Measurement of Infectious Titers and Total Viral Particle Counts to Fully Characterize Your Viral Process and Product

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LumaCyte®

Introduction

Accurate assessment of viral quality is paramount for ensuring the efficacy and safety of viral-based therapies, including vaccines, viral vectors, and oncolytic viruses. In manufacturing environments, the growth and stability of viruses can be profoundly influenced by various environmental conditions during the production and purification process, including temperature fluctuations, pH changes, oxidative stress, and osmotic pressure alterations. Even minor deviations from optimal growth temperature and pH can impede viral replication rates and alter protein folding, structure, and function, potentially compromising viral infectivity and therefore product quality[1]. Because of this, meticulous control and monitoring of environmental conditions is an essential part of manufacturing quality control to ensure optimal viral growth, productivity, and quality. However, even under the most stringent conditions, unintended events may occur that negatively affect viral production and/or viral titer, including the production of defective interfering particles (DIPs)[2], ultimately reducing product quality. This possibility underscores the need for an accurate assessment of viral particle counts and infectious titer throughout the manufacturing process.

Total viral particle concentration can be used as a metric to quickly gauge a viral sample's suitability during production and before use. While quantifying the number of viral particles provides valuable insights into sample purity and yield, it does not capture an essential aspect of viral quality or function: virulence. Assessing the ability of viral particles to infect host cells is crucial for evaluating their functional potency and thus the infectious capacity of viral particles is a critical aspect that must be evaluated in viral manufacturing. Traditional techniques such as plaque assays and endpoint dilution assays remain widely utilized for determining viral infectivity. However, these methods are labor intensive, inherently prone to variability and subjective interpretation, and most importantly, incredibly time-consuming, not allowing for the assessment of product quality in a useful time. Innovative technologies such as Laser Force Cytology™ (LFC™) seek to address these limitations by offering label-free, high-throughput, and quantitative analysis of viral infectivity[3-5]. By utilizing LFC™ for single-cell analysis, LumaCyte's Radiance® instrument enables direct measurement of viral infectivity without the necessity for laborious staining and subjectivity associated with traditional methods.

Radiance® delivers real-time, dynamic insights into viral infectivity, enabling scientists and developers to track viral samples across various stages of the biomanufacturing process. This capability allows for the assessment of viral potency and stability, streamlining the evaluation of viral quality and enhancing the reliability, reproducibility, and scalability of viral-based processes and products.

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To this aim, here we demonstrate the impacts of various process insults, namely elevated temperature, low pH, and freeze-thaw cycles on the virulence of viral particles and the capability of Radiance® to assess these changes quickly. The Malvern Panalytical Nanosight Pro NS300 is also used for viral particle analysis prior to infection in order to better understand the viral starting material and how the ratio of total to infectious viral particles might change with various insults. The combination of these methods allows for the full characterization and function of a viral product.

Experimental Results and Discussion

In order to gauge the effect of the selected process insults, previously produced stocks of both vesicular stomatitis virus (VSV) as well as baculovirus (BV) were thawed and then analyzed for both physical count (NanoSight Pro) as well as infectious viral titer (Radiance®) both pre- and post-treatment. Results for VSV for both assays are provided in **Figure 1** and **Table 1**. Starting with the physical count (**Figure 1**, solid bars), there is minimal statistical change in the total viral particle count as a result of the insults. Specifically, the average viral particle count of the untreated samples was 2.51E9±2.80E8 (particles/mL) versus 1.81E9±1.80E8 for the Freeze Thaw samples, 1.74E9±4.70E8 for the low pH samples, and 2.78E9±3.60E8 for the High Temp samples. This indicates there was no appreciable change in the total number of particles due to the sample insults. However, when looking at the infectious viral titer (**Figure 1**, dotted bars), there are clear changes of varying magnitude as a result of some treatments.

