Optimizing Bioprocess Parameters and Rapid Viral Infectivity Quantification with Laser Force Cytology™

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LumaCyte®

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Introduction

The development and manufacturing of advanced therapies, including vaccines and cell and gene therapies, demands rapid, precise, and scalable analytical technologies capable of providing actionable insights in real time. Traditional methods for assessing cell health, viral infectivity, and product potency often rely on time-consuming, labor-intensive protocols that require fluorescent labels, antibodies, or extensive sample preparation. These approaches can introduce variability, delay critical decisions, and consume valuable resources during process development and production. In the development of viral vaccines, where rapid evaluation of viral titer is essential, these traditional approaches can significantly bottleneck optimization efforts.

LumaCyte's Radiance® instrument, powered by Laser Force Cytology™ (LFC), offers a transformative alternative to conventional analytical techniques. LFC is a label-free, cellular process analytical technology (PAT) that integrates advanced optical and hydrodynamic forces to probe the biochemical and biophysical properties of individual cells. By eliminating the need for exogenous labels, LFC enables real-time, high-precision analysis while preserving cellular integrity. Radiance® allows for rapid, direct measurement of viral infectivity and production metrics, accelerating the screening and optimization process of culture conditions critical for improving viral vaccine yields and manufacturing efficiency.

Radiance® measures over 20 biochemical and biophysical parameters per cell, providing robust, multiparametric datasets that enable the detection of subtle early changes in cell state, offering a powerful tool for real-time monitoring and optimization of biomanufacturing processes. Applications of Radiance® span a wide range of use cases including rapid viral infectivity and neutralization assays, cell health and fitness monitoring during stress conditions (e.g., cryopreservation or media adaptation), T-cell characterization, and potency measurements—all critical for ensuring product quality and consistency.

In this technical note, we demonstrate the utility of Radiance® for real-time viral infectivity and cell health analysis in the context of Vero cell adaptation and virus production. Vero cells (kidney epithelial cells of an African green monkey), originally cultured in serum-containing medium, were adapted to each of the four different serum-free growth medias from the Gibco™ Adherent Kidney Media Panel available from Thermo Fisher Scientific. Following adaptation, the cells were infected with vesicular stomatitis virus (VSV) at varying multiplicities of infection (MOIs) and

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cultured in specially formulated production medias, specifically with the intent of increasing viral productivity. By sampling cells and supernatants at multiple times post-infection (24, 48, and 72 hours), we were able to capture dynamic biological responses across different media conditions. Traditional infectivity assays, such as plaque and TCID50, are laborious, have a long time-to-result, and are difficult to standardize. As a result, they are cumbersome for use as a tool for rapid screening of process conditions.

Radiance® was used to analyze harvested cells in a label-free manner, providing insight into viral production without requiring fluorescent antibodies, additional overlay reagents or subjective assay readouts. Subsequently, supernatant samples were used in downstream infectivity assays to assess functional viral output. This approach enabled the correlation of single-cell biophysical profiles post-infection with traditional infectivity outcomes, showcasing Radiance®'s potential as a real-time process analytical technology (PAT) for upstream viral production monitoring.

By leveraging LFC's sensitivity to subtle biophysical changes, this study underscores how Radiance® can accelerate the optimization of bioprocess parameters, such as media selection and infection timing, while reducing reliance on slow, error prone, and resource-intensive assays. The result is a streamlined, data-rich workflow that supports both the development and manufacturing of viral-based therapies and vaccines.

Experimental Results

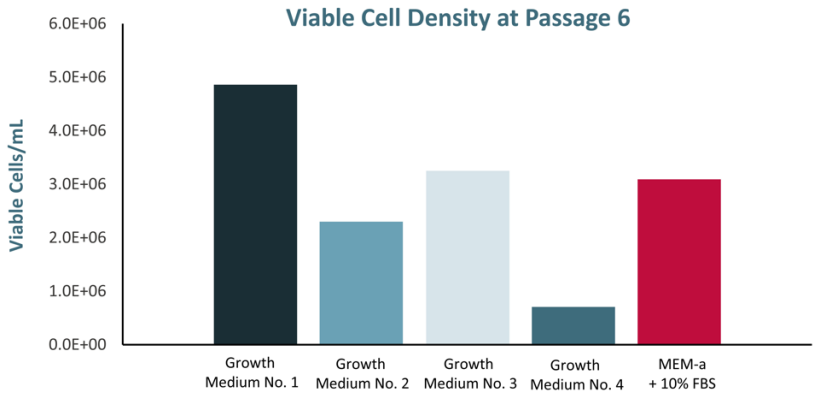

Figure 1: Viable cell density of Vero cells at passage 6 following adaptation to different serum-free growth media. Cells adapted to Growth Media 1 and Growth Media 3 demonstrated higher viable cell densities compared to the traditional serum-containing control (MEM-α + 10% FBS). Growth Media 4 supported the lowest viable cell density among the tested conditions.

