EXECUTIVE SUMMARY

Are Real-time Gene Therapy Results Possible?

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Catalent® CELL & GENE THERAPY

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INTRODUCTION

Gene therapies are delivering therapeutic results 30 years after the start of their development. The FDA approval of the second adeno-associated viral (AAV) vector-based gene therapy treatment in May of 2019 and the more than 420 gene therapy clinical trials (1) in progress is indicative of the potential of the industry.

As science and technology companies continue to make advancements in vaccine and viral vector-based gene therapy development, there is an increasing need for analytical techniques that can help assess quality parameters, especially in real-time. In a step toward increasing the speed of process development and improvement, Catalent collaborated with LumaCyte in the evaluation of its Radiance® Laser Force Cytology™ (LFC) instrument for more rapid and accurate measurement of quality attributes, such as viral titer and infectivity, and monitoring of the cell culture process, such as transfection efficiency.

NOVEL ANALYTICAL TOOLS AND METHODS

LumaCyte's LFC instrument was designed to detect subtle phenotypic changes in cells used in vaccine R&D, biomanufacturing and cell and gene therapy. This label-free analysis measures more than 20 parameters per cell including velocity, shape and deformability. These single-cell measurements allow for robust, multivariate data analytics. One or more cellular response metrics can be identified that change proportionally and consistently with infection or transfection and be used to quantify TCID50, or infective titer. The metric or metrics used are referred to as the Radiance Response Metric (RRM).

Traditional infectivity-based assays can include long development and execution timelines, are qualitative, can result in false positives, require prior knowledge and development, are error prone, and remain time consuming and expensive.

Label-free means that the innate cellular response is protected throughout the measurement process. This contributes to reduction in errors in assay results, enables discoveries of new cell phenotypes, and delivers higher quality results with less time and money.

As an example of its sensitivity, LFC was compared to flow cytometry in differentiating between macrophages that had/had not engulfed silica beads. LFC was able to detect the different phenotypic species, achieving almost baseline separation, while the two populations of macrophages could not be resolved using forward and side scatter measurements in a flow cytometer.

LFC can be used for rapid viral infectivity measurements, monitoring transfection and transduction, potency assays, cell banking characterization and many other analyses to help support process optimization.

> "For in-process cell infection monitoring, the Radiance® measurement is **800 to 3,000** times faster than the traditional method."

LFC VERSUS STANDARD ASSAYS FOR VIRAL VECTORS

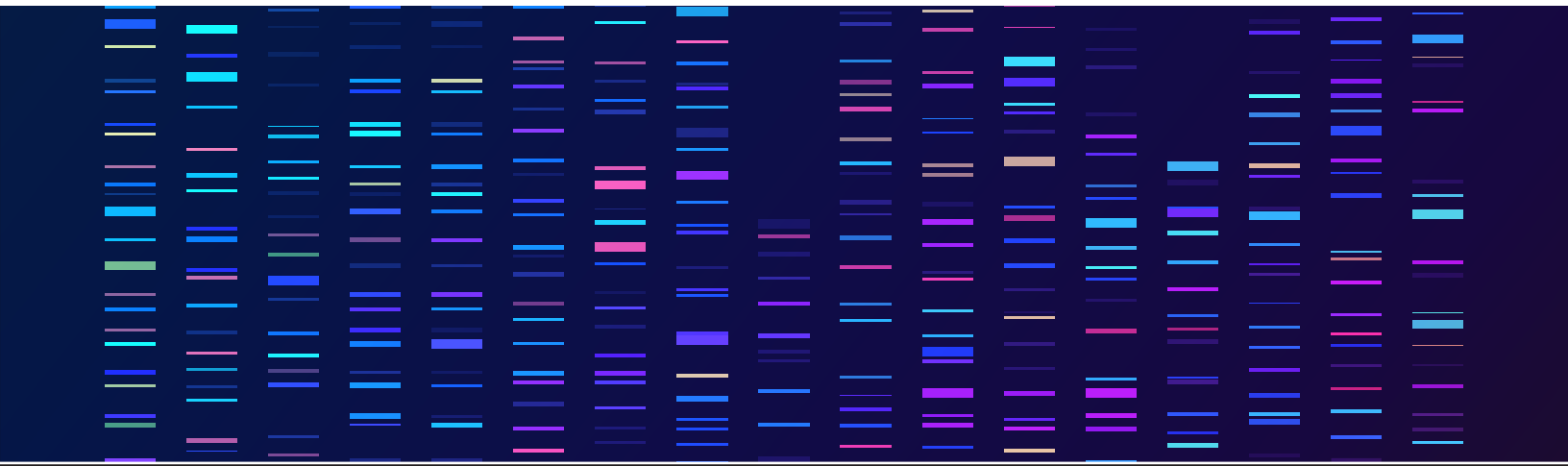
Traditional infectivity quantification methods, including TCID50 and viral plaque assays, can take days up to weeks to produce results, often suffer from subjectivity and require significant resources. New, alternative methods offer potential improvements. For instance, comparing the LFC process side-by-side with that of TCID50 for a viral titer assay, the biggest difference is in the incubation times: 1-3 days for LFC versus 3-15 days for TCID50.

