BioReliance®
Pharma & Biopharma
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White Paper

Accelerating the Time to Market of Gene Therapies through use of a Platform Approach to Characterization and Safety Testing

The Alliance for Regenerative Medicine cites a total of 362 active gene therapy clinical trials and a similar number for gene-modified cell therapies. With recent approvals in both modalities, expansive and growing pipelines and substantial global financings of companies in the field, the pace at which these important new therapeutics will reach the market will continue to accelerate. It has been estimated that by 2025, the FDA will be approving 10 to 20 cell and gene therapy products per year (1).

As gene therapies and gene-modified cell therapies show increasing promise, the need for innovative and proficient viral vector manufacturing continues to grow. Increased regulatory guidance governing the manufacturing and testing of viral vectors adds complexity and increases the timelines to proficient viral vector manufacturing and analytical testing. Using adeno-associated virus (AAV) as one example, this white paper will describe how the implementation of platform characterization and safety assays for gene therapy vectors can increase the likelihood of success in process validation and accelerate the timeline to commercialization for gene therapy products and delivery to patients in need. These platform assays allow for specific parameters to be customized (e.g. gene of interest) and require minimal additional qualification work to maintain the validated state. Because these assays are pre-qualified, they reduce the variability inherent in assay validation and subsequently the time needed to establish readiness for regulatory compliance. While this approach increases the standardization across the testing workflow, it remains flexible and able to address the specific needs of developers and manufacturers. Additionally, the approach is as future-proof as possible, allowing for adaptability as the regulatory landscape of gene therapies evolves.

Characterization and Safety Testing

AAV is a non-enveloped virus that can be engineered to deliver DNA to target cells and is one of the most frequently used gene therapy vehicles. The ability to generate recombinant AAV particles lacking any viral genes and containing DNA sequences of interest for various therapeutic applications has proven to be one of the safest strategies for developing gene therapies. That being said, the viral nature of the therapy and the route of administration – directly into the patient's bloodstream or tissue – sets a high bar for characterization and safety testing (**Figure 1**).

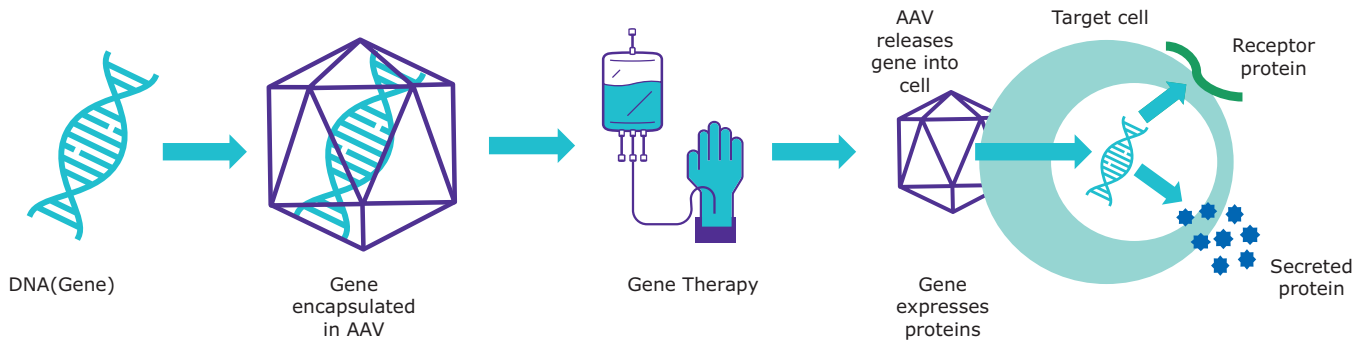

Figure 1. With the route of administration of a gene therapy being directly into the patient's bloodstream or tissue, specialized testing is needed to ensure drug safety.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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Due to the uniqueness of gene therapy products, customized methods are developed and validated to address safety and characterization, extending timelines in the progress towards the clinic. In a therapeutic area where time is of the essence for patients with limited or no therapeutic alternatives, or with a limited window for the therapy to deliver the desired benefit, development of new analytical methods remains frustratingly slow and costly. In contrast, utilization of pre-developed, platform assays to assess safety and characterization eases the burden of ensuring product quality and shortens overall commercialization timelines. This approach, which combines a fully validated generic method with product-specific elements, can shorten the time to get to a GMP assay while still being compliant.

