Accelerating path to clinic through an end-to-end solution focused on supply chain, regulatory, and a robust manufacturing process for viral vectors

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Abstract

Recombinant adeno-associated viral (rAAV) vectors are currently one of the most widely used gene therapy products in development due to their lack of pathogenicity, gene expression persistence, and the presence of various serotypes that enable a diverse cell tropism. Lentiviral vectors are mostly used to stably transfer and express genes in gene therapy for monogenic diseases. The rapidly growing demand challenges arising from long process development timelines, lack of cGMP-suitable fit-for-purpose components, and uncertainty in regulatory approval have led to a critical need for scalable and cost-effective standardized manufacturing platforms. Thermo Fisher Scientific has developed a high-quality, scalable, and robust end-to-end rAAV & Lentivirus (LV) manufacturing process that uses fit-for-purpose materials with matched analytics. These processes were designed to be standardized and, therefore-can accommodate various biochemical and biophysical differences between target therapeutics, including different serotypes for adeno-associated virus (AAV). Utilizing this characterized process will help enable the delivery of products to the clinic on a de-risked and accelerated pathway.

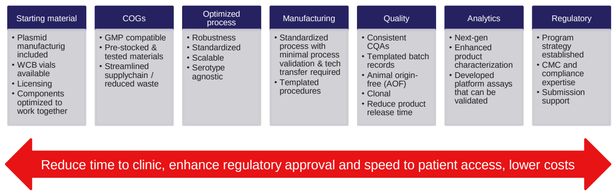
The Patheon™ Quick to Clinic™ viral vector program is a standardized, all-inclusive solution for suspension-based adeno-associated viral (AAV) and lentiviral (LV) vector manufacture, offering a variety of benefits compared to a standard process development (PD) program and other platform process solutions on the market (Figure 1), including:

- ✓ Serotype agnostic (minor fine-tuning for the gene of interest changes)
- ✓ Next-gen analytics: chemistry-based, enhanced precision and accuracy, absolute quantification
- ✓ Robust process control and consistent critical quality attributes (CQAs), including optimized purification process to address known challenges
- ✓ ≥30% reduction in project timelines
- ✓ ≥90% of manufacturing materials pre-stocked and tested
- ✓ Phase-appropriate regulatory support throughout the product lifecycle
- ✓ Inclusion of relevant license rights to Thermo Fisher Scientific assets

Combining optimized and tested processes with platform-qualified analytics supports robust suitability assessment in early development and subsequent scale-up. Moreover, the program timeline includes expedited plasmid manufacture, suitability, and scale-up, providing an overall time savings of more than six months compared to standard process development and nine to twelve months when including plasmid manufacturing.

Quick to Clinic viral vector: reach milestones faster, reduce supply chain and regulatory risk, built for commercial success

Figure 1. Overview of Quick to Clinic end-to-end solution



Viral vector manufacturing process and analytics: Quick to Clinic end-to-end solution

The Quick to Clinic viral vector manufacturing process involves using a Working Cell Bank (WCB) derived from a suspension-based HEK293 clonal and documented cell line (VPC & VPC 2.0) that is expanded via standard cell culture processes using single-use technologies. Viral vector production initiates via transient transfection. Critical parameters and reagents have been optimized to work together, resulting in optimum transfection efficiency and vector yield. Vector harvest and purification are achieved by scalable and robust depth filtration, endonuclease treatment, and chromatography-based vector purification and enrichment processes (Fig 2). Overall process performance for AAV and LV vectors using the Quick to Clinic vector manufacturing process demonstrates high vector yield and recoveries compared to industry standards. Additionally, the consistency of critical quality attribute results across multiple batches, serotypes, and product conditions indicate the process's robustness, including an isocratic chromatography-based full particle enrichment process for AAV that consistently demonstrates 2-3x enrichment (Fig 3, 4). The scalability of the process has been confirmed from ambr®15 to 200L scale and can further be scaled out or up, supporting additional product yield requirements.

Figure 2. Quick to Clinic AAV vector manufacturing process overview

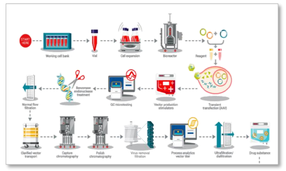
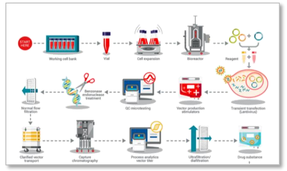
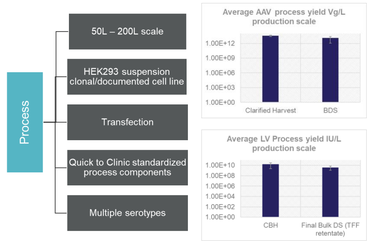


