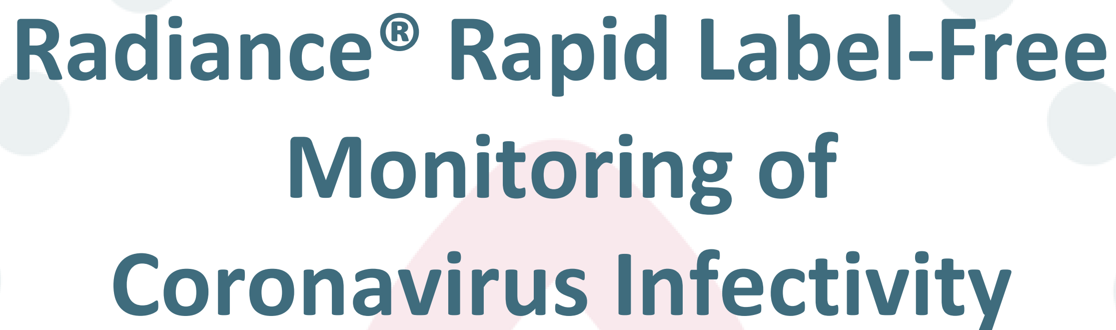

Radiance® Rapid Label-Free Monitoring of Coronavirus Infectivity

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Introduction

Human coronaviruses (HCoVs) 229E and OC43 were first described in the 1960s when they were isolated from persons suffering from upper respiratory illness[1,2]. Since their initial discovery, about 40 species have been discovered that cause infection in different hosts, including humans as well as birds and other mammals. However, it was not until nearly 40 years later that they gained their infamy with the emergence of the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012[3,4]. The growing coronavirus disease-2019 (COVID-19) pandemic, caused by the SARS-CoV-2 virus, first discovered and reported in Wuhan, China in December, 2019, has underscored the need to develop not only rapid testing and robust countermeasures, but also advanced tools and technologies for improving the speed of vaccine and antiviral therapy development and production.

A crucial measurement that is needed throughout the vaccine development cycle is the rapid quantification of viral infectivity. Specific stages include upstream process optimization and production monitoring, downstream cleanup and purification, formulations, release assays, adventitious agent testing (AAT), and neutralization assays for clinical vaccine efficacy testing. Traditional viral titering methods such as plaque assays and TCID50 (end-point dilution) are slow, labor-intensive, time consuming, operator dependent (subjective) and error prone. It is therefore important to develop a replacement that rapidly and accurately measures viral infectivity.

LumaCyte's label-free Laser Force Cytology™ (LFC) Radiance® instrument can accomplish this using a combination of optical force and microfluidics to measure intrinsic biophysical and biochemical changes in cells due to viral infection[5]. Radiance® uses this data generated to accurately, quickly, and cost effectively quantify viral infectivity.

Experimental Results

To demonstrate LFC's effectiveness in quickly quantifying HCoV-OC43 cell-based viral titer, Vero cells were infected at a range of dilutions and analyzed using Radiance® in order to develop a correlation between LFC data and the initial amount of virus added (MOI). Cells flow single file through a microfluidic channel in direct

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opposition to the laser, which exerts an optical force on the cell that resists the drag force of the flow. Viral infection causes an increase in refractive index and other optical changes within a cell which results in higher optical forces experienced by the cell that Radiance® is able to measure without the need for antibody labels at very low MOIs. **Figure 1** demonstrates that as cells are infected with HCoV-OC43, their optical force index and cell focal positions increase.

