AAV Capsid ELISA Automation for High-throughput AAV Titer Determination

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AAV Capsid Titer | Automation| High-throughput

Introduction

The AAV gene therapy community is facing major challenges like the competitive go-to-market time pressure as well as increasing numbers of samples that need to be processed and analyzed within a very limited time span. Consequently, there is a growing demand for more time-efficient solutions with less hands-on time for analytical characterization of AAV samples.

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used tool, well-known for its accuracy and reliability. Nevertheless, the time-consuming nature may hinder rapid analysis, which is essential for meeting tight production schedules or addressing urgent quality control issues. An automated ELISA processing system might significantly reduce potential human errors and variability while allowing fast and high-throughput analyses.

To show that PROGEN's AAV capsid ELISA is suitable for high-throughput processing, we transferred our established manual PROGEN AAV9 ELISA (PRAAV9) to a fully automated system (DSX® Dynex®). We analyzed plate homogeneity, intra- and inter-assay variance, as well as recovery obtained by the automated system and compared the data to the same key performance indicators obtained by manual processing. Using a fully automated system significantly reduced hands-on time and allowed higher sample throughput, whilst maintaining robustness and accuracy of the conventional AAV ELISA. With this data we show a high-throughput protocol for our AAV ELISAs, thus can support our customers to accelerate efficiency of AAV titer determination.

Plate Homogeneity

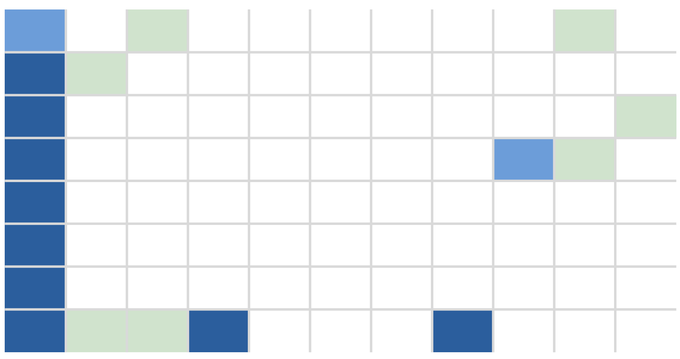

Figure 1. Edge effects of automated ELISA. The colored plate layout refers to the CV values, each calculated by two neighboring plate strips. Edge effects were defined as values whose CV was greater than 10%. Strip 1 showed higher ODs on several plates whereas other wells were affected unsystematically and less frequently. Analysis of manual ELISA did not show any edge effects.

Edge effect	Abberance on one plate	Edge effect on multiple plates	CV
no edge effect			< 8%
slight abberance			8 – 10%
edge effect			11% –15%

Table 1. Comparison of plate homogeneity. To test the homogeneity, a virus solution was prepared and distributed in each well of the 96-well plate. The mean OD and %CV were calculated for each plate. *For plate 7, the conditions were identical to the automated plates, to be able to compare CVs and to rule out a dependency on OD heights. For plates 8 and 9, capsid concentrations were adjusted to reach OD values comparable to the automated system. The mean for the manual ELISA was calculated using plate 8 and 9 only.

	Automated ELISA						Manual ELISA*			p-value
Plate No.	P1	P2	P3	P4	P5	P6	P7	P8	P9	
Mean OD	0.753	0.736	0.764	0.728	0.816	0.823	0.522	0.762	0.759	
CV %	4.9	5.1	8.6	6.8	8.4	7.3	3.5	1.9	2.4	
Mean CV %	6.85							2.15		<0.001

Plate homogeneity of the automated system was tested in comparison to our manual ELISA. The general procedure (e.g. dilution and incubation) was performed as described in the manual. The automated assay showed some edge effects, which were mostly limited to the first and bottom strip (Figure 1). The mean CV of the automated ELISA was significantly higher compared to the manual ELISA (Figure 1), but still within the accepatble range (<10%) (Table 1).

