

Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline—Second Edition

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.

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



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Abstract

Clinical and Laboratory Standards Institute document 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* is written for laboratorians/clinicians (users), manufacturers (producers), and governmental regulators (inspectors, legislators, reviewers). The guideline summarizes the current state of technology, biological specimens that are routinely tested, practical methods for the evaluation of reagents, and clinical interpretation of results. Emphasis is placed on allergen nomenclature and methods for evaluating allergen-containing assay reagents and human IgE-specific, immunologic reagents. Procedures are included for evaluating assay precision, analytical accuracy, sensitivity, parallelism, interference, and documenting the quantitative status of an assay. Guidelines for supplier validation and quality assurance, intralaboratory quality control practices, and interlaboratory proficiency testing are discussed. Finally, performance targets are outlined with recommendations for manufacturers, laboratorians, and users.

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Foreword

New developments in immunological assays for IgE antibodies of defined specificity continue to occur at a rapid pace. The 1997 I/LA20 (first edition) consensus guideline¹ has provided an initial framework from which IgE assay reagent validation and quality control, assay calibration, and quality assurance have been defined. This second edition has been written to serve as a more comprehensive compendium for laboratorians/clinicians (users), manufacturers (producers), and governmental regulators (inspectors, legislators, reviewers). Its primary goal is to foster uniformity and enhance the quality of IgE antibody measurements that are performed in clinical laboratories throughout the world. As such, the second edition expands upon the issues covered in the first edition and adds a discussion of the clinical utility of IgE antibody measurements, with a focus on their potential for supporting the clinical history in the diagnosis of allergic disease. This document provides the laboratorian/clinician with practical information that relates to the specimen, reagent validation, assay calibration and quality control, intra- and interlaboratory quality assurance, and the clinical utility of IgE antibody measurements. Emphasis has been placed on the harmonization of definitions, allergen descriptions and codes, proficiency testing survey protocols, and quality assurance methods that are used by manufacturers, regulators, and users of IgE assays throughout the world. With this document, diagnostic kit manufacturers have benchmark targets for the validation and improvement of IgE antibody assays. Regulators and inspectors will find useful targets for assessing the quality of diagnostic allergy laboratory performance. This document incorporates the most current evidence-based information that is related to IgE antibody analyses. As such, the working committee invites comment and critique.

Key Words

Allergen, allergy, assay methods, analyte-specific reagents, clinical utility, human, IgE, IgE-antibody of defined specificity, performance, quality assurance, sensitivity, specificity, total IgE, type-1 hypersensitivity

Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline—Second Edition

1 Scope

This guideline defines the current state of serological assay technology that is used to measure total IgE and IgE antibodies of defined allergen specificities. The focus of this document includes IgE assay design and calibration, methods used in the validation and quality assurance of component reagents, quality control strategies, and clinical applications.

The document is designed for use by laboratorians/clinicians (users), manufacturers (producers), and governmental regulators (inspectors, legislators, reviewers). The guideline summarizes the current state of technology, biological specimens that are routinely tested, practical methods for the evaluation of reagents, diagnostic allergy laboratory quality control strategies, and the clinical interpretation of IgE antibody results. This guideline emphasizes achievable qualification practices that have been employed by manufacturers to validate the quality and test the performance of component reagents and configured assay systems. I/LA20 also outlines methods that can be performed by clinical laboratory workers to supplement manufacturer testing and quality control practices with the ultimate goal of optimizing overall quality assurance. In addition, the document serves as a guideline for inspectors and regulators who are involved in qualifying diagnostic laboratories for IgE analysis.

The specific goals of this guideline can be summarized as follows:

- To define performance criteria and list current methods for qualification of component reagents (source allergens, final allergen-containing reagents, antihuman IgE) and configured immunoassays (analytical sensitivity and specificity, limits of detection, parallelism, precision, reproducibility) that are designed to quantify human total IgE and IgE antibody of defined allergen specificities.
- To define reasons why different IgE antibody assays may not yield comparable results.
- To outline practical manufacturer, intralaboratory, and interlaboratory quality control measures that can be incorporated into an overall quality assurance scheme to ensure valid and reproducible IgE antibody measurements.

Total serum IgE assays are discussed in this guideline to illustrate the high degree of standardization and interlaboratory agreement that can be achieved when immunoassays use a common calibration system and an internationally recognized reference preparation as a primary calibrator. All efforts have been made to provide recommendations that do not conflict with good manufacturing practices or with guidelines currently in use by regulatory agencies for clearance of new or modified allergy products.

The clinical utility of IgE antibody measurements has over the past 40 years clearly been demonstrated by research and clinical studies. The primary goal of this document is to provide the reader with unambiguous guidelines as to how reagents are validated and configured into optimized clinical assays for the measurement of total serum IgE and human IgE antibody. Potential solutions to technical challenges yet remaining are discussed in a manner that is intended to facilitate improved intermethod standardization and interlaboratory agreement in IgE antibody measurements among diagnostic allergy laboratories using the commercially available assay methods. A new feature of the revised guideline is an overview of recent evidence that supports the predictability of IgE antibody measurements in the assessment of clinical allergic disease.

2 Introduction

The term allergy, which stems from two Greek words, *allos* (different, changed) and *ergos* (work or action), was first used by Clemens von Pirquet in 1906 to describe an unusual, adverse, or heightened reactivity to a normally harmless stimulus. As early as 1921, investigators showed that local itching and swelling that was surrounded by a zone of erythema occurred when serum from an allergic person was injected intradermally into the skin of an unsensitized (nonallergic) person, followed 24 hours later by the injection of specific allergen into the same skin site. This passively transferred allergic or PK (Prausnitz Kustner) reaction² reached a maximum within 10 minutes, persisted for about 20 minutes, and then gradually disappeared. In 1967, the antibody responsible for this was identified as belonging to a new human immunoglobulin class and designated 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, physical examination, and 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 confirmatory tests.^{8,9} For over 40 years, the diagnostic allergy laboratory has played a key role in facilitating the diagnosis of human allergic diseases by using commercially available assays to measure total IgE and allergen-specific IgE antibodies. The first assay for allergen-specific IgE was developed in 1967.¹⁰ Since then, technical improvements have led to enhanced analytical sensitivity, quantitation, and reproducibility of some currently available assay methods. The most significant technical advance has been the high degree of quantitation and interlaboratory standardization that has been possible with automation, and widely adopted calibration methods traceable to an international IgE reference preparation. For the most part, the diverse calibration schemes with their confusing units that have little in common with each other have been replaced by a common IgE antibody unit: kIUa/L. As the number of allergen specificities offered in these IgE antibody kits has continued to expand, redundant and often conflicting coding schemes for the allergens have increased in use. This document represents the first effort at harmonization of allergen codes used by manufacturers of allergen-specific IgE antibody assays.

