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Application Note

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Sartoclear® Depth Filters for the Clarification of AAV

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Abstract

Depth filtration is a critical step in the production of cell and gene therapies, particularly for the clarification of adeno-associated virus (AAV) vectors.

In this application note, we evaluated the performance of Sartoclear® depth filters in the clarification of AAV serotype 8 (AAV8) products from HEK293 cell cultures. Small-scale experiments demonstrated the filters' high performance in terms of capacity, protection of subsequent sterilizing filters, and product recovery. Process-scale performance was also assessed, showing that the Sartoclear® DL75 filters provide robust and scalable clarification, maintaining high product recovery, even with a more challenging feed material.

The results of this study confirm the suitability of Sartoclear® filters for suspension-based AAV processes and their competitive performance, with implications for improving patient access to cell and gene therapies.

Introduction

Depth filtration is an established unit operation used in the bioprocessing industry for the clarification of bioreactor offload, especially in mammalian batch- or fed-batch processes. The introduction of multilayered single-use depth filter systems has met the industry's need for a flexible solution that addresses the clarification task of fed-batch processes with increased cell densities from bench to 1,000 L scales. Single-use depth filters also facilitate key process requirements like achieving high product recovery and ensuring the integrity of subsequent microfilters and sterilizing grade filters. A two-stage depth filtration with multi-layered depth filters of decreasing retention rate in-line with a sterilizing grade filtration is nowadays a standard approach in monoclonal antibody (mAb) processes.

Depth filters act via a dual filtration mechanism: Physical retention of particles on the surface and within the filter matrix, and adsorptive retention of especially finer particles and sub-micron colloids. The latter mechanism is primarily determined by the materials and chemistry used to make the depth filters. The clarification step in biopharmaceutical processes is dominated by depth filters made of fibrillated cellulosic fibers, an inorganic filter aid such as diatomaceous earth (DE) or perlite, and a binder providing wet strength to the filter matrix. The adsorptive capacity is mainly determined by the exact type of filter aid and the amount and type of binder used.

Sartoclear® filters are cellulose- and DE-based depth filters from Sartorius. The portfolio includes a range of double-layered depth filter grades covering coarse filtration for cell and cell debris removal, to fine filtration and protection of microfiltration and sterilizing grade filtration steps. Filter sizes are available for applications ranging from bench- to manufacturing-scale processes.

Depth filtration with Sartoclear®-type depth filters has also become the favored technology for the clarification of lysed mammalian cell suspension feed material. This application is hugely important in the production of adeno-associated virus (AAV) processes which are the leading platform for delivering gene therapies. During AAV manufacturing, assembled viral vectors are released and subsequently purified from cells. Therefore, robust and scalable AAV clarification processes are critical to the timely development of cell and gene therapies, ultimately improving patient access to these next-generation therapies.

Based on preliminary data, we hypothesized that Sartoclear® depth filters could facilitate single-stage depth filtration of such material with high filtration capacity, sterilizing grade filter protection, and product recovery. To address this, we tested the performance of Sartoclear® depth filters DL75 and DL60. We further demonstrate the performance of Sartoclear® Cassettes at process scale and scalability from bench to process in this application. Here, we focus on AAV serotype 8 (AAV8). These experiments were performed at both small scale and large scale. Data for AAV2 and AAV9 are available upon request.

Materials and Methods

AAV Production

For the first test series, HEK293 suspension cells were cultivated in shake flasks. AAV production was achieved by triple transfection of plasmids encoding AAV serotype 8 (AAV8) proteins and GFP as the gene of interest. Cells were lysed three days after transfection.

For the second test series, pre-cultures were seeded and expanded in shake flasks and used to inoculate a 50 L stirred-tank bioreactor. AAV production was initiated by triple transfection of plasmids encoding for AAV8 proteins and GFP as the gene of interest. Cells were lysed three days after transfection.

On the day of harvest, cells were lysed using 1% Tween 20, 50 mM Tris pH 8.0, 50 U/mL Benzonase, and 2 mM $MgCl_2$ and incubated at 37 °C and with agitation for 1.5 hours (h). Then, 500 mM NaCl was added, and the material was further incubated for 0.5 h.

Filtration

Filtration experiments were carried out by first using a depth filter, Sartoclear®, followed by a sterilizing grade filter, Sartopore® 2 XLG.

