ROBUST HARVEST CLARIFICATION FOR ADENO-ASSOCIATED VIRAL VECTORS VIA DEPTH FILTRATION

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Merck demonstrates that depth filters clarify AAV vectors, helping to overcome the unique separation challenges presented by these important vectors for gene therapy.

GROWING DEMAND FOR VIRAL VECTORS

Viral vectors are used to manufacture gene therapies and gene-modified cell therapies (e.g., chimeric antigen receptor or CAR-T cell therapies), and hundreds of clinical trials are currently underway to investigate potential new gene and cell therapy drug candidates. As a result, the demand for viral vector manufacturing capacity is growing rapidly, with estimates for the compound annual growth rate ranging from 18% to 20%.[1–3] The value of the market is also predicted to grow, reaching nearly $815 million by 2023[3] and just over $1.01 billion by 2026.[1]

CLARIFICATION IS KEY

As with recombinant proteins and antibodies, recombinant adeno-associated viruses (rAAV) are manufactured using cell culture processes, typically using insect (Sf9) or mammalian (HEK293) cells. During early clinical phases or for low-dosage applications, rAAV production tends to be performed using adherent cell cultures; however, for larger late-stage clinical- and commercial-scale processes (>200 L), suspension cell culture in bioreactors is preferred.

After transfection or infection to produce rAAV, lysis is typically performed to release the virus from the cells. While mechanical or salt-based lysis is possible, detergent lysis is often accomplished using a surfactant, such as Triton X-100 or Tween® 20. Breaking open the cells releases large quantities of DNA into the bioprocess fluid, which generally is degraded with the addition of a nuclease such as Benzonase® endonuclease.

After these steps are executed, rAAV must be harvested from the bioprocess solution. Clarification is performed to separate undesirable materials from rAAV to ensure good capacity utilization during downstream operations. The primary clarification step removes larger particles, such as cells and cellular debris, and, if necessary, a secondary step removes colloids, submicron particles, and other contaminants, including host-cell proteins (HCPs), host-cell DNA, and macromolecular DNA complexes. A final polishing filtration step is then performed with a bioburden reduction filter.

IMPORTANCE OF DEPTH FILTRATION

There are several methods for achieving clarification. Centrifugation is the traditional technology and is commonly used in production. It can handle high solid loads and is typically used at the research scale for viral vectors. However, centrifugation has high capital costs for large-scale commercial use and is difficult to scale due to lack of a robust scale-down model, and as such is not generally used for cGMP production of rAAV. Microfiltration tangential flow filtration (MF-TFF) is another option but has not been widely adopted for viral vector clarification, as the unit operation can be complex relative to other approaches, and single-use options are limited.

Normal flow filtration (NFF) using depth and membrane filters is the most popular approach for clarification of rAAV, as NFF generally provides good yields for rAAV vectors at a reasonable cost with minimal process development complexity. Depth filters can clarify a wide range of cell culture feed streams in a single-use format. It is also easy to link a polishing membrane filter to a depth filter in series to provide a fully single-use flow path and efficient operation.

There are several types of depth filters available, including conventional, graded density, and newer synthetic products. For clarification, they have nominal pore sizing of 0.1–60 μm and are often multilayer. Depth filter devices are available in a wide range of sizes to accommodate process development scale (1–10 L) through commercial manufacturing scales (200 L to >2,000 L), and individual devices can be stacked together in a non-product contact holder to provide linear scalability. Depth filters are thus true "plug-and-play" solutions, as these single-use devices require no clean-in-place solution.

THREE MECHANISMS OF SEPARATION

While the performance of depth filters is typically thought to depend on their micron rating — or how tight the filter is for size-exclusion purposes — depth filters also operate by two additional mechanisms. Depth filters can retain particles larger than the micron rating, but they also can remove contaminants via the tortuous path through the depth of the filter structure. In addition to these sieving mechanisms, they remove unwanted contaminants via adsorption.

Adsorption is achieved because the materials from which they are manufactured (diatomaceous earth/cellulose and polyacrylic fibers/silica for traditional and synthetic filters, respectively) have electrostatic and hydrophobic properties. In addition, many depth filters utilize charged resin binders to create the filter matrix, which can also interact with the feed. The tortuous path mechanism exists because the filter medium is porous, but not in a consistent manner. It makes turns and twists that create nooks and pockets, in which smaller particles can be trapped. Due to these additional mechanisms, it is possible to remove particles smaller than the micron rating, such as DNA. It should be acknowledged that depth filters with the same nominal range will not have the exact same pore size distribution, adsorptive, and tortuous path properties, leading to differences in their overall performance.

