Proposed DRAFT Guidance for FDA Consideration: Testing of Adeno-Associated Viral (AAV) Vector-Based Human Gene Therapy Products for Empty Capsids During Product Manufacture

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Proposed Draft Guidance

I. INTRODUCTION

Reports of serious and life-threatening adverse events in clinical trials involving systemic administration of high doses (~1 x 10^{14} vector genomes per kilogram body weight or higher) of adeno-associated viral (AAV) vector-based human gene therapy products have been the subject of an FDA Cellular, Tissue, and Gene Therapies Advisory Committee Meeting.[1] It is considered likely the reported adverse events may be linked to patient total AAV capsid exposure. One potential contributor to the immunotoxicity observed is the presence of empty AAV capsids in the final gene therapy product. Empty AAV capsids are considered a product impurity as they do not carry genomic material intended to provide a therapeutic effect. This guidance provides sponsors of AAV vector-based human gene therapy products with the recommendation to establish a maximum release criterion for empty AAV capsids to better control immunogenicity that may be due to empty capsid product impurity and provide for improved product safety in the context of systemic administration. This guidance applies to human gene therapy products and to combination products.[2]

This guidance supplements the following final guidance: "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs); Guidance for Industry" dated January 2020 (CMC Guidance).[3]

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[1] Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting #70: Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy, September 2-3, 2021; Available at https://www.fda.gov/media/151599/download

[2] Combination products are comprised of any combination of a drug and a device; a device and a biological product; a biological product and a drug; or a drug, a device, and a biological product; see 21 CFR 3.2(e) for the complete definition of combination product. Combination products are assigned to a lead center for review; see 21 CFR 3.4.

[3] The CMC Guidance is available at this website: https://www.fda.gov/media/113760/download

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance documents describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidance documents means that something is suggested or recommended, but not required.

II. Background

Recent preclinical and clinical experiences with some systemically administered AAV vector-based human gene therapy products highlight the need for additional specific guidance on process-related impurities and product-related impurities. In prior guidance on Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (Ref 1) FDA outlined broad recommendations for reporting manufacturing process and control information on drug substance molecular structure (section 3.2.S.1.2), and impurities (section 3.2.S.3.2) resulting from both the manufacturing process and the product. **Table 1** below summarizes a list of product-related and process-related impurities described in CMC Information for Human Gene Therapy Investigational New Drug Applications (Ref 1) as well as representative examples of additional identified impurities that merit consideration. Here, we wish to expand on the assessment of empty capsid content in AAV products as this may relate to overall vector safety, particularly at higher vector doses administered systemically.

Table 1: Identified AAV Vector Impurities and their Classification

Identified AAV Vector Impurities	Referenced in GT CMC Guidance (Yes / No)	Impurity Classification
Empty capsids	Yes	Product Impurity
Noninfectious AAV	Yes	Product Impurity
Aggregated AAV	Yes	Product Impurity
Replication-competent AAV	Yes	Product Impurity
Encapsidated host-cell DNA	Yes	Product Impurity
Encapsidated helper plasmid DNA	Yes	Product Impurity
Residual host-cell DNA	Yes	Process Impurity
Residual host-cell protein	Yes	Process Impurity
Residual helper plasmid	Yes	Process Impurity
Residual manufacturing reagents (e.g., nuclease)	Yes	Process Impurity
Residual helper virus (e.g., baculovirus, adenovirus, or herpesvirus)	Yes	Process Impurity
Encapsidated partial genome	No	Product Impurity
Encapsidated mutated or methylated full genome	No	Product Impurity
Capsid post-translational modifications (e.g., deamidation, glycosylation, and methylation)	No	Product Impurity