Creating a Platform Approach

Regulatory authorities expect the critical attributes of identity, purity, potency and freedom from residuals to have been addressed in characterization and release of gene therapy products in order to assure product quality and safety (**Figure 2**). As described below, there are well-established, platform approaches for addressing each of these attributes, and these approaches can be modified as needed for specific applications. Importantly, genomic titer, infectious titer and product purity assays can be shifted from a fully custom to a semi-custom approach while maintaining the validated state and can be easily modified to meet the requirements of a specific AAV vector. These semi-custom assays strike the appropriate balance of speed and customization for the biosafety testing programs supporting the manufacture of gene therapies.

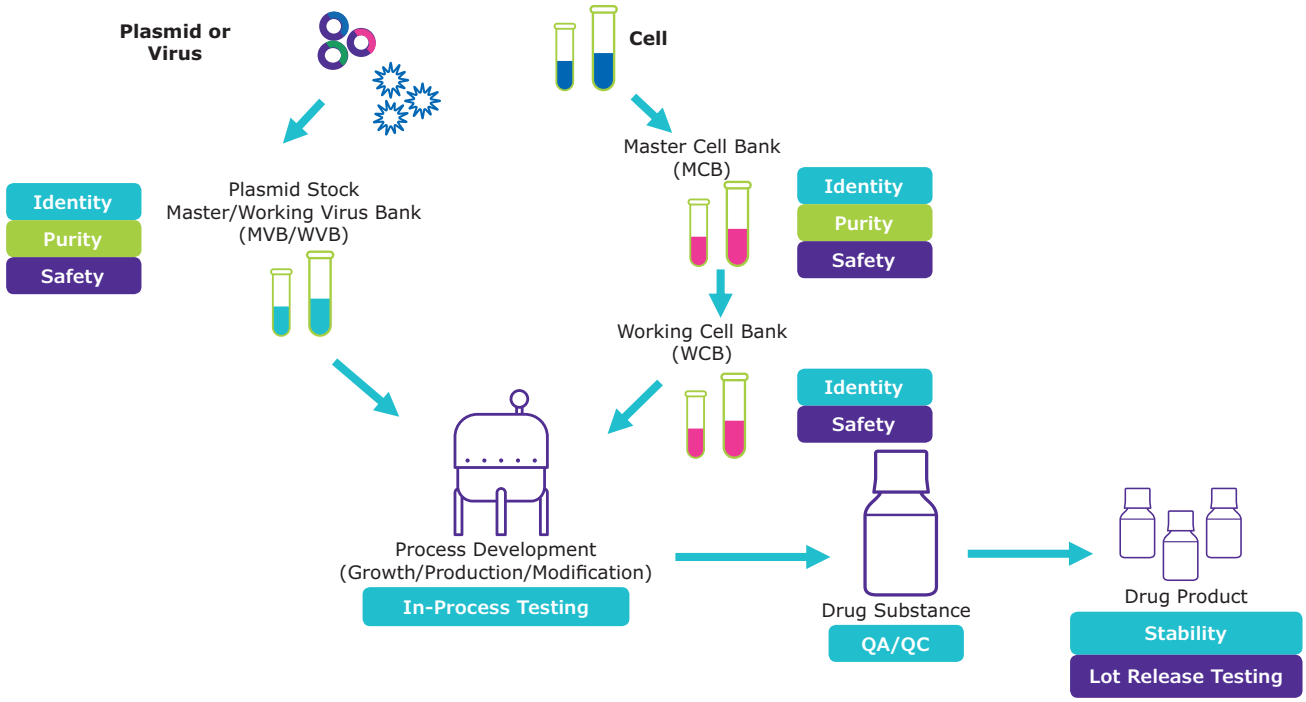

Figure 2. At each step of the gene therapy manufacturing process, critical attributes must be assessed.

Identity

Simple methods to confirm identify of the viral vector and transgene may use a common element such as serotype-specific capsid proteins for ELISA or similar antibody-based detection methods, while a promoter or region of the vector common across the platform may be used for genotypic identification (**Figure 3**). As the gene therapy company moves into later development and toward filing, the gene of interest or transgene identity should be confirmed. Use of a platform approach to PCR-based methods allows for rapid change of primers and probes within a validated method to meet the specificity required. In this instance, the PCR method has been validated as an identity assay in compliance with ICH Q2 requirements and a change in primers for specific regions can be achieved by qualification and demonstration of meeting the method specifications.

Genotypic	Phenotypic
Custom PCR	ELISA
Generic PCR	Mass spec
Sanger	HPLC
NGS	Cell surface markers (e.g. FACS)
DNA fingerprinting	
Short tandem repeat analysis	

Figure 3. Genotypic and phenotypic assays to assess and confirm identity of the viral vector and transgene.

Purity

Assays used to assess purity provide verification that the product is free from impurities and adventitious agents including bacteria, fungi, and mycoplasma (**Figure 4**).

