APPLICATION NOTE

Novel approach for quantitative real-time particle analysis of lentiviral vectors

Authors

aratinga.bio

Victoire Barailles, Alice Pailleret, Aurélie Perier, Nicolas Delacroix, Cécile Bauche, Renaud Vaillant

Myriade

Matthieu Greffet, David Poizat

Abstract

Lentiviral vectors are efficient vehicles for stable gene transfer in dividing and non-dividing cells. They tend to be increasingly used as a powerful tool to introduce genes into cells *ex vivo,* for instance in CAR-T cell therapies.

During manufacturing and production of lentiviral vectors, relevant quality control is necessary to allow batch release (1). Among standard quality control methods that can be used, quantification of lentiviral vector particles – or physical titer – is one of the most important. Up to now, this characterization can be achieved either indirectly with p24 protein quantification or with physical methods like Tunable Resistive Pulse Sensing (TRPS) for example, both methods implying prior preparation of samples (lysis, dilution or filtration). These two methods thus show important limitations as they cannot accurately reflect the true nature of the product, in addition to being relatively time-consuming (2). Myriade, a French company created in 2017, is developing Videodrop, a new optical technique performing real-time, user-friendly, and label-free measurement of lentiviral vector physical titer. This method, based on full-field interferometry (3), was tested on various lentiviral vector samples: in a context of Drug Product (DP) release as well as in-process controls.

We compared three lentiviral physical titration methods on aratinga.bio productions: p24 ELISA, qNano and Videodrop – Myriade instrument. The correlation between Videodrop analysis and the other two methods appeared to be robust, with high R^2 values. These results suggest that Myriade technology is relevant for DP release as well as in-process controls, offering the ability to be a tool for continuous improvement. It is an easy-to-use and fast alternative to the standard more complex and time-consuming physical titration methods.

Introduction

aratinga.bio is a preclinical stage biotechnology company developing an off-the-shelf CAR-T therapy allowing the in vivo targeting and transduction of T-cells, relying on the use of lentiviral vectors.

In the context of the production of pilot batches and GMP batches of lentiviral vectors, aratinga.bio is looking for innovative and relevant solutions to better characterize its viral particles. To quantify the physical viral titer, two techniques are used as internal references: p24 ELISA and Tunable Resistive Pulse Sensing (TRPS) with qNano. Myriade is a French company developing Videodrop: a new type of microscope that measures the concentration of nanoparticles from a single drop of sample (4,5). The interest of this method lies in its simplicity: the measurement is real-time, label-free, filtration-free, non-destructive and only requires 5 to 10 µL sample volume. This technology can therefore be particularly adapted to evaluate a lentiviral vector titer.

Here we compare back to back Myriade technology with p24 protein quantification and TRPS on two identified needs: characterization of the final lentiviral vector product – after purification and concentration – and in-process controls during Upstream Process (USP) and Downstream Process (DSP) steps.

Methods

Three techniques were used to evaluate the lentiviral vector physical titer.

Myriade technology

Videodrop consists of a custom microscope relying on an interferometer phenomenon to produce images where nanoparticles in solution can be detected (3). The instrument records videos where nanoparticles and microparticles can be seen. Figure 1 shows diffraction limited spots of lentiviral vectors – approximately 110 nm diameter. The recorded films can be used to count the nanoparticles and estimate the sample concentration as well as tracking their movement to estimate their hydrodynamic radius. The microscope magnification and camera speed allow for small sample volumes (down to 5 µL) and fast measurement, under one minute. The samples do not need either labeling or filtration even in the presence of large cellular debris. The instrument is essentially a microscope, therefore objects in the micrometer range will be seen the same way as with a conventional microscope, allowing to control the purity of the sample. The samples were diluted in DPBS (Gibco), and measurements were performed on 7 µL droplets, in triplicate.

