intertek
Total Quality. Assured.

CELL AND GENE THERAPIES ANALYTICAL DEVELOPMENT SERVICES

CMC Development Support, Characterisation, Stability and Quality Control Services

GLP / GCP / GMP laboratories at Intertek's Centre of Excellence for Biologics and Gene Therapy Development Support

Analytical Development and Chemistry, Manufacturing and Control (CMC) Support

The complexity of cell and gene therapies and their mode of action present many challenges to development. Our experts provide analytical development and routine testing to help you meet regulatory expectations for safety and efficacy.

Cell and gene therapies are complex medicines for human use that are based on genes, tissues or cells. In addition, these may also be incorporated into a delivery technology or a medical device, for example, cells embedded in a biodegradable matrix or scaffold and are referred to as combined advanced medicinal therapy products. The wide variety of products within this category and their inherent complexity means that each will present different analytical development challenges, and so specific characterisation, potency, purity and identity assays are required for each product.

Regulatory requirements for these classes of medicines are evolving in the EU and US currently and further guidance documents are expected in the near future. From a best practice perspective, there is an expectation for orthogonal analytical techniques to be applied to characterisation, stability and release testing, with methods adapted to deal with, typically, small sample volumes.

CELL AND GENE THERAPIES

>800
INDs ON FILE WITH FDA

>200
ANTICIPATED INDs RECEIVED PER YEAR TO 2025

18
APPROVED TO DATE

SOURCE: FDA.GOV [1,2]

Expert analytical services for Cell and Gene therapies

Our bespoke, purpose-built GLP / GCP / GMP laboratories have supported developers and manufacturers for over 20 years through the provision of advanced characterisation programs. We have worked on multiple studies involving cell or gene therapies, mRNA and plasmid DNA based products. With specialist laboratory facilities, we can handle Class I and Class II Biological Agents. The facility has been designed to handle recombinant genomic materials for the purposes of research and compliant testing.

Our solutions:

- Cell and virus characterisation
- Virology assays
- Host cell and residual plasmid DNA
- Cell-based assays / potency testing
- General compendial testing
- GMP analysis
- GCP/GLP bioanalysis
- ICH stability storage and testing
- Method development and validation
- QC release testing
- Advanced delivery technology analytical support

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 CMC packages and specific services.

Our 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)
- Digestion of isolated proteins followed by LCMS and / or MALDI-MS to give detailed information to assist identification of viral proteins
- Infectious Genome Titre (qPCR, ddPCR)
- Viral Titre (TCID50)
- Host Cell DNA (qPCR, ddPCR)
- Residual Impurities
- Next-Generation Sequencing

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 for each particular class of cell and gene therapy. 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.

CASE STUDY - COMPARISON OF qPCR AND ddPCR FOR ANALYSIS OF RESIDUAL DNA

A challenge with some viral based gene delivery systems, such as Adeno-associated viruses (AAVs), is that they can package host cell and plasmid residual DNA (rDNA) inside the viral capsid. To support the development of safe and effective therapies, and in line with regulatory requirements, a sensitive, fast, and cost-efficient method of quantification is essential. Using HEK293 and E. Coli rDNA quantification assays, a comparison was undertaken, to assess quantitative PCR (qPCR), an industry standard approach, and digital droplet PCR (ddPCR), a more recent alternative, to assess each technique in terms of sensitivity, reproducibility and technical challenges (Table1).

	qPCR	ddPCR
Detection Principle	Measures PCR amplification as it occurs.	Measures the fraction of negative replicates to determine absolute copies
Absolute quantification	No, relative to the standard curve	Yes, sample partition eliminates the need for std curve
Multiplexing	Duplex assays Wide selection of detection chemistry	2 to 4
Reproducibility	Good	Excellent
Prep. Time	>1.5hours	>5.5hours
Cost/ sample	Low	High

Table 1: Comparison of qPCR and ddPCR methods for rDNA quantification

In summary, both qPCR and ddPCR are robust techniques for analysing rDNA. qPCR has high sensitivity, accuracy, precision, and time-saving benefits, whilst ddPCR offers improved precision without the dependence on a calibration and reduced interference of PCR inhibitors. Download the webinar "Comparison of qPCR and ddPCR techniques for residual DNA quantification " for full insights to the methodologies and results of this case study.

WEBINAR: Comparison of qPCR and ddPCR techniques for residual DNA quantification.
Download here: http://bit.ly/rDNA-quantification

CASE STUDY - PURITY: RATIO OF EMPTY TO FULL CAPSIDS

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 as they present a source of unnecessary, potentially antigenic material, possibly inducing or elevating capsid-triggered anti-AAV immune responses. There are a range of methods that can be applied.

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.

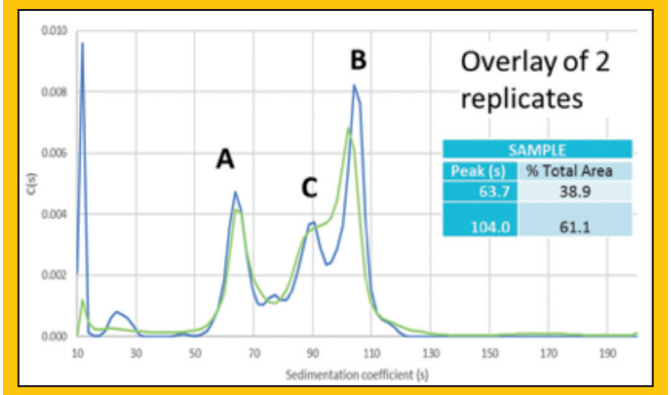

Figure 1: AUC data for an AAV sample containing (A) empty capsids, (B) full capsids and (C) other species are partially filled capsids containing an incomplete DNA load

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).

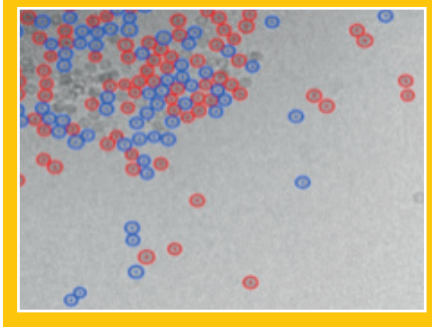

Figure 2: Cryo-TEM Image analysis of filled (red) and empty (blue) capsids allowing for statistical evaluation

References

1. Gottlieb, S., Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies, January 2019, http://www,fda.gov.uk

2. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products, 24 July 2020

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