Increased regulations have also placed new demands on manufacturers, regulators, and users of total and allergen-specific IgE antibody assays for more effective documentation of quality assurance. This guideline was written with the goal of providing consensus on these issues and creating two sets of operationally achievable procedures: one that can be used by assay manufacturers to validate the quality and test the performance of the component reagents and configured assay systems, and a second that can be used by investigators in clinical laboratories to ensure maximal quality of their reported IgE antibody results.

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 blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.¹¹ 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 M29.¹²

4 Terminology

4.1 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 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 *accuracy*, in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, and comprises both random and systematic effects. Precision and trueness refer to the average of values obtained from a large (infinite) series of results of measurements. In particular, *trueness* means the closeness of agreement between this average value and a true value (or a reference value) while *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/LA20-A2, 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*, because in Europe, the term “clinical” often refers to clinical studies of drugs under stringent conditions.

Users of I/LA20-A2 should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in Section 4.2 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.

4.2 Definitions

accuracy (measurement accuracy//result accuracy) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (VIM07).¹³

affinity – in immunology, a measure of the attraction or force of association between a single antigenic site and a single antibody to that site (K_a).

affinity constant (K_a) – in immunology, the equilibrium constant for the receptor + ligand reaction¹⁴;
NOTE 1: Strictly, the term only applies to homogeneous receptors and their ligands, eg, monoclonal antibodies. However, polyclonal antibody preparations, which are heterogeneous in their affinity for

homogeneous ligands, are often used; **NOTE 2:** The term also expresses the intrinsic binding strength of a receptor-ligand pair.

affinity-independent assays – an assay is affinity-independent whenever the product of K_a and R_f (molar concentration of binding sites) is greater than 10; when this occurs, the assay is considered to be in receptor excess.

allergen – an immunogen that when introduced into a host elicits the formation of IgE antibodies; **NOTE:** Appendix A contains a list of identified and partially characterized native and recombinant allergens.

allergen extract – a mixture of molecules, typically, proteins, glycoproteins, lipoproteins, or protein-conjugated chemicals/drugs that have been solubilized from a defined (usually biological) source and that a portion of which can elicit an IgE antibody response in exposed persons; **NOTE:** Appendix A contains a list of available allergen extracts.

allergen potency – the composite concentration of all of the allergenic epitopes within an allergen that together produce a defined biological (type 1 hypersensitivity, IgE-mediated) response in allergic persons.

allergen reagent – the component of the IgE antibody assay that contains allergen either bound to a solid phase (immobilized allergen) or in solution phase (liquid allergen); **NOTE:** These reagents, like the allergen extracts from which they are derived, are typically composed of heterogeneous mixtures of proteins or chemicals/drugs that are conjugated on carrier proteins. More recently, purified components of native allergens or recombinant allergens have been successfully used. Ideally, the allergen reagent should contain all of the allergenic epitopes of that specificity that elicit IgE antibodies in humans.

allergen source (raw) material – the starting raw material from which allergenic extracts are obtained; **NOTE:** This material may or may not have been physically processed to remove extraneous, nonbiological materials, and it is typified by materials such as raw pollen, animal hair, mold cultures, drugs, venoms, food stuffs, or recombinant expressed proteins.

allergenic epitope – a submolecular structure primarily responsible for IgE antibody binding.

allergenic molecule – a single component (typically, a protein, glycoprotein, or lipoprotein) of a biological substance that is highly purified from extracts of native, biological material or produced by recombinant molecular biology techniques or other means for artificial epitope production methods.

allergosorbent – a solid phase material to which allergenic molecules are attached by covalent coupling methods or adsorption.

analyte – component represented in the name of a measurable quantity (ISO 17511)¹⁵; **NOTE 1:** The analyte is the constituent or characteristic of the sample to be measured; **NOTE 2:** This includes any element, ion, compound, substance, factor, infectious agent, cell, organelle, activity (enzymatic, hormonal, or immunological), or property, the presence or absence, concentration, activity, intensity, or other characteristics of which are to be determined; **NOTE 3:** For the diagnostic allergy laboratory, allergen-specific IgE molecules may be considered the primary analyte of interest.

analyte-specific reagents (ASRs) – antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents, which, through specific binding or chemical reactions with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens¹⁶;

NOTE: ASRs can be viewed as having the following characteristics: used to detect a single ligand; no instructions or performance claims; and not promoted for use on specific instruments or in specific tests or test systems. The ASR rule was designed to accomplish several policy objectives: 1) ensuring the quality of materials used as components of in-house laboratory tests, 2) providing appropriate labeling so that health care users would understand the level of test validation.¹⁶

ASRs are intended to be unique reagents that are not marketed in a form that is combined with other reagents or support materials and have not been optimized to work on a proprietary or preoptimized analytical system (one in which the software and hardware has been specifically designed to allow for detection of signals generated by that reagent). Where reagents are marketed in this manner, the reagent is not considered an ASR but a part of a total test system and it should be submitted to an applicable regulatory agency for premarket review. Although it may be possible for an ASR to be used for purposes of allergen-specific IgE testing, to date most if not all existing test systems are configured in such a manner that precludes use of ASRs. Of note, use of ASRs for testing of rare allergens is a particularly problematic practice because it is unlikely that most laboratories could assemble analytical and clinical materials needed to adequately validate such as assay.

In contrast to an ASR, a “General Purpose Reagent” (GPR) is “a chemical reagent that has general laboratory application, that is used to collect, prepare, and examine specimens from the human body for diagnostic purposes, and that is not labeled or otherwise intended for a specific diagnostic application.”¹⁶ Like ASRs, GPRs are not labeled for a specific clinical or diagnostic use. Because GPRs are not analyte-specific, they should be able to be combined with, or used in conjunction with more than one ASR. In contrast, as stated above, an ASR is a specific chemical component, probe, or antibody that can detect an individual chemical substance or ligand. An ASR is considered the “active ingredient” or “building block” of a laboratory-developed test.

antibody – 1) any of numerous Y-shaped protein molecules produced by B cells as a primary immune defense, each molecule and its clone having a unique binding site that can combine with the complementary site of an antigen, as on a virus or bacterium, thereby signaling other immune defenses; **2)** the functional component of antiserum, often referred to collectively as a population of molecules, each member of which is capable of reacting with (binding to) a specific antigenic determinant; **NOTE 1:** An antibody molecule is, by definition, monospecific, but it might also be *idiospecific*, *heterospecific*, *polyspecific*, or of *unwanted specificity*. It cannot be nonspecific, except in the sense of nonimmunochemical binding; **NOTE 2:** These proteins are immunoglobulins and bind by means of specific binding sites to a specific antigenic determinant.

