Radiance® Rapid Viral Quantification of Adenovirus Infection of HEK293 Cells with Label-Free Laser Force Cytology™

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LumaCyte®

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Introduction

Since its discovery in 1953[1], adenovirus has developed into an important tool for biotechnology research, development, and production. It has been the focus of numerous clinical trials as a gene delivery vector and was one of the first viruses to be used for this purpose as a result of its well-defined biology, broad tropism, and large-scale producibility[2]. In fact, despite the recent rise in popularity of Adeno-Associated Virus (AAV) as a gene therapy vector[3] (the discovery of which was also a result of adenovirus research), adenovirus still accounts for more than 20% of the worldwide registered gene therapy clinical trials as of 2017[2]. Adenovirus is also widely used as a vector for viral vaccine development, including as part of a prime-boost strategy in combination with modified vaccinia virus Ankara (MVA) and other viruses[4,5]. Various adenovirus strains also serve as the backbone for numerous candidates against COVID-19, including both the AstraZeneca and Johnson & Johnson vaccines[6].

For each of these applications involving adenovirus, a rapid and accurate viral infectivity measurement is needed to maximize yields, reduce costs, minimize out-of-specification (OOS) failures, and reduce time to market. As such, the precise and accurate quantification of infectious viral particles is a critical aspect of its use. However, traditional methods such as the plaque assay or $TCID_{50}$ (end-point dilution) are slow, labor-intensive, and subjective as they can vary greatly depending upon the operator. More recent antibody-based methods still require significant time, labor, and costly reagents while being limited to specific adenovirus serotypes and host species.

In contrast, LumaCyte's Laser Force Cytology™ (LFC) instrument, Radiance®, is a rapid and sensitive tool that uses a combination of optical force and microfluidics to examine the intrinsic biochemical and biophysical changes in cells due to viral infection. By providing an objective and automated method for measuring cell-based viral infectivity, Radiance® provides game changing improvements in time to result (TTR), throughput and resource efficiency while at the same time providing highly precise and accurate results.

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Experimental Results

In order to demonstrate LFC's ability to quantify adenovirus infection in HEK293 cells, cells were infected two hours post seeding and then harvested for analysis with Radiance® at 48 hours post infection in two separate experiments. Cells were infected at multiplicities of infection (MOIs) of 0.00, 0.082, 0.25, 0.74, 2.22, 6.67, and 20.00 (IU/cell[8]) to generate a correlation curve between Radiance® data and viral titers. As each cell flows through the microfluidic channel, the laser within Radiance® exerts an optical force on the cell, reducing its velocity. 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. As shown in **Figure 1**, cell populations infected with adenovirus shifted to a lower velocity, with their histograms broadening overall and including a higher percentage of cells in the lower velocity tail due to heterogeneity in the infected population. Because of the inversely proportional relationship between velocity and optical force, the lower velocity of these cells indicates an increase in the optical force due to infection.

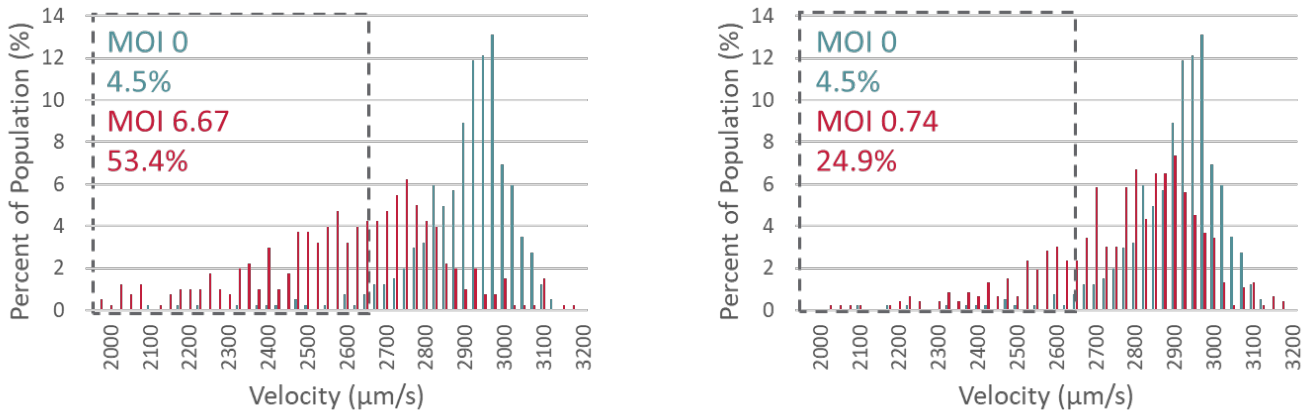

Figure 1: **Velocity histograms comparing uninfected HEK 293 cells (MOI 0) to cell populations infected with adenovirus at MOI 6.67 and MOI 0.74.** There is a clear shift to a lower velocity due to infection, and a greater increase in the low velocity tail of the MOI 6.67 population compared to MOI 0.74 population, as illustrated by the increased percentage of cells with a velocity less than 2650 µm/s (53.4% vs 24.9%).