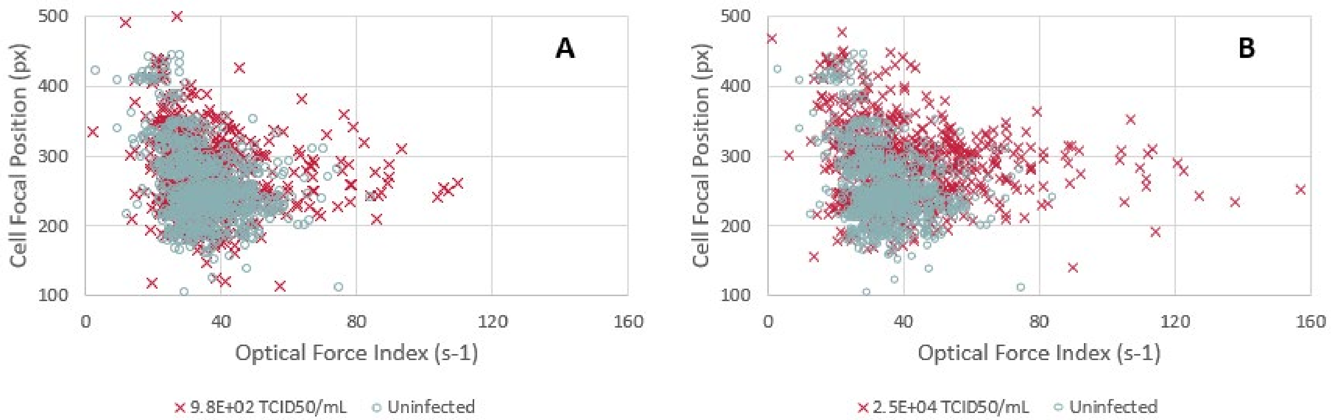

Figure 1: Cell focal position versus optical force index scatter plots comparing Vero cell populations infected with two different but low titers of HCoV-OC43, A) 9.8eE+02 TCID50/mL and B) 2.5E+04 TCID50/mL. The infected Vero cells have a greater optical force index and cell focal position as compared to uninfected Vero cells, demonstrating a change in their optical properties.

A higher optical force index indicates an increase in the size-independent optical force for a cell, while a higher cell focal position indicates that the infected cell reaches its minimum velocity at a position further from the laser. Changes in these metrics indicate more significant optical forces which may be a result of increased refractive index within the cell due to viral infection. A bivariate metric can be developed from the product of these two Radiance® parameters in order to describe the separation or detection between the uninfected and the infected populations. **Figure 2** shows this metric for each of the viral dilutions as well as uninfected cells. From the bar graph, it is clear that the unique Radiance® bivariate metric amplifies as the amount of initial virus added to the cells increases.

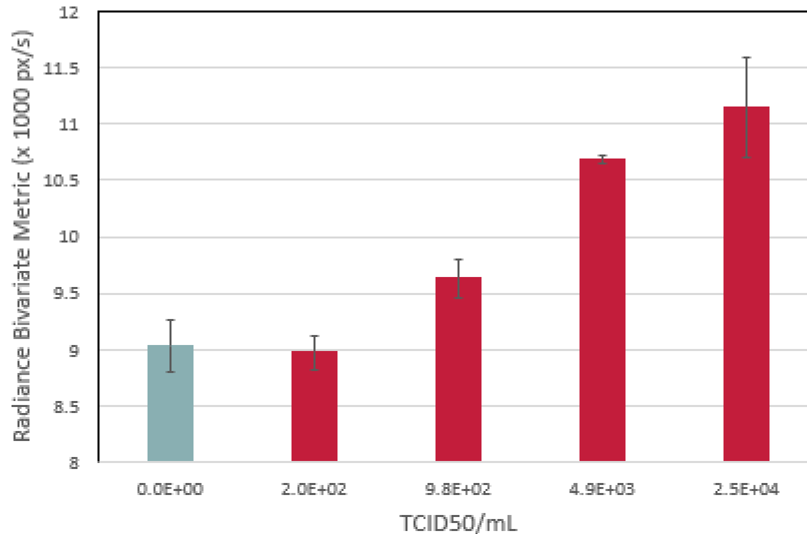

Figure 2: Bar graph of the Radiance® bivariate metric (cell focal position x optical force index). The bivariate metric rises significantly as the initial amount of virus added increases, indicating that this metric can be used to correlate well with and quantify viral titer.

The data shown in **Figure 2** can be used to generate a correlation curve shown in **Figure 3** with the Radiance® data on the x-axis and the TCID50/mL on the y-axis. By developing this strong correlation curve, the titer of HCoV-OC43 samples in subsequent assays can be directly calculated from the real-time LFC data as the infected cells are analyzed on Radiance®. Radiance® results were obtained in 4 days post infection versus 13 days with the existing standard TCID50 method. Based upon recent published data on SARS-CoV-2 and the expected performance of Radiance®, it is estimated that SARS-CoV-2 could be detected and quantified in 15-22 hours without the need for fluorescent or antibody labels, versus the 48-72 hours currently required for standard infectivity assays[6,7]. The reduced time when compared HCoV-OC43 is likely due to the higher infectivity of the SARS-CoV-2 strain.

For vaccine production, in-process bioreactor monitoring where infected cells are taken directly for analysis on Radiance®, yields results in minutes as opposed to days or even weeks. LFC provides advanced scale up and production monitoring capabilities, with near real-time analytics allowing processes to be optimized sooner and corrective action taken, ensuring consistent vaccine quality and product yields. Used as a rapid process analytical tool (PAT), Radiance® can save millions of dollars in lost materials, resources, and time. This is incredibly important, particularly if a vaccine needs to be produced quickly in response to an emerging pandemic. Additionally, reducing the R&D time necessary for vaccine development for current or emerging human coronaviruses is incredibly valuable when time is of the utmost importance.

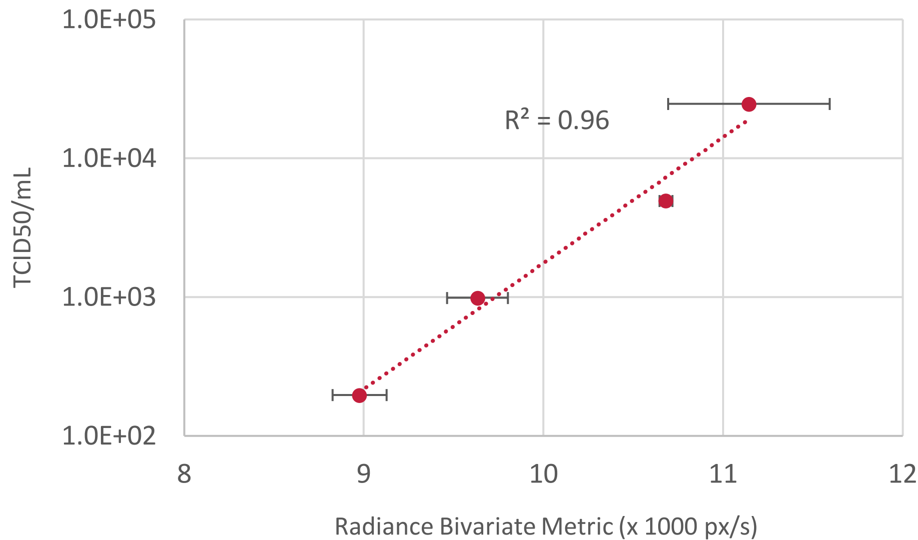

Figure 3: Correlation curve between the Radiance® bivariate metric and TCID50/mL added. The equation for the curve above can be incorporated into the Radiance® software, allowing for real-time viral quantification results in the future.

Laser Force Cytology™can be used to increase the speed and precision of viral titer measurements, enabling the compression of important R&D efforts and improving scale-up and production timelines, as well as formulations, release assay, and viral safety testing timelines across the vaccine development life cycle.

Cell Culture, Infection, and Harvest

Vero cells (ATCC CCL-81) were grown in 24-well plates in Minimum Essential Media alpha (MEM α) supplemented with 10% fetal bovine serum at 37oC in a humidified incubator with 5% CO2. The HCoV-OC43 (ATCC VR-1558) stock was stored in the vapor phase of liquid nitrogen. Cells were seeded at a density of 250,000 cells/mL and incubated for 24 hours prior to infection. The cells were infected with HCoV-OC43 in MEM supplemented with 2% fetal bovine serum and then incubated at 35oC with 5% CO2. Cells were harvested 4 days post infection using a dissociation buffer, centrifuged for 5 minutes at 200g, and resuspended at a concentration of 600,000 cells/mL in LumaCyte Stabilization Fluid (SF-1600) and 0.5% paraformaldehyde (PFA). Cell samples were then loaded into a Radiance® +Autosampler 96-well plate for analysis with a Radiance® instrument.

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