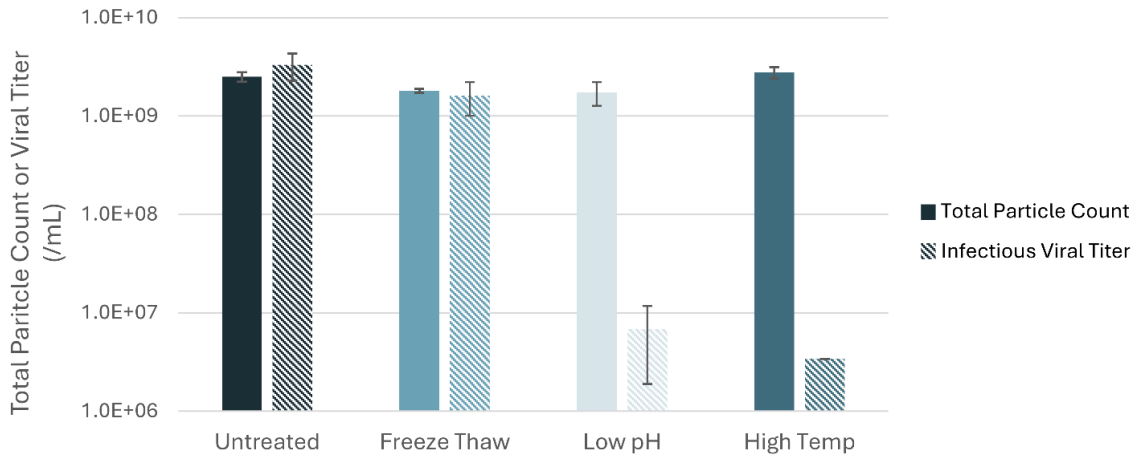

Figure 1: **Total Particle Count versus Titer for VSV Samples**. Comparison between the total particle count (solid bars) to the infectious viral titer (diagonal bars) for untreated VSV samples as well as those subjected to various treatments including freeze thaw, low pH, and high temperature.

Treatment	Total Particle Count (particles/mL)	Infectious Viral Titer (TCID50/mL)	Log_{10} Reduction from Untreated
Untreated	2.51E9±2.80E8	3.31E9±1.03E9	N/A
Freeze Thaw	1.81E9±1.80E8	1.61E9±6.02E8	0.31
Low pH	1.74E9±4.70E8	6.83E6±4.95E6	2.69
High Temp	2.78E9±3.60E8	3.40E6	2.99

Table 1: **Viral Titer Reduction due to Sample Insults.** Total particle count and infectious viral titer for untreated VSV samples as well as those subjected to various treatments including freeze thaw, low pH, and high temperature. The final column indicates the reduction in viral titer as a result of each treatment.

The Freeze Thaw samples showed the smallest change, reducing to 1.61E9±6.02E8 (TCID50/mL) versus the untreated sample at 3.31E9±1.03E9. This represents a 0.31 log_{10} reduction as a result of the treatment. Both the low pH and High Temp treatments resulted in a much larger reduction in viral titer, dropping to 6.83E6±4.95E6 and 3.40E6, respectively. This represents a nearly 3-log reduction in titer for both treatments, illustrating the importance of quantifying the viral titer in addition to the particle count. If only the particle count is used, the ratio of total to infectious viral particles can change with sample treatment, and therefore incorrect assumptions could be made regarding product quality and potency.

A similar analysis was performed for baculovirus samples, as illustrated in **Figure 2**. Initially, a total particle count was conducted for the untreated samples and samples subjected to freeze thaw cycles or high temperature. As shown in **Figure 2A**, minimal change is seen in the total particle count for both treatments. However, when looking at the viral infectivity of the samples, there is a clear reduction in the viral titer for the heat-treated samples, as shown in the LFC™ analysis in **Figure 2B**, which shows the reduction in average velocity due to infection for a population of Sf9 cells at 46h post infection. When looking at the untreated viral sample, there is a reduction in velocity for both levels of virus added when compared to the uninfected control cells, at 153 µm/s and 133 µm/s for 16 µL and 4 µL of sample added, respectively. This is the expected behavior for baculovirus infection and the reduction in velocity is proportional to the amount of virus added. For the treated samples, a velocity decrease similar to that of the untreated sample is seen for the freeze thaw samples, at 165 µm/s and 129 µm/s for 16 µL and 4 µL, indicating no apparent reduction in viral titer due to the treatment. However, when looking at the high temperature treated viral samples, there is a significantly smaller reduction in velocity, at only 78 µm/s and 56 µm/s for 16 µL and 4 µL. This indicates at least a 10-fold reduction in the infectious viral titer for these samples due to the heat-treatment, even though there is no appreciable decrease in the total particle count.

