The Path to IDE/IVDR: Navigating the Evolving Regulatory Guidelines for Gene Therapies

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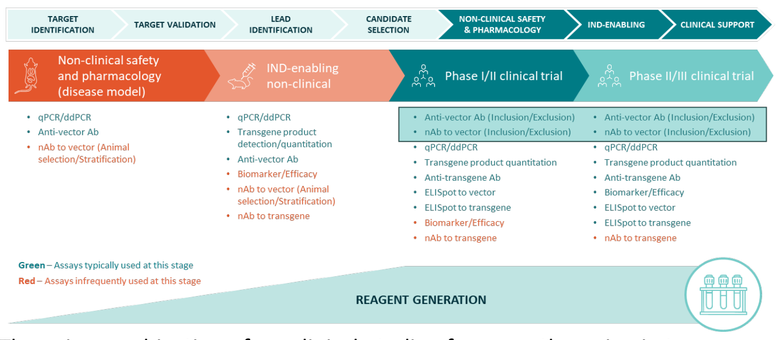
INTRODUCTION

Immunogenicity testing has become a key parameter of patient enrollment in Gene Therapy clinical trials. This is due to the potential for pre-existing immunogenicity to adeno-associated virus (AAV) vectors which are a leading platform for gene delivery. Pre-existing immunogenicity may inhibit the effectiveness of AAV-based gene therapies through immune clearance and neutralization of cell transduction functions. Therefore, many gene therapy clinical trials screen patients for the presence of pre-existing immunogenicity to AAVs prior to treatment with the intent of excluding patients with anti-AAV antibodies. Immunogenicity to AAVs can be determined through bioanalytical assays such as an anti-AAV total antibody assay (TAb) or a functional cell-based assay for detecting neutralizing antibodies (NAb) to the AAV serotype. The regulatory expectations for these assays have been rapidly evolving; current expectations are that assays used for inclusion/exclusion of patients from enrollment in clinical trials fall into the IVD companion diagnostic category where the assay is contemporaneously developed with the therapeutic drug for marketing approval. Since the result from these assays is used to enroll patients and the assays have not already received marketing authorization for that specific intended use, the IVD use in that context becomes subject to requirements of the Investigational Device Exemption (IDE) regulation for U.S.-based clinical trials and falls in scope for the IVDR guidelines for EU-based clinical trials.

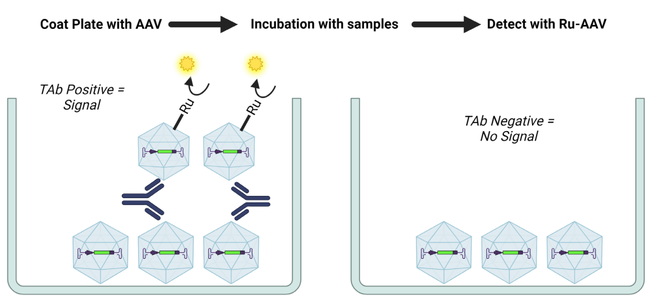
AAV Gene Therapy Assay Strategy



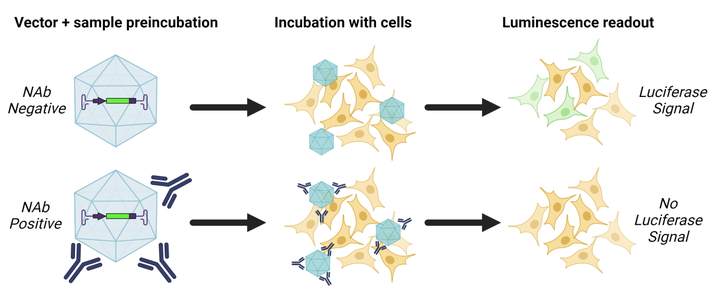
The primary objective of preclinical studies for gene therapies is to measure the biodistribution and shedding of the vector, expression of the transgene protein, and potentially using immunogenicity screening such as the NAb test to screen animals onto study. Once in the IND-enabling and clinical space, immunogenicity testing against both the vector and transgene product becomes increasingly critical part of the bioanalytical strategy.

METHODS FOR TESTING IMMUNOGENICITY TO AAV

A. Total Antibody Assay (TAb/BAb)



B. Neutralizing Antibody Assay (NAb)



C. Pre-Existing Immunogenicity to AAV Serotypes

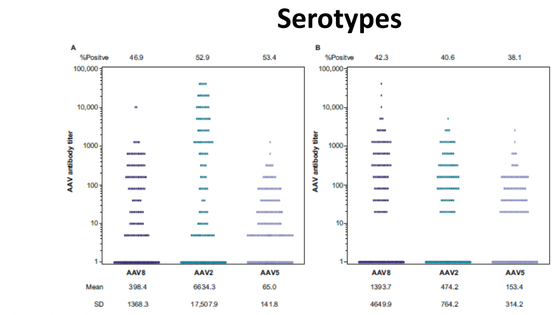


Fig. 3 NAb and BAb titers at baseline. A NAb titers and **B** BAb titers in enrolled participants at baseline. An NAb value of 163,840 for one participant (AAV2) has been removed because it was too high and skewed the output.

