APPLICATION NOTE

VIDEODROP: Ideal tool for lentiviral vector bioproduction follow-up

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Abstract

By their ability to transduce a wide range of cell types and to integrate host genome in dividing and non-dividing cells, lentiviral vectors tend to be increasingly used as a powerful tool for gene and cell therapy. The lentiviral vector R&D field is growing, and with it, the need for bioproduct characterization tools. For instance, process optimization and stability studies are crucial steps in the development of new drug products. Furthermore, during production of lentiviral vectors, relevant quality controls – as lentiviral vector quantification and size distribution – are necessary to allow batch release (1).
Hence, in the context of the production of pilot batches and GMP batches of lentiviral vectors, Ixaka is looking for innovative and relevant solutions to rapidly control their bioprocesses and easily characterize their bioproducts.

Myriade, a French company, developed the Videodrop, a new optical device that performs real-time, user-friendly, and label-free measurements of lentiviral vector physical titer and size distribution. This method, based on Interferometric Light Microscopy (ILM) (2), was tested on various lentiviral vector samples: in a context of Drug Product (DP) release, as well as in-process controls.

We compared ILM to three well-known physical titration methods: p24 ELISA, RTqPCR, and Nanoparticle Tracking Analysis (NTA). We also compared NTA and ILM size measurements through a thermal stress-induced study on lentiviral vector DP. The correlation between Videodrop analysis and the other three methods appeared to be robust, with high R^2 values. These results suggest that Videodrop is relevant for DP release, and in-process controls. as well as enabling continuous method and process improvements.
Videodrop is an easy-to-use and fast alternative to the current more complex and time-consuming physical titration and biophysical characterization techniques.

Introduction

Ixaka is a private UK-based cell and gene therapy company with operations in the UK, Spain, and France. Ixaka develops immuno-oncologic therapeutic products relying on the use of lentiviral vectors.

The rise of gene and cell therapies development, at preclinical and clinical stages, comes with scale up challenges, including costs & time reduction requirement (3). Ixaka, like every biotech company focusing on gene and cell therapy using lentiviral vector, needs a fast and simple solution to monitor lentiviral vector production along the production process.

To address these needs, Myriade developed a new device – the Videodrop - designed to provide lentiviral vectors concentration and size distribution and suitable for in-process controls, as well as for final product characterization. The interest of this method lies in its simplicity: the measurement is real-time, label-free, non-destructive, and the cleaning takes only a couple of seconds. Therefore, this new methodology is particularly well adapted for laboratories where a large number of samples must be characterized. Moreover, it only requires a small drop of sample, which is ideal for BSL 2 Labs.

> *« The measurement is real-time, label free, non-destructive and the cleaning takes only a couple of seconds"*

Here we compare back-to-back ILM with p24 ELISA and NTA on lentiviral vector physical titer quantification, and we compare ILM and NTA on size measurement through a thermal stress-induced stability study.

Material and Methods

Interferometric Light Microscopy (ILM)

Videodrop is a custom microscope that uses interference phenomenon to detect the light scattered by individual nanoparticles in solution. Videos recorded by the Videodrop are processed to reveal the interference patterns created by the nanoparticles moving in the light path. Using this interferometric signal, nanoparticles are automatically detected and tracked to compute concentration and hydrodynamic diameters. Counting particles allows to measure the concentration, while tracking their Brownian motion allows to measure their hydrodynamical diameters.

The microscope magnification and camera speed allow to perform analysis of small sample volumes (down to 5 µL) in less than one minute.

The instrument is essentially based on a microscope. Therefore, objects in the micrometer size range can also be imaged, thus also allowing to control the purity of the sample. (i.e. direct visualization of cell debris, impurities, and large aggregates). This innovative way of detecting nanoparticles through interference allows the analysis of size polydisperse samples, without glare nor clogging. Figure 1 shows the diffraction of limited interference patterns of lentiviruses and their corresponding size distribution.

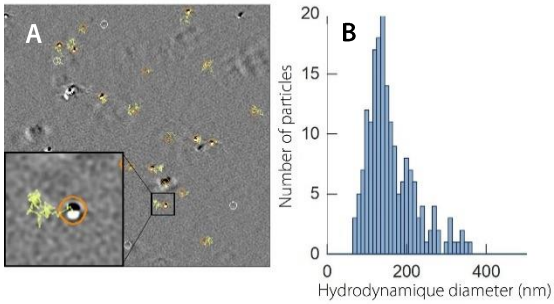


Figure 1. Purified lentiviral vectors images obtained with ILM **A.** Nanometric image where interference patterns of lentivirus can be detected and tracked to compute concentration and hydrodynamic diameter. B. Lentivirus size distribution histogram from tracked particles.

