AAV Capsid Titer Determination of AAV Standard Material by Orthogonal Methods

AAV Characterization | Capsid Titer | Orthogonal Methods | Standard Material

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Introduction

Within the field of AAV gene therapy, the use of reliable AAV standards and orthogonal methods is indispensable to ensure consistency and meet current regulatory guidelines.

Since there is a lack of commercially available reference material and the production of internal standard material is not feasible for many companies, PROGEN offers comprehensively characterized AAV standard materials. During our characterization process, several parameters (e.g. vg and capsid titer, filling grade, purity and aggregation) are tested. However, to ensure the utmost level of accuracy and reliability of our data, we additionally analyzed our standard material with a set of suitable orthogonal methods in this study.

Here we present PROGEN's AAV ELISA comparison data for blinded orthogonal measurements of our eGFP-filled AAV standards (AAV2, AAV5, AAV8 and AAV9) with current biophysical methods such as SEC-MALS and DLS, and alternative immunoassays, e.g. BLI and CE. We used the following criteria for acceptance (*see Table 1*).

Titer Recovery	Classification
> 130%	Rejected
120% – 130%	Conditionally accepted
80% – 120%	Accepted
70% – 80%	Conditionally accepted
< 70%	Rejected

Table 1. Acceptance criteria of different capsid titer recovery ranges.

Conclusion

In summary, we observed that the AAV2 titers compared to the ELISA data were underestimated by three out of four orthogonal methods (*Figure 5*). It is generally known, that aggregation can have an influence on immunoassays and probably on biophysical methods as well. Since AAV2 specifically is known for its tendency to aggregate, this might be a possible explanation for the diverse results obtained for AAV2. Intriguingly, none of the methods systematically over- or underestimated the capsid titers determined by ELISA, indicating that there are several factors influencing the final result, e.g. serotype-specific and methodological factors that still need to be assessed.

Here, methods using the same standard material (ELISA and BLI) showed mainly a good correlation. These results demonstrate a high precision of both methods as well as high consistency of data when using the PROGEN standard material. In contrast, methods using a third party standard (CE) showed lower correlation with ELISA. This clearly demonstrates that using the same standard material across different methods allows higher precision and consistency of data from start to finish.

Thus, reliable standards and their use in orthogonal methods and throughout the whole process ensures not only the most precise result but also the safety of the final product in compliance with current regulatory requirements.

Biolayer Interferometry (BLI)

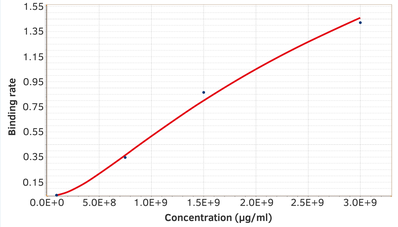

Figure 1. BLI standard curve using the binding rate at different concentrations of AAV8 standard material.

Table 2. Comparison of capsid titers determined by Gator® BLI and ELISA.

Serotype	ELISA [capsids/ml]	BLI HS [capsids/ml]	CV%* [BLI HS]	Titer Recovery [%]
AAV2	1.2E+13	1.2E+13	9.0	98
AAV5	3.5E+12	2.4E+12	4.8	69
AAV8	5.1E+12	4.2E+12	7.3	83
AAV9	7.7E+12	6.7E+12	10.3	86

*CVs were calculated of eight measurements per serotype

BLI, performed on the Gator®Pro device, measures the change in wavelength of the reflected beam due to capsid binding in comparison to a standard. PROGEN's empty capsid standards have been used as standard material for these experiments. The analysis by BLI resulted in an acceptable correlation with the titers determined by ELISA, except for AAV5 (*Table 2*).

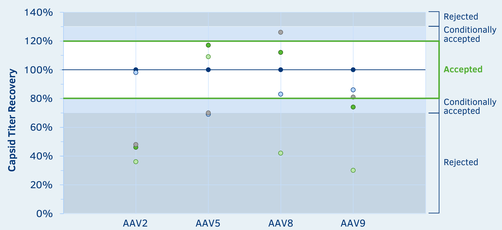

Figure 5: Capsid titer recovery of orthogonal methods compared to ELISA.

Capillary Electrophoresis (CE)

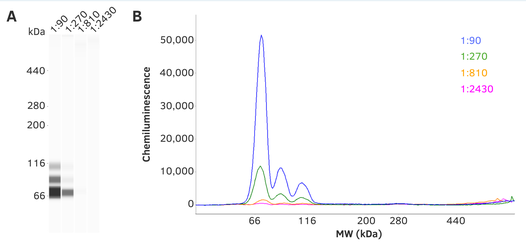

Figure 2. CE results of AAV8 showing (A) the chemiluminescence intensity for the three different VP proteins and (B) its quantification.

