DEVELOPMENT OF AN OPTIMIZED STATISTICAL METHOD AND FULL VALIDATION OF THE ELECTROCHEMILUMINESCENCE IMMUNOASSAY FOR DETECTING ANTI-AAV ANTIBODIES IN HUMAN SERUM

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PURPOSE

As one of the most widely used gene delivery vectors, AAVs are being used in many clinical trials for the treatment of a variety of diseases. Anti-AAV antibody determination is important for participant screening in AAV-based gene therapy and can provide valuable information for drug safety and efficacy evaluations. However, the majority of the population have pre-existing anti-AAV antibodies, which raises the thorniest challenge in cut point determination of anti-AAV antibody assays.

Traditional statistical methods can't distinguish samples with pre-existing antibodies from high-background negative samples, resulting in high screening and confirmatory thresholds. In addition, there is a paucity of published anti-AAV antibody detection methods with comprehensive validation data following guidance for bioanalytical method validation.

In this study, an appropriate statistical method was used to establish cutoff. Based on this approach, we developed Meso Scale Discovery (MSD) methods for anti-AAV antibody detection in clinical trials using AAV products (AAV2, AAV8, AAV9).

METHODS

In the assays, the presence of anti-AAV antibody is determined by comparing the signal in the sample or control to a statistically derived threshold, the assay cut point. Biotinylated AAV is coated on a streptavidin (SA)-plate, followed by the addition of the samples and ruthenium-labeled AAV. Anti-AAV antibodies in the samples can form a bridging complex between the two labeled components.

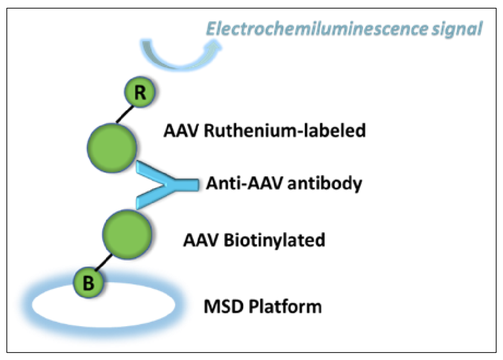

Figure 1. *Principle diagram for detecting Anti-AAV antibodies*

RESULTS

Cut Point

A large number of samples (≥100) were used for negative control selection for statistical cut-point calculations and samples that produced outliers, including analytical and biological outliers, were excluded from statistics through multiple rounds of analysis. As such, more reasonable cut points were obtained.

All data statistical analysis was performed by SAS software.

Cut point	AAV2	AAV8	AAV9
SCPF	1.17	1.17	1.23
TCPF	1.29	1.30	1.40
CCP	26.7%	20.6%	28.7%

Table 1. *Cut point factor in anti-AAV antibody assays*

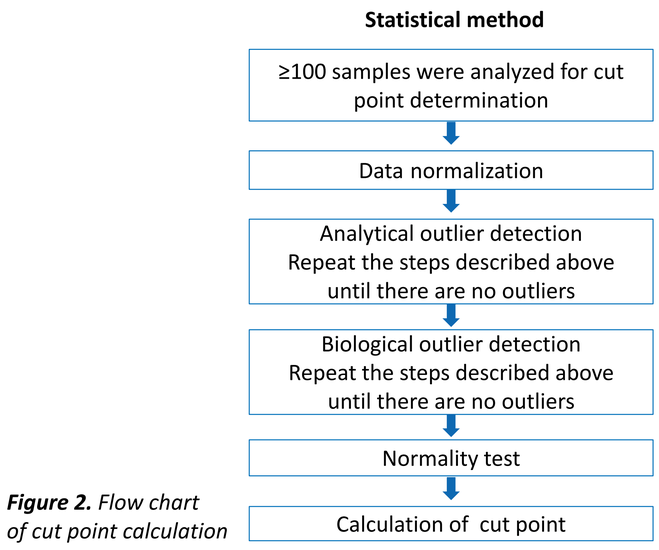

Figure 2. *Flow chart of cut point calculation*

Sensitivity

The anti-drug antibody was serially diluted in pooled normal human serum to prepare the sensitivity samples.

Sensitivity samples were analyzed with and without drug.

Acceptance criteria for screening and confirmatory sensitivity: the mean sensitivity should be less than or equal to 100 ng/mL.

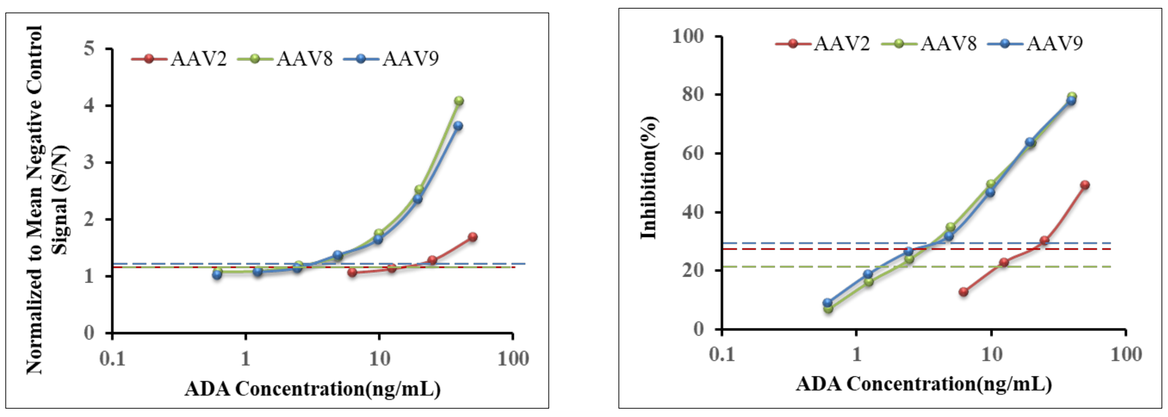

Figure 3. *Sensitivity curve of anti-AAV antibody assays*

Drug Tolerance

For drug tolerance of anti-AAV2, anti-AAV8, anti-AAV9 antibody assays, at 100 ng/mL ADA concentration, the method can tolerate 1.0E+10 vp/mL, 5.0E+10 vp/mL, 5.0E+10 vp/mL, respectively.

