required constant manipulation of crossflow rate and permeate flux as a result of significant fouling and polarization and no stable operating condition could be found. Based on these results, the HyStream 100 kD SIUS® Cassette was selected for the AAV-HSC concentration/diafiltration process. The yield was 85% following the 20 X concentration with no product observed in the permeate.

### Flux optimization through excursion

Flux excursions were performed with the 100 kD membrane to determine the optimal TMP for maximum permeate flux without causing excessive membrane polarization or fouling (Figure 3). An operating TMP of 15 - 20 psi and a crossflow flux rate (CFF) of 7.0 LPM/$m^2$ were found to be optimal, providing an initial permeate flux of 100 - 120 LMH.

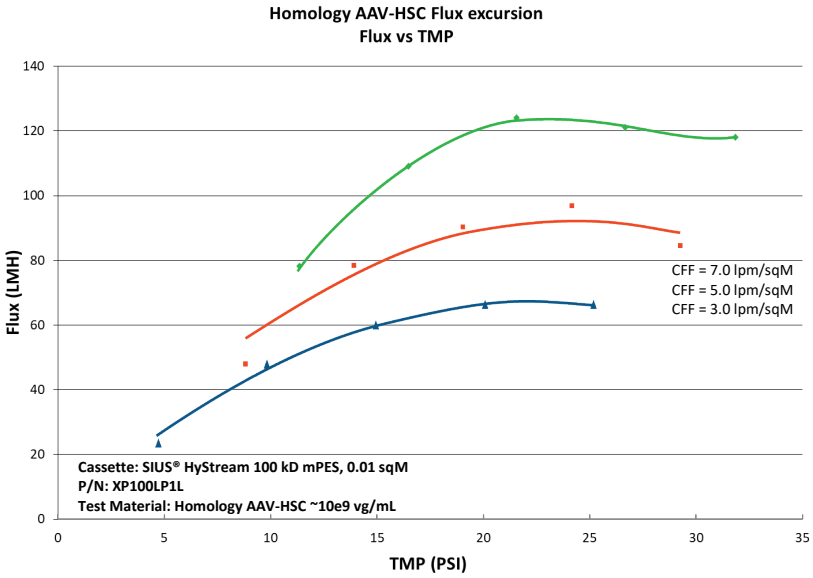


**Figure 3.** Flux excursions curves determined the optimal TMP and CFF conditions.

## Concentration and diafiltration scale-up

As a 10X scale-up from 1L confirmation experiment, 10 L of AAV-HSC starting material was concentrated 20 X - 0.5 L followed by a 10 DV constant volume buffer exchange. During the 20X concentration from 1.0 x $10^{10}$ vg/mL to 2.0 x $10^{11}$ vg/mL, flux started at approximately 90 LMH and decreased to 40 LMH as the concentration factor approached 10 - 20 (Figure 4). The expected decreasing flux trend stems from the increasing viscosity and/or increasing density of the polarization layer with increasing concentration. Trendline extrapolation of the plot of flux versus concentration provides the theoretical value of $C_G$, the gel layer constant. $C_G$ enables calculation of the concentration at which to perform a diafiltration buffer exchange ($C_{DV}$), which balances process time and expended buffer volume.

