I/LA29-P
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Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Proposed Guideline

PLEASE

This proposed document is published for wide and thorough review in the new, accelerated Clinical and Laboratory Standards Institute (CLSI) consensus-review process. The document will undergo concurrent consensus review, Board review, and delegate voting (ie, candidate for advancement) for 60 days.

Please send your comments on scope, approach, and technical and editorial content to CLSI.

Comment period ends

15 February 2008

The subcommittee responsible for this document will assess all comments received by the end of the comment period. Based on this assessment, a new version of the document will be issued. Readers are encouraged to send their comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; Fax: +610.688.0700, or to the following e-mail address: customerservice@clsi.org

COMMENT

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.

(Formerly NCCLS)

Clinical and Laboratory Standards Institute

Advancing Quality in Health Care Testing

Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the health care community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related health care issues. Our process is based on the principle that consensus is an effective and cost-effective way to improve patient testing and health care services.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, we provide an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

A document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

CONSENSUS PROCESS

The CLSI voluntary consensus process is a protocol establishing formal criteria for:

- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate consensus level.

Proposed A consensus document undergoes the first stage of review by the health care community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Approved An approved standard or guideline has achieved consensus within the health care community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (ie, that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

Our standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following CLSI's established consensus procedures. Provisions in CLSI standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any document. Address comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

Health care professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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Abstract

The current and emerging technologies for detecting and characterizing HLA alloantibodies provide powerful tools for predicting the risk of immunological response to a transplant. Clinical and Laboratory Standards Institute document I/LA29-P—*Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Proposed 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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Proposed Guideline

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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.

Invitation for Participation in the Consensus Process

An important aspect of the development of this and all CLSI documents should be emphasized, and that is the consensus process. Within the context and operation of CLSI, the term "consensus" means more than agreement. In the context of document development, "consensus" is a process by which CLSI, its members, and interested parties (1) have the opportunity to review and to comment on any CLSI publication; and (2) are assured that their comments will be given serious, competent consideration. Any CLSI document will evolve as will technology affecting laboratory or health care procedures, methods, and protocols; and therefore, is expected to undergo cycles of evaluation and modification.

The Area Committee on Immunology and Ligand Assay has attempted to engage the broadest possible worldwide representation in committee deliberations. Consequently, it is reasonable to expect that issues remain unresolved at the time of publication at the proposed level. The review and comment process is the mechanism for resolving such issues.

The CLSI voluntary consensus process is dependent upon the expertise of worldwide reviewers whose comments add value to the effort. At the end of a 60-day comment period, each subcommittee is obligated to review all comments and to respond in writing to all which are substantive. Where appropriate, modifications will be made to the document, and all comments along with the subcommittee's responses will be included as an appendix to the document when it is published at the next consensus level.

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, the term *precision* is defined as the "closeness of agreement between independent test/measurement results obtained under stipulated conditions." As such, it cannot have a numerical value, but may be determined qualitatively as high, medium, or low. For its numerical expression, the term *imprecision* is used, which is the "dispersion of results of measurements obtained under specified conditions." In addition, different components of precision are defined in I/LA29-P, primarily *repeatability,* ie, "the closeness of the agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement; while *reproducibility* describes the closeness of agreement of results of measurements under changed conditions.

The term *measurand* (a particular quantity subject to measurement) is used in combination with the term *analyte* (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix; and the term *measurement procedure* is combined with *analytical method* for a set of operations, used in the performance of particular measurements according to a given method.

The term *diagnostic sensitivity* is combined with the term *clinical sensitivity,* and correspondingly the term *diagnostic specificity* is combined with the term *clinical specificity*, because in Europe, the term "clinical" often refers to clinical studies of drugs under stringent conditions.

Users of I/LA29-P should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in the Definitions section 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.

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; Proposed 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 Definitions

absorbance – (A) the logarithm of the ratio of radiant power (I_o) incident on the sample to the radiant power (I) transmitted by the sample; $A = \log I_o / I$; **NOTE 1:** Alternative terms sometimes used are "extinction" and "optical density"; **NOTE 2:** The wavelength at which the absorbance is measured can be shown as a superscript, the component of which the absorbance is measured as subscript, eg, $A^{540}{}_{HiCN}$ = absorbance of hemiglobincyanide at 540 nm.

affinity (purified) chromatography – a method for separating specific molecules from a heterogeneous mixture by capturing the molecule of interest (target) with a molecule for which the target has a high affinity or binding constant; **NOTE:** The capture molecule is attached to a solid phase cross-linked dextran gel material.

allele – **1)** one of the alternative forms of a gene that may occupy a given locus; **2)** *in genetics,* any of several forms of a gene that is responsible for hereditary variation; **3)** one of the alternate forms of a polymorphic DNA sequence that is not necessarily contained within a gene.

alloantibodies – antibodies directed at epitopes that are present in some but not all members of the same species; **NOTE:** In the HLA setting, the antigens encoded by HLA genes are very polymorphic, with many variations of the genes found at the same loci. An individual can make an immune response against the epitopes that differ in another individual.