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Scaling AAV Production: Transitioning From 250 mL to 200 L

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Abstract

Cost effective, seamlessly scalable upstream manufacturing remains a bottleneck in adeno-associated virus production. Ascend Advanced Therapies, in collaboration with Sartorius, demonstrated a scalable upstream manufacturing platform for adeno-associated virus vectors. Using HEK293 cells, proprietary media, and the two-plasmid EpyQ® AAV Production System, consistent productivity, quality, and potency were demonstrated from 250 mL to 200 L across Ambr® 250 Modular, Univessel® SU, Univessel® Glass, and Biostat STR® bioreactors. Successful scale-up has also been achieved with a traditional triple-plasmid transfection approach. Critical quality attributes—including viral genome and capsid titers, host cell and plasmid DNA impurities, and percentage of full capsids—remained consistent across scales. Combining a standardized, fully scalable bioreactor platform with the two-plasmid approach accelerates development timelines and delivers a cost-effective, robust path from laboratory to manufacturing volumes.

Introduction

Adeno-associated viruses (AAVs) have a clearly demonstrated ability to successfully deliver gene therapies. Characteristics including minimal immunogenicity and limited ability to replicate offer important advantages, as does the varied tropism of an ever-expanding array of AAV serotypes.[1]

Large-scale AAV production remains in the early stages of adoption within the pharmaceutical industry. Cost-effective and seamlessly scalable AAV manufacturing processes, as well as industry standards, are still needed. Understanding this, Ascend Advanced Therapies has built a flexible manufacturing platform, including a well-characterized portfolio of lab- to production-scale bioreactors from Sartorius. Excellent results have been achieved with both the EpyQ® AAV Production System and conventional triple-transfection processes, from 250 mL to 200 L scale.

Materials

Sartorius equipment used in this study:

- Ambr® 250 Modular
- Univessel® SU 2 L
- Univessel® Glass 5 L
- Univessel® SU 10 L Essential
- Biostat STR® 50
- Biostat STR® 200

Ambr® 250 Modular

Univessel® SU 2 L

Univessel® Glass 5 L

Univessel® SU 10 L

Biostat STR® 50

Biostat STR® 200

Methods

The data were generated using HEK293 cell cultures producing AAV vectors with proprietary media and Ascend Advanced Therapies' EpyQ® AAV Production System, which employs efficient split two-plasmid designed to deliver high yield and quality. The same process parameters were used across all bioreactors, and scale-up was performed with guidance from Sartorius.

Transient transfection was performed with PEIPro® transfection reagent. Crude lysate titers were measured after cell lysis and endonuclease treatment using digital droplet PCR (ddPCR) to quantify viral genomes and a Gyros immunoassay to determine capsid titers.

Impurity analysis was performed using a rapid purification protocol with spin columns. For the 50 L and 200 L bioreactors, an affinity-based chromatography purification was employed. Impurity levels were quantified with ddPCR, and the percentage of full AAV particles was determined using a Refeyn TwoMP mass photometry system. Potency was determined using a cell-based assay with a functional read-out.

Results and Discussion

A strategy to overcome AAV process scaling challenges

Transient transfection processes for the production of AAV vectors require control of numerous starting material attributes and process parameters. Design of experiment (DoE) approaches performed in robust, scale-down devices, such as Ambr® small-scale bioreactors, enable rapid and cost-effective optimization of product-specific modular platform processes.

Many critical process parameters are scale dependent, making it difficult to consistently maintain multiple factors—such as mixing time, power input, impeller tip speed, and oxygen transfer rate (OTR)—across process scales | operating volumes. Different seed train volumes can influence cellular performance, impacting product yield and quality. Critical quality attributes (CQAs) related to product impurities—including full | empty capsid ratios and residual plasmid and host cell DNA (hcDNA) packaging levels—are often strongly influenced by the biological starting materials used and the design of upstream process steps, such as transfection.