Filtrations were carried out with peristaltic pump | tubing systems accommodated to match the required ranges of flow rates. The systems were equipped with one or two in-line single-use pressure sensors.

All filters were pre-flushed according to Sartorius' recommendations, with conditioning buffer matching the lysate conditions. Depth filtrations were executed with a flux of 200 L/m²/h [LMH] unless stated otherwise. All small-scale runs were stopped at 1 bar (blockage) or a predetermined maximum load as stated to facilitate the required throughput of experiments.

Filter loads | capacities are always reported in L/m² referring to the frontal area of the filter used unless stated otherwise.

The filters used in this study are presented in Table 1.

Analytics

All analytics were performed using internal protocols based on the manufacturers' recommendations using the following materials and systems:

- Cell concentration and viability — Cedex® HiRes analyzer
- Total AAV capsid concentration — ELISA (Progen)
- ds-DNA concentration — Picogreen (Thermo Fisher)
- HCP concentration — ELISA (Cygnus)
- Total protein concentration — BCA (Thermo Fisher)
- Turbidity: Hach turbidimeter

Table 1: *Summary of the Depth Filters and Sterilizing Grade Filter Used in This Study*

Filter	Retention Rating [µm]	Filtration area [cm²]	Construction	Filter Components	Order Codes
Small-Scale Experiment					
Sartoclear® DL60	8 | 0.8	25	2 Layers (depth filters)	Cellulose & DE	29CDL60-CACHH--M
Sartoclear® DL75	10 | 2	25	2 Layers (depth filters)	Cellulose & DE	29CDL75-CACHH--M
Sartopore® 2 XLG	0.8 | 0.2	4.5	2 Layers (membranes)	Polyethersulfone	5445307GV--LX--C
Large-Scale Experiment					
Sartoclear® DL75	10 | 2	2,000	2 Layers (depth filters)	Cellulose & DE	29XDL75-FCA
Sartopore® 2 XLG	0.8 | 0.2	1,300	2 Layers (membranes)	Polyethersulfone	5445307G8--FF--A

Results and Discussion

Small-Scale Experiments

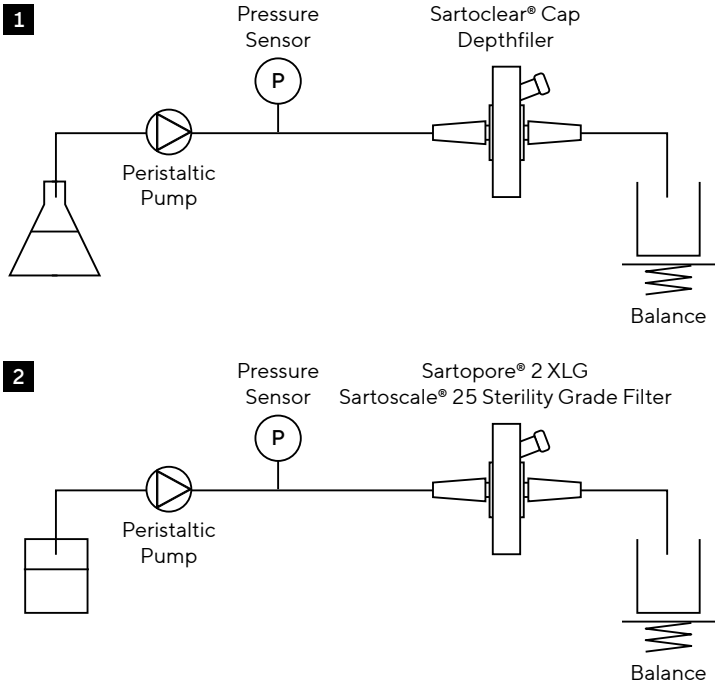
In the first experimental setup, we assessed the performance of Sartoclear® in the clarification of HEK293 suspension cells producing AAV8. The cell culture material was generated in two production runs by orbital shaking incubation. Upon harvest, we observed a range of 3.9 – 4.2 million cells/mL for the total cell density (viable and non-viable cells), 204 – 236 NTU for the feed turbidity, cell viabilities of 68% – 73%, and titers of 0.8×10^{12} – 1.1×10^{12} virus particles (VP)/mL. Figure 1 shows the high consistency of the feed material used in the tests.