Inter-assay and Intra-assay Variance

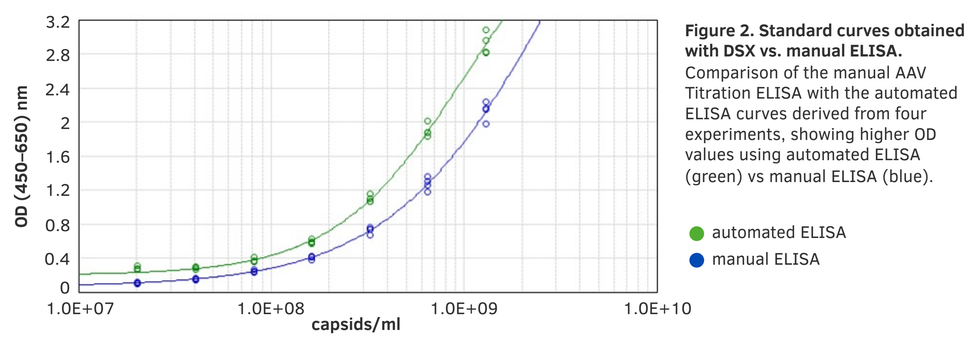

Figure 2. Standard curves obtained with DSX vs. manual ELISA. Comparison of the manual AAV Titration ELISA with the automated ELISA curves derived from four experiments, showing higher OD values using automated ELISA (green) vs manual ELISA (blue).

Table 2. Intra-assay variance.
The intra-assay variance was tested by applying three dilutions (S1, S2, S3) of the same sample in 16 or 24 replicates to the same ELISA plate.

	Automated ELISA			Manual ELISA		
Sample no.	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Tested replicates	n=24	n=16	n=24	n=24	n=16	n=24
Sample conc. [capsid/ml]	3.7E+08	2.6E+08	1.9E+08	3.7E+08	2.6E+08	1.9E+08
Average OD	1.024	0.895	0.688	0.842	0.629	0.468
Average reading [capsids/ml]	3.8E+08	2.6E+08	1.9E+08	3.6E+08	2.6E+08	1.8E+08
Recovery	**101%**	**101%**	**102%**	**98%**	**99%**	**97%**
Recovery range	87–117%	89–113%	91–122%	95–107%	93–106%	90–110%
CV sample reading [%]	7.1	6.8	8.5	2.9	3.9	4.4

Table 3. Inter-assay variance.
The inter-assay variance was tested by measuring established internal standards with four ELISA kits of the same lot on four different days.

	Automated ELISA			Manual ELISA		
Sample no.	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
No. of test days	n=4	n=4	n=4	n=4	n=4	n=4
Sample concentration [capsids/ml]	3.7E+08	2.6E+08	1.9E+08	3.7E+08	2.6E+08	1.9E+08
Average reading [capsids/ml]	3.6E+08	2.5E+08	1.8E+08	3.8E+08	2.6E+08	1.9E+08
CV [%]	**4.5**	**5.8**	**5.8**	**4.4**	**4.1**	**2.9**

Testing assay variances are critical for ensuring the precision and reliability of the automated assay process. Therefore, samples were measured at different positions on the same plate (intra-assay variance) as well as on different test days on different plates (inter-assay variance). As a reference, the manual ELISA was performed using components of the same lot.

The OD values received by automated pipetting were again higher than obtained with manual pipetting (Table 2, Figure 2). Notably, the intra-assay experiment showed good recovery of all samples (+/- 25%) for both systems. In addition, the inter-assay experiment demonstrated a high reproducibility at different test days, comparable to the manual ELISA (Table 3).

Conclusion

The automation of our PROGEN AAV Titration ELISA has proven to be a powerful tool for high-throughput AAV titer analyses. The data demonstrates that accuracy and reproducibility can be maintained while enhancing efficiency by the automated system.

However, the preparation of standard dilutions could not be performed in a serial manner as described in the manual, due to instrument-specific restrictions. Thus, hands-on time was slightly increased since the dilutions had to be prepared and transferred manually into the instrument. We also observed potential for improvement of the automated protocol e.g. first experiments showed that a more stringent washing and adjustment of incubation temperature reduces OD values to a level comparable to the manual ELISA.

With these insights, we have moved closer to fully utilizing automation of our AAV ELISAs to enhance processes for the gene therapy community.

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