As a result of heterogeneity in the infection process mentioned above, using a threshold metric to calculate the percentage of cells in the population below a certain velocity can be an effective way to describe the mean shift and distribution broadening that occurs with varying levels of adenovirus infection. **Figure 2** shows the bar graph of a Radiance Infection Metric defined as the fractional percent of cells with a velocity less than or equal to 2650 µm/s for each MOI for both experiments.

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In **Figure 2**, the percent of cells with a lower velocity increases with an increasing MOI. However, at MOI 20 the infection metric values begin to drop off or plateau while MOI 0.082 points are statistically different from the negative controls. Therefore, the linear dynamic range (highlighted in the dashed line box) is defined for this infection covers 2 logs of usable instrument response.

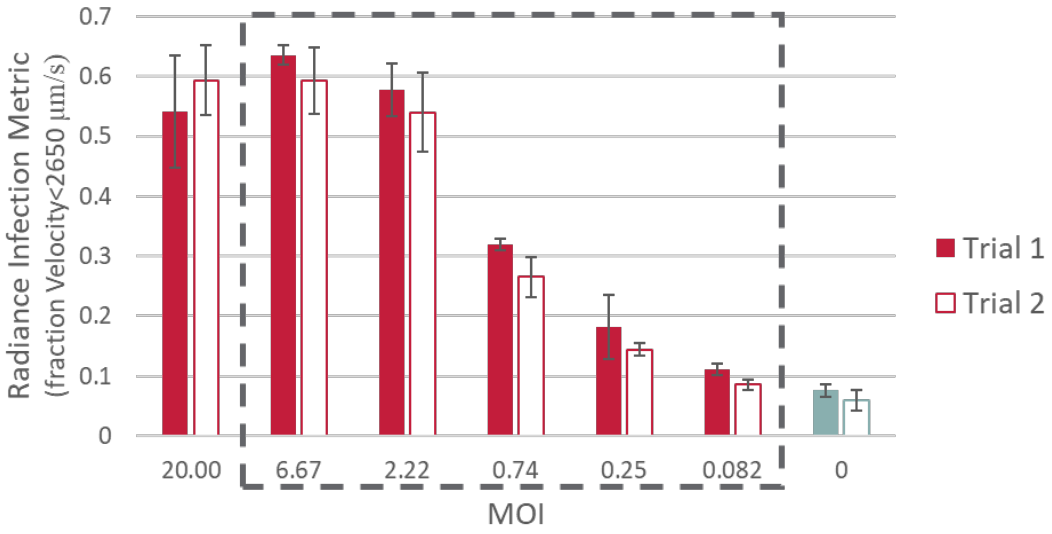

Figure 2: Velocity bar graph showing the Radiance Infection Metric (fraction of cells in the population for each MOI with a velocity less than 2650 µm/s). The Radiance Infection Metric increases with respect to MOI with the linear dynamic range occurring between MOIs 0.082 and 6.67.

A calibration curve can be generated to quantify the adenovirus infection by correlating the MOIs in the linear dynamic range to their respective Radiance Infection Metric. This calibration curve can be utilized to calculate the effective MOI of samples with unknown levels of adenovirus using Radiance®, which allows for rapid and accurate titer calculation. Using the equation,

$$Titer\left(\frac{IU}{mL}\right) = \frac{Cell\ Count\ per\ Vessel\ at\ Infection\ (cell)}{Volume\ of\ Undiluted\ Viral\ Stock\ Added\ (mL)} \times MOI\left(\frac{IU}{cell}\right)$$

the calculated MOIs from the calibration curve can quickly be converted to a traditional titer value. This equation, along with the calibration curve equation can be used to calculate unknown sample viral titers, using Infection Metric data collected directly from Radiance® in near real time.

To demonstrate this Radiance® capability, the two trials, measured two weeks apart, have been used to predict each other. The two calibration curves in **Figure 3** have been created using the five MOIs in the linear dynamic range highlighted in **Figure 2** from the two different trials. Using Trial 1 as the calibration curve and the associated equation correlating MOI with Radiance Infection Metric and treating Trial 2 as a set of unknown samples, the MOIs for Trial 2 can be easily calculated.

