Comparative Detection of Pre-existing Anti-AAV8 Antibodies in Normal Human Serum by a Total Antibody Assay versus a Transduction Inhibition Assay

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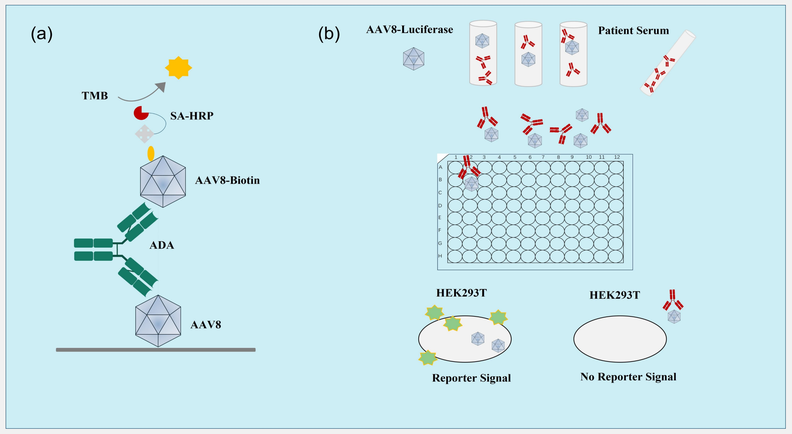
PURPOSE

Pre-existing antibodies to AAV exist in certain human subjects and these antibodies may impact the effectiveness of AAV gene therapy. Thus, patients are usually screened for pre-existing AAV antibodies with a transduction inhibition assay (TI assay) to detect neutralizing antibodies at entry. However, the TI assay is a cell-based assay and it is technically challenging to conduct. Using AAV8 as an example in this study, we explored if an immunoassay-based total antibody assay (TAb assay) could be used in lieu of the TI assay for patient screening.

METHOD(S)

In the TAb assay, AAV8 is directly coated onto a 96-well microplate (Figure 1 a). Following incubation of human serum samples, a biotinylated AAV8 is added to form a bridging structure for detection of anti-AAV8 capsid antibodies. The TAb assay is performed in two tiers – screening and confirmation, with the confirmed positives as final positive results. In the TI assay, the AAV8 virus harboring luciferase gene is employed, which expresses luciferase after successful transduction into a host cells (Figure 1 b). The virus is incubated with serum samples, and then added to a 96-well cell plate pre-seeded with HEK293T host cells. Following an overnight incubation, the luciferase activity is measured chemiluminescently. Upon assay validation, the same set of human serum samples from 36 normal subjects are analyzed with the two assays and the results are compared for concordance.

Figure 1. Design of TAb Assay and NAb Assay



RESULT(S)

Assay Validation Results

A total of 36 normal individual human serum samples were employed for assay cut point evaluation. The TAb screening cut point factor was determined to be 1.23 based on 5% false positive rate, and the confirmatory cut point was 17.13% based on 1% false positive rate. For the NAb assay, we took 50% of inhibition of luciferase activity compared to negative control serum samples as the assay cut off, as suggested by Gorovits et al. Following cut point determination, the TAb assay sensitivity was assessed to be 14.0 ng/mL, and the NAb assay sensitivity was 204.4 ng/mL. The calculated intra- and inter-assay precision (%CV) for both assays were all ≤20%.

Selectivity was assessed at LPC and NC levels. Results from all 10 individual serum samples met the acceptance criteria and there was no matrix effect in both TAb and NAb assay. No interference was observed with highly lipemic serum samples (up to 150 mg/dL triglycerides) or severely hemolyzed serum samples (up to 5% hemolyzed) as well. A summary of the assay validation parameters is provided in Table 1.

