Testing Approaches For Virus Vectors Used In Gene Therapy: Novel Methods And Regulatory Expectations

Leyla Diaz, Krista Spreng, Alison Armstrong. Global Development Services, Rockville, Maryland, US. **Merck**

Introduction

Ensuring the biosafety and quality of virus vectors used in gene therapy is achieved through a multi-tiered approach that examines several factors to establish product safety and manufacturing consistency. The manufacture of viral vectors is complex and challenging with a number of key process goals that need to be maintained to achieve scalable processes and ensure reproducibility of product. The steady increase in the use of virus vectors to produce ground-breaking gene-based therapies has intensified the need for novel approaches to both manufacturing and virus testing that improves upon well-established techniques and streamlined testing.

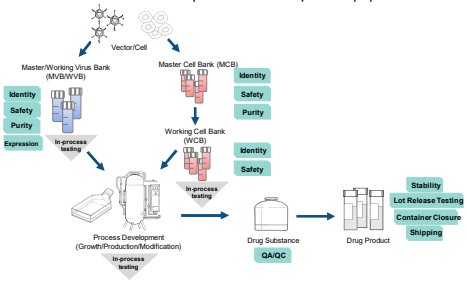
Based on the innovative nature of many gene therapy processes, customization of test methods has been critical for success. The use of state-of-the-art techniques to improve and expand existing testing methods that examine process product related impurities, identity and viral safety provides a level of quality assurance that addresses current regulatory expectations. We provide an analysis of the regulatory requirements for cell substrate testing and characterization of gene therapy virus vectors. Current testing methods are reviewed and testing challenges for viral vectors are discussed. This presentation will focus on innovative techniques, address critical quality attributes and address the limitations of small lot size, lack of terminal sterilization, limited availability of starting materials and continued supply of reference standards.

Process

Testing Requirements of Any Medicinal Product

The ultimate goal of a testing campaign for any medicinal product is to demonstrate that it is safe, potent, effective and stable. Figure 1 outlines the typical testing requirements at different stages of cell based therapeutic development. The following attributes are taken into account when designing a testing strategy and interpreting results:

- Quality
  - Physical characteristics of the manufacturing process and finished product
- Safety
  - Relative risk from harm
- Efficacy
  - Benefit provided to the patient
- Risk/Benefit Ratio
  - The degree to which risk is acceptable given the amount of benefit provided to the patient population



Typical testing requirements at each stage of development
Biosafety testing is required at various stages of product development including starting materials such as cell and virus banks. The testing usually involves sterility and purity and in instances of modified viruses or cells, the stability of the modification must be defined. Methods to test in process samples often form the basis for product specific assay used in lot release testing along with additional sterility and purity of the final product

Testing challenges associated with viral and gene-based therapies

Conventional testing methods are often not suitable for cell-based therapies due to unique characteristics of these novel products

- Limited availability of starting materials for process and method development
- Limited sample volume in final product
- Limited shelf life
- Regulatory landscape

Objective

Design assays suitable for viral vectors while maintaining quality of conventional methods and regulatory compliance.

- Faster results than conventional methods
  - Automated, high-throughput
  - Enhanced sample and data processing
- Increased accuracy, reproducibility and sensitivity

Rapid Microbiological Methods

- Microbial detection faster than traditional techniques
  - Increased sensitivity, accuracy, and reproducibility
  - Automation will maximize process efficiency by eliminating steps (labeling samples, paperwork, counting plates)
  - Can provide faster interim results – may help with a proactive response to avoid larger product issues

Regulatory Acceptance

- US FDA Amendments to sterility testing requirements for biological products (21 CFR 600, 610, 680, Federal Register, 3rd May 2012)
- Eliminates specified sterility test methods, culture media requirements, specified membrane filtration procedure.
- Replaces sample size requirement with a requirement that the sample be appropriate to the material being tested

Provides manufacturers of biological products greater flexibility and encourages use of the most appropriate and state-of-the-art test method.

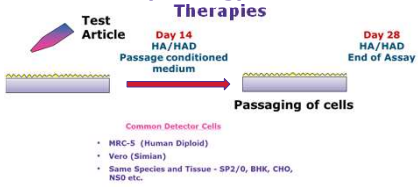
BacT/ALERT® 3D Rapid Microbial Detection System

- Early detection of metabolic bi-products
- Increased sensitivity
- Increased readout objectivity
- Reduced Sample Requirement
- Reduced testing time

Organism	Average Time to Positive (Days)	Detection Limit (CFU)
B. subtilis	0.49	1
C. sporogenes	0.64	2
P. aeruginosa	0.70	6
S. Aureus	0.61	2
C. albicans	1.03	2
A.brasiliensis	2.17	2
Y. enterocolitica (IAST)	0.92	6
Y. enterocolitica (INST)	0.90	16
S. pyogenes	0.60	6
P. acnes	3.69	6
Micrococcus sp.	3.07	2

Time to positive and detection limit for various microbial organisms using the BacT/ALERT® 3D Detection System.
The average time, in days, necessary to detect a positive result is shown along with the detection limit for each of the organisms listed. The organisms were tested in the context of a cellular matrix to simulate a viral vector test sample.

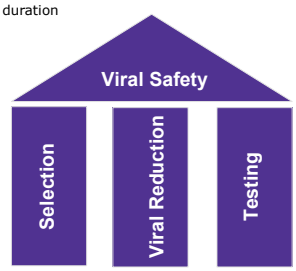
Virus Safety Strategy for Gene Based Therapies



Current *in vitro* adventitious virus testing method.
The test article is inoculated onto plates containing a human cell line (MRC-5) a simian cell line (Vero) and a cell line of the same species as that used in the production of the test article.
The detector cells are incubated for 14 days and observed for cytopathic effects (cpe), hemadsorption (HAD) and hemagglutination (HA). The cell supernatant is then inoculated onto fresh cells for an additional 14 days and the cpe, HAD and HA observations are repeated at 28 days.

Considerations for Gene Therapy Vectors for the *in vitro* adventitious agent assay

- Sample Volume
- Lack of Neutralizing antibody
- Assay duration



Risk Reduction Approach for Viral Safety

A full understanding of potential sources of virus contamination and product specific testing considerations is necessary to develop a robust virus safety strategy.

Selection of Source Materials
- Safe sourcing and testing of raw materials

Testing
- Verify absence of viral contaminants at appropriate process stages
  - In vitro adventitious virus testing for broad spectrum coverage
  - Virus specific PCR assays to account for known risks.
  - Alternative/complimentary assays such as Next Generation Sequencing
  - Replication competent virus testing

Evaluation of Viral Clearance
- Verify capacity of manufacturing process to remove or inactivate potential viral contaminants
  - Non-enveloped Viral Therapies
    - Some inactivation steps may be implemented
    - Large pore viral filters may be used
  - Lipid enveloped viral and cell therapies
    - Viral inactivation procedures may denature the product
    - Virus filtration may not be a viable option

Summary

Biosafety testing for vector and cellular based therapies can present a number of challenges depending on the nature of the therapy. Regulatory agencies are aware of these challenges and are revising guidelines to allow for greater assay flexibility and encourage the use of state-of-the-art methodology to overcome testing roadblocks.

A comprehensive risk management strategy encompassing all stages of development is necessary to provide assurance that the final product meeting the safety attributes of a medicinal product. This requires a multi-prong approach that includes material selection, contaminant removal or reduction and testing that incorporates state of the art methods when appropriate.