

Review

## Risk Assessment in Clinical Histocompatibility Testing: Challenges, Strategies, and Future Directions

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### Abstract

Though the role of clinical histocompatibility testing is fundamental to successful transplant, systematic risk assessment and mitigation strategies remain underutilised in this domain. This review addresses risk identification, analysis, and control measures across the pre-analytical, analytical, and post-analytical phases of clinical histocompatibility testing, in line with international standards such as ISO 15189:2022. A structured, risk-based quality management system (QMS) is imperative for ensuring accurate testing, timely reporting, and subsequently optimal transplant outcomes. Future directions include leveraging digital tools and fostering a culture of continuous improvement in histocompatibility laboratories.

### Keywords

Histocompatibility testing; risk assessment; RCA; QMS; ISO 15189



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## 1. Introduction

For successful transplant outcomes in both solid organ and hematopoietic stem cell transplantation (HSCT), clinical histocompatibility testing plays an important role. Histocompatibility testing includes tests like human leukocyte antigen (HLA) typing, anti-HLA antibody screening and identification, complement-dependent cytotoxicity (CDC), and flow cytometry cross-match (FCXM), which provide crucial immunological compatibility between recipients and prospective donors [1-3]. Hence, any incorrectness in these can have significant clinical consequences on the transplantation outcomes. While several processes exist to improve analytical accuracy, the systematic application of risk assessment, covering risk identification, analysis, evaluation, control, monitoring, and review, is still limited in clinical histocompatibility testing. Despite risk assessment being a fundamental component of routine clinical laboratory testing, histocompatibility laboratories usually depend more on retrospective error tracking than on proactive risk identification and mitigation [4-6]. In histocompatibility testing, the risk is often assessed considering both the chance of an event happening and how serious its potential consequences [7, 8]. This review attempts to bridge that gap by critically examining the possible sources of risk across all phases of histocompatibility testing, evaluating established tools such as failure modes and effects analysis (FMEA) and risk matrices, and highlighting practical strategies alongside relevant regulatory expectations. These expectations will help in implementing comprehensive risk management systems in transplant immunology laboratories.

## 2. Sources of Risk

The source of risk in histocompatibility testing generally arises during the pre-analytical, analytical, and post-analytical phases, as mentioned in Table 1, which includes HLA-specific examples relevant to molecular and antibody-based assays.

**Table 1** Sources of Risk in Histocompatibility (HLA) Testing.

| Phase                 | Risk Factors                                                                                             | HLA-Specific Examples                                                                                                                                                                                                 | Potential Impact                                                                                |
|-----------------------|----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| <b>Pre-Analytical</b> | Sample mislabeling, wrong ID, hemolyzed/clotted samples, temperature fluctuations, and delayed transport | Delay in lymphocyte isolation, reducing cell viability; improper anticoagulant for molecular typing; degraded DNA affecting PCR amplification                                                                         | Sample rejection, invalid or inaccurate typing/crossmatch results                               |
| <b>Analytical</b>     | Reagent variability, instrument malfunction, human error, SOP deviation                                  | Lot-to-lot variation in commercial HLA typing or antibody kits; incorrect bead/serum ratio in Luminex assay; allele misassignment due to outdated software/database; pipetting or calibration errors in NGS platforms | Inaccurate HLA typing or antibody identification; false donor-specific antibody (DSA) reporting |

|                        |                                                                                            |                                                                                                                                  |                                                                                         |
|------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| <b>Post-Analytical</b> | Reporting delays, transcription errors, LIS issues, and poor communication with clinicians | Transcription errors in the HLA report, failure to communicate positive crossmatch or DSA result, and delayed result validation. | Delayed or inappropriate transplant decision, increased risk of rejection or graft loss |
|------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|

Where, LIS: Laboratory Information System; SOP: Standard Operating Procedure; DSA: Donor-Specific Antibody; NGS: Next-Generation Sequencing.

