AAV2 Transduction Analytics with Laser Force Cytology™

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com

LumaCyte®

AAV2 Transduction Analytics with Laser Force Cytology™

Introduction

The field of gene therapy has advanced significantly over the past decades, from initial speculation about inserting DNA in patient's cells to the approval of over 27 therapies for range of diseases as of 2023. Adeno-associated virus (AAV)-based gene therapy is a promising and constantly evolving field that applies to a small but growing number of diseases. The use of AAV contributes to minimal immunogenicity, cytotoxicity, and lack of replication in the host cell. This safety advantage makes AAV an attractive tool for gene therapy applications[1].

Yet, the tool does not come without its own analytical measurement challenges. The inability to replicate, the need for helper viruses and cell lines containing *rep* and *cap* genes, and the stock variation in *r*AAV due to its manufacturing difficulties embody ongoing analytical problems for manufacturing AAV-based therapies[2]. The regulatory agencies governing the manufacturing of cell and gene therapies emphasize consistent analytical assessments for AAV quality control and potency reporting, which can impact efficacy of the product[3]. Since AAV infection does not result in cytopathic effect, plaque assays cannot be used to determine the infectious titer. $TCID_{50}$ is a widely used assay to titer infectious unit, however, this method applied to AAV also depends on helper virus and cell lines with *rep-cap* genes[4]. The results are very imprecise and far from accurate as the rAAV infection *in vivo* solely depends on the host cells[5]. Reports of physical titer measured through qPCR can add to consistency of analysis, however, it does not represent the efficacy of the product.

LumaCyte's Laser Force Cytology™ (LFC) Radiance® instrument uses a combination of optical pressure and microfluidics to interrogate individual cells. LFC measures the intrinsic biophysical and biochemical properties of single cells and has the potential to detect and characterize the rAAV transduced cells without relying on helper viruses or cell-lines containing *rep-cap*.

Experimental Results

A549 cells were seeded into 48-well tissue culture plates and transduced the following day with AAV2 expressing eGFP at MOIs ranging from 980 to 65 $TCID_{50}$/cell, as measured using a standard PCR based $TCID_{50}$ measurement.

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com

Non-transduced cells were included as a negative control, and no additional helper virus was added. Cells were then harvested at 72h post transduction and measured with Radiance®. As shown in **Figure 1(a-c)**, as cells are transduced with AAV2 particles, they experience higher optical forces that can be measured by Radiance®, likely due to refractive index changes within the cell.

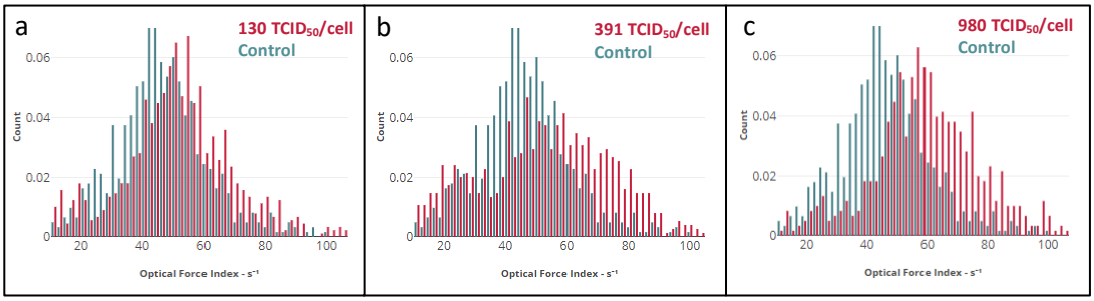

Figure 1. (a-c): **Optical force index reflects the optical force experienced by the cell, normalized by the cell size**. A progressively larger increase in optical force is seen at 72h post infection with the addition of a higher concentration of AAV2.

As each cell is exposed to the laser, its velocity in the fluid flow is decreased in proportion to its optical force or pressure. Thus, cells with a higher force will travel at a lower velocity and vice versa[6]. This change in velocity is due to the transfer of momentum from the photons in the laser as they scatter and refract through the cell and is a function of the biophysical and biochemical state of the cell[7]. Using Optical Force Index as the metric, the reported detection limit for AAV2 is 65 $TCID_{50}$/cell, based upon multiple dilution studies.

To demonstrate Radiance® precision and capability, over 20 independent experiments throughout three different lots of AAV with different titer ($TCID_{50}$/ml or Vg/ml) were conducted. Multivariate analysis using partial least squares regression (PLS) was employed to create a calibration curve using the single cell data generated by Radiance®. Data representing five independent experiments (one lot) is presented in **Figure 2**. A range of known dilutions (**green**) was used to develop the curve, which can then be used to predict unknown samples (**red**). This model produced accurate and precise prediction for unknown samples with an average log_{10} difference of 0.045 with a CV of 14% (**Table 1**) and a limit of quantification of ~130 $TCID_{50}$/cell.