Figure 1: *Small-Scale Experiment Setup*

Trial Part 1:

- Small scale (Sartoclear® Caps, Sartopore® 2 XLG Sartoscale® 25)
- Splitted into two parts

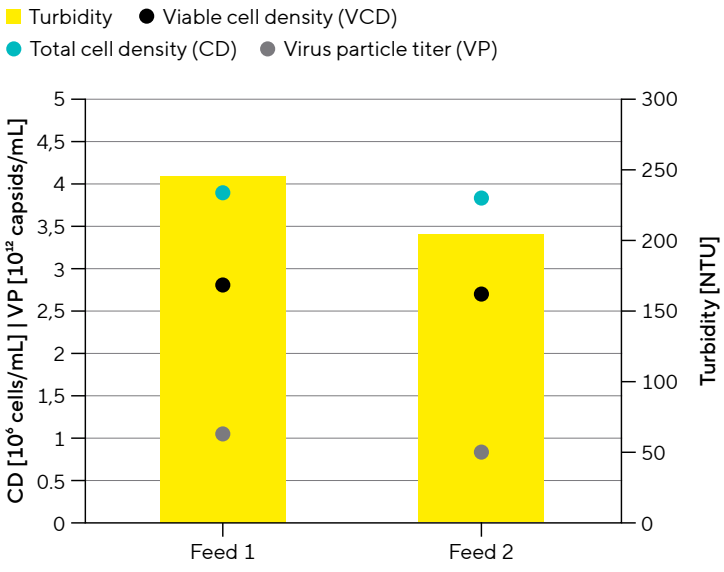
1. Depth Filtration (Harvested | Lysed AAV batch from shake flasks)
2. Sterile Filtration (Clarified AAV material from 1st clarification step)



We then tested the filtration performance of Sartoclear® depth filters at constant flux conditions of 200 LMH. As stop criteria, we used a maximum inlet pressure of 1 bar for the given flow rate (filter blockage) or a maximum filter load of 400 L/m², which we expected to be sufficient to achieve filter blockage for both filter types tested. In one DL75 run, the filtration had to be stopped before reaching 1 bar.

Using the filtrate from the depth filtration steps, we evaluated the filtrate quality and the protection of a subsequent sterilizing grade filtration step in tests with Sartopore® 2 XLG filters in the Sartoscale 25 format. For some runs, the filtrations were extended beyond 500 L/m² before reaching 1 bar.

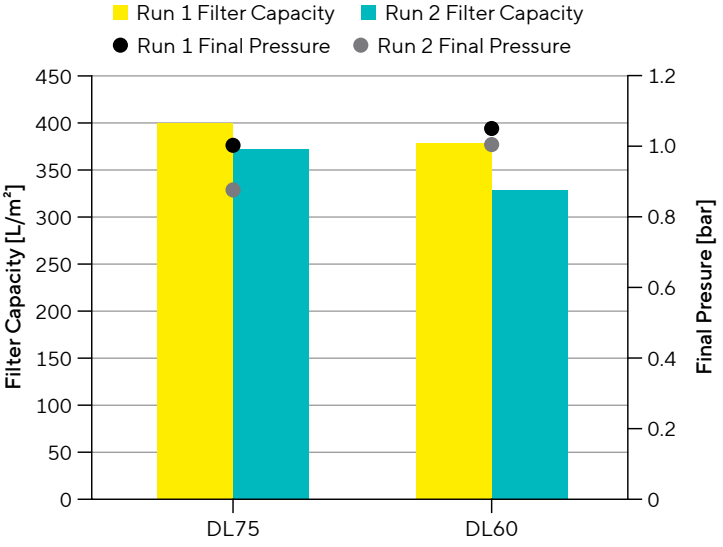
Figure 2: *Analysis of AAV8 Feed Material*



Note. The material was produced in two production runs.

