CLINICAL AND LABORATORY STANDARDS INSTITUTE®

June 2011

I/LA34-A

Design and Validation of Immunoassays for Assessment of Human Allergenicity of New Biotherapeutic Drugs; Approved Guideline

This document provides guidance for the design, validation, analytical performance, and quality assurance of laboratory assays used in the measurement of human immunoglobulin E antibodies specific for new biotherapeutic drugs.

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

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I/LA34-A
ISBN 1-56238-755-3 (Print) Vol. 31 No. 12
ISBN 1-56238-756-1 (Electronic) Replaces I/LA34-P
ISSN 0273-3099 Vol. 30 No. 19

Design and Validation of Immunoassays for Assessment of Human Allergenicity of New Biotherapeutic Drugs; Approved Guideline

Volume 31 Number 12

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Abstract

Clinical and Laboratory Standards Institute document I/LA34-A—*Design and Validation of Immunoassays for Assessment of Human Allergenicity of New Biotherapeutic Drugs; Approved Guideline* provides a framework for the design and validation of a qualitative immunoassay that detects human immunoglobulin E (IgE) antibody to new drugs in various body fluids and tissue extracts. It addresses technical challenges that are uniquely associated with the development of an assay that detects drug-specific IgE antibody in human blood and tissue extracts. It provides an approach for validation of an assay in the absence of a positive drug-specific human IgE antibody serum, which involves a feasibility study phase and then development and validation, using a concomitantly established drug-specific human immunoglobulin G antibody assay as part of its quality control program. This guideline is intended for use by clinical and laboratory investigators who are involved in generating preclinical data and performing clinical trials involving new biotherapeutic drugs. It is also intended as a guideline for administrators of manufacturer safety programs, and government regulators who are required to critique IgE antibody assay methods and assess the validity of allergenicity data that have been submitted by innovator pharmaceutical investigators as part of a governmental licensing process for a new drug.

Clinical and Laboratory Standards Institute (CLSI). *Design and Validation of Immunoassays for Assessment of Human Allergenicity of New Biotherapeutic Drugs; Approved Guideline*. CLSI document I/LA34-A (ISBN 1-56238-755-3 [Print]; ISBN 1-56238-756-1 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2011.

Suggested Citation

CLSI. *Design and Validation of Immunoassays for Assessment of Human Allergenicity of New Biotherapeutic Drugs; Approved Guideline*. CLSI document I/LA34-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

Previous Edition:
September 2010

Reaffirmed:
March 2013

Archived:
January 2017

ISBN 1-56238-755-3 (Print)
ISBN 1-56238-756-1 (Electronic)
ISSN 0273-3099

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Foreword

When a new drug is developed and evaluated in clinical trials, pharmaceutical companies establish assays early in the drug development process to monitor subjects for possible drug immunogenicity. The development of a drug-specific antibody response is used as the principal indicator for a drug's ability to induce a humoral immune response in humans. The design and validation of drug-specific human antibody immunoassays has been thoughtfully chronicled in a number of consensus-based documents.[1-4] Two 2008 documents in particular by Shankar et al.[1] and Koren et al.[2] extend the 2004 recommendations of Mire-Sluis et al.,[3] and provide detailed guidelines for the design, validation, and performance specifications of assays for the detection of human antibodies specific for new biotherapeutics. The availability of a human antibody assay has the benefit of identifying human sera containing antibodies reactive with the drug if there are early human trials with pre- and post-treatment specimens. Alternatively, the drug can be administrated to a number of animals (eg, rabbits, goats, sheep), sometimes haptenized onto a carrier protein, to produce hyperimmunized animal antisera. These human and animal sera contain immunoglobulin G (IgG) antibodies that serve as critical quality control reagents for facilitating the IgG, immunoglobulin E (IgE), immunoglobulin A, and immunoglobulin M antibody assays' development and validation.

Development of an assay to detect human IgE antibodies is more challenging than its companion drug-specific IgG antibody assay. This is because there are rarely positive drug-specific human IgE control sera available at the time of development, and hyperimmunized animal drug-specific antisera are not useful in documenting the IgE specificity of human antibody assays. However, there is a need for assays that can detect, and if present, semiquantify human IgE antibody responses to new therapeutic drugs. This is especially important because biotherapeutics are being designed for repetitive human administration, often with interspersed intervals of months. These administration conditions facilitate secondary humoral immune responses, sometimes of the IgE isotype. *In vivo* drug interference, such as the blocking or neutralization of drug action, can occur when IgG antidrug responses reach microgram per milliliter levels in blood. However, this is rarely a problem with human IgE antibody responses that occur typically at nanogram per milliliter levels. There is, however, a need for exceptional assay sensitivity, and one should address the potential concern of IgG antibody interference in an IgE antidrug assay. This guideline is intended to complement existing comprehensive immunogenicity (IgG assay)-based recommendations by selectively addressing unique aspects of therapeutic drug-specific human IgE antibody assay development, validation, and performance specifications. Where possible, validation techniques presented in the IgG antibody documents are used to minimize redundancy.