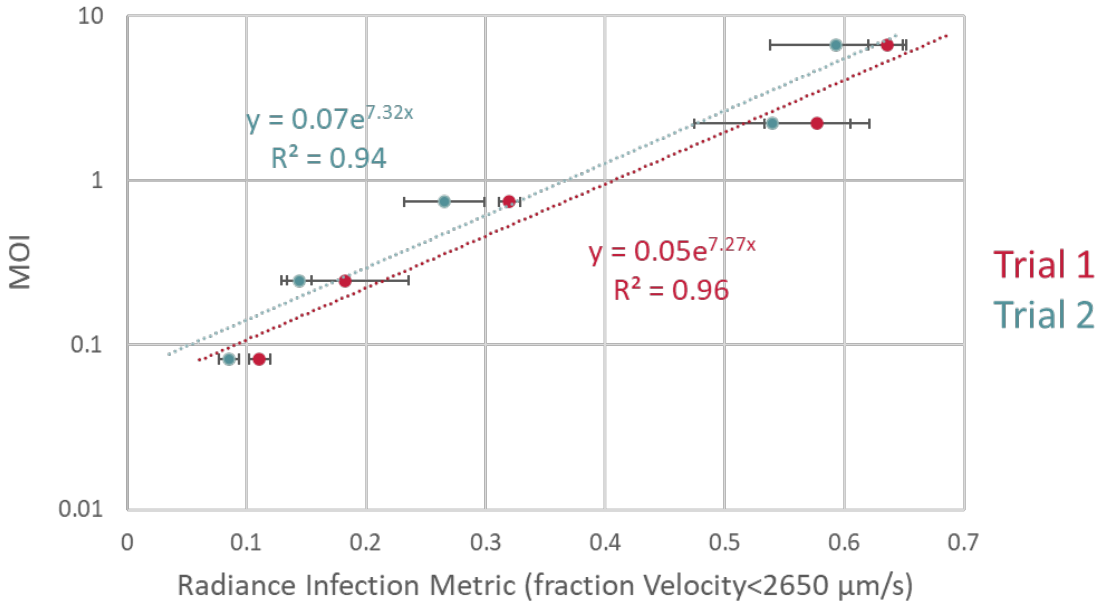

Figure 3: Calibration curves from two separate adenovirus infections of HEK293 cells with the MOI plotted against the Radiance Infection Metric (fraction of cells with a velocity below 2650 µm/s). Both curves result in a strong correlation between the infection metric and MOI and are also very similar to one another, demonstrating the ability of Radiance® to produce accurate and precise calibration curves.

The same can be done using Trial 2 to predict Trial 1; the results of both sets of predictions are shown in **Table 1**. **Table 1** also lists the $\log_{10}$ differences of the measured MOIs versus the known MOIs for both experiments, and the resulting $TCID_{50}$/mL, which resulted in average $\log_{10}$ differences of 0.15 or less, which is far superior to the industry standard of 0.3-0.5 $\log_{10}$ error, demonstrating the accuracy of viral quantification using LFC method with Radiance®.

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	Actual MOI (IU/cell)	Predicted MOI (IU/cell)	Predicted Titer (IU/mL)	Log_{10} Difference
Using Trial 1 to Predict Trial 2	6.67	3.89	5.84E+08	0.233
	2.22	2.64	1.19E+09	0.075
	0.74	0.358	4.84E+08	0.315
	0.25	0.148	6.00E+08	0.221
	0.082	0.097	1.18E+09	0.072
		Average	**8.07E+08**	**0.093**
Using Trial 2 to Predict Trial 1	6.67	3.91	1.08E+09	0.231
	2.22	3.24	2.10E+09	0.164
	0.74	0.38	9.61E+08	0.284
	0.25	0.16	1.05E+09	0.193
	0.082	0.13	1.87E+09	0.186
		Average	**1.41E+09**	**0.150**

Table 1: Table of actual versus predicted MOI and titers when using the equation from the other trial to predict each MOI. The low log_{10} difference for both experiments demonstrates the ability of Radiance® to produce accurate and precise calibration curves.

In conclusion, LFC™ and Radiance® can be used to rapidly quantify adenovirus infection by correlating significant shifts in multiple optical force metrics with titer within 2 days post infection. Compared to traditional methods (plaque assay or $TCID_{50}$), this represents a three-to-five-fold improvement in time to result while removing subjectivity, and lowering errors substantially (<0.2 log_{10} error). The magnitude of the velocity differences and therefore, optical force, between uninfected and infected cell populations is tightly correlated with the amount of infectious virus.

This enables users of LFC™ to generate robust and highly reproducible models correlating adenovirus titers to measurements taken by Radiance®. Once an experimental protocol and model are established, the correlation curve can be used for every future experiment in order to precisely and accurately measure unknown titers, while greatly reducing the time to viral quantification.

Cell Culture, Infection, and Harvest

HEK293 cells (ATCC CRL-1573) were grown in either 12 or 24 well plates in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37° C in a humidified incubator with 5% CO2. Adenovirus

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reference material stocks (ATCC VR-1516) with a known concentration reported in IU/mL[8] were stored in the vapor phase of liquid nitrogen and thawed just prior to use. Cells were seeded at a density of 400,000 cells/mL two hours prior to infection and incubated either 24 or 48 hours before analysis. Cells were then detached using a cell dissociation buffer, centrifuged for 5 minutes at a speed of 150-200g, and resuspended in LumaCyte Stabilization Fluid with 0.5% paraformaldehyde (PFA) to a final concentration of 500,000 cells/mL. Cell samples (200 µL) were then loaded into the 96-well plate for analysis with a Radiance® instrument.

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