

Sterile Filtration of Small AAV solutions

Abstract

Adeno-associated virus (AAV) vectors are central to gene therapy, yet their instability under conventional sterilization necessitates sterile filtration to ensure product integrity. We systematically evaluated four 0.22 μm syringe filters differing in diameter (15 mm vs. 25 mm) and membrane chemistries (polyethersulfone vs. regenerated cellulose) to maximize AAV recovery and minimize hold-up volume. Across replicate filtrations, 15 mm devices exhibited markedly lower volume loss than the 25 mm devices. Quantification of AAV particles demonstrated the highest recovery for the 15 mm PES filter at 96.3%, compared with 86.5% (25 mm PES), 88.7% (15 mm RC), and 84.6% (25 mm RC). To ensure highest particle recovery, purging the filter with air after filtration is highly recommended. These data identify the Minisart® 15 mm PES syringe filter as the optimal choice for sterile filtration of AAV solutions. Based on these findings, we recommend using the Minisart® PES15 (1776D-----ACK), which showed the best performance in terms of volume loss as well as AAV8 particle recovery.

- Tested different syringe filter for sterile filtration of AAV8
- Successful filtration with all tested devices
- Exceeding bubble point of membranes ensures low volume loss (see How To section below)
- Highest recovery rates for Minisart® PES15 (1776D-----ACK)



Figure 1: Minisart® PES15 (1776D-----ACK) showed the best results in terms of viral particle recovery in combination with volume loss during AAV8 sterile filtration.

Introduction

Adeno-associated virus (AAV) vectors are a cornerstone of gene therapy, valued for their safety, efficiency, and durable expression. Because oral delivery is impractical for these viral vectors, AAV products are typically administered by injection, making sterile preparation mandatory for in vivo studies, clinical use, and cell-based assays. Conventional terminal sterilization (heat or irradiation) is incompatible with AAV stability, so sterilizing-grade filtration is the method of choice. Across major regulatory frameworks—the FDA Guidance for Aseptic Processing (2004), EU GMP Annex 1 (rev. 2023), and Chinese GMP/Chinese Pharmacopoeia—sterile injectable products are expected to be filtered through $\leq 0.22 \mu\text{m}$ membranes with validated processes, including pre- and post-use filter integrity testing, demonstrated microbial retention such as *Brevundimonas diminuta*, and control of endotoxins, particulate matter, and potential extractables/leachables.

Practically, for sterile filtration of AAV solutions the challenge is to maximize vector recovery while ensuring sterility. Although AAV capsids ($\sim 20\text{--}25 \text{ nm}$) easily pass $0.22 \mu\text{m}$ pores, they can be lost to adsorption or leftover volume within the filtration device. Membrane chemistries like polyethersulfone (PES) and regenerated cellulose (RC), device geometry and operating conditions like flux and pressure can influence yield and throughput. Syringe filters are widely used at lab scale for formulation and fill-finish simulation; PES and RC are considered low-binding but differ in surface properties.

This application note evaluates four different $0.22 \mu\text{m}$ syringe filters—two diameters (15 and 25 mm) and two membrane chemistries (PES and RC)—to quantify AAV recovery and practical performance. Our goal is to provide data-driven guidance on filter selection and operating practices that preserve high AAV recoveries while meeting sterile filtration expectations.

How To: Minimize volume loss during sterile filtration

To recover residual liquid (“hold-up volume”) from a syringe filter after filtration, purge the wetted membrane with air. Proceed as follows:

- Perform the sterile filtration of your liquid using a Luer-lock syringe and the syringe filter as usual.
- Disconnect the used syringe from the filter.
- With the same sterile syringe, draw in air, then reconnect the syringe to the used filter.

- Slowly depress the plunger to push air through the filter. For sterile filtration applications: Do not apply more pressure than specified in the Bubble Point Value of the Minisart. Otherwise, the integrity of the filter cannot be secured.

Recommendations and notes:

- For 15 mm and 25 mm Minisart® syringe filters, applying approximately 5 mL of air is typically sufficient to expel the residual volume.
- Press the plunger steadily, not abruptly. Ensure a secure Luer-lock connection.

Results

To evaluate the performance of different syringe filters for the sterile filtration of an AAV solution, four different syringe filters were tested. Besides two different membrane chemistries (PES vs. RC), also two different membrane diameters 15 mm and 25 mm were compared.