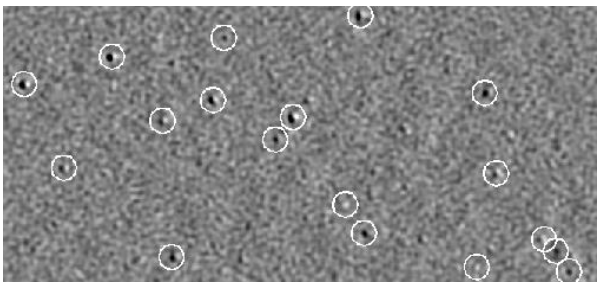

Figure 1. Lentiviral vector sample image obtained with Myriade technology.

p24 protein quantification

Cell Biolabs' QuickTiter™ HIV Lentiviral Quantitation Kit (HIV p24 ELISA) was used for virus quantification. It is an enzyme immunoassay developed for detection and quantitation of the HIV-1 p24 core protein. A mouse monoclonal antibody to HIV-1 p24 is coated onto strip wells of microtiter plate. The quantity of HIV p24 antigen is determined by comparing its absorbance with that of known recombinant p24 antigen standard curve[1].

TRPS

qNano is an equipment relying on tunable resistive pulse sensing (TRPS). It is based on the Coulter principle, which states that particles pulled through a pore, while an electric current is applied, produce a change in impedance that is proportional to the volume of the particle traveling through the pore (6). The samples were analyzed using NP150 nanopores (Izon Science Ltd., New Zealand), after prior dilution in DPBS (Gibco) and 0,22 µm filtration. Measurements were performed on 35 µL of sample, in triplicate.

All three techniques were performed on the samples of interest, stored at -80°C after production and thawed at room temperature.

[1] The calculation is based on the fact that there are approximately 2000 molecules of p24 per Lentiviral Particle (LP), therefore, 1 LP contains: 2000 x 24 x 103 / (6 x 1023) g of p24 = 8 x 10-5 pg of p24

Results and discussion

Characterization of the final lentiviral vector product

Prior to the release of raw materials used for CAR-T cell products production, appropriate tests must be carried out to ensure that the products fulfil regulatory criteria (1). When a lentiviral vector batch is released, the titer of the fraction of interest – thereafter called Drug Product (DP) – must be precisely determined. It can also be compared with that of previous batches, in order to demonstrate reproducibility.

The physical titer of 8 Drug Products was measured with p24 ELISA, qNano and Videodrop. The results of p24 ELISA and Myriade instrument show a strong correlation together (correlation coefficient $R^2 = 0.95$), and slightly less with the qNano (correlation coefficient of $R^2 = 0.85$).

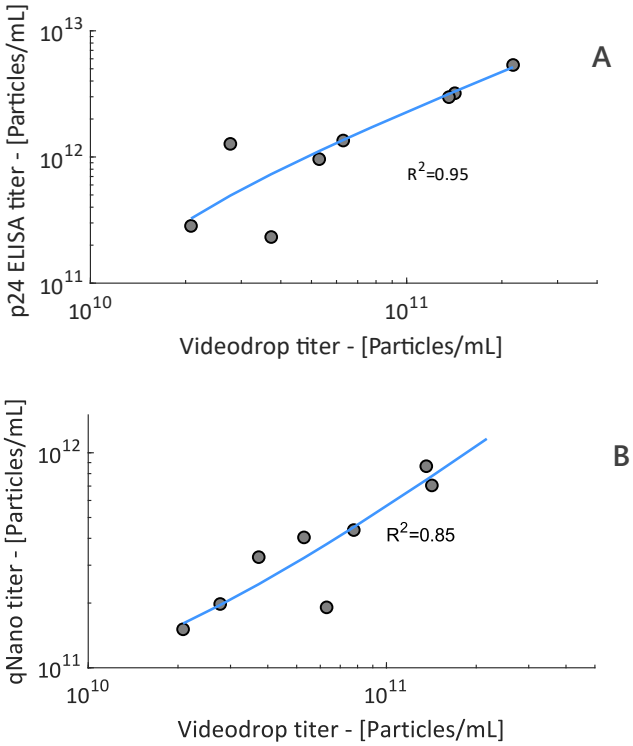

Figure 2. Comparison of methods measuring physical titer on Drug Products. (A) Correlation between p24 ELISA and Videodrop. (B) Correlation between qNano and Videodrop.

