Performance Qualification of a System Measuring Size and Concentration of Nanoparticle Samples: Corning® Videodrop

CORNING

Application Note

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Abstract

Viral vector applications in gene and cell therapy have been showing great potential for a variety of diseases. This involves the need for quality and safety control of the gene therapy product throughout production and quality control to ensure its progress to clinical trials. One of such regulations includes the validation of the analytical methods that are not yet standardized to be compatible with the good manufacturing practices (GMP). For instance, quantification and characterization of viral vectors production are important quality controls to perform.

Myriade is a French company that developed a new analytical device for nanoparticles characterization: Videodrop. The instrument is based on Interferometric Light Microscopy (ILM), enabling fast measurement of live concentration and size of biological nanoparticles (e.g., viral vectors) in a single drop (5 to 10 µL) of a sample.

In this application note, are presented a set of tests that have been conducted on Videodrop performance for its validation as an analytical tool. The concentration and size measurements were assessed through linearity, accuracy and precision based on monodisperse samples of NIST size standard polystyrene nanobeads.

The validation procedure suggests that Corning Videodrop is a suitable tool for quick characterization of viral vectors. It is an easy-to-use and fast alternative to the standard more complex and time-consuming methods.

Introduction

Cell and gene therapy has recently become one of the most rapidly evolving fields in bioengineering due to its outstanding prospective in therapeutical strategies for a huge variety of diseases. Viral vectors tend to be increasingly used as a powerful tool to introduce genes into cells *ex vivo*, for instance in CAR-T cell therapies. However, the success of this advanced therapeutical approach is extremely dependent on the design of standardized protocols and analytical techniques in accordance with Good Manufacturing Practice (GMP)[1].

The control of the production process and analytical methods used throughout manufacturing and production of viral vectors is crucial to allow final batch release. As described in EMA guidelines on the quality, non-clinical and clinical aspects of gene therapy medicinal products, the number of particles, the particle size average and distribution and aggregation levels should be determined[2].

By rapidly measuring the size and physical titer of nanoparticles in solution, the Videodrop can be integrated in such quality control strategy for Drug substance (DS) and Drug Product (DP).

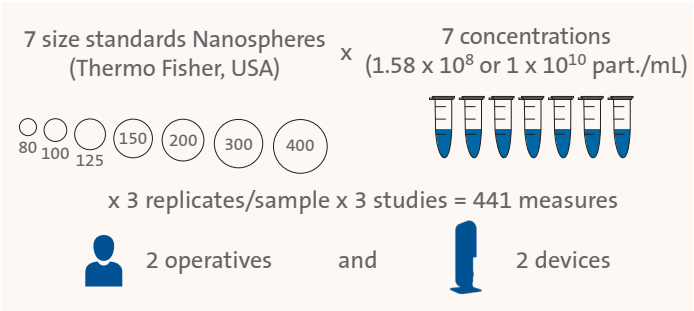
Validation of an analytical method for viral vector production must be done according to the International Conference on Harmonization Q2 (ICH Q2) Guidelines. The validation procedure of the method performance, includes testing of various parameters like specificity, linearity, range, accuracy, precision, detection limit, quantification limit and robustness[3].

Videodrop, being one of the analytical methodologies for viral vector characterization during production, is therefore subject to such validation. In this application note, we proposed to study linearity, accuracy and precision of the Videodrop methodology regarding nanoparticles concentration and size. Those parameters are evaluated with size calibrated polystyrene nanoparticles.

Materials and Methods

Sample preparation

The study was performed with standard size polystyrene nanobeads (3000 Series Nanosphere™ size standards, Thermo Fisher, USA). 7 sizes of beads were tested (80, 100, 125, 150, 200, 300, 400 nm) in 7 concentrations each (1.56 x 10^8, 3.13 x 10^8, 6.25 x 10^8, 1.25 x 10^9, 2.5 x 10^9, 5 x 10^9, 1 x 10^{10} particles/mL) summing up to 49 samples in total per study. Measurements were taken in triplicates for each sample and the study was repeated 3 times, involving 2 operators and 2 devices.



Theoretical concentrations were estimated with information given by the nanospheres' supplier. Dilutions were performed in distilled water. Following suppliers' recommendations and to avoid nanoparticle aggregation, each sample was sonicated before the measurement.

Interferometric Light Microscopy (ILM)

Corning® 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 diffraction 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 diameter. Counting particles allows to measure the concentration, while tracking their Brownian motion allows to measure their hydrodynamical diameter.

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

The Videodrop does not need any calibration or settings adjustment. It is a turnkey solution to quickly measure size and concentration of viral vector samples.

Measurement protocol

Samples were systematically diluted in distilled water (successive dilutions: x2, x4, x8, etc.) to obtain the 7 studied theoretical concentrations based on the initial supplier's one. All measurements were performed on videos of 5 to 10 blocks of 100 frames each, depending on the sample's concentration; saturation between 90 and 95%; 7 µL sample droplets. Measurements were processed by qvir 2.5.5 software, including doublet detector. The default detection threshold was set to value 4.2 for all assays, except for 80 nm beads studies where the threshold was 3.8.

