

Viral Reference Materials at American Type Culture Collection

Heather Couch, David Einfeld, Rebecca Bradford, Joseph Leonelli

AFS ATCC FEDERAL SOLUTIONS



ABSTRACT

Over twenty years ago, the members of the Adenovirus Reference Material Working Group (ARMWG) collaborated to develop the first viral reference standard material (VR-1516) for gene therapy that was distributed by the American Type Culture Collection (ATCC), a non-profit biological resource and standards organization.

VR-1516, adenovirus serotype 5 (Ad5), was produced in 2001 and is still used worldwide to define particle titer, vector genome titer, and infectious titer for adenovirus-based gene vectors. Adenoviral vectors have been widely assessed as recombinant vaccines since they have many advantages as vaccine delivery vectors, including the ability to be manufactured in mammalian cell culture systems. These are safe and have relatively low production costs and high viral titers. ATCC has embarked on a replenishment production effort to restore the distribution inventory of this critical reference material.

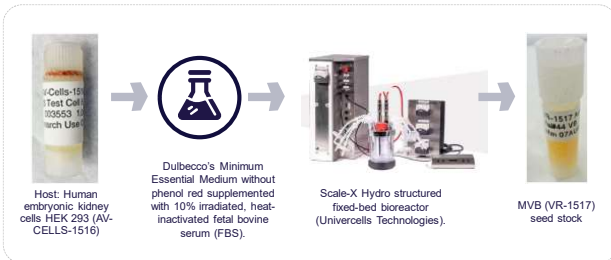
ATCC, in collaboration with the National Institute of Standards and Technology (NIST) and other members of the ARMWG, including IsBioTech, will ensure that the reference material meets all previously established characterization specifications. The bulk material was grown in the cGMP-produced HEK293 master cell bank infected with the virus from the cGMP master virus bank in a Scale-X Hydro structured fixed-bed bioreactor (Univercells Technologies). The harvested virus was purified according to the original protocol using tangential flow filtration (TFF) and anion exchange chromatography (AEC). Characterization of the material and purification of additional bulks are in progress.

In addition to Ad5 (VR-1516) production, ATCC is replenishing the distribution inventory of the adeno-associated virus reference materials, VR-1616 and VR-1816.

ATCC is collaborating with National Research Canada, NIST, FDA, and IsBioTech to support the production of a Lentiviral Vector Reference Material (LVVRM) distributed in the ATCC catalog as VR 3382. The material was produced in a bioreactor by the National Research Council Canada, and the bulk material will be sent to ATCC for sterile filtration and vialing. ATCC and NIST will perform characterization of the material. Like the adenovirus and adeno-associated virus reference materials mentioned above, the LVVRM will be used by the research community and industry to validate quantification methods for LVVs used in gene therapy for life-threatening diseases, including cancer, Parkinson's disease, and immunodeficiency disorders.

All reference materials mentioned above are expected to be available in the ATCC Catalog by fall 2023.

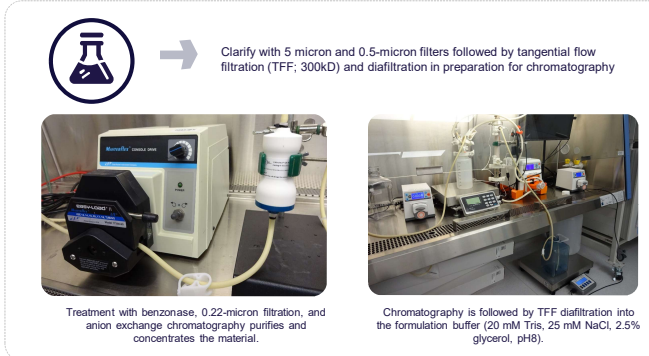
AD5 REFERENCE MATERIAL



The bioreactor probes continuously monitor temperature and dissolved oxygen, carbon dioxide, and pH during production.
Cell seeding: 2.4 m² bioreactor seeded at 2.3 x 10⁶ cells/cm².
Infection: Based on density cells were inoculated on day 2 to day 4.
Incubation: 3-4 days at 37°C, pH 7.0 and 50% dO₂.
Harvest: Cells are lysed with a detergent treatment, and the virus is harvested in a volume of approximately 5 L (total) or 1.5 L lysate.



AD5 REFERENCE MATERIAL



Dilution Before Vialing:

The final material is analyzed for virus particles per mL (vp/mL) and diluted to a concentration of 5E11 vp/mL. Following dilution, the material is filtered again, 0.22 micron, and stored at -80°C until ready to vial.

