BIOTECH

Application Note

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Clarification of Recombinant Adeno-Associated Virus (rAAV)

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1 Introduction

In recent years, development in the gene therapy industry has grown rapidly. As of 2021 there are over 20 gene and gene-modified cell therapies approved by regulatory bodies across the world with hundreds more in clinical trials[1]. The largest class of viral vectors in development today is recombinant Adeno-Associated Virus (rAAV). rAAV is non-enveloped virus ~20 nm in diameter. It can package ~4.7 kilobases of DNA and shows relatively low immune response compared to retroviruses and adenoviruses. Furthermore, it is relatively stable under standard bioprocessing conditions.

Recombinant AAV is produced in host cells which can be grown either on substrates in adherent bioreactors, or in suspension in stirred-tank bioreactors. In typical rAAV production the product can be found both intra- and extracellularly, and many processes therefore include a cell lysis step to maximize product recovery. Similar to recombinant protein processing, the next step is clarifying the product from the complex mixture of impurities which includes cell debris, host-cell proteins, and DNA.

Depth filtration is a well-established technology for clarifying a product from a complex cell culture stream. Depth filters are typically made up of a mixture of cellulose, inorganic filter aids, and resin. They provide the 'depth' of filter media and wide pore size distribution necessary to retain the amount and wide size range of impurities present in the bioreactor. Depth filters are often followed by membrane filters to further remove fine particles as well as any bioburden in the process stream. Depth and membrane filters offer a robust, cost-effective solution for clarification over a wide range of scales.

In this application note we evaluate combinations of depth and membrane filters from Pall's portfolio for clarification of rAAV. The impact of feed stream characteristics (rAAV serotype, adherent vs suspension, turbidity), filter characteristics (chemistry, pore size) and scale are evaluated for impact on clarification performance.

2 Materials and Methods

2.1 Crude Harvest Supply

All rAAV5 used in this work was supplied through transient transfection of HEK293T cells. Adherent cultures were either produced with Corning® CellSTACK® chambers or in iCELLis® Nano bioreactors. At the end of production, the culture supernatant was removed, the cells were lysed using a detergent, and the lysate collected from the bioreactor. The supernatant, wash, and lysate were combined and treated with an endonuclease prior to clarification. rAAV5 concentration in the crude harvest averaged 7.4 ± 2.0 x 10^9 gene copies (gc)/mL. Suspension cultures producing rAAV5 were grown in benchtop stirred tank bioreactors. At the end of production, the cells were lysed using a detergent and the pool was endonuclease treated prior to clarification. rAAV concentration in the crude harvest averaged 2.7 ± 0.64 x 10^{10} gc/mL.

Cells producing rAAV8 and rAAV9 were procured from Vector BioLabs. Treatment of the cells was designed to mimic that of the rAAV5 harvest from the iCELLis Nano bioreactors. Cells were lysed with a detergent buffer, diluted into culture medium, and endonuclease treated prior to clarification.

2.2 Clarification

The depth filters used in this work are shown in Figure 1A and include several sheets from Pall's Seitz™ P-series and Bio-series filter lines. P-series filter sheets are made of a combination of cellulose, inorganic filter aids, and a binding resin. Note that the V100P is a sheet designed specifically for virus work that is low-charge and free of diatomaceous earth. These filters are available in single-layer format, or in dual-layer configurations in Pall's HP series (product key shown in Figure 1B). The Bio-series filter line is made of cellulose and a resin and is ideal for low binding applications. For more information including permeability and retention rating of each sheet refer to Pall documents reference USD 2463f[2] and USD 2590[3]. All depth filters were tested in Supracap™ 50 or Supracap 100 capsule formats. Pall's Supor® EKV membrane was used as a sterilizing-grade filter after depth filtration. It is a dual -layer PES filter with an asymmetric pre-filtration upstream layer and a downstream symmetric 0.2 µm layer. All sterile filters were tested in Mini Kleenpak™ syringe filter, Mini Kleenpak 20 capsule, or Mini Kleenpak capsule formats. More information can be found in Pall document reference USD 2461b[4].

All filtration work described here was run at constant flux on PendoTECH♦ NFF control systems with peristaltic pumps on the feed lines. Pressures and filtrate volumes were recorded over time. In all trials, filters were equilibrated using a 1x phosphate buffered saline (PBS) solution prior to loading. We recommend flushing 100 L/m^2 at 300 L/m^2/h (LMH) for the filter grades used here to ensure full wetting of the pores (more information on flushing can be found in the validation guide found in Pall's Accelerator Documentation Center). A post-use buffer chase of 1.5x hold-up volumes was also employed to maximize virus recovery.

