BioAgilytix

Screening Assays for Anti-AAV Antibodies in Support of Gene Therapies: TAb or NAb?

Ivonne Bernal, Robert Nelson, Roman Wernyj

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BACKGROUND

Adeno-associated virus (AAV) vectors are a leading platform for gene delivery for the treatment of a variety of human diseases. Immunogenicity assessments as inclusion/exclusion criteria to predict investigative drug safety and efficacy are critical in the development of Adeno-Associated Virus (AAV)-based gene therapies. Although multiple assay formats which measure distinct endpoints (i.e., binding versus neutralizing antibody activity) are applicable and are already being used in this context, it is not clear which assays should be selected and at what stage of drug development these methods should be implemented. In this presentation, we compare results obtained from testing screening samples for anti-AAV8 and anti-AAV2 total antibodies (TAb) and a neutralizing antibodies (NAb). Additionally, the suitability of both methods as a screening assay for patient enrollment into AAV8 and AAV2 gene therapy clinical studies will be analyzed.

METHODS – TAb ASSAYS

Sequential bridging ELISA setup: AAV capsids are coated on microwell plates. After sample incubation, an HRP-conjugated anti-human antibody is used for detection of binding antibodies. The signal is proportional to the TAbs in the sample.

Table 1. Validation Results TAb assays

	AAV2	AAV8
MRD	1:20	1:20
Cut Point	NC + 0.174	NC + 0.042
Sensitivity	1.4 ng/mL	15.6 ng/mL
Precision	CV ≤ 20%	CV ≤ 20%

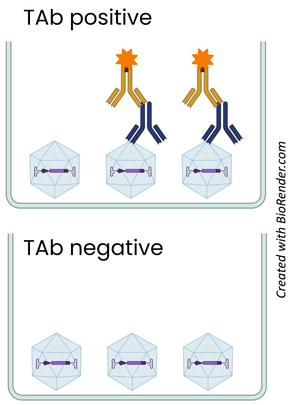


Figure 1. TAb assays scheme

METHODS – NAB ASSAYS

Cell-based assay setup: Huh7 cells are seeded and pre-incubated with Ad5-DL309 (transduction helper vector). AAV vectors carry a f-luciferase reporter gene (AAV-Luc). AAV-Luc and sample mixes are added to the cells for transduction. Luciferase expression is detected in absence of NAbs.

Table 2. Validation Results NAb assays

	AAV2	AAV8
MRD	1:5	1:5
Cut Point	NC x 0.5	NC x 0.5
Sensitivity	39.1 ng/mL	22.4 ng/mL
Precision	CV ≤ 30%	CV ≤ 30%

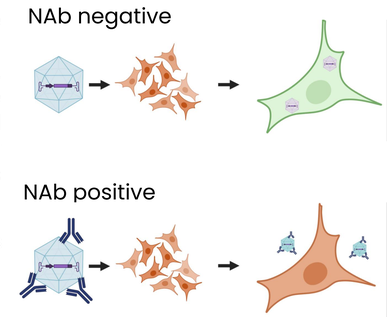


Figure 2. NAb assays scheme

RESULTS – AAV2

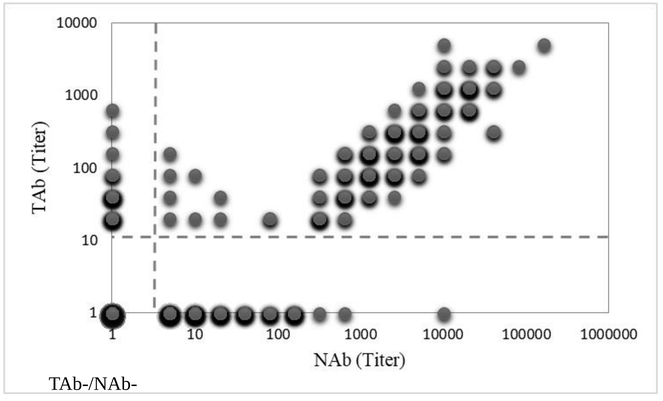
461 serum samples were screened in both assays. Of these, 42% (192 samples) screened negative in both assays (TAb-/NAb-), whereas 32% (150 samples) screened positive in both assays (TAb+/NAb+). 26 samples (6%) that screened positive in the TAb assay did not demonstrate positivity for NAbs to AAV2 (TAb+/NAb-), while 93 samples (20%) that screened positive in the NAb assay were negative in the TAb assay (TAb-/NAb+, Table 1). The TAb and NAb titer responses are shown in Figure 3.