The pre-analytical phase primarily recognises all activities that occur before testing begins, such as sample collection, labelling, packaging, and transport to the testing laboratory [9, 10]. Sample mislabeling, incorrect patient identification, hemolyzed or clotted samples, inappropriate collection tubes, and transportation delays are the various risks associated with the pre-analytical phase. Temperature control during transport is crucial, as even minor fluctuations, especially in cellular assays, can compromise sample integrity. The impact of these risks is significant; all of them may contribute to invalid test results and unnecessary repeat sampling. In the specific context of HLA laboratories, pre-analytical factors such as sample quality, anticoagulant choice, and time between sample collection and testing are critical, as delayed processing may reduce lymphocyte viability and affect molecular typing or crossmatch accuracy. For molecular HLA typing, factors like reagent lot-to-lot variability, quality of extracted DNA, and calibration of PCR or sequencing platforms can influence allele assignment [11, 12]. Similarly, for antibody screening and identification, deviations in incubation time or bead storage conditions may alter mean fluorescence intensity (MFI) values and lead to misinterpretation. Furthermore, incorrect software versioning or mismatches in allele databases can introduce typing discrepancies, highlighting the need for ongoing validation and version tracking. In the absence of risk identification measures, they may be vulnerable to patient safety [13]. Further, the analytical phase is the actual testing phase, which consists of all types of histocompatibility testing. Lot-to-lot variability in reagents, instrument malfunctions, calibration errors, and operator errors, such as deviations from defined standard operating procedures (SOPs), can also contribute to inaccuracies in results, ultimately impacting critical transplant outcomes [14]. The post-analytical phase includes result validation, reporting, and communication with the clinical team. This phase is susceptible to delays in reporting, transcription errors, or data entry errors, and communication gaps between laboratory personnel and clinicians. Such issues can directly affect timely clinical decision-making and may compromise transplant outcomes, including delayed procedures or inappropriate therapeutic interventions. Implementing structured reporting protocols, dual result verification, and timely clinician notification can mitigate these risks [14, 15].

### 3. Systemic Risks

Systemic risks refer to issues related to laboratory infrastructure, workflows, and environmental facilities that can affect overall histocompatibility testing across all phases. Malfunctioning of the laboratory information system (LIS) is a critical systemic risk that can lead to data loss and ultimately delay reporting. Similarly, risk-associated power backup and failure in environmental facility control (like deviations in temperature or humidity) also compromise various laboratory activities (like sample stability and assay performance) [16, 17]. Further, insufficient personnel training and frequent turnover of laboratory personnel are significant systemic risks, as inadequate

staff competency directly increases the likelihood of errors and misinterpretation of results. Dependency on a manual system increases human error. A communication gap between laboratory personnel and clinicians can also introduce systemic inefficiencies and safety risks. Resource limitations may exacerbate systemic risks, including limited access to backup equipment, delayed reagent procurement, and inadequate ongoing education and quality improvement initiatives. Within HLA laboratories, systemic risks are often linked to staff competency and communication between laboratory and clinical teams. Insufficient training of HLA technologists or high personnel turnover can increase the likelihood of analytical or interpretive errors. Maintaining close coordination between the HLA laboratory director and transplant clinicians is essential for ensuring accurate result interpretation and timely clinical decision-making. Periodic competency assessments, participation in external proficiency testing (EPT), and regular interdisciplinary meetings help minimise systemic risk and strengthen quality assurance. A defined procedure should be in place to address the systemic risks, which include robust infrastructure, well-trained and competent personnel, continuous quality control (QC) monitoring, and strong interdepartmental communication channels [13].

#### 4. Tools and Methodologies for Risk Assessment

Various tools and methodologies are available to proactively identify, quantify proactively, and control sources of error within histocompatibility laboratories. Table 2 outlines key approaches, including FMEA, risk matrices, and RCA, with examples specific to HLA typing and antibody screening workflows.

**Table 2** Tools for Risk Assessment in Histocompatibility (HLA) Laboratories.

| Tool                                             | Purpose                                                                                | Application Example                                                                                                          |
|--------------------------------------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| <b>FMEA (Failure Modes and Effects Analysis)</b> | Proactive identification of failure points and prioritization by RPN                   | Incorrect serum-to-bead ratio in Luminex antibody screening, leading to high RPN; improved by automation and retraining      |
| <b>Risk Matrix</b>                               | Visual categorization of likelihood vs. severity to guide immediate control measures   | Classifying the risk level of delayed reporting of crossmatch or DSA results                                                 |
| <b>RCA (Root Cause Analysis)</b>                 | Retrospective analysis of errors to determine underlying causes and prevent recurrence | Incorrect HLA allele assignment traced to outdated database version; mitigated by dual verification and software validation. |

Where, RPN: Risk Priority Number; FMEA: Failure Modes and Effects Analysis; RCA: Root Cause Analysis; HLA: Human Leukocyte Antigen; DSA: Donor-Specific Antibody.

