Setting the Standard: A Lentiviral Vector Reference Material to Support Assay Validation Across Platforms

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INTRODUCTION

The field of gene therapy is rapidly evolving, offering promising new approaches to treat a wide range of genetic diseases, including inherited immune disorders, neurodegenerative conditions, and certain cancers. Central to many gene therapy platforms are viral vectors, which deliver therapeutic genes to target cells. However, advancing these therapies requires standardized reference materials to validate reproducibility, demonstrate effectiveness, and support regulatory approval. In collaboration with the National Research Council (NRC), ATCC has recently added a new Lentiviral Vector Reference Material (LVVRM; ATCC® VR-3382™) to its collection. This material serves as an important standard for gene therapy research and development, enabling reproducibility, quality assurance, and regulatory alignment across academic, industrial, and pharmaceutical sectors. The LVVRM was produced by colleagues at NRC using fed-batch bioreactors, followed by purification via Tangential Flow Filtration (TFF) and monolith chromatography. ATCC then pooled, filtered, vialed, and preserved the material under controlled conditions. Prior to distribution, ATCC performed extensive quality control (QC) and characterization, including assessments of physical and functional titer, sterility, purity, and replication competence. This reference material is now accessible to researchers worldwide, representing a trusted standard for gene therapy research and development.

METHODS

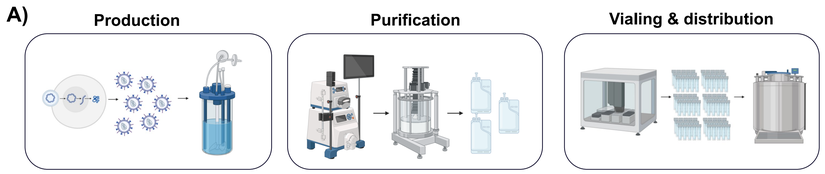

Figure 1. Production of LVVRM. The LVVRM producer cell line, HEK293SF-LVP-GFP, was generated by NRC. The cell line was engineered to produce a VSV-G pseudotyped lentivirus harboring a GFP transgene when induced by tetracycline and cumate switches. Large-scale batches of LVVRM were produced and purified by NRC using process innovations, including suspension-adapted cells, serum-free medium, and purification by TFF and monolith chromatography. The bulk material was frozen and transferred to ATCC, where it was thawed using the proprietary SmartThaw™ system, pooled, sterile filtered, and dispensed using an automated dispenser. Vials were frozen under controlled conditions and stored for distribution. Figure created with biorender.com.

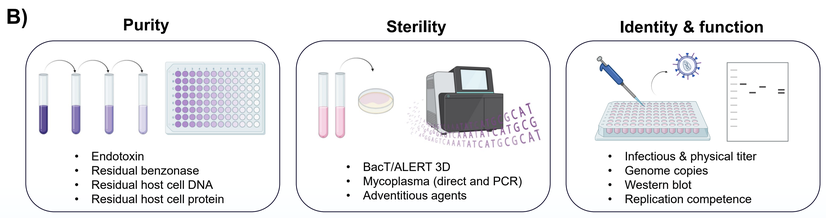

Figure 2. QC testing of LVVRM. Following dispense and preservation of LVVRM, the material underwent comprehensive QC testing and characterization. Over a dozen assays were performed to verify the purity, sterility, as well as physical and functional titers. Testing employed multiple orthogonal methods, including ELISA, western blot, ddPCR, next generation sequencing (NGS), and cell-based infectivity assays (see Table 1). Figure created with biorender.com.

RESULTS

Table 1. Overview of QC testing methods and representative lot-specific results for LVVRM (VR-3382™)

Test	**Method**	**Result**
Sterility	BacT/ALERT 3D	No Growth
Mycoplasma	Direct Culture and PCR	Not Detected
Adventitious Agents	PCR and NGS	Not Detected
Purity		
Test	**Method**	**Result**
Endotoxin	Chromogenic LAL	0.186 EU/mL
Residual Benzonase	Endonuclease Quantification	Below Limit of Detection
Residual Host Cell DNA	RNaseP ddPCR	Below Limit of Detection
Residual Host Cell Protein	HEK293 HCP ELISA	196.0 ng/mL
Function		
Test	**Method**	**Result**
Identity	Western blot	Viral GAG Proteins Detected
Physical Titer	p24 ELISA	6.6 x 10^9 LP/mL
Genome Copies	Genome Copies ddPCR	1.7 x 10^9 GC/mL
Infectious Titer	Transduction Assay	2.3 x 10^7 TU/mL
Replication Competence	RCL Assay	Not Replication Competent