Table 3. TAb – NAb AAV2 correlation. Sample count

		NAb Result		
		Negative	Positive	Total
TAb Result	Negative	192	93	285
	Positive	26	150	176
	Total	218	243	461

The number of assays in agreement (either both positive or both negative) is 342 of 461 (74%). The Cohen's kappa correlation coefficient is 0.49, which denotes a moderate agreement.

Figure 3. TAb – NAb AAV2 correlation. Sample Titer



RESULTS – AAV8

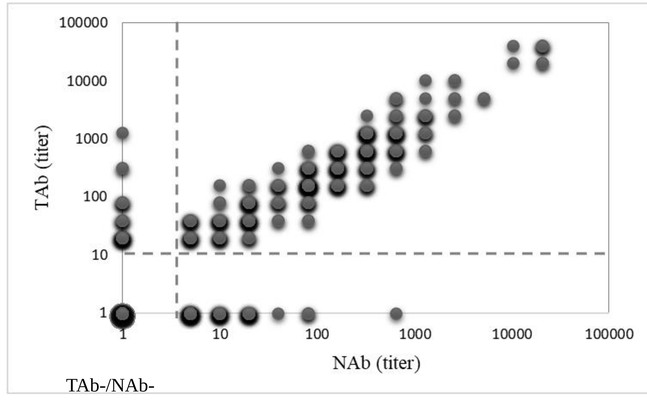
474 serum samples were screened in both assays. Of these, 49% (233 samples) screened negative in both assays (TAb-/NAb-), whereas 38% (179 samples) screened positive in both assays (TAb+/NAb+). 18 samples (4%) that screened positive in the TAb assay did not demonstrate positivity for NAbs to AAV8 (TAb+/NAb-), while 44 samples (9%) that screened positive in the NAb assay were negative in the TAb assay (TAb-/NAb+, Table 2). The TAb and NAb titer responses are shown in Figure 4.

Table 4. TAb – NAb AAV8 correlation. Sample count

		NAb Result		
		Negative	Positive	Total
TAb Result	Negative	233	44	277
	Positive	18	179	197
	Total	251	223	474

The number of assays in agreement (either both positive or both negative) is 412 of 474 (87%). The Cohen's kappa correlation coefficient is 0.74, which denotes a substantial agreement.

Figure 4. TAb – NAb AAV8 correlation. Sample Titer



CONCLUSION

The concordance data we present herein shows a substantial agreement between TAb and NAb assay results for AAV8 and a moderate agreement for AAV2. Some samples, 4% for AAV8 and 6% for AAV2, presented binding antibodies and did not have neutralizing activity. The TAb-/NAb+ samples, 9% for AAV8 and 20% for AAV2, may reflect the presence of non-antibody or other uncharacterized neutralizing serum components; the clinical relevance of these factors is unknown.

These data demonstrate the suitability of either method as a screening assay for patient enrollment into AAV8 or AAV2 gene therapy clinical studies. Using the TAb assays could be beneficial: short turn-around time, high throughput and robustness, detection of antibodies that impact transduction by different mechanisms. NAbs are assumed to be better predictors of transduction outcomes and clinically more relevant. However, they are labor intensive, have high variability with potential false positive results due to non-NAb inhibitors.

The method suitability for improved selection of patients during clinical study enrollment should be determined for every AAV serotype and evaluated on a case by case basis.