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During the initial phase of the study, Vero cells frozen under standard conditions with serum-containing media were thawed and subsequently adapted into serum-free media over a period of six passages. Vero cells successfully adapted to all serum-free media conditions in the Gibco kidney media panel by passage 6, with no observable issues during the transition from serum-containing media. When comparing viable cell densities across media, Growth Media 1 and Growth Media 3 outperformed the serum-containing control (MEM-α + 10% FBS), exhibiting higher viable cell concentrations at the time of infection. These results indicate that these serum-free formulations not only support robust growth but may offer a performance advantage over traditional serum-supplemented conditions.

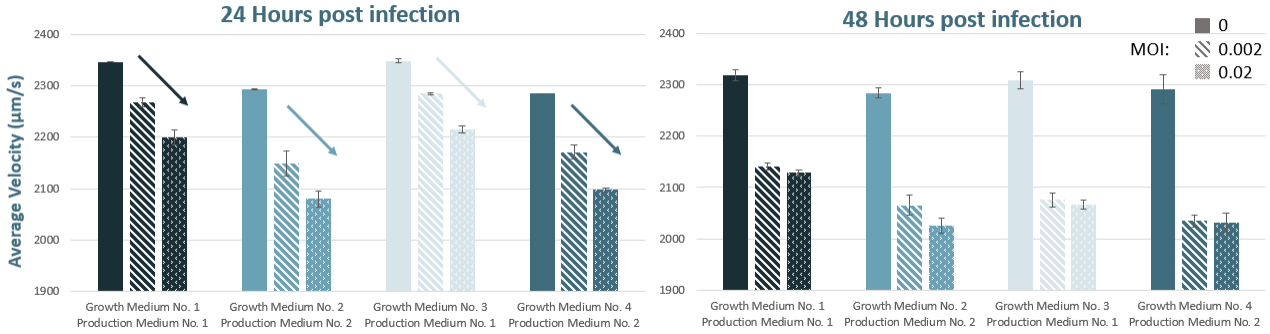

Figure 2: **Average velocity (∝ optical force) of Vero cells 24- and 48-hours post-infection across different growth and production media combinations.** A clear MOI-dependent decrease in average cellular velocity was observed in all conditions, indicating successful infection. For each media pairing, cells infected with VSV at MOI 0.02 showed a lower velocity than MOI 0.002, while uninfected controls (MOI 0) maintained the highest velocities. Infection trends observed at 24 hours persisted at 48 hours. Error bars represent the standard deviation of individual wells (n=3).

Radiance® was used to evaluate the viral infection by measuring the average cellular velocity, a biophysical indicator that decreases upon successful viral infection due to the presence of viral inclusion bodies within the cells. Vero cells were seeded in one of four serum-free growth media formulations, and following overnight incubation, the media was replaced with the corresponding production media for viral infection; Production Media 1 paired with Growth Media 1 and 3, and Production Media 2 paired with Growth Media 2 and 4. Cells infected with vesicular stomatitis virus (VSV) at two multiplicities of infection (MOI 0.002 and 0.02) exhibited a clear, MOI-dependent decrease in velocity at both 24- and 48-hours post-infection, confirming successful viral entry and replication. Control samples (MOI 0) maintained higher average velocities, as expected. This pattern was consistent across all media conditions tested. Notably, some media pairings demonstrated more pronounced velocity shifts, potentially reflecting enhanced viral

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replication based on media composition. These findings reflect infection-driven biophysical changes detectable with Laser Force Cytology™.