antigen – in immunology, any substance that can stimulate the production of antibodies by an organism and combine specifically with them.

atopy – the inherited tendency to develop immediate-type hypersensitivity to common and generally harmless substances.⁹

avidity – the combined intensity of attraction of all antigenic sites on an antigen molecule and all antibodies to those sites^{14,17}; **NOTE:** Avidity depends on the antibody isotype (valence), the size and conformation of the antigen molecules, and the number of antigenic epitopes.

binding capacity – within the context of this document, the binding capacity refers to the number of human IgE antibody molecules that an allergen-containing reagent (eg, allergosorbent, liquid-phase allergen) can bind reproducibly under standardized assay conditions (pH, ionic strength, protein matrix, time, temperature); **NOTE:** The binding capacity of a reagent is highly dependent on the number of immunoreactive allergen molecules and individual epitopes attached to the solid phase or present in the solution phase reagent. For manufacturers, the goal is to identify allergen-containing reagent preparation

conditions that produce maximal binding of allergen to the reagent solid phase in a relevant and reproducible manner.

calibrated assays – three levels of calibrated IgE antibody assays are proposed as follows: 1) titration assays that use an arbitrary reference system to define a class or arbitrary value; this category of calibration can reliably discriminate between doubling dilutions, but it does not ensure parallelism; 2) calibrated titration assays, such as IgE antibody assays that are calibrated with a heterologous serum IgE dose-response curve and that generate reliable, reproducible results; these assays behave consistently with respect to parallelism; 3) quantitative assays as represented by the total serum IgE assay but which are not commercially available for IgE antibody quantification; **NOTE:** These are assays that use a homologous calibrator.¹⁷ They produce reproducible and accurate results, and behave appropriately with respect to parallelism and recovery. The number of calibrators must be in context to the measurement system. Factory-calibrated procedures, which are often stored in random access memory, may have one or more “adjusters” that normalize response data and can reproducibly and accurately determine analyte concentrations for many immunoassay runs.

calibration material/calibrator – a material (eg, solution) or device of known quantitative/qualitative characteristics (eg, concentration, activity, intensity, reactivity) used to calibrate, graduate, or adjust a measurement procedure or to compare the response obtained with the response of a test specimen/sample; **NOTE 1:** The quantities of the analytes of interest in the calibration material are known within limits ascertained during its preparation and may be used to establish the relationship of an analytical method’s response to the characteristic measured for all methods or restricted to some; **NOTE 2:** The calibration material must be traceable to a national or international reference preparation or reference material when these are available; **NOTE 3:** Calibration materials with different amounts of analytes may be used to establish a calibration or response “curve” over a range of interest; **NOTE 4:** The term “standard,” commonly used in clinical laboratory protocols and having there a meaning as described here, has a specific and different meaning in the US Federal Register, February 28, 1992); **NOTE 5:** The terms “primary” and “secondary standard” are used by WHO and ISO to refer to calibration materials.

conjugate – an assay reagent that is produced by covalently attaching two (or more) substances to each other such as an antibody with a second biolabel (enzyme [horseradish peroxidase, alkaline phosphatase] or biotin), radiolabel, or fluorophor; **NOTE:** In the solid-phase, two-site immunometric assays that have been used historically in diagnostic allergy laboratories, the conjugate is commonly a labeled antihuman IgE reagent. In some recent fluid-phase assays, the conjugate may be a biolabeled allergen reagent.

control/control material – a device, solution, lyophilized preparation, or pool of collected human or animal specimens, or artificially derived materials, intended for use in the quality control process; **NOTE:** The control serum should possess a matrix similar in ionic charge, density, pH, and protein content to that of the test specimens. It serves as the primary quality control check on the validity of the calibration-reference curve, the assay reagents, and any required equipment. Moreover, it allows computation of interassay variation using values obtained from more than 10 individual assay runs. A range of values (2 SD, 95% confidence interval) are computed for the control specimen and should be used in Levey-Jennings quality control charts to identify assays that are in or out of control.

cross-reactivity – *in immunology*, the reaction of an antibody with an antigen other than that which elicited its formation, as a result of shared, similar, or identical antigenic determinants; **NOTE:** Within the context of this document, cross-reactivity has two meanings. First, it refers to a human IgE antibody that binds to an allergenic epitope that is structurally similar to, but not identical with, the molecule that elicited its formation. Cross-reactivity results from shared, similar, or identical allergenic determinants. There are many illustrations of cross-reactive allergen molecules—for example, among the Hymenoptera (vespid) venoms (see allergen section below). Second, cross-reactivity can refer to the degree to which the monoclonal or polyclonal antihuman IgE detection reagents bind to other human immunoglobulin isotypes (IgG, IgA, IgM, IgD).

data reduction algorithm – a mathematical process that converts assay-response data (eg, counts per minute [CPM]-bound, absorbance units) into interpolated dose results; **NOTE:** The dose-response relationship in the assay is defined by the standard, reference, or calibration curve.

depletion analysis – a procedure used to estimate the quantity of IgE antibody in the calibration process of a prospective reference serum; **NOTE:** This technique has not been widely used for estimating the quantity of IgG, IgA, or IgM antibody of defined antigen specificities in other prospective reference sera because the percentage of the total immunoglobulin of these isotypes that is specific antibody directed to a particular antigen is low generally in comparison to the percentage of IgE that is specific for one allergen. This leads to inaccuracies that prevent the successful use of this method in calibrating reference sera. Using short ragweed as an illustrative allergen specificity, a potent IgE antiragweed containing serum is first optimally preabsorbed with either ragweed-solid phase or a sham-solid phase. Three sera (unabsorbed, sham-absorbed, and ragweed-absorbed) are then analyzed in a total serum IgE for their IgE content. Difference in the total IgE levels between the sham- and ragweed-absorbed serum represents the amount of specific IgE that was depleted or removed from the serum by the solid-phase allergen.^{17,18} The sham-adsorbed IgE antibody levels should be equivalent to unabsorbed serum levels. Elution of the ragweed-solid phase adsorbed IgE antibody can be added to the procedure to validate the efficiency of the extraction procedure and provide an additional level of confidence in the specific antibody estimation.

