Comparison of Gator® BLI and Progen ELISA for Quantitation of AAV Serotypes

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INTRODUCTION

Determination of total capsid titer is one of the critical quality attributes for AAVs used in gene therapy.

This poster presents quantitation of AAV serotype 2, 8 and 9 using Gator® Prime system and Gator® AAVX probes and compares the performance with Progen Xpress ELISA assay for the same serotypes.

ELISA was run on SpectraMax iD5, Molecular Devices. The AAV serotype standards were purchased from Virovek, Newark, CA.

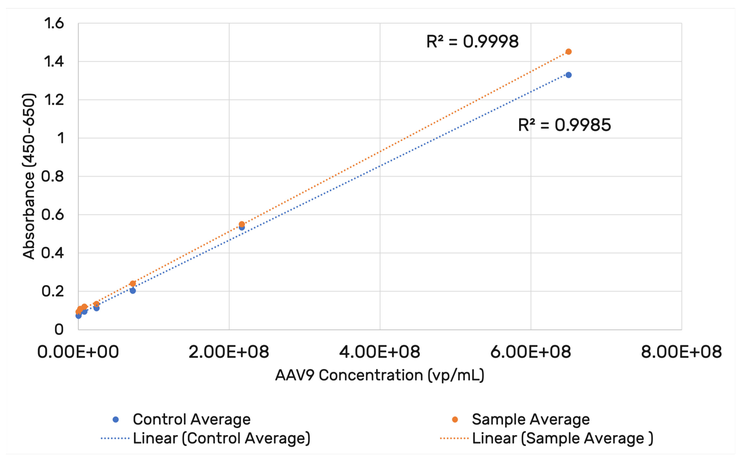
AAVX PROBE FEATURES

- Total capsid quantitation for serotypes AAV1-10
- Dynamic range up to 1E+14 vp/mL (for most serotypes)
- Less than 30 min analysis time
- LOD: 1E+09 vp/mL
- Crude sample tolerant
- Stable over broad pH range
- Cost effective
- Easy to use with minimal hands-on time

QUANTITATION PRINCIPLE AND WORKFLOW

- Gator® probe uses CapSelect™ AAVX nanobody as a ligand to enable direct measurement
- Samples containing AAV particles are pipetted into the 96-well plate and captured on the probe
- The total virus capsid concentration determined using rate of binding of the AAV serotype of interest to the probe
- Different AAV serotypes bind at different rates
- Gator® software calculates the binding rates from standards with known concentrations to generate a standard curve

VERIFICATION OF AAV9 ELISA LINEARITY



Linearity in the dynamic range specified for the kit.

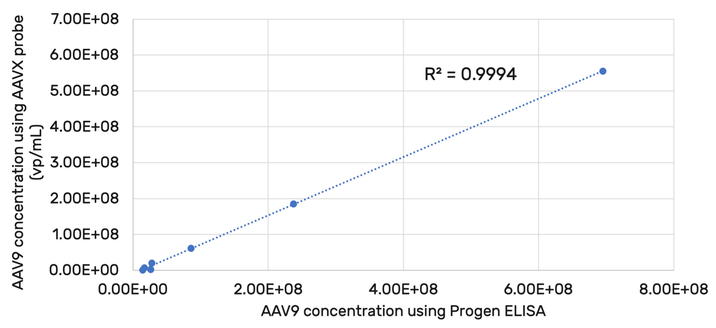
COMPARISON OF GATOR® BLI AND PROGEN ELISA FOR AAV

Method: The performance of Gator® BLI platform for quantitation of various AAV serotypes was evaluated using an established and relatively commonly used Xpress ELISA kit.

The Virovek AAV 2, 8 and 9 standards were at a highest concentration of 1E+11, 2E+11 and 2E+11 vp/mL, respectively. For ELISA, the stocks were diluted 100x, 10x, and 300x, respectively, to bring into the ELISA assay.