Experiments used to determine filter capacity were run in constant pressure (Pmax) format to a terminal pressure of 10 psi (0.7 bar). Adherent rAAV capacity trials were run at 200 LMH on the depth and 1000 LMH on the sterile filters. Suspension rAAV trials were run at 75 LMH on the depth and 500 LMH on the sterile filters. It's worth noting that depth filter flux rates at clarification can range from ~50 – 200 LMH and are often in the ~75 – 100 LMH range. It's well known that operating flux can impact depth filter performance (including capacity), however that was not a focus of this work.

2.3 Analytics

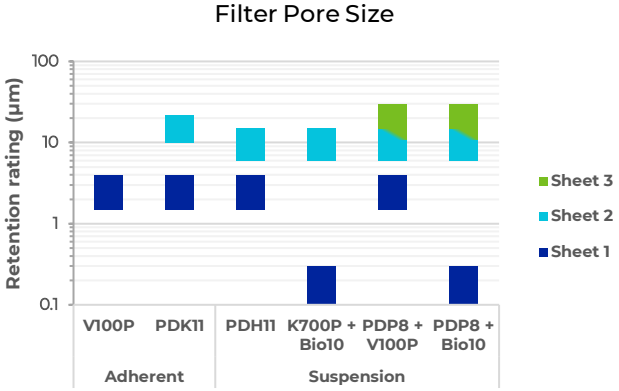
Pool turbidities were measured offline on a Hach♦ 2100Q portable turbidimeter. rAAV concentrations were measured by a digital droplet polymerase chain reaction (ddPCR) method. Step yields were calculated using Equation 1 below where V_f and V_p refer to feed and filtrate pool volumes and C_f and C_p refer to feed and filtrate pool concentrations respectively.

Equation 1

$$Yield\ \% = \frac{(V_p * C_p)}{(V_f * C_f)} * 100$$

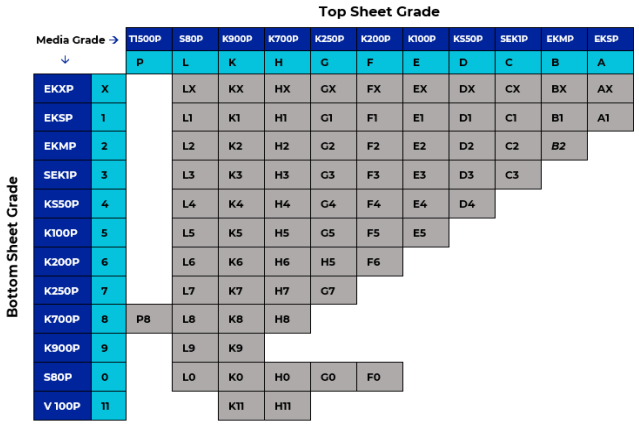
Figure 2

A: Retention ratings of filters evaluated in this work.



Note the overlap in color between sheets 3 and 2 indicates the overlap in retention ratings between the sheets

B: Product key for filter grades present in dual-layer HP filters.



As an example, a PDP8 filter is a combination of a T1500P layer ('P') and K700P layer ('8').

3 Results and Discussion

3.1 Filter Selection

Initial screening work was done with both adherent and suspension rAAV5 crude harvest feed streams to identify an appropriate filter train for clarification. Select single-, double-, and triple-layer filter combinations were tested; pore sizes are described in Figure 1A. Filters were evaluated with the same feedstock run under the same flux conditions in Pmax studies to a terminal pressure of 10 psi (0.7 bar).

