

I/LA20

Analytical Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Immunoglobulin E Antibodies of Defined Allergen Specificities

This report provides guidance for the design, analytical performance, standardization, quality assurance, and clinical application of laboratory assays used in the measurement of human immunoglobulin E antibodies of defined allergen specificity.

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Analytical Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Immunoglobulin E Antibodies of Defined Allergen Specificities

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Abstract

Clinical and Laboratory Standards Institute report I/LA20—*Analytical Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Immunoglobulin E Antibodies of Defined Allergen Specificities* is written for laboratorians, clinicians, manufacturers, and governmental regulators (inspectors, legislators, reviewers). The report summarizes the current state of immunoglobulin E (IgE) antibody assay technology, routinely tested biological specimens, practical methods for the evaluation of human IgE antibody assay-specific reagents, QA methods, and clinical interpretation of total and allergen-specific IgE antibody results.

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Foreword

Allergen molecule–based IgE antibody results supplement allergen extract–based analyses. They lower the allergen-specific immunoglobulin E (IgE) assay’s lower limit of quantitation and increase the analytical specificity (selectivity) and thus permit more effective assessment of cross-reactivity and risk assessment in support of a clinical history–based diagnosis of allergic disease.

This report provides the laboratorian or clinician with practical information on the appropriate specimen type, reagent validation algorithms, assay calibration and QC strategies, intra- and interlaboratory QA plans, and an overview of the clinical utility of IgE antibody measurements. It emphasizes that the presence of IgE antibody is strictly a biomarker for allergic sensitization. Furthermore, allergic sensitization is a risk factor but not equivalent to the definitive diagnosis of allergic disease in humans, which also requires a positive clinical history.¹ I/LA20 also clarifies that in commercially available allergen-containing reagents, the user does not need to repeat the extensive validation testing that includes verification of specificity that has been performed by the manufacturer. An extensive well-documented serum bank is required for frequent repetitive testing of new lots of IgE antibody reagents provided by the manufacturer. Once validated and released to the user, this testing need not be repeated to successfully use the reagent in the user’s IgE antibody autoanalyzer on which the performance of reagents has been evaluated.

Additionally, this report was written to create operationally achievable procedures that can be used by assay manufacturers to validate the quality and test the performance of the allergenic extracts and individual molecular allergens that are used as reagents in the different assay systems. This revision discusses in detail the increasing availability of allergenic molecules as assay reagents and represents a continuing effort by users, manufacturers, and regulators to promote harmonization of allergen codes, QC procedures, and licensing guidelines for allergen-specific IgE antibody assays.

Finally, this report aims to create procedures that can be used by investigators in clinical laboratories to ensure maximal quality of reported IgE antibody results. Regulators are encouraged to view the IgE antibody autoanalyzers in clinical use as single systems into which individual allergen reagents are added as they are identified as clinically important and qualified by evidence-based data.

Overview of Changes

Immunological assays for IgE antibodies of defined specificity continue to manifest improved performance. Previous editions of I/LA20 provided a framework from which IgE assay reagent validation and QC, assay calibration, and QA have been defined. This report has been updated to serve as a more comprehensive resource for laboratorians/clinicians, manufacturers, and governmental regulators (inspectors, legislators, reviewers). The primary goal of this report is to foster harmonization and enhance the quality of IgE antibody measurements that are performed in diagnostic immunology laboratories throughout the world. As such, this report expands upon the technical and clinical utility issues covered in the first two editions. It also includes an examination of allergenic molecules used in molecular-based allergy diagnosis.

Harmonization of definitions, proficiency testing survey protocols, and QA methods have been updated. With this report, diagnostic kit manufacturers are given fundamental benchmark targets that can be used for the validation and performance improvement of IgE antibody assays. Useful strategies for assessing the quality of IgE reagents and the clearance of IgE antibody assays for use in licensed diagnostic allergy laboratories are provided for regulators and inspectors. This report incorporates the most current evidence-based information related to IgE antibody analyses.

NOTE: The content of this report is supported by the CLSI consensus process, and does not necessarily reflect the views of any single individual or organization.

Key Words

Allergen, allergy, assay methods, clinical utility, human IgE, IgE antibody, molecular allergens, molecule-based allergy diagnosis, performance, quality assurance, total IgE, type 1 hypersensitivity

SAMPLE

Analytical Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Immunoglobulin E Antibodies of Defined Allergen Specificities

Chapter 1: Introduction

This chapter includes:

- Report's scope and applicable exclusions
- Background information pertinent to the report's content
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the report
- Abbreviations and acronyms used in the report

1.1 Scope

This report defines the current state of reagents and serological assay technology used to measure total immunoglobulin E (IgE) and IgE antibodies of defined allergen specificities in human blood. This report focuses on IgE assay design and calibration, validation methods, QA of assay reagents, QC strategies, and clinical applications.

The report is designed as a general reference for laboratorians, clinicians, manufacturers, and governmental regulators (inspectors, legislators, reviewers). It provides consensus on the current state of assay technology, the appropriate biological specimens that are routinely tested, practical methods for the validation of allergen and immunological reagents, diagnostic allergy laboratory QC strategies, consensus guidelines for clearance of allergen-containing reagents by governmental regulatory agencies, and a brief examination of the clinical interpretation of IgE antibody results. It also emphasizes achievable qualification practices that have been used by manufacturers to validate the quality and test the performance of reagents and configured assay systems. Once validated by the manufacturer, additional new lot specificity testing of allergen-containing reagents purchased by the user for one of the IgE antibody autoanalyzers does not need to be repeated. I/LA20 outlines strategies and procedures that have been successfully used by clinical laboratory workers to supplement manufacturer testing and QC practices with the goal of optimizing the laboratory's overall QA program. In addition, this report serves as a resource for inspectors and regulators who are involved in qualifying diagnostic reagents and assays and clinical immunology laboratories that perform IgE analyses.