Our strategy to effectively scale modular platform processes was as follows:

- Step 1: Employing lab- to commercial-scale bioreactors for which vessel geometry is consistent across the portfolio ensures process scalability by design.
- Step 2: Identifying the optimal starting materials.
- Step 3: Rapidly developing a platform process that targets comparable quality and productivity across all scales.
- Step 4: Using robust, fit-for-purpose analytics for in-depth product and process characterization.

This approach enables consistent productivity, quality, and potency across scales—from 250 mL laboratory runs to 200 L commercial production—corresponding to an 800-fold scale-up.

Flexible and consistent performance across scales

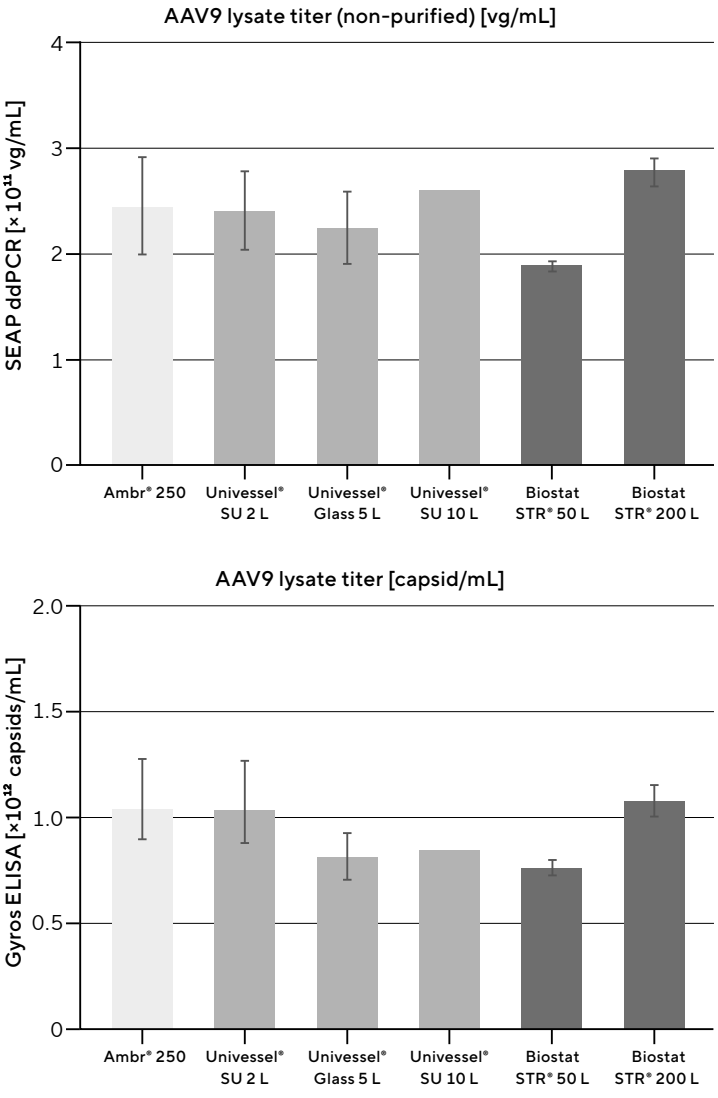
Along with the proprietary split two-plasmid EpyQ® AAV Production System, the Sartorius bioreactor portfolio enabled the development of robust processes that balance CQAs with yield for multiple serotypes. These results were also confirmed using traditional triple-transfection processes (data not shown). In-house purification protocols developed by Ascend Advanced Therapies were established to fit various scales and application needs, considering material quality and offering optional polishing steps for impurity reduction and removal of empty capsids. An unmatched range of in-house and outsourced analytics (>50 assays) informed early process and formulation development, regulatory discussions, and product release.

