

# The Characterization of Adenovirus 5 Reference Material (VR-1516™)

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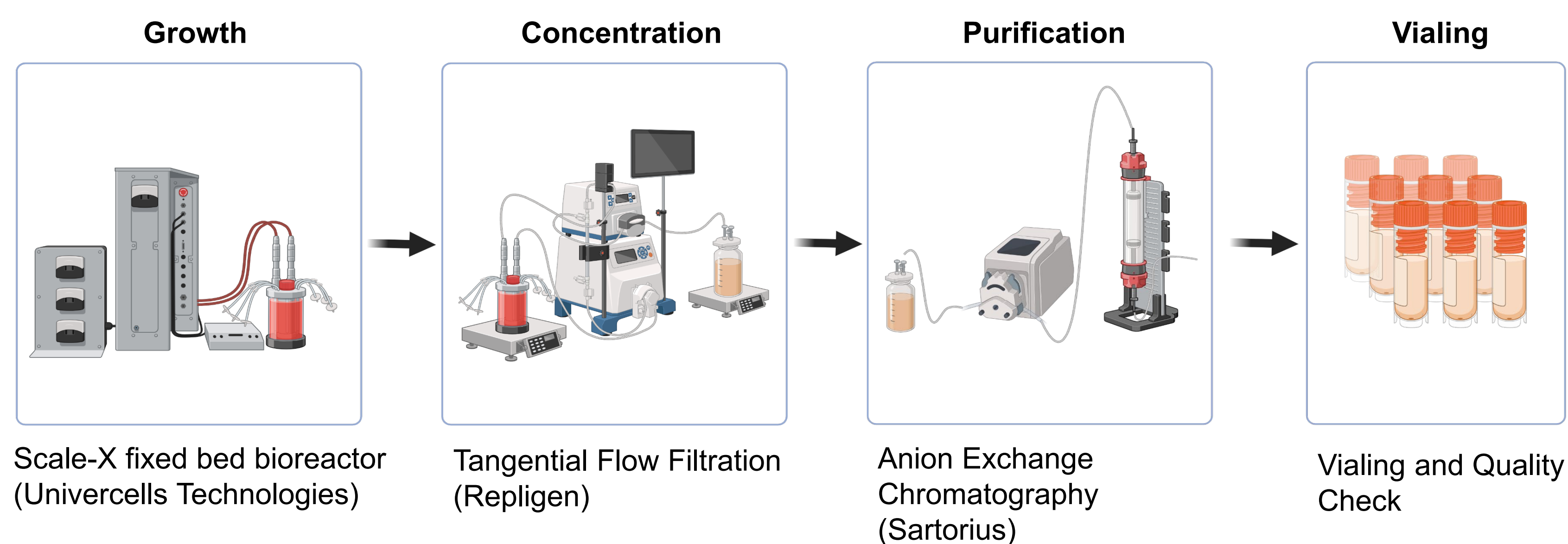
## Abstract

First-generation adenoviral vectors are some of the most widely used recombinant vectors in biomedicine, with applications in cancer therapy, gene therapy, and vaccine development.<sup>1</sup> Many of these vectors are based on adenovirus type 5, and are useful for their high transgene packaging capacity, recombination ability, and capacity to grow to a high titer.<sup>2</sup>

The Adenovirus Type 5 Reference Material (ATCC<sup>®</sup> VR-1516™) is derived from a wild-type adenovirus (ATCC<sup>®</sup> VR-5™) and was first generated as a product of the Adenovirus Reference Material Working Group (ARMWG). The development of this reference material was recommended in the January 2002 report of the NIH Recombinant DNA Advisory Committee. It is used to establish a common reference standard to define the particle counts, vector genome titer and infectious units for adenovirus-based gene vectors. New batches of the reference material are currently being produced at ATCC; here, we discuss the state-of-the-art technologies we use to develop and characterize replenishment batches of the reference material.

## Ad5 Reference Material Production

Replenishment batches of the Adenovirus Type 5 Reference Material were developed using state-of-the-art scale-up technologies, Scale-X bioreactors, concentration via Tangential Flow Filtration, purification processes using anion exchange chromatography, and optimized cryopreservation and vialing.



