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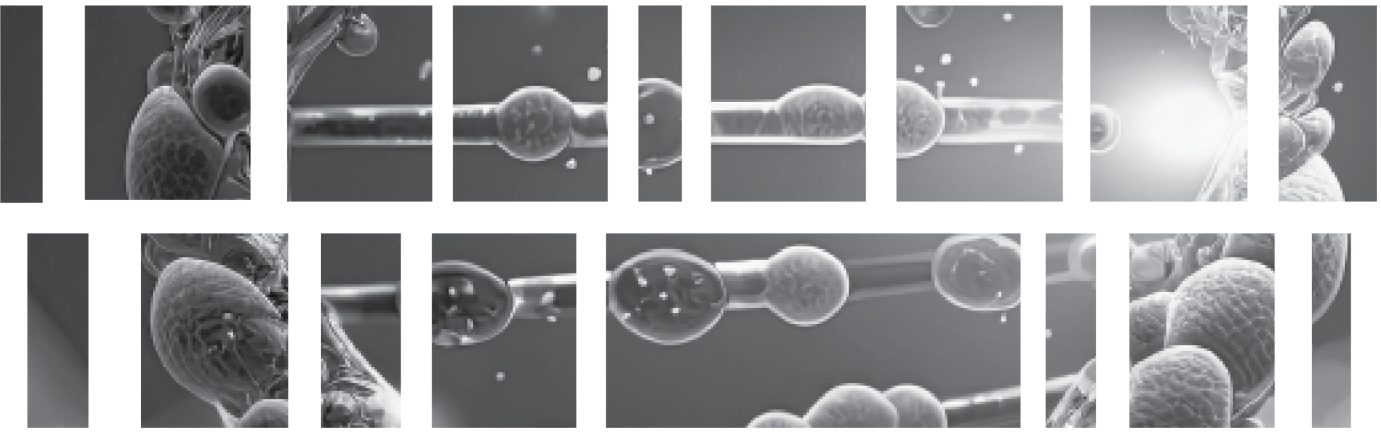

AN INDUSTRY BRIEF FROM INSTITUTE@PRECISION

Understanding Immunogenicity and AAV Gene Therapy

Best Practices for Designing Robust Assays and Navigating Regulatory Pathways

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Executive Summary

Adeno-associated virus (AAV) vectors are increasingly prominent in gene therapy, making immunogenicity assays key for assessing patient safety and therapeutic efficacy when used for patient selection. Developing immunogenicity assays within an appropriate regulatory framework and implementing design control within a robust quality system are essential for both compliance and efficiency.

Early-phase planning and non-human primate (NHP) studies provide valuable insights to support a smooth transition of the assays into clinical applications. Adhering to a high regulatory standard remains a best practice for AAV immunogenicity assay development for those assays used for patient selection.

In this white paper, we explore key considerations and best practices for developing immunogenicity assays from feasibility through commercialization.

Introduction

Gene therapy holds great promise as a game-changer for treating genetic disorders by addressing their root causes. Rather than merely alleviating symptoms, these advanced therapeutics have the potential to cure genetic disorders by delivering transgenes that can replace the function of defective genes.

In 2024, the US Food and Drug Administration (FDA) approved 8 novel cell and gene therapies (CGTs), along with at least 6 new indications for existing CGTs.[1] As of January 2025, the American Society of Gene and Cell Therapy (ASGCT) and Citeline estimate that more than 2,000 CGT therapies are in some stage of development globally, including approximately 938 gene therapies, 1,008 genetically modified cell therapies, and 902 non-genetically modified cell therapies.[2,3] Of these, 11 are in the preregistration phase and 35 are in Phase 3 trials, though this includes both cell and gene therapies. At the current pace, the FDA is on track to meet its projection of approving 10 to 20 CGTs per year.[3-5]

Gene therapy typically comprises a vector, a promoter, and a transgene, and may include other elements to:

- Enhance gene expression
- Prevent unwanted interactions with the host genome
- Increase the efficiency of gene delivery
- Maintain stability

Understanding immunogenicity and pre-existing immunity throughout the development continuum, beginning at the earliest stages, is critical to the success or failure of any gene therapy. A strong immune response to any component of these complex therapeutics may cause a safety issue including activation of the complement cascade, leading to potentially severe outcomes. Additionally, the efficacy of gene therapy may be affected by a pre-existing immune response. Identifying patients who are unlikely to benefit from the therapy is important not only from a physician's perspective but also from a payer's perspective, given the high cost of gene therapies.