To ensure consistent results at the process scale, Ascend Advanced Therapies employed a range of extensively characterized bioreactors from Sartorius, spanning from laboratory to commercial production. Ascend Advanced Therapies' current deployment of Sartorius bioreactors includes Ambr® 250 Modular, Univessel® SU 2 L, Univessel® Glass 5 L, Univessel® SU 10 L, Biostat STR® 50 L, and Biostat STR® 200 L systems. In developing these systems, Sartorius has considered various factors, including the gassing rate, liquid volume, stirring speed, specific power input, and mixing time. Ascend Advanced Therapies used the Ambr® 250 platform as a high-throughput, scale-down model to first optimize process conditions, before demonstrating platform scalability by transferring the optimized process to the Univessel® bench-scale and Biostat STR® large-scale bioreactor platforms. Process scaling was enabled through a geometrically-consistent bioreactor portfolio, and supported by a comprehensive bioreactor characterization data package and Sartorius scaling tools.

To demonstrate comparable yield and quality across scales, a 3 kb AAV9 vector containing the gene for the secreted embryonic alkaline phosphatase (SEAP) reporter protein was produced in the Sartorius bioreactor portfolio at Ascend Advanced Therapies. Titers (viral genomes/mL and capsids/mL), levels of packaged hcDNA, plasmid DNA, impurities, percentage of full capsids, and potency—all key CQAs mainly defined by plasmid design and upstream process parameters—were determined.

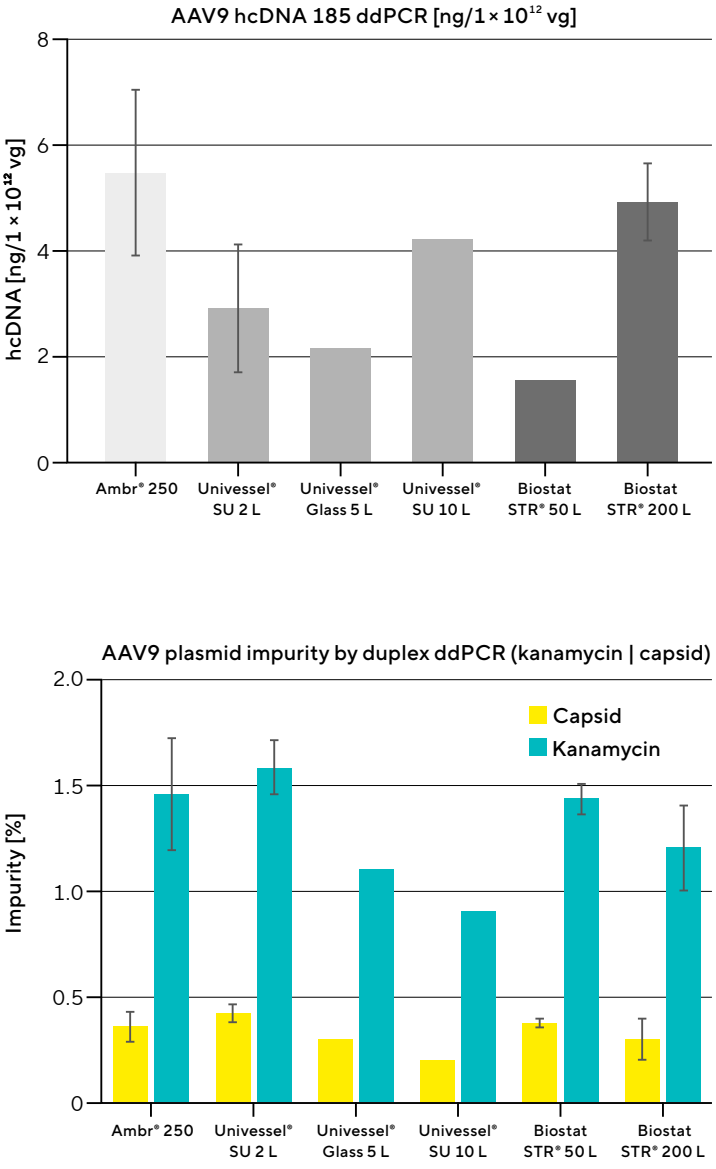
Non-purified lysate titers are presented in Figure 1. The maximum variation around average viral genome (vg) values was within an acceptable range at +32%, and the highest yield was observed at the 200 L scale. The variations in capsid yields were even lower than that for the vg titer.