Failure modes and effects analysis is one of the most structured tools for risk assessment. This systematically reviews each step within the defined laboratory process, identifies potential failure points (known as “failure modes”), and evaluates them based on three criteria: severity of impact, likelihood of occurrence, and ease of detection. Further, each failure mode is assigned a risk priority number (RPN) [RPN Severity × Occurrence × Detectability], allowing the laboratory to grade the

issue by RPN and accordingly address high-risk issues first [4, 14, 17]. To strengthen the practical application of risk management tools, the following examples illustrate their use in routine histocompatibility laboratory settings. For example, during FMEA of a Luminex-based antibody screening assay, a laboratory identified that an incorrect serum-to-bead ratio caused by a pipetting error carried a high RPN. This prompted the introduction of an automated pipetting system and additional competency-based staff retraining, which subsequently reduced assay variability and error frequency. Similarly, a root cause analysis (RCA) of a discrepant HLA typing result revealed that the error originated from a database mismatch in the allele assignment software. The issue was mitigated by implementing dual result verification and periodic database validation checks, thereby preventing recurrence.

A “risk matrix” is another valuable tool that visually plots risk by likelihood and severity, typically using a colour-coded grid to indicate whether a risk is low, moderate, or high. This tool is useful during decision-making processes when immediate control measures are required [18].

Root cause analysis is an integral part of the laboratory system and plays a valuable role in risk assessment. RCA is often applied retrospectively following an incident or error. This usually explains what went wrong and why, and frequently reveals process deficiencies [19]. It commonly uses the “Five Whys” technique or Fishbone (Ishikawa) diagrams to trace contributing factors. A fishbone diagram helps visually map complex multifactorial problems into a structured manner for both prospective and retrospective investigations. All these tools can be applied in routine laboratory work to improve risk assessment. By embedding these methodologies, the histocompatibility laboratory can transition from reactive to proactive quality management, reducing error rate and increasing patient safety. Routine use of risk assessment matrices during quality review meetings can help track trends and facilitate timely mitigation [20, 21].

## 5. Risk Mitigation Strategies

An effective risk mitigation strategy is pivotal to ensuring test consistency, reliability, and patient safety. Risk mitigation should combine proactive and reactive control measures, integrating preventive and contingency plans. Table 3 summarises key HLA laboratory-specific strategies and their associated benefits.

**Table 3** Risk Mitigation Strategies and Associated Benefits for Histocompatibility (HLA) Laboratories.

| Strategy                               | Description                                         | HLA-Specific Application                                                                        | Benefit                                                   |
|----------------------------------------|-----------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------|
| <b>SOP Standardization</b>             | Uniform procedures across all testing steps         | Harmonised protocols for DNA extraction, PCR/NGS typing, antibody screening, and cross-matching | Reduces procedural variability, improves reproducibility. |
| <b>Dual Result Verification</b>        | The second person reviews results and reagent logs. | Independent review of ambiguous allele calls or antibody specificity before release             | Minimises interpretation and transcription errors         |
| <b>Staff Training &amp; Competency</b> | Structured onboarding and                           | Regular competency testing for HLA technologists;                                               | Ensures consistent technical performance                  |

|                                                |                                                                     |                                                                                                 |                                                                            |
|------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
|                                                | periodic skill assessment                                           | training on database/software updates                                                           |                                                                            |
| <b>Automation &amp; LIS Integration</b>        | Use of automated pipetting, barcode tracking, and digital reporting | Integration of HLA software with LIS for data traceability from sample to report                | Reduces manual errors, enhances traceability                               |
| <b>Internal &amp; External Quality Control</b> | Routine monitoring of assay performance and participation in EPT    | Use of internal positive controls and involvement in EQAS for HLA typing and antibody detection | Maintains assay reliability and accreditation compliance                   |
| <b>Internal Communication Protocols</b>        | Structured reporting and a clinician feedback system                | Immediate notification of positive crossmatch or new DSA findings to transplant clinicians      | Facilitates timely clinical decision-making and improves team coordination |

Where, SOP: Standard Operating Procedure; LIS: Laboratory Information System; EQAS/EPT: External Quality Assessment Scheme/Proficiency Testing.

Maintaining standardisation of protocols across the laboratory is vital for reducing risk. A double-check system, such as dual review of results and reagent verification logs, is essential in minimising errors. Personnel training, competency assessments, and ongoing education programs are also critical risk mitigation strategies [22, 23]. A mentoring system can strengthen individual accountability and reduce subjective interpretation errors [23]. For HLA laboratories, risk mitigation must include continuous reagent lot verification, routine internal quality control (IQC) for both molecular and antibody assays, and documentation of all instrument calibration and maintenance activities [14]. Dual verification of HLA typing results, particularly in cases of ambiguous allele calls or low-level donor-specific antibodies (DSA), helps prevent reporting errors. Integration of laboratory information systems (LIS) with HLA software ensures traceability from sample accessioning to report generation and facilitates effective communication with the clinical transplant team.

By implementing automation systems such as automated pipetting stations and digital data management through a LIS, laboratories can minimise manual handling errors. In addition, structured and controlled inventory management, along with environmental monitoring systems, can further reduce errors and enhance traceability. A well-defined internal communication system between laboratory personnel and the clinical team is a critical mitigation strategy, particularly for managing essential alerts of value and urgent reporting [24, 25]. Finally, all the risk mitigation strategies within the laboratory must be regularly monitored for effectiveness through quality indicators, incident reports, turnaround time (TAT), etc. In case of any issue or deviation identified, corrective and preventive action (CAPA) should be promptly implemented and reviewed [14]. By adopting a layered, data-driven approach to risk mitigation, histocompatibility laboratories can build resilient systems that support safe and timely transplantation outcomes.

## **6. Regulatory Expectations and Accreditation Requirements**

The integration of risk assessment and mitigation into laboratory testing practices is not only a quality imperative but also increasingly a regulatory compliance requirement. The international standard for medical laboratories, ISO 15189:2022, explicitly incorporates risk-based thinking into its framework [26]. This edition also shows improved alignment with other ISO standards addressing various requirements, including ISO 22367, which focuses on risk management. Notably, ISO 15189:2022 includes five requirements related to risk analysis, compared to only one in the previous edition (ISO 15189:2007) [14, 26]. Overall, the rights, requirements, and safety of patients are more strongly emphasised in this updated version of ISO 15189:2022 [26]. Histocompatibility testing laboratories are expected to assess risks in accordance with their quality management system (QMS), conduct RCA of mitigation actions, and monitor their effectiveness [14, 26, 27].

College of American Pathologists (CAP) accredited histocompatibility laboratories are required to document quality indicators, perform RCA for significant errors, and show continuous quality improvement; these are essential parts of a risk-based approach [28]. Similarly, the European Federation for Immunogenetics (EFI) and the American Society for Histocompatibility and Immunogenetics (ASHI) guidelines emphasise the need for validated SOPs, proficiency testing (PT), and ongoing personnel competency assessment [29, 30]. All these practices rely on strong risk management processes. Further, national transplant authorities, such as the Organ Procurement and Transplantation Network (OPTN) in the United States (US) and the National Organ and Tissue Transplant Organisation (NOTTO) in India, depend on accredited testing laboratories to meet strict risk and reliability standards [31-33]. As transplantation becomes more regulated and standardised, risk-based QMS will be crucial for compliance, audit readiness, and institutional reputation. To meet these expectations, histocompatibility testing laboratories must establish formal risk assessment procedures and systems and incorporate risk awareness into daily laboratory operations. Furthermore, risk assessment and mitigation activities should encompass CAPA implementation, training modules, and regular SOP updates. Accreditation is no longer just about checking off tasks; it involves fostering a culture of risk-aware decision-making that supports patient safety and regulatory compliance.

## **7. Conclusions and Future Directions**

Risk assessment and mitigation are now essential for a laboratory's QMS in clinical histocompatibility testing. Each step in the testing process, from pre-analytical (sample handling) to reporting results (Analytical), carries risks that can influence transplant outcomes. Therefore, building a culture of risk awareness, along with structured mitigation methods like FMEA and RCA, not only improves reliability but also helps laboratories meet international accreditation standards. In the future, digital innovations and global teamwork will be crucial for keeping risk management in line with modern transplantation needs. By proactively managing risk, histocompatibility laboratories can enhance their position as trusted partners in providing safe, timely, and effective transplant care.

## Author Contributions

V.C.M.: Conceptualisation, original draft preparation, review, and editing. D.C.: Data collection, technical assessment, review, and editing. V.R.: Conceptualisation, visualisation, and supervision. All authors have read and approved the final version of the manuscript.

## Competing Interests

The authors have declared that no competing interests exist.

## AI-Assisted Technologies Statement

During the revision of the manuscript, and following the reviewers' suggestion for language editing, the author used Grammarly to improve readability and language clarity. After using this tool, the author thoroughly reviewed and edited the content as needed and takes full responsibility for the final version of the manuscript and all of its content.

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