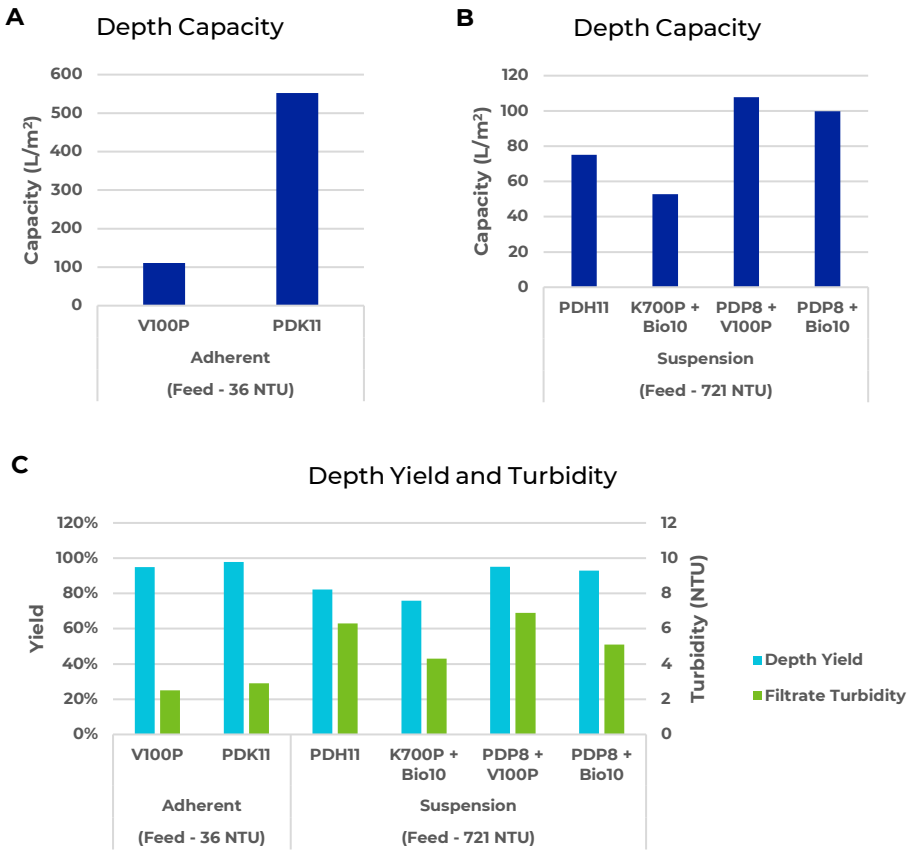
The turbidity of the adherent rAAV5 crude harvest was 36 Nephelometric Turbidity Unit (NTU). For clarification, both V100P and PDK11 filters demonstrated high yields (≥95%) and strong turbidity reduction

(<3 NTU in the filtered pool; Figure 2C). Here we saw a significant capacity benefit from the dual-layer PDK11 (Figure 2A), reaching >500 L/m^2 at 10 psi (0.7 bar). However, we note that for cleaner feedstream the single-layer V100P may also be a good option. Both depth-filtered pools were taken offline and used to measure capacity on Supor EKV sterilizing-grade filters with both showing capacities >1700 L/m^2 and rAAV5 yields >99%.

The turbidity of the suspension rAAV5 crude harvest was 721 NTU. For clarification, the triple-layer filter combinations of PDP8 with either V100P or Bio10 provided the highest capacity (≥100 L/m^2; Figure 2B) and rAAV5 yield (≥93%; Figure 2C). Using the tighter Bio10 as a bottom layer filter provided slightly better turbidity reduction (5 NTU in the filtered pool; Figure 2C), but also slightly lower capacity. Depth-filtered pools were taken offline and used to measure capacity on Supor EKV sterilizing-grade filters. Unfortunately, there was not have enough feed material to reach the pressure limit on the sterile filters, but both showed >700 L/m^2 throughput with pressure drops of ≤5 psi (0.3 bar). The data shows both filter trains would provide a strong clarification solution for this feed. The best option for suspension rAAV will likely depend on small differences in process or feedstream as well as any manufacturing constraints.

Figure 3

Depth filter capacities (A-B), yields, and pool turbidities (C) from select filters run with adherent and suspension rAAV5 crude harvests.



3.2 Process Robustness

Performance of any clarification step can be influenced by many factors, including control over process conditions and variability in the feedstock. The latter can be particularly challenging as it is often difficult to measure and control important feed characteristics such as cell density, cell viability, and particle size distribution and concentration. This highlights the importance of a robust clarification process that can accommodate some of that variability.

Turbidity of the crude harvest is often used as a rough measurement to encompass the characteristics described above. Using an adherent rAAV5 we ran seven replicate trials with feedstocks ranging from 29 – 129 NTU. The feed turbidity will impact the capacity on the depth filters, and this should be accounted for when developing a process. In this work we consistently reached >250 L/m^2 throughput using a PDK11 + EKV filter train. The key finding was across the range of feed turbidities where we observed strong robustness for turbidity reduction (Figure 3A) and yield (Figure 4A) with pool turbidities at 3.0 ± 1.3 NTU and yields at 104% ± 9.6%.

Similar process robustness was seen over two trials with suspension rAAV5 material. In these trials the feed turbidity ranged from 721 – 984 NTU. The pools clarified over PDP8/V100P + EKV had turbidities at 4.0 ± 1.8 NTU (Figure 3B) and yields at 92% ± 6.1% (Figure 4B).

Finally, we evaluated three different rAAV serotypes produced in adherent culture. Here we saw no significant difference in pressure curves on the PDK11 or EKV when run with an rAAV8 or rAAV9 feed compared to an rAAV5 feed with a similar turbidity (data not shown). We also saw strong robustness to serotype for turbidity reduction (Figure 3A) and yield (Figure 4A) with all clarified pools at or below 10 NTU and yields >93%.