**Figure 1: Adenovirus Type 5 Reference Material production process overview.** Figure created using BioRender.com.

## Ad5 Reference Material Characterization

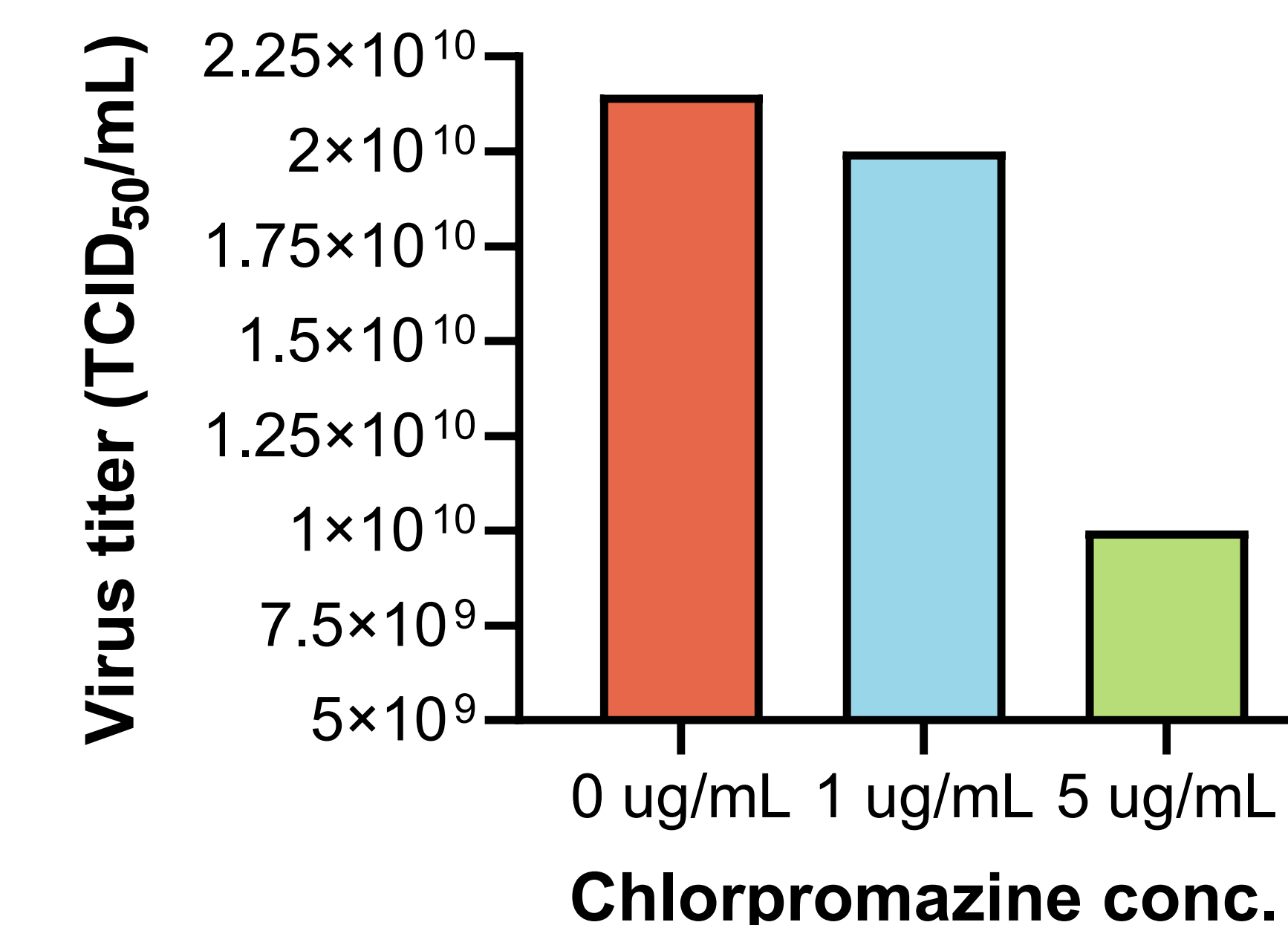
The reference material has undergone rigorous characterization of virus particles, infectious titers, kinetics, genome concentration, authentication, residual host cell protein and DNA concentrations, and substrate endotoxin and BSA titers. Final release criteria for the reference material also include tests for sterility and mycoplasma. Using novel technologies that were not available with the first production batch of the Adenovirus Type 5 Reference Material, we have enhanced the characterization of the replenishment material. These technologies include the use of Laser Force Cytology to accurately assess the infectious titer of the adenovirus material and Real-Time Cell Analysis assays to determine viral infection kinetics.