For monogenic diseases, AAV vectors are the leading platform for transgene delivery. More than 50% of the general population has some degree of pre-existing immunity to one or more serotypes of AAV; thus, the assessment of pre-existing anti-AAV antibodies is a significant consideration in the development of any systemically administered gene therapy.[6]

Assessing pre-existing immunity can help mitigate the risk and expense of treating patients who may face treatment complications or suboptimal outcomes. This assessment should begin in the preclinical stage and continue throughout the clinical stages of development. Immunogenicity assays are commonly used to determine patient eligibility for treatment in clinical trials, which entails a higher regulatory burden for assay validation and performance. When used for patient selection, these assays may ultimately transition to companion diagnostics (CDx), although the regulatory environment is evolving. For instance, in March 2025, the US District Court for the Eastern District of Texas vacated the FDA's Final Rule on Laboratory Developed Tests (LDTs), ruling that the FDA lacks statutory authority to regulate LDTs under the Federal Food, Drug, and Cosmetic

medical devices, and therefore fall under the jurisdiction of the Centers for Medicare & Medicaid Services (CMS) via the Clinical Laboratory Improvement Amendments (CLIA) framework. The FDA chose not to appeal this decision, effectively halting its efforts to regulate LDTs as devices.[7]

There is also potential for varying regulatory classifications and requirements across countries. These assays could be regulated either as CDx or as traditional in vitro diagnostics (IVDs). Given the cost, time, and strategic planning involved in scaling up to a commercially available CDx, understanding the regulatory pathways and requirements at each phase of development is critical. The key is to ensure that the right assay is ready at the right time at every stage of development.

Whether or not a CDx is ultimately required, designing and validating immunogenicity assays within a regulated framework is recommended for streamlining development, reducing delays, and managing risk.

Developing AAV Immunogenicity Assays in a Regulated Context

All clinical investigations of devices or assays are covered under the Investigational Device Exemption (IDE) regulation (21 CFR Part 812) and must have an approved IDE, an abbreviated IDE, or be exempt from the IDE regulation. The level of regulatory control depends on the risk profile of the assay's use in the clinical trial, based on its intended use. If the use of the assay as part of the clinical trial is considered significant risk, then an IDE application will be required.

The approved IDE allows a medical device that has not yet received marketing clearance or approval to be shipped for use in a clinical study without complying with other regulations of the Federal Food, Drug, and Cosmetic Act. Sponsors of approved IDEs are also exempt from the Quality System (QS) Regulation, except for the requirements for design controls (21 CFR 820.30).[8]

Any laboratory test used in an investigational study to establish its clinical utility in a specific patient population is considered a clinical trial assay (CTA). CTAs that evaluate primary or secondary endpoints are subject to regulatory oversight. Immunogenicity assays used to determine treatment eligibility for gene therapy, such as a "screening assay," will also be regulated. However, the actual requirements will depend on whether the use of the assay as part of the clinical protocol is assessed as significant risk or non-significant risk.

Taking a regulated approach to immunogenicity assay development ensures that assays are fit-for purpose and adhere to standards for quality, reliability, accuracy, reproducibility, and robustness. Immunogenicity assays developed in a regulated framework—even without a CDx designation— encompass the following characteristics:

- Adherence to design control principles developed under formal Quality Management Systems including FDA 21 CFR Part 820 and ISO 13485
- Compliance with regulatory guidance and guidelines for analytical and clinical validation criteria
- Clear documentation of assay development, validation protocols and reports, performance metrics, reagent sourcing, and stability

While not all regulated assays become CDx, if they are developed under design control and rigorously validated, they can transition seamlessly if clinical data or regulatory requirements necessitate future CDx requirements.

Methods for Detecting Antibodies

The most common immunogenicity assays are total antibody (TAb) and neutralizing antibody (NAb) assays (see Table 1). TAb assays are designed to detect antibodies that bind to the viral capsid, while NAb assays are designed to determine whether there are antibodies present in the sample that neutralize the ability of an AAV vector to transduce cells.

Table 1. Comparison of TAb and NAb assays

	Total Binding Antibody (TAb)	Neutralizing Antibody (NAb)
Assay Type	Binding	Functional
Format	Immunoassay	Cell-based
Throughput	1-day assay, many samples per plate	Modern, human-readable formats (JSON, XML).
Sensitivity	+++	+/++
Selectivity (Interference)	Little to moderate	Moderate to significant
Precision	Low %CV	High %CV
Antigen	Drug or empty capsid	Reporter vector (eg, luciferase)*
Positive Control	May be commercially sourced (monoclonal or polyclonal)	

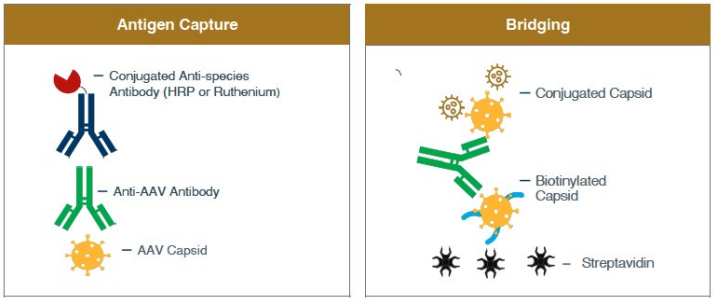
*Ideally made in the same expression system as the drug product.

