Restoring a Critical Resource: Biomanufacturing of Adenovirus Reference Material

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INTRODUCTION

Viral reference materials are essential for gene therapy, vaccine development, and diagnostic testing because they provide standardized benchmarks for quantifying and characterizing viral properties such as titer, purity, and functionality. Adenoviruses, in particular, serve as critical reference standards in these applications; however, the availability of standardized adenovirus reference materials has been limited since the depletion of legacy stocks. To address this gap, ATCC recently initiated replenishment of Adenovirus type 5 reference material (ATCC® VR-1516™), originally developed in the early 2000s under the guidance of the Adenovirus Reference Material Working Group (ARMWG). ATCC used the scale-X™ hydro fixed-bed bioreactor (Univercells Technologies) to enable large-scale adenovirus production, leveraging real-time monitoring of dissolved oxygen (DO), pH, and temperature to maintain optimal cell health and virus yield. Following harvest, the material was concentrated and purified using Tangential Flow Filtration (TFF) and Ion Exchange Chromatography (IEX). Final characterization and quality control (QC) testing included functional titer and physical titer, as well as various other assays to assess purity, identity, and sterility. Additionally, LumaCyte Radiance™ technology was employed as a novel, label-free approach to assess viral infectivity and titer. The results from these assays confirm the scalability and robustness of the scale-X™ platform for adenovirus manufacturing and reinforce ATCC's commitment to restoring a critical reference resource that supports the advancement of gene therapy and vaccine development.

METHODS

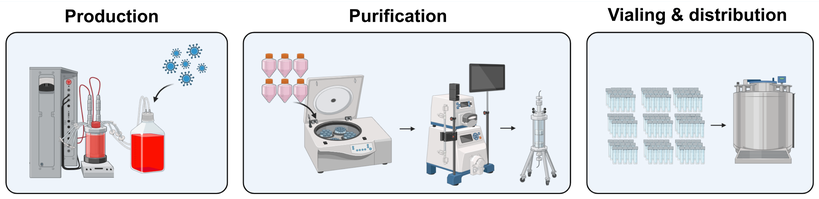

Figure 1. Large scale production of adenovirus reference material. HEK 293 cells (ATCC® CRL-1573™) are scaled-up and seeded in the bioreactor. After reaching an appropriate density, the cells are infected with the adenovirus stock and then incubated for an additional 4 to 5 days. The viral material (~5L) is harvested from the bioreactor, pooled, and clarified by centrifugation. Downstream purification processes include tangential flow filtration (TFF) and ion exchange chromatography (IEX). The material is frozen under controlled conditions and stored for distribution. Figure created with biorender.com

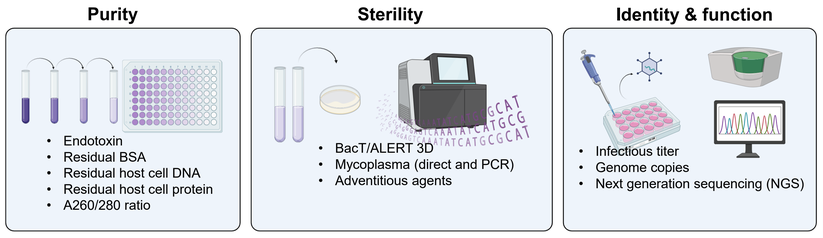

Figure 2. QC testing and characterization. Following preservation, comprehensive QC testing and characterization assays were performed. Over a dozen assays were employed to verify the purity, sterility, identity, and titer. Multiple orthogonal methods, including ELISA, PCR, next generation sequencing (NGS), and cell-based infectivity assays were used (see Table 1). Figure created with biorender.com.

RESULTS

Table 1. Overview of QC testing methods and representative lot-specific results for Adenovirus type 5 reference material (VR-1516™).

Sterility		
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	0.164 EU/mL
Residual BSA	ELISA	3.45 ng/mL
Residual Host Cell DNA	RNaseP ddPCR	Below Limit of Detection
Residual Host Cell Protein	HEK293 HCP ELISA	63.05 ng/mL
A260/280 ratio	UV spectroscopy	1.44
Identity and function		
Test	**Method**	**Result**
Identity	NGS	100% identity to reference sequence (AY339865.1)
Physical titer	Particle concentration	6.23 x 10^{10} particles/mL
Genome Copies (GC)	ddPCR	3.90 x 10^{9} GC/mL
Infectious titer	$TCID_{50}$/mL on HEK293 cells	3.13 x 10^{9} $TCID_{50}$/mL
Infectious titer (LumaCyte Radiance™)	Absolute titer	1.10 x 10^{9} IU/mL

