LENTIVIRUS

VIDEO DROP

APPLICATION NOTE

Videodrop: a suitable system for in-process controls of lentiviral vectors in GMP environment

A quality control method for physical titer monitoring of lentiviral vectors

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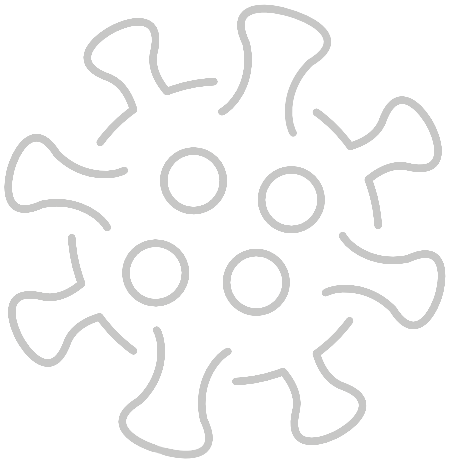
myriade



Introduction

Viral vector applications have been showing great potential for cell and gene therapies. Over the years, clinical research has seen more and more clinical trials involving viral vectors. Based on the Wiley database on Gene Therapy trials Worldwide, more than 3500 vector-based therapy clinical trials are referenced including for instance 10% of lentiviral vectors (LV), 15% of retrovirus, 16% of adenovirus[1].

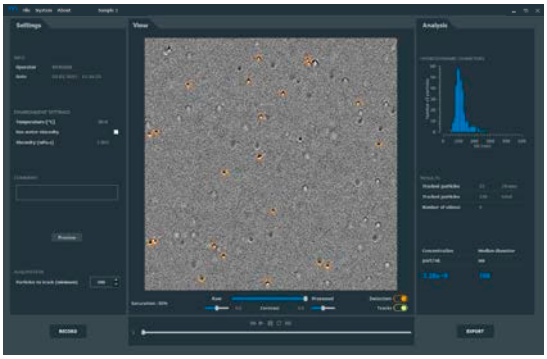
Viral vector clinical applications involve large-scale **Good Manufacturing Practice (GMP),** including production, purification and quality controls[2].
By rapidly measuring the size and physical titer of nanoparticles in solution, Videodrop can be integrated in lentiviral quality control strategy. Indeed, Videodrop proved its value for lentiviral vector physical titer measurement[3]. GMP environment implies analytical method validation. Validation of an analytical method for viral vector production must be done according to International Conference on Harmonization Q2 (ICH Q2) Guidelines[4]. The validation procedure of the method performance includes testing of various parameters like specificity, linearity, range, accuracy, precision, detection limit, quantification limit and robustness.

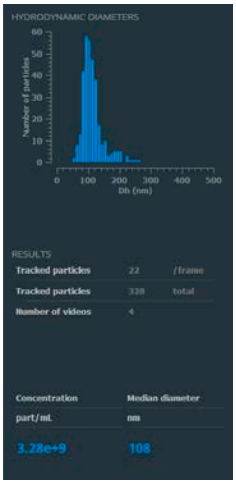
In this application note, we demonstrate how Videodrop is suitable for GMP environments, as a quality control method for physical titer monitoring of lentiviral vectors. The demonstration shows the three main topics implied in GMP environment: **Software, Hardware and Method.** More than 150 measures of samples collected during biomanufacturing of lentiviral vector batches (purified, intermediate and starting material) were used to evaluate the method validation of physical titer analysis using Videodrop.

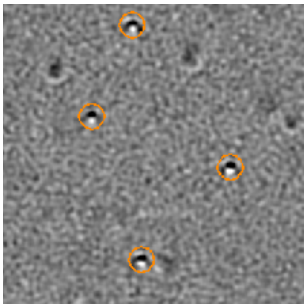
Software

Computerized systems used for the manufacture of medicinal products should be validated according to the requirements of GMP Annex 11[5]. Videodrop is available with a Regulated environment license software (QVIR) that will help the lentiviral manufacturer among the computerized system Validation.