**Table 1: Adenovirus Type 5 Reference Material Specifications**

Test/Method	Specification	Result
<b>Particle Concentration</b> (Absorbance at 260 nm in the presence of 0.5% SDS)	Report Results	3.60 x 10 <sup>10</sup> particles/mL
<b>Genome Copies</b> (ddPCR)	Report Results	1.27 x 10 <sup>10</sup> gc/mL
<b>Infectivity</b>	TCID <sub>50</sub> /mL on HEK293 cells with CPE readout	5.00 x 10 <sup>8</sup> TCID <sub>50</sub> /mL
<b>Infectivity – LumaCyte Radiance</b>	Absolute Titer	6.11 x 10 <sup>8</sup> TCID <sub>50</sub> /mL
<b>Authentication</b>	Virus identity verified by sequencing	99.95% identity to the reference sequence (AY339865.1)
<b>RNaseP</b> (digital-based PCR)	Report Results	4.9 copies/μL or 3.44 x 10 <sup>-13</sup> ng/μL
<b>HEK293 Host Cell Protein</b> (ELISA)	Report Results	48.6 ng/mL
<b>260 nm to 280 nm ratio</b> in the presence of 0.5% SDS	Report Results	1.04
<b>Bovine Serum Albumin</b> (ELISA)	Report Results	1.92 ng/mL
<b>Mycoplasma Contamination</b> (Broth and Agar direct culture method)	No growth	In Progress
<b>Mycoplasma Contamination</b> (DNA detection by PCR)	None detected	In Progress
<b>Sterility Test – BacT/ALERT 3D – iAST</b> bottle (aerobic) at 32.5°C, 14-day incubation	No growth	In Progress
<b>Sterility Test – BacT/ALERT 3D – iNST</b> bottle (aerobic) at 32.5°C, 14-day incubation	No growth	In Progress
<b>Adventitious Agents – Human, Mouse, Rat Panels</b> (IDEXX)	None detected	In Progress
<b>Adventitious Agent detection</b> (NGS)	None detected	In Progress
<b>Endotoxin</b> (Chromogenic LAL)	Report Results	In Progress

## Ad5 Virus Kinetics

After binding to a primary cellular receptor, adenoviruses are internalized, triggering an endocytic event via clathrin-coated pits. Chlorpromazine inhibits AP2-derived clathrin pit formation on the cell surface, thereby reducing the adenovirus-derived internalization and subsequent productive infection.<sup>3</sup> To assess the effect of chlorpromazine on the viral kinetics of the Adenovirus Type 5 Reference Material, we used an xCELLigence assay (Agilent) to measure differences in viral titer in the presence of increasing amounts of chlorpromazine.



**Figure 2: Adenovirus Type 5 Reference Material titer reduction depends on chlorpromazine concentration.** The presence of increasing amounts of chlorpromazine resulted in a concentration-dependent reduction in Ad5 titer, indicative of lower levels of productive Ad5 infection of HEK293 cells.

## Conclusion

As an important standard for adenovirus vector-based development, a well-characterized reference material is a necessary requirement to ensure accurate and safe adenovirus vectors in cell and gene therapy applications. We describe here the replenishment of the Adenovirus Type 5 Reference Material and improvements to both the process and analytical methods.

### References

- Russel W. C. (2000) Update on Adenovirus and its vectors. *J. Gen. Virol.* 81 (11), 2573-2604.
- Deal C., Pekosz A., Ketner G. (2013). Prospects for oral replicating adenovirus-vectored vaccines. *Vaccine* 31 (32), 3236–3243.
- Kanerva A., Raki M., et. al. (2007). Chlorpromazine and apigenin reduce adenovirus replication and decrease replication associated toxicity. *J. Gene Med.* 9, 3-9.