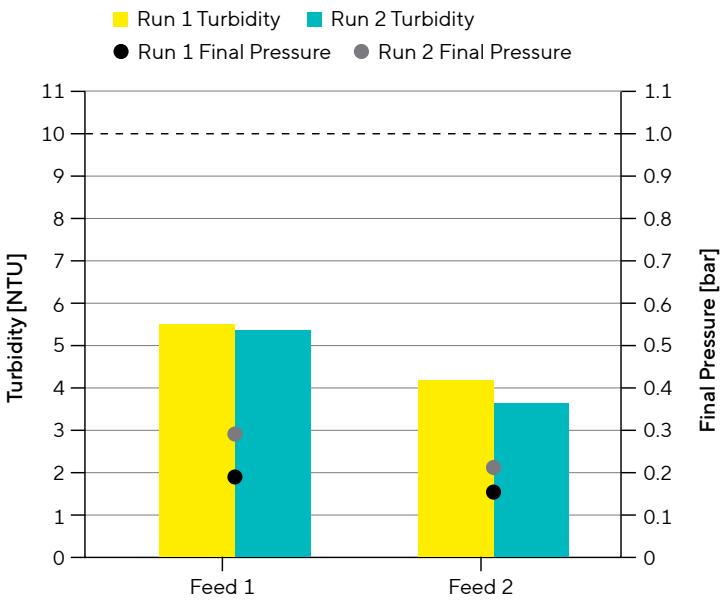
Figure 2 shows both Sartoclear® depth filter types tested reached close to the pre-set maximum expected filtration capacity of 400 L/m² for AAV8. In one of the first runs, the Sartoclear® DL75 filter was stopped at 400 L/m² at 0.88 bar, indicating, the potential for an even higher filtration capacity.

Figure 3: *Depth Filter Capacity | Final Pressure*



Note. The bars show depth filter capacity (primary axis) and the dots show the final pressure (secondary axis) when the filtration was stopped. The depth filtration tests were halted when the capacity reached 400 L/m², or if the pressure reached 1 bar. For both filter types, runs were executed in duplicate.

Figure 4: *Filtrate Turbidity | Sterilizing Grade Filter Protection*



Note. The bars show turbidity after depth filtration (primary axis) and the dots show the final pressure of the Sartopore® 2 XLG sterilizing grade filtration tests (secondary axis) when the filtration was stopped. The membrane filtration tests were halted when the capacity reached 500 L/m², or if the pressure reached 1 bar (indicated by a dotted line). Notably, the pressure threshold of 1 bar was not met during the tests, although the capacity of 500 L/m² was slightly surpassed. For both filter types, runs were executed in duplicate.

The Sartoclear® DL60 filter shows a slightly lower filtration capacity compared to the Sartoclear® DL75 as expected from its lower nominal retention rate. Both filter types provide excellent filtrate turbidity below 10 NTU. Here, the DL60 filtrates show a slightly lower filtrate turbidity, which again is consistent with the lower nominal retention rate.

As expected from the very low turbidity values after depth filtration, the protection of the Sartopore® 2 XLG sterilizing grade filter is excellent (see Figure 3). In each run the sterilizing grade filter reached the target filtration capacity of higher than 500 L/m² (range: 540 – 584 L/m²) at very low pressure profiles. The final pressures are in the range of 0.2 – 0.3 bar, promising very high filtration capacities before filter blockage. The DL60 filter shows a slightly better protective effect on the sterilizing grade filter.

In summary, both Sartoclear® depth filter types, in combination with the Sartopore® 2 XLG sterilizing grade filter, demonstrate very high filtration performance in the clarification of the AAV8 feed material.

Table 2 shows that all tested depth filters consistently show a high yield of the AAV target product in the range of 80% – 100% for AAV8 and absolute titer. Taking into account the accuracy of the ELISA test method used, no relevant loss of virus particles can be detected. The mean recovery and total protein is in the range of ~80% – 100% for all filtrates, and that of total DNA is in the range of ~70% – 80%. No trend for a particular filter type can be observed. Overall, a low depletion of total DNA and total protein is achieved by clarification filtration under the selected test conditions. The analytical results are listed in Table 2.

Following depth filtration with both Sartoclear® DL60 and DL75 filters, sterilizing grade filtration achieved very high viral titer recoveries. Taking into account the accuracy of the ELISA test, it can be concluded that there is no significant loss of the virus product.

The Sartoclear® DL75 filter shows the highest performance; the Sartoclear® DL60 filter offers a slightly lower filtration capacity in comparison, but even better protection of the following membrane filter.

