Automation of AAV Capsid ELISA on Tecan Fluent

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SOLID BIOSCIENCES

Scan for Capsid Information

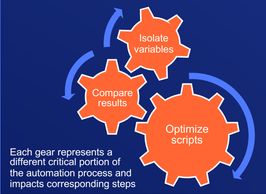
INTRODUCTION

Enzyme-linked immunosorbent assay, or ELISA, is a widely employed analytical method for measuring a recombinant AAV product's capsid titer. Capsid titer results, particularly their role in determining empty/full capsid ratios, help to evaluate upstream and downstream processes and can be used as process control tests. Additionally, Health Authorities expect that capsid titer will be routinely assessed on AAV gene therapies. There is an increased demand for capsid titer data, especially during process development where large numbers of samples are generated. However, due to the assay's high sensitivity and low throughput, it is challenging for scientists to meet this demand. Therefore, the use of automated liquid handler platforms can address many of the method's challenges. Automation of capsid ELISA reduces potential for human error and increases sample throughput to ultimately improve the AAV gene therapy development process.

APPROACH

An iterative approach was taken to automate AAV capsid ELISA. An initial script was programmed onto the automated liquid handler and refined with simulations and dry runs. Samples were then run on the liquid handler, and capsid titer results were compared to manual results. Potential sources of discrepancies between results were identified, and subscripts were created to isolate these variables and test their impact on results. This process was repeated, as necessary.

Figure 1. Iterative Approach to Automation of Capsid ELISA



Each gear represents a different critical portion of the automation process and impacts corresponding steps

METHODS AND MATERIALS

An in-house AAV-SLB101 capsid ELISA method was programmed onto a Tecan Fluent using Fluent Control scripting. The automated assay requires manual preparation of samples and reagents, while the Tecan Fluent performs steps involving serial dilutions, pipetting of reagents, timing incubation periods, and plate washing. Reagents are manually prepared and added to the Tecan Fluent immediately prior to their use to minimize the effects of a degraded reagent on the assay. The absorbance levels of the ELISA plate are then measured in a SpectraMax i3x and analyzed in Microsoft Excel.

Figure 2. Workflow of Automated AAV-SLB101 Capsid ELISA on Tecan Fluent

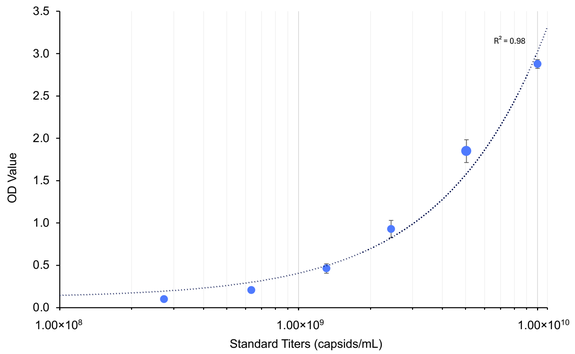
Automated steps in orange and manual steps in blue.
HRP, horseradish peroxidase; PBST, Phosphate Buffered Saline with TWEEN® 20; TMB, 3,3',5,5'-Tetramethylbenzidine.

ACCURACY AND PRECISION

MEETING SYSTEM SUITABILITY CRITERIA

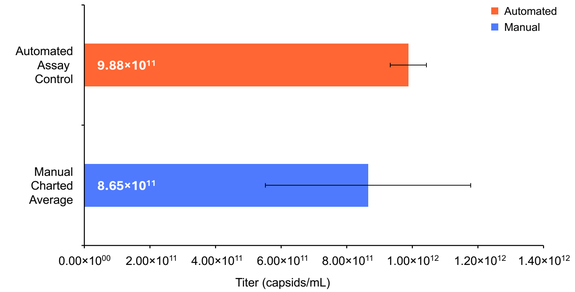
Capsid ELISA plates must meet a set of system suitability criteria to determine whether sample results are reportable. Standards must be within a pre-defined optical density range, and meet goodness of fit, replicate precision, and level of recovery criteria. The assay control must meet precision criteria and be within +/- 3 standard deviations of a historically charted average. Results from a sample capsid titer may only be reported if they meet replicate and inter-dilutional precision criteria. Automated AAV-SLB101 capsid ELISA passed all system suitability criteria.

Figure 3. Automated AAV-SLB101 Capsid ELISA Standard Curve



Standard curve of an automated AAV-SLB101 capsid ELISA used to determine goodness of fit and optical density range. Error bars represent +/- 1 standard deviation (n=9). R^2 value of 0.98. Average OD value of the first standard of 2.877. OD, optical density.

Figure 4. Automated AAV-SLB101 Capsid ELISA Assay Control



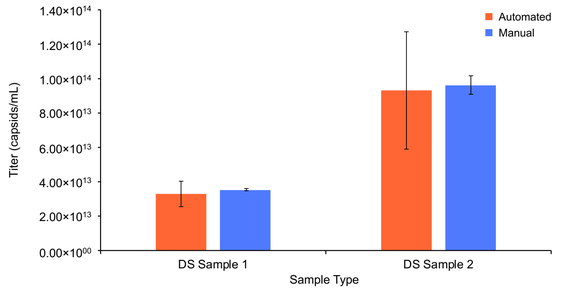
AAV-SLB101 capsid ELISA assay control results. Error bars of automated assay control represent +/- 1 standard deviation (n=6). Error bars of manual charted average represent +/-3 standard deviations (n=239). Automated assay control is within +/- 3 standard deviations of the manual charted average.