There are a variety of factors that influence AAV vector capsid composition. For example, in both transfection-based and baculovirus-based AAV manufacturing systems, 30-95% of AAV vectors can lack the desired genome at harvest (Ref 2, 3). The packaging efficiency may be higher for helper virus-based infection systems with <5% of all AAV capsids lacking the desired genome at harvest. Various process-related factors, as well as the length of the desired payload, can impact the encapsidation efficiency (Ref 4-7). Typical product-related impurities may include noninfectious AAV particles, unincorporated free AAV viral proteins, aggregated capsid particles, empty capsid particles that lack any genome, capsid particles containing mutated, incomplete, or partial genomes of the desired product, encapsidated impurities (e.g., plasmid DNA, host cell DNA/RNA, helper virus sequences), and capsid particles with non-desired capsid protein post-translational modifications. In prior guidance (Ref 1), we suggested that product-related impurities should be measured as the presence of these impurities may have adverse effects on product quality and safety. Characterization of these impurities early in product development provides important information pertaining to product quality, safety, and consistency in manufacture. If methods for characterizing impurities are not established during the early stages of product development, Sponsors are encouraged to collect sample retains for retrospective measurement when implementing impurities test methods as well as assessing the impact of manufacturing process changes. Here we expand our recommendations for testing AAV vector capsid composition, with a focused emphasis on performing assessments for the presence of empty capsids.

III. ASSESSMENT OF AAV VECTOR COMPOSITION

A. Establishing a Release Criterion for AAV Vector Empty Capsid Impurity

Detailed product characterization involves assessing specific attributes that predict likely clinical benefit as well as ensure product safety. With respect to AAV vector-based gene therapy products, evaluation of vector capsid composition can be informative for both product efficacy and safety based on the proportion of the product that consists of full capsids (carrying therapeutic vector genome / benefit) versus the portion of the product consisting of empty capsids (safety). Given empty AAV vector capsids are incapable of conveying direct clinical benefit as they are devoid of intended payload genetic material, while contributing to the overall immunogenicity of the final product administered to patients, empty capsids are considered a product-related impurity. Furthermore, empty capsids may pose challenges to consistency in product formulation at high capsid concentrations since vector aggregation is driven by total capsid concentration rather than vector genome concentration. It is generally acknowledged that the presence of impurities may adversely impact both product quality and safety.

1. Empty Capsids as a Product-related Impurity

As indicated in the CMC Guidance for Human Gene Therapy Investigational New Drug Applications (Ref 1), it is recommended that Sponsors design an AAV vector manufacturing process capable of eliminating product-related impurities such as empty capsids, and that tests are in place to measure residual impurity levels with

the setting of appropriate limits to the amount of an impurity in the product. It is recommended that impurities be measured throughout the product development life cycle and reported. This will promote product safety, contribute to an overall understanding of the manufacturing process, and provide a baseline reference for performing product quality comparisons following implementation of a manufacturing process change, as warranted.

2. Setting a release criterion that limits the level of empty capsids in AAV vector Drug Substance / Drug Product

Clinical preparations of AAV can vary in empty capsid impurity levels, impacting a patient's total AAV capsid exposure. Empty capsids can exacerbate the innate and adaptive immune responses elicited by the AAV capsid (Ref. 8). Empty capsids may also pose CMC challenges to product formulation. For example, AAV2 is prone to aggregation in low ionic strength formulations (Ref. 9). In volume limited dosing applications where high concentrations of AAV2 are needed (e.g. local administration in the central nervous system), it is advantageous to remove empty capsids to allow for a higher proportion of full capsids (i.e., vector genomes) at soluble capsid concentrations. With these considerations in mind, a manufacturing process should be designed to yield a Drug Substance / Drug Product with as few empty capsids as is achievable.

Analogous to empty capsids in an AAV vector-based product, are non-viable / dead cells in a cell-based product. Dead cells do not contribute a therapeutic benefit when administered and could pose a safety risk when infused due to collection in the microvasculature of vital organs such as the lung impairing their critical physiologic function. Consequently, a minimum release criterion for cellular product viability was established.[4] For somatic cellular therapies, the minimum acceptable viability criterion is generally set at ≥ 70% viable cells for infused, systemically administered products (Ref. 10). When this level of viability cannot be achieved, data should be submitted that support a lower viability specification demonstrating the presence of dead cells and cellular debris do not adversely affect the safe administration of the product and/or its therapeutic effect.

