Technology Evaluation: Quicker way of Lentiviral Vector Physical Titer Determination

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Introduction

The measure of HIV-1 p24 antigen by ELISA is a well-established method for lentivirus quantitation. However, this process can be laborious, involving numerous hands-on steps and long incubation times. At Genezen, we produce large- and small-scale Lentiviral vector in both adherent and suspension platforms. We sought to decrease the cost and time spent on p24 evaluation and evaluated two alternative options, **ELLA from Bio-Techne** and **VideoDrop from Myriade**.

ELLA is a cartridge-based system that streamlines and automates the immunoassay by using capture antibodies and a fluorescent label that binds to the target. To evaluate the method, we compared it to our standard p24 ELISA kit by running Lentiviral vectors obtained from our internally developed process. The correlation coefficient between the two methods was determined to be r=0.97, demonstrating a strong positive linear correlation.

The VideoDrop measures particles ranging from 80 nm to 500 nm in size, making it suitable for analyzing lentiviruses. In less than a minute, the VideoDrop can analyze 7 µL of sample and produce concentration values in particles/mL. When compared to Genezen ELISA data, the correlation coefficient was 0.94, indicating a strong positive linear correlation.

ELLA and VideoDrop are promising alternatives to the traditional ELISA method for evaluating lentiviral vectors. Ella is more specific testing method, measuring HIV-1 p24 antigen like our internal ELISA based platform. The VideoDrop method can be used as a real-time in-process analysis during upstream and downstream processing, reducing time and costs.

Analytical Testing Platform improvement

Two different Platforms were used for comparison with our current ELISA based LVV p24 physical Titer platform: ELLA instrument from Bio-Techne and VideoDrop from Myriade. We sought to answer the following questions:

- Which method measures LVV p24 at a **lower cost**?
- Which method measures LVV p24 in a **shorter duration** of time?
- Whether the method can be used during **in-process testing in PD and GMP**?
- Can this be accomplished **while retaining robust results**?
  - - Maintain sensitivity.
  - - Quantitative analysis.
  - - Method can be qualified/ validated.
  - - 21 CFR Part 11 compliant.

Lentiviral p24 Assay Platform Comparison

ELLA HIV-1 Gag p24 Cartridge

Current p24 Platform

VideoDrop by Myriade

- The ELLA instrument from Bio-Techne is an immunoassay that is performed in a microfluidic cartridge.
- Genezen's current platform is Lentiviral titration by p24 ELISA.
- The VideoDrop from Myriade is a platform to measure the concentration of a particle in a (Particles/mL).

Large scale Lentiviral Vector Material Generation at Genezen

Genezen's adherent platform was used to obtain samples from:

- Upstream processes.
- Downstream processes.

Results- Physical Titers Comparison

Table-1: Titers obtained from Genezen's current ELISA method was compared with two physical titer methods, ELLA p24 assay and VideoDrop.

Samples	Current ELISA (VP/mL)	ELLA (VP/mL)	VideoDrop (Particles/mL)
Harvest #1Day 2	2.16E+09	9.73E+07	2.68E+09
Pooled Harvest	1.12E+09	5.24E+07	1.41E+09
Clarified Pooled Harvest	1.10E+09	4.97E+07	1.79E+09
Mustang Q Flowthrough	4.27E+08	6.05E+06	No detection
TFF Retentate (retentate)	1.15E+10	1.36E+09	3.11E+10
Sartopore 2 with 17 cm^2	6.82E+09	8.09E+08	1.32E+10
Sartopore 2 XLG with 17 cm^2	7.36E+09	5.93E+08	1.30E+10
Sartopore Platinum with 17 cm^2	6.63E+09	6.19E+08	1.28E+10
EKV with 20 cm^2	9.31E+09	7.75E+08	1.38E+10

Results- Physical Vs Biological Titers Comparison

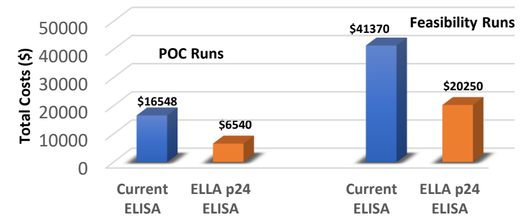
Table-2: Physical titers obtained from ELLA p24 assay was compared with Genezen's Flow and ddPCR based biological titer assay.

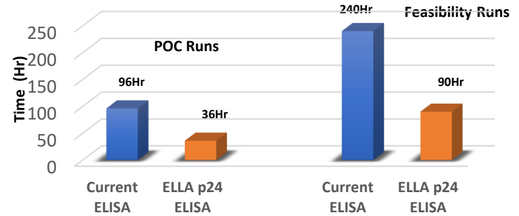
Samples	ELLA(VP/mL)	Flow (TU/mL)	ddPCR (IU/mL)
Harvest #1Day 2	9.73E+07	1.43E+07	5.29E+07
Pooled Harvest	5.24E+07	7.04E+06	3.56E+07
Clarified Pooled Harvest	4.97E+07	4.62E+06	2.85E+07
Mustang Q Flowthrough	6.05E+06	1.55E+04	No detection
TFF Retentate (retentate)	1.36E+09	2.45E+08	5.74E+08
Sartopore 2 with 17 cm^2	8.09E+08	2.11E+08	3.10E+08
Sartopore 2 XLG with 17 cm^2	5.93E+08	2.30E+08	3.23E+08
Sartopore Platinum with 17 cm^2	6.19E+08	3.56E+08	2.94E+08
EKV with 20 cm^2	7.75E+08	3.33E+08	3.77E+08

Comparison of Platforms by Correlation (r)

Test Methods	ELLA p24	VideoDrop
Current ELISA	0.97	0.94
Flow	0.80	0.69
ddPCR	0.98	0.97

Total Assay Costs and Time Savings





Summary

- Genezen will have a method that quantitatively measures LVV p24 at a much lower cost than our current ELISA -based platform with the adaptation of ELLA.
- Genezen can evaluate LVV physical titers of intermediate and final products at a much shorter processing time than the current ELISA based method.
- Genezen can quantitatively analyze viral particles with ELLA while maintaining the same sensitivity of the current qualified method. ELLA is 21 CFR Part 11 compliant.
- VideoDrop is a robust method for determining rapid titers during process development steps.
- VideoDrop should be used in conjunction with traditional ELISA based assays since is doesn't quantify p24 proteins, rather it measures all viral particles present in a sample that are within its limits.

References

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2. Tona R, Shah R, Middaug K, Roszell B, Jung C, Steve J, Marques J 2023.02.017; Process intensification for lentiviral vector manufacturing using tangential flow depth filtration; Mol Ther Methods Clin Dev. 2023 Mar 3; 29:93-107. doi:10.1016/j.omtm.2023.02.017.eCollection 2023 Jun 8. PMID: 36994313, PMCID: PMC10041461