A. The total antibody assay format measures antibodies that bind AAV (Bab) by using the AAV capsid to bridge the antibody. In presence of TAbs, a positive signal is measured. B. The neutralizing antibody (NAb) assay format for AAVs uses a transduction inhibition cell-based assay where the AAV capside is added to cells with a luciferase reporter. In the absence of NAbs, the AAV is internalized by the cell activating the luciferase report, which is read out as a positive signal. In presence of NAbs, the AAV is not internalized, resulting in a decreased luciferase signal. The NAb assay detects only functionally active antibodies and results may be more predictive of impact on efficacy. Cell-based NAb assays may be more variable than plate-based TAb assays, and can also detect non-antibody transduction inhibitors in addition to NAb. C. Both of these assay formats have been used to measure pre-existing immunogenicity to AAV serotypes, which are common and exist at varying titers.

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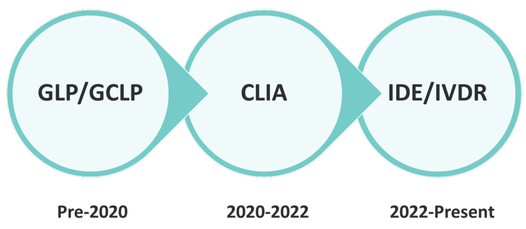
REGULATORY EXPECTATIONS OF INCLUSION/EXCLUSION CRITERIA FOR GENE THERAPY



As the number of gene therapy candidates have advanced through the clinic and undergone review by health authorities, the regulatory expectations around immunogenicity assays being used for inclusion/exclusion have evolved. Pre-2020, it was acceptable to use GCLP validated assays for inclusion/exclusion of patients. However, there was a shift toward using a CLIA-validated assay in 2020, which further evolved into the current expectation of using an IDE/IVDR validated assay. I

IDE- Investigational Device Exemption (US)

- **Essentially treats an assay as if it is a medical device**
  - Required when Study Risk Determination from the FDA deems the assay to present a significant risk
  - Q-Sub or Pre-Sub may be submitted to receive CDRH feedback on proposed approach
- **Strategies pursued with assays at BioAgilytix:**
  - Full CLSI validation (details proposed in Pre-Sub) prior to IDE
  - IDE application with GCP/GCLP validation report alone
  - CDRH responses: unconditional approval, conditional approval with additional validation, requirement for full CLSI validation
  - Remove assay result from enrollment criteria and change assay to exploratory endpoint

IVDR – In vitro Diagnostic Regulation (EU)

- **Essentially treats an assay as if it is a medical device**
  - Requires CE marker for assay testing samples from EU in the US
- **Member nation-specific regulations apply as well as general EU**
  - Germany seems to be the strictest jurisdiction
- **Strategy pursued with assays at BioAgilytix:**
  - In-house exemption allows laboratory based in EU to use non-CE marked assay, if validated to CLSI standards
  - ISO 15189 accreditation still needed
  - BioAgilytix Hamburg lab is validating multiple NAb and TAb assays (originally validated in BAL's Durham lab) for use under ISO15189 and in-house exemption

	GCP/GCLP	CDx
	Immunogenicity (FDA CBER/CDER, 2019; EMA)	**CLSI (Clinical Laboratory Standards Institute) (FDA CDRH refers to this)**
Specificity by Analyte Depletion	Confirmatory assay for TAb	Immunodepletion with Protein A/G/L- or AAV-coupled beads
Inter- and Intra-assay Precision	20% CV (higher for cell-based) Controls tuned to cut point Low positive control near cut point (1% failure rate)	15% CV (higher may be acceptable at agency discretion) Same plate map as to be used in sample analysis Controls ~25% above and ~25% below clinical cut-off Clinical cut-off vs cut point needs to be very clear If clinical cut-off changes, precision needs to be re-validated at new cut-off
Sensitivity	Needs mass/volume concentration	Mass/volume concentration not needed
Stability	Samples only Freeze-thaw, short-term (limited LTS for CLIA) Two time/treatment points sufficient	Samples and critical reagents Freeze-thaw, short-term, long-term, in-use, transportation Sufficient time/treatment points to allow detection of drift
Interfering Substances	Hemolysis and lipemia	Panels of endogenous interferents (particularly rheumatoid factor, cholesterol, triglycerides, hemoglobin) plus OTC and concomitant medications, assessed at clinical cut-off Minimum ~12 interferents
Matrix	Matrix as close as possible to samples	Matrix as close as possible to samples
Surrogate Positive Control	Purified with defined concentration Can be (and usually is) animal-derived	Purified/defined concentration not required (native serum preferred) Must only be human-derived
Critical Reagents	One lot is sufficient No requirements for regulated manufacturing	Multiple lots recommended Labeling requirements Manufacturing controls may be needed

CONCLUSION

Regulation of custom assays used for clinical trial enrollment has increased with reclassification as investigational devices. Assay validations originally intended for use in clinical trials may be leveraged for achieving investigational device status. Additional validation for device status includes the use of fully human samples tuned to the clinical assay cut-off, testing interfering substances, using same plate map as to be used in analyzing samples, and following CLSI guidances. Design control principles and processes are needed to govern the development, validation, and manufacturing of assays regarded as devices.