Table 1. A subset of assay parameters evaluated during validation

Parameters	Result	
	AAV8 TAb Assay	AAV8 NAb Assay
MRD	10	20
Cut Point	1.23 (SCP); 17.13% (CCP)	50%
Sensitivity	14.0 ng/mL of a monoclonal anti-AAV8	204.4 ng/mL of a monoclonal anti-AAV8
Inter-assay Precision(%CV)	SCP: HPC 8.8, MPC 14.5, LPC 14.1 CCP: HPC 6.3, MPC 7.0, LPC 18.0	HPC 0.06, MPC 0.06, LPC 2.4
Intra-assay Precision (%CV)	SCP: HPC 1.7, MPC 2.8, LPC 4.4 CCP: HPC 1.7, MPC 2.6, LPC 13.3	HPC 0.05 , MPC 0.14, LPC 13.7
Selectivity	10/10 passed at 0 ng/mL anti-AAV8 10/10 passed at 50 ng/mL anti-AAV8	10/10 passed at 0 ng/mL anti-AAV8 10/10 passed at 250 ng/mL anti-AAV8
Hemolysis (5% and 2%)	100% Pass at 0 ng/mL anti-AAV8 100% Pass at 50 ng/mL anti-AAV8	100% Pass at 0 ng/mL anti-AAV8 100% Pass at 250 ng/mL anti-AAV8
Lipemia (150 mg/dL and 60 mg/dL)	100% Pass at 0 ng/mL anti-AAV8 100% Pass at 50 ng/mL anti-AAV8	100% Pass at 0 ng/mL anti-AAV8 100%Pass at 250 ng/mL anti-AAV8

Consistency of AAV8 TAb and NAb Test Results

This study aimed to compare the results from TAb versus the Nab assay towards AAV8 to determine if the TAb assay can be used for patient enrollment. Given viral nature of AAV8, we expect most of confirmed TAb positive serum samples to exhibit neutralizing capability (NAb positive). TAb and NAb assay results were compared as shown in Figure 2. In 36 normal subjects, 13 subjects (36.1%) were NAb-positive and subjects (63.9%) were NAb-negative.

Of the 13 NAb-positive subjects, 84.6% were positive for TAb confirmation, and the sensitivity of the TAb assay was therefore 84.6%. Of the 23 NAb-negative individuals, 95.7% were confirmed negative for TAb, and the specificity of the TAb assay for prediction was 95.7% (Table 2). Overall percentage agreement was 91.7% (Table 3), indicating a high concordance between AAV8 TAb confirmatory assay and NAb assay.

Figure 2. Comparison of test results from AAV8 TAb screening, TAb confirmation (CCP) versus NAb (TI) assays

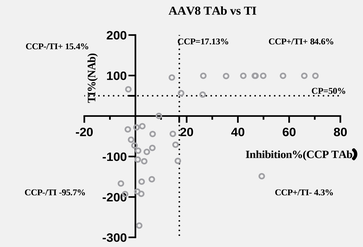


Table 2. Positive and negative results from TAb confirmation assay vs Nab assay

Assay		TAb Confirmation		Total
		Positive	Negative	
NAb	Positive	11	2	13
	Negative	1	22	23
Total		12	24	36

Table 3. Concordance analysis between TAb confirmation and NAb assays

	Calculation	Concordance, % (95% CI)
TAb Assay (Positive)	PPA	91.7 (78.2 ~ 97.1)
TAb Assay (Negative)	NPA	91.7 (78.2 ~ 97.1)
NAb Assay (Positive)	PPV	84.6 (69.6 ~ 93.0)
NAb Assay (Negative)	NPV	95.7 (83.5 ~ 99.0)
Overall Results	OPA	91.7 (78.2 ~ 97.1)

NAb: Neutralizing antibody; NPA, Negative percentage agreement; NPV; Negative predictive value; OPA, Overall percentage agreement; PPA, Positive percentage agreement; PPV, Positive predictive value; TAb: Total antibody.

CONCLUSION(S)

An immunoassay (TAb assay) and a cell-based assay (TI assay) have been developed and validated for detection of pre-existing anti-AAV8 antibodies. Results from the two assays are highly concordant, leading to the higher diagnostic sensitivity (84.6%) and the higher diagnostic specificity (95.7%). Based on these results, it is reasonable to use the TAb confirmation assay in lieu of the TI assay for screening patients' samples for an AAV8 gene therapy.