Given the similitude of empty AAV vector capsids to non-viable dead cells as a product impurity, it is recommended that a maximum release criterion of ≤ 30% consisting of empty capsids be established for drug product. Accordingly, ≥ 70% of the product should consist of primarily full genome capsids. In many cases, a small fraction of the product will consist of partial genome capsids. See Part D of this guidance document for more information on analytical techniques for measuring % empty and partial genome capsids. When the level of empty capsids cannot be achieved, data should be submitted demonstrating the presence of empty capsids do not adversely affect patient safety beyond a reasonable risk and/or impair the

[4] Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications: Final Guidance for Reviewers and Sponsors; April 2008. Available at https://www.fda.gov/media/73624/download

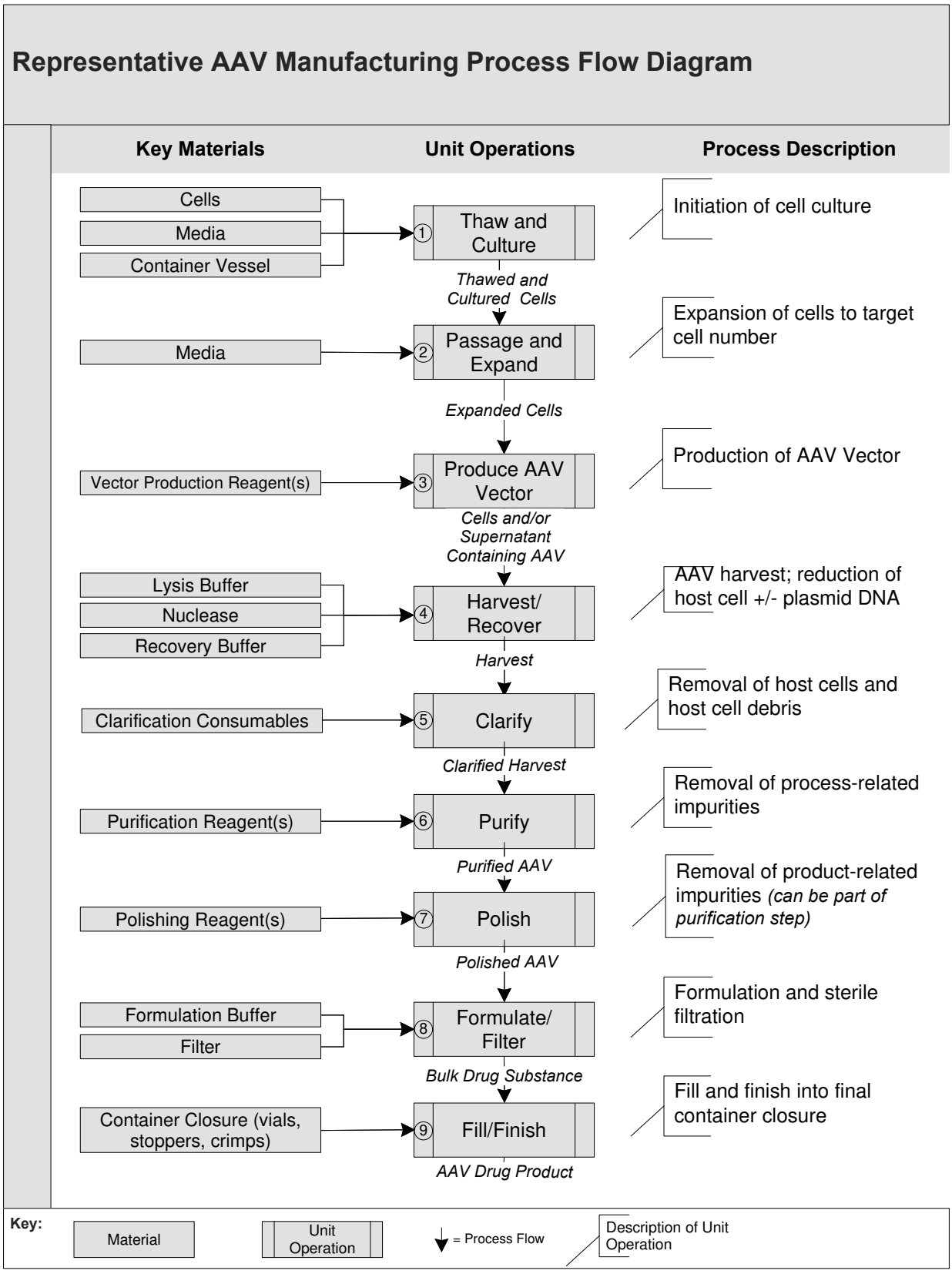
therapeutic effect.

