M1230-01-05

RAPID AND REPRODUCIBLE TAB/NAB AAV ASSAYS ACROSS SEROTYPES AND SPECIES TO SUPPORT GLOBAL PRECLINICAL PROGRAMS

Quentin Osseman, Dhruti Dave, Evguenia Nepotchatykh, Melanie Lambert, Jerry Fenelon, Geoffroy Danieau, Wangisa Dunuwille, Ranjithkumar Ravichandran, Olga Malykhina, Manoranjan Sahoo, Sophie Corbeil, and Danielle Salha

Altasciences, Laval, QC, Canada

CONTACT INFORMATION: qosseman@altasciences.com

aaps PharmSci 360

Click here to listen to the recorded poster presentation

PURPOSE

AAV vectors are among the most commonly used tools in gene therapy due to their high efficacy and diverse tropisms, which broaden their applicability across a wide range of disease indications.

AAV-vector gene therapies depend on vector transduction efficacy, which is significantly reduced in the presence of AAV neutralizing antibodies (NAb).

OBJECTIVES

The development of Total Antibody (TAb) and NAb assays is required to evaluate preexisting immunity and treatment-induced/boosted responses, thereby supporting gene therapy programs. Key challenges include detecting low-level immunity, distinguishing treatment effects, and ensuring assay robustness.

Fast, reliable AAV TAb/NAb assays developed across various serotypes, species, and sites.

METHODS

TAb AAV Bridging ELISA

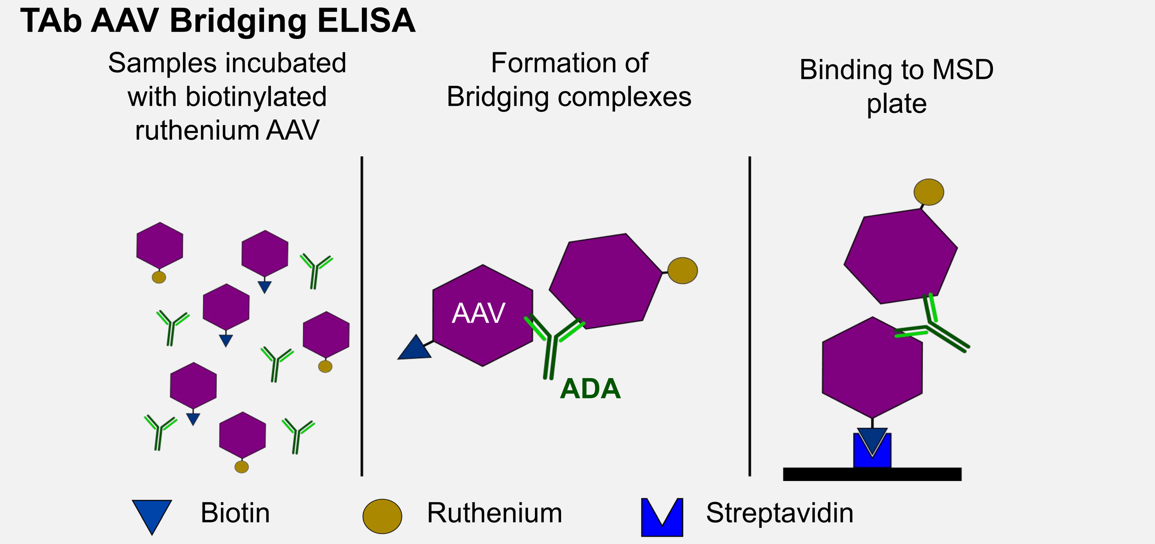

Figure 1: Incubation of the samples with AAV labeled to either biotin or ruthenium leads to the formation of bridging complexes. These complexes are captured on a streptavidin-coated plate, resulting in electroluminescence upon addition of reading buffer.