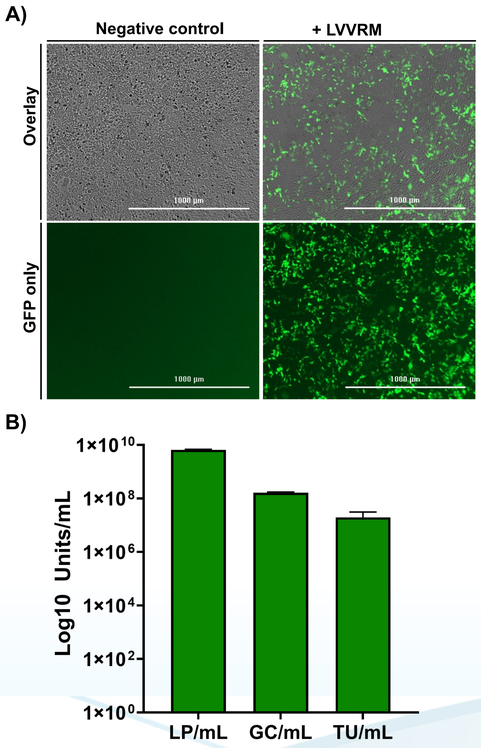

Figure 3. Physical and functional titer of LVVRM. A) The functional titer was evaluated by transduction of HEK293 cells (ATCC® CRL-1573™) with serial dilutions of LVVRM and assessing GFP expression via fluorescent microscopy. Representative images (phase contrast and GFP) were taken two days post-infection following a 24-hour adsorption period. Each dilution was tested in triplicate. **B)** LVVRM titers were quantified using three different methods. Physical titer (lentiviral particles (LP)/mL) was calculated from p24 capsid protein levels measured by ELISA; lentiviral vector genome copies (GC) per mL was calculated by ddPCR; transducing units (TU) per mL was calculated from readouts of the functional assay in panel A using the following equation: TU/mL= ((Number of cells transduced)*(percentage of cells expressing GFP)*(dilution factor)) ÷(mL transduction volume).

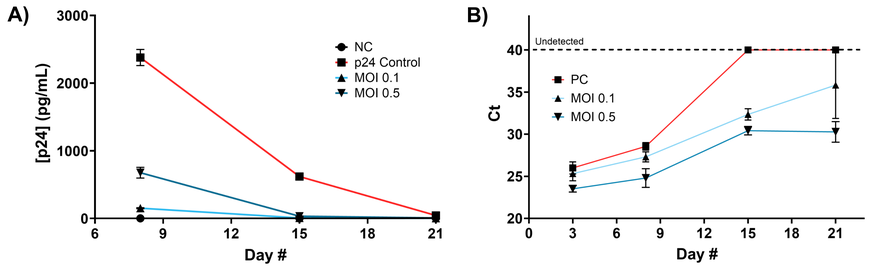

Figure 4. Replication competence. To verify product safety, a Replication Competent Lentivirus (RCL) assay was performed to demonstrate that LVVRM is unable to replicate in human cells. For this assay, the highly permissible cell line, C8166 (BEI Resources catalog # ARP-404) was exposed to LVVM and cultured for an additional 21 days. Samples were collected on days 3, 8, 15, and 21. Cell pellets and supernatants were separated and frozen for **A)** p24 ELISA and **B)** qPCR, respectively. A replication-incompetent VSV-G pseudotyped positive control was included for comparison. Each condition was cultured in duplicate, while ELISA and qPCR assays were performed in triplicate. The qPCR primers were designed to target the Psi packing signal region of the lentiviral vector genome.

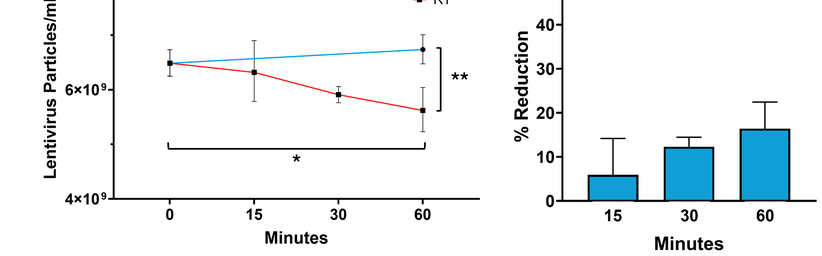

Figure 5. Short-term LVVRM stability. A short-term stability study was conducted to evaluate the impact of different temperature conditions relevant to the production process. For this experiment, vials were thawed on ice, then incubated at either room temperature for 15, 30, and 60 minutes, or at 4°C for 60 minutes. **A)** Samples were processed by p24 ELISA. Each sample was run in triplicate. **B)** LVVRM samples stored at room temperature exhibited a 5.9% reduction in LP/mL after 15 mins, then 12.3% and 16.4% after 30 and 60 minutes, respectively, relative to the 4°C sample. Statistical significance determined by One-way ANOVA with Tuckey's post-hoc test (*p <0.05, **p < 0.005).

CONCLUSIONS

ATCC is advancing the production and distribution of highly characterized reference materials to support gene therapy research and development. This work demonstrates the successful development of the LVVRM ATCC® VR-3382™. In collaboration with NRC, ATCC has introduced this LVVRM to the scientific community as a standardized resource to support assay development, comparability studies, and quality control. By producing quality standards like LVVRM, ATCC continues to reinforce its position as a trusteed partner in biomedical research.

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