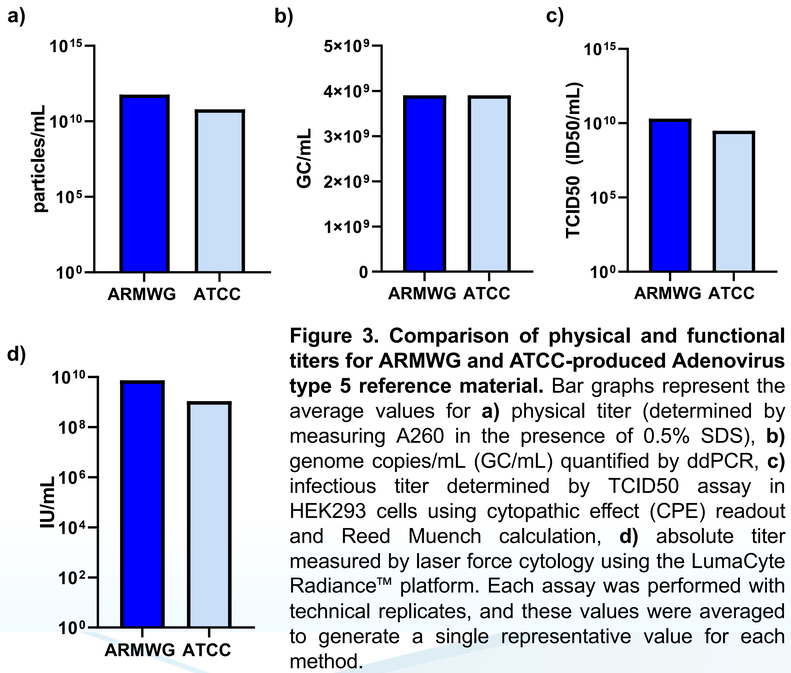

Figure 3. Comparison of physical and functional titers for ARMWG and ATCC-produced Adenovirus type 5 reference material. Bar graphs represent the average values for **a)** physical titer (determined by measuring A260 in the presence of 0.5% SDS), **b)** genome copies/mL (GC/mL) quantified by ddPCR, **c)** infectious titer determined by TCID50 assay in HEK293 cells using cytopathic effect (CPE) readout and Reed Muench calculation, **d)** absolute titer measured by laser force cytology using the LumaCyte Radiance™ platform. Each assay was performed with technical replicates, and these values were averaged to generate a single representative value for each method.

Table 2. NGS results and variant analysis of Adenovirus type 5 reference material.

Virus	Identification	Variant Analysis
ATCC-produced	Identity confirmed; 99.92% identify to reference sequence	4 variants, all SNPs ≤ 10%
ARMWG-produced	Identity confirmed: 99.15% identity to reference sequence	4 variants, all SNPs ≤10%

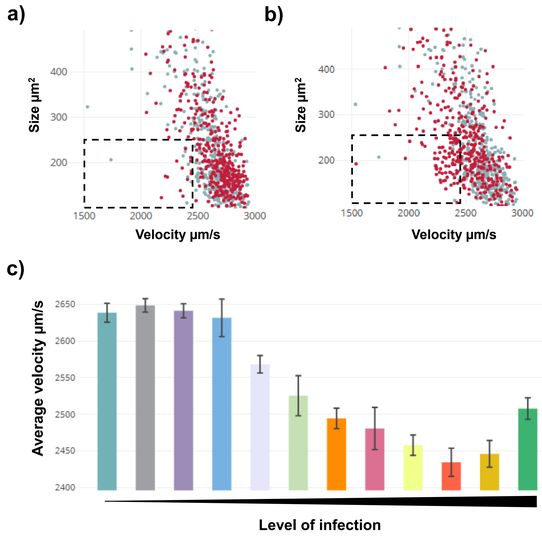

Figure 4. Absolute titer analysis of VR-1516™ by LumaCyte Radiance™. Cells were seeded in 24-well plates, incubated overnight, then infected with 3-fold serial dilutions of virus stock (each dilution in triplicate). After ~44 hours cells were harvested and loaded onto Radiance™. Each sample had a minimum of 300 single cells analyzed per well. Raw data was analyzed using the system's built-in analysis platform ReportR®. Representative scatter plots show the biophysical profiles of individually infected cells across **a)** low level of infection and **b)** high level of infection. **c)** census chart summarizing the biochemical changes of cells across the full dilution series. Each bar represents the average velocity for a given infection level (from low to high). As infection increases, average velocity decreases, reflecting virus-induced changes. This trend allows Radiance™ to determine the dilution at which infection reaches a defined threshold, enabling calculation of absolute viral titer.

CONCLUSIONS

The depletion of viral reference materials creates a critical gap for assay standardization in gene therapy and vaccine development. To address this need, ATCC is advancing the production and distribution of highly characterized viral reference materials that enable comparability and regulatory confidence. This effort includes the successful restoration of the Adenovirus type 5 reference material (VR-1516™), a foundational standard originally developed to support harmonization of analytical methods and quality control. Comprehensive characterization confirmed that the restored material is consistent with historical specifications for infectivity, purity, and genomic integrity. Additionally, analysis by Lumacyte Radiance™ was incorporated as a novel methodology for estimating absolute viral titer, expanding the characterization toolkit for adenoviruses. By restoring and distributing this critical standard, ATCC reinforces its position as the leading provider of trusted reference materials to advance assay comparability and reproducibility.

ACKNOWLEDGEMENTS

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