NAb AAV Cell-Based Assay

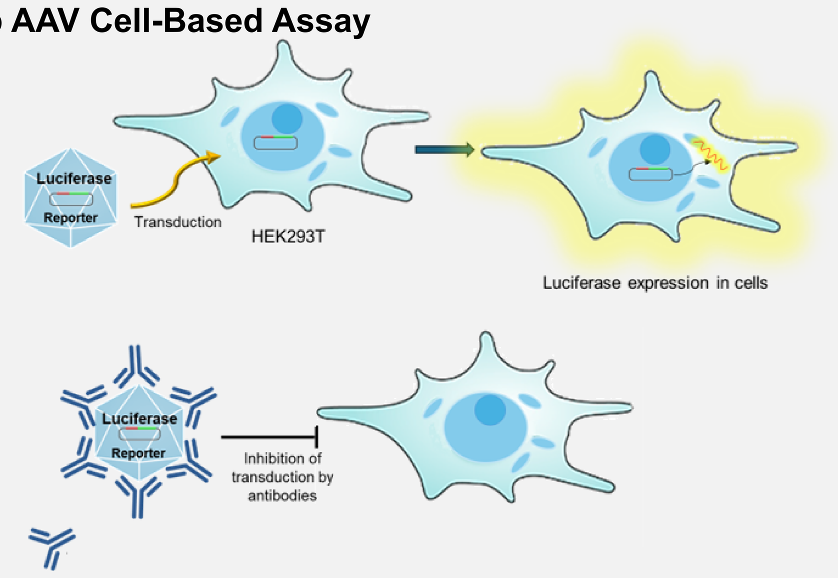

Figure 2: Viral transduction of HEK293T cells, in the absence of NAb, will result in bioluminescence detection. On the contrary, viral entry is inhibited in the presence of NAb, preventing luciferase expression, resulting in no bioluminescence signal.

RESULTS

Enhanced Cell Susceptibility Response Using Compound C

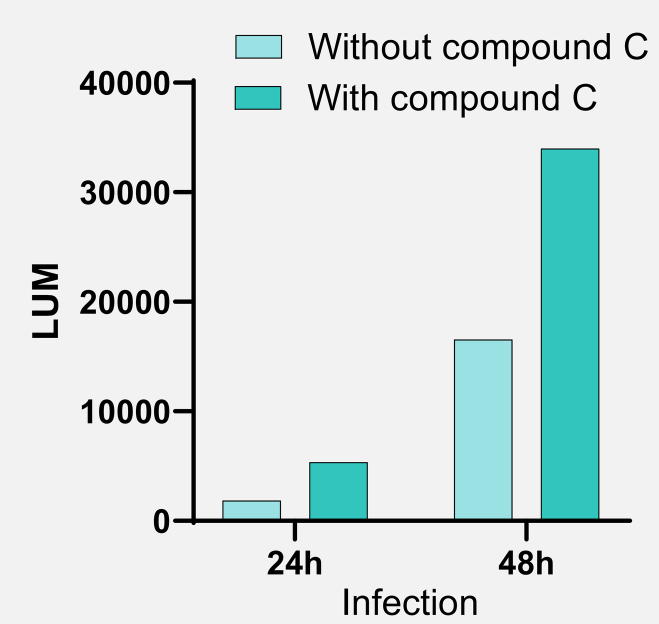

Figure 3: Cells seeded for 24h before transduction with an AAV8 vector, prior to transduction, with or without Compound C. Bioluminescence (LUM) was measured 24h and 48h after transduction. Similar results were obtained with AAV9.

Compound C increases bioluminescence as early as 24 hours post-transduction. The luminescence signal is higher at 48 hours compared to 24 hours, indicating a time-dependent effect. Notably, the increase is significantly more pronounced with Compound C than without. **These results demonstrate that Compound C significantly enhances the transduction efficiency of HEK293T cells as early as 24 hours post-transduction.**

