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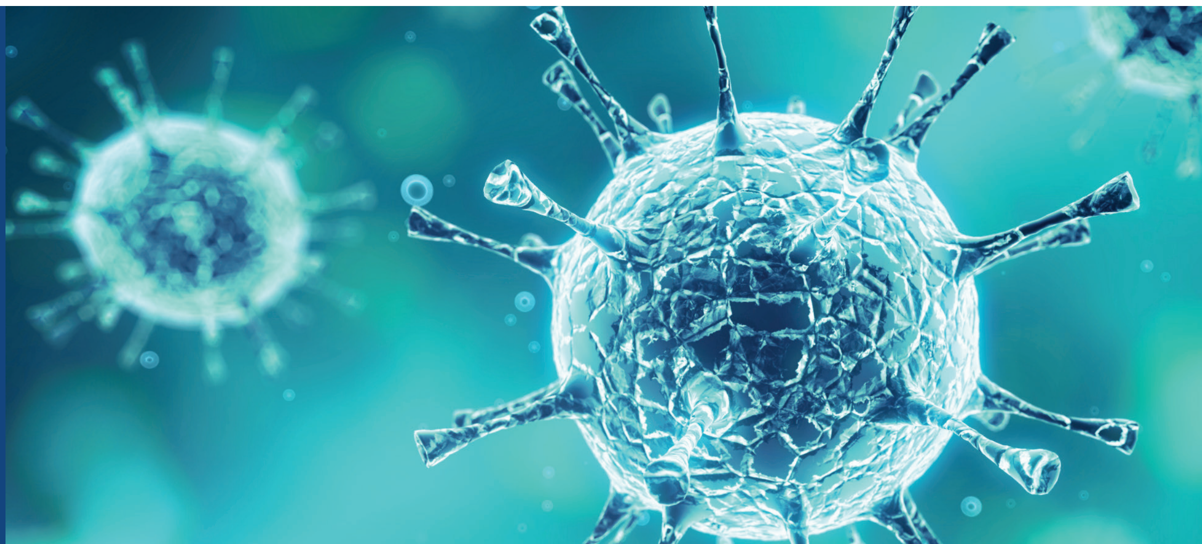
Summary
Recombinant AAV products are becoming increasingly important in the realm of gene therapies. Charles River provides specialized testing packages for the characterization and quality control testing of these products.



BIOLOGICS TESTING SOLUTIONS

Recombinant Adeno-Associated Virus Product Support Services

Recombinant adeno-associated viruses (rAAVs) are becoming the vector of choice for delivering therapeutic transgenes. The resulting products are currently being investigated for the treatment of various conditions, including cancer and ocular, pancreatic and central nervous system diseases. rAAV therapies work by introducing new genes into a patient's cells to replace a malfunctioning gene. rAAV-mediated gene therapies are ideal since, after infection of the host, the delivered genes do not incorporate into the host genome, but instead, the DNA remains in the extrachromosomal state. This DNA is then transcribed as the other genes in the cell but does not replicate when the cell undergoes division. Especially of interest to the CNS field is the rAAVs' ability to infect not only replicating but also quiescent cells, particularly neurons. Recombinant AAV gene therapies have shown strong evidence of efficacy and safety in a large number of animal models and clinical trials.

This unique therapeutic approach requires a specialized testing package for the characterization and quality control (QC) testing of AAV particles and constituent capsid protein assemblies. Some of these methods are already available for use, while others may require optimization and ICH validation prior to being implemented in a quality control testing program. Testing that is part of a standard characterization package for rAAV gene therapies is described below.

Mass Spectrometry

Many different mass spectrometry methods can be employed for the characterization of rAAV therapies, spanning from strain confirmation to virus particle (VP) quantitation and glycan profiling.

Peptide mapping with LC-MS/MS of the entire digested product is commonly used for both characterization of the rAAV therapy and as a stability-indicating QC method. Quantitation of each virus particle (VP) may be performed via a digestion/mapping approach, in which a unique VP peptide is quantified after normalization by means of a spiked synthetic heavy peptide as an internal standard (IS). This type of analysis is performed using a triple quadrupole MS and may be preferable over amino acid analysis testing (AAA). Additionally, once the viral capsid proteins are reduced/dissociated from one another, intact molecular weight (MW) via LC-MS provides useful characterization data.

EVERY STEP OF THE WAY

Glycan profiling of viral envelope proteins (e.g., VSV-G) on the surface of the enveloped virus particles should be included in characterization package. This profiling can include O-linked glycosylation as well as N-linked. Quantitative sialic acid analysis and quantitative monosaccharide analysis are also commonly employed to characterize the glycan population of gene therapy products.