Table 3. Comparison of capsid titers measured by CE and ELISA.

Serotype	ELISA [capsids/ml]	CE [capsids/ml]	CV%* [CE]	Titer Recovery [%]
AAV2	1.2E+13	5.8E+12	8.5	48
AAV5	3.5E+12	2.4E+12	14.4	70
AAV8	5.1E+12	6.4E+12	13.6	126
AAV9	7.7E+12	6.2E+12	5.7	81

*CVs were calculated of eight measurements per serotype

For CE, performed on the Simple Western Jess® device, standards provided by a third party were used for calibration curves for all 4 serotypes to analyze the titers of the unknown samples. Additionally, capsid protein ratio (VP1:VP2:VP3) can be determined by this method. The recovery of the AAV9 capsid titer determined by CE showed an acceptable correlation with the ELISA data. In contrast, the CE data obtained with AAV8, AAV2 and AAV5 was over-/ underestimated (*Table 3*).

Dynamic Light Scattering (DLS)

DLS, performed on the DynaPro® Nanostar® II, provides rapid, low-volume, non-destructive AAV quantification to determine size, size distribution and physical titer. This technology does not require a standard. The AAV2, AAV8 and AAV9 capsid titer recoveries are in the rejected correlation range, whereas the mean AAV5 titer showed an acceptable correlation to the ELISA result (*Table 5*). The low values of DLS are caused by the presence of oligomers in the samples, which increases the average hydrodynamic radius measured by DLS and reduces measured particle concentration.

Serotype	ELISA [capsids/ml]	DLS [capsids/ml]	CV%* [DLS]	Titer Recovery [%]	Titer Recovery Range [%]
AAV2	1.2E+13	4.4E+12	34.7	36	22–46
AAV5	3.5E+12	3.8E+12	23.2	109	82–132
AAV8	5.1E+12	2.1E+12	32.4	42	32–52
AAV9	7.7E+12	2.3E+12	10.3	30	28–34

*CVs were calculated of eight measurements per serotype

Table 5. Results of DLS measurement compared to ELISA titers.

Size Exclusion Chromatography-Multi Angle Light Scattering (SEC-MALS)

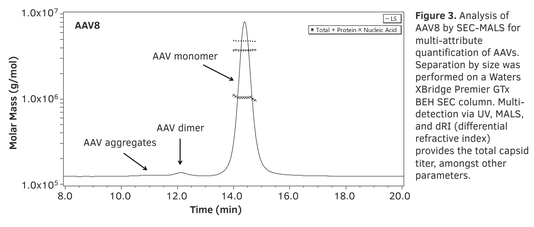

Figure 3. Analysis of AAV8 by SEC-MALS for multi-attribute quantification of AAVs. Separation by size was performed on a Waters XBridge Premier GTx BEH SEC column. Multi-detection via UV, MALS, and dRI (differential refractive index) provides the total capsid titer, amongst other parameters.

Table 4. Results of SEC-MALS measurement compared to ELISA titers.

Serotype	ELISA [capsids/ml]	SEC-MALS [capsids/ml]	CV% * [SEC-MALS]	Titer Recovery [%]
AAV2	1.2E+13	5.6E+12	2.7	47
AAV5	3.5E+12	4.3E+12	3.5	123
AAV8	5.1E+12	5.5E+12	3.0	108
AAV9	7.7E+12	5.6E+12	1.4	73

*CVs were calculated of five measurements per serotype

SEC-MALS was performed on a system equipped with a DAWN™ MALS detector and an Optilab™ differential refractive index detector. It does not require any standard material. The sample is separated by size followed by multiple detectors to measure several AAV quality attributes simultaneously, e.g. capsid titer, empty/full ratio, and aggregation. While the analysis by SEC-MALS showed an acceptable correlation with the ELISA data in case of AAV8, AAV2 and AAV9 were underestimated. The AAV5 titer was slightly overestimated by SEC-MALS (*Table 4*).

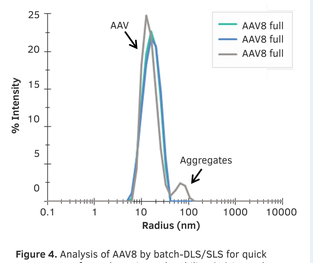

Figure 4. Analysis of AAV8 by batch-DLS/SLS for quick assessment of sample purity and stability. SLS is used to determine total capsid concentration. The Graph shows the intensity of molecules of a certain radius.

Acknowledgement We would like to thank our partners at Waters | Wyatt Technology for performing SEC-MALS and DLS, Gator Bio for BLI, and Bio-techne for CE analyses.