Increase in Signal With No Seeding

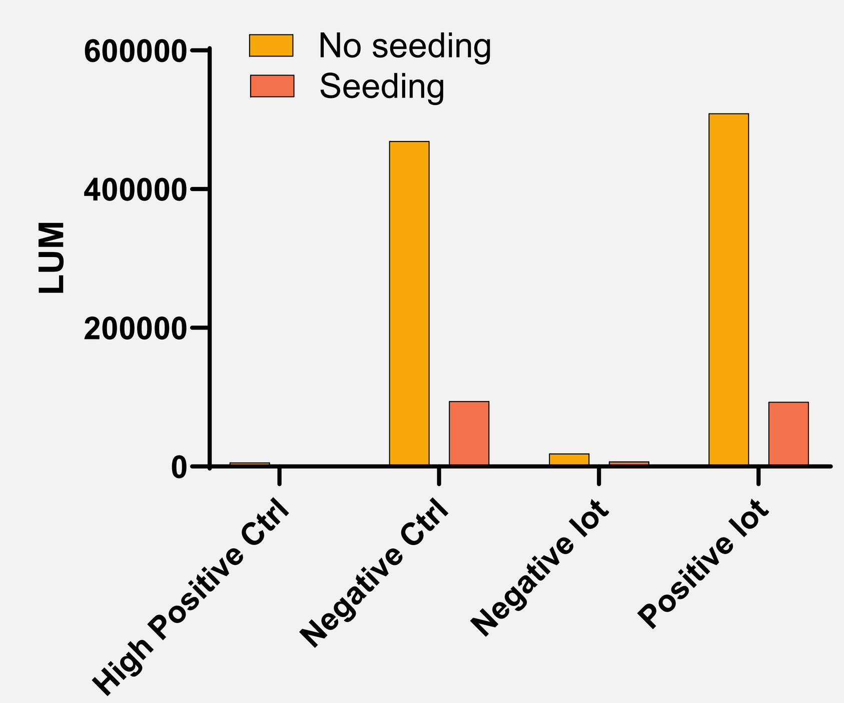

Figure 4: Cells were seeded or thawed, stimulated with Compound C prior to 22-24h of transduction with an AAV8 vector. Bioluminescence (LUM) was then measured. Similar results were obtained with AAV9.

Negative Control was used as signal control, while High Positive Control was included to evaluate the entry signal inhibition. Nab positive and negative monkey serum lots to AAV8 were selected. Across both seeded and no seeded conditions, assays performed with direct transduction of cells ("No seeding") consistently resulted in higher overall bioluminescence signals compared to the "Seeding" conditions. **This suggests that the absence of a pre-seeding step may enhance viral uptake or luminescence expression efficiency.**

Increase in Signal in Transduction With Component C for Both AAV8 and AAV9

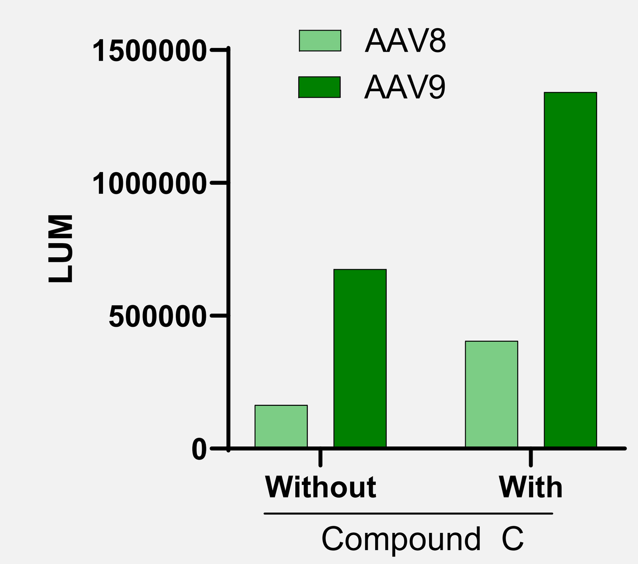

Figure 5: Cells were thawed and transduced 24h with AAV8 or AAV9 with or without co-incubation with compound C, prior to bioluminescence (LUM) measurement.

Without Compound C, cells infected with AAV9 exhibited higher bioluminescence than those infected with AAV8. Co-incubation with Compound C did not eliminate this difference but increased the bioluminescence signal for both serotypes. **These results demonstrate that Compound C enhances the transduction efficiency of HEK293T cells across multiple AAV serotypes with a similar fold increase.**

Key Parameters of the Tab and Nab Assays

	AAV8	
Key parameters	**TAb**	**NAb**
Sensitivity	1 ng/mL	30 ng/mL
MRD	1/50	1/32
Sample volume (screening)	20.0 µL	20.0 µL
Sample volume (Titration)	40.0 µL	40.0 µL
Precision of titer	Within 2-fold	Within 2-fold
Selectivity	Met acceptance criteria	Met acceptance criteria
Specificity		
Hook Effect		
Homogeneity		
Robustness		

Table 1: Key parameters for TAb and NAb methods.