TAb assays

TAb immunogenicity tests are simpler to design, develop, and implement as they are standard enzyme-linked immunosorbent assays (ELISAs) for detecting binding. Multiple samples can be placed on a plate and the assay can be completed in hours using technology that is available in most labs. The 2 most common formats for TAb assays are:

1. **Antigen capture,** where the AAV capsid is bound to a plate to capture any anti-AAV antibodies present in a specimen and a conjugated anti-species antibody is used to detect that binding.
2. **Bridging,** where a biotinylated AAV capsid is bound to a streptavidin-coated plate. Human serum is applied to the plate and, if anti-AAV antibody is present, it will bind to the capsid and can be detected with a conjugated capsid.

Figure 1: TAb assay formats



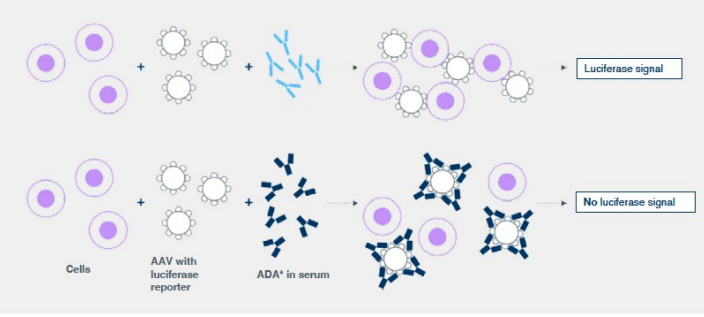
NAb assays

Anti-AAV NAb assays are functional, cell-based tests that require a reporter vector such as a luciferase construct. Because these assays involve both a cell line and a live reporter virus, they are subject to biological variation. Consequently, NAb assays are more complicated than TAb assays in terms of both development and clinical testing.

The most common NAb assay format involves incubating cells with the luciferase reporter vector and serum overnight. Presence of a luciferase signal indicates that the reporter gene has been delivered into the cells and no NAbs are present. Conversely, absence/reduction of a luciferase signal indicates the presence of NAbs.

Figure 2. NAb assay formats

*Anti-drug antibody



Selecting an immunogenicity assay format

The FDA has released guidance on Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection but has not provided specific guidance on how to select the appropriate immunogenicity assay for gene therapy studies.8 Deciding which type of immunogenicity assay to use can be challenging. A subset of binding antibodies will also neutralize, so TAb and NAb assays are not mutually exclusive. To further complicate the decision, there are also reports in the literature of non-antibody factors inhibiting transduction, therefore, some refer to NAb assays as transduction inhibition assays.

Though the "ideal" assay continues to be a subject of debate, key factors to consider when selecting an immunogenicity assay include the following:

Technical considerations

- Assay throughput. TAb assays are 1-day assays that can accommodate many samples per plate. NAb assays are typically multi-day assays that require more replicates and controls, thus allowing fewer samples per plate
- **Sensitivity.** TAb assays are generally more sensitive, though reagents, assay design, and platform all impact sensitivity. Assay sensitivity will determine seroprevalence, so, in any given population, differing assays—even those of the same type—can give markedly different seroprevalence results depending on underlying assay sensitivity
- **Selectivity.** As cell-based assays, NAb assays will be more prone to interference by endogenous factors such as hemoglobin, lipids, and certain medications than TAb assays
- **Reagent requirements.** TAb assays often utilize an empty capsid or the drug substance, while NAb assays require a relevant cell line and a reporter vector that involves a separate manufacturing process

Clinical considerations

- The type of assay used is less important than correlation with efficacy
- Existing animal or other preclinical data may provide insight on which type of assay is most relevant
- Depending on the application and relative sensitivity of the assay, a TAb assay may exclude more samples than a NAb assay, potentially limiting its clinical utility
- If feasible from time and cost perspectives, running both TAb and NAb assays in preclinical or early clinical studies may help clarify which assay is more clinically relevant

Only a subset of binding antibodies exert neutralization; thus, a TAb assay could potentially pick up antibodies that may not have a clinical effect. On the other hand, non-antibody factors may inhibit transduction, so a NAb assay may pick up artifacts that do not have clinical relevance. It is generally wise to bank extra, appropriately consented samples in case additional assays need to be run in subsequent studies. Discussions with regulatory agencies can help to clarify their requirements and expectations.

Best Practices for Assay Development and Validation

Planning early and with a long-term perspective is essential for ensuring that the assay is fit for its intended purpose at every stage of clinical investigation. However, any assay development strategy must be flexible, as changes to assay format, clinical cutoff, or even the level of validation required may arise as study data are generated and regulatory strategies evolve.

