CLINICAL AND LABORATORY STANDARDS INSTITUTE®

4th Edition

CLSI I/LA20™

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

CLSI I/LA20 provides recommendations 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.

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

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

Robert Hamilton, PhD, D.ABMLI
Ida Unhammer Njerve, MD, PhD
Michael Allmann, PhD
Sic Chan, PhD
Tom Chuang
Pradip Data, PhD, DABCC
Michael Dubrovsky, PhD
Guruprasath Durairajan, MSc, C(ASCP)^{CM}
Glynis Frans, PhD
Christian Harwanegg
Debra Hovanec-Burns, PhD
Jonas Lidholm, PhD
Christian Lupinek, MD
Carina Magnusson, PhD
Per Matsson, PhD
Gunnar Nordin, MD
Ilja Ovsiy, PhD
Fabien Rebeaud, PhD
Harald Renz, Prof. Dr.
Lina Souan, MSc, PhD, D.ABMLI
Sanja Stankovic, PhD
Elaine Taine, MD, PhD
Yang Tran, MBBS, BMedSc, FRACP, FRCPA
Andre Valcour, MBA, PhD, DABCC, FADLM
Iswariya Venkataraman, PhD

Abstract

CLSI I/LA20—*Analytical Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Allergen-Specific Immunoglobulin E Antibodies* defines the current state of allergen reagents (extracts, molecules, and epitopes) and assay technology (ie, singleplex and multiplex, manual and autoanalyzer based) used to measure total immunoglobulin E (IgE) and allergen-specific IgE antibodies (Abs) in human blood. CLSI I/LA20 examines IgE assay design, calibration, validation, and verification methods; QA of assay reagents; internal QC strategies; external proficiency testing; and clinical applications. It contrasts the advantages and disadvantages of IgE Ab serological results with those obtained by *in vivo* skin testing and provocation testing. Mentioned but not covered in detail is a general discussion of the clinical history-based diagnostic algorithm for human allergic disease, cell-based assays (eg, basophil and eosinophil), and immunoglobulin G (IgG)/IgG4-blocking Ab assays (eg, facilitated allergen binding assay). Important trends in IgE Ab assay technology are highlighted; these include the use of molecular allergens, multiplexed bead, fluid-phase and chip-based arrays, and allergen-specific IgE monoclonal antibodies as reference and QC reagents. CLSI I/LA20 serves as a reference for laboratory professionals, clinicians, assay manufacturers, and regulatory bodies aiming to harmonize practices and improve the accuracy of IgE Ab measurements globally.

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Foreword

Detection of the presence of immunoglobulin E (IgE) antibody (Ab) remains a biomarker for allergic sensitization. Allergic sensitization, in turn, is a risk factor for, but not equivalent to, the definitive diagnosis of allergic disease in humans, which also requires a positive clinical history. Immunological assays for total IgE and allergen-specific IgE Abs continue to manifest improved performance with the advent of new assay technologies and the increasing use of molecular allergens and defined allergen epitopes.

Previous editions of CLSI I/LA20 have provided an initial framework from which IgE assay reagent validation and QC, assay calibration, and QA have been defined. CLSI I/LA20 has been updated to serve as a more current and comprehensive resource for laboratorians, clinicians, manufacturers, and governmental regulators (eg, inspectors, legislators, reviewers). The primary goal of CLSI I/LA20 is to foster harmonization and improve the quality of IgE Ab measurements that are performed in diagnostic immunology laboratories throughout the world. As such, CLSI I/LA20 expands upon the technical and clinical utility issues covered in the first 3 editions. It also includes an expanded examination of allergenic molecules and epitopes used in molecular-based allergy diagnosis. In this edition, harmonization of definitions, proficiency testing survey protocols, and QA methods have been updated. With CLSI I/LA20, diagnostic kit manufacturers and governmental regulators are given improved targets that can be used for the validation and performance of new and improved IgE antibody autoanalyzer and chip-based assays. Useful strategies for assessing the quality of IgE reagents and the clearance of IgE Ab assays for use in licensed diagnostic allergy laboratories are provided for regulators and inspectors. CLSI I/LA20 incorporates the most current evidence-based information related to IgE Ab analyses and contrasts this information with comparable peer-reviewed literature that discusses the performance of *in vivo* skin testing and (oral) provocation tests.

CLSI I/LA20 is designed as a reference for laboratorians, clinicians, manufacturers, and governmental regulators. It provides consensus on the current state of both *in vivo* and *in vitro* assay technology, the appropriate biological specimens that are routinely tested, practical methods for the validation (by manufacturer) and verification (by laboratory) of allergen and immunological reagents, diagnostic allergy laboratory QC strategies, consensus guidance for clearance of allergen-containing reagents by governmental regulatory agencies, and an examination of the clinical interpretation of IgE Ab results.

CLSI I/LA20 includes new information on the clinical utility of molecular allergen testing that has been gained by massive work in molecular allergology during the past 5 years. Also, novel allergen epitope assays are introduced. Such assays, as well as established forms of molecular allergen testing, may reduce the need for diagnostic food challenges.

Overview of Changes

CLSI I/LA20-Ed4 replaces CLSI I/LA20-Ed3, published in October 2016. Several changes were made in this edition, including:

- Adding a discussion of new molecular allergen and allergenic epitope-based measuring system and micro- and macro-arrays for the detection of allergen-specific IgE Ab
- Enhancing the discussion of strategies for governmental clearance of multiplex measuring systems and chip-based assays
- Updating definitions, proficiency testing survey protocols, and QA methods
- Adding a discussion of the utility of the new companion CLSI I/LA37[1] Diagnostic Allergen Database

NOTE: The content of CLSI I/LA20 is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

allergenic proteins
allergens
allergy
human immunoglobulin E
specific immunoglobulin E assays
type 1 hypersensitivity

Chapter 1

Introduction

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

1 Introduction

1.1 Scope

CLSI I/LA20 provides recommendations on the current state of reagents and assay technology (ie, serological and molecular) used to measure total immunoglobulin E (IgE) and allergen-specific IgE antibodies (Abs) in human blood. CLSI I/LA20 focuses on IgE assay design and calibration, validation and verification methods, QA of assay reagents, QC strategies, and clinical applications. It contrasts serological results with those obtained by *in vivo* skin testing and provocation testing.

The intended users of CLSI I/LA20 are health care professionals, governmental regulators and inspectors, and industrial manufacturers of allergens (extracts, molecules, epitopes) and IgE Ab assays.

CLSI I/LA20 is not intended to cover cell-based assays involving basophils (eg, basophil activation test [BAT]) or eosinophils or immunoglobulin G (IgG)/IgG4-blocking Ab assays (eg, facilitated allergen-binding assay).

1.2 Background

As early as 1921,[2] investigators showed that local sites of itching and swelling 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 Ab responsible for this reaction was identified as belonging to a new human immunoglobulin class and designated as IgE.[3-5] Scientific observations leading to the discovery of IgE are presented elsewhere.[6,7]

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 as confirmatory tests for the detection of allergen-specific IgE Abs in tissue or serum to confirm allergic sensitization. The presence of IgE Abs 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 requires a positive history involving objective allergic symptoms that are induced following a known objective allergen exposure. Since 1967, the diagnostic allergy laboratory has promoted evidence-based diagnosis of human allergic disease by using commercially available serological assays to measure total IgE and allergen-specific IgE Abs.

The first serological assay for allergen-specific IgE was developed in 1967.[8] The radioallergosorbent test was initially introduced for the detection of IgE Abs with a defined allergen specificity. This noncompetitive, heterogeneous, immunoradiometric assay used allergen immobilized on paper discs (allergosorbent) to bind specific Abs of all isotypes from serum, a buffer wash to separate free and bound human Ab, and a radiolabeled antihuman IgE Ab to detect bound IgE Abs. Subsequently, many commercial variants on this theme have been developed,[9] and many of these have subsequently been discontinued. Since then, major technological improvements have led to the development of measuring systems that exhibit an enhanced lower limit of quantitation (LLoQ), making for excellent Ab quantitation reproducibility. Significant technological advancements

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