Linearity

Linearity regions are identified for both concentration and hydrodynamic diameter measurements based on the coefficient of determination R^2 evaluation. For size, linearity was evaluated for 7 different bead sizes studied. The concentration linearity range was determined for 5 concentration values, excluding two of the studied concentrations (1.56×10^8 and 1×10^{10} particles/mL). Then, the subsequent accuracy and precision analyses were conducted on these linearity regions.

Accuracy

Accuracy of measurements is addressed through the recovery rate to assess the proximity of the experimentally obtained values to the theoretical ones. It is expressed as a percentage with 100% being the ideal recovery. The recovery rate is therefore calculated as follows.

$$\text{Recovery rate} = \frac{\text{Experimental}}{\text{Theoretical}} \times 100\%$$

Precision

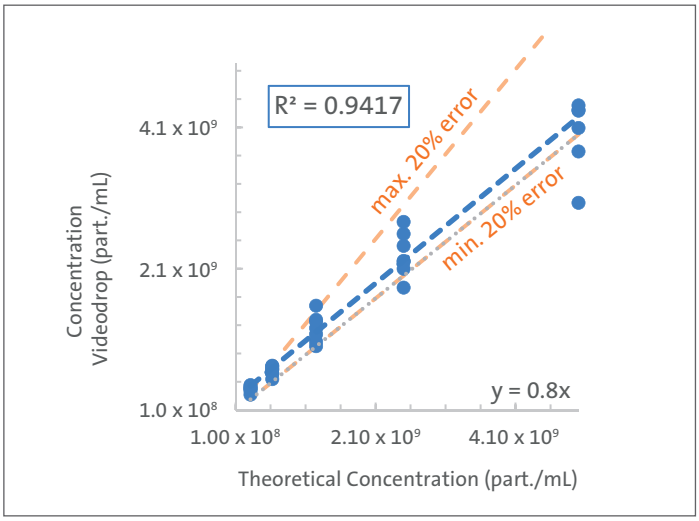
Precision study is performed to estimate the degree of scatter between a series of measurements acquired from repetitive sampling of the individual sample under the same operating conditions. Each sample, therefore, is measured 3 times during 1 study session and 3 such study sessions are performed. Here, the precision is expressed through a coefficient of variation (CV).

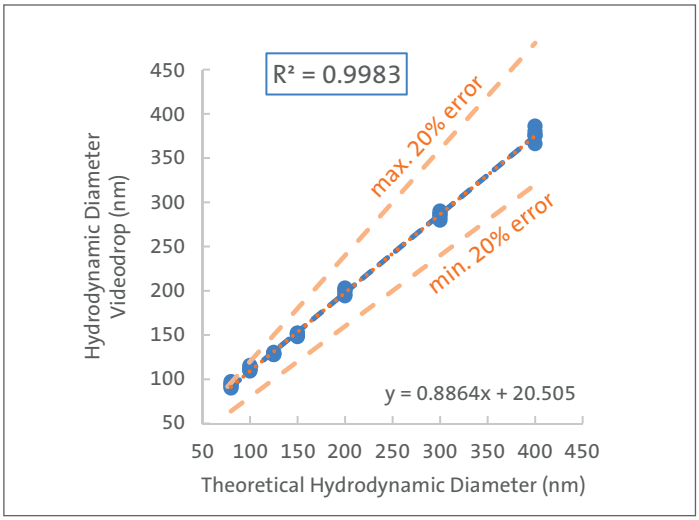
$$CV = \frac{\text{Standard deviation}}{\text{Mean}} \times 100\%$$

Results and Discussion

Linearity

For linearity study performance, the experimentally obtained values for both concentration and size were plotted versus the theoretical ones, and the linear fit was performed.