Early-Phase Considerations

NHP immunogenicity data are helpful for informing the design and optimization of immunogenicity assays intended for human gene therapy trials. NHP models offer a biologically relevant bridge due to their genetic, physiological, and immunological similarities to humans. Consequently, NHP studies help to predict the type, timing, magnitude, and persistence of potential immune responses, providing insights into assay sensitivity and

specificity parameters. These insights provide robust preclinical evidence of assay relevance and facilitate a seamless transition to the clinical phases of development, minimizing the risk of redevelopment costs and accelerating regulatory approvals.

While preclinical studies can be used to determine a technical cutoff that can be used for further testing, clinical trials are needed for identifying a clinical cutoff for pre-existing immunity that maximizes a gene therapy product's therapeutic potential.

Successful incorporation of immunogenicity assays into early phase studies for further development as a CTA or a marketed CDx requires meticulous planning and execution. Three key considerations for streamlining assay development are:

1. Determine an appropriate assay format

The presence of pre-existing immunity to the vector can be detected using either a TAb or NAb assay. Both types of assays can be designed as either qualitative or semi-quantitative, and each approach has its pros and cons (see Figure 3).

With a qualitative assay, the result is either a positive or negative Planning early and with a long-term perspective is essential for ensuring that the assay is fit for its intended purpose at every stage of clinical investigation. However, any assay development strategy must be flexible, as changes to assay format, clinical cutoff, or even the level of validation required may arise as study data are generated and regulatory strategies evolve.

Figure 3. Qualitative vs semi-quantitative NAb assays

	Qualitative Pos/Neg response based on signal relative to background	**Semiquantitative** Titer value
PROS	• Not necessary to titer samples • Can fit many samples per plate	• Correlation between titer and efficacy • Can set data-driven cut-off for enrollment
CONS	• No opportunity to correlate titer to efficacy or adverse events • Performance verification challenges when the cut-off is limit of detection (LoD) • Hard to change cut-off if initially select LoD; fewest patients eligible	• Titration requires considerable space on plate so can only fit a few samples per plate • Performance verification challenges due to high%CV and panel members required within 20%of cut-off (above and below), moderate/high positive, and low negative

2. Allow time to complete prerequisite studies for assays where use is considered to involve significant risk

If a sponsor is pursuing marketing authorization as a CDx, the assay must be used in an investigational study to establish its clinical utility in a specific patient population. For each protocol, the sponsor must conduct a risk assessment for assay use, and a traditional IDE may be required. Often, gene therapy assays are used for inclusion and exclusion criteria, and this can be considered significant risk by the FDA. In such cases, an IDE must be granted by the FDA/ Center for Devices and Radiological Health (CDRH) prior to sample testing as part of a clinical trial, regardless of the trial phase.

If an IDE application is required due to a significant risk determination, analytical validation compliant with the CLIA regulations may not be sufficient to support IDE approval. The studies needed to support IDE approval for a gene therapy CTA typically require more samples or replicates, a more rigorous assessment of endogenous and exogenous interference, and evaluation of sample and control stability. Planning ahead is essential for conducting these studies, and careful preparation may allow some of the data from the IDE application to be repurposed for the final analytical data package submitted for premarket approval (PMA) or a Humanitarian Device Exemption (HDE).

3. Avoid screening in early-phase studies if possible

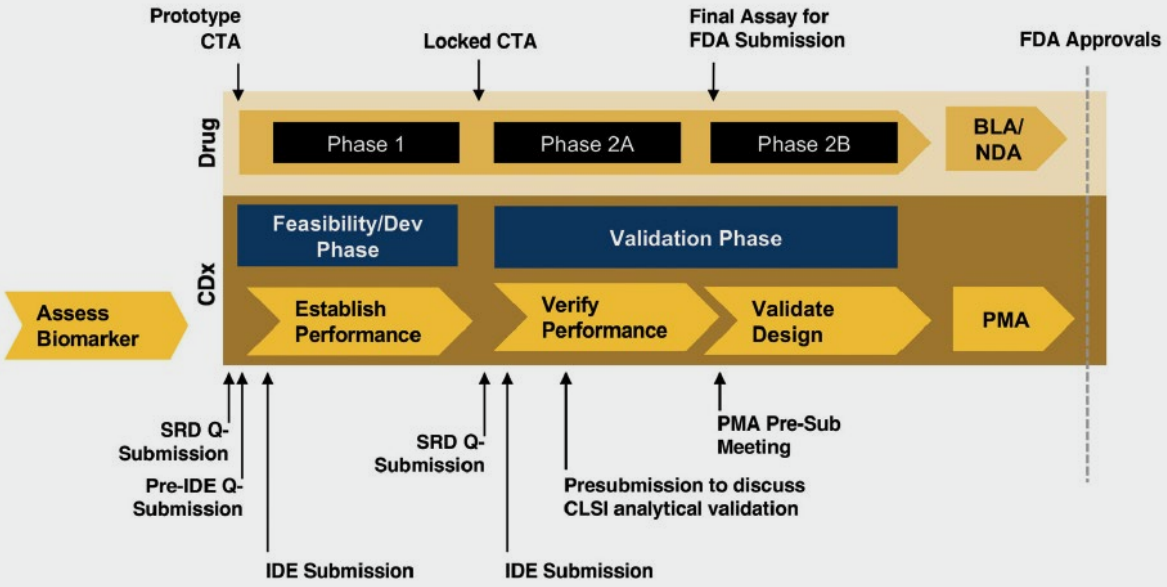
If possible, using an all-comers testing strategy would be preferred in Phase 1 to allow selection of a cutoff based on clinical efficacy. Further, this will allow the necessary time for regulatory submissions and communications to take place prior to Phase 2 or pivotal trials.