These assays also identify possible contaminants such as related vectors or replication competent viruses, which must be confirmed to be absent.

Sterility

- BACT/ALERT® 3D Microbial Identification System
  - Detects changes in pH due to bacterial growth
  - Real time sample monitoring
  - Objective readout

Mycoplasma

- PCR
  - Equivalent sensitivity and specificity to compendial method
  - GMP and EP 2.6.7 compliant
  - TAT and sample requirements better suited for cell therapies

Adventitious Virus

- Detection and identification of adventitious agents
- Circumvents toxicity and neutralization issues
- Can be combined with ID test

Replication Competent Virus

- Cellular assay
- Three or more rounds of amplification
- CPE, qPCR or QPERT endpoint

Figure 4. Many of the methods used in biopharmaceutical testing for impurities are applicable to gene therapy products.

A number of methods can reduce the time required to ensure the purity of and release for AAV therapies, as well as the critical components of the manufacturing process; these include rapid sterility testing and nucleic acid-based mycoplasma testing. The BacT/ALERT® 3D system has been validated for both the compendial, two media sterility test and the recently published EP 2.6.27 method suitable for Microbiological Control of Cellular Products; both methods shorten the overall time-to-result by half. The qPCR method for mycoplasma detection has been validated in accordance with the EP 2.6.7 guidance; this GMP method speeds the time to result to almost a quarter of the time required to perform the traditional, compendial culture method.

These methods, as well as next generation sequencing for identification and detection of adventitious agents, are available for use in any gene therapy project. In addition, freedom from replication-competent virus can be demonstrated using the *in vitro* procedure for amplification of replication-competent Adeno-Associated Virus (rcAAV) in cell culture.

During manufacturing of viral vectors for gene therapy, incomplete particles are also produced. These empty particles lack recombinant viral genomes and consist of capsid proteins only. Empty capsids increase the required dose of virus for medical applications and are thought to cause immunological reactions against the vector capsid, leading to unwanted side effects.

It is important to distinguish between these variants and ensure similarity from lot to lot. More importantly, regulatory agencies request quantitation of empty vector particles in a formulation for product characterization and before release for patient dosing. Analytical ultracentrifugation can differentiate and quantify empty versus full particles in an AAV prep.

Additional analytical methods for assessment of the purity of AAV lots include qPCRs against host cell sequences and helper virus, replication-competent virus tests for helper virus including HSV and dynamic light scattering to evaluate the degree of aggregation in a sample. The latter can also be used to aid buffer formulation and in stability testing. A summary of product-related impurities and related tests is provided in **Table 1**.

Product Related Impurity	Nature of Lot Release Test Used
Empty Capsids	Chromatography (IEX), Ultracentrifugation; EM
Nuclease resistant Host cell DNA (encapsidated)	qPCR to target generic host cell sequences or specific sequences of concern e.g. AdE1
Nuclease resistant helper DNA (plasmid)	qPCR to target generic helper virus sequence
Replication competent Virus	Various depending on Vector system
Non-infectious particles	Total viral particle (VP): infectious unit (IU)
Aggregated, oxidised, degraded vector	Size exclusion chromatography, electrophoresis, DLS and others.

Table 1. Gene therapy product-related impurities and options for lot release testing.

Potency

Potency is specific to each gene therapy and is the determination of how well the product performs *in vitro* and may be based on the mechanism of action of the transgene product or an effect of the transgene in the target tissue. Assessment of potency is critical to show consistency in the product and manufacturing process and for lot release. In some cases, the assay is used to determine dosing amounts for clinical trials and commercial products.

Potency assays must be:

- Sensitive enough to discriminate small differences in biological activity and stability
- Quantitative over a range of treatment concentrations
- Easy to use and robust, suited to consistently and accurately measuring the biological effect
- Available with relevant controls and appropriate data analysis methods

One method for measuring potency is to assess the amount of infectious titer present in the virus stock either by biological activity (median tissue culture infectious dose, $TCID_{50}$) or particle enumeration by PCR. While potency assays must be customized for each individual product, their design and validation can be accelerated with use of a platform approach.

$TCID_{50}$ is used to determine the dose of virus that when applied to cell cultures, leads to 50% of the cultures becoming infected. The assay utilizes HEK293, CI886 or HeLa cell lines grown in 96-well plates and infected with replicate 10-fold serial dilutions of AAV vector. After infection, various endpoint methods can be used to measure activity including qPCR, immunofluorescence and plaque formation. Several elements of the $TCID_{50}$ assay are universal in nature and as such, can be standardized; these platform elements include the serial dilution, cell culture parameters, inoculation, harvesting and analysis. To allow flexibility with different vector modalities, the AAV serotype, AAV reference, cell lines susceptible to the viral vector and qPCR primers can all be varied in the generically validated method. Building a custom assay upon these universal, platform elements allows the validation qualification of the final method to be as streamlined as possible.

