CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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I/LA29-A

Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Approved Guideline

This guideline describes criteria for optimizing methods that utilize flow cytometry and other conventional and multiplex platforms.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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For further information on committee participation or to submit comments, contact CLSI.

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consensus process. However, because of its usefulness to segments of the health care community, it is available for its informational content.

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Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Approved Guideline

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Abstract

The current and emerging technologies for detecting and characterizing human leukocyte antigen (HLA) alloantibodies provide powerful tools for predicting the risk of immunological response to a transplant. Clinical and Laboratory Standards Institute document I/LA29-A—*Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Approved Guideline* describes criteria for optimizing methods that utilize flow cytometry and other conventional and multiplex platforms. The intended audience includes solid organ and stem cell transplant laboratories, manufacturers of systems for histocompatibility testing, and organizations that manage organ sharing.

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Foreword

The current technologies for detecting and characterizing human leukocyte antigen (HLA) alloantibodies in patients awaiting transplants are among the most significant advances in the field of clinical histocompatibility. The benefit of these advances is clear and measurable. In the early days of organ transplantation, hyperacute rejection (where the graft is lost immediately in the operating room) was not uncommon. A transplant surgeon training today may never experience such a devastating event in his or her career. The impact on transplanting sensitized patients is even greater. In contrast to the historically poor prognosis for organ survival, it is now normal for sensitized patients—in particular, those undergoing retransplantation—to have graft survival rates the same as primary transplant recipients.

Current technologies allow a donor organ to be matched with a sensitized patient by predicting the crossmatch using detailed specificity analysis of the patient's HLA antibodies. However, even with the predictive value of HLA antibody profiling, flow cytometric crossmatching remains an important procedure in the histocompatibility laboratory. The ability to gate out dead cells on the flow cytometer using light scatter often allows interpretable results to be obtained even when lymphocyte viability is low.

Appropriate utilization of these powerful tools allows clinical transplant centers to transplant the most difficult patients and provide them with excellent chances for a successful outcome. By standardizing methods, quality control, and clinical interpretations, transplant centers can more readily identify optimal donor-recipient pairs and encourage organ sharing. The optimal use of precious donor organs is the overarching goal of this consensus guideline.

Key Words

Alloantibodies, avidity, crossmatch, cryptic epitope, donor-specific antibody (DSA), flow crossmatch/ flow cytometric crossmatch, HLA, multiantigen

Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Approved Guideline

1 Scope

This guideline describes criteria for optimizing flow cytometry crossmatching and the detection of human leukocyte antigen (HLA) alloantibody by solid-phase methods in conventional and multiplex platforms. Specific areas include technical consideration for instrument setup and staining procedures, screening methods, single-antigen and multiantigen approaches, reporting formats, clinical interpretation, and multicenter quality assurance. The guideline does not address cytotoxicity assays or standard methods for lymphocyte immunophenotyping, which are covered in CLSI document H42.[1] The intended users of this guideline are: 1) laboratories conducting tests of histocompatibility for solid organ and stem cell transplants; 2) manufacturers of reagents and systems for conducting such tests; and 3) organizations that promulgate organ sharing between transplant centers.

2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.[2] For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to CLSI document M29.[3]

3 Terminology

3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all challenges to harmonization. In light of this, CLSI recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In order to align the usage of terminology in this document with that of ISO, I/LA29-A describes *reproducibility* as the closeness of agreement of results of measurements under changed conditions.

Users of I/LA29-A should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in Section 3.2 of this guideline.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.