$$C_{DV} = C_G / e$$
$$= C_G / 2.72$$

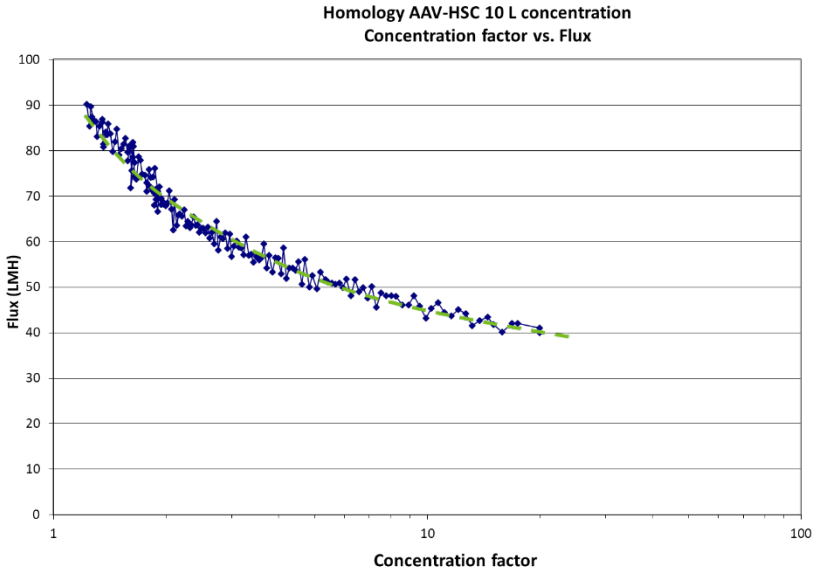


**Figure 4**. Plot of concentration versus flux during a 20 X concentration of 10 L - 0.5 L using a 0.1 $m^2$ TangenX® SIUS® Cassette.

Using the extrapolated trendline from the plot of flux versus concentration factor grow, $C_{DV}$ was determined to be significantly greater than 100X, indicating that diafiltration can be performed after completion of the 20X concentration step. Plotting flux vs. time for both the concentration and diafiltration steps enables a visual analysis of flux over the entire unit operation (Figure 5). Flux at the end of the concentration step reached 40 LMH and then slightly increased to 50 LMH at the onset of diafiltration. Flux then decreased slowly over the course of diafiltration, returning to 40 LMH at the end of the process. Gaps in the plot of flux vs concentration indicate sampling from the product reservoir for off-line analysis. The complete process resulted in an average process flux of 50 LMH with completion in 2.2 hours.

To demonstrate stability and control during the concentration and diafiltration processes, TMP and feed channel pressure drop were monitored throughout the course of the process (Figure 6). Feed channel pressure drop measures resistance to flow through the cassette. TMP drives flow through the membrane and retained mass to the surface of the membrane. Both TMP and the pressure drop, remained fairly constant throughout the entire process.

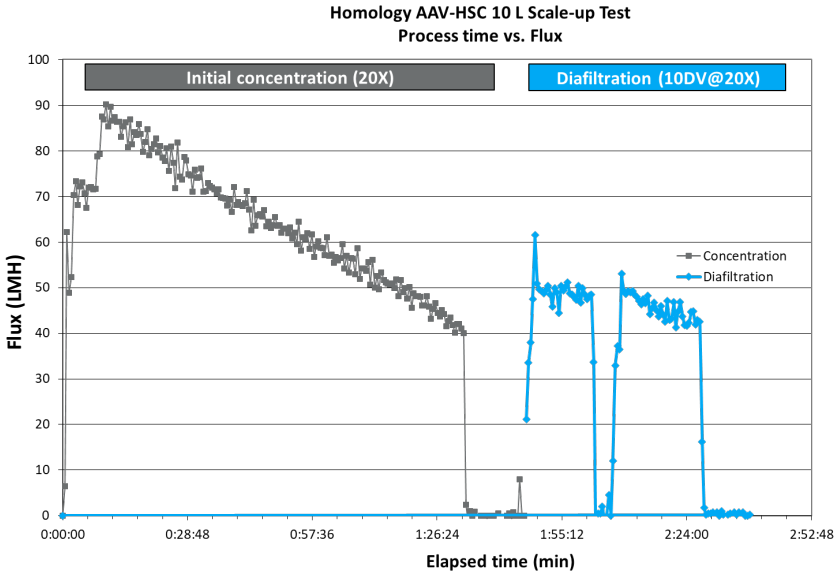


**Figure 5**. Flux tails from 90 - 40 LMH during concentration and then remains steady at 50 LMH during diafiltration. Gaps in recording indicate sampling from the product reservoir for off-line analysis.