detection limit/limit of detection – lowest amount of analyte in a sample that can be detected with (stated) probability, although perhaps not quantified as an exact value (revised from WHO-BS/95.1793)¹⁹; **NOTE 1:** Detection limit is the smallest quantity of an analyte that can be reproducibly and statistically distinguished from the background (including variation in background), or a zero calibrator in a given assay system; **NOTE 2:** It is usually defined at the 95% confidence interval and has also been called the lower detection limit or positive threshold of the assay; this term is not synonymous with analytical sensitivity; **NOTE 3:** Assuming an adequate number of samples and that their distributions are similar, an equivalent distribution between the 0 calibrator and low positives, the 95% confidence interval means 3.3 standard deviations between the mean levels produced by the 0 calibrator and the low positives. The positive cutoff level should, therefore, be greater than 3.3 SD from the mean of the 0 calibrator. See CLSI/NCCLS document EP17.²⁰

diagnostic (clinical) sensitivity – as used in this guideline, the ability of a test to correctly identify samples in which allergen-specific IgE antibody is present; it correlates the presence of allergen-specific IgE antibody in the serum of patients who are defined by clinical criteria and/or gold standard laboratory tests as truly allergic; **NOTE:** Sensitivity for IgE antibody is ideally determined by testing a panel of serum samples from subjects with a positive history of allergic disease and evidence of IgE antibody positivity by an alternative accepted method such as skin testing, provocation testing, and/or an accepted reference serology method (provided its sensitivity and specificity are known). The serum panel should reflect patients from a variety of allergic disease states relevant to that allergen specificity as well as healthy nonatopic controls.

diagnostic (clinical) specificity – as used in this guideline, the ability of a test to correctly identify samples in which allergen-specific IgE antibody is absent; it correlates the absence of allergen-specific IgE antibody in the serum of patients who are defined by clinical criteria and/or gold standard laboratory tests as truly not allergic; **NOTE:** Specificity for IgE antibody is ideally determined by testing a panel of serum samples from subjects with a negative history of allergic disease and evidence of the absence of IgE antibody positivity by an alternative accepted method such as skin testing, provocation testing, and/or an accepted reference serology method (provided its sensitivity and specificity are known). The serum panel should reflect patients from a variety of allergic disease states relevant to that allergen specificity as positive controls as well as healthy nonatopic negative controls.

efficiency – a statistical parameter that defines the percentage (number fraction multiplied by 100) of results that are true results as measured by an analytical method.

epitope/determinant – **1)** the minimum molecular structure of the antigenic site that will react with a monoclonal antibody; **2)** any site on an antigen molecule at which an antibody can bind; the chemical structure of the site determining the specific combining antibody; **NOTE:** In the context of IgE assays, allergenic epitopes are regions on allergens that bind directly to the IgE binding site. They can be detected by monoclonal antibodies to (1) determine the level of allergens of a particular specificity in an environmental specimen (eg, *Der p 1* and *Der f 1* in house dust); and (2) demonstrate identity and qualify extracts during the manufacturing of allergen-containing reagents.

free IgE – human IgE that circulates in blood in an unbound state, free of therapeutically administered humanized IgG anti-IgE, soluble IgE receptors, or other binding factors; **NOTE 1:** Research assays for free IgE that use the alpha chain of the high affinity FcεR1 receptor as the IgE detection reagent have been developed²¹ but are not yet commercially available; **NOTE 2:** See **IgE** below.

heterologous interpolation – a calibration scheme in which the standard or reference (calibration) dose-response curve is constructed using reagents that have a different (heterologous) specificity from those being used to measure the analyte of interest¹⁷; **NOTE:** This assay involves the performance of two (or more) simultaneously performed assays with different sets of reagents. The first is the calibration portion of the assay that can be illustrated by a total serum IgE standard (calibration) curve. The second portion of the assay involves the measurement of IgE antibody to a defined allergen specificity (eg, *Ambrosia artemisiifolia*, common ragweed). Once the analyte (IgE) is bound in both the calibration and antibody detection sections of the assay, the same conjugated, antihuman IgE detection reagent is added to both. The response results (CPM-bound, optical density, fluorescence signal units) generated in the IgE antishort ragweed portion of the assay are interpolated from the (heterologous) total serum IgE calibration curve in international units per milliliter (IU_A/mL) or mass units per milliliter (ng/mL) of IgE, which are calibrated back to an IgE primary reference standard. Parallelism between the heterologous calibration curve and dose-response curve of test specimens analyzed at multiple dilutions is a requirement for the successful use of the heterologous interpolation calibration scheme.

heterophilic antibodies – antibodies in test sera that can bind to immunoglobulins from other species (eg, human antimouse immunoglobulins); **NOTE:** The observed reactivity may be specific, as seen with sera from animal handlers or subjects that have received a therapeutic monoclonal antibody drug or immune serum, or nonspecific as a result of human autoantibody cross-reactivity (eg, rheumatoid factor). In either case, these heterophile antibodies can induce false-positive or false-negative clinical test results depending on the assay design. See CLSI document I/LA30.²²

homologous interpolation – a calibration scheme in which the standard or reference (calibration) dose-response curve is constructed using reagents that have the same (homologous) specificity from those being used to measure the analyte of interest¹⁷; **NOTE 1:** In this assay, both the calibrator and test specimens are analyzed using the identical assay reagents; **NOTE 2:** In an assay that measures ragweed-specific IgE antibody, for instance, the calibration curve is constructed with multiple dilutions of a serum containing a predefined amount of ragweed-specific IgE antibody. Test sera are simultaneously analyzed using the same reagents for IgE antiragweed. When complete, the response results (CPM-bound, absorbance, fluorescence signal units) generated for the test sera are interpolated from the IgE antishort ragweed calibration curve that has been precalibrated in arbitrary units or mass units per volume (eg, ng/mL). Depletion analysis, with or without elution, can be used to prepare an IgE antibody reference serum that has an assigned mass/volume quantity of allergen-specific IgE antibody^{18,23}; **NOTE 3:** Homologous interpolation is the conventional calibration scheme used by most clinical immunoassays; however, it has been rarely applied to IgE antibody assays, because large amounts of serum-containing IgE antibodies of all clinically relevant specificities are difficult to prepare. Moreover, since epitope specificities are rarely defined and they may differ with respect to the reference and test sera, homologous interpolation within the context of IgE assays is impractical.