Figure 4

Turbidity reduction over clarification from adherent (A) and suspension (B) rAAV crude harvests. Where multiple trials were run error bars represent a 95% confidence interval.

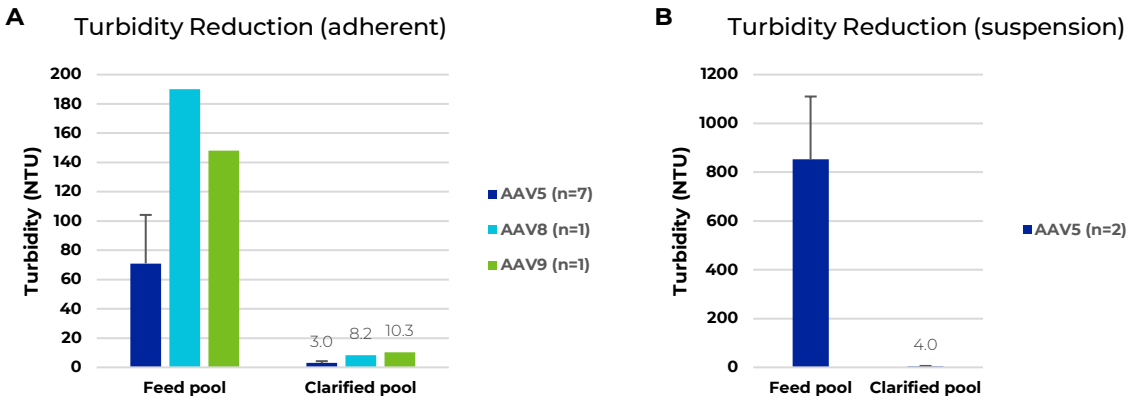
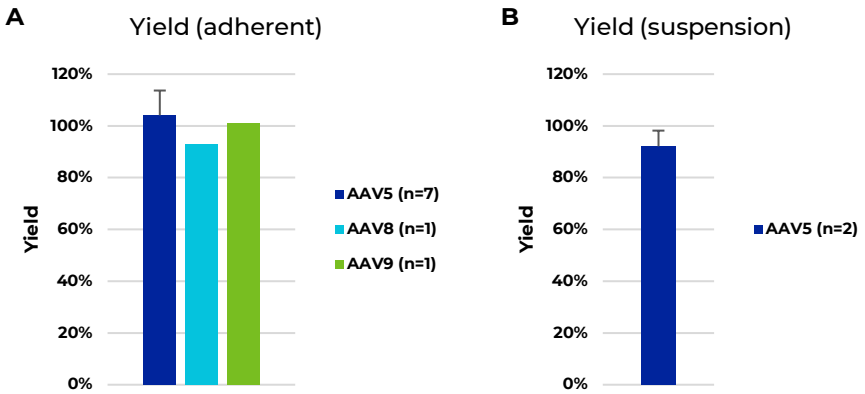


Figure 5

Virus yields over clarification from adherent (A) and suspension (B) rAAV crude harvests. Where multiple trials were run error bars represent a 95% confidence interval.



3.3 Scalability

Assessing scalability is another critical step in the development of a clarification process. Using the adherent rAAV5 material, performance of the PDK11 + EKV filter train was evaluated across process development and pilot-scale capsules. Figure 5 shows there was no significant difference seen in pool turbidities or rAAV yields between the

development-scale PDK11 Supracap 50 capsule + EKV Mini Kleenpak syringe filters or Mini Kleenpak 20 capsules and the pilot-scale PDK11 Supracap 100 + EKV Mini Kleenpak capsules. Additional information on scale-up to production scale using Pall's Stax™ depth filter capsules can be found in the referenced literature below[2,3,5,6].

Based on the filter capacities found in this work we can predict a manufacturing footprint based on common manufacturing scales for rAAV. Predictions are shown in Table 1 for a 500 L adherent batch as well as a 500 L or 2000 L suspension batch. A representative setup is shown in Figure 6 to help conceptualize the footprint.

Figure 6

Turbidity reduction (A) and yields (B) from adherent rAAV5 crude harvest clarified over a range of depth and sterile filter scales. SC50 indicates PDK11 Supracap 50 depth filters run over Supor EKV media in Mini Kleenpak syringe filters or Mini Kleenpak 20 filter capsules. SC100 indicates PDK11 Supracap 100 depth filters run over Supor EKV media in Mini Kleenpak filter capsules. Error bars represent a 95% confidence interval.

