Quality by design for AAV production: a fast track to AAV manufacturing success

Adeno-associated virus (AAV) is a popular and effective viral vector used in the design of gene therapies, many of which are now rapidly progressing through clinical trials toward approval and commercialization. However, the industry is currently experiencing a viral vector shortage, with existing processes failing to produce vectors at the volumes and quality required for sustained commercial production. As such, manufacturers are seeking approaches like quality by design (QbD) to secure their commercial potential. A significant driver for the adoption of this principle is the A-gene initiative, which endeavors to bring best practices and standardized methodologies to the gene therapy industry, emulating similar efforts in the monoclonal antibody and vaccine spaces [1]. With careful consideration and application of this approach early in process development, manufacturers can help smooth their transition to large-scale production.

Critical quality attributes for AAV

The first priority when implementing QbD in your AAV vector development is identifying and characterizing the vector's critical quality attributes (CQAs). These physical, chemical, or biological CQAs correspond to quantifiable characteristics that demonstrate a product's safety and efficacy and typically relate to a product's identity, purity, and potency. Setting acceptable limits relative to a specific process and fully characterizing and monitoring any changes are critical to maintaining consistent product quality throughout process development and into clinical trials and beyond.

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While CQAs can vary from product to product, there are several CQAs that are shared across the majority of AAV manufacturing processes, including capsid titer, genome titer, genome integrity, and aggregate content. Accurately quantifying both capsid and genome titers is key to determining the dosage of a therapy. Dosage and potency are both also directly related to genome integrity—notably the empty-to-full capsid ratio but also encapsidation of partial genomes and small impurities. The presence of empty or partial capsids, as well as aggregate content, can have a dramatic impact on the efficacy and potential immunotoxicity of the therapy. As AAV therapies are intended for long-term gene expression, any impurities generated in the manufacturing process that could elicit a negative immune reaction should be controlled. The importance of the control of impurities has been well established, as AAV capsid–specific $CD8^+$ T cells have been shown to have a detrimental effect on the efficiency of AAV-based gene therapies [2]. In addition, low capsid stability and solubility can result in an ineffective gene therapy [3].

Measuring CQAs

Being able to accurately measure these CQAs is key to implementing a successful QbD approach to the manufacturing of AAV vectors. There are a variety of methods to measure these key CQAs that should be evaluated for their suitability at different stages or scales of production. For example, factors such as the method's throughput and accuracy can be used to determine if and when it should be used in the process. Table 1 outlines some key methods used for the measurement of AAV vector CQAs [4,5,6].

It should be noted that variation in the sample preparation method can also have an impact on the accuracy of the measurement method [4]. Often, sample preparation is not standardized and is overlooked as a source of variability and error in both analytical and process methods. As such, standardization of analytical sample preparation should be implemented as much as possible with clearly defined steps, controls, and checks to help reduce variability and potential inaccuracy.

Currently, many analytical workflows used for AAV vector development and manufacturing processes are low-throughput with long turnaround times. For this reason, there are widespread initiatives to implement more efficient high-throughput and in-process analytical technologies—often referred to as process analytical technologies (PAT) [7]. The implementation of robust scalable PAT methods allows for in-line or on-line measurement and control of factors that can affect end-product quality measurements. PAT methods optimally operate without the need to withdraw, manipulate, or dilute samples. These methods effectively decrease result turnaround times, but usually sacrifice some level of precision and accuracy. However, if acceptable levels of precision and accuracy are achievable for the purpose of factor monitoring and control, these in-process methods can allow for the rapid identification of issues with critical process parameters (CPPs), and better control of product variation, and ultimately achieve a more consistent high-quality end product.

Table 1. Measurement methods for key AAV vector CQAs.

CQA	Impact on final AAV vector product	Method of measurement*
Capsid titer	Potency and dosing	ELISA, AEC, SEC-MALS, SLS-DLS
Genome titer	Potency and dosing	qPCR or ddPCR
Genome integrity	Efficacy and potential immunotoxicity	• Empty-to-full capsid ratios: qPCR, ELISA, TEM, AUC, SEC-MALS • Encapsidated impurities: NGS, CE, MS
Aggregate content	Efficacy and potential immunotoxicity	TEM, AUC, SEC-MALS
Biological activity	Potency	*In vitro* transduction of a disease-relevant cell line, infectivity (e.g., $TCID_{50}$)

*ELISA = enzyme-linked immunosorbent assay, AEC = anion-exchange chromatography, SEC-MALS = size-exclusion chromatography–multi-angle light scattering, SLS-DLS = static light scattering–dynamic light scattering, qPCR = quantitative polymerase chain reaction, ddPCR = digital droplet polymerase chain reaction, TEM = transmission electron microscopy, NGS = next-generation sequencing, CE = capillary electrophoresis, MS = mass spectrometry, $TCID_{50}$ = median tissue culture infectious dose.

Factors affecting CQAs

With a QbD approach, it is important to identify and control potential sources of variability in your AAV development and manufacturing processes—these could include cell culture media and supplements, choice of transfection reagent, and plasmid purity.

The choice of cell culture medium and supplements is an important factor to consider in AAV vector production. Many AAV manufacturers prefer to use chemically defined (CD) culture media and supplements that do not contain serum or other animal-origin (AO) components. Due to their heterogeneous and variable composition, AO and undefined components in an AAV workflow can introduce the need for additional testing and control steps to minimize end-product variability and maintain high product quality. Consideration of media and supplement product formats can also be an important factor for reducing potential process variability, and thus streamlining scale-up. Dry media formats that don't require pH or osmolality adjustments upon reconstitution are currently available. These dry formats are often preferred for larger production due to smaller space requirements, longer shelf-life, and the flexibility to reconstitute the needed amount as required to meet production demand.

Transfection is another area of the development and manufacturing processes that should be carefully monitored and controlled; variation could be introduced at this stage depending on the purity of the plasmids and type of reagent used. Understanding and considering the most appropriate type of transfection for your process will help improve product quality, and early consideration of scale-up requirements can reduce the need for transfection process changes when production demand increases. The adoption of alternative cell lines such as packaging or producer cell lines could further reduce the potential for variability by removing the transfection process altogether.

Although there will ultimately be variability to control throughout the development and manufacturing processes, achieving a consistent start point for each run can be key to maintaining consistency throughout. Accordingly, the qualification of your raw material suppliers is a critical step in controlling variability at the start of the process. Choosing a trusted supplier with its own rigorous quality management systems, including robust raw material testing protocols and strict supplier qualification, can help secure the quality and consistency of your raw materials. Highly sensitive testing protocols are particularly important for enabling the identification of low-level raw material contaminants, such as trace metals, that could have a significant impact on AAV vector CQAs.

Lastly, it should be noted that variability and product quality degradation can also be introduced after production, during storage and use. These factors are often not considered until clinical trials have begun. For example, AAV vectors stored in solution can be degraded by a variety of factors, including changes in pH due to low buffering capacity, raw material contaminants, and external factors such as temperature, repeated freeze/thaw cycles, shear stress, and exposure to light.

A simple approach for a complex problem

The benefits of implementing QbD in AAV vector manufacturing processes cannot be overstated. As changes are unavoidable during therapeutics development, this approach is the cornerstone in developing a sound comparability strategy [8]. Undertaking the early consideration, identification, and control of critical factors that can contribute to AAV vector CQA variability is key to building a robust AAV vector production process. This, in turn, can generate sustained high product quality as well as streamline scale-up to help the industry address the accelerated timelines faced by developers.

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