Figure 1: Consistent non-purified lysate titers across bioreactor scales



Data for hcDNA and total plasmid DNA (kanamycin and capsid) impurity packaging levels for vector production at different scales are shown in Figure 2. Analyses were performed on samples collected after purification. The levels of both types of impurities were at the lower end of the ranges reported in the industry for AAV vectors (approximately 20 ng/1 × 10^{12} vg and 5–10%, respectively) for all process scales.

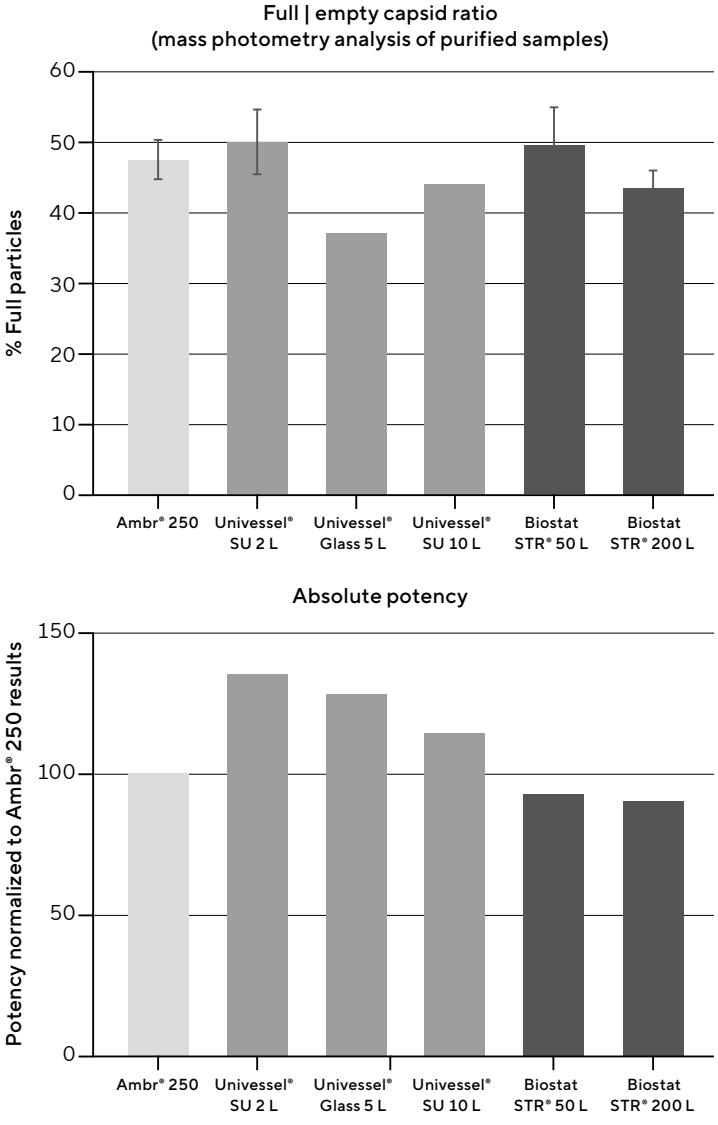
Figure 2: Low levels of DNA impurity packaging across bioreactor scales



The presence of empty capsids in AAV vectors intended for use as gene therapies has come under increasing scrutiny due to rising concerns about their impact on safety and efficacy. The percentage of full capsids, or full | empty ratio, is therefore an important CQA. The Ascend Advanced Therapies platform process, using EpyQ®, was designed to yield a high percentage of full capsids even without the performance of a specific chromatographic purification step to remove empty capsid impurities.

Importantly, the ability to produce a high percentage of full capsids is retained across scales. As shown in Figure 3 (top), after purification (not including any steps to remove empty capsids), a high percentage of full capsids (37 – 55%) was observed for the AAV9-SEAP vector runs performed from 250 mL to 200 L as determined via Refeyn TwoMP mass photometry analysis. The observed variation may be attributed to analytical method variance (suggesting a need to perform both titering and cell-based assays) or differences in the purification protocol (quick-spin for small-scale, one-step affinity chromatography at medium-scale, and full downstream purification for the two large-scale runs). Given the similar high percentages of full capsids obtained for the different runs, it is not surprising that the vectors produced at different scales exhibited comparable potency (Figure 3, bottom).