Biophysical Characterization (Higher Order Structure Analysis)

Biophysical methodologies may be used for characterization as well as QC testing of rAAV based products. Specially developed analytical ultracentrifugation (AUC) methods can be used to determine empty versus full capsid ratios, as this is a key quality measurement for gene therapy products.

Stability-indicating methods will focus on particle quality and size, using methods which can resolve particle aggregation and particle degradation. Differential scanning calorimetry (DSC) is used as a stability-indicating method, demonstrating stability as a function of formulation and for comparability of batch-to-batch. Dynamic light scattering (DLS) is also useful for particle size distribution and characterization.

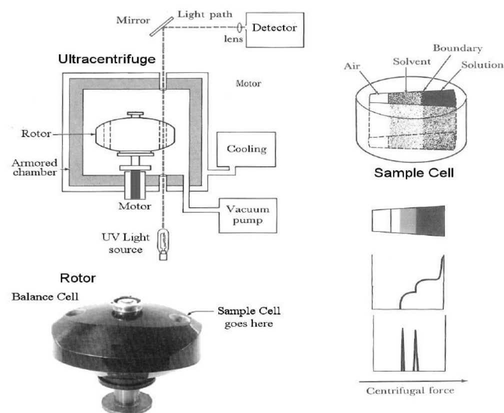


Figure 1: Sedimentation velocity analytical ultracentrifugation (SV AUC) is a promising method as applied to measurement of empty-full AAV ratios, because both empty and full particles are in principle resolved in the same experiment, and requires minimal sample manipulation. *Image courtesy of Beckman Coulter.*

Analytical Testing

Along with mass spectrometry and biophysical methods, there are a variety of other analytical techniques that can be used for analysis of rAAV-based gene products. These include chromatographic, electrophoretic and ELISA-based assays. The unique nature of rAAV products requires a customized testing package to meet clients' specific needs. Some testing that may be included in this package is described below.

A variety of HPLC methods can be incorporated into an rAAV testing plan. Reverse-phase high-performance liquid chromatography (RP-HPLC) may be used for QC and characterization of capsid proteins. Following reduction/dissociation of capsid proteins from one another, RP-HPLC may be used to generate percent ratios of virus particles (VPs). Anion exchange HPLC is employed for separation and quantification of supercoiled versus non-supercoiled plasmids. Percent supercoiling is often requested by the FDA, as it is believed to correlate with plasmid infectivity.

ELISA assays may be applied as orthogonal methods to quantify viral coat or capsid proteins. This can be coupled with a prior step to fractionate the analyte based on size to remove monomers or fragments of the capsid protein which may give a positive ELISA response. Quantitation of proteins by amino acid compositional analysis (AAA) is also common.

N-terminal sequencing by Edman degradation is an industry standard method commonly used to verify the N-terminal integrity of capsid proteins. Initial separation of capsid proteins via SDS-PAGE or RP-HPLC may be used, and additional information such as capsid N-terminal truncations may be evaluated.

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Other techniques that can be incorporated into testing plans include SDS-PAGE or CE-SDS for analysis of non-reduced versus reduced virus-like particles (VLPs). Residuals testing should also be performed for these gene therapy products. Polyethylenimine (PEI) is often used as a transfection agent in gene therapy to promote plasmid entry into cells, and it is important to analyze for residual linear as well as branched PEI, along with other residuals and impurities that may be introduced during your process.

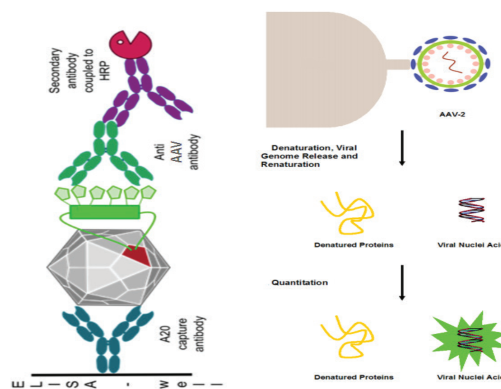

Figure 2: Present methods for quantifying the amount of AAV protein and nucleic acid, and therefore the empty-full ratio. Protein is often quantified by ELISA, while nucleic acid by qPCR. A more recent method utilizes the A260/A280 ratio of processed AAV samples [1]. *Image courtesy of www.progen.de.*