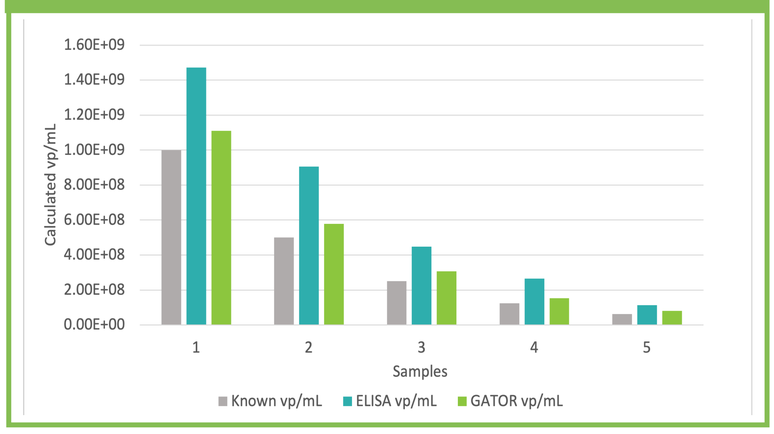
The ELISA kit performance was verified on SpectraMax iD5. As can be seen in AAV9 CORRELATION - GATOR® AAVX PROBES VS AAV9 ELISA, the AAVX probes show a good correlation to ELISA kit. Also, as can be seen in VERIFICATION OF AAV9 ELISA LINEARITY, the ELISA measurements were linear as specified for the kit.

AAV9 CORRELATION - GATOR® AAVX PROBES VS AAV9 ELISA

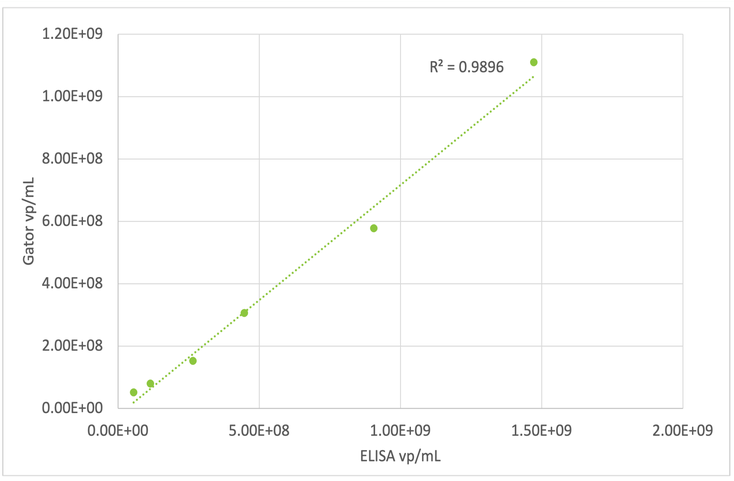


Correlation between Gator and ELISA assay for AAV9

AAV2 KNOWN VS ELISA VS GATOR®



CORRELATION OF ELISA AND GATOR® FOR AAV2

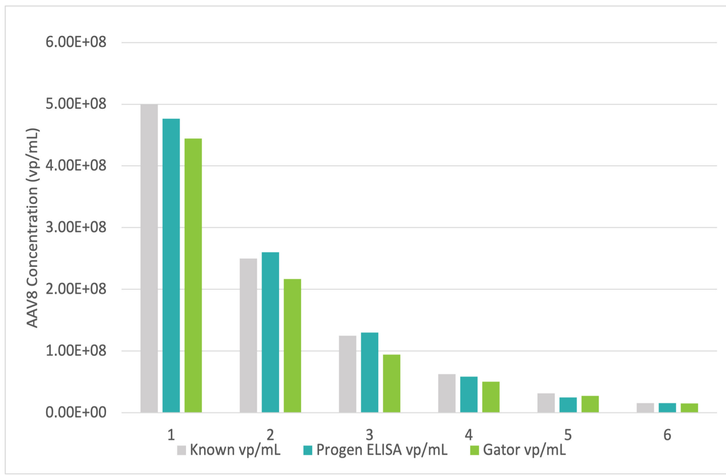


Correlation of ELISA and Gator calculated concentrations for AAV2.

