

# The Evolution of HLA Testing in Clinical Trial Design



Advances in molecular testing, next-generation sequencing (NGS), and informatics have expanded what human leukocyte antigen (HLA) data can reveal, transforming it from a technical requirement for clinical transplantation into a strategic input that increasingly shapes discovery, clinical trial design, regulatory strategy, and therapeutic development. With unique clinical, laboratory, and regulatory considerations, the question is no longer whether HLA testing matters in clinical trials, but how intentionally it is being integrated, and how prepared trial teams are for the complexity it introduces.

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## Uses and Application of HLA Testing

Each individual possesses a unique set of HLA, half inherited from each parent.<sup>1</sup> HLA typing remains essential before organ or hematopoietic cell transplantation (HCT) to assess compatibility between donor and recipient cells. Typing may also be used to identify markers for specific diseases. While this testing is primarily used for investigating immune compatibility in the clinical patient care setting, in research and trial applications, HLA testing is increasingly used as a strategic tool that can shape study design, patient selection, and data interpretation.

Testing Applications	
<b>Clinical Laboratory Diagnostics</b>	<b>Clinical Trial and Research Applications</b>
<ul style="list-style-type: none"><li>• Solid Organ and HCT Transplantation</li><li>• Post-Transplant Monitoring</li><li>• Disease Association &amp; Risk Stratification</li><li>• Pharmacogenomics/Drug Safety</li></ul>	<ul style="list-style-type: none"><li>• Patient Selection and Stratification</li><li>• Biomarker Development</li><li>• Immunogenicity and Safety Assessment</li><li>• Cellular Therapy Research</li><li>• Population Genetics and Equity Studies</li></ul>

## Technological Evolution

Initially, HLA testing relied on serologic methods. While foundational, these approaches were limited in resolution and sensitivity, leaving ambiguity in antigen identification and interpretation. The transition to molecular typing marked a turning point, dramatically improving allele-level discrimination and reducing uncertainty.

NGS accelerated this evolution further. High-resolution sequencing generates vastly richer datasets, revealing the extraordinary polymorphism of HLA genes and enabling a level of precision that was

previously unattainable. At the same time, practical innovations in sample collection and validation such as the move from fresh blood samples to cheek swabs have made testing more scalable and accessible, supporting broader participation and global reach.

The cumulative effect has been transformative: more data, easier logistics, and a foundation for precision medicine. But increased resolution has revealed a new challenge: interpretation.



## From Genotyping to Biological Meaning

The field has become adept at identifying HLA variants via emerging technology, but there continues to be a critical shift in focus. Classical HLA class I genes are polymorphic at nearly every amino acid position in the peptide binding region, and human populations actively maintain this diversity. Simply cataloging variants is no longer sufficient. Increasing attention is now focused on identifying the meaning of each polymorphism. The emerging question becomes functional: which differences matter biologically and clinically?

Answering this requires looking beyond genotype to factors such as expression levels, alternative splicing, and epistatic effects within haplotypes. It also requires abandoning binary thinking around matching. In hematopoietic stem cell transplantation, mismatched procedures have increased substantially reflecting a more nuanced understanding that not all mismatches carry equal risk. This evolution requires enhanced frameworks that can compare types and degrees of mismatch, rather than defaulting to just 'match' versus 'mismatch' as a proxy for risk.

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## Nuances in Antibody Testing

Advances in antibody testing have been equally consequential. Virtual crossmatching and solid-phase antibody assays are now highly predictive and central to transplant decision-making, yet antibody testing remains one of the most challenging areas to standardize and automate due to human interpretation of machine signals. In some cases, results vary based on reagents, assay conditions, and interpretation thresholds. As a result, expert human judgment remains indispensable.

While artificial intelligence holds promise for streamlining interpretation, meaningful automation will require systems capable of reflecting immunologic context—not just pattern recognition. Therefore, antibody interpretation sits at the intersection of data and expertise, reinforcing the need for skilled laboratories, expert guidance, and thoughtful integration into clinical trial workflows.

## Epitope and Eplet Considerations

As understanding deepens, the field is moving beyond allele-level comparisons toward epitope and eplet analysis. This shift highlights growing recognition that molecular differences are not equally immunogenic, and that chemically and structurally informed comparisons offer great predictive value. As datasets grow and correlations with outcomes strengthen, these approaches are expected to play a larger role—not only in clinical decision-making, but in how trials define eligibility, stratify risk, and interpret immune responses.

## HLA in the Modern Clinical Trial Landscape

Today, HLA testing is embedded across clinical trials in ways that extend far beyond donor–recipient matching. It is used for immune risk stratification, immunogenicity assessment, antibody monitoring, and safety signal interpretation, particularly in immune-modulating and HLA-directed therapies.

One of the most consequential shifts has been the evolution of HLA testing into a formal inclusion–exclusion biomarker in drug development. When therapies are HLA-directed, aligning biomarker strategy with the therapeutic or drug's mechanism of action best supports precision medicine diagnoses. As a result, testing must be integrated early to ensure appropriate patient selection, preserve efficacy signals, and reduce adverse outcomes.

Retrofitting HLA strategy mid-trial introduces scientific, operational, and regulatory risk. As a result, regulatory scrutiny has increased, and HLA tests that determine therapeutic eligibility often function as companion diagnostics—even when embedded within broader development programs.

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## Regulatory Considerations

Regulatory considerations for HLA testing differ primarily based on how the test is used within a study. In transplant studies where HLA testing supports donor-recipient matching or immune risk assessment, HLA testing is considered standard of care. Accordingly, assuming the assay itself is not the subject of investigation, the FDA allows the use of appropriately validated LDTs conducted in accredited laboratories.

