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Rapid quantification of vesicular stomatitis virus in Vero cells using Laser Force Cytology

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ABSTRACT

The ability to rapidly and accurately determine viral infectivity can help improve the speed of vaccine product development and manufacturing. Current methods to determine infectious viral titers, such as the end-point dilution (50% tissue culture infective dose, TCID50) and plaque assays are slow, labor intensive, and often subjective. In order to accelerate virus quantification, Laser Force Cytology (LFC) was used to monitor vesicular stomatitis virus (VSV) infection in Vero (African green monkey kidney) cells. LFC uses a combination of optical and fluidic forces to interrogate single cells without the use of labels or antibodies. Using a combination of variables measured by the Radiance™ LFC instrument (LumaCyte), an infection metric was developed that correlates well with the viral titer as measured by TCID50 and shortens the timeframe from infection to titer determination from 3 days to 16 h (a 4.5 fold reduction). A correlation was also developed between in-process cellular measurements and the viral titer of collected supernatant, demonstrating the potential for real-time infectivity measurements. Overall, these results demonstrate the utility of LFC as a tool for rapid infectivity measurements throughout the vaccine development process.

1. Introduction

Many processes across the biopharmaceutical industry require quantification of viruses. Applications range from vaccine manufacturing, to serum neutralization assays for clinical efficacy, to viral clearance measurements of raw materials or finished goods. The most commonly accepted methods include the plaque and endpoint dilution (TCID50) assays, followed by immunological methods [1–8]. Plaque and TCID50 assays are labor intensive and can take up to two weeks (Table 1), depending on the pathogen [9,10]. Since the assays and their interpretation can be subjective and highly variable, a sufficient number of replicates must be performed to obtain statistically significant results [11,12]. This extends the timeline of pharmaceutical process development and can cause considerable delays to the release of finished goods. For vaccines this rate-limiting step is of particular concern, as it can be challenging to detect peak titer and delay the time between harvest and final product release, which requires accurate infectivity measurements [13–15].

Novel vaccine development process can take decades, with reports of the average development time ranging from 18 to 30 years [16–18]. A number of process parameters can be modified to accelerate time to launch. Recent advances in cell culture media development have improved the manufacturing process to the point where an optimized serum-free medium formulation can increase growth performance and vaccine titers, product quality, and product purification requirements [19–21]. High throughput screening methods are available to improve development of new cell culture media for growth and protein production. Along those lines, a rapid screening method for virus titers would greatly facilitate media and process optimization for vaccine production.

Several virus quantification techniques use instruments to accelerate virus quantification and analysis (Table 2), including qPCR, ELISA, immunofluorescence foci assay, and flow cytometry. Methods based on antibody or fluorescent labeling face several limitations. They are often limited by the type of virus that can be quantified, may not distinguish between non-infectious and infectious virus particles, and can have a reduced limit of detection as a result of background from other substances in the sample matrix [22]. In this study, we have evaluated the use of Laser Force Cytology (LFC) to determine the infectivity of vesicular stomatitis virus (VSV) produced in Vero cells. The Radiance LFC instrument uses a unique combination of advanced optics and microfluidics to analyze suspended cells based upon their intrinsic properties, while simultaneously taking high resolution video of each cell. LFC does not require fluorescent antibody or dye labeling to differentiate cell phenotypes, but instead uses optical force or pressure to impact cellular structures and transfer momentum. The

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Table 1
Example timeline for a 12-arm experiment. Total time includes culture and infection, assay setup, titer determination, and data analysis.

	Culture and infection	Assay setup (per sample)	Titer determination	Total time
Plaque assay	3–8 days	2 h	3–10 days	9–21 days
TCID50	3–8 days	1 h	3–8 days	8–18 days
Laser Force Cytology	1–3 days	20 min	10 min	2–4 days

Table 2
Comparison of viral quantification techniques [42,45].