IgE – human IgE is an immunoglobulin of the approximate molecular weight of 190 000, which exists normally in monomeric form and constitutes approximately 0.0005% of the total serum immunoglobulins; **NOTE:** It binds with high affinity to FcεR1 receptors mainly expressed on mast cells and basophils and FcεR2 receptors on a number of other cells. IgE mediates the production and release of vasoactive mediators following the binding of allergen. (See Table 1 for the physical and chemical properties of IgE antibodies.)

immunoassay – any laboratory method for detecting a substance by using an antibody reactive with it (RHUD2CD); **NOTE:** Immunoassays can be competitive or noncompetitive, solid or liquid phase, isotopic or nonisotopic, labeled antigen or immunometric (labeled antibody), single or dual site, homogeneous (no separation step), or heterogeneous (separation step). The majority of clinically used total IgE and allergen-specific IgE assays are noncompetitive heterogeneous immunometric assays.

immunodiffusion – an immunological method for measuring antigens (eg, serum proteins) in which a small amount of serum (eg, 5 µL) is pipetted into a well cut into a porous agarose gel; **NOTE 1:** In the single immunodiffusion or Mancini assay, the gel contains antibody specific for the analyte of interest. As the analyte migrates through the gel by diffusion, it binds to antibody and forms a precipitin line at a point of optimal antigen-antibody binding or equivalence. In the double immunodiffusion assay, antigen and specific antibody are pipetted into separate wells in the same gel and they diffuse in all directions. A precipitin line forms at equivalence (the point of maximal antigen-antibody cross-linking). The shape and location of lines and diameter of precipitin rings provide information about the analyte's quantity and quality; **NOTE 2:** Immunodiffusion is not used to measure IgE antibodies in serum because of its inadequate lower limit of detection. Rather, it can be used by allergen manufacturers to qualify allergen extracts as part of a quality assurance program.

immunoglobulin – a glycoprotein composed of two heavy and two light chains that functions as an antibody; **NOTE:** Human immunoglobulins have been subdivided into different classes or isotypes (IgM, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE), each of which possesses a unique set of antigenic markers, physiochemical properties, and each of which produce a different pattern of effector functions (receptor binding, complement activation, opsonization). All antibodies are immunoglobulins, but it is not certain that all immunoglobulins possess antibody function. (IgE and IgG antibodies are contrasted in Table 1.)

isoallergens – proteins (allergens) that are essentially identical except for minor differences in their primary amino acid composition or substituted side chains.³⁶

laboratory developed test (LDT) – a test developed by a laboratory for which the laboratory takes responsibility for the quality control and the appropriate validation of any claims made.

linearity – the ability (within a given range) to provide results that are directly proportional to the concentration (amount) of the analyte in the test sample (WHO-BS/95.1793)¹⁹; **NOTE:** Linearity typically refers to overall system response (ie, the final analytical answer rather than the raw instrument output (see CLSI/NCCLS document EP06 and CLSI document EP10^{24,25}). See “parallelism.” It can be defined by evaluating the slope of a dose response curve on a semilog plot over a defined working range.

matrix – all components of a material system, except the analyte (ISO 15193)²⁶; **NOTE:** The matrix of the calibrator, standard, and the test specimens should be comparable to maximize parallelism and ensure that the degree of nonspecific binding is comparable between the calibration and test specimen portions of the assay. Serum from a nonatopic person that is devoid of IgE is considered an ideal diluent or protein matrix for IgE antibody assays.

measurand – quantity intended to be measured (VIM07)¹³; **NOTE:** The term “measurand” and its definition encompass all quantities, while the commonly used term “analyte” refers to a tangible entity subject to measurement.

multiplexed assay – a test delivering more than one test result based on a single addition of a test specimen; **NOTE:** Multiplex assays can be discriminated from singleplex assays where one addition of a specimen results in IgE antibody measurement to one allergen specificity. A multiallergen screen that involves multiple allergens in one allergen-containing reagent generates a single result, and thus should be considered a singleplex assay.

MUXF3 – Bromelain is a glycoprotein extracted from pineapple, which has been used for checking the cross-reactivity between a glycan and other glycoproteins. Its carbohydrate epitope MUXF3 is found in many plant proteins; however, a true allergy to bromelain is rare. MUXF3 is a carbohydrate digested from bromelain that is used as a reagent that contains the carbohydrate epitope and thus avoids the detection of IgE antibody binding to other bromelain epitopes.

native allergenic molecules – allergenic proteins, glycoproteins, and lipoproteins that have been purified by chemical, chromatographic, electrophoretic and/or immunoaffinity techniques from allergen extracts of natural biological materials and that can elicit an IgE response.

nonspecific binding (NSB) – attachment not attributable to specific binding to a receptor (see CLSI/NCCLS document LA01²⁷); **NOTE:** Typically, the NSB is defined as the background signal in an immunoassay that is derived from the nonspecific association of the signal generator with other reactants or the reaction vessel itself. The NSB of an assay plays a critical role in determining its analytical sensitivity characteristics. In IgE antibody assays, background binding can also be affected by the total level of IgE antibodies present in a specimen, because IgE may also associate nonspecifically with reactants, giving a falsely high measure of specific IgE antibody levels.

omalizumab – for asthma and allergy patients who receive subcutaneous injections of omalizumab, which is a humanized IgG1-kappa antihuman IgE Fc therapy, their IgE will exist in two states: free IgE (unbound with omalizumab) and complexed omalizumab-bound IgE, which is blocked by the anti-IgE from binding to the alpha chain of the high affinity Fc-εR1 receptor; **NOTE:** Caution should be exercised when analyzing blood from allergy patients receiving omalizumab, since the therapeutically administered anti-IgE can alter the accuracy of some total and allergen-specific IgE assays.²⁸

parallelism – extent to which the dose-response relationship between two materials (ie, calibrator vs unknown specimens) is constant for the examined range of concentrations (see CLSI/NCCLS document LA01)²⁷; **NOTE:** Parallelism is a property (and a requirement) of quantitative immunoassays in which the calibrator and test sera produce parallel dose-response curves. As a consequence, the same estimate of analyte concentration in the test specimen is generated by the assay once it is corrected for dilution, irrespective of the actual dilution of the specimen analyzed. Good parallelism can be demonstrated by analysis of multiple dilutions of serum in the same assay run. Low interdilutional “dose” coefficients of variation provide evidence that the calibrator and test portions of the immunoassay are parallel. See “linearity.”

point-of-care testing (POCT) – testing that is performed near or at the site of the patient care with the result leading to possible change in the care of the patient (ISO 22870)²⁹; **NOTE 1:** Point-of-care testing is diagnostic tests that are performed at the point of care (eg, bedside, outpatient clinic, community pharmacy, or home); **NOTE 2:** These tests are often miniaturized hand-held devices that produce a rapid analytical result with less than ideal analytical sensitivity and quantitation.

