REACTION BIOLOGY

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Establishment of a fast assay for identifying neutralizing antibodies in primate sera to support viral gene therapy stratification

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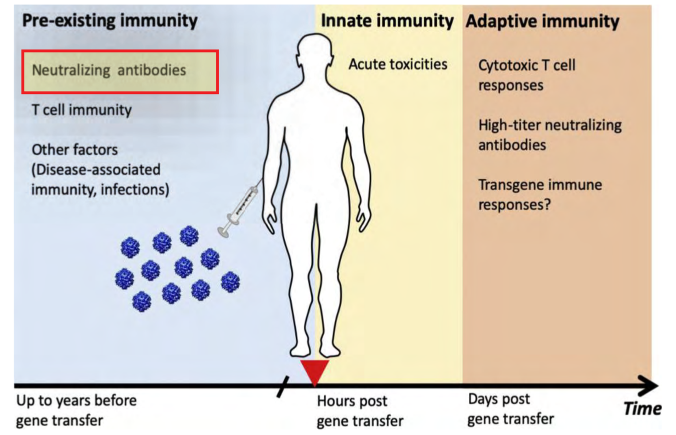
Procedure of analysis of sera for anti AAV antibodies

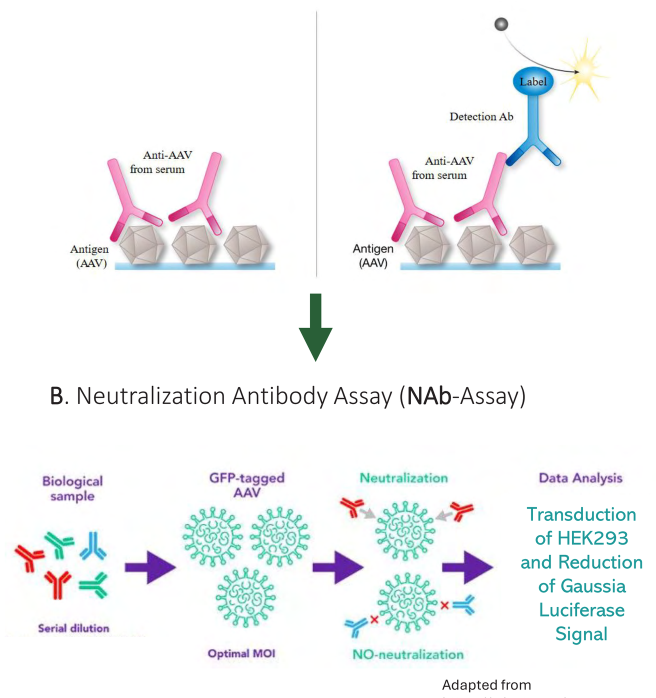
Gene therapy using viral vectors is gaining traction as a promising approach for treating a range of diseases, including cancer. A key determinant of therapeutic success is the absence of pre-existing neutralizing antibodies against the viral serotype, which may have developed through prior natural infections. Such antibodies pose a risk of inactivating the therapeutic virus, thereby compromising efficacy. This concern is particularly pronounced in treatments involving adeno-associated viruses (AAVs), as immunity to various AAV serotypes is widespread.

In this study, we present the development and validation of a simple and robust assay to detect functional neutralizing antibodies in primate serum. The method utilizes AAVs of distinct serotypes to transduce a human cell line with a readily quantifiable reporter gene. This assay enables effective stratification of primates and patients based on the presence of neutralizing antibodies prior to initiating AAV-based therapy. Furthermore, the approach holds potential for broader application in other viral therapeutic platforms.



Adapted from Verdera et al. Molecular Therapy. 2020; 28 (3):723-746

Fig 1: Host immune response against AAV vectors
Prior to vector administration, humans are exposed to wild-type AAV and therefore can develop humoral immunity to the vector. Exposure to wild-type AAV can occur years prior to gene transfer, and together with host-specific factors can determine the overall immunological context of AAV vector delivery.



Adapted from https://virtaat.com/

Fig 2: Serum test procedure to stratify patients for AAV therapy.
A: First, sera are tested for anti-AAV antibodies in a binding assay (BAb) using MSD-ELISA: AAV (of the different serotypes) are coated and incubated with test sera to detect potential binders with an indicator-labeled anti-human-IgG-antibody
B: To exclude to miss adverse antibodies, that may not be detected in the BAb assay, the neutralizing activity of sera is analyzed in a functional NAb assay. Luciferase coding AAV (of different serotypes) are incubated with test sera to allow interaction with potential binders in solution. Use mixture to transduce HEK293 cells and look for reduced transgene expression.