P24 protein quantification

Cell Biolabs' QuickTiter™ HIV Lentiviral Quantitation Kit (HIV p24 ELISA) is 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 a microtiter plate. The quantity of HIV p24 antigen is determined by comparing its absorbance with that of known recombinant p24 antigen standard curve. Following supplier recommendations, the titer calculation is determined by using the standard conversion factor. There are approximately 1.25 x 10^4 physical particles of lentivirus for every picogram of p24 antigen(5).

NTA

ViewSizer™3000 from Horiba is used for physical titer characterization. The NTA is a device used for particle visualization and determination of particle concentrations and sizes. The instrument characterizes nanoparticles size by analyzing their thermal-induced motion (Brownian motion). The optical system includes innovative multispectral illumination and detection techniques.

RTqPCR

The RTqPCR is performed with a kit from Takara. The Lenti-X qRT-PCR Titration Kit use a quick RNA purification step and DNAse I treatment before quantifying the number of lentiviral genome copies using real-time PCR. The qRT-PCR procedure entails dilutions of the sample and a control RNA of known copy number to one-step qRT-PCR. The lentiviral copy number contained in the sample can then be determined by comparing its Ct value to the standard curve. RTqPCR is only performed on bulk fraction (B) and Final product (DP).

Lentiviral vector production process

Lentiviral vectors production is divided in two main steps: upstream process and downstream process. Upstream process consists of virus production with cells; downstream process is described as purification steps.

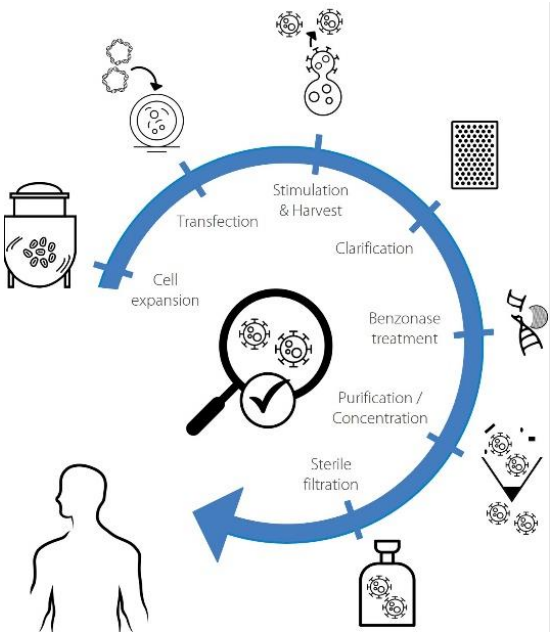


Figure 2. Lentiviral bioproduction process – upstream & downstream process

The study is conducted on 3 batches from two different processes, which involved different steps and different media. The physical titer of 5 fractions of interest in 3 different batches was measured with p24 ELISA, NTA and ILM. The evaluated fractions are:

- Bulk (B),
- Clarified Bulk after Benzonase treatment (CBBT),
- different Purification steps (PS)
- and final drug product (DP) – after 0,22 µm filtration.

Stress-induced viral vector samples

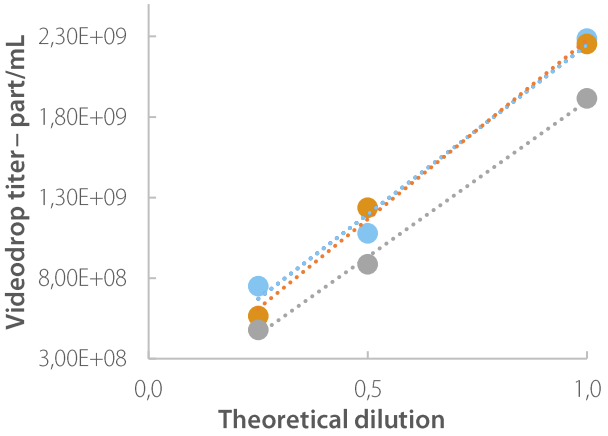
Viral particles tend to form aggregates in what can be defined as a survival mechanism that helps viruses resist environmental stress. This complex mechanism can be influenced by biochemical properties but also environmental factors like temperature.

Viral vector aggregation and particle formation need to be measured to evaluate stability upon extended storage. Analytical techniques are required to study the propensity of solutions to form aggregates and to allow the investigation of the conditions encountered during manufacture and storage. The ability of the Videodrop to detect size differences is studied in comparison with the NTA on thermal stress-induced aggregated samples. Aggregated samples are generated on two DPs at two different temperatures (25°C and 50°C). Samples are analyzed by the two methodologies at four time points (T0, T24h, T48h, T72h). In this study we aim to confirm the tendency of both devices to detect size increase with complex samples.