Figure 3: High percentage of full AAV capsids with comparable potency

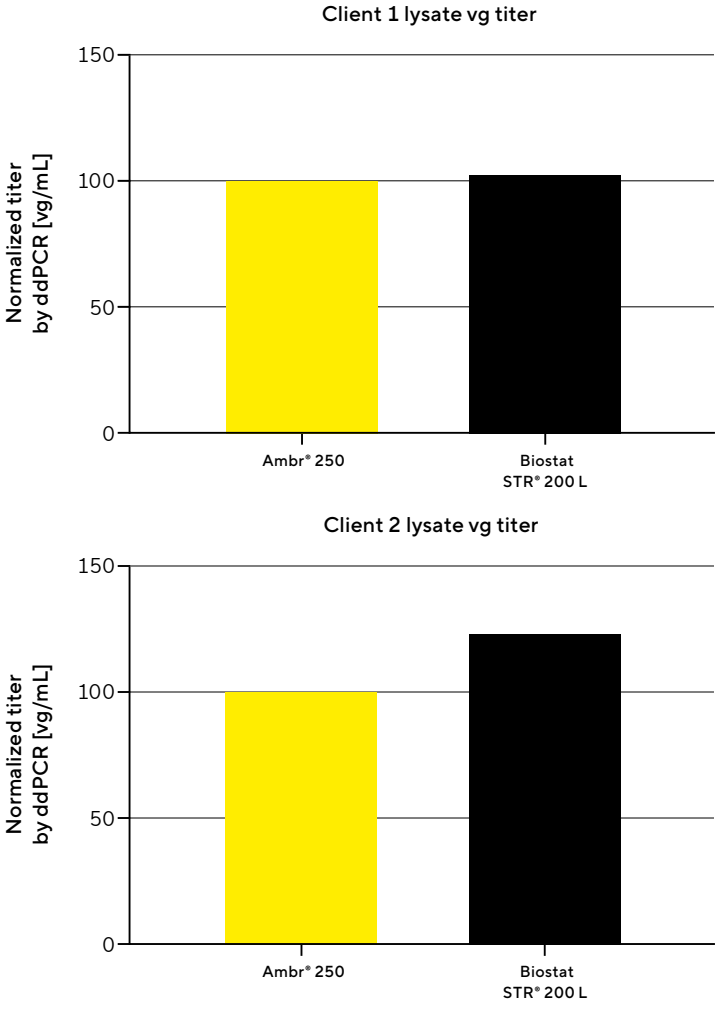


Expediting projects with tailored solutions

The production of the AAV9-SEAP vector in this study was achieved using the EpyQ® AAV Production System. It is important to note, however, that the platform also supports scalable AAV vector production with a similar balance of yield and quality via more conventional triple-transfection processes (data not shown here).

Figure 4 presents the results for two different client processes when directly scaled from the Ambr® 250 Modular system to the desired final scale (either 50 L or 200 L) using the client's triple plasmid system. Despite this customization, the viral titer of the non-purified lysate obtained at the higher scale was, in one case, nearly the same as that obtained at the lab scale, while in the other, it was measurably higher.

Figure 4: Direct scale-up of client processes from lab (250 mL) to production scale



Conclusion

Ascend Advanced Therapies has designed a gene-to-GMP offering in the United States and Europe that supports a wide spectrum of AAV-based gene therapies. Working with partners like Sartorius, they aim to reduce development timelines, increase quality and safety, and ultimately break down barriers blocking patient access to advanced therapies. The Sartorius bioreactor portfolio ensures process scalability through consistent bioreactor design, within a powerful, flexible platform that scales from bench to 200 L while meeting stringent quality targets—an important enabler of these goals.

References

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