practice parameters – strategies for patient management developed to assist medical professionals in clinical decision-making; **NOTE:** Practice parameters may also be called practice options, practice

guidelines, practice policies or practice standards. They are documents that define, from the perspective of the health care provider, effective means of diagnosing, treating, and managing various conditions and diseases. They are usually based upon literature review (scientific research in the form of randomized, controlled clinical trials, reports of series, or case studies) or “expert clinical experience and opinions.” They are prepared by individuals with expertise in the field.

precision – 1) the closeness of agreement between independent test results obtained under prescribed conditions (ISO Guide 30)³⁰; **2)** closeness of agreement between a series of measurements, under specified conditions, of a substance or biological product (WHO-BS/95.1793)¹⁹; **3)** the closeness of agreement between independent test results obtained under stipulated conditions (ISO 3534-1)³¹; **4)** agreement between replicate measurements; **NOTE 1:** Precision has no numerical value but is expressed in terms of imprecision—the SD or the CV of the results in a set of replicate measurements (see CLSI/NCCLS document H26)³²; **NOTE 2:** Intra-assay precision refers to agreement within an individual assay run. Interassay precision refers to results obtained from different sets of assays.

precision profile – the precision of an assay across the analyte concentration range of interest; **NOTE:** A precision profile is constructed by determining the standard deviation (or coefficient of variation) of replicate measurements (within assays, between assays, or between specimen dilutions within an assay) spanning the entire analyte concentration range, albeit without the exact knowledge of the true analyte concentration that is contained in the serum specimens.³³ When the CV_{dose} (Y-axis) is graphed against the dose (X-axis), a precision profile plot is generated. The precision profile is also referred to as the “imprecision profile” by some investigators.

predicate device – an assay or device for the *in vitro* measurement of an allergen-specific IgE antibody that has been cleared for marketing by the applicable government regulation or that was in commercial distribution before 1976; **NOTE:** The performance of a new assay or device can be measured and reported in terms of increased or decreased analytical sensitivity and analytical specificity, and limitations for use in direct comparison with a predicate assay.

proficiency testing (PT) – a program in which multiple samples are periodically sent to members of a group of laboratories for analysis and/or identification, in which each laboratory’s results are compared with those of other laboratories in the group and/or with an assigned value, and reported to the participating laboratory and others; **NOTE 1:** PT is an independent (nonmanufacturer sponsored) program in which challenge specimens (eg, serum) are sent to participating clinical laboratories to be evaluated in assays that measure a spectrum of analytes; **NOTE 2:** The purpose of PT is to evaluate the laboratory’s proficiency to measure a particular analyte. The results are returned to the sponsoring agency, which computes the performance of a given laboratory for a particular analyte using a particular method. Issues related to PT include computation of method performance using mean, standard deviation, coefficient of variation measures to assess accuracy, and interlaboratory reproducibility of peer groups (that use the same and different assay methods).

qualitative assay – an assay system that produces an indication of the presence or absence of an analyte but does not provide a precise estimate of the concentration of that analyte; a positive test result implies only that the assay signal exceeds the analytical threshold or positive cutoff point that has been set to obtain an arbitrary combination of diagnostic sensitivity and specificity; **NOTE:** A positive assay signal should relate to a certain presence of IgE antibody specific to the allergen tested in the subject’s blood.

quantitative assay – an assay system that produces an accurate and reproducible estimate of the concentration of an analyte, such as IgE antibody, in the test specimen; its analysis can employ homologous or heterologous interpolation from a calibration curve, which is referenced to a readily available standard reference preparation; **NOTE:** The units reported from quantitative assays for total serum IgE are traceable to a defined serum standard (eg, World Health Organization [WHO] standard material 75/502) that is available to all assay manufacturers. Because of the general absence of calibrated

homologous human IgE antibody standards, current quantitative assays for IgE antibody can be calibrated by heterologous interpolation from a total human serum IgE reference curve. Quantitative assays tend to be among the most complex tests (eg, immunoassays) and their results can be reported in gravimetric (ng/mL [total serum IgE assays]) or international units (IU/mL [total IgE assays] or IUa/mL [allergen-specific IgE antibody assays]), where 1 IU is equivalent to 2.42 ng of IgE.

The first allergen-specific IgE assay developed in 1967 was the radioallergosorbent test (RAST),¹⁰ which employed a calibration system based on arbitrary units (eg, PRU/mL, AU/mL) and produced, at best, semiquantitative IgE antibody results. These assays were not automated. Gradually, allergen-specific IgE assays evolved with manual pipetting and a defined accessible calibrator that is based on a widely available reference material (eg, WHO standard material 75/502). This assay quantified IgE in units that were directly related to the amount of IgE antibody that was present in the specimen. Current assays have evolved into quantitative assays that involve full “walk away” automated platform instrumentation. These assays represent the most precise, rapid, and quantitative assay designs available for IgE antibody measurement.

radioallergosorbent test (RAST) – the acronym “RAST” should now be considered a historic term, since radioisotopes are rarely used today in clinical assays for IgE antibody; **NOTE:** In 1967, the RAST was developed as the first immunoassay system available for the measurement of human IgE antibodies to defined allergen specificities in serum.¹⁰ In its original form, the RAST employed a paper disc solid phase to which allergen was covalently attached (allergosorbent) to bind allergen-specific antibodies of all isotypes (primarily IgE, IgG, IgA) from serum. Following a buffer wash to remove unbound serum proteins, bound IgE was detected with I¹²⁵-labeled polyclonal antihuman IgE. Results were reported in log-related classes or in arbitrary units by interpolation from a heterologous IgE antibody pollen reference curve. Many minor modifications of this original assay format have been commercialized and they are discussed in the text. When referring to serological IgE antibody assays, the term RAST should no longer be used as a generic descriptor to refer to an IgE antibody assay unless it describes a radioisotopic allergosorbent assay for human IgE antibody.

recombinant allergens – allergenic molecules produced in a heterologous cell system using a recombinant gene construct encoding the allergenic protein; **NOTE:** A description of the process of cloning, sequencing, transfecting expression vectors, and expressing allergenic peptides and proteins is beyond the scope of this document. Some IgE antibody assays currently use some recombinant allergens to supplement natural allergen extracts and replace labile, low abundance, or absent allergens (eg, rHev b 5 in the *Hevea brasiliensis* latex containing reagent).

recovery – the measurable increase in analyte concentration in a sample after adding a known amount of an identical substance (see CLSI/NCCLS document LA01)²⁷; **NOTE:** Unspiked and spiked serum specimens are then analyzed in the assay, and the proportion of the analyte that is detected over preexisting analyte levels is compared to the amount added. Accuracy in identifying the amount of analyte added is essential to a recovery study. A recovery of 100% is considered ideal. An alternative form of recovery studies called dilution-recovery analysis is presented in Appendix B.