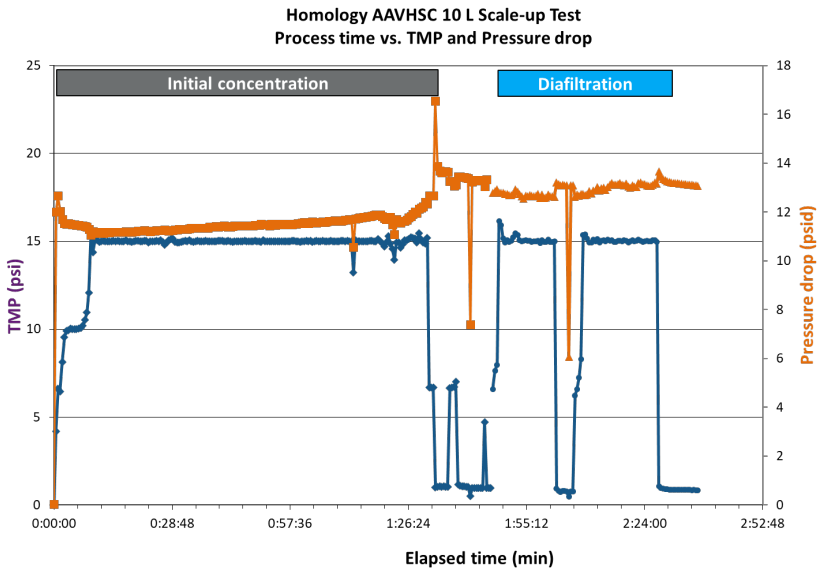


**Figure 6.** Plot of TMP versus elapsed time indicates robustness towards fouling.

## Equivalence of non-irradiated and Gamma-irradiated TFF devices

Both the non-irradiated SIUS® Cassette and the irradiated SIUS® Gamma Device successfully completed a 50X concentration of 70 L with equivalent performance. Both processes concentrated 70 L of clarified AAV-HSC product 50 X to 1.5 L at constant TMP (10 ± 2 psi) and CFF (7.0 L/min/$m^2$) (Figure 7). With equivalent values and trends for flux, TMP, and $C_G$, no adjustment of operating conditions was required to transfer the concentration process from the non-irradiated SIUS® Cassette to the irradiated SIUS® Gamma Device.

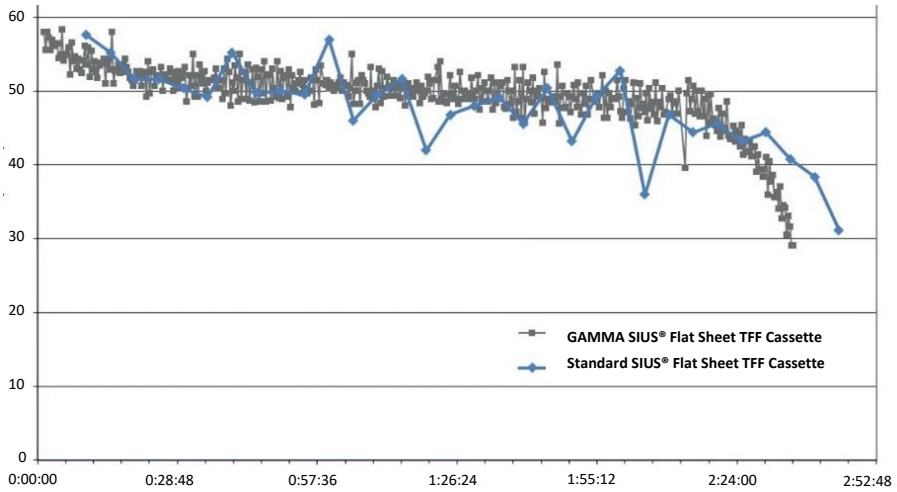


**Figure 7.** 50 X concentration of non-irradiated TangenX® SIUS® Cassette and the irradiated TangenX® SIUS® Gamma Devices.