Figure 3. Quick to Clinic LV vector manufacturing process overview



Developing a robust viral vector production process is only-possible with having the appropriate analytical methods established to support product and process understanding while maintaining visibility over the potency, purity, and safety of the vector produced. This enables the characterization of critical quality attributes (CQAs). Generally, for recombinant adeno-associated virus (rAAV) vector processes, analytics such as vector genome titer, capsid titer, empty vs. full capsid ratio, infectivity, purity, residuals, and aggregation are the most common attributes evaluated. Historically, the analytical methods associated with measuring these attributes are impacted by long turnaround times, high variability, and the need for more specificity. Here, we highlight the methods developed and used in the Quick to Clinic (Table 1). Some examples of these next-generation analytics are Droplet Digital PCR (ddPCR), size-exclusion chromatography multi-angle light scattering (SEC-MALS), analytical ultracentrifugation (AUC), capillary electrophoresis (CE), and others. The key benefits of utilizing these methods are high-throughput, rapid in-process control (IPC), absolute quantification, increased precision, sensitivity, and robustness. These enable product characterization early in the development and manufacturing process, while delivering insight into process performance and product quality.

Figure 3. Quick to Clinic process performance



Attribute	Quick to Clinic™ average - AAV	Quick to Clinic™ average - LV
Purified bulk vector substance yield from nominal culture volume	2.0E13 vg/L	4.0E09 IU/L 7.0E12 VP/L
Host cell protein (ng/mL)	< LLOQ (6.25)	<1E4
HEK DNA	< 200 ng / E12	<LLOQ
Residual benzonase	< LLOQ	<LLOQ
Residual plasmid DNA (ng/mL)	< 250 ng / E12	<2.0
Product recovery at DS (overall)	15 – 40 %	10 – 40%

Table 1. Next generation analytics supporting enhanced AAV vector product characterization

Attribute	Current industry analytics	Quick to Clinic analytics	Benefits of QtC analytics
Vector Genome titer(Vg/mL)	qPCR	ddPCR & SEC-MALS	• Absolute quantification & increased precision and robustness • SEC-MALS= semi automated & absolute quantification
Capsid titer: Vector genome titer	ELISA	ELISA & SEC-MALS	• SEC-MALS = rapid IPS, Serotype/GOI agnostic
% Genome containing (AUC)	qPCR/ELISA Calculation	AUC & SEC-MALS	• AUC-Gold Standard for empty/partial/full analysis • SEC-MALS= rapid IPC, serotype/GOI agnostic
Infectivity	TCID50	TCID50	-
Residual HEK DNA	qPCR	ddPCR	• Absolute quantification & increased precision and robustness
Residual plasmid	qPCR	ddPCR	• Absolute quantification & increased precision and robustness
Purity	SDS-PAGE	CE-SDS	• Increased sensitivity, precision & Data integrity

Robust AAV Purification Platform Tolerant of Changes in Harvest Material: Quick to Clinic end-to end-solution

Most rAAV vector manufacturing processes in the industry today need to be standardized, where varying production methods, cell lines, culture systems, and technologies are used to produce rAAV-mediate gene therapies. The lack of standardization in the upstream production process directly impacts the outcome of the downstream vector purification performance. Additionally, different AAV serotypes and transgenes can demonstrate unique behaviors during the purification process, typically due to varying particle density, isoelectric point, aggregation, or stability profiles. The Quick to Clinic process for viral vectors has been developed to help address these potential challenges and provide a robust purification platform tolerant of varying upstream process conditions. Optimizing buffer compositions and process conditions directly impacts product purification and enrichment of viral particles containing the entire/complete therapeutic gene. The Quick to Clinic purification process was evaluated for different serotypes and vector production systems, as outlined in Figure 4. As a result of similar yields and full-particle enrichment, the process tolerates a variety of serotypes and upstream platforms. Additionally, similar purity profiles were observed (data not shown here). These results support the robustness of the process and the ability to improve process understanding and modeling toward the path of commercialization.

Figure 4. Overview of AAV vector purification and full capsid enrichment across multiple vector types

	Step recovery (vg)		% Full particles (post AEX step)			
	AAVX	AEX	VG:VP(PCR/ELISA)	AUC	SEC-MALS	Fold Enrichment
HEK AAV2	85%	56%	37%	87%	ND	~2-3X
HEK AAV9	65%	59%	60%	80%	75%	2X
Sf9 AAV6	81%	87%	66%	ND	90%	1.5-2X

- ➢ Capsid ELISA overestimates vp titer → artificially low % Full numbers
- ➢ Consistent fold enrichment (~2X across all material)

Summary

The Quick to Clinic end-to-end solution consists of a standardized, all-inclusive program for 200L suspension-based AAV and LV vector manufacturing, product release, and regulatory support. The vector production process was developed based on significant expertise in vector process and product development. The data outlined herein demonstrates proven robustness and scalability with consistent product quality and yield from batch to batch and across varying conditions or product types.
This all-inclusive solution provides the critical starting material and optimized and tested vector production process in combination with next-generation analytics, suitability assessment, and optimization for your transgene. Additionally, in-stock process consumables, licenses, expertise, and regulatory support are necessary to de-risk your clinical path and help accelerate product access to patients.

References

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