Table 2: *Summary of Analytical Results for Depth Filtration and Sterilizing Grade Filtration*

Feed	Filter	Depth Filtration (1. Step)					Sterile Filtration (2. Step)		
		Virus Particles Pre-Filtration [VP/mL]	Virus Particles Post-Filtration [VP/mL]	VP Recovery [%]	DNA Removal [%]	Protein Removal [%]	VP Pre-Filtration [VP/mL]	VP Post-Filtration [VP/mL]	VP Recovery [%]
Feed 1	DL75	10.3×10^{11}	8.7×10^{11}	84	25	-15	8.7×10^{11}	8.0×10^{11}	92
Feed 2	DL75	8.4×10^{11}	7.3×10^{11}	87	29	22	7.3×10^{11}	6.9×10^{11}	94
Feed 1	DL60	10.3×10^{11}	1.2×10^{12}	117	20	0	1.2×10^{12}	7.5×10^{11}	63
Feed 2	DL60	8.4×10^{11}	7.8×10^{11}	93	31	24	7.8×10^{11}	7.2×10^{11}	93

Process-Scale Performance

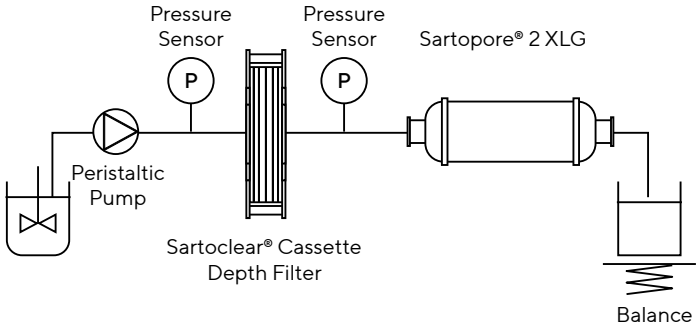
We then wanted to answer the question of how Sartoclear®-based clarification filtration strategy works at process scale.

For this purpose, we produced AAV8 viruses in HEK293 suspension cells by transient transfection on a 50 L scale in a stirred-tank bioreactor and carried out a filtration experiment analogous to the first test series after cell lysis. The total cell concentration on the day of harvest was 5.4 million cells/mL and cell viability 83%. The AAV8 virus particle titer of 1.2×10^{12} vg/mL is in the upper range of the titers obtained in the first series of tests. The lysate produced has a turbidity of 345 NTU. This means that the feed material used for this series of tests has ~50% higher biomass and turbidity values. The DNA and protein concentration is about twice as high compared to the first series of tests. Therefore, this feed material is a significantly higher challenge for clarification and membrane filtration.

Figure 5: *Process-Scale Experiment Setup*

Trial Part 2:

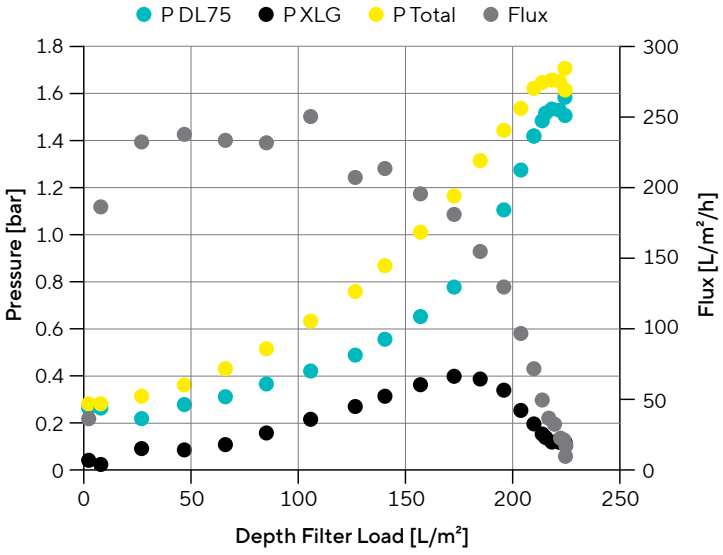
- Large scale (Sartoclear® Cassette, Sartopore® 2 XLG Midicaps®)
- Depth Filtration + Sterile Filtration in one run
- Harvested | Lysed AAV batch from STR®
  (to show that the STR® was linked to the clarification filter directly)



To address the application of a Sartoclear®-based AAV clarification filtration at process scale, a filter train consisting of a 0.2 m² Sartoclear® DL75 cassette was used in series with a 0.13 m² Sartopore® 2 XLG size 8 Midicap. The hypothetical filtration capacity based on the data of the first series of tests is 80 L for the depth filter cassette and > 65 L for the membrane filter, which would be sufficient for complete filtration of the feed material. However, due to the previously expected higher biomass and turbidity of the available feed material, a lower filtration capacity was to be expected.