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com

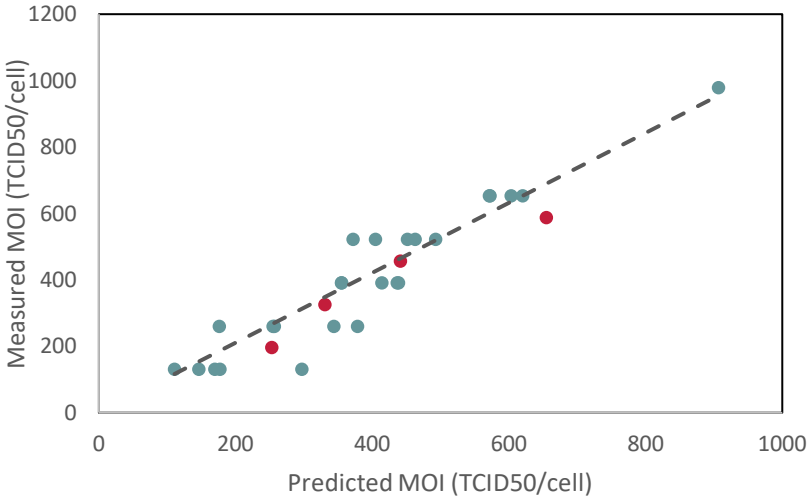

Figure 2: Radiance® Calibration Curve. Graph showing the correlation between Radiance® LFC data (Predicted MOI) and a known reference standard using a traditional $TCID_{50}$ (Measured MOI) to calculate the infectivity of AAV-2 samples. A range of known samples was used to create the calibration curve (**green**) that was then used to calculate the MOI for an unknown sample (**red**).

Known Stock titer: 9.14E+09 TCID50/mL				
	Measured MOI ($TCID_{50}$/cell)	Predicted MOI ($TCID_{50}$/cell)	Predicted Titer ($TCID_{50}$/cell)	Log_{10} Difference
Calibration Curve (Knowns)	979.5	906.4	8.5E+09	0.034
	653.0	568.5	8.0E+09	0.060
	522.4	436.8	7.6E+09	0.078
	391.8	399.9	9.3E+09	-0.009
	261.2	282.0	9.9E+09	-0.033
	130.6	180.1	1.3E+10	-0.140
		Average	**9.31E+09**	**0.059**
Unknown 1	587.7	654.4	1.02E+10	-0.047
Unknown 2	457.1	441.6	8.83E+09	0.015
Unknown 3	326.5	331.1	9.27E+09	-0.006
Unknown 4	195.9	253.3	1.18E+10	-0.112
		Average	**1.00E+10**	**0.045**
		Standard Deviation (%CV)		**1.32E+09 (14%)**

Table 1: Table comparing the $TCID_{50}$ values of dilutions of a known stock titer to predictions using LFC data collected by Radiance. Values are shown for knowns used to make the calibration curve as well as independent unknown predictions that show an average log_{10} difference of 0.045 with a CV of 14%.

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com

LFC and Radiance® enable users to predict the titer for unknown AAV2 samples independent of helper virus and add accuracy and robustness to their final gene therapy products. Once an experimental protocol, multivariate model, and calibration curve are established, users can measure unknown samples precisely, accurately, and rapidly.

Cell Culture, Infection, and Harvest

A549 cells (CCL-185) were seeded in 48-well plates at a density of 75000cells/well in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37° C in a humidified incubator with 5% CO2 and incubated overnight. Cells were transduced with rAAV2 at indicated MOI range and incubated for 72h without media change. Cells were then detached using a cell dissociation buffer, centrifuged for 5 minutes at a speed of 250-300g. Pellet was then resuspended in LumaCyte Stabilization fluid plus 0.5% PFA to a final concentration of 800,000 cells/ml. Cell samples (200 µL) were then loaded into the 96-well plate for analysis with a Radiance® instrument.

References

1. Pupo, A., et al., *AAV vectors: The Rubik's cube of human gene therapy.* Mol Ther, 2022. **30**(12): p. 3515-3541.
2. François, A., et al., *Accurate Titration of Infectious AAV Particles Requires Measurement of Biologically Active Vector Genomes and Suitable Controls.* Mol Ther Methods Clin Dev, 2018. **10**: p. 223-236.
3. Gavin, D.K., *FDA statement regarding the use of adeno-associated virus reference standard materials.* Hum Gene Ther Methods, 2015. **26**(1): p. 3.
4. Zen, Z., et al., *Infectious titer assay for adeno-associated virus vectors with sensitivity sufficient to detect single infectious events.* Hum Gene Ther, 2004. **15**(7): p. 709-15.
5. Ellis, B.L., et al., *A survey of ex vivo/in vitro transduction efficiency of mammalian primary cells and cell lines with Nine natural adeno-associated virus (AAV1-9) and one engineered adeno-associated virus serotype.* Virol J, 2013. **10**: p. 74.
6. McCracken, R., et al., *Rapid In-Process Measurement of Live Virus Vaccine Potency Using Laser Force Cytology: Paving the Way for Rapid Vaccine Development*. Vaccines (Basel), 2022. **10**(10).
7. Hebert, C.G., et al., *Viral Infectivity Quantification and Neutralization Assays Using Laser Force Cytology*, in *Vaccine Delivery Technology: Methods and Protocols*, B.A. Pfeifer and A. Hill, Editors. 2021, Springer US: New York, NY. p. 575-585.

 1145 River Road | Charlottesville, Virginia, 22901, USA
www.lumacyte.com | Global Support: +1 888-472-9295 | info@lumacyte.com