Results and Discussion

Linearity and repeatability

Videodrop aims to be a reference device for Quality Control (QC) in viral vector bioproduction. As an analytical instrument used for its ability to measure the concentration of nanoparticles, Videodrop should show good performances in linearity and repeatability. A preliminary study is conducted for the purpose of a future method validation following ICHQ2. In this preliminary study, we propose to analyze 3 DPs especially on linearity and repeatability. Each DP is analyzed on 3 dilutions, and 3 replicates by dilution.



Sample	R^2
DP 1	0,983
DP 2	0,995
DP 3	0,997

Figure 3. Linearity analysis of 3 lentiviral vectors DP.

Concerning the 3 DPs produced from two different production process, the correlation coefficients are high (R^2>0.98) showing an excellent linearity of the methodology.

CV (%)	Dilution 1	Dilution 0,5	Dilution 0,25
DP 1	8,4	23,3	3,3
DP 2	18,8	12,9	3,5
DP 3	2,1	13,0	0,90

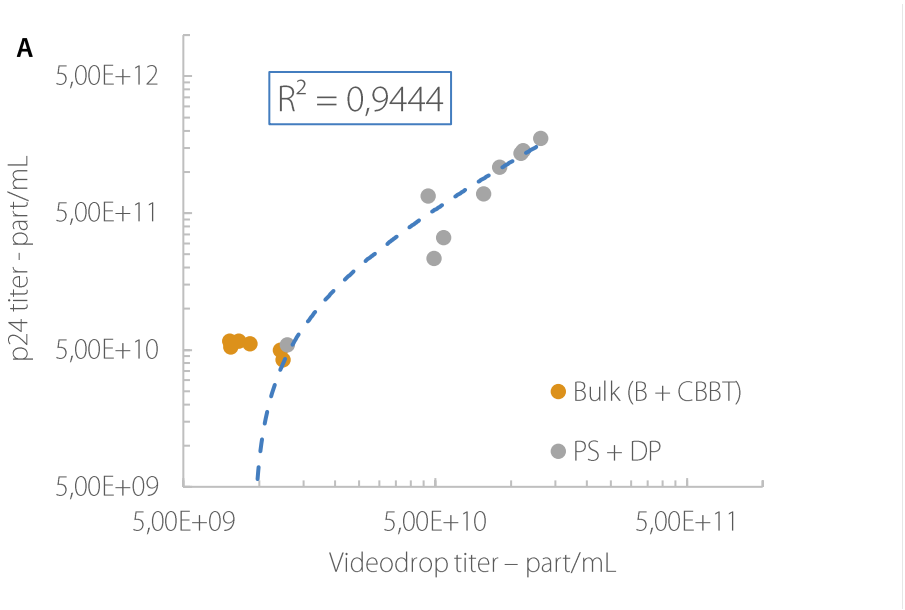
Figure 4. Repeatability analysis of 3 lentiviral vectors DPs.

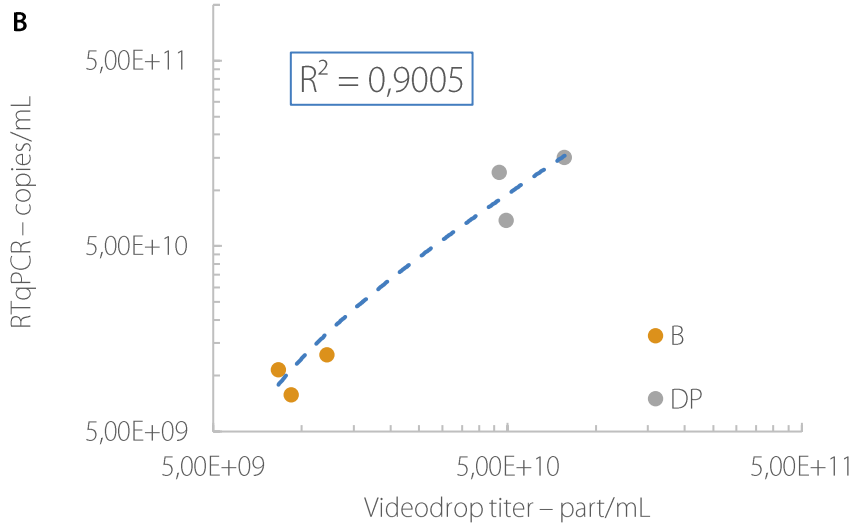
The coefficients of variation calculated for the 3 DPs and the 3 dilutions (3 technical replicates per dilution), are showing values mostly <20%. It shows a good repeatability of the methodology. Those results are very promising for the purpose of validating the method following ICH guidelines.

Concentration determination of 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 must be precisely determined. Moreover, 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 (6). This approach also allows process improvement in real-time and ensures reproducibility over time.

The physical titer of 5 fractions of interest - from the harvest to the final purified product - in 3 different batches was measured with p24 ELISA, RTqPCR, NTA and ILM. The results of ILM (Videodrop) show a strong correlation with p24 ELISA (correlation coefficient R^2 = 0.94), NTA (correlation coefficient of R^2 = 0.91) and RTqPCR titers (correlation coefficient of R^2=0.90).