Key Words

Assay methods, biotherapeutic drugs, human IgE antibody, performance, quality assurance, sensitivity, specificity, type I hypersensitivity, validation

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1 Scope

This guideline provides an overall strategy for the design and validation of assays to measure human immunoglobulin E (IgE) antibodies specific for new biotherapeutic drugs in the serum of subjects enrolled in clinical trials. This document builds on past drug-specific antibody–focused recommendations,[1-4] incorporating new analytical technologies. Because IgE antibody responses are typically in the nanogram per milliliter range, human serum should be analyzed in IgE antibody assays minimally diluted. This guideline addresses the technical challenges associated with nonspecific binding (NSB) that can occur when specimens such as serum or tissue extracts are analyzed undiluted or concentrated. It also provides an approach for addressing potential assay interference that can result when nanogram per milliliter levels of IgE antibody attempt to bind to limited immobilized drug in the presence of microgram per milliliter quantities of drug-specific non-IgE antibody (eg, immunoglobulin G [IgG], and sometimes immunoglobulin A [IgA] and immunoglobulin M [IgM]). The guideline overviews supplemental serological analyses that can support the interpretation of human IgE antidrug assay results (eg, atopy screens, total IgE). It presents practical methods for the quality control (QC) of assay reagents.

This document does not discuss the relationship between analytical measurement of IgE antibody quantity in blood or tissue and a subject's clinical risk for type I hypersensitivity. It does not discuss assays used to assess adverse reactions to the active ingredients of vaccines. The I/LA34 document does not duplicate information in published recommendations for IgG antibody assays,[1-4] except as needed for clarity. These published consensus strategies for defining the positive cut-point and validating IgG antidrug assays assume to be operative for IgE antibody assays as well, unless discussed in this document as not technically feasible due to the absence of a positive human IgE antidrug control. Assays that can be used to monitor cellular immune responses or the release of mediators and cytokines (eg, interleukin [IL]-4, IL-5, IL-6, transforming growth factor beta [TGFβ]) from basophils and mast cells are also not discussed.

The document is designed for use by academic and industrial laboratory scientists and clinicians, and drug manufacturers who are involved in generating preclinical data and performing clinical trials of new biotherapeutic drugs. Clinical laboratories involved in developing drug-specific IgE antibody assays for use in monitoring subjects involved in clinical trials or companies manufacturing IgE antibody assay kits for monitoring marketed drug effectiveness may need to meet applicable international, national, accreditation, local, and organizational requirements. Others reside in companies that are involved in developing IgE antibody assay kits for use in monitoring drugs following federal licensure. This guideline is also intended for use by administrators involved in establishing manufacturer safety programs and government regulators who are required to critique assay methods and assess the validity of allergenicity data that have been submitted by innovator pharmaceutical investigators as part of the licensing process for a new drug.

2 Introduction

The drug-specific human IgE antibody assay development process involves an initial feasibility study phase and second validation phase during which detection and confirmatory assays are established. This process relies on the prior development of a companion drug-specific human antibody assay using previously established recommendations.[1-4] Unique challenges are discussed involving the validation of a drug-specific IgE antibody assay in the absence of a positive IgE antibody control serum. Additionally, there is a need to address possible interference caused by microgram per milliliter levels of IgG antibody in the detection of nanogram per milliliter levels of IgE antibody, and the potential for high NSB when human test specimens are analyzed undiluted. These conditions can compromise the IgE antibody assay's

analytical sensitivity. The proposed testing algorithm involves not testing sera from clinical study subjects who have no clinical evidence of an immediate-type hypersensitivity reaction following receipt of the drug.

