Unlocking the potential of viral vectors through functional titer assays

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Introduction

Ensuring the quality of virus vectors used in gene therapy is achieved through a multi-tiered approach that examines several factors to establish manufacturing consistency and product safety. The steady increase in the use of virus vectors to produce ground-breaking gene-based therapies has intensified the need for novel approaches to virus testing that improve upon well-established techniques and streamline testing.

Understanding the functional characteristics of gene therapy vectors is crucial in establishing their potential as an effective gene delivery system. In particular, consistent delivery of the appropriate amount of virus vector to the correct tissue is critical in designing accurate dosing strategies to ensure efficacy and safety. Based on the unique nature of virus vectors used for gene-based therapies, careful design and often customization of test methods is necessary. In this presentation, we discuss two virus-based gene delivery platforms – adeno-associated virus (AAV) and lentivirus (LV) – focusing on the development, validation, and implementation of titration methods used to measure functional titer. We will review factors to consider for method selection and discuss strategies to accelerate method development and validation.

Vector Measurement

Physical Titer

The measurement of the total amount of virus in a virus vector preparation. Physical titer is often measured in one of two ways:

- Quantitative PCR to measure the number of virus genomes
- ELISA based assays to measure virus proteins (capsid).

Infectious Titer

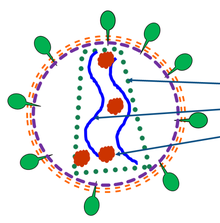
The concentration of virus particles that can transduce cells. This is measured using an infectivity assay appropriate for the specific virus and has two main components.

- Appropriate cell line that allows for virus infection
- Virus measurement using a variety of techniques such as ELISA, PCR, flow cytometry, or plaque/foci formation.

Virus Measurement Targets

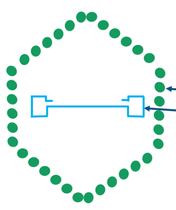
AAV and lentivirus vectors contain a number of suitable targets that can be used to design methods to measure both physical and infectious titer.

Lentivirus



Assay Targets	Copies/virion	Method
Capsid Protein	1000-5000	P24/p30 ELISA
Genomic RNA	2	RT-qPCR/ddPCR
Reverse Transcriptase	100-250	Enzymatic Activity

AAV



Assay Targets	Copies/Virion	Method
Capsid Protein	60	ELISA
Genomic DNA	1	qPCR/ddPCR

A combination of methods may be used to determine particle number and infectious titer.

Accurate and consistent virus measurement is important for production process development and critical in designing dosing strategies.

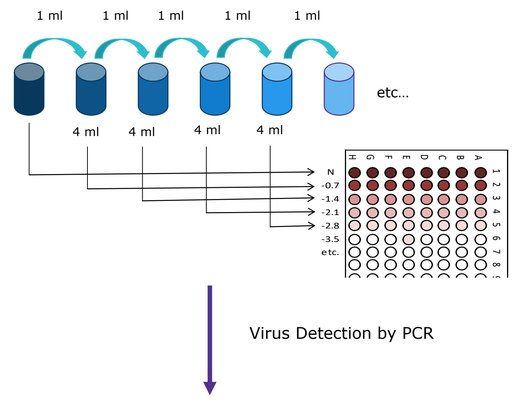
Method Development

Infectious Titer

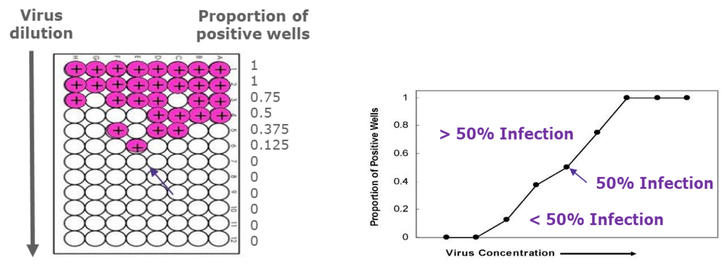
Given the unique characteristics of virus vectors used for gene delivery, there is no "one size fits all" approach to method design. Nevertheless, there are common factors that apply to the measurement of both lentivirus and AAV based vectors that need to be considered as demonstrated in the infectivity assay description below.

AAV Infectivity Assay: $TCID_{50}$

Infect replicate wells with serial dilutions of sample



Wells are scored as positive (+) or negative (-)



One approach to an infectivity method for AAV uses a cell culture system to determine the median tissue culture infectious dose ($TCID_{50}$).

The method requires several components that could be applied to a number of AAV vectors:

- A cell line or cell culture system that allows for the replication of the AAV taking into consideration that the virus is unable to replicate on its own therefore, helper genes or a helper virus are necessary.
- A detection method such as quantitative PCR measurement targeting a gene sequence within the vector.
- A positive control to track method performance.

Method validity criteria should be established to demonstrate accuracy, precision, specificity, linearity, and range, consistent with validation requirements for a quantitative assay.

Given the variety of AAV serotypes and gene inserts used to produce AAV-based therapeutics, it may be necessary to customize the method for each AAV vector. However, the method can be designed to allow for customization while maintaining the established assay parameters.

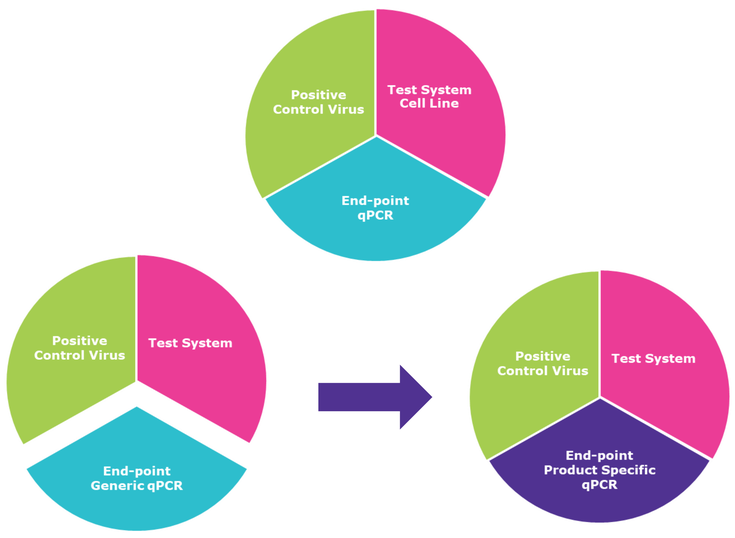
Designing and validating a vector specific method can be time consuming.

Leveraging the validation performed with a generic method can provide a way to streamline this process.

A platform strategy for method development and validation can be established by generalizing method components so they apply to multiple AAV vectors.

Platform Method: Flexibility by Design

Designing and validating a vector specific method can be time consuming. Leveraging the validation performed with a generic method can provide a way to streamline this process. A platform strategy for method development and validation can be established by generalizing method components so they apply to multiple AAV vectors.



The platform AAV $TCID_{50}$ method is represented by a circle where each slice corresponds to the three main components listed above.

Each component can be removed and replaced with one that is more appropriate for a specific product.

The custom method still requires development and validation, however leveraging the experience and documentation generated during the design of the generic method offers several advantages:

- Existing development plans and documentation
- Pre-determined validation strategy
- Simplified validation protocol and reporting

This streamlined process allows for faster development and validation of a product-specific method.

Summary

- **Measurement of virus-based gene therapy vectors is critical to ensure efficient processing, product quality, and define patient dosing.**
- **Consistency of results is essential highlighting the need for a well-designed validated method.**
- **Platform method development and validation can streamline product-specific method implementation.**

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