AAV8 Immunogenicity Status Using the Tab and Nab Assays

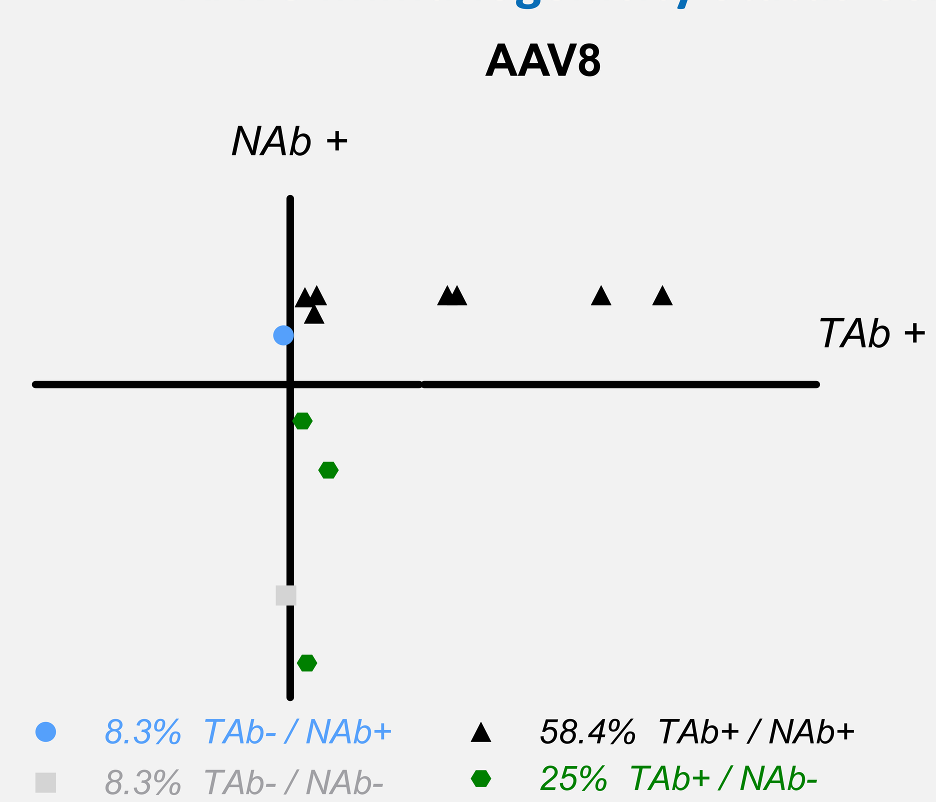

Figure 6: Screening status of AAV8 TAb and NAb in 12 NHP serum lots. Similar results were obtained with AAV9.

Results align with the published prevalence of AAV8 in NHP serum lots for Tab and Nab.

- 83% of lots were Tab-positive, as indicated by black triangles and green dots.
- 66% of lots contained Nab, represented by a black triangle and blue dots.
- 8% of lots demonstrated no detectable antibodies against AAV8.

In one sample, neutralizing activity was observed (blue dot) despite the absence of detectable Tab antibodies (Tab-), suggesting a potential non-antibody-dependent mechanism of action.

A similar pattern was observed with the AAV9 serotype. Higher prevalence in female lots was observed with the AAV9 serotype.

CONCLUSIONS

Prevalence of AAV8/AAV9 TAb and NAb in NHP serum is consistent with previous published findings.

Complementarity of TAb and NAb assays

The TAb and NAb assays offer complementary insights: The Tab assay provides a **broad overview of binding antibody responses,** while the Nab assay measures specifically **functional neutralization capacity.** Together, they enable a comprehensive evaluation of immunity.

Rapid Turn-Around Time

AAV Tab and Nab assays deliver results within 30 hours, streamlining the decision-making process with speed and efficiency.

NAb Transferability: Across Serotypes and Species

Nab assay parameters established to maintain high transduction efficiency across various **AAV serotypes and species.**

NAb Transferability: Across Sites

Successful validation across different sites supports global standardization and application to study sample analysis. For example, the Anti-AAV8 NAb Predose sample screened in two studies showed prevalence rates of 75% (185 animals) and 87% (120 animals).

Broad Application

Together, these features highlight the assay's **reliability, flexibility, and readiness** for broad expansion in gene therapy development.

Building on its cross-species and serotype versatility, current efforts are directed toward enhancing assay performance in human serum to enable broader clinical application.

FUNDING / GRANTS / ENCORE / REFERENCE OR OTHER USE

ALTASCIENCES