semiquantitative assay – a semiquantitative assay system provides an additional option over the qualitative assay in terms of defining the magnitude of the response. The variations in the positive signal detected by the assay are commonly presented in terms of a series of increasing grades or classes (eg, I to VI, low to high); in arbitrarily defined units per milliliter determined relative to a supplier-specific heterologous dose-response curve or an end-point dilution at which the assay signal becomes negative (eg, titer); or in comparison to a qualitative grading scheme (eg, color chart); **NOTE:** Allergen-specific IgE assays that use normalized counts generated in two-point calibrated, modified, or alternative scoring systems are considered semiquantitative assays unless demonstrated otherwise.

sensitivity (analytical) – **1)** the sensitivity of a method as the slope of the calibration curve and the ability of an analytical procedure to produce a change in the signal for a defined change of the quantity; **2)** change in the response of the measuring system divided by the corresponding change in the quantity or property of the analyte; **NOTE:** This term should not be used synonymously with *detection limit*.

specificity (analytical) – ability of a measurement procedure to measure solely the measurand (ISO 17511)¹⁵; **NOTE 1:** Specificity is the ability of an analytical method to determine only the component it purports to measure; the extent to which the assay responds only to (all subsets of) a specified analyte and not to other substances present in the sample; **NOTE 2:** Within the context of this guideline, specificity refers to an assay that is specific for IgE and shows no cross-reactivity of the antihuman IgE reagent with other classes of human antibodies (eg, IgG, IgA, IgM, and IgD). Tests of reagent specificity shall demonstrate that the antibody being measured is IgE and that it is specific for the allergen of interest based on soluble allergen inhibition studies. The specific IgE assay system should be tested for interfering substances, including but not limited to, lipids, hemoglobin, and medications commonly used by allergic patients, and any known interference should be identified in the manufacturer's product literature.

specimen – the discrete portion of a body fluid (typically in the case of this report: serum/plasma) taken for analysis of one or more quantities or characteristics to determine the character of the whole (modified from ISO/DIS 18113-1).³⁴

trueness (measurement) – closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value (VIM07)¹³; **NOTE 1:** Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725 (VIM07)¹³; **NOTE 2:** Measurement trueness is inversely related to systematic measurement error, but is not related to random measurement error (VIM07)¹³; **NOTE 3:** Measurement accuracy should not be used for 'measurement trueness' and vice versa (VIM07)¹³; **NOTE 4:** In the case of total serum IgE assays, the WHO IgE Standard 75/502^{35,36} (1 IU = 2.42 ng) and the United States IgE Standard³⁷ have been used as primary reference material to promote trueness. For IgE antibody assays of defined allergen specificity, there are currently no universally accepted IgE antibody standards with calibrated levels of allergen-specific IgE. Heterologous interpolation of allergen-specific IgE antibody results from a total IgE dose response curve has become a universally accepted calibration method. Over the years, several research-based secondary IgE antibody standards have been prepared with mass per volume unit estimates using antibody adsorption or depletion methods.^{18,23}

validation – confirmation, through the provision of objective evidence, that specified requirements have been fulfilled (modified from ISO 9000)³⁸; **NOTE:** A one-time process completed to determine or confirm test performance characteristics before the test system is used for patient testing.

4.3 Abbreviations/Acronyms

ASR	analyte specific reagent
CAP	College of American Pathologists
CCD	cross-reactive carbohydrate determinant
DBPCFC	double-blind, placebo-control food challenge
ELISA	enzyme-linked immunoabsorbent assay
FEIA	fluorescent enzyme-linked immunoassay
kiU/L	kilo international units per liter of IgE: 1 IU of IgE = 2.44 ng of IgE
KIU _A /L	kilo international units per liter of allergen-specific IgE: 1 IU of IgE=2.42 ng of IgE
MUXF3	a cross-reactive carbohydrate determinant derived from bromelain
POCT	point-of-care tests
RAST	radioallergosorbent test
RIST	radioimmunosorbent test

5 Biochemical and Clinical Properties of IgE

In 1967, IgE was identified as a unique human immunoglobulin class or isotype.³⁻⁷ Table 1 contrasts the known biochemical and physical properties of IgE (reaginic) antibodies with those documented for the four subclasses of IgG antibodies that may be involved in the modulation of the human allergic reaction. Structurally, human IgE molecules contain two light chains (kappa, lambda) that are indistinguishable from the light chains of IgG, IgM, IgA, and IgD. IgE's two heavy (epsilon) chains contain five structural domains (V_H , $C_{\epsilon}1$, $C_{\epsilon}2$, $C_{\epsilon}3$, and $C_{\epsilon}4$), which possess its unique antigenic attributes that confer its special biological properties. IgE has 18% carbohydrate and a reported molecular weight of 190 000 daltons. Total IgE levels in serum are at the lowest concentration of any of the five human immunoglobulin isotypes (0 to 0.00004 g/L, 0.0005% of the total adult serum immunoglobulin in nonatopic persons). Approximately 50% of IgE is distributed in the extravascular space. Its short biological half-life of one to five days in peripheral blood is partly due to IgE's relatively high fractional catabolic rate and partly due to its exodus from the plasma into tissue. While IgE does not pass the placenta or activate the classical complement pathway, its reaginic (mast cell sensitizing) activity is dependent on its ability to bind to the alpha chain of the high affinity IgE Fc-epsilon receptor ($Fc_{\epsilon}RI$), which resides on the membrane surface of basophils and tissue mast cells.

Table 1. Physical and Chemical Properties of IgE and IgG Antibodies.³⁹ Reprinted with permission from Taylor & Francis Group LLC.