1.2 Background

As early as 1921, investigators showed that local itching and swelling that were surrounded by a zone of erythema occurred when serum from an allergic person was injected intradermally into an unsensitized (nonallergic) person, followed 24 hours later by the injection of specific allergen into the same skin site. This passively transferred allergic or Prausnitz-Küstner reaction maximized within 10 minutes, persisted

for about 20 minutes, and then gradually disappeared. In 1967, the antibody responsible for this reaction was identified as belonging to a new human immunoglobulin class and designated as IgE.²⁻⁴ Scientific observations leading to the discovery of IgE are presented elsewhere.^{5,6}

The diagnosis of human allergic diseases involves the combined use of a carefully recorded clinical history and physical examination. Additionally, it involves the use of *in vivo* and *in vitro* assay methods for the detection of IgE antibodies of defined allergen specificities in tissue or serum as indicative of sensitization.^{7,8} The presence of IgE antibody in the skin or blood is referred to as “sensitization,” and it remains an important risk factor for, but not synonymous with, the presence of allergic disease. The definitive diagnosis of allergic disease also requires a positive history involving objective allergic symptoms that are induced following a known allergen exposure.⁹ Since 1967, the diagnostic allergy laboratory has promoted evidence-based diagnosis of human allergic disease through the use of commercially available serological assays to measure total IgE and allergen-specific IgE antibodies.

The first serological assay for allergen-specific IgE was developed in 1967.¹⁰ Since then, major technological improvements have led to the development of autoanalyzers that exhibit an enhanced lower limit of quantitation (LLoQ), unsurpassed antibody quantitation, and excellent reproducibility. One significant technological advancement has been the high degree of quantitation and interlaboratory standardization that has been possible with automation, and the use of a common calibration scheme with a common international unit (kUa/L) that is traceable to an international IgE reference preparation.¹¹⁻¹³ The number of allergen extract-based specificities that are available for clinical use in these IgE antibody assays continues to expand. Conflicting coding schemes for the allergens have been essentially eliminated by a consensus method fostered by the first and second editions of I/LA20. A second significant technological advance involves the increasing availability of allergenic molecules as assay reagents. IgE antibody is a single analyte that uses several hundred different allergen-containing reagents. All the assay formats have an IgE calibration system and they use allergen-containing reagents that are prepared with different sources of allergen extracts or recombinant or native allergen molecules. Once released by the manufacturer for sale, each allergen-containing reagent is considered to have undergone QC by the manufacturer, and the user need not repeat this validation testing. The user should simply validate the calibration curve and analytical threshold limit of the qualitative assay or the calibration portion of the semiquantitative and quantitative assays in each assay run. This validation can be accomplished with the analysis of three sera-containing IgE antibody with a few representative allergen specificities at different levels (high, medium, and low) of IgE antibody. The low control should be between 0.1 and 1.0 kUa/L to evaluate variation in the region of the minimal detectable dose of the assay.

The goals of this report are:

- Summarize the current state of clinically used assay autoanalyzer technology for quantifying total IgE and IgE antibody levels in human serum.
- Define performance criteria and outline methods for qualification of assay reagents (including source allergens) that are used to prepare the allergen extracts, characterization of individual molecular allergens, finalized allergen-containing reagents, and antihuman IgE conjugates performed by the manufacturer before sale.
 - The manufacturer and user participate in evaluation of the routine performance assessment of the finalized regulatory-cleared singleplex and multiplex immunoassays designed to quantify human total IgE and IgE antibody of defined allergen specificities. Consensus methods are provided to evaluate the assay’s LLoQ, assay specificity, parallelism, total precision, and repeatability of routinely performed assays.
- Discuss potential causes for quantitative result discordance among the different clinically used IgE antibody assays.

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) 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 QMS 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:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

I/LA20 covers 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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29			C24	X C24 EP05 EP06 EP07 EP09 EP12 EP17 EP25 EP28					

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

I/LA20 covers the medical laboratory path of workflow process indicated by an “X.” For a description of the other document listed in the grid, please refer to the Related CLSI Reference Materials section.

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt and processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
				C24	X			

Related CLSI Reference Materials*

- C24** **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed., 2016.** This guideline provides definitions, principles, and approaches to laboratory quality control design, implementation, and assessment.
- EP05** **Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed., 2014.** This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures and for laboratories that develop or modify such procedures.
- EP06** **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. 1st ed., 2003.** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.
- EP07** **Interference Testing in Clinical Chemistry. 2nd ed., 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.
- EP09** **Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed., 2013.** This document addresses the design of measurement procedure comparison experiments using patient samples and subsequent data analysis techniques used to determine the bias between two *in vitro* diagnostic measurement procedures.
- EP12** **User Protocol for Evaluation of Qualitative Test Performance. 2nd ed., 2008.** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.
- EP17** **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.
- EP25** **Evaluation of Stability of *In Vitro* Diagnostic Reagents. 1st ed., 2009.** This document provides guidance for establishing shelf-life and in-use stability claims for *in vitro* diagnostic reagents such as reagent kits, calibrators, and control products.
- EP28** **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. 3rd ed., 2010.** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- I/LA37** **Supplemental Data for Allergen Specificity of IgE Antibody Autoanalyzers. Version 1.0, 2016.** This database provides a harmonized listing of the allergen codes currently used by immunoglobulin E antibody assay manufacturers along with their common name, Latin name, and allergen grouping.
- M29** **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** 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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