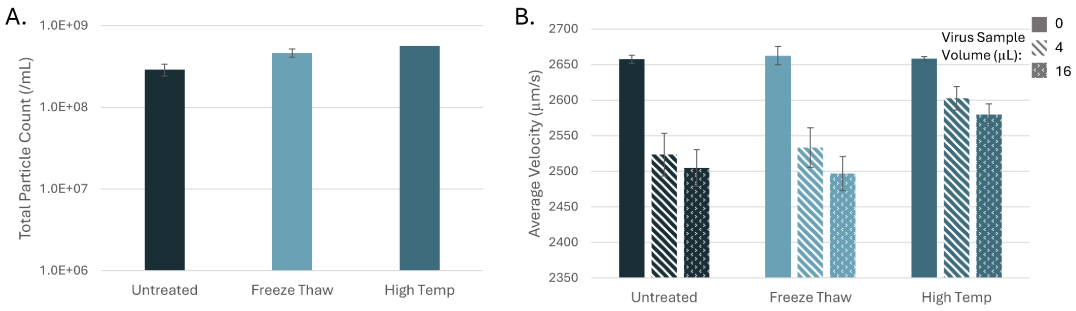

Figure 2: Total Particle Count and Viral Titer for Baculovirus Samples. A. Total particle count for untreated baculovirus samples compared to samples subjected to either freeze thaw cycles or high temperature treatment. **B**. LFC™ analysis of viral samples as shown by the average velocity (∝ optical force) of Sf9 cells at 46h post infection. Uninfected cells are compared to two volumes of virus sample added, 16 µL and 4 µL. Note that the same volume of virus was added to both the untreated and treated samples.

Taken together, these data for treated samples of both VSV and baculovirus illustrate the value of tracking both the total particle count and the infectious viral titer. Various treatments that may not have an effect on the total particle count can have a profound effect on the infectious capacity of viral samples and thus measuring both are important. Assays that measure physical count or provide a non-infectious measure of the amount of virus can provide a quick time to result, but they should always be paired with the essential measurement of infectious titer when evaluating product quality for viral products such as vaccines and viral vectors.

Laser Force Cytology™ offers a rapid and precise solution for potency assessment in viral-based therapies due to its numerous advantages over traditional methods. The capability to directly measure viral infectivity with high-throughput in a label-free manor consumes less time and materials compared to assays that require specific stains or labeling procedures. LFC™ can provide a pivotal assay in biomanufacturing processes with its rapid and accurate assessment of viral potency.

Methods

Previously collected and frozen samples of Vesicular Stomatitis Virus (VSV) and baculovirus were thawed and subjected to multiple insults including elevated temperatures and freeze thaw cycles prior to analysis for both physical count and infectious titer. VSV was additionally subjected to a low pH environment for 30 minutes as an additional treatment.

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Insults were administered as follows: Freeze-Thaw samples were thawed to room temperature and then immediately placed back into a -80°C freezer for 30 minutes. This process was repeated for three freeze thaw cycles. High temperature samples were added to a well plate, wrapped in aluminum foil, and heated to a temperature of 50°C for 30 minutes using a hot block before being allowed to return to room temperature. Finally, low pH samples were treated with 0.1M HCl until reaching a pH of 2 as indicated by pH probe and left at room temperature for 30 minutes. The pH was then returned to approximately 7.0 using 1M NaOH before use.

Viral starting material size and concentration was measured at room temperature using a Nanosight Pro NS300 instrument equipped with a 532 nm laser. 10X dilutions of the samples were prepared and loaded in the flow cell. The exposure time, contrast gain, and focus were set automatically by the software and the syringe pump flow rate was set at 3 µL/min. Five repeat measurements of 750 frames were collected for each sample.

For the VSV infectivity studies, Vero cells were harvested and then seeded into 24 well plates at a concentration of 100,000 cells/well in MEM-α media with 10% FBS and 2% L-Glutamine. Cells were then incubated overnight and counted at the time of infection to ensure accurate cell counts for infectivity and MOI determination. After determination of the cell count, growth medium was removed from each well and replaced with Advanced MEM medium with 10% FBS containing VSV virus to create MOI of 1, 0.1, 0.01, and 0.001 for both experimental and control samples. Control virus received no insult prior to infectivity. Cells and virus were incubated at 37°C for 24 hours and then harvested for infectivity analysis using Radiance®.

Sf9 cells were seeded into 50 mL conical tubes and incubated at 25°C for baculovirus infectivity. Baculovirus was added to the tubes to create MOIs of 2 and 0.5 when referenced to the untreated samples, which corresponds to 16 µL and 4 µL of sample, respectively. Cells and virus were harvested at 24 hours and 46 hours post infection for infectivity analysis using Radiance®.

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