COMPARISON TO MANUAL ELISA

COMPARISON OF CAPSID TITER RESULTS

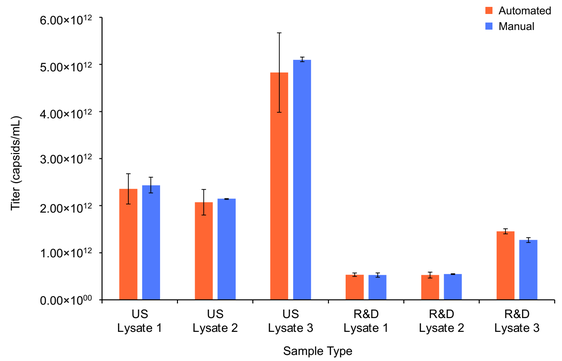
Capsid titer data were compared between AAV-SLB101 capsid ELISA samples run on the automated liquid handler versus manually. Automated sample titers had an average variability of 4% when compared to the manual sample titers. Automated sample titers had lower precision than manual sample titers, potentially due to differences in manual sample prep between runs. Further optimization of the liquid handler script may correct this issue. Samples included downstream, upstream, and research and development (R&D) with expected titers ranging from 1×10^{11} to 1×10^{14} capsids/mL.

Figure 5. Downstream Sample Comparison



Downstream (DS) sample titer results. Error bars of automated results represent +/- 1 standard deviation (n=6). Error bars of manual results represent +/- 1 inter-dilutional standard deviation (n=2). Axis scaled for 1×10^{13} to 1×10^{14} titers.

Figure 6. Lysate Sample Comparison



Upstream (US) and R&D lysate sample titer results. Error bars of automated results represent +/- 1 standard deviation (n=6). Error bars of manual results represent +/- 1 inter-dilutional standard deviation (n=2). Axis scaled for 1×10^{11} to 1×10^{12} titers.

TIME SAVINGS ANALYSIS

COMPARISON OF HANDS-ON TIME

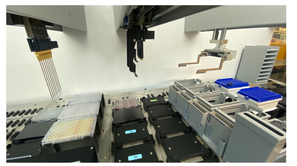
The amount of hands-on time spent running the automated and manual AAV-SLB101 capsid ELISA was measured and compared. Overall, there was a 94-minute total reduction in hands-on time spent running the automated AAV-SLB101 capsid ELISA as opposed to the manual version of the assay. The amount of hands-on time saved by automating the assay also allows scientists to steer their attention towards alternative, non-automatable tasks. Time spent on reagent preparation was the same in both versions of the method. Lastly, due to the Tecan Fluent worktable set-up, it is possible to run up to three AAV-SLB101 capsid ELISA plates in parallel, thereby tripling the sample throughput of the automated assay and further reducing the amount of hands-on time spent running the method. Further testing is necessary to determine the consistency in meeting system suitability across plates run in parallel.

Table 1. Hands-On Time (minutes)

AAV-SLB101 Capsid ELISA Step	AUTOMATED	MANUAL	REDUCTION
Reagent preparation	21	21	0
Equipment preparation	5	3	-2
Serial dilutions	10	40	30
ELISA plate washing/pipetting	0	48	48
OD measurements + analysis	7	25	18
TOTAL	**43**	**137**	**94**

Side-by-side table comparison of the amount of hands-on time (in minutes) spent performing each group of steps of AAV-SLB101 capsid ELISA with the automated versus manual version of the assay. Highlighted box indicates the version with the shorter hands-on time. Reduction column (in minutes) is the calculated difference between the automated and manual columns. OD, optical density.

Figure 7. Tecan Fluent Worktable Set-up for AAV-SLB101 Capsid ELISA



Reagents in grey troughs, dilution and ELISA plates, and plate lids on worktable in black nests. Fixed pipetting, disposable pipetting, and plate moving arms above the worktable, and plate washer (not pictured) underneath the worktable. Empty nests can be utilized in the future to run multiple ELISA plates simultaneously.

CONCLUSIONS

- AAV capsid ELISA is a strong candidate for method transfer onto the Tecan Fluent.
- Automated AAV-SLB101 capsid ELISA met system suitability criteria, capsid titer data were within an average 4% variability to manual capsid titers, and hands-on time was reduced by up to 94 minutes while maintaining the same cost per sample.
- Automated AAV-SLB101 capsid ELISA plates have the potential to be run in parallel, thereby simultaneously increasing sample throughput while reducing human error and allowing scientists to redirect their time towards non-automatable tasks.
- Further investigation will be conducted into the cause of lower precision in the automated AAV-SLB101 capsid ELISA samples and ways to further decrease hands-on time.
- Advance your research with AAV-SLB101 & Solid's AAV Gene Therapy Development Kit. Contact our BD team at businessdevelopment@solidbio.com.

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