Advancing Purification Technologies and Process Innovations to Support High rAAV Upstream Productivity and Meet Growing Industry Demand

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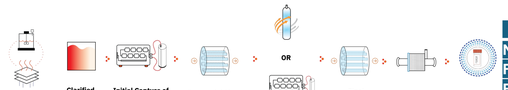
Introduction

Background

One of the key challenges in rAAV manufacturing is developing optimized, efficient, and scalable processes that support a wide range of rAAV serotypes and diverse transgenes. This includes novel and engineered capsids and meeting the needs of expanding pipelines and large patient populations. Forge Biologics has launched a manufacturing platform termed "FUEL™" for AAV gene therapy production. The FUEL™ platform is designed to improve process efficiency, productivity, and safety by combining its proprietary pEMBR™ 2.0 Ad helper plasmid, the Ignition™ cell line, and optimized rep/cap plasmids, all working together for an optimized, efficient, and scalable production process. As rAAV upstream productivity continues to increase, new challenges have emerged in downstream processing, where higher vector titers along with novel serotypes put increased demand on the loading capacity of resins, throughput, and scalability of traditional purification operations. The tight integration of Forge's high yield upstream process with its tailored downstream workflows has demonstrated improved rAAV recovery and directly increases the number of patients who can be dosed per batch. The platform integrates clarification of crude lysate by using systematically evaluated depth-filtration strategies. Vector capture utilizes established resin–buffer systems derived from multi-serotype experience, with customized buffer optimization and affinity resins applied as needed to enhance recovery and product quality. Anion exchange chromatography (AEX) was evaluated for full capsid enrichment under relevant conditions to assess impact on product recovery, impurity clearance and loading capacity. AEX was assessed in parallel to benchmark cesium chloride enrichment and inform full to empty capsid separation strategies that support scalable and consistent processing. Further, viral retentive filtration was evaluated to assess throughput, yield and robustness supporting a fit-for-use clearance strategy and scalable process design. This process concludes with final formulation and fill/finish using state-of-the-art capabilities, yielding highly pure rAAV suitable for preclinical studies, toxicology, or clinical applications. The advancements enabled by the FUEL™ platform and optimized manufacturing methods have increased vector yield compared to standard processes. Forge has systematically purified the rAAV products generated by these advancements across multiple serotypes and scales with more targeted optimization of key steps to improve process consistency. Each purification step is verified through a suite of advanced analytics spanning potency, purity, and safety. This presentation will highlight data generated with the FUEL™ platform and optimizations, demonstrating high rAAV yield and the exceptional quality attributes of the final drug product and setting up a foundation for process characterization and production geared towards late-stage batches.

FUEL™ Platform Process

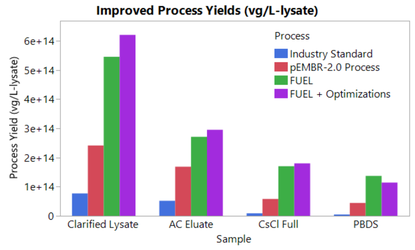
Improvements to upstream production necessitate modifications to the downstream purification process to accommodate higher productivities. Particularly, higher vector yields may lead to challenges with clarification and filtration; higher in-process titers can lead to issues with vector stability; and higher load factors may lead to product loss, particularly in the affinity capture unit operation and other scalability issues. Here we show that improvements to the upstream productivities are carried through to the drug product (DP) stage of purification seamlessly using established Forge Platform process.



	%Full*	CE %Purity
Non-FUEL™	86.6%	97.2%
FUEL™	98.8%	97.2%
FUEL+ Optimizations	98.3%	96.0%

* %full determined by mass photometry

Figure 1: *Schematic of Forge rAAV platform purification process*



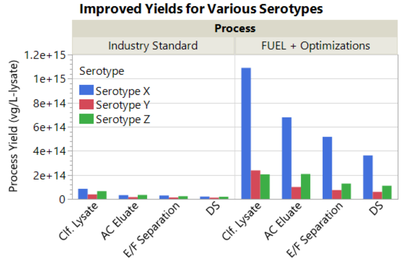
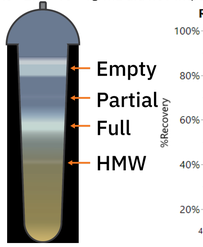