Scenarios in which administering AAV vector-based gene therapy products with an empty capsid impurity level that exceeds the recommended threshold could include directed delivery of lower doses to targeted anatomic locations, or the use of strategies that reduce immunogenic potential of the AAV vector.

B. AAV Empty Capsid Composition: Manufacturing Considerations

Multiple strategies are pursued for the manufacture of AAV, with each platform following a common set of processes/unit operations for vector production and purification. A representative set of processes/unit operations is provided in reference **Figure 1**. Sponsors may choose to include additional processing steps, including the concentration of vector prior to final formulation.

Figure 1: A Representative Process Flow Diagram (PFD) for AAV Manufacturing



AAV product purification can involve techniques, including but not limited to, normal-flow and tangential-flow filtration, affinity chromatography, ion-exchange chromatography, size-exclusion chromatography, hydrophobic interaction chromatography, and ultracentrifugation. Often, a combination of orthogonal purification techniques are employed to provide distinct modes of selectivity based on the physiochemical properties of the vector.

Ultracentrifugation represents one example method often employed for the removal of impurities such as empty capsids for clinical AAV manufacture. Ultracentrifugation utilizes cesium chloride or iodixanol density gradients to separate desired AAV products from impurities, including empty capsids. In general, density-based ultracentrifugation can better resolve and enrich for full capsids compared to chromatography-based methods. However, density-based ultracentrifugation methods have several disadvantages relative to chromatography-based methods. First, ultracentrifugation-based methods are labor intensive and involve several manual processing steps requiring skilled operators. This makes ultracentrifugation difficult to scale and standardize for indications requiring high doses and consisting of large patient populations. Second, centrifuges can generate airborne particles and a suitable facility must be identified to maintain the appropriate cleanroom environment/a low airborne particle count for GMP manufacture. Density-based ultracentrifugation may be better suited for smaller scale studies.

Another method for removal of impurities is chromatography-based purification which relies on the physiochemical properties of the vector, such as the affinity to a ligand, charge, size, and/or hydrophobicity to separate desired AAV products from impurities. Affinity chromatography, for example, is used to purify AAV particles from process-related impurities, including residual host-cell DNA or plasmid DNA in the case of transient transfection-based production platforms. Post-purification, a polishing step, involving ion exchange chromatography, for example, can be implemented for the enrichment of full capsids.

C. Material for Testing

Testing for empty capsids should be instituted early in product development to ensure the manufacturing process is capable of consistently reducing impurity levels such as empty capsids. We recommend characterizing empty capsid composition for the Bulk Drug Substance and/or the Drug Product as part of an overall evaluation of vector impurities which in aggregate may impact product safety. Sampling of Drug Substance process intermediates post-harvest and pre-purification/polishing may provide additional process knowledge regarding the efficiency of full capsid enrichment. When considering whether additional Drug Substance process intermediates will be tested for empty capsids, it is critical to confirm the analytical test method selected is suitable for performing the evaluation as sample matrices can impact the accuracy and precision of a test method.