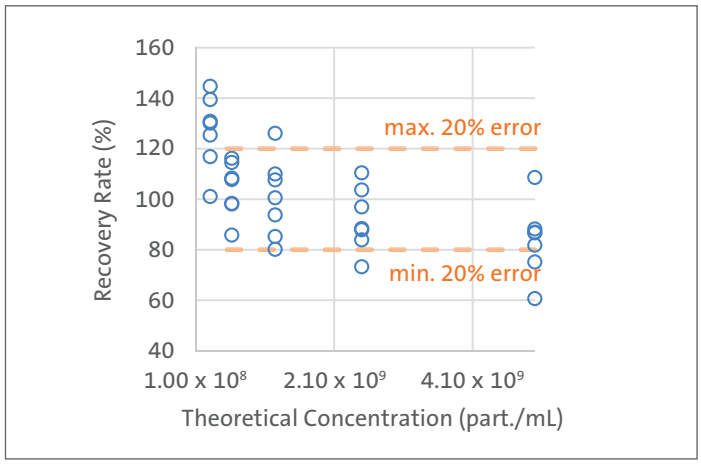


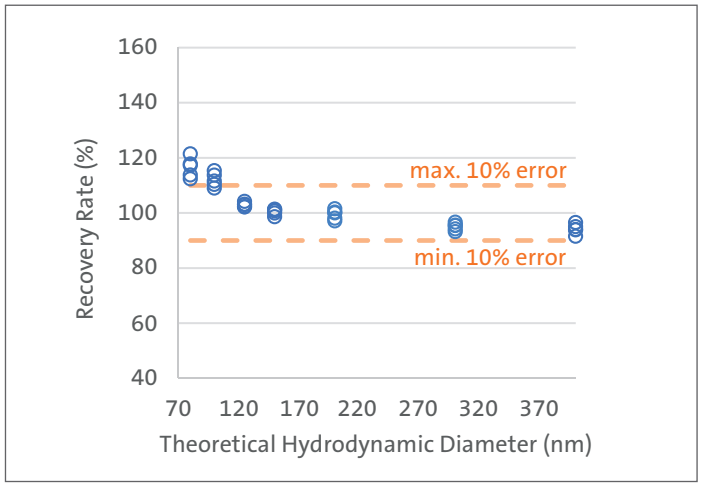
The linear fit for concentration measurement based on 5 studied concentrations resulted in $R^2 = 0.9417$. For size measurements, the linear fit was performed on 7 studied sizes with resulting coefficient of variation $R^2 = 0.9983$. Both analyses show an excellent linearity on the range of concentration and size, both with a slope superior to 0.8.

Accuracy

Accuracy was evaluated for both size and concentration measurements plotting the recovery rate as a function of experimental results. Thus, for size measurements the ideal value or 100% recovery is represented by the supplier's indicated values. For concentration, these values correspond to theoretically derived values based on the information provided by the supplier and the subsequent successful serial dilutions. For hydrodynamic diameter accuracy study, the majority of the measurements were within 10% error, except for the two lowest sizes (80 nm and 100 nm) for which the error was within 20%.

For concentration results, most of the measurements are included in between ±20% error, with a slight tendency to overestimate the concentration around low concentration and underestimate the concentration around higher concentration of the range.

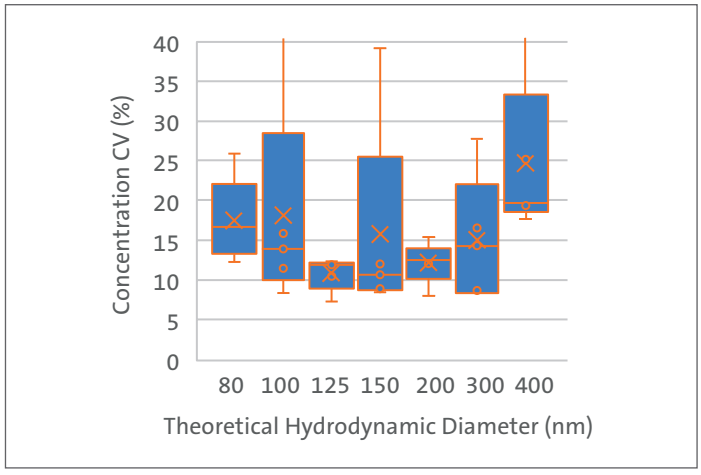


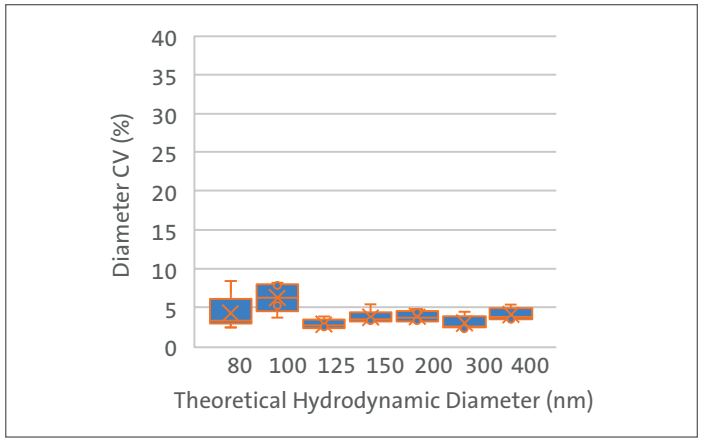


Precision

Precision was estimated through coefficient of variation (CV) based on 9 measurements of each individual sample (3 measurements/session x 3 sessions).

To represent the coefficient of variation for size and concentration measurements, the studied beads were grouped according to their size and the CV was plotted for concentration and size measurements accordingly.





CV of concentration measurement are mostly under 20%, whereas CV of size measurement are always under 10%. Precision results are very good and promising for method validation.

Conclusions

EMA guidelines for quality control of gene therapy recommend controlling the size and number of particles of viral vectors DS, DP, and critical intermediates during the process. The Corning® Videodrop, measuring size and concentration of nanoparticles easily and rapidly, can naturally be integrated in quality control strategy. For this specific objective, analytical methods should be validated following ICH Q2 guidelines. In this application note, we proposed to determine linearity, accuracy and precision by analyzing polystyrene standard beads. Results of this performance study are very good and promising for future method validation on viral vectors for gene therapy medicinal products.

	Concentration	Size
Linearity	R^2=0.9417	R^2=0.9983
Accuracy	Maj ±20% error	Maj ±10% error
Precision	CV mostly <20%	CV<10%

References

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