Property	IgE	IgG1	IgG2	IgG3	IgG4
Heavy (H) chain class	epsilon	gamma1	gamma2	gamma3	gamma4
H chain molecular weight	70 000	50 000	50 000	60 000	50 000
H chain-carbohydrate average %	18	3-4	3-4	3-4	3-4
H chain-# oligosaccharides	5	1	1	1	1
Light chain type	k and l	k and l	k and l	k and l	k and l
Average light chain K/L ratio		2.4	1.1	1.4	8.0
Molecular weight (D) of secreted form*	190 000	150 000	150 000	160 000	150 000
H chain domain number	5	4	4	4	4
Hinge (amino acids)	none	15	12	62	12
Tail piece	NO	NO	NO	NO	NO
Allotypes	Em1	G1m: a(1),x(2), f(3), z(17)	G2m: n(23)	G3m: b1(5), c3(6), b5(10), b0(11) b3(13),b4(14) s(15), t(16), g1(21), c5(24), u(26),v(27), g5(28)	G4m Gm4a(i) Gm4b(i)
Distribution in secretions	+	—	—	—	—
Distribution: % intravascular	50	45	45	45	45
Biological half-life (days)	1–5	21–24	21–24	7–8	21–24
Fractional catabolic rate (% intravascular pool catabolized/day)	71	7	7	17	7
% total immunoglobulin in adult serum (nonatopic population)	0.0005%	45-53%	11-15%	0.03-0.06%	0.015-0.045%
Approximate adult range: age 16-60 in serum grams per liter	~0.0001 nonatopics	5-12	2-6	0.5-1	0.2-1
Functional valency	2	2	2	2	1-2
Transplacental transfer	0	++	+	++	++
Binding to basophils and mast cells	+++	?	?	?	?
Complement activation classical pathway	0	++	+	+++	0

5.1 Biological Activity of IgE

Development of an IgE antibody mediated “allergic” state involves two discrete phases: the sensitization phase and a subsequent challenge.^{40,41} One of hundreds of foreign substances (allergens) initially enters the blood, skin, respiratory tract, or the gastrointestinal system. Macrophages and T-cell lymphocytes interact with and digest the allergen, presenting portions of it in conjunction with major histocompatibility antigens to B-cell lymphocytes, which subsequently differentiate into plasma cells. In predisposed persons, some activated plasma cells produce IgE antibodies, which bind to high affinity IgE Fc receptors on the surfaces of basophils in the blood and mast cells in the connective tissue of the skin, respiratory,

and digestive tracts, and around blood vessels. IgE also binds to low affinity FcεRII receptors on other (immune) cells. These events comprise the *sensitization phase*.

Upon a subsequent exposure of the sensitized person to an allergen (*challenge*), allergen cross-links IgE antibodies attached to basophil and/or mast cell surface receptors, inducing degranulation and the release of multiple chemical mediators, including histamine, leukotrienes, neutrophil chemotactic factor of anaphylaxis, platelet-activating factor, kinin-producing enzymes (kallikrein), and in the case of mast cells, prostaglandin D₂. These chemical mediators induce dilation of small blood vessels, increase vascular permeability and postcapillary leakage of fluid, constrict smooth muscle in lung and blood vessels, increase mucous gland secretion, and activate blood platelets and other postinflammatory events. All these events may ultimately lead to an immediate type I hypersensitivity response, which manifests as a spectrum of reactions ranging from localized swelling and erythema in the skin (wheal and flare), to mucous and fluid accumulation in the nasal mucosa and bronchoconstriction, to more systemic manifestations such as urticaria (hives), angioedema, and in severe cases, anaphylaxis and death.^{40,41}

5.2 Algorithm for the Diagnostic Evaluation of an Allergic Patient

5.2.1 Clinical History and Physical Examination

The diagnostic algorithm for human allergic disease begins with a detailed clinical history.^{8,9,42} A questionnaire is often used to record the patient's chief complaint and history of the present illness. The individual's past history, family history, and environmental exposure history at home and work or school are all relevant.⁴³⁻⁴⁶ Details should include when the symptoms were first noted, eg, age, suspected cause, circumstances, place, course, and treatment; the frequency of recurrence; average duration of symptoms or exacerbations; and relationship to certain activities, places, exposures, food, infections, time of day, or season. Questions focus on symptoms related to the nose, eyes, ears, chest, skin, gastrointestinal tract, and adverse reactions to foods, drugs, or insects.

A physical examination provides more objective evidence of symptoms related to the eyes (lacrimation, erythema); nose (edema and discoloration, discharge); sinuses (tenderness and drainage, oropharynx [mouth breathing, postnasal drip]); ears (redness); chest (audible wheezing); and skin (rashes, dermographism). Because many of the signs and symptoms of allergic disorders are not pathognomonic, nonallergic causes should be considered in the differential diagnosis. Details on differential diagnoses relevant to human allergic disease are presented elsewhere.^{8,9,42-55}

It should be noted that the clinical history consists of a mental process that uses probabilistic reasoning with imperfect information. As such, its accuracy varies with experience and personal judgment. In addition, it is susceptible to a number of well-documented cognitive errors described as anchoring, representativeness, availability, and fear of regret. These cognitive factors can result in errors in judgment, which when used to judge test results, can result in false impressions of test efficiency. Thus, it is conceivable that diagnosis by history may not be an ideal standard to judge clinical test performance.^{56,57}

5.2.2 Postphysical Examination

Once the suspicion of allergic disease has been substantiated by the medical history and physical examination, the next step in the diagnostic algorithm is to undergo one or a combination of *in vivo* provocation and *in vitro* confirmatory tests to support the clinical diagnosis of allergic disease.⁵⁸ The actual tests used ultimately depend upon the nature of the disease process under investigation (eg, IgE mediated asthma, urticaria/angioedema, rhinitis/sinusitis, or anaphylaxis). They involve both *in vivo* and *in vitro* tests. Practice parameters⁴²⁻⁵⁵ outlining the importance of a comprehensive clinical history and physical examination in the diagnostic algorithm for human allergic disease are recommended reading.

5.2.3 *In Vivo* Confirmatory Tests

Both the skin test and nasal/bronchial/gastrointestinal provocation tests are useful *in vivo* tools for the confirmation of immediate-type hypersensitivity reactions associated with allergic disease. These are often used as reference methods from which the performance of serological assays are judged. They can also aid in the identification of the offending allergens, which can lead to recommendations of avoidance, or management with pharmacotherapy, immunotherapy, or anti-IgE therapy. For these reasons, the methods are briefly discussed.

5.2.3.1 Skin Test

In vivo tests such as skin testing have an important place in confirming a diagnosis of allergic disease that is suggested by a clinical history, especially because they are biologically relevant (occur in the patient) and results can often be obtained the same day. However, they can suffer from variable performance due to the use of different techniques and methods of scoring and interpretation,⁵⁹ and variability associated with the quality and stability of allergen sources used in provocation testing and extracts used in skin testing. Possibly, most importantly, these techniques are not risk free and they are sometimes not tolerated well, especially by children. For this reason, serum-based assays for the detection of allergen-specific IgE have become important in the diagnostic algorithm as an equivalently effective means to the *in vivo* methods of confirming the clinically based diagnosis of human allergic disease.

The skin test can be described as a three-phase response (Lewis triple response) in the skin following the administration of a stimulus (allergen or histamine-positive control). First, a bluish-white area appears that involves the constriction of capillaries and which typically disappears within minutes. