I/LA30-P
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Immunoassay Interference by Endogenous Antibodies; Proposed Guideline

PLEASE

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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 (i.e., candidate for advancement) for 60 days.

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

Comment period ends

25 May 2007

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 the 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

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COMMENT

This guideline discusses the nature and causes of interfering antibodies, as well as their effects on immunoassays and mechanisms by which interference occurs. Methods to identify and characterize the interferences will be addressed along with assessment of methods used to eliminate interference.

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

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Clinical and Laboratory Standards Institute

Advancing Quality in Healthcare Testing

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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.

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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 healthcare 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 healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., 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

Healthcare 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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Immunoassay Interference by Endogenous Antibodies; Proposed Guideline

Joan H. Howanitz, MD
Johan Bjerner, MD, PhD
Nina M. Chace, MS
Bernard C. Cook, PhD, DABCC, FACB
Pradip Datta, PhD, DABCC
Robert C. Doss, PhD
Steven C. Kazmierczak, PhD, DABCC, FACB
Stanley S. Levinson, PhD
Vadiraja V. Murthy, PhD
Robert M. Nakamura, MD
Wadid Sadek, PhD
Jennifer A. Snyder, PhD

Abstract

Clinical and Laboratory Standards Institute document I/LA30-P—*Immunoassay Interference by Endogenous Antibodies; Proposed Guideline* presents information on the origin, nature, and prevalence of circulating endogenous antibodies, which cause interference with immunoassay results. The mechanisms of the interference along with some specific examples are included. To address the problem, recommendations for regulatory bodies, reagent manufacturers, and laboratorians are provided.

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Committee Membership

Area Committee on Immunology and Ligand Assay

Dorothy J. Ball, PhD
Chairholder
Abbott
Irving, Texas

Robin G. Lorenz, MD, PhD
Vice-Chairholder
University of Alabama at Birmingham
Birmingham, Alabama

Joan H. Howanitz, MD
SUNY Brooklyn
Brooklyn, New York

Marilyn M. Lightfoote, MD, PhD
FDA Center for Devices and Radiological Health
Silver Spring, Maryland

Tom H. Stahlberg
Wallac Oy
Turku, Finland

Robert F. Vogt, Jr., PhD
Centers for Disease Control and Prevention
Atlanta, Georgia

Ronald J. Whitley, PhD
University of Kentucky Med. Ctr.
Lexington, Kentucky

Advisors

W. Harry Hannon, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia

Gerald E. Marti, MD, PhD
FDA Center for Biologics Evaluation and Research
Bethesda, Maryland

Per N. J. Matsson, PhD
Phadia AB
Uppsala, Sweden

Robert M. Nakamura, MD
Scripps Clinic & Research Foundation
La Jolla, California

Thomas A. O'Brien, PhD
Ortho Biotech Products LP
Bridgewater, New Jersey

Jelili Ojodu, MPH
Association of Public Health Laboratories
Silver Spring, Maryland

Robert F. Ritchie, MD
Foundation for Blood Research
Scarborough, Maine

Donald R. Tourville, PhD
Zeus Scientific, Inc.
Raritan, New Jersey

Daniel Tripodi, PhD
The Sage Group
Branchburg, New Jersey

Robert W. Veltri, PhD
Johns Hopkins Hospital
Baltimore, Maryland

Philip R. Wyatt, MD, PhD
York Central Hospital, Toronto North York, Ontario, Canada

Subcommittee on Interference by Heterophile Antibodies

Joan H. Howanitz, MD
Chairholder
SUNY Brooklyn
Brooklyn, New York

Johan Bjerner, MD, PhD
Norwegian Radium Hospital
Oslo, Norway

Nina M. Chace, MS
FDA Center for Devices and Radiological Health
Rockville, Maryland

Bernard C. Cook, PhD, DABCC, FACB
Beckman Coulter, Inc.
Chaska, Minnesota

Pradip Datta, PhD, DABCC
Bayer HealthCare
Tarrytown, New York

Robert C. Doss, PhD
Abbott Diagnostics Division
Abbott Park, Illinois

Steven C. Kazmierczak, PhD, DABCC, FACB
Oregon Health and Science University
Portland, Oregon

Stanley S. Levinson, PhD
Department of Veterans Affairs Medical Center
Louisville, Kentucky