For the standard viral titer assays, areas that need improvement include near real-time process measurements to support Process Analytical Technology (PAT) and Quality by Design (QbD) approaches, assay accuracy and consistency, automation, and a reduction in subjectivity.

To evaluate if LFC could address the desired improvements, Catalent conducted three studies comparing LFC to a standard method: two studies looking at viral titer measurements (baculovirus in Sf9 cells and adenovirus in HEK293 cells) and one monitoring the AAV transfection efficiency of three different transfection reagents in HEK293 cells.

Catalent used LumaCyte's ReportR™ platform to expedite analysis, reduce time to results and generate fully automated custom reports. Using the system, more than 20 unique parameters were measured and trended per cell. For the baculovirus and adenovirus infection experiments, cell velocity was selected as the RRM to correlate with multiplicity of infection (MOI). In the AAV transfection experiment, velocity was also selected to develop the RRM to correlate with ddPCR results.

Below is a summary of the experiments and their results. The data and a full review of the results for the three studies are available in an on-demand webinar. Additionally, the AAV transfection study is included in a technical note from Catalent and LumaCyte.



BACULOVIRUS AND ADENOVIRUS EXPERIMENTS

Cells were infected with a range of MOIs and LFC measurements were taken at several timepoints post infection: between 24-48 hr for the baculovirus infected Sf9 cells and between 24-72 hours for the adenovirus infected HEK293 cells – duplicate tests were done for each.

For baculovirus, the timepoint with the strongest correlation between the 2 trials was at 24 hrs; for adenovirus it was 48 hrs. The data from each independent trial was used to create MOI calibration curves (RRM vs MOI). The range for the baculovirus curve was 1 to 0.008 MOI; the range for the adenovirus curve was 20 to 0.25 MOI. Stock solutions of baculovirus and adenovirus with titers quantified by TCID50 were diluted and tested with LFC. The MOI from the RRM data (cell velocity) was estimated with the corresponding viral LFC calibration curves and a TCID50/ml titer was calculated for each dilution.

The resulting titers from the LFC measurements were comparable to the stock TCID50 titers, as shown in Table 1. Both trials demonstrated accurate model prediction, with a precise fit across their dynamic ranges.

Table 1

Study	Stock TCID50 titer/ml	Average predicted LFC titer	Log 10 difference	Std Deviation	% CV
Baculovirus	2.30E+7	3.08E+07	0.127	6.7E+06	22%
Adenovirus	3.56E+8	3.85E+08	0.034	4.50E+07	12%

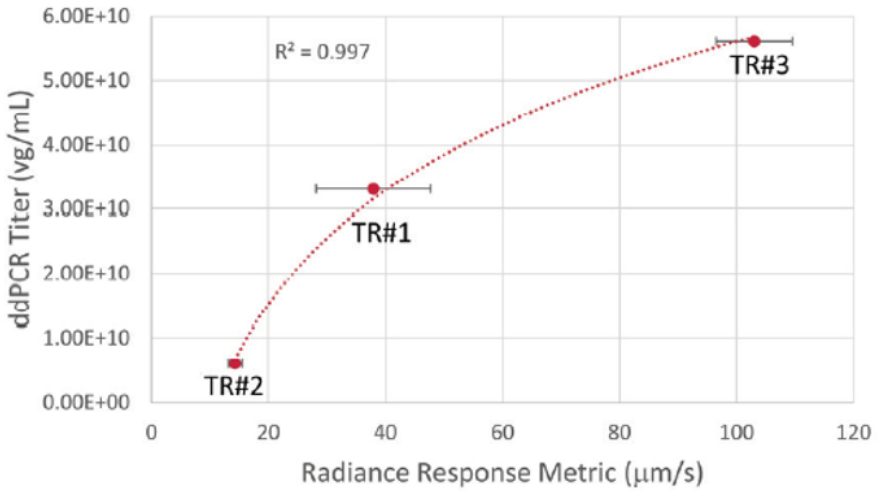
AAV TRANSFECTION MONITORING EXPERIMENT

HEK293 cells were transfected with AAV using three different reagents. The cells were harvested three days post transfection and analyzed via LFC using cell velocity as the RRM. ddPCR titers were determined in parallel for comparison.

Changes in cell velocity were observed based on the level of transfection. Transfected populations showed an increase in high optical force (low velocity) cells compared to non-transfected cells. LFC was able to provide evidence of transfection efficiency and data for the development and optimization of the transfection process. The LFC data was comparable to ddPCR results in predicting which transfection reagent (TR) yielded better results (Figure 1). Both methods predicted TR#3 as the better reagent. The advantage for LFC is the results were available in near real-time with a 5-minute analysis time per sample.

As seen with the experiments on viral titer and transfection monitoring, several improvements were achieved with LFC including rapid analysis, enabling of in-process measurements, and reduced sample load and labor. LFC can provide rapid answers that can help streamline process development activities.

Figure 1



Are real-time gene therapy test results possible? The LumaCyte Radiance® platform provided results in near real-time, 5 minutes of analysis time per sample, a significant time and labor savings over the ddPCR method. And with at-line bioreactor sampling, ongoing bioprocesses can be monitored to help manufacturing teams make decisions in near-real time.

WHY PARTNER WITH CATALENT?

Catalent Cell and Gene Therapy strives to incorporate the most advanced analytics in its processes in order to provide high quality, innovative solutions for its customers. This includes looking for new tools to offer clients an improved understanding of results early in process development.

Catalent partners with innovators to develop manufacturing solutions for their viral vector-based gene therapies. Catalent is dedicated to enabling a better and faster customer journey from product development to patient.

Radiance® LFC Offers:

- Significant time and resource allocation savings
- Accelerated assay workflows (weeks to minutes)
- Cost savings across time, reagents and resources
- Reduced errors through improved accuracy

References:

1. https://alliancerm.org/sector-report/2020-annual-report/
2. https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics

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Catalent Cell & Gene Therapy is an industry-leading technology, development and manufacturing partner for advanced therapeutics. Our comprehensive cell therapy portfolio includes a wide range of expertise across a variety of cell types including CAR-T, TCR, TILs, NKs, iPSCs, and MSCs. With deep expertise in viral vector development, scale-up and manufacturing for gene therapies, Catalent is a full-service partner for plasmid DNA, adeno-associated viral (AAV), lentiviral and other viral vectors, oncolytic viruses, and live virus vaccines. An experienced and innovative partner, Catalent Cell & Gene Therapy has a global network of dedicated, small- and large-scale clinical and commercial manufacturing facilities, including an FDA-licensed viral vector facility, and fill/finish capabilities located in both the U.S. and Europe.