D. Assays for Measurement and Sample Preparation

Provided in **Table 2** below are potential analytical techniques for characterization of vector capsid composition in Bulk Drug Substance and Drug Product. Note, several of these methods may not give accurate and reproducible data with unpurified samples. The recommended release criterion of ≤ 30% empty capsids is based on specific analytical techniques such as analytical ultracentrifugation (AUC) or charge detection mass spectrometry (CDMS). AUC and CDMS provide accurate and precise measurements of capsid content in purified samples (Ref. 11). See **Table 1 in Appendix 1** for a description of the relative advantages and disadvantages of each method listed for characterizing empty capsids in the Drug Substance and/or Drug Product.

When selecting a method for characterization, sponsors should demonstrate the assay is fit for purpose by assessing attributes such as accuracy, precision, range, linearity, limit of quantification, limit of detection, and specificity.

An important feature to the success of assays used to measure empty capsids in AAV products is quality of the sample preparation. Careful consideration should be made to identify sample preparation steps that will convert the sample into a form that is compatible with the intended analytical method and suitable for measurement. For example, sample preparation steps can include the use of nuclease to reduce the presence of residuals such as host-cell DNA that may interfere with sample measurement.

Table 2: Potential Analytical Techniques for Measuring AAV Empty Capsids

Assay	**Common Abbreviation**
Charge Detection Mass spectrometry	CDMS
Enzyme-linked immunosorbent assay + quantitative / (droplet) digital polymerase chain reaction	ELISA + qPCR/(d)dPCR
Size exclusion chromatography with multi-angle light scattering (in combination with UV and differential refractive index)	SEC-MALS
Transmission electron microscopy	TEM
Analytical ultracentrifugation	AUC

IV. REFERENCES

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V. APPENDIX 1: ASSESSMENT OF ANALYTICAL TECHNIQUES FOR MEASURING FULL AND EMPTY AAV CAPSIDS IN PRODUCT

Table A1: Comparison of Several Analytical Techniques Used to Measure Full and Empty AAV Capsids in Drug Substance and/or Drug Product[1]

Evaluation Criteria	Throughput	Ease of Implementation	Material Requirement	Partial Genomes	Accuracy/Precision
	Sample preparation and throughput	*Technical difficulty of method and availability of equipment*	*Low material requirement/assay sensitivity*	*Resolves partial genomes*	*Accuracy and Precision of the analytical technique*
CDMS	(+); requires a purified sample that is buffer exchanged into a compatible solvent for mass spectroscopy	(-); relatively new technique requiring specialized equipment	(++); low volume and capsid concentration requirement	(++); can resolve partial genomes based on mass	(++); measurement is accurate and precise
ELISA: qPCR/(d)dPCR	(+); qPCR/(d)dPCR requires DNAse treatment and protein denaturation	(++); analytical techniques are commonly a part of an AAV testing panel	(++); low material requirement for ELISA and qPCR/(d)dPCR	(-); can't resolve partial genomes	(-); ratio of two methods that each have their own coefficient of variance
SEC-MALS	(++); no additional preparation needed	(+); relatively common equipment	(+); high capsid concentration needed	(-); can't resolve partial genomes	(+); relatively accurate and precise
TEM	(-); requires sample staining and is low throughput	(-); requires specialized equipment	(++); relatively low volume requirement	(-); can't resolve partial genomes	(-); small sample size
AUC	(-); limited number of samples run in one experiment	(-); requires specialized equipment	(+); specific capsid concentration range required	(++); can resolve partial genomes based on density	(++); accurate and precise technique

[1]Table adapted from Werle, et. al., "Comparison of Analytical Techniques to Quantitate the Capsid Content of Adeno-Associated Viral Vectors" (2021). https://doi.org/10.1016/j.omtm.2021.08.009 and Gimpel, et. al., "Analytical methods for process and product characterization of recombinant adeno-associated virus-based gene therapies" (2021). https://doi.org/10.1016/j.omtm.2021.010
(-) = disadvantage to the assay; (+) = advantage to the assay.