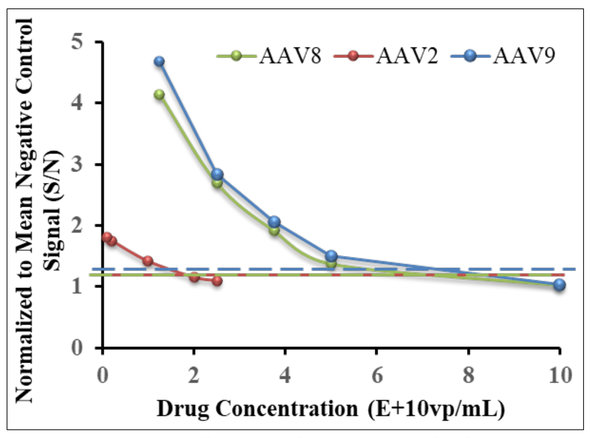

Figure 4. *Drug tolerance of anti-AAV antibody assays*

HOOK Effect

Hook effect is defined as a decrease in assay signal with increasing anti-AAV antibody.

The positive control samples were serially diluted and analyzed in the confirmatory assay.

No hook effect was observed up to 20,000 ng/mL for AAV2, AAV9 assays and up to 10,000 ng/mL for AAV8 assay.

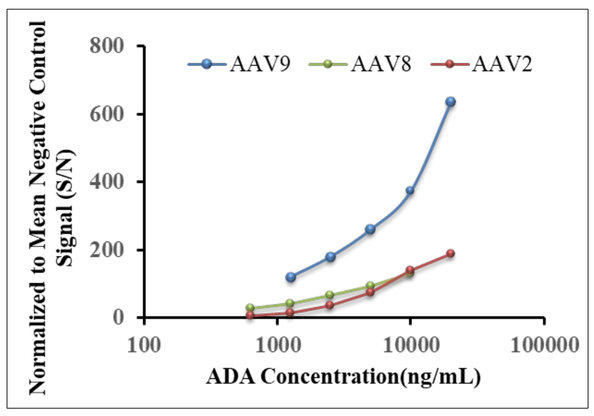

Figure 5. *Hook effect of anti-AAV antibody assays*

Selectivity

For AAV2, AAV8 and AAV9 assays:
No matrix effect was observed.

- Normal individual serums: HPC, LPC and NC samples were prepared with ten lots of normal serum and analyzed in the confirmatory assay. ***10/10 Passed***
- Lipemic human serum: HPC, LPC and NC samples were prepared with three lipemic serum and analyzed in the confirmatory assay. ***3/3 Passed***
- Hemolysis human serum: HPC, LPC and NC samples were prepared with three hemolytic serum and analyzed in the confirmatory assay. ***3/3 Passed***

Precision

Precision runs were tested by 6 sets of PCs and NCs in each run of six runs by 2 analysts over 3 separate days.

Intra- and inter-assay precision of PCs were all within 20%.

Positive Control	AAV2				AAV8				AAV9			
	Ratio		%Inhibition		Ratio		%Inhibition		Ratio		%Inhibition	
	Inter CV%	Intra CV%	Inter CV%	Intra CV%	Inter CV%	Intra CV%	Inter CV%	Intra CV%	Inter CV%	Intra CV%	Inter CV%	Intra CV%
HPC	10.7	2.3-5.3	0.2	0-0.1	11.3	2.0-8.4	0.4	0.1-0.3	9.2	8.3-10.1	0.1	0-0.1
MPC	13.2	3.3-6.7	0.9	0.2-0.5	10.5	2.7-6.3	0.5	0.2-0.3	7.7	6.8-7.8	0.8	0.6-0.7
LPC	18.3	4.7-7.7	13	2.2-6.7	4.9	1.0-3.5	7.6	1.7-5.3	5.0	3.2-4.4	15.3	8.1-13.4

Table 2. *Precision results of anti-AAV antibody assays*

Stability

Three sets of stability samples for each level (HPC, LPC, NC) were prepared independently and analyzed for each stability test.

Conditions	AAV2	AAV8	AAV9
2-8℃	72 hours	72 hours	48 hours
RT	72 hours	72 hours	48 hours
F/T(-20℃ and -70℃)	9 cycles	9 cycles	9 cycles

Table 3. *Stability results of anti-AAV antibody assays*

It showed that short term stability (2-8℃, RT), 9 cycles freezing/thawing testing all passed.

CONCLUSION AND NOVELTY

In this study, more reasonable cut points were obtained. Based on this approach, we developed affinity capture and elution Meso Scale Discovery (MSD) methods for anti-AAV antibody detection in clinical trials using AAV products (AAV2, AAV8, AAV9). The methods were fully validated by evaluating sensitivity, precision, robustness, selectivity, hook effect and stability and met the acceptance criteria. The established statistical methodology and thorough method validation results are valuable references for future clinical investigations of AAVs.

REFERENCES

1. Weber T. Anti-AAV Antibodies in AAV Gene Therapy: Current Challenges and Possible Solutions. Front Immunol. 2021;12:658399.
2. Schulz M, Levy DI, Petropoulos CJ, et al. Binding and Neutralizing Anti-aav Antibodies: Detection and Implications for Raav-mediated Gene Therapy. Mol Ther. 2023;31(3):616-630.