FIGURE 1. Data mining output showing depth filter loading, protection of sterilizing grade filters, and step yield for various serotypes of rAAV produced in SF9 or HEK293 cell cultures. No significant differences were noted between serotypes, though SF9 cell cultures generally provided higher depth filter loading and sterilizing grade filter capacity post depth filtration versus HEK293.

Filter	Depth Filter Loading	Sterilizing Grade Filter Capacity Post DF	Yield
Clarisolve® 20MS	> 250	500–1000	> 80
Millistak® D0SP	> 250	> 1000	> 80 / 70–80
Millistak+® D0HC	150–250	500–1000	> 80
Millistak® C0SP	150–250	> 1000	70–80
Millistak® C0HC	100–150	> 1000	> 80

Depth Filter Loading (L/m^2)	Sterilizing Grade Filter Capacity Post DF (L/m^2)	Yield
> 250	> 1000	> 80
150–250	500–1000	70–80
100–150	< 500	< 70

rAAVs PRESENT UNIQUE HARVEST CHALLENGE

rAAV processes present unique harvest challenges, not only due to the commonly used lysis step but also because of their physiochemical characteristics. Depth filters have been traditionally optimized for use with recombinant protein and antibody process streams, in which the target products are relatively high in concentration and mostly have near neutral isoelectric points. rAAV vectors, on the other hand, have slightly acidic isoelectric points and are thus negatively charged at typical harvest pH ranges and have different hydrophobic interactions than most antibodies, which creates different watch-outs for the use of adsorptive filters.[4] While it can be beneficial to have some adsorptive interactions, there must not be too many or the virus may be retained by the filter, leading to reduced yields. Additionally, the larger size of rAAV relative to monoclonal antibodies can alter harvest considerations. Finally, rAAV production processes can vary significantly, as different cell lines and bioreactor types are used, as well as various viral serotypes produced with

WITH THE GOAL OF REDUCING THE WORK REQUIRED FOR EACH rAAV PROJECT, MERCK REVIEWED INTERNAL DATA **TO UNDERSTAND TRENDS IN DEPTH FILTER PERFORMANCE FOR rAAV CLARIFICATION FROM Sf9 AND HEK293 CELL LINES.**

THOUGH ADHERENT CELL LINES CAN USUALLY BE CLARIFIED USING ONLY A MEMBRANE FILTER, OUR **STUDIES SUGGEST THAT FILTRATION CAPACITY AND CONSISTENCY OF THE CLARIFICATION STEP GREATLY IMPROVE WITH THE USE OF A DEPTH FILTER.**

competing transfection or infection methodologies. This heterogeneity brings into question the feasibility of a platform clarification unit operation.

MERCK DEPTH FILTER PORTFOLIO

Merck offers a wide range of depth filters. For rAAV clarification, our options include traditional and fully synthetic filters. Millistak+® traditional filters are composed of diatomaceous earth and cellulose and are available in a wide range of micron ratings. These filters have been heavily adopted for recombinant protein harvest clarification and purification. Clarisolve® depth filters are produced using polypropylene/cellulose and diatomaceous earth and possess open micron ratings. They have gradient layering that provides a capacity increase over conventional filters and were originally designed for clarification of high-cell-density cell cultures.

Millistak+® HC Pro fully synthetic depth filters are made using polyacrylic fibers and silica and have the advantage of lower water flushing requirements relative to conventional depth filters due to lower levels of extractables and absence of beta-glucan. Those designed for primary clarification, Millistak+® HC Pro DOSP and COSP, also have four layers – rather than the two layers commonly found in other depth filters – providing a significant boost in capacity. These filters also exhibit different adsorptive properties relative to traditional media, which can enable higher DNA and HCP impurity clearance.

MILLISTAK+® AND CLARISOLVE® AS A PLATFORM FILTER TECHNOLOGY FOR rAAV CLARIFICATION

Because there are many different depth filter options and rAAV vectors present unique challenges, process development requires thorough screening and investigation of depth filters before selection for rAAV clarification. With the goal of reducing the work required for each rAAV project, Merck reviewed internal data to understand trends in depth filter performance for rAAV clarification from Sf9 and HEK293 cell lines.