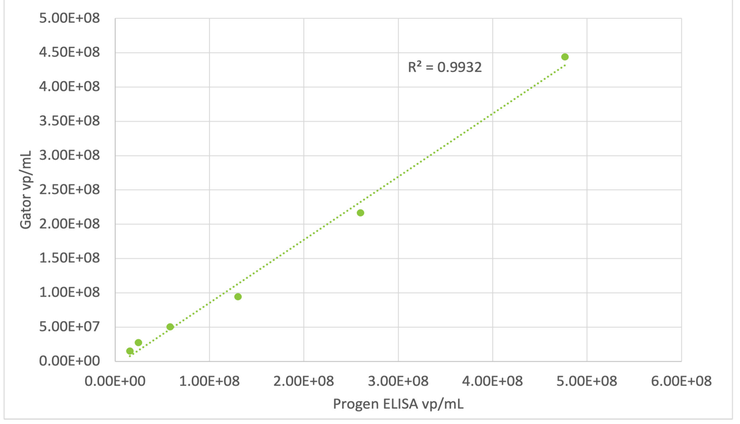
CALCULATED CONCENTRATION OF ELISA AND GATOR® FOR AAV2

Known vp/mL	Progen ELISA vp/mL	Gator vp/mL
1.00E+09	1.47E+09	1.17E+09
5.00E+08	9.06E+08	6.12E+08
2.50E+08	4.47E+08	3.24E+08
1.25E+08	2.65E+08	1.63E+08
6.25E+07	1.14E+08	8.59E+07

AAV8 KNOWN VS ELISA VS GATOR®



CORRELATION OF ELISA AND GATOR® FOR AAV8



Correlation of ELISA and Gator calculated concentrations for AAV8.

CALCULATED CONCENTRATION OF ELISA AND GATOR® FOR AAV8

Known vp/mL	Progen ELISA vp/mL	Gator vp/mL
5.00E+08	4.77E+08	4.44E+08
2.50E+08	2.60E+08	2.17E+08
1.25E+08	1.30E+08	9.43E+07
6.25E+07	5.84E+07	5.02E+07
3.13E+07	2.45E+07	2.72E+07
1.56E+07	1.56E+07	1.49E+07

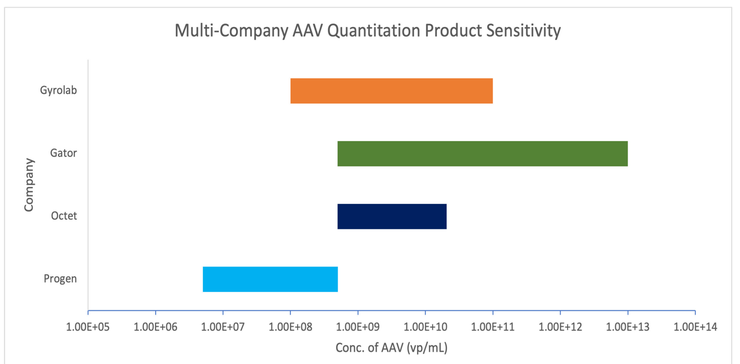
COMPARISON WITH OTHER METHODS

Gyrolab- Wide dynamic range but indirect measurement with multiple steps

Gator- Wider dynamic range, ready to use probe, much less dilution

Octet- Application note shows narrow range and self prepare the probes

ELISA- Highly sensitive, too many steps, multiple kits needed and multiple dilutions



GATOR® AND ELISA WORKFLOW COMPARISON

CONCLUSION

- The Gator® solution comprising of Gator® AAVX probes and Gator® Plus system is capable of accurate and reproducible quantitation of AAV serotypes 2, 8 and 9
- The accuracy of Gator® platform is superior to ELISA
- The analysis time of 26 min /96 samples is much shorter than ELISA
- Good correlation with ELISA kit
- Plug and play with little hands-on time
- Eliminates errors associated with dilutions performed for ELISA