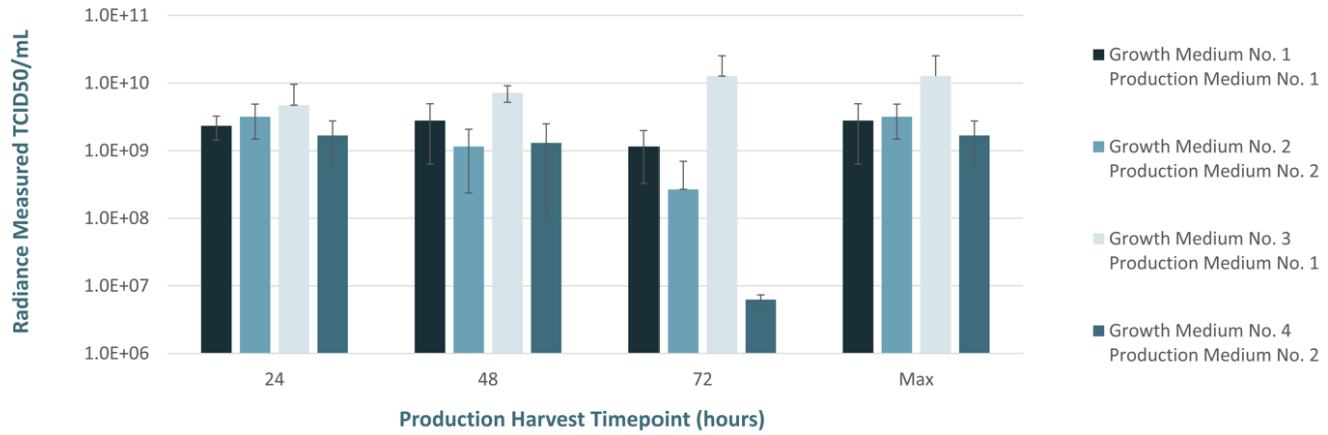

Figure 3. Radiance® measured viral titer ($TCID_{50}$/mL) across production harvest timepoints for a variety of serum-free Gibco™ kidney media panel pairings. Vero cells were used for assessing viral production using supernatants from the MOI 0.002 samples from Figure 2. Growth Media 3, paired with Production Media 1 consistently outperformed other conditions, yielding the highest titer at all timepoints and achieving the greatest maximum titer overall. Notably, this condition showed sustained productivity even at 72 hours, while other conditions exhibited a sharp decline. Error bars represent standard deviation (n=3).

Supernatants from the lower MOI condition (0.002) were analyzed at 24-, 48-, and 72-hours post-infection to quantify viral output using Radiance®. As most of the cells were lysed by the 72h time point, Radiance® samples were analyzed at only 24 and 48 hours. However, the supernatant titer analysis covered the additional 72h time point to ensure we captured the maximum amount of variation in titer between groups. Among all combinations tested, the pairing of Growth Media 3 with Production Media 1 yielded the highest viral titer ($TCID_{50}$/mL) across all time points. While most conditions showed a plateau or decline at later timepoints, this optimal pairing maintained its productivity, highlighting the value of media optimization and the rapid analytical power of Radiance® for upstream process development. At the 72-hour production harvest timepoint, this media pairing had a viral titer of 1.27E+10 $TCID_{50}$/mL. Final viral titer of this combination was over sixfold higher than those typically observed in production using the serum-containing control (2.08E+09 $TCID_{50}$/mL as seen in Figure 5), underscoring the potential of this serum-free pairing for enhanced viral production.

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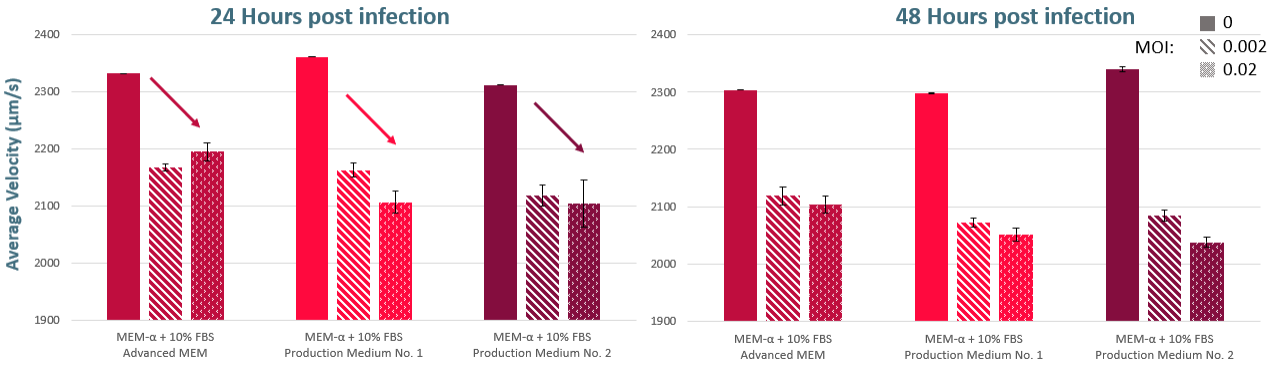

Figure 4. Average velocity (∝ optical force) **of Vero cells 24- and 48-hours post-infection using serum-containing growth media with serum-free production media.** Similar to previous conditions, a clear MOI-dependent decrease in average cellular velocity was observed in all conditions, indicating successful infection. At each media pairing, cells infected with VSV at MOI 0.02 showed the lowest velocities, while uninfected controls (MOI 0) maintained the highest velocities. Infection trends observed at 24 hours persisted at 48 hours. Error bars represent standard deviation (n=3).