New drugs have the potential to induce immune responses in humans. The overall area of drug immunotoxicology involves the study of a number of special areas including evaluation of a drug's ability to generate antibody responses (immunogenicity) and more specifically IgE antibody responses that are a risk factor for the development of hypersensitivity or allergic reactions (see Figure 1). In 1967, the antibody isotype responsible for mediating allergic reactions was identified as a new human immunoglobulin class, and it was called IgE (fifth human immunoglobulin class, fifth letter of the alphabet, and the antibody that can induce erythema).[5-7] Scientific observations leading to the discovery of IgE are chronicled elsewhere.[8,9]

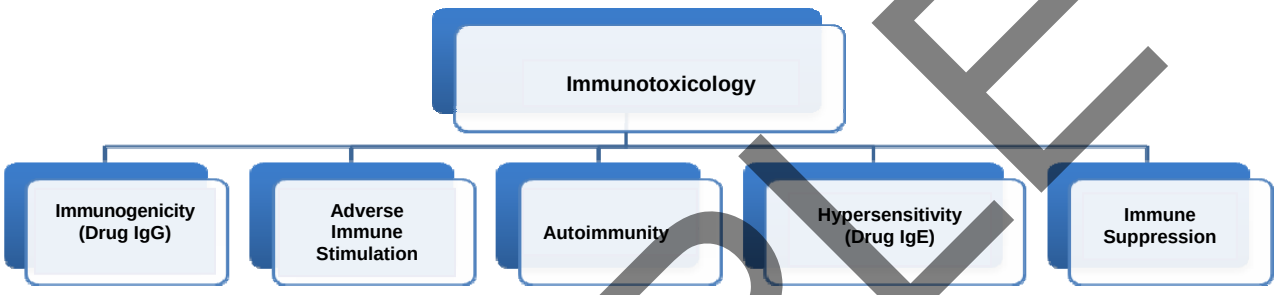

Figure 1. Drug Immunogenicity and Hypersensitivity Fit Into the Overall Context of Immunotoxicology

Clinically, IgE antibody measurements are used in the diagnostic process to confirm sensitization in an individual who provides a history of allergic symptoms following exposure to a defined allergen source.[10] IgE antibody needs to be considered a risk factor for, but not an absolute marker of, clinically manifested allergic symptoms. Together with a positive clinical history, IgE antibody measurements are useful in making the diagnosis of human allergic disease. IgE antibody can be detected by *in vivo* skin test using an epicutaneous (puncture) or intradermal administration of allergen, or by *in vitro* means in serum or extracts of tissues using immunoassay. Each method has its advantages and limitations.

Skin tests are attractive because they involve a biological response in the individual's skin, and results can be obtained within 15 to 20 minutes following allergen administration. A histamine positive control and saline negative control serve to identify patient-associated problems related to false-negative results stemming from interference by antihistamine premedication and false-positive reactions resulting from dermographism. The major limitations associated with skin testing methods relate to the potential risk for eliciting allergic reactions following administration of allergen, challenges associated with optimizing the skin test conditions (eg, device, drug concentration, mode of application), and ensuring the drug is in a form (eg, hapten conjugated to a carrier protein, metabolite form) that possesses sufficient allergenic epitopes to crosslink IgE on mast cells and elicit a wheal and erythema response in the skin. In clinical studies of therapeutic drugs, the skin test may be required in the study protocol for anyone who experiences classic symptoms that are typically associated with allergic disease.

Serological evaluation of drug-specific IgE antibody requires development of an assay that typically involves the binding of antibody to immobilized drug and subsequent detection of a particular isotype or class of antibody (eg, IgE). An alternative assay configuration that involves IgE capture and labeled drug detection is generally considered less attractive for reasons discussed subsequently. This guideline addresses the issues associated with determining the feasibility and subsequent development and validation of an IgE antibiotherapeutic drug assay. The attractive feature of monitoring IgE sensitization serologically is that serum from clinical study subjects can be collected pre- and post-treatment as part of

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in CLSI document HS01—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are as follows:

Documents and Records	Equipment	Information Management	Process Improvement
Organization	Purchasing and Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessment—External and Internal	Facilities and Safety

I/LA34-A addresses the QSE indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessment—External and Internal	Process Improvement	Customer Service	Facilities and Safety
					X EP07 EP17 I/LA20				EP07		M29

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

I/LA34-A does not address any of the clinical laboratory path of workflow steps indicated in the grid below. For a description of the document listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
					I/LA20			

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

EP07-A2 **Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (2005).** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.

EP17-A **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits.

I/LA20-A2 **Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline—Second Edition (2009).** This document provides guidance for the design, analytical performance, standardization, quality assurance, and clinical application of laboratory assays used in the measurement of human IgE antibodies of defined allergen specificity.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

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PRINT ISBN 1-56238-755-3

ELECTRONIC ISBN 1-56238-756-1