1. Establishment of Antibody binding (BAb)-Assay

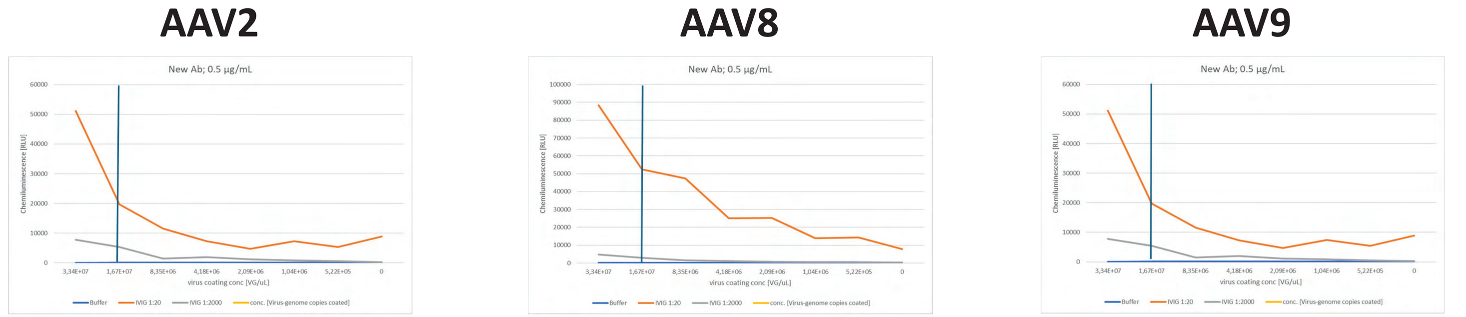


Fig 3: BAb assay establishment The BAb binding assay was established by coating increasing concentrations of AAV-serotypes, which are detected +/- two concentrations of IVIG "AAV-Abs"-positive control, finally detected by anti-human IgG antibody in MSD. The green line indicates the chosen AAV coating concentration for antibody detection.