To further explore the impact of serum-free production conditions, a second experiment was conducted using serum-containing growth media (MEM-α + 10% FBS) for all groups followed by infection and production in either one of the serum-free production media (Production Medium 1 or 2) or using serum-free Advanced MEM as a control condition. Similarly to the first experiment, cells were analyzed at 24- and 48-hours post-infection, while supernatant titers were analyzed at 24, 48, and 72 hours post-infection. Radiance® measurements again showed a decrease in average velocity with an increasing MOI, consistent with productive infection. Viral titer analysis of the supernatants from the lower MOI (0.002) condition revealed that both production media 1 and 2 outperformed the standard serum-containing control, particularly at the earliest (24 hour) time point. Remarkably, Production Media 2 achieved a viral titer approximately 14-fold higher than the control,, where the viral titer was 2.88E+10 $TCID_{50}$/mL for production media 2 compared to 2.08E+09 $TCID_{50}$/mL for the control. These results highlight the performance advantage of the Gibco™ Kidney Media Panel in optimizing viral productivity even in workflows retaining serum during the growth phase.

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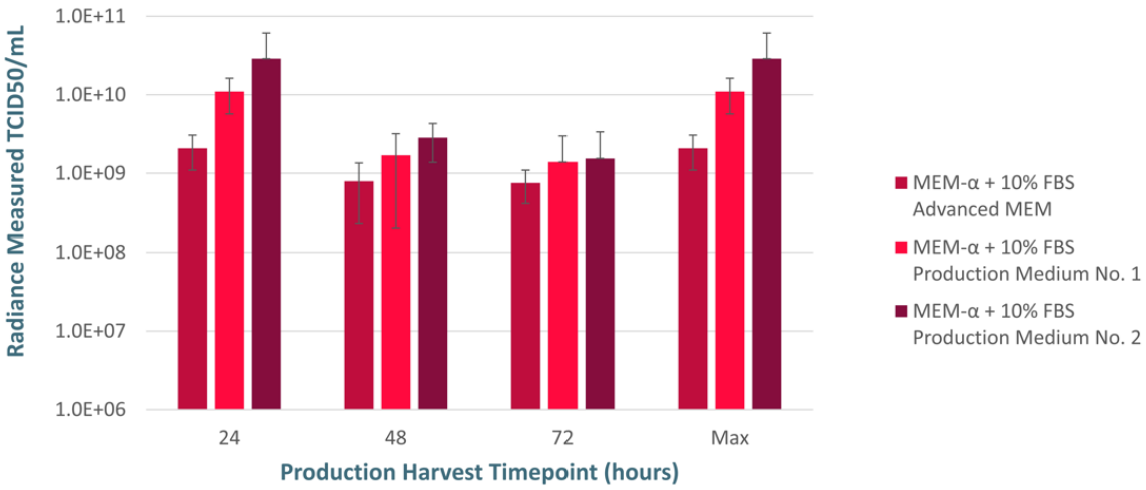

Figure 5: **Radiance® measured viral titer ($TCID_{50}$/mL) using serum-containing growth media with serum-free production media.** Vero cells cultured in MEM-α + 10% FBS were paired with either Advanced MEM or Production Media 1 or 2 during the viral production phase. Viral titers were assessed at 24, 48, and 72 hours post-infection. The pairing of MEM-α + 10% FBS with Production Media 2 consistently yielded the highest titers across all timepoints and achieved the greatest maximum titer under these conditions. Error bars represent standard deviation (n=3).

Discussion

The findings presented in the study highlight the utility of Laser Force Cytology™ (LFC) via the Radiance® instrument as a powerful real-time process analytical technology (PAT) for evaluating viral infectivity and optimizing upstream conditions in cell-based bioprocesses. Using Vero cells as a model system, we successfully demonstrated that Radiance® can sensitively detect infection related changes across various media conditions in a rapid manner, without the need for labeling or time-consuming and subjective assays such as $TCID_{50}$.

Adaptation of Vero cells to the Gibco™ Adherent Kidney Media Panel was rapid and effective, with Growth Media 1 and Growth Media 3 supporting higher viable cell densities than traditional serum-containing media. These enhanced growth conditions provided a strong foundation for viral production studies and support the industry trend toward development of serum-free media conditions.