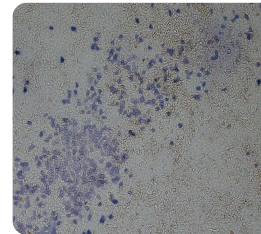
Vialing: Before vialing, the purified virus is filtered again. Vialing is completed in an ISO 5 rated biosafety cabinet. Exposure to possible contaminants is limited through ATCC aseptic technique. Purified virus is vialled in standard cryovials. Each cryovial will contain approximately 0.5 mL.

AD5 REFERENCE MATERIAL CHARACTERIZATION

Concentration: Particle concentration (~10¹¹ particles per mL) (by OD₂₆₀)

Infectious titer (~7 x 10¹⁰ NAS Infectious Units (NIU)/mL on HEK 293 cells) (NAS- normalized adjusted standard)

Sequencing: Next-Generation Sequencing will be performed on the virus preparation to verify the sequence and identify potential variants to the reference sequence (AY339865.1). The ATCC Sequencing and Bioinformatics Center (S.B.C.) will perform the library preparation, sequencing, and bioinformatics analysis.



Adenovirus infected A549 cells treated with anti-hexon antibody

Adventitious Agent Testing:

Vialled material will be submitted for testing to determine if the material is free of human, mouse, rat, and hamster adventitious agents.

Mycoplasma:

ATCC will test for mycoplasma via DNA detection by PCR of extracted Test Article nucleic acid as well as agar and broth culture (14-day incubation at 37°C).

Endotoxin:

ATCC will test for endotoxin using the chromogenic LAL testing kit.

Stability Testing will be completed by ATCC and NIST: The reference material produced by ATCC will be stored within cGMP quality assured, temperature-controlled, and monitored storage units at ATCC. Stability will be analyzed using assays for particle concentration and infectious titer since the reference material is used to standardize those measurements. Test material will be analyzed for OD260 and NAS titer differences relative to the baseline material stored at -80°C.

ADENO-ASSOCIATED VIRAL VECTOR REFERENCE MATERIAL

Following the example set by the adenoviral reference material working group (ARMWG) for developing the adenovirus reference material (ARM), the Adeno-associated Virus Reference Standard Working Group (AAVRSWG) was organized to develop a high-quality AAV reference material. ATCC distributes Recombinant Adeno-associated virus 2 Reference Standard Stock (rAAV2-RSS) (VR-1616) and Recombinant Adeno-associated Virus 8 Reference Standard Stock (rAAV8-RSS) (VR-1816).

LENTIVIRAL VECTOR REFERENCE MATERIAL

ATCC is also participating in the production of Lentiviral Vector Reference Material (LVVRM) that will be distributed in the ATCC catalog as VR-3382. The material was produced in a bioreactor by the National Research Council (NRC) Canada.

The LVVRM will be shipped in 500 mL bags, each containing 200mL of LVVRM. ATCC will also store the production cell line for future production lots.

ATCC will receive, thaw, sterile filter, vial, store and distribute the LVVRM in the ATCC catalog.

SUMMARY

Viral vectors play a central role in gene therapy. Ad5, AAV and LV vector gene therapy applications include cardiovascular, metabolic, neurological, immunodeficiency, muscular and hematology diseases. Examples of successful therapeutics include: Gendicine™, a replication-deficient Ad vector expressing the p53 gene, was used to treat more than 30,000 patients with head and neck cancer; the AAV-based Onasemnogene AAV (Zolgensma) has been approved for the treatment of spinal muscular atrophy; the AAV based Voretigene neparovect (Luxturma) was accepted for the treatment of inherited retinal dystrophy¹.

The global availability of adenovirus, adeno-associated virus and lentivirus reference materials permits the standardization of quantification techniques between research and manufacturing organizations and makes interpretation of preclinical and clinical data easier to compare across the field. These standards are critical to the development of new therapeutics and ATCC is excited to take the lead in the production/distribution of these valuable reference materials.

(1) Lundstrom, K. Viral Vectors in Gene Therapy: Where Do We Stand in 2023? *Viruses* 2023, 15, 698. <https://doi.org/10.3390/v15030698>.

ACKNOWLEDGEMENTS

ATCC would like to acknowledge Keith Carson and IsBioTech for his role in organizing the manufacture of these important reference materials, NIST for their participation in quality control testing and information sharing. ATCC acknowledges all the aid provided by the many donors of time and materials for these projects.

© ATCC 2023. The ATCC trademark, trade name, any and all ATCC catalog numbers listed in this publication are trademarks of the American Type Culture Collection unless indicated otherwise.