An Automated Assay Platform for Influenza Virus Titration

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INTRODUCTION

Seasonal influenza virus affects up to one billion individuals globally each year, contributing to an estimated 650,000 deaths. Vaccination remains the most effective strategy for preventing severe illness and mortality. To support global surveillance, therapeutic development, and research efforts, institutions worldwide rely on timely access to high-quality reagents. ATCC plays a critical role in this network by providing well-characterized live influenza virus and derivative materials essential for these initiatives.

Traditional methods for determining virus titers, such as ELISA, are often labor-intensive and time-consuming, requiring significant hands-on time and resources. In this study, we evaluated an automated approach for titrating live influenza virus using the LumaCyte Radiance® system, comparing its performance to the conventional ELISA method. Radiance® technology utilizes Laser Force Cytology™, a novel, label-free platform for single-cell analysis that measures the intrinsic biophysical and biochemical properties of individual cells. This approach allows for high-throughput, real-time analysis of infection dynamics without the need for staining or complex sample preparation.

Our findings demonstrate that virus titers generated using the Radiance® system for both Influenza A and B were consistent with those obtained via ELISA, validating the accuracy of this label-free approach. Notably, the Radiance® method reduced hands-on time by over 50% when compared to ELISA. This gain in operational efficiency supports faster decision-making in production settings, helping to streamline processes and maintain throughput without compromising data quality.

METHODS

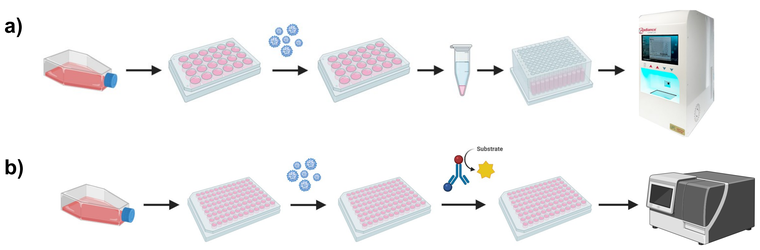


Figure 1. Assay workflows. a) For Radiance® assays, cells were seeded in 24-well plates, incubated until confluent, and infected with 3-fold serial dilutions of virus stock (up to 8 dilutions, each dilution in triplicate), followed by a 24-hour incubation. Infected cells were harvested, counted, and normalized to 8.5 × 10^5 cells per mL in stabilization fluid containing 0.5% paraformaldehyde (PFA). 200 µL of samples were loaded into corresponding wells of the 96-well Radiance® assay plate. Each sample had a minimum of 300 single cells collected and analyzed per well. Raw data was analyzed using the system's built-in analysis software platform ReportR®. **b)** For ELISAs, cells were seeded in 96-well plates, incubated until confluent, and infected with 3-fold serial dilutions of unknown virus stock (five replicates per dilution) alongside a positive control (PC). Following fixation, viral antigen was detected using an ELISA-based assay and plates were read on a SpectraMax. Tissue Culture Infectious Dose 50% ($TCID_{50}$) values were calculated based on the dilution at which 50% of wells showed positive signal. **Table 1** summarizes the viruses tested in this study.

Table 1. Viruses tested.

Type	Origin	Strain number/Year	Lineage or Subtype
Influenza B	Alabama	07/2023	B/Victoria
Influenza B	Bangladesh	5972/2007	B/Yamagata
Influenza A	Wisconsin	67/2022	(H1N1) pdm09

RESULTS

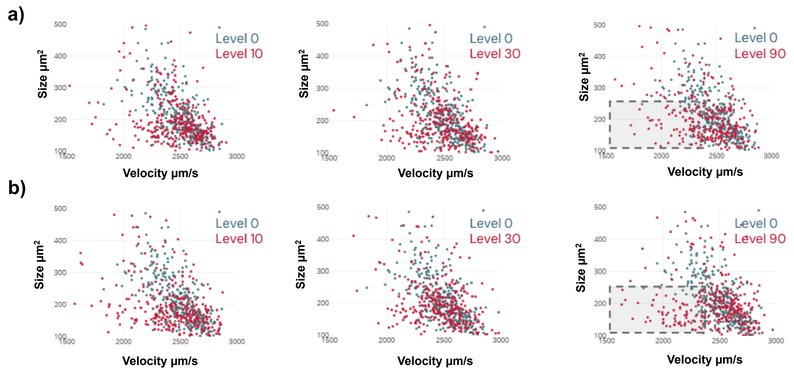


Figure 2. Size vs. velocity scatter plots. Representative scatter plots generated from ReportR® show the biophysical profiles of cells infected with **a)** B/Alabama/07/2023 and **b)** B/Bangladesh/5972/2007 across different levels of infection. Each point represents an individual cell, with size (y-axis) and velocity (x-axis) derived from laser-based measurements. A shift to lower velocity and a slight decrease in size is observed with increasing volume of virus. These changes can be quantified using velocity and optical force index (OFI) parameters, providing a biophysical assessment of viral infectivity and particle quality.