Using the CTA for inclusion/exclusion in Phase 1 studies significantly affects the timing for assay development and validation, as well as the associated regulatory submissions. Screening in early phase studies can delay the start of the trial

because the associated validation to support an IDE application must be conducted prior to the Phase 1 study if use of the assay is deemed a significant risk (see Figure 4). Without efficacy data to support selection of a clinical cutoff, it may be necessary to set the most conservative cutoff (the limit of detection), which may deprive therapy to individuals who could respond to the gene therapy despite low levels of antibodies.

Figure 4. Using a CTA for inclusion/exclusion in a Phase 1 study

CTA: Clinical Trial Assay; BLA: Biologics License Application; NDA: New Drug Application; PMA: Premarket Approval; SRD: Study risk determination; SRD Q: Special Review Device Question; Pre-IDE Q: Pre-Investigational Device Exemption Question CLSI: Clinical and Laboratory Standards Institute



If the CTA is to be used for screening, the sponsor may choose to participate in the Pre-IDE Q-submission process, which includes a 70-day review period by the FDA, plus an additional 5 days for a meeting. While Q-submissions are not generally required by the FDA, they offer sponsors an opportunity to communicate and collaborate with the agency to ensure that the appropriate data package is submitted. This is especially important for an IDE application, as the requirements or recommendations for the analytical validation data package to support IDE approval are not published.

Analytical Validation

Core parameters for analytical validation of immunogenicity assays include:

- Accuracy, which demonstrates assay performance against known reference standards or validated comparator assays
- Precision, which ensures consistency of assay results within and across runs, operators, reagent batches, and equipment
- Specificity, which confirms that the assay detects only the anti-AAV antibodies of interest without interference from related substances, matrix effects, or irrelevant antibodies
- Linearity and range, which demonstrate consistent, proportional responses across defined assay ranges
- Robustness, which confirms assay performance in response to minor variations in protocol, reagents, equipment, and environmental factors

Each of these parameters should be assessed against predefined acceptance criteria to minimize variability and provide confidence in consistent assay performance to support regulatory submissions.

Planning ahead for critical reagent requirements

Since it is expected that the assay will be used for many years, it is critical for gene therapy and diagnostic developers to ensure a reliable source of critical reagents, such as reporter vectors, cell lines, negative matrices, and positive controls.

- For a CTA not being used to select subjects for enrollment, a single reagent lot should be sufficient and good manufacturing practice (GMP) is not required
- For a CTA being used to select subjects for enrollment, multiple lots are generally required and, while GMP is not a mandate, having GMP material at this stage would be advantageous
- For an IVD study intended to support a PMA submission, at least 3 reagent lots are necessary to demonstrate lot-to-lot reproducibility and to conduct stability studies

While it is not required for the material to be produced under GMP conditions, rigorous documentation and testing are essential. Precision for Medicine recommends that manufacturing be performed by a facility that complies with a robust quality management system and has been audited by us. The material should also be accompanied by a certificate of analysis.

Clinical Validation

Clinical validation confirms that an assay is suitable for real-world, patient-derived samples by assessing its performance and suitability for clinical decision-making.

Selecting the clinical cutoff

Unlike an analytical cut point for an ADA assay, a clinical cutoff for an immunogenicity assay is a result that drives a medical decision. The cutoff for a qualitative assay is essentially the limit of detection. For a semi-quantitative assay, the cutoff should be a specific titer greater than the minimum required dilution. Further, the assay validation to support clinical trial use will involve a panel of samples, including samples that are within 20% above and below the clinical cutoff. For a clinical diagnostic, these samples must be human in origin. Generating these samples can be a significant challenge, particularly for cell-based NAb assays that often have ~20% variability. Thus, it is essential to have a robust release process when preparing and evaluating these samples prior to use in clinical validation studies.

