# ASSESSING CRITICAL QUALITY ATTRIBUTES OF VIRAL VECTORS

intertek
Total Quality. Assured.

Mark Redfern - Cell and Gene Therapy Team Leader, Intertek Pharmaceutical Services, describes key considerations for the characterization of viral vectors

## Challenges

The inherent complexity of viral vector-based products makes their physical and biological characterization highly challenging. From a regulatory perspective, an understanding of viral vector critical quality attributes that impact product safety, purity, and potency, mean that specific, robust analytical assays are needed.

## Addressing key attributes

Adeno-associated viruses (AAVs) are the most commonly used type of viral vector applied in gene therapies in trials to date [1] and determining the presence of capsids that are either empty or contain DNA other than the desired full-length vector genomes (including wild-type AAV [wtAAV] DNA sequences) is key to ensuring purity and for improving the downstream process of large scale vector production. Empty capsids present a source of unnecessary, potentially antigenic material, possibly inducing or elevating capsid-triggered anti-AAV immune responses [2].

There are a range of methods that can be applied to quantify full versus empty capsids (Table 1) which include Analytical Ultra Centrifugation (AUC), cryo-TEM, qPCR/ELISA, A260/A280/ absorption at 260nm, and HPLC [SEC, Ion exchange and affinity columns].

| METHOD | ADVANTAGE | DISADVANTAGE |
|---|---|---|
| Electron microscopy (EM) / Cryo TEM | Single experiment Robust, Accurate good for comparative studies | Labour intensive, potential for complexity |
| qPCR/ELISA | | Indirect, relies on data from two different methods plus uncertainty stems from whether the ELISA is only detecting complete vector particles or if it also interacts even weakly with free capsid proteins |
| A260/A280 / absorption at 260nm | Rapid | Measures DNA to protein ratio, assumes all DNA/ Protein is associated to the capsids therefore requires highly purified capsids |
| HPLC [SEC, Ion exchange and affinity columns] | Single experiment | Very sensitive to impurities and the nature of buffer formulation |
| Analytical Ultra Centrifugation (AUC) | Superb mass-resolution – can distinguish / quantify the different AAV species Does not require standards | Validation can be challenging Low throughput |

Table 1: Analytical methods to quantify full versus empty capsids

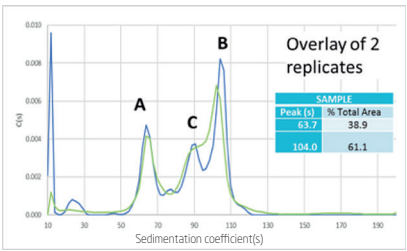


Figure 1: AUC data for an AAV sample containing (A) empty capsids, (B) full capsids and (C) other species are partially filled capsids containing an incomplete DNA load.

## Analytical techniques

Analytical ultracentrifugation (AUC) enables characterisation of the homogeneity of a vector preparation and determination of complete, empty, and partially packaged capsids (Figure 1). Benefits of AUC include good mass resolution with samples analyzed directly in a buffer/formulation of interest so there are no interactions of sample with chromatography media to consider when analysing data. It is possible to look at noncovalent/ weak associations and provide information about particle shape and conformation. Results depend on effective fitting of a model, input parameters and fitting procedures so these must be carefully controlled.

Cryo-TEM can accurately discriminate and quantify particles containing partial genomes from full/empty particles and is now routinely used to characterize the composition of AAV vector preparations. Cryo-TEM can clearly visualize viral vectors in their native state and distinguish between full and empty capsids allowing for accurate statistical analysis (Figure 2).

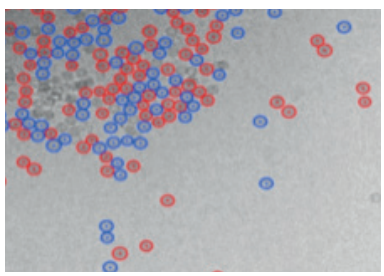

Figure 2: Cryo-TEM Image analysis of filled (red) and empty (blue) capsids allowing for statistical evaluation

### References


1. Ginn, S.L. et al, Gene therapy clinical trials worldwide to 2017: An update, J Gene Med., 2018;20, :e3015
2. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Draft Guidance for Industry Last accessed April 01 2019


## Intertek's viral vector characterisation expertise

Our experts have worked on multiple studies involving Adenovirus, Adeno- Associated Virus (AAV) and Lentivirus based products. With a wide range of expertise and technology in-house, we deliver comprehensive characterisation packages or specific services:

- Aggregation analysis (AUC, DLS, SEC/HPLC, Cryo-TEM)
- Empty vs full capsid analysis (Cryo-TEM)
- AAV capsid purity (CE-SDS)
- icIEF – Charge heterogeneity
- Transgene expression (RT qPCR, ELISA, Flow cytometry)
- Infectious genome titre (qPCR, ddPCR)
- Viral titre (TCID50)
- Host cell DNA (qPCR, ddPCR)
- Residual impurities
- Next-generation sequencing
- Cell-based potency with a range of read outs (Biochemical endpoints and/or flow cytometry)

## Your Total Quality Assurance partner

We can support your product development from early-stage, through to in-process control and product release assays. Our experts are adept at developing, optimising, qualifying and validating methods. We also have significant experience in method transfer. With a heritage of supporting advanced pharmaceutical product development, coupled with a comprehensive range of analytical technology, our experts offer Total Quality Assurance expertise to help you ensure the safety, efficacy and quality of your product.


### About our expert

**Mark Redfern Cell and Gene Therapy Team Leader**

Mark has 20 years' experience in the analysis of biopharmaceuticals and vaccines, including method development, validation, and QC release testing of licensed products. His wide range of experience covers live viral and bacterial products, therapeutic DNA together with classical recombinant proteins and monoclonal antibodies