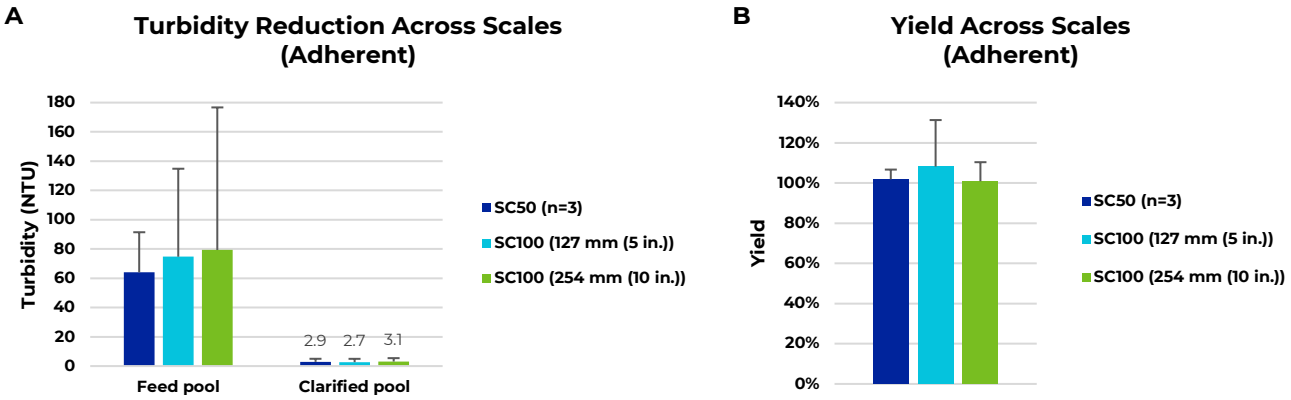


Table 1

Clarification scale-up.

		Depth Filtration		Sterile Filtration	
Upstream Process	Batch Size (L)	Required EFA* + 20% SF** (m^2)	Footprint	Required EFA + 20% SF (m^2)	Footprint
Adherent	500	1.09	2x Large Stax capsules (1x chassis)	0.23	1x 127 mm (5 in.) Kleenpak Nova capsule
Suspension	500	5.57	6x Large Stax capsules (1x chassis)	0.82	1x 508 mm (20 in.) Kleenpak Nova capsule
	2000	22.28	23x Large Stax capsules (3x chassis)	3.30	2x 762 mm (30 in.) Kleenpak Nova capsules

*EFA = effective filter area
**SF = safety factor

Figure 6

Representative image of a clarification setup including 4x large Stax capsules in one Stax chassis and 1x 254 mm (10 in.) Kleenpak Nova capsule

4 Conclusions

Pall's P-series and Bio-series depth filters and Supor membrane filters are proven technologies providing cost-effective, scalable, and robust performance for bioprocess clarification. This work demonstrated that their use can extend to clarification of rAAV grown in either adherent or suspension cell culture with multiple serotypes. The data showed consistent yields well above 90%, robust turbidity reduction to prepare the process stream for further downstream purification, and capacities that allow both depth and sterile filtration steps to comfortably scale to manufacturing batch sizes.

The exact filter combination that will optimize product quality, process economics, and footprint will vary based on factors such as feedstream characteristics and process constraints. Nonetheless, this data should provide some guidance for developing an rAAV clarification process. In this work we found a combination of PDK11 and Supor EKV membranes can provide a strong clarification solution for adherent rAAV. For suspension cultures we demonstrated success using a PDP8 followed by either a V100P or Bio10 depth filter combination connected with a Supor EKV sterile filter.

5 Further Information

For more information on filter permeability, retention ratings, scalability, and ordering information please refer to the product brochures identified in the reference section. To request support in developing a clarification step please reach out to your local Pall sales representative or our technical support team.

6 References

1. Barlow, J. F. *et al.* Insights on Successful Gene Therapy Manufacturing and Commercialization, (2020): 1-6.
2. USD 2463f - Supracap Depth Filter Capsules Scalable Single-use Depth Filtration.
3. USD 2590a - Stax Disposable Depth Filter Systems.
4. USD 2461b - Pall's Supor EKV Sterilizing Grade Filters.
5. Collins, M. *et al.* Investigating Flow Distribution and Its Effects on Scale-Up. *Bioprocess Int.* 46–51 (2009).
6. Krupp, A. U., Please, C. P., Kumar, A. & Griffiths, I. M. Scaling-up of multi-capsule depth filtration systems by modeling flow and pressure distribution. *Sep. Purif. Technol.* 172, (2017): 350-356.

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