Correlating immunogenicity with efficacy

Currently, immunogenicity assays are primarily used to exclude patients with detectable immunity to any component of an investigational gene therapy, minimizing the risk of adverse events and maximizing the likelihood of detecting a treatment effect. Going beyond safety to correlate pre existing immunity with efficacy of a gene therapy is more nuanced. Determining which level or type of immunogenicity is clinically relevant is challenging and requires clinical studies. To date, there is some—but limited—evidence that elevations in NAb to AAV are relevant to the efficacy of gene therapy. In an ideal world, the immunogenicity data being gathered now would inform the future of this space; however, there are few studies with systemic AAV gene therapy administration that are accepting all-comers into the clinical trial regardless of pre-existing immunity.

Regulatory Landscape and Compliance

The regulatory path from immunogenicity assay development to CDx approval is complex and requires multiple steps.

Investigational Device Exemption

In the US, there are 3 regulatory pathways available for CTAs:

1. **Significant risk,** which requires an IDE application approval prior to sample testing
2. **Non-significant risk,** which falls under abbreviated IDE regulations where an IDE application is not required, but other requirements such as adverse event reporting and record keeping must be met
3. **IDE exempt,** which is typically reserved for assays used for research or exploratory analysis

The requirement for an IDE for a CTA is determined based on how the CTA is used in the context of the clinical study, as well as the level of risk introduced by use of the CTA as part of the clinical trial (see Figure 5).

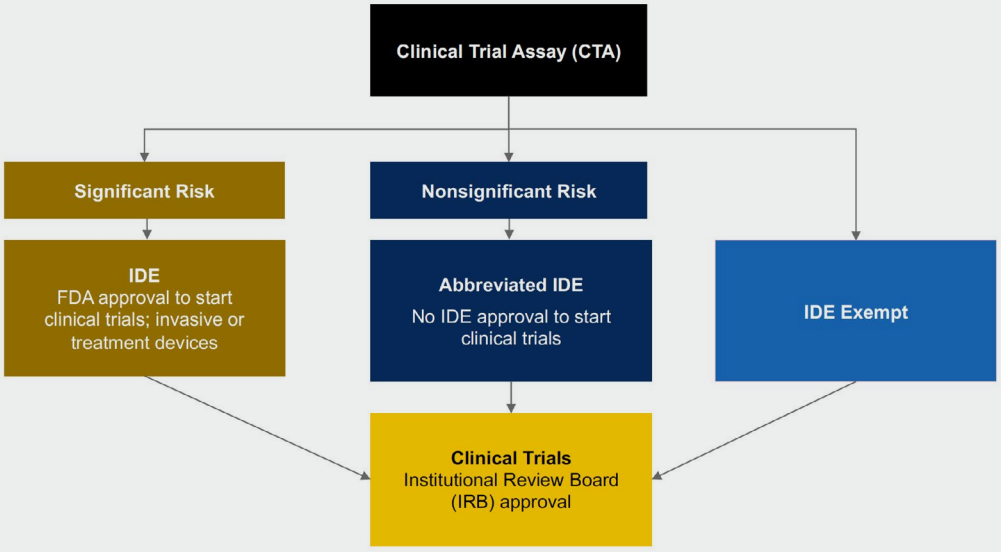


Figure 5. Risk assessment and regulatory requirement for a CTA

Key questions to help assess the level or risk presented by use of the device in the context of the clinical study include:

Study Considerations

- Are the test results used to determine whether the patient receives treatment?
- Would a false-positive result lead to the patient not receiving a known and effective therapy or standard of care (SOC)?
- How does the safety profile of the experimental therapeutic compare to the SOC?
- Have patients exhausted all SOC options?

Device Considerations

- Has the device been used in prior investigations with available safety data?
- Is the test being used prospectively or retrospectively?
- Which sort of sample is required for the test and does it involve an invasive procedure?
- If a fresh biopsy is required, is it a significant risk procedure, such as a biopsy of the brain, lung, or pancreas?

Content and timing of IDE submission

The content of an IDE, which will be required if the device is deemed significant risk, includes the following:

- Background information on the study treatment and disease state
- Summary of the investigational plan, including the informed consent forms and final clinical study protocol
- Information on prior studies involving the study treatment and diagnostic
- A description of the methods, facilities, and controls used for the assay
- Device description
- Validation studies, including concordance data

For developers with an assay that has not yet been validated or with questions about how to validate a CTA, it is highly recommended to do a pre-IDE Q-submission, which allows for feedback from the CDRH on a few specific topics prior to submitting an IDE. Ideally, the pre-IDE Q-submission would include draft protocols—or at least detailed summaries of the proposed study designs. CDRH can provide feedback either in written form or via teleconference as to the adequacy of the proposed study designs to support an IDE application approval.