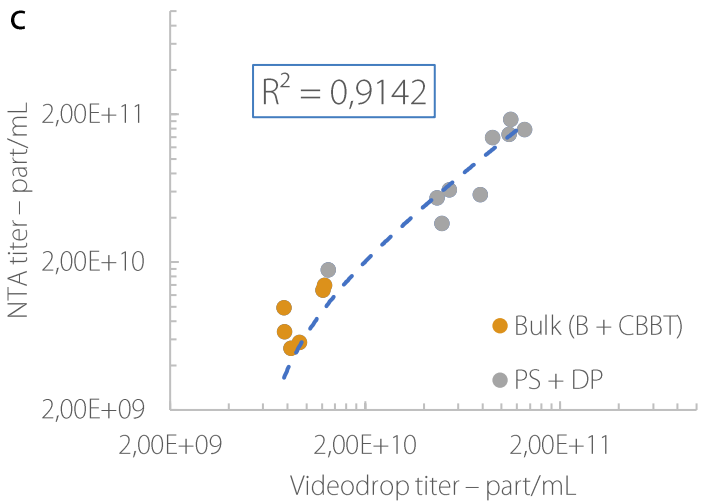


Figure 5. Lentiviral vector quantification method comparison **A.** Comparison between p24ELISA and Videodrop titers **B.** Comparison between RTqPCR and Videodrop titers.**C.** Comparison between NTA and Videodrop titers

The results show the suitability of the Videodrop to be used for physical titer determination. The ease of use, and rapidity of the Videodrop compared to the two described techniques, make it an ideal tool for bioproduction follow-up.

> *« [Videodrop] is an ideal companion for production process optimization and quality controls"*

Diameter measurement of complex samples: stress-induced lentiviral vector

Lentiviral vector aggregation is a complex mechanism that impacts the quality of the product. In this study we propose to generate aggregated samples to challenge both abilities of NTA and ILM to analyze complex samples. Both techniques size measurement is based on Brownian motion analysis. Here we analyzed the median diameter of the 2 DPs at four time points of the thermal stress study at 25°C and 50°C.

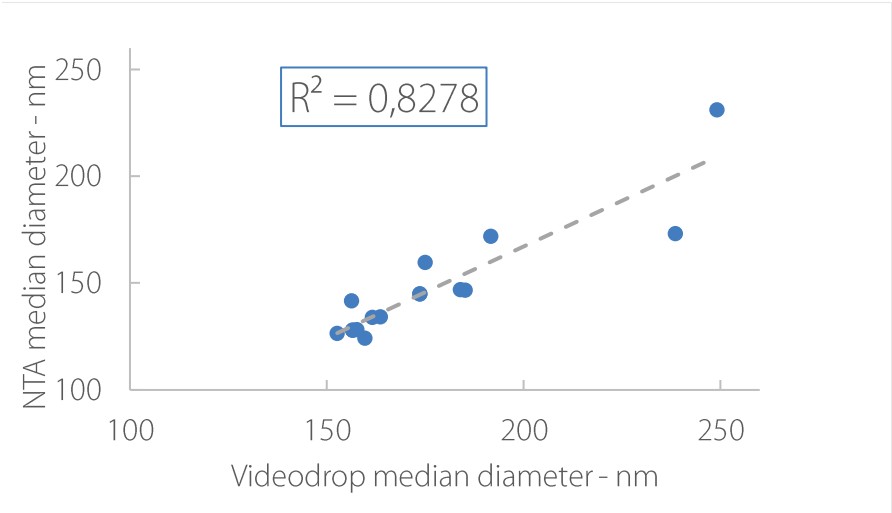


Figure 6. Median diameter measurement comparison between NTA and Videodrop on complex stress samples

The comparison between NTA and ILM median diameter estimation shows a good correlation with a R^2 at 0.83. Both devices are following the same tendency when analyzing complex aggregated samples. This study aims to confirm the ability of both devices to detect size increase with complex samples. This result is very promising and presents the Videodrop as a reliable tool for lentiviral vectors samples characterization.

Conclusion

In this study, a comparison of four methods to measure lentiviral vector physical titer at every step of the production process has been performed. Quantification achieved with Videodrop correlate strongly with p24 ELISA, RTqPCR and NTA with high correlation coefficients R^2=0.94, R^2=0.82, and R^2=0.91 respectively. The thermal stress-induced study was helpful to evaluate the ability of the Videodrop to characterize the aggregated and non-aggregated lentiviral vector and shows a consistent size correlation with the NTA.

Videodrop appears suitable for lentiviral vector quantification and characterization. It is an ideal companion for production process optimization and quality controls.

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