Figure 2a: *Progressive upstream yield improvements are carried through to increased PBDS yield, demonstrated with AAV9/eGFP vector. FUEL + Optimizations consists of FUEL platform parameters plus transfection enhancer.* ***Figure 2b:*** *Coordinated improvements to upstream and downstream processes resulted in higher productivities and yields across various serotypes regardless of which full capsid enrichment technique was used.*

Accommodating Higher Upstream Productivities

Optimization of CsCl UC tube Loading

Increased upstream productivity for several serotypes has introduced new challenges in downstream purification, especially in scaling empty/full capsid separation. Full capsid enrichment is commonly achieved using CsCl equilibrium density ultracentrifugation (UC), which is serotype-agnostic and historically favored for its high recovery and superior full capsid yield. To accommodate higher productivity, one strategy is to maximize CsCl ultracentrifuge tube loading, while an alternative approach involves the use of anion exchange (AEX) chromatography. FORGE improvements to CsCl UC increased throughput; tube loading up to ~2.5 × 10^{14} vg/mL did not impact recovery (below) and showed a positive correlation with step recovery (p = 0.0177).



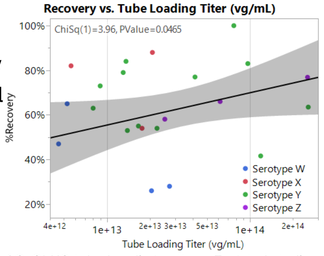


Figure 3a: *Schematic of CsCl UC tube banding pattern. Targeted studies demonstrated improved recoveries across multiple serotypes.* ***Figure 3b:*** *This approach preserves the traditional method while enabling faster processing and progress within shorter timelines.*

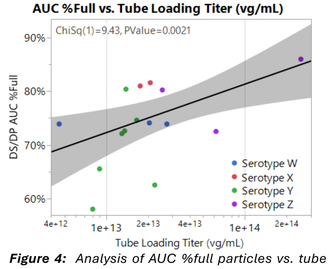
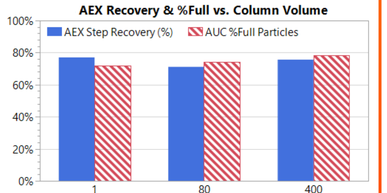
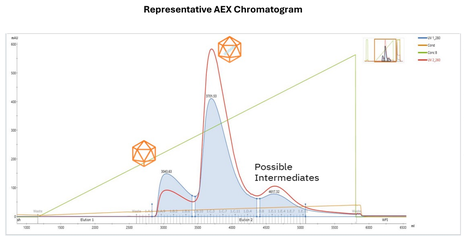


Figure 4: *Analysis of AUC %full particles vs. tube loading titer shows that higher tube loading correlates with higher %full at PBDS with p = 0.0021.*

AEX Development and Scale up

Anion exchange (AEX) chromatography is an attractive alternative to CsCl ultracentrifugation; compared to ultracentrifugation, AEX offers reduced operator variability, improved scalability, and is well suited for high-throughput optimization. **Figure 4** shows a representative chromatogram for AEX separation of full and empty capsids. AEX chromatography has been successfully demonstrated with consistent recoveries and full capsid enrichments over a wide range of scales (**Figure 5, top**). Further, step recoveries and % full particles (AUC) were consistent across production scales of 4–50 L (**figure 5, bottom**), regardless of whether CsCl UC or AEX were utilized for full capsid enrichment.





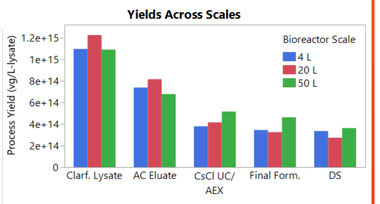
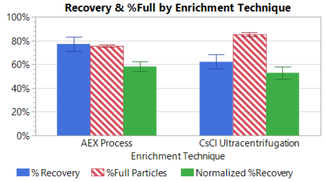


Figure 4*: Representative AEX chromatogram for showing clear separation of different capsid species. First peak corresponds to empty capsids; second peak corresponds to full capsids.*

Figure 5a: *Comparable recoveries and product quality are seen at different scales.* ***Figure 5b:*** *shows consistent recoveries across various scales, regardless of full capsid enrichment technique. CsCl UC was used at the 4 L scale, AEX were used at the 20 and 50 L scales.*