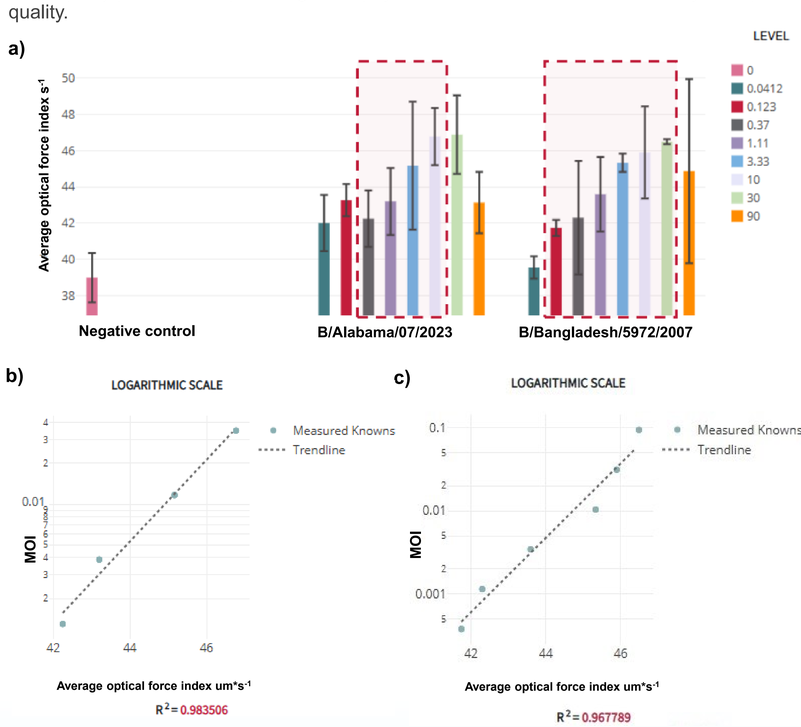
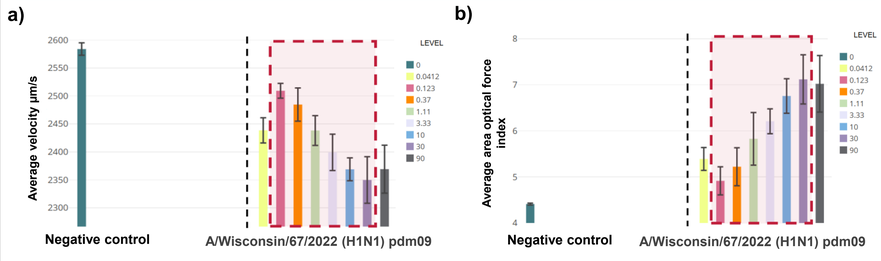


Figure 3. Census data and calibration curves for Influenza B. MDCK cells were seeded and infected at 8 different multiplicity of infection (MOIs) per virus. Samples were harvested 24 hours post-infection. **a)** Representative bar plots show the average optical force index (OFI) across MOIs for B/Alabama/07/2023) and B/Bangladesh/5972/2007. A consistent shift in OFI was observed with increasing MOI, reflecting increasing levels of infection for both viruses. Calibration curves were generated for **b)** B/Alabama/07/2023 and **c)** B/Bangladesh/5972/2007 by plotting average OFI values against the corresponding MOIs.

Table 2. Representative virus titer for B/Bangladesh/5972/2007. Titers were derived per MOI using ReportR® and a calibration curve; the final value reflects the mean across MOIs

Virus	Level	Known MOI	Calculated MOI	Calculated Titer (IU/mL)
B/Bangladesh/5972/2007	0.123	0.000383	0.000467	3.17E+06
	0.37	0.0012	0.000828	1.87E+06
	1.11	0.0035	0.0186	2.36E+06
	3.33	0.0104	0.0332	4.67E+06
	10	0.0311	0.0332	2.77E+06
	30	0.0933	0.0612	1.71E+06
Average Calculated Titer (IU/mL)				2.76E+06



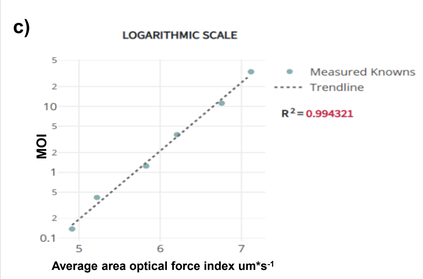


Figure 4. Census data and calibration curve for Influenza A. MDCK cells were seeded and infected at 8 different MOIs. Samples were harvested 24 hours post-infection. Representative bar plots show excellent response for both **a)** average velocity and **b)** average OFI across multiple MOIs. **c)** A robust calibration curve with an R^2 of 0.99 was generated using the average OFI and corresponding levels 30-0.123. This curve can be used to predict the titer of future unknown samples.

CONCLUSIONS

As a trusted biological resource center, ATCC plays a critical role in supporting global influenza research and production through the development and validation of reliable virological tools. Our study demonstrates that the LumaCyte Radiance® platform offers a robust, label-free alternative to traditional titration methods, delivering consistent results for both Influenza A and B viruses. In our studies, titers for both Influenza A and B were consistent with ELISA results (**Table 3**), while the workflow significantly reduced hands-on time. This efficiency promotes faster, data-driven decision-making in production settings. Collectively, these findings support the future integration of Radiance into influenza production workflows, where rapid, reproducible, and scalable titering methods are essential, further reinforcing ATCC's commitment to advancing high-quality, scalable solutions for the global scientific community.

Table 3. Comparison of virus titers calculated by ELISA and Radiance®.

Average calculated titer (IU/mL)		
Virus	**ELISA**	**Radiance**
B/Alabama/07/2023	1.89E+06	2.43E+06
A/Wisconsin/67/2022	2.40E+08	2.43E+08
B/Bangladesh/5972/2007	2.62E+06	2.76E+06

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