Parameter	QVIR software options
Auditing all actions	• Audit trail • Export of audit trail
Access control	• Access control via windows (possible implementation of password and access)
Data integrity	• Data cannot be edited from qvir software • Automatic saving of raw data (qvirx) • One measurement session per sample
Change configuration Management	• Notification for any settings change by user

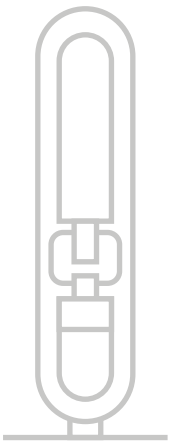






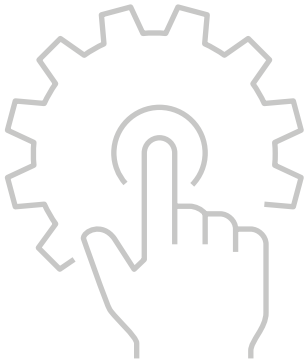
Videodrop Software

Validation kit
IQ OQ PQ documentation



Hardware

Qualification of equipment are key requirements in GMP guidelines[6]. Videodrop system is available with Regulated environment kit including IQ/OQ/PQ (Installation Qualification / Operation Qualification / Performance Qualification) documentation. This option will guide lentiviral manufacturer among the Analytical Instrument Qualification.



Method Validation

Videodrop, being one of the analytical methodologies for lentiviral vector characterization during production, is therefore subject to method validation. International Conference on Harmonization provides guidelines (ICH Q2) on Validation of analytical procedures and describes the need to define method parameters as accuracy, precision, linearity, range. In this application note, we proposed to study **linearity, accuracy, and precision** of the Videodrop methodology regarding lentiviral vectors concentration.

Protocol

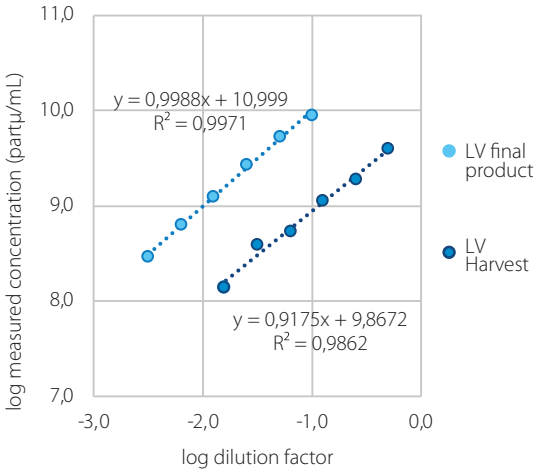
This study was performed on lentiviral vectors produced by Ixaka, now Alaya.bio. Standard lentiviral vector bioproduction includes a virus production process (upstream process) and **purification process (downstream process).** In this case, downstream process is composed by a clarification of the harvest, a DNAse treatment, then an ion exchange chromatography (AEX), a TFF (Tangential Flow Filtration) and a final sterile filtration. For this study, we chose to evaluate ICH Q2 parameters with two bioprocess-extreme samples of the LV bioprocess: harvest after clarification (LV Harvest) and sample after the complete purification process (LV Final product). The accuracy compared with orthogonal method is evaluated with samples from each step from 2 batches (LV1 and LV2). The validation protocol can be summarized by the following table.

ICH Q2 parameter	Samples	Dilution factor	Replicate number
Linearity	LV1 Final product	1/10; 1/20; 1/40; 1/80; 1/160; 1/320	3
	LV1 Harvest	1/2; 1/4; 1/8; 1/16; 1/32; 1/64	3
Precision	LV1 Final product	1/80	9
	LV1 Harvest	1/4	9
Accuracy	LV1 Final product	1/10; 1/20; 1/40; 1/80; 1/160; 1/320	3
Accuracy compared with orthogonal method	LV1: 6 fractions LV2: 3 fractions	Linear range	3

Linearity

The linearity is defined by ICH Q2 as the ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample[4].