AEX/CsCl UC Comparison

Performance of AEX was evaluated against the Forge platform CsCl UC process, demonstrating higher recovery. Both methods achieved high % full exceeding 75%. When we only consider full capsid yield as determined by AUC, we see no significant difference in recovery; this suggests differences in percent full between methods may be attributable to choice of AEX peak cutting and CsCl UC band pulling technique. Product quality testing showed lower or comparable levels of endotoxin, host cell DNA (HCDNA), host cell protein (HCP) and other residuals when purified by AEX. Both CsCl UC and AEX are viable options for full capsid enrichment and can be tailored to meet specific client needs.



ANOVA
%Recovery: p=0.1334
%Full: p=0.0043
%Rec. Normalized: p=0.4561

Residuals Comparison by Enrichment Technique

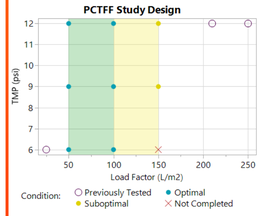
Residual	Process 1 (CsCl UC)	Process 2 (AEX)
Endotoxin (EU/mL)	<0.5	0.072
HCDNA (Alu) [+] (pg/1E+9 vg)	2.20	0.28
HCDNA (Alu) [-] (pg/1E+9 vg)	2.72	0.29
pDNA [+] (copies/mL)	1.24E+10	3.03E+9
pDNA [-] (copies/mL)	1.25E+10	3.06E+9
E1a [+] (copies/µL)	< 5.0	7.27
E1a [-] (copies/µL)	< 5.0	8.94
HCP (ng/mL)	0.73	BLOQ

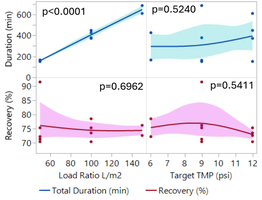
Figure 6a: *Performance of AEX was evaluated against the standard CsCl UC in purifying AAV; these demonstrate higher recovery with AEX with slightly lower % full.* ***Figure 6b****: One-way ANOVA on process recoveries shows no significant difference in recovery (p=0.1334). When percent recovery is normalized (%Full × %Recovery) we see no significant difference in recovery (p=0.4561).* ***Figure 6c:*** *Comparison of endotoxin, HCP, pDNA (KanR), E1a and HCDNA levels in PBDS. [+]/[-] indicates whether the sample was treated with DNAse; purified vector met quality specifications regardless of full capsid enrichment technique.*

Continuing Process Improvements

Other Platform Improvements

Further improvements to Forge platform process is an ongoing endeavor to adapt to increasing productivity and facilitate seamless transition to late-stage clinical manufacturing. Concentration of clarified lysate via post-clarification TFF (PCTFF) is an attractive option to reduce Affinity chromatography loading duration and overall process turnaround time with reduction in impurities. Here we show that process duration is significantly dependent on load factor [p<0.0001] but not TMP set point; load factor and TMP did not significantly affect recovery (***Figure 7a***). At load factors ≤ 100 L/m², 10x concentration followed by 6 DV UF/DF can be achieved in under 4 hours. In addition, Viral Retentive Filtration (VRF) using Planova VRF filters has been successfully demonstrated in two serotypes ***(Figure 8)*** with consistent recoveries obtained at scales ranging from 10 cm² to 1200 cm²; roughly correspond to bioreactor scales up to 500 L.





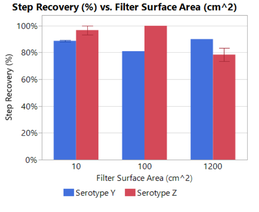


Figure 7a: *Parameters tested for PCTFF optimization. Green region shows optimal PCTFF parameters; yellow region shows suboptimal conditions (achievable with extended processing time).* ***Figure 7b:*** *Plots of PCTFF step recoveries and process durations vs. filter load factor and TMP set point.*

Figure 8: *VRF step recoveries were consistent across different scales with two different serotypes.*

Conclusions: Forge's optimized downstream processes and flexible, regulatory-ready purification strategies enable more tailored, efficient, scalable AAV therapy development. By improving overall productivity and enabling targeted optimizations, these advancements deliver meaningful benefits for both clients and the patients they serve.

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