The initial flux in this experiment is 200 LMH relative to the depth filter. Filtration was performed with a peristaltic pump. Pressure sensors are positioned before the first and second filters. Figure 4 shows the filtration process.

Figure 6: *Scaled-up AAV8 Clarification With Sartoclear® DL75 0.2 m² Cassette*



The pressure curves of the Sartoclear® DL75 depth filter and the Sartopore® 2 XLG membrane filter increase in parallel up to a load of approximately 150 L/m², at which the pressure level of the Sartoclear® DL75 is about 0.2 bar higher than that of the Sartopore® 2 XLG. At approximately 150 L/m² the total pressure is over 0.8 bar, so that — due to the selected pump system — the effective flow rate decreases slowly at first, and then faster. Accordingly, the pressure before the second filter decreases again. The filtration is then stopped at the maximum possible total pressure of 1.7 bar. At this point, the pressure is almost entirely caused by the blocked Sartoclear® DL75 depth filter. Table 3 summarizes the analytical results of the filtration.

Table 3: *Analytical Results for Depth Filtration and Sterilizing Grade Filtration*

Initial Turbidity	Final Turbidity	Initial DNA [µg/mL]	Final DNA [µg/mL]	Initial Protein [mg/mL]	Final Protein [mg/mL]	Initial VP [VP/mL]	Final VP [VP/mL]
342	7.6	32.9 ± 4.1	17.9 ± 1.3	5.8 ± 1.0	4.9 ± 0.9	$(1 \pm 0.2) \times 10^{12}$	$(1.1 \pm 0.2) \times 10^{12}$

The turbidity of the filtrate is less than 10 NTU, and the recovery of AAV is again about 100%. The reduction of the protein concentration to approximately 80% is at a comparable level compared to the first test series. The reduction of the DNA concentration to 54% is slightly higher than in the first test series, but can be explained, for example, by a stronger retention due to the higher degree of blockage at the end of the filtration.

Overall, with this filter setup, a very good filtration capacity of 45 L (corresponding to 225 L/m²) and high product recovery can be achieved for this more challenging feed solution at process scale.

In order to obtain direct scale-up filtration data, we conducted parallel filtration tests with small-scale Sartoclear® DL75 Caps in series with Sartopore® XLG sterilizing grade filters in Sartoscale 25 format. The target flux is 130 LMH, the filtrations are stopped at 1 bar final pressure. The tests are carried out in triplicate. A part of the lysate described above from the 50 L STR cultivation is available as feed material in these tests, the filter trains reached a filtration capacity of 138 L/m² (data not shown), which is very comparable to process filtration at the same pressure level (158 L/m²). The slightly higher capacity on a process scale can also be explained by the more favorable filter-area ratio of depth filter to sterilizing grade filter (1.54 instead of 5.6).

In summary, this test demonstrates that the Sartoclear® DL75 depth filter shows a very good filtration performance with high product recovery at the process scale in the clarification filtration of process-relevant AAV8 feed material and that there is a very good scalability from the screening scale to process scale.

Conclusion

This study clearly demonstrates the suitability and competitiveness of Sartoclear® depth filters for the clarification step in suspension-based AAV processes.

The key takeaways from this application note are that Sartoclear® depth filters showed:

- High filtration capacity of 45 L per 0.2 m² Sartoclear® cassette (corresponding to 225 L/m²)
- High AAV8 recovery (over 80%)
- Scalability

The two Sartoclear® filter grades DL75 and DL60 used in this study show very similar performance, with DL75 having a slightly higher capacity and DL60 showing slightly better membrane filter protection.

Sartoclear® DL75 was selected for the scale-up experiment with a more challenging feed material, and the results demonstrated scalable performance from bench to process scale for AAV8 clarification, which was then followed by a sterilizing grade filtration using Sartopore® 2 XLG.

Small-scale tests have already been carried out for AAV2 and AAV9, and results are comparable and expected to transfer to large-scale formats. Small-scale data for AAV2 and AAV9 available upon request.

In conclusion, Sartoclear® depth filters are an excellent choice for clarification in AAV production processes, independent of serotype.

For more information about Sartoclear® Depth Filters, visit sartorius.com/en/products/process-filtration/cell-harvesting/sartoclear-depthfilters

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