Technique	Method of detection	In-process assay time	Labor	Cost per sample	Reproducibility	Virus specific?	Limit of detection
Plaque assay [46,47]	Infectivity assay	3–21 days	High	Low	Poor	No, CPE required	Low
TCID50 [47,48]	Infectivity assay	3–21 days	High	Low	Poor	No	Low
Immunofluorescence foci assay [49,50]	Infectivity assay	1–3 days	High	High	Moderate	Yes, viral antigens	Moderate
ELISA [49,51]	Viral protein	2–6 h	Moderate	Moderate-high	Good	Yes, viral antigens	Moderate
qPCR [52,53]	Viral nucleic acid	2–4 h	Moderate	Moderate-high	Good	Yes, viral DNA/RNA	Low
Viral flow cytometry [1,46]	Viral particle	3–6 days	High	High	Good	No	Moderate
Hemagglutination assay [49,54]	Viral Protein (Limited applicability)	<2 h	Moderate	Low	Moderate	No	Moderate
Transmission electron microscopy [48,55]	Viral particle	1–2 days	High	High	Moderate	No	High
Virus counter [55,56]	Viral particle	<1 h	Low	Moderate	Moderate	Optional: specific with viral antigens	High
LFC/Radiance	Infectivity assay	<1 h	Low	Low	Good	No	Low

combination of optical and fluidic forces has been used to characterize a number of features, including cell differentiation, viral infection, and cell deformability [23–27]. Specifically, subtle cellular changes in membranes, cytoplasmic organelles, and nuclear features manifest themselves as changes in the velocity, size, shape, and orientation of cells as they pass through the region of laser photon pressure. These biochemical and biophysical interactions can be quantified to characterize cells in a label-free manner.

Vero cells were isolated from African green monkey (*Ceropithecus aethiops*) kidney cells in 1962 and are commonly used for the production of human vaccines, including polio, EV-71, rabies, influenza, and rotavirus [28–30]. They are adherent cells that can be cultured in flasks, cell factories, and in bioreactors supported by microcarriers. VSV belongs to the *Rhabdoviridae* family and is characterized by a rapid lytic growth cycle [31]. It is zoonotic, can cause flu-like symptoms in humans, and foot and mouth disease in cattle, horses, and pigs. Because of its broad host range, the envelope glycoprotein (VSV G) is commonly used as a coat protein for lentiviral vectors [32]. VSV was chosen as an analog for rabies virus as both viruses share many structural and functional characteristics but differ in pathogenicity.

In this work, we identified the optimal infection period to generate a standard curve that allowed us to correlate the instrument's multi-parameter data to the viral titer. The optimized process reduced the incubation and analysis time to 16 h post infection over a wide range of multiplicities of infection (MOIs). In addition, a correlation was developed between the cell-based LFC data and the TCID50 of corresponding viral supernatants, demonstrating the ability to make in-process infectivity measurements directly on infected cell populations with no further incubation. This capability could substantially increase the speed of iterative process development in vaccine manufacturing.

2. Materials and methods

2.1. Cell culture

Vero cells (ATCC CCL-81) were cultured in MEM α with 10% FBS or VP-SFM (Gibco™), supplemented with 6 mM glutamine, in a humidified incubator at 37 °C with 5% CO_2. Cultures were passaged with Trypsin-EDTA (0.05%) or TrypLE™ (Gibco). Trypsin activity was quenched with Defined Trypsin Inhibitor (Gibco) and cells were washed with medium before determination of viable cell densities with a Vi-CELL XR Cell Counter (Beckman Coulter).

2.2. Virus propagation and infection

Vero cells were grown in T-75 flasks or 6-well plates in MEM α with 10% FBS or VP-SFM (Gibco). For infection, the growth medium was removed, the cell monolayer washed with DPBS (Gibco), and the medium replaced with Advanced MEM or VP-SFM (Gibco). All media were supplemented with 6 mM glutamine. Cultures were infected with vesicular stomatitis virus (ATCC VR-1415) at the MOIs indicated. Cultures were observed daily for signs of cytopathic effect (CPE). The culture supernatant was used for virus quantification with a $TCID_{50}$ assay, and the cells were detached for analysis with the Radiance (LumaCyte).

2.3. Sample preparation

Infected cells were harvested using Trypsin-EDTA and Defined Trypsin Inhibitor and washed before resuspension in Sample Dilution Buffer (LumaCyte). Cells were diluted to a concentration of approximately 500,000 cells/mL. 200 µL of the cell suspension was measured on a Radiance instrument (LumaCyte). Radiance employs optical force (laser photon pressure) and image capture together with microfluidics to image and analyze single cells. The instrument processes and analyzes images to generate a multi-parameter description of each cell. These data are stored in a database describing the analyzed cell population.

2.4. Virus titer determination (TCID50)

Virus production in the infected supernatant was determined by measuring the TCID50 using the endpoint dilution assay. In short, Vero cells were seeded in MEM α with 10% FBS in 96-well plates at densities that reached near 100% confluence in 24 h. The cells were then washed with DPBS and medium was