Similarly, development of assays used to measure genomic titer – the concentration of viral particles containing viral genomes – can also benefit from a platform approach as described in the example below using droplet digital PCR.

Droplet digital PCR (ddPCR) is a hybrid methodology which utilizes aspects of both standard PCR and qPCR and produces data of superior accuracy of quantitation. Absolute quantitation is achieved by utilizing fluorescent-based technology coupled with reading at reaction end-point, instead of during the exponential amplification.

Assays for AAV titering have been validated using representative viral serotypes of known titer and generic primer sets developed for AAV targeting the CMV promoter. Vectors containing this target may utilize this off-the-shelf method after performing a suitable phase-appropriate qualification of the assay on the specific product. Vectors which do not contain these common elements may undergo product specific qualification whereby a product specific primer set may be introduced into the process and appropriately qualified.

Residuals

Manufacturing processes are designed to minimize or remove process- and product-related impurities. Regulatory agencies recommend monitoring residual impurities using appropriate methods throughout product development to define product specifications and assure product safety. Testing for process-related impurities may include cell substrate proteins and nucleic acids as well as reagents used in the manufacturing process. For certain impurities such as cell substrate DNA, regulatory guidance documents have specific recommendations on the limits of amount and size of DNA that can be present per dose. Examples of tests used to monitor various impurities are listed in **Table 2**.

Product Related Impurity	Nature of Lot Release Test Used
Host cell protein	Immunoassay using HCP specific antibodies (ELISA, chemiluminescent IA)
Host cell DNA	qPCR to target generic host cell sequences or specific sequences of concern e.g. AdE1
Residual cell culture related components e.g. BSA, HSA	Various approaches, however immunoassay common for abundant proteins such as BSA, HSA
Residual process reagents e.g., Benzonase® nuclease, chromatography ligands, Polyethylenimine (PEI)	Various approaches, however immunoassay common
Residual plasmid DNA	qPCR targeting plasmid sequence (non-vector)

Table 2. A range of tests are available to measure levels of process- and product-related impurities.

Conclusion

Unlike monoclonal antibodies and other protein-based biologics for which testing methods are common and have been standardized, methods for the different viral vectors used in gene therapies often have differing non-standardized requirements. And while the number of assays needed for safety and characterization of the viral vectors may seem overwhelming and time consuming to develop and validate, use of platform assays can accelerate timelines and serve as the foundation for custom assays (**Table 3**).
This approach allows the developer to get to a GMP method much faster, which translates to increased speed into the clinic.

Unpurified bulk	Purified bulk	Formulated & vialed final lotsw
Identity: GOI	**Identity:** GOI, ELISA, Vector genome	**Identity:** GOI
Titer: $TCID_{50}$ of viral vector	**Titer:** $TCID_{50}$ of viral vector, Genomic titer	**Titer:** $TCID_{50}$ of viral vector, Genomic titer
Purity: • Bioburden • Mycoplasma • Mycobacterium • Adventitious viruses (*in vitro* & *in vivo*) • Replication competent AAV (rcAAV)	**Potency:** Expressed protein, function **Purity:** Sterility, Endotoxin, rcAAV **Residuals:** • Residual host cell DNA • Residual DNA size distribution • Host cell protein, Residual BSA • Residual AAV Affinity Ligand • Purity by SDS PAGE • Empty: Full Capsid	**Potency:** Expressed protein, function **Purity:** Sterility, Endotoxin **Product characteristics:** • Vector aggregates • Osmolality • pH • Extractable volume • Appearance • Particulates

Table 3. A full testing panel for AAV bulk and final lots. Assays listed in blue represent those for which a platform strategy can be applied, saving time and reducing costs.

To reduce the complexity of analytical method development for gene therapies, tests that were typically semi-customized have now been standardized, and some methods that previously required complete customization for each project are now platform assays. The resulting flexibility, combined with the application of advanced technologies such as ddPCR, reduces the burden of assay qualification and accelerates commercialization timelines. Most importantly, these assays protect the process, the product and ultimately the patient.

REFERENCE

1. Statement from Scott Gottlieb, M.D. former FDA Commissioner 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 15, 2019

14920 Broschart Road
Rockville, MD 20850
USA

Contact us for more information

EMDMillipore.com/gene-therapy-testing

info@bioreliance.com

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