Recovery values for viral genomes and total capsids with the SIUS® Cassette reached 75% and 88% respectively (Figure 8). Recovery values for viral genomes and total capsids with the SIUS® Gamma Cassette were highly comparable with values of 78% and > 100% respectively.

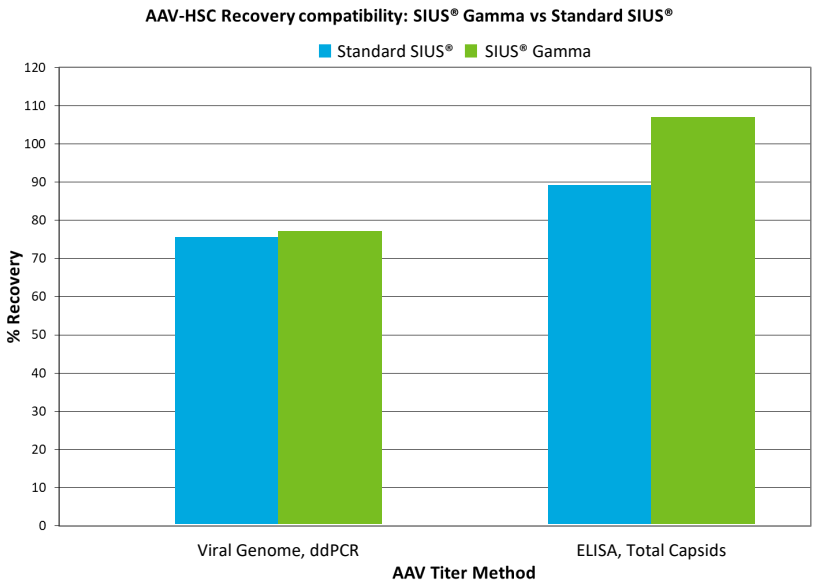


**Figure 8.** Comparison of recovery during concentration using a SIUS® Cassette and SIUS® Gamma Device.

## Discussion and conclusions

Ultrafiltration/diafiltration represents a key unit operation during the manufacturing of AAV particles, such as AAV-HSC, that may be optimized for productivity gains using new technologies. This study evaluates the closed SIUS® Gamma Device, motivated by the potential of accessing the established SIUS® single-use membrane efficiency gains in a format that also mitigates risk to the product, environment and operator. The scope of the study included 100 kD and 300 kD screening studies for membrane MWCO selection, flux excursion experiments for process optimization and scale-up from 0.1 L - 70 L.

Screening the 100 kD L-Screen, 300 kD L-Screen and 300 kD J-Screen HyStream configurations indicated that the 100 kD L-Screen device was the most appropriate, producing an average, constant flux of 50 LMH with negligible fouling.

Excursion experiments determined optimal conditions of 15 - 20 psi TMP and 7.0 LPM/$m^2$ CFF that would deliver 100 - 120 LMH with minimal generation of a gel layer. Scale-up of these conditions to 10 L with a 0.1 $m^2$ filter was accomplished in a straightforward manner with maintenance of the 40 - 50 LMH and robustness towards membrane fouling.

With successful scale-up of concentration and diafiltration to 10 L using the SIUS® Cassette, a 20X concentration from 70 L - 1.5 L was repeated to directly compare the SIUS® Cassette and SIUS® Gamma Device. Flux, TMP and time, and recovery were found to be equivalent, indicating irradiation does not affect membrane performance. The ddPCR method, which quantitates viral genomic copies, agreed very closely between SIUS® Cassette and SIUS® Gamma Device with results between 75 - 80%. The ELISA method, which quantitates viral coat protein concentration, showed more variance, but still held values greater than 75% for both SIUS® formats. ELISA methods frequently have larger variance, especially with large and more complex analytes, such as viruses, and therefore the conclusion was made that yield for the SIUS® Cassette and SIUS® Gamma Device runs were equivalent with values of approximately 75 - 85%.