SRD Q-Submission

If the CTA is used to determine who receives treatment—either via prospective stratification or prospective enrollment of only assay-positive patients—an SRD Q-submission can be submitted to the FDA to determine the risk associated with the use of the assay in the clinical trial.

Based on the feedback from the FDA, the assay will be deemed non-significant or significant risk which determines whether an IDE will be required (see Table 2).

Table 2. Summary of the SRD vs IDE for clinical trial assays

Submission Type	Purpose	General Content	FDA Review Time
SRD Q Submission	Ask FDA for a risk determination to assess whether IDE is required	• Background on study compound, disease state, etc • Final clinical study protocol • Informed consent form(s) (ICFs) • Risk assessment (answers to 4 questions)	90 days
IDE	Allow investigational device to be used in a clinical study	• Background information on the study compound, disease state, etc. • Device description • Supporting analytical validation • Investigational plan, study protocol, and ICF(s) • Information/report on prior studies involving the study compound and diagnostic • A description of the methods, facilities, and controls used for the assay	30 calendar days

An SRD can also be obtained by going directly to an IRB and providing a risk-benefit assessment justifying why an assay is a non-significant risk or inclusion of the risk determination in the pre-IND benefit assessment justifying why an assay is a non-significant risk. However, the FDA strongly briefing book. However, the FDA strongly recommends an SRD Q-submission since its thinking on

recommends an SRD Q-submission since its thinking on gene therapy assays has evolved significantly gene therapy assays has evolved significantly over the past few years and IRBs may not be aligned with that thinking.

Content and timing of SRD Q-submission

Compared to an IDE, an SRD Q-Submission is a streamlined submission that does not include the analytical validation data for the CTA. This approach can be favorable and preferred for the risk assessment if a strong argument can be made that use of the device in the context of the clinical study is low risk. The SRD submission includes:

- Background information on the study treatment and disease state
- Summary of the investigational plan, including the informed consent forms and final clinical study protocol
- Intended use for the CTA and a description of the device
- Risk assessment that answers 4 questions from FDA draft guidance demonstrating non-significant clinical study protocol risk

The FDA can take as many as 90 days to issue an SRD, so this review period should be built into the overall development timeline to avoid delays.

Premarket Approval

The FDA Guidance, Human Gene Therapy for Rare Diseases, acknowledged the risk associated with pre-existing antibodies and recommended that developers “strongly consider contemporaneous development of a [CDx] to detect antibodies” to the gene therapy product, even if the AAV has been modified to make it less immunogenic or if the serotype used is not commonly seen in humans because there is significant cross-reactivity across serotypes.9

Among the 6 AAV–based gene therapies currently on the market in the US, the FDA has required immunogenicity testing—whether an LDT or a CDx—as a condition to approval for the 5 most recently approved gene therapies. However, evolving regulatory guidance now allows greater flexibility and a CDx may not always be mandatory for regulatory approval.

The FDA considers CDx to be high-risk devices that usually require PMA. The modular PMA is preferred by the FDA and typically includes 4 modules: Analytical Validation, Software, Manufacturing and Quality, and Clinical Validation. It is

recommended that developers use the pre-submission program with CDRH to align on the table of contents for the modular PMA, content for each module, and review timelines prior to the submission of the first module.

For developers who are adding a CDx indication to an already approved PMA, the appropriate regulatory submission mechanism is a PMA Supplement (sPMA), which typically follows a 180-day review timeline. The sPMA is a streamlined submission focused on supporting the analytical and clinical validation for the CDx indication of interest.

Developing a Global CTA Regulatory Strategy

For CTAs that will be used in global clinical trials, start with an internal risk assessment of how the assay will be used as part of the study. Questions to think through in this risk assessment include:

- Is the assay being used prospectively, or retrospectively?
- How is the assay being used? Does the assay have a medical purpose? For exploratory endpoints, stratification, monitoring or inclusion/exclusion?
- Has the assay ever been used in prior investigations with safety data?
- Which type of sample is required and does it involve an invasive procedure?
- Are the test results used to determine whether the patient receives treatment? If so:
  - Would a false-positive result lead to a patient not receiving a known and effective therapy or SOC?
  - How does the safety profile of the experimental therapeutic compare to SOC?
  - Have patients exhausted all SOC options?

Risk will impact both regulatory requirements and the level of assay validation required.

EU regulatory framework for clinical trials

Keep in mind that the regulatory framework, submission requirements for enabling testing with a clinical trial assay, and review or approval timelines for these submissions vary by country between the US and the EU under the In Vitro Diagnostic Regulation (IVDR). It is important to recognize this distinction, as it will impact the regulatory strategy and roadmap for sponsors seeking therapeutic approval in both markets.