2. Sample BAb screen

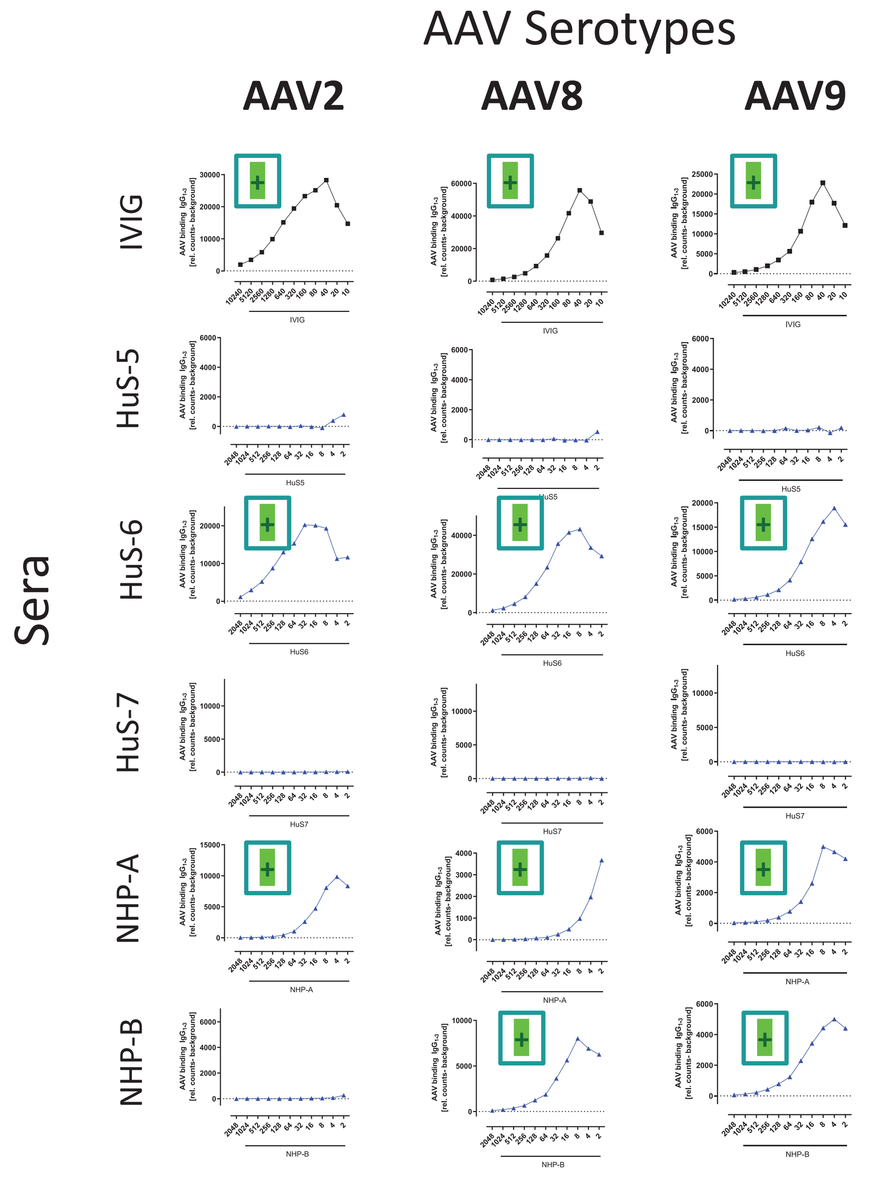


Fig 4: BAb screening reveals differential detection pattern of anti-AAV antibodies in primate serum samples
3 human sera (HuS), 2 non human primate sera (NHP) and 1 human positive control (IVIG) were tested in different concentrations for their ability to detect coated AAV of indicated sero-type as established in panel 1.
Undesirable AAV-ab-positive primate samples detect the given AAV serotype, and result in a <u>high</u> MSD signal with increasing concentration to the right side of the x-Axis (i.e. less diluted), These are indicated with a +.
Only BAb negative sera would next be tested in the NAb assay for confirmation of negativity.
For validation here we test all samples.

3. Establishment of Antibody-Neutralization (NAb)-Assay

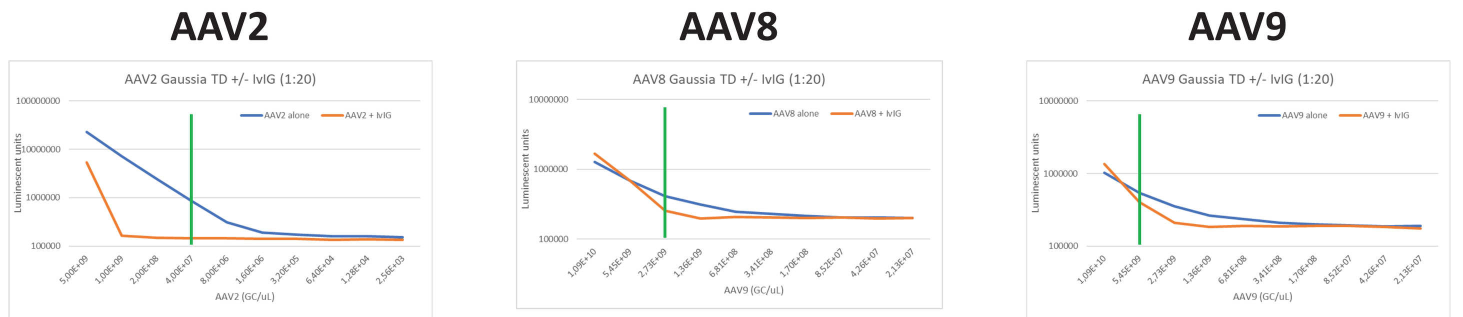


Fig 5: NAb assay establishment The NAb neutralization assay was established by preincubating different concentrations of luciferase-coding AAV-serotypes with a high concentration of IVIG "AAV-Abs"-positive control, before transducing target cells. The green line indicates the chosen AAV concentration to detect neutralizing AAV Ab in serum.

4. Sample NAb screen

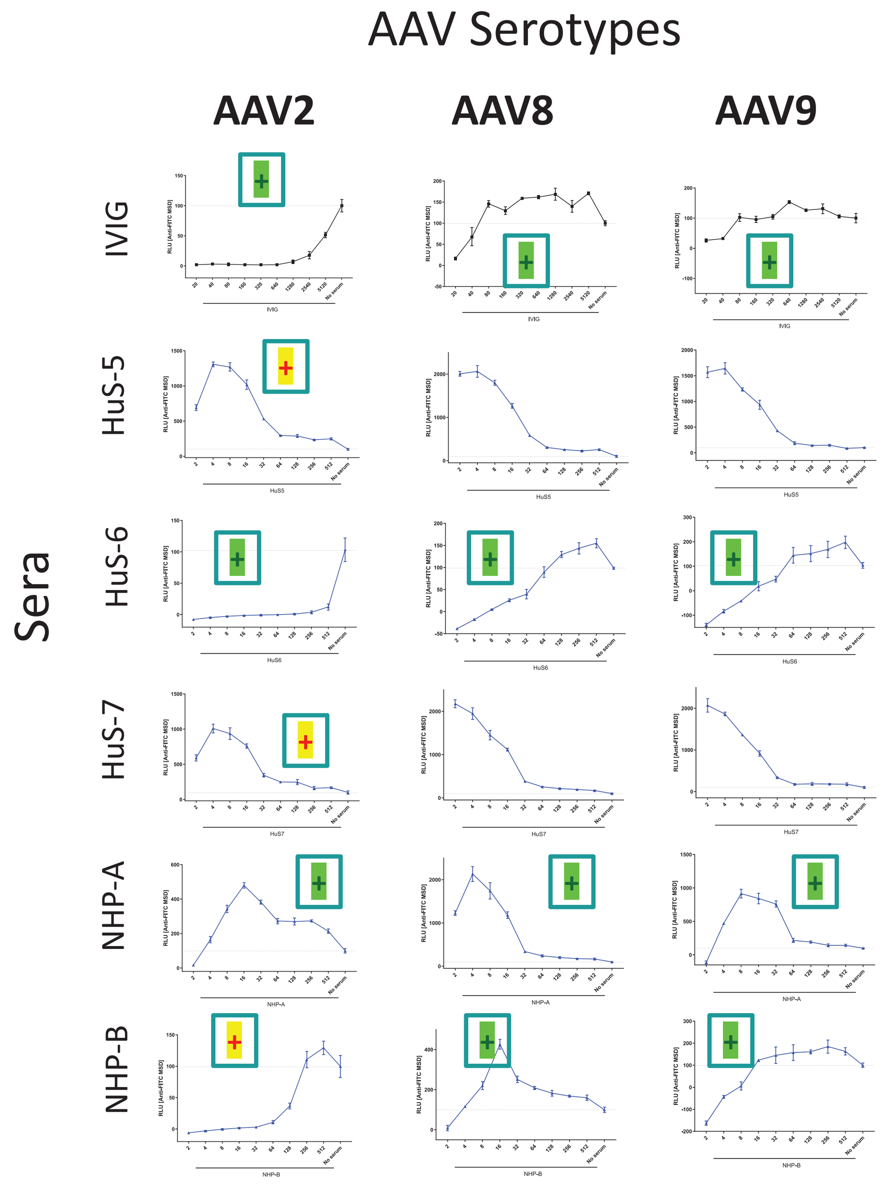


Fig 6: NAb screening identifies further anti-AAV antibody positive samples
All samples tested in panel 2 were tested here in different concentrations (descending from left to right) for their ability to neutralize the transducing activity of AAV as established in panel 3.
Undesirable AAV-Ab-positive primate samples neutralize the given AAV serotype, resulting in a <u>reduced</u> luminescence signal in the highest concentration. Interpretation of results is difficult, as serum components knowingly cause increase of the transduction signal. Thus, all samples that show a decline of the transduction signal at the highest sample concentration would be considered positive. Samples additional positive to the BAb assay are indicated with a +.

Material and Methods

Material: Adeno-associated virus serotypes (AAV2, 8 and 9) genetically engineered to encode Gaussia Luciferase, were obtained from Vector Biolabs. Non-human plasma (NHP) was obtained Cynomolgus Mauritius provided by LabCorp. Human Serum (HuS) was obtained from healthy volunteers provided by Freiburg University Clinic. For antibody detection by MSD, a sulfo-tag labeled anti-human antibody from Mesoscale was used.
Method: For the Antibody binding assay (BAb), AAV were coated on a MSD plate, sera added and subsequently bound antibodies detected using a sulfo-tag-labeled anti-human antibody from Mesoscale.
For the Antibody neutralization assay (NAb), sera were preincubated with the AAV, and subsequently added to HEK293 cells seeded the day before. After incubation, supernatants were harvested and analyzed with Gaussia Luciferase Glow Assay (Pierce).

Conclusion

- We have sucessfully established two assays for the detection of anti-AAV antibodies in primate serum samples, applicable for stratification of individuals for AAV gene therapy
- The straight forward BAb assay is sensitive and identifies many of AAV-antibody positive samples.
- Although the NAb assay is disturbed by the transduction enhancing serum effect, it reveals additional individual that would not be suited for AAV gene therapy.

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