First the volume loss during the filtration process of 3.35 mL purified AAV8 solution was determined and is shown in Figure 2. Irrespective of the membrane, the smaller 15 mm syringe filter devices showed a volume loss below $< 50 \mu\text{L}$, respectively. For all four syringe filters tested, the volume lost to the 5 mL Luer-lock syringe was between $39.7 \mu\text{L}$ and $23.3 \mu\text{L}$. Relative to the total amount of 3,35 mL filtered volume, the leftover volume within the syringe was below 1 %. For both 25 mm filters, we could show that the leftover volume within the syringe filter is $\sim 2.6\times$ higher compared to those of the smaller 15 mm diameter devices, irrespective of the membrane chemistry. The overall volume loss for the two 15 mm syringe filters after syringe driven filtration lay between 2.2% and 2.1% for the 15 mm PES and the 15 mm RC filters, respectively. Viral quantification analytics using the Octet® BLI system equipped with AAVX Biosensors allowed to determine the viral particle titer (vp/mL) before and after the sterile filtration with the four different syringe filter devices. Figure 3 shows that the absolute AAV titer before filtration was $1.20\text{E}+12 \text{ vp/mL}$. After sterile filtration of 3.2 mL of the respective AAV solution, the 15 mm PES syringe filter showed the highest particle titer with $1.18\text{E}+12 \text{ vp/mL}$. Also, for the other three tested syringe filters, high virus particle titers of $1.09\text{E}+12 \text{ vp/mL}$, $1.09\text{E}+12 \text{ vp/mL}$, and $1.07\text{E}+12 \text{ vp/mL}$ were found for the 25 mm PES, 15 mm RC and 25 mm RC filters, respectively.

To determine the relative particle recovery for the AAV8 particles filtered through the different syringe filter devices, the absolute filtrate volume (mL) was multiplied with the measured viral capsid titer (vp/mL) and compared with the total amount of loaded viral particles. Figure 4 shows that the 15 mm PES filter shows the highest AAC particle recovery of 96.3 %. In other words, only 3.7 % of the viral particles were lost during the filtration process. For the other three syringe filters tested, AAV particle recoveries of 86.5%, 88.7% and 84.6% were found for the 25 mm PES, 15 mm RC and 25 mm RC syringe filters, respectively.

We therefore can conclude that all the tested syringe filter devices showed a high recovery of AAV virus particles during syringe driven sterile filtration. Out of the four tested different devices, we can recommend using the Minisart® 15 mm PES syringe filter for sterile filtration of AAV solutions. To ensure a maximum recovery and lowest possible volume loss, it is recommended to overcome the bubble point of the membrane by applying at least 5 mL of air using a Luer-lock syringe (also see section “How To: Minimize volume loss during sterile filtration”).

Virus Particle Titer After Filtration

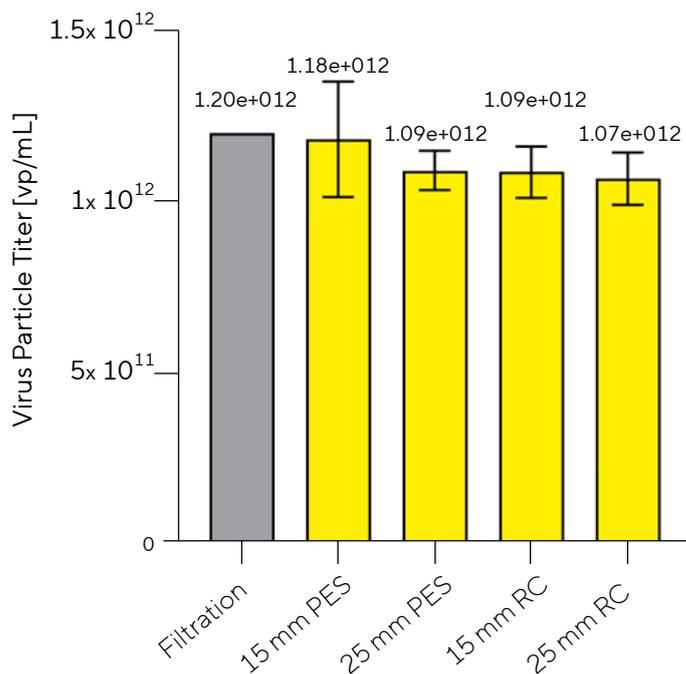


Figure 3: Absolute viral particle titer before and after filtration. The grey bar represents the viral particle titer (vp/mL) of the AAV solution before sterile filtration was applied. The yellow bars show the viral particle after filtration with the four respective syringe filters. Error bars represent the standard deviation of n=3 filtrations.