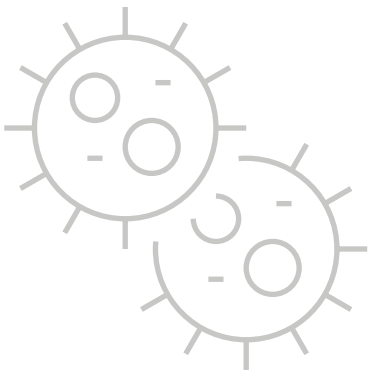
The linearity parameter is evaluated on two selected samples from the process: the clarified harvest bulk (LV Harvest) and the final purified product (LV final product). Each sample is diluted 6 times with filtered PBS.

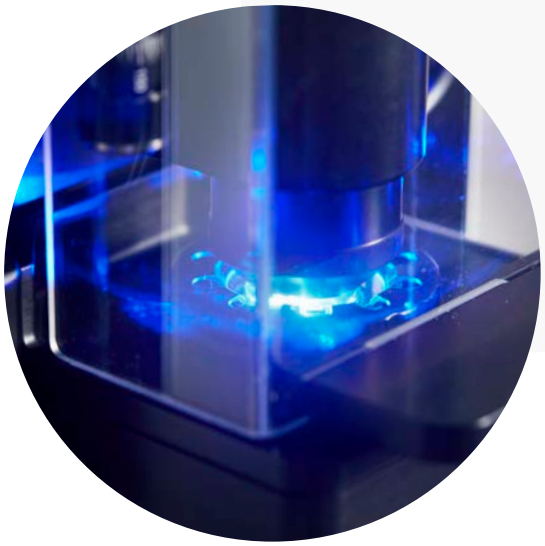


For each sample, the dilution curve (linear regression) presents a correlation **coefficient (R^2) superior at 0.98** and **a slope superior at 0.9** – meaning the dilution curve is linear on the evaluated range.

Those results show a strong linearity on two types of samples, purified and non-purified lentiviral vector.

For the LV harvest and the LV final product, the linearity is evaluated between respectively [1E8 – 5E9 part/mL] and [2E8 – 9E9 part/mL].

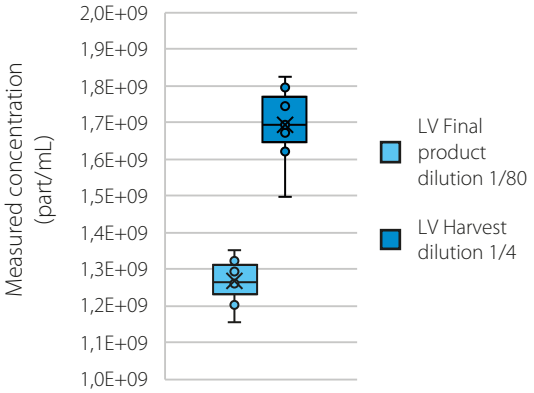




Precision

The precision (or repeatability) is defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the same conditions[4].

The repeatability is evaluated on two selected samples and one selected dilution in the mid (LV Harvest:1/4 and LV final product: 1/80).



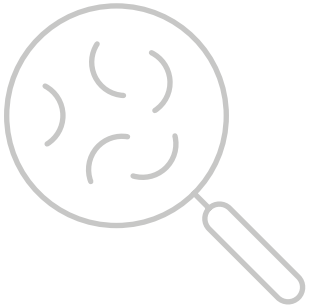
For each sample, the coefficient of variation is calculated as follow:

$$CV(\%) = \frac{Std}{Mean} \times 100$$

Sample	CV (%)
LV Final Product, dilution 1/80	10.8
LV Harvest, dilution 1/4	11.8

For each sample, **coefficient of variation (CV) is inferior at 12%.**

The repeatability of measurement, with purified and non-purified lentiviral vector is excellent.



Accuracy

The accuracy is defined by ICH Q2 as the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[4].