The biochemical and biophysical readouts captured by Radiance®, specifically average cellular velocity, have proved to be reliable early indicators of viral infection. In all conditions, velocity decreased as multiplicity of infection (MOI) increased, reflecting viral entry and replication. Importantly, these changes were apparent as early as 24 hours post-infection and were

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maintained at 48 hours, demonstrating the instrument's capacity to provide rapid insights into viral infectivity.

The infectivity assay revealed significant variability in viral production across different media pairings, highlighting the capability of Radiance to rapidly screen a wide range of process conditions, including harvest time and media. Notably, when using a serum-free medium for both growth and production, Growth Media 3 combined with Production Media 1 consistently delivered the highest viral titer at all time points and maintained productivity through 72 hours of infection. This combination outperformed the serum-containing control over sixfold, illustrating the value of systematic media screening in upstream optimization.

Furthermore, when cells were grown in serum-containing media and transferred to serum-free production conditions, we again observed superior performance with Production Media 1 and 2, where Production Media 2 yielded a viral titer approximately 14-fold higher than the standard control. These results underscore the critical role of media composition not only in supporting cell viability, but also in driving efficient viral production.

Collectively, these results validate the use of Radiance® for highly sensitive, label-free analysis that can streamline process development by enabling rapid screening of media and infection parameters. The ability to correlate single-cell biophysical changes with infectivity outcomes demonstrates Radiance® as a transformative instrument for vaccine development, cell and gene therapy manufacturing, and other bioprocess applications. By delivering actionable data in near real time, LFC supports faster, more informed decision-making and reduces the development timelines for vaccines as well as other advanced therapies.

Cell Culture and Experimental Methods

Vero Cell Culture and Media Adaptation

Vero cells originally banked in traditional culture medium, Gibco™ MEM-α (Gibco-12571-048) supplemented with 10% fetal bovine serum (Corning-35-016-CV) were thawed and cultured under standard conditions (37°C and 5% CO^2) for two passages. Following expansion, cells were directly transitioned into four serum-free growth media formulations from the Gibco™ Adherent Kidney Media Panel along with a control condition (Gibco™ MEM α + 10% FBS). Throughout the adaptation phase, all cultures were passaged synchronously at consistent seeding densities. Cells were considered fully adapted by passage six.

To assess cell viability at the end of adaptation, cells were dissociated using TrypLE™ Express (Gibco-12604-021), stained 1:1 with trypan blue (Corning-25-900-CI), and counted using a hemacytometer. Subsequently, Vero cells were used for viral production experiments.

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Viral Production and Infection

Adapted Vero cells were seeded into 48-well plates and allowed to adhere overnight in their respective serum-free growth media. The following day, cells were infected with vesicular stomatitis virus (VSV) at two multiplicities of infection (MOIs: 0.002 and 0.02). At the time of infection, growth medium was replaced with the corresponding production media. The media conditions were assigned as follows:

- Production Media 1: paired with Growth Media 1 and 3
- Production Media 2: paired with Growth Media 2 and 4

Cells and supernatants were harvested at 24- and 48-hours post-infection. Harvested cells were analyzed on the Radiance™ instrument (LumaCyte), and supernatant samples were stored for downstream viral titer analysis.

Viral Titer Assay (Radiance®-Based Infectivity Analysis)

To further evaluate viral production, fresh Vero cells were seeded into 48-well plates, incubated overnight, and then infected the following day. Cells were exposed either to a VSV reference stock of known titer or to previously collected supernatant from MOI 0.002 samples. Infections were carried out for 24, 48, and 72 hours. At each time point, cells were harvested and analyzed using the Radiance® instrument to quantify infection levels, measured as $TCID_{50}$/mL.

Serum-Containing Growth Media Workflow

The above experiment was repeated using traditional culture medium, Gibco™ MEM-α (Gibco-12571-048) supplemented with 10% fetal bovine serum (Corning-35-016-CV) as the growth medium. Vero cells were seeded into 48-well plates, incubated overnight, and infected with VSV the following day at the same two MOIs. At time of infection, growth medium was replaced with either Production Media 1, Production Media 2, or the standard serum-free control, Gibco™ Advanced MEM (Gibco-12492-013). Harvested cells and supernatants at 24- and 48-hours post-infection were analyzed as described above. The viral titer assay was also performed on the supernatant from the MOI 0.002 samples as previously described.

Data Collection and Analysis

All Radiance® measurements were analyzed using the integrated ReportR™ software for real-time cell analysis. Viral titers were quantified based on Radiance®-measured infectivity. All conditions were tested in triplicate unless otherwise specified.

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