Absolute Volume Loss During Filtration

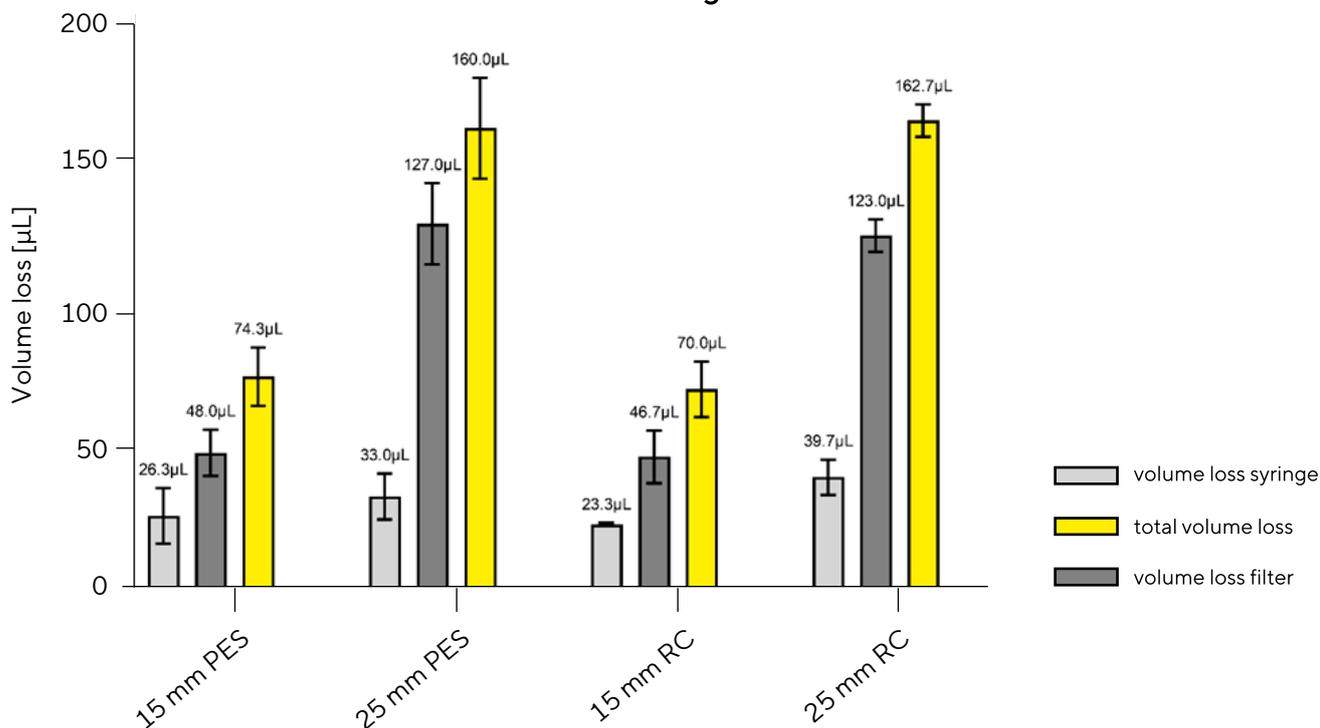


Figure 2: Absolute volume loss during filtration process. For all filtrations a total volume of 3.4 mL was applied to the syringe filters of 15 mm and 25 mm diameter, respectively. The lost volume was determined by determining the weight of the filters as well as the used 5 mL Luer-lock syringes before and after the filtration. The light grey bars show the volume loss to the syringe; the dark grey bars show the volume loss to the used syringe filters. The yellow bars represent the overall volume loss and are the sum of the light and dark grey bars. Error bars represent the standard deviation of n=3 filtrations.

AAV Recovery After Filtration

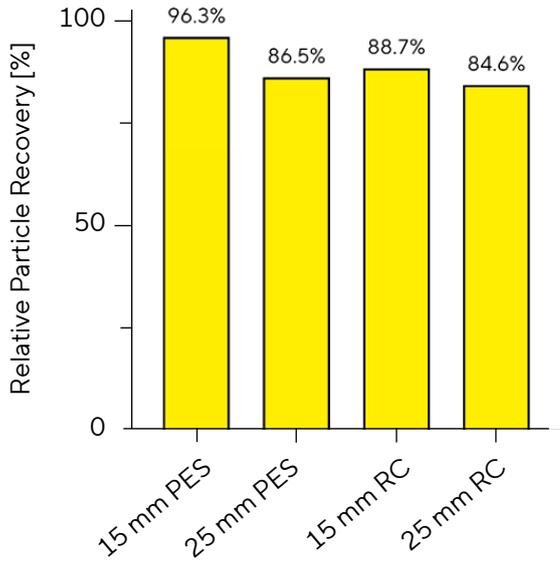


Figure 4: Relative particle recovery after sterile filtration. The yellow bars represent the relative viral particle recovery after sterile filtration with four respective syringe filters. Numbers represent the recovery of the sterile filtrate and are relative to the total amount of applied viral particles.

Conclusion

In this application note we could show that our Minisart® PES15 syringe filter is the best option for sterile filtration of AAV containing liquid solutions. In an experimental approach we could show that the use of a PES membrane for sterile filtration results in a lower particle loss compared to those of a RC membrane. Furthermore, we could show that the use of a smaller diameter (15 mm) of the syringe filters significantly lowers the particles loss during the filtration process. To minimize sample loss due to residual liquid within the filtration device it is highly recommended to purge the filter by applying air, overcoming the so-called bubble point of the respective filter.

Based on these findings we can highly recommend the Minisart® PES15 syringe filter (1776D-----ACK) as the optimal product for sterile filtration low-volume AAV solutions. Minisart® PES25 and Minisart® RC25 are more applicable to slightly larger-volume filtration of more than 10 ml when throughput is more important than residual liquid within the filtration device.

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