The accuracy is estimated on LV final product, including the 6 dilutions.

The accepted reference value (or theoretical value) is the mean of dilution factor-corrected concentration of the 6 dilutions (LV final product=1.004*E*11 part/mL) divided by the corresponding dilution factor.

$$Theroretical\ concentration = \frac{1.004E11}{dilution\ factor}$$

Recovery is calculated as follow:

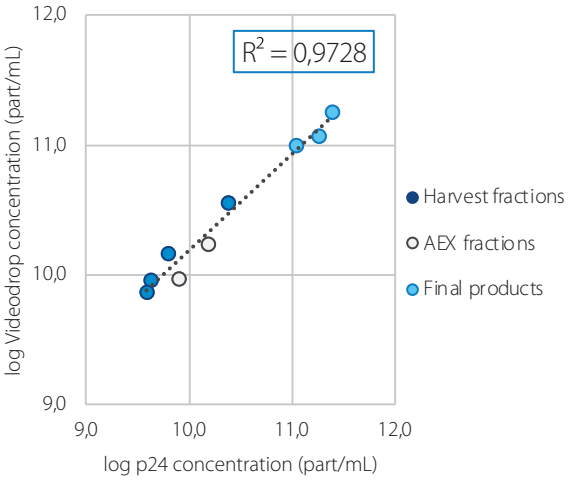
$$Recovery = \frac{Measured\ concentration}{Theoretical\ concentration}\ x\ 100$$

Dilution factor	Measured Concentration (part/mL)	Theoretical concentration (part/mL)	Recovery (%)
1/10	9.00E+09	1.00E+10	89.7
1/20	5.34E+09	5.02E+09	106.4
1/40	2.71E+09	2.51E+09	108.1
1/80	1.26E+09	1.25E+09	100.8
1/160	6.30E+08	6.27E+08	100.4
1/320	2.97E+08	3.14E+08	94.7

For each dilution, **recovery is included in [90%-110%].**

Accuracy: comparison with orthogonal method

As described on a previous application note (VIDEODROP: ideal tool for lentiviral vector bioproduction follow-up - Myriade), Videodrop is especially suitable for lentiviral vector bioproduction in-process controls with consistent linearity with lentiviral physical titer orthogonal methods (p24 ELISA, RT-qPCR, NTA). With those two new batches of lentiviral vectors, we compare concentrations measured by p24 ELISA and Videodrop on samples from different steps of the process.



With a R^2=0.97, these results confirm the accuracy and relevance of the Videodrop as a predictive measure of physical titer for lentiviral batches.

Conclusion

Videodrop shows a great interest for physical titer analysis in lentiviral vector bioproduction. Videodrop is intensively used in bioproduction process development. And the interest grows for lentival vector biomanufacturing in-process controls. With this extensive study, we explore the suitability of the Videodrop in GMP environment. Both software and hardware are suitable **for qualification.**

The validation characteristics considered in the ICH Q2 guidelines as linearity, precision and accuracy show very good performances for LV physical titer analysis using Videodrop. Videodrop can fit in **GMP bioprocessing of lentiviral vectors,** that are commonly used for ex-vivo engineered cell therapy as CAR-T cells[7] or Hematopoietic Stem Cells gene therapy[8].

Lentiviral vector physical titer method using Videodrop can be integrated in Quality section of **IMPD (Investigational Medicinal Product Dossier)** for a Clinical Trial Application, and Quality section of **CTD (Common technical document)** for a Marketing Authorization Application.

References

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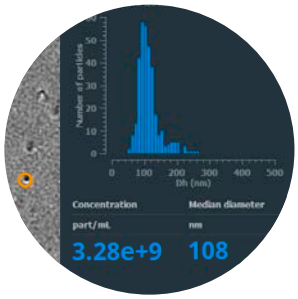
VIDEO DROP

Measuring size & concentration
of nanoparticles

In a single drop
(5-10 µL)

In real time
(40 s)



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