By contrast, in HLA-directed therapy studies, HLA testing is typically functioning as a companion or complementary diagnostic where the test is used to support assessments of safety and efficacy of the corresponding therapeutic. In this scenario, the HLA assay is considered an investigational device and is subject to the FDA's investigational device exemption (IDE) requirements, which include the need to file an IDE application with the FDA and receive authorization prior to initiating use of the HLA test in the drug study. However, if the study sponsor can demonstrate the investigational study of the HLA test meets the criteria of a non-significant risk study, then the sponsor is not required to file an application with the FDA but must comply with the remainder of the IDE requirements. This is also known as an abbreviated IDE.

## Regional Regulatory Divergence

Regulatory expectations differ markedly by region. In the United States, under FDA framework, there is flexibility with how HLA testing is governed, depending on the intended use of the test. In transplant studies where HLA testing is typically used in line with standard of care and is not itself the subject of the study, the FDA permits the use of validated LDTs on the condition the test and the laboratory performing the test comply with CMS regulations. When HLA testing functions as a companion or complimentary diagnostic in an HLA-directed therapy trial, the test meets the definition of an IVD and is therefore subject to FDA regulations rather than CMS regulations.

In the European Union under In Vitro Diagnostic Regulation (IVDR), there exists increased complexity and operational burden. In the context of a clinical study, including transplant studies and HLA-directed therapy studies, the sponsor is expected to use a CE-marked HLA test for the intended use required for the study, if one already exists. Alternatively, the sponsor can use an "In-House" HLA test performed by Health Institute

residing in the EU, assuming the Health Institute and HLA test comply with the corresponding section of the IVDR reserved for these types of tests. If neither a CE-marked assay nor an In-House test is available for the required intended use, then the study sponsor will need to develop their own test and file a performance study application (similar to an IDE application) with the National Competent Authority in the member state where the study is being conducted. It is worth noting that the IVDR does not have an equivalent to FDA's non-significant study exemption. Rather, the IVDR requires performance studies for all Class C and Class D IVDs which includes HLA tests for donor matching purposes and HLA tests for companion diagnostic purposes.

<b>Planning for Regulatory Review</b>	
United States Regulatory Environment (FDA)	European Union (IVDR)
<b>Transplant Studies</b>	
<ul style="list-style-type: none"> <li>• HLA testing for donor matching is considered standard of care</li> <li>• Laboratory-Developed Tests (LDTs) commonly acceptable</li> <li>• IDE not required if the HLA test is used in line with standard of care</li> </ul>	<ul style="list-style-type: none"> <li>• Preference for CE-marked assays when available</li> <li>• Health institution exemptions available</li> <li>• Performance studies required for all investigational HLA tests used for donor matching</li> <li>• Drug and diagnostic approvals must proceed in parallel</li> </ul>
<b>HLA-Directed Therapy Studies</b>	
<ul style="list-style-type: none"> <li>• Functions like a CDx</li> <li>• IDE Framework applies</li> <li>• Formal IDE submission depends on risk determination</li> <li>• IDE applications and IND applications are filed in parallel with the different FDA centers</li> </ul>	<ul style="list-style-type: none"> <li>• HLA tests functioning as companion diagnostics require performance study</li> <li>• There is no pathway for performance study exemption</li> <li>• Performance study applications and CTA applications are filed in parallel with the National Competent Authority in the member state where the trial is taking place</li> </ul>

## The Next Frontier for HLA Testing

Looking ahead, HLA testing is moving beyond its historical role as a static laboratory measurement and becoming a core enabler of precision therapeutics and clinical trial design. This evolution is shaped as much by regulatory reality as by technology. In Europe, IVDR has introduced friction—particularly for HLA-directed therapies—where performance study requirements may delay trials and limit patient access. In response, sponsors are exploring hybrid approaches, enrolling patients through available health institution exemptions while regulatory approvals proceed in parallel. This highlights a broader need for regulatory frameworks that align more closely with the intent of modern precision medicine.

From a testing and technology standpoint, HLA genotyping is fundamentally a form of DNA sequencing. This raises an opportunity to define test classes or standing approvals for categories of HLA-directed therapies, rather than requiring repeated validations for similar diagnostic strategies across individual trials. Antibody testing remains a challenge, as immune profiling reflects a biological continuum, and current reliance on semi-quantitative thresholds can oversimplify complex immune realities. Moving away from rigid binary thresholds toward outcome-linked and standardized frameworks may better reflect immune risk and support more informed clinical decision-making.

## Designing with HLA in Mind

HLA testing has moved beyond its historical role as a technical prerequisite and is now a strategic consideration that influences clinical trial design, regulatory planning, and therapeutic success. Advances in molecular testing and informatics have expanded what HLA data can reveal, while growing use in patient selection and risk stratification has increased both its impact and its complexity. Designing with HLA in mind requires early, intentional integration across scientific, operational, and regulatory domains, ensuring that testing strategies align with study objectives and therapeutic mechanisms. As clinical trials continue to evolve toward more precise, immune-informed approaches, thoughtful HLA strategy will be essential to reducing risk, preserving interpretability, and enabling meaningful clinical outcomes.



This piece complements a *Precision Medicine Online Lunch and Learn* webinar hosted by **GenomeWeb** and sponsored by **Versiti Clinical Trial Services**. The full webinar recording is available via the QR code for readers seeking a deeper exploration of the discussion.

## References

1. Berger A. HLA typing. *BMJ*. 2001 Jan 27;322(7280):218. <https://pmc.ncbi.nlm.nih.gov/articles/PMC1119473/>