In the EU, a CTA is referred to as a performance study device and is considered to have a medical purpose for managing trial participants if it is used for inclusion or exclusion of subjects, treatment arm allocation, or monitoring the safety and efficacy of the treatment during the study. However, if the assay is used for patient stratification to ensure equal distribution of selected variables across treatment arms, or for exploratory endpoint analysis that typically does not impact the medical management of trial subjects, it would not be considered to have a medical purpose in the trial.

Under the IVDR, for a device performance study of a CDx, if the assay has a medical purpose in the clinical trial and its results impact the medical management of a patient, the sponsor must comply with IVDR Annex XIII, Annex XIV, and Article 58(2). If it is an in-house test developed and performed in an EU-based facility that qualifies as a health institution, it can be used if all requirements of Article 5(5) of the IVDR are met. In the EU, if a lab has been established as a health institution meeting the requirements of Article 5(5)(a) through (i), the test may be exempt from competent authority submissions in each EU country under the IVDR.

Challenges associated with gene therapy assays used for patient selection in clinical trials conducted in the EU include unclear assay validation requirements, the absence of a pre-submission process to clarify analytical validation expectations, and long review timelines for Annex XIV and ethics committee submissions. All of these factors should be considered in a global regulatory strategy. Therefore, internal timelines should include parallel workstreams for regulatory submissions for both the CTA and the therapeutic across different global regions.

Best practices for developing a global regulatory strategy

Validating an assay is expensive and time-consuming, so it is critical to plan early, to think through all the scenarios, and to talk to regulatory agencies about the strategy. Performing well-designed animal studies will help to inform human assays and saving samples from all stages and studies with the appropriate consent to allow additional testing can help to mitigate risk.

When developing a global regulatory strategy for a gene therapy immunogenicity assay:

- Consider how the assay is being used in the clinical study and, where feasible, consider an all comers trial which offers the potential to be relieved from additional regulatory burden
  - Identify the markets in which clinical studies will be conducted and evaluate the average CTA submission approval timelines for each country
  - Develop and analytically validate an assay from the start that would meet both US and EU requirements
- Tailor the level of validation to study phase and intended use
- Understand the exportation laws of human samples in the countries where the study will be conducted
- Plan ahead if the CTA will eventually need to become a CDx
- Interact with regulatory authorities early and often
- Begin development of the regulatory documentation required for performance study device in the EU as early as possible

Conclusion

Rigorous immunogenicity assay development that complies with regulatory requirements is critical, even if a full CDx pathway is not the ultimate goal. Incorporating regulatory input at the earliest stages of assay planning helps ensure that the assay is fit for its intended purpose, thereby streamlining development.

Synergy among sponsors, regulatory authorities, and clinical research organizations (CROs) is shaping the future of immunogenicity assay development, particularly regarding whether a CDx is required for gene therapy approval. Early investment by sponsors in robust assay validation can help demonstrate to regulators that safety and efficacy are adequately addressed without requiring a formal CDx, leading to greater regulatory flexibility.

Recently, regulatory authorities have adopted more flexible stances on CDx in recognition of the diversity and complexity of gene therapy approaches and the cost-benefit considerations associated with CDx development. CROs bridge the scientific and operational gaps between sponsors and regulators, leveraging their technical knowledge and regulatory experience to develop, validate, and standardize assays, and to facilitate informed dialogue. Through this collaboration, the necessity of a CDx can be evaluated on a case-by-case basis, aligning regulatory requirements more closely with scientific justification, clinical utility, and practical feasibility—ultimately accelerating patient access to innovative therapies.

Choosing an immunogenicity CTA and CDx partner early and wisely is critical. Ideally, the diagnostic partner should have the capability to support development from assay selection through commercialization, as switching labs midstream would require repeat assay development and performance validation. To succeed in a new, rapidly developing field involving complex assays, that partner must have deep laboratory experience in assay optimization and CLSI validation, as well as extensive regulatory and quality expertise—including relevant certifications such as CLIA, ISO 15189, ISO 13485, and 21 CFR Part 820—and an experienced IVD regulatory team to support all clinical trial-enabling submissions and registrations. It is also essential that they have the appropriate instrumentation, software, and infrastructure to support studies in all desired geographies.

Precision for Medicine provides global support for complex innovations through 6 specialty labs in North America and Europe, 12 sample processing labs, and more than 3,000 employees. Precision's IVD Regulatory team has supported more than 350 IVD and CDx regulatory filings in countries around the world. We have supported more than 15 AAV-focused gene therapy companies and their projects across AAV serotypes, assay types, and therapeutic areas, including rare diseases. Precision for Medicine also developed, validated, and serves as the sole site for the global LDT for HEMGENIX®.

> Learn more about how Precision for Medicine can help with compliant, future-ready strategies for immunogenicity assays in AAV gene therapy.

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