Implementing closed systems can be challenging due to vendor restrictions. Some vendors require purchase of the complete flow path with purchase of the filtration cassette. Other vendors have developed proprietary connectors, which may function well, but can complicate execution if a

multiple connector types are used. The SIUS® Gamma Device is the first example of a closed and irradiated TFF product that remains “open” for connectivity. The ability to modularly integrate flow paths with the closed SIUS® Gamma Device using non-proprietary connectors provides an unprecedented combination of single-use process efficiencies, contamination risk mitigation and system design flexibility.

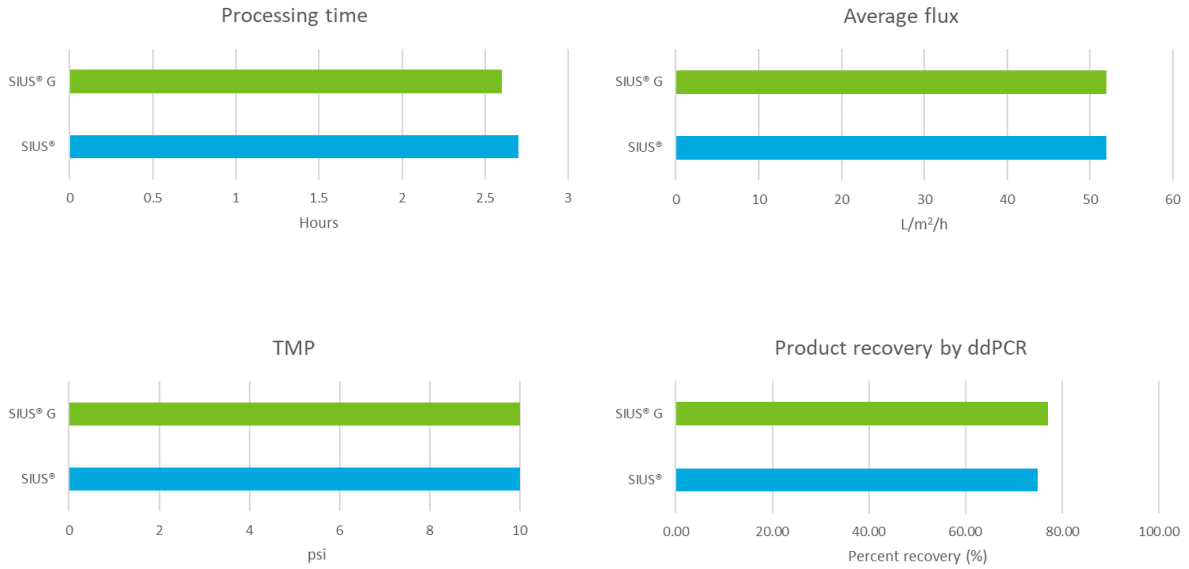


**Figure 9.** Equivalency of TangenX® SIUS® Gamma Device and SIUS® Cassettes. Process parameters for average flux, processing time, TMP and product recovery were highly comparable for the batch split processing using SIUS® and SIUS® Gamma configurations.

In conclusion, closed and irradiated technologies play an important role in the biotherapeutic trend towards multi-product facilities with flexible scales while also maintaining strict quality standards. The TangenX® SIUS® Gamma Device was shown to perform equivalently to the SIUS® Single-use TFF Cassette in terms of flux, TMP scalability and recovery for AAV viral vector manufacturing (Figure 9). In addition to performance, its connectivity options play a crucial role with regards to the logistics of implementation. The simplicity of three connections with negligible contamination risk helps reduce contamination risk significantly. Use of closed and irradiated devices does incur increased cost on a per run basis, but those costs need to be weighed against the cost savings of reduced deviations, overall facility utilization and multi-product enablement. That cost-benefit and risk assessment is unique to each group, their facility and business goals.