Our internal database was mined for clarification data to create an rAAV platform, considering several factors, including cost, footprint and ease of use (depth filter capacity and sterilizing grade filter capacity), yield (product binding), and flushing and recovery protocols. Controlled experiments were then conducted to confirm the findings.

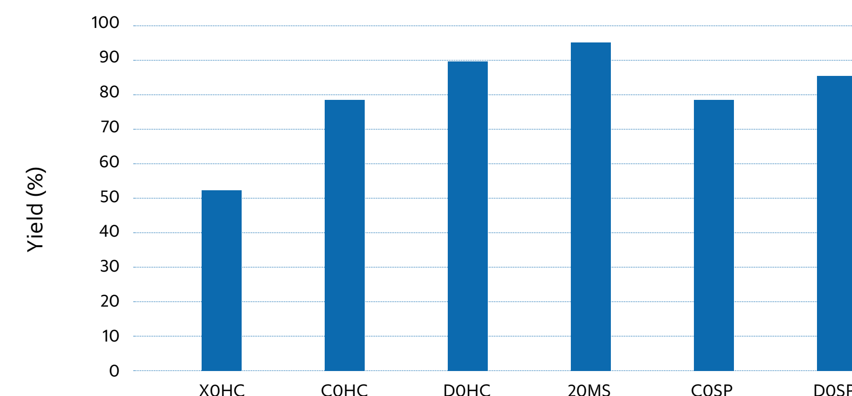
Data from more than 50 studies involving clarification of eight different AAV serotypes produced in either Sf9 or HEK293 cells using Clarisolve® 20MS, Millistak+® C0HC, C0SP, and D0HC, and Millistak+® HC Pro D0SP depth filters were then evaluated. Filter screening experiments included the Pmax™ constant flow rate test for depth filters and membrane filters to measure the increase in pressure and turbidity as a function of volumetric throughput and the Vmax™ constant pressure test for membrane filters to measure the decrease in flow as a function of volumetric throughput. rAAV yield was also determined using typical ELISA or qPCR analytical methods.

The results indicate that Millistak+® and Clarisolve® depth filters provide high depth filter loading, protection of sterilizing grade filters, and high yield for rAAV, including consistent results between multiple different serotypes from both Sf9 and HEK293 cell cultures (Figures 1 and 2). We also demonstrated the scalability of our depth filters across bench, pilot, and process scales (within plus or minus 10%). Furthermore, a comparison of results for two-stage and single-stage filtration solutions indicated that, in 90% of cases, a single-stage filtration solution provided at least comparable yield, loading, and sterilizing grade filter protection. This result is in line with those from other bioprocesses with similar cell densities. Though adherent cell lines can usually be clarified using only a membrane filter, our studies suggest that filtration capacity and consistency of the clarification step greatly improve with the use of a depth filter.

OPTIMIZATION STILL NECESSARY

This study clearly demonstrates that the robust performance, ease of use in manufacturing, and scalability of Merck depth filters make them ideal choices for clarification of rAAV vectors. It is still essential to recognize that unit operation outputs can vary between processes due to differences in feed characteristics and pretreatments, such as cell culture attributes, lysis technique, and nuclease treatment. Parameters such as feed flux, pressure endpoint, and recovery flush should all be considered in order to achieve consistent quality attributes of particle reduction, yield, and purification. For example, the appropriate loading level will depend on the total cell density, feed flux, the exact lysis conditions used, and other pretreatments to the process stream. Additionally, product yield is likely the most key quality attribute of the unit operation and can depend on background buffer characteristics, product aggregation, differential pressure endpoint, and the filter used for clarification.

FIGURE 2. Average step yield calculations across various depth filters for clarification of rAAV from SF9 and HEK293 cell culture.



PARTNERING WITH MERCK

Merck's global team has expertise in production and purification of viral vectors and can support customers in every region where viral vector bioprocessing is relevant. We offer onsite process development support, as well as remote process development consulting.

Our R&D team is deeply involved in both product and application development for rAAV vectors and viral-based gene and cell therapies. These efforts are focused both on adapting existing products to viral vector production and purification, and specific product development targeting the industry.

With all of these resources dedicated to the support of customers in the viral vector /gene therapy space and the advancement of technology to support their process development and manufacturing efforts, Merck is in a unique position to help our customers develop and implement efficient, cost-effective, fit-for-purpose bioprocesses that overcome the specific manufacturing challenges presented by rAAV and other viral vectors. P

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