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ADA Assay for AAV-Based Gene Therapies

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Introduction

Gene therapies generally utilize viral vectors as delivery tools, with adeno-associated viruses (AAV) proving to be effective vectors. AAV have a relatively good safety profile compared to other viruses, but their unintended immune responses remain a major concern. Studies have shown that preexisting antibodies to AAV exist in certain percent of human subjects and that these preexisting antibodies may impact efficacy of AAV gene therapy. Thus, patients are usually screened for preexisting AAV antibodies at entry screening and only the patients with ADA signal below a cut point get enrolled. In this study, an bridging assay was developed for the detection of anti-AAV antibodies in human serum against AAV8, a frequently used serotype of AAV.

Methods

In the assay, AAV8 is directly coated on 96-well microplate. Samples are added to the microplate and the anti-AAV8 antibodies in the sample bind to the AAV8. Subsequently, biotin-labelled AAV8 is added to form a bridging structure. This is followed by the addition of SA-HRP and TMB for detection. The assay design is illustrated below.

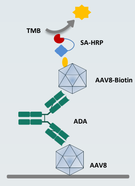


Figure 1 Assay Design

Assay Results

Cut-Points

According to the literature, proportion and intensity of pre-existing antibodies to AAV can vary among individuals. A total of 65 individual human serum samples were analyzed in the screening assay, and the serum samples with a signal-to-noise ratio of less than 1.1 were selected as negative serum samples for cut point establishment. The ADA signal distribution profile of these individual samples is presented in Figure 2A.

Subsequently, the pre-selected 30 individual human samples were analyzed one or two times, resulting in 52 data points. After outlier removal, 95th percentile of one-sided confidence interval was screening cut point factor values. After outlier removal, 99th percentile of one-sided confidence interval was confirmatory cut point values. Screening cut point factor values was 1.15 and confirmatory cut point values was 15.69%. A summary of the calculation results was presented in Figure 2B and 2C.

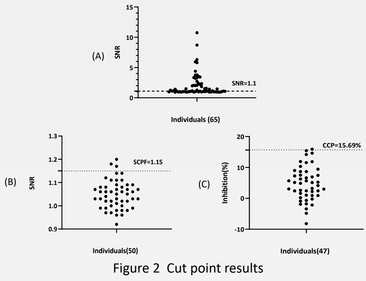


Figure 2 Cut point results

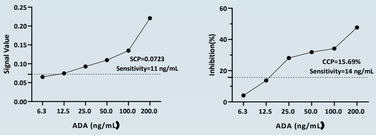
Sensitivity

Anti AAV8 antibody was used as positive control and assay sensitivity was determined using Excel function FORECAST(x, known_y's, known_x's), where x is CP, known_y's is two concentrations that span CP, known_x's is the values spanning CP , the y value calculated by this function is the sensitivity. Based on the calculation formula, screening assay sensitivity was determined to be 11 ng/mL and confirmatory assay sensitivity was 14 ng/mL. A summary of the calculation results was presented in Table 1 and Figure 3.

Table 1 Sensitivity data

Assay type	Unit	ADA positive control (ng/mL)						
		NC	6.3	12.5	25.0	50.0	100.0	200.0
Screening	Signal Value	0.0629	0.0652	0.0746	0.0929	0.1097	0.1349	0.2208
Confirmatory	Inhibition (%)	/	4.14	13.74	28.11	31.87	34.19	47.66

Figure 3 Sensitivity results



Assay Precision

A total of 5 precision runs were performed by 2 analysts over 4 different days. Data from an intra-assay precision run was used for intra-assay precision calculation, while all accepted precision runs were used to calculate inter-assay precision. To assess precision of the method, 1000 ng/mL ADA was set as HPC, 400 ng/mL as MPC, and 50 ng/mL as LPC. The calculated intra- and inter-assay precision are listed in Table 2. Precision are all within the acceptable range: CV ≤ 20%.

Table 2 Precision results

Assay type	Unit	HPC	MPC	LPC
		Intra-run CV		
Screening	SNR Ratio	1.7	2.8	4.4
Confirmatory	%inhibition	1.7	2.6	13.0

Assay type	Unit	HPC	MPC	LPC
		Intre-run CV		
Screening	SNR Ratio	8.8	14.5	14.4
Confirmatory	Inhibition	6.3	7.0	18.0

Selectivity

Selectivity was assessed by preparing ADA at LPC and NC levels in 10 individual human serum samples. The results showed that 9 out of 10 (90%) LPC individual serum samples tested positive(Individual 7 CV was greater than 20%), and 9 out of 10 (90%) NC individual serum samples tested negative(Individual 3 CV was greater than 20%), meeting the pre-set acceptance criteria. A summary of the calculation results was presented in Table 3.

Table 3 Selectivity results

Matrix	Screening				Confirmatory			
	LPC (SCP=0.1139)		NC (SCP=0.1139)		LPC		NC	
	Signal values	Results	Signal values	Results	Inhibition %	Results	Inhibition %	Results
Indiv 1	0.1682	Positive	0.0705	Negative	34.90	Positive	-25.48	Negative
Indiv 2	0.1642	Positive	0.0762	Negative	32.70	Positive	5.18	Negative
Indiv 3	0.1580	Positive	0.0783	Negative	24.18	Positive	-28.29	N/A
Indiv 4	0.1545	Positive	0.0747	Negative	29.84	Positive	-4.35	Negative
Indiv 5	0.1398	Positive	0.0908	Negative	28.19	Positive	-0.72	Negative
Indiv 6	0.1524	Positive	0.0857	Negative	25.57	Positive	1.28	Negative
Indiv 7	0.1543	Positive	0.1002	Negative	29.79	N/A	-11.68	Negative
Indiv 8	0.1504	Positive	0.0994	Negative	33.33	Positive	-25.11	Negative
Indiv 9	0.1410	Positive	0.0698	Negative	33.38	Positive	-41.83	Negative
Indiv 10	0.1348	Positive	0.0700	Negative	17.32	Positive	-31.52	Negative

Hook effect

The ADA positive control was 3-fold serially diluted from 15000.0 ng/mL to 555.6 ng/mL to evaluate if there was a hook effect in the screening assay. The results showed that the signal values of hook samples (15000.0 ng/mL to 1666.7 ng/mL) were higher than that of HPC, no hook effect was concluded. A summary of the calculation results was presented in Table 4.

Table 4 Hook effect results

Assay type	Unit	ADA (ng/mL)				
		HPC (1000.0)	555.6	1666.7	5000.0	15000.0
Screening	Signal Value	0.6851	0.5548	0.81625	0.8771	0.8404

Conclusion

An ADA assay has been established for anti-AAV8 antibody analysis in human serum involving AAV8 gene therapies. This assay is used to detection clinical samples of AAV8 gene therapy to guide the inclusion and screening of clinical patients.

The results of the validation experiments demonstrated sufficient assay sensitivity and precision, and selectivity for the intended purpose. No hook effect was observed.

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