Jennifer A. Snyder, PhD
Dade Behring, Inc.
Newark, Delaware

Advisors

Vadiraja V. Murthy, PhD
Albert Einstein College of Medicine
Teaneck, New Jersey

Robert M. Nakamura, MD
Scripps Clinic & Research Foundation
La Jolla, California

Wadid Sadek, PhD
St. Helen Medical Center
S. Burlington, Vermont

Staff

Clinical and Laboratory Standards Institute
Wayne, Pennsylvania

John J. Zlockie, MBA
Vice President, Standards

Lois M. Schmidt, DA
Staff Liaison

Donna M. Wilhelm
Editor

Melissa A. Lewis
Assistant Editor

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Foreword

This guideline describes methods for identification and potential elimination of immunoassay interference caused by antibodies in patient specimens. These circulating endogenous antibodies can cause falsely increased or decreased results for analytes measured by immunoassay. Interferences are assay-dependent and often go unrecognized, thus leading to misinterpretation of results. When results falsely signify an underlying medical condition, unnecessary follow-up testing or treatment can occur. Assay interferences also can cause failure to recognize disease. Even though in the design and development of immunoassays, the issue of interfering antibodies has been addressed, complete elimination of interference has not been possible. Clinicians thus need to be aware of the limitations of immunoassays. Test results that are inconsistent with other sources of medical information and do not fit the clinical picture should be considered suspect. This requires awareness of this type of problem and good communication for both laboratory personnel and the patient's physician.

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 healthcare procedures, methods, and protocols; therefore, it 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 that 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.

Key Words

Antianimal antibodies, autoantibodies, endogenous antibodies, heterophile antibodies, immunoassay, interference

Immunoassay Interference by Endogenous Antibodies; Proposed Guideline

1 Scope

This guideline discusses the nature and causes of interfering antibodies as well as their effects on immunoassays and mechanisms by which interference occurs. Methods to identify and characterize the interferences will be addressed along with assessment of methods used to eliminate interference. This document suggests guidelines for regulatory bodies, manufacturers, and laboratorians in their roles identifying and eliminating endogenous interfering antibodies in patient specimens. Although examples of specific assay interferences are included, the document does not intend to describe all methods or analytes where antibody interference has been reported. The guideline does not address other types of immunoassay interferences, such as hemolysis, cross-reacting substances, and drug interference except when the drug is an antibody. The intended users of the guideline are organizations responsible for regulatory oversight of immunoassay reagent production, manufacturers of immunoassay reagents, and laboratorians performing immunoassays.

2 Introduction

Because of their sensitivity and specificity, immunoassays are important diagnostic tools allowing measurement of a wide variety of analytes. Immunoassays, however, are subject to a number of interferences including those caused by circulating endogenous antibodies. Interference can occur because of heterophile antibodies, antianimal antibodies, or autoantibodies. The interfering antibodies can give rise to falsely high or, less commonly, falsely low results. The erroneous result is recognized as being inconsistent with the patient's clinical picture, but often it is clinically difficult or impossible to recognize an assay result as spurious. Additionally, it may be difficult to ascertain by commonly used laboratory procedures that a given result is erroneous. The laboratory procedures generally used to identify interfering antibodies are demonstration of a nonlinear response to dilutions, addition of nonimmunoglobulin protein to block the interfering antibody, or use of an alternate immunoassay. None of these commonly used procedures, however, can identify interference reliably in all cases. The magnitude of the problem of antibody interference is unknown with certainty, because wide variation in prevalence has been described depending on the detection methods used and the populations studied. Circulating endogenous antibodies may arise from incidental or occupational exposure to foreign protein, use of antibodies as diagnostic or therapeutic agents, following infection or vaccination, or for unknown reasons. The interference is variable, complex, and unpredictable because of the wide range of affinities and avidities found among the various endogenous antibodies that can be encountered. The antibodies may react with the analyte, the reagent antibodies, or both. There are also reports of antibodies interfering with the immunoassay detection systems. Interfering antibodies are not only difficult to recognize but are problematic to eliminate. Nonlinear response to dilutions cannot always be identified in the presence of interfering antibodies. The interfering antibodies can have high titer or avidity and thus, it may be difficult to eliminate the interference with blocking agents. Interfering antibodies may react with various types of assay antibodies and thus may interfere in different assay types. The intent of this document is to increase awareness of the problem of interfering antibodies and to suggest approaches to minimize their impact on patient care.

3 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