In-process controls

In-process control tests are now the most widely used tests/criteria to monitor the progress during manufacturing of active pharmaceutical ingredients (APIs) and intermediates (7). Researchers should characterize the key production steps in the process control and define the limits of critical parameters in order to avoid process deviation (1). This approach also allows process improvement in real-time and ensures reproducibility over time.

During USP/DSP steps – involving production, clarification, purification, concentration, diafiltration and filtration – the lentiviral vector physical titer, as well as the infectious titer, can be strongly impacted. Therefore, in order to monitor in real time the production quality and to calculate yields, the titer evolution has to be evaluated during the process through in-process control.

The physical titer of 6 fractions of interest in 4 different batches was measured with p24 ELISA, qNano and Videodrop. The evaluated fractions were: harvested bulk (HB), clarified harvested bulk (HCB), clarified harvested bulk after benzonase treatment (HCBB), AEX eluate (E2), drug substance (DS) – after tangential flow filtration – and drug product (DP) – after 0,22 µm filtration.

p24 ELISA and qNano titers correlate particularly well with Myriade instrument results (correlation coefficient $R^2 = 0.96$ and $R^2 = 0.94$ respectively).

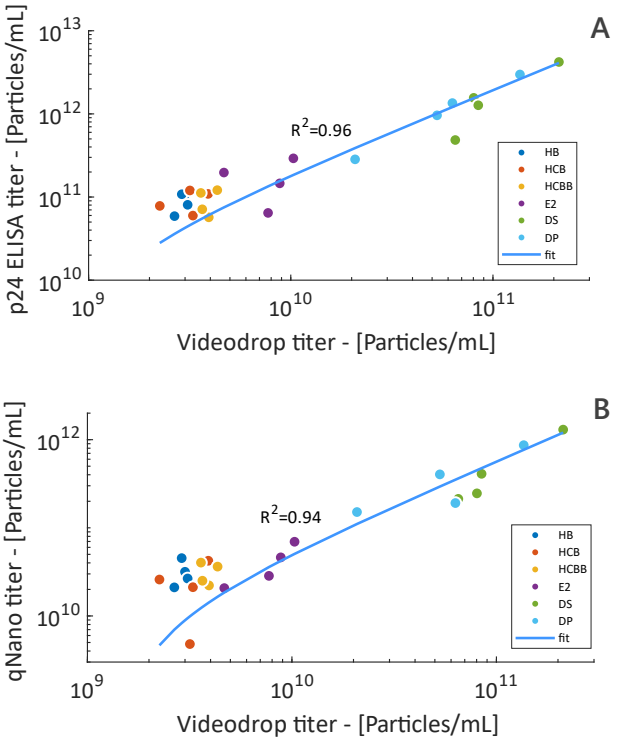

Figure 3. Comparison of methods measuring physical titer on Drug Products. (A) Correlation between p24 ELISA and Videodrop. (B) Correlation between qNano and Videodrop.

Conclusion

In this study, a comparison of three methods to measure lentiviral vector physical titer for DP release and USP/DSP in-process control has been performed. Measurements achieved with Videodrop correlate well with qNano, with correlation coefficients R^2 = 0.95 and R^2 = 0.85 for in-process controls and DP release respectively. The correlation was even higher with p24 ELISA results, with R2 = 0.96 for DP and R^2 = 0.93 for in-process control on various USP/DSP fractions of interest.

Results obtained with Videodrop are consistent with the standard lentiviral vector physical titration methods. Furthermore, Videodrop seems to be particularly adapted to API control, since it is a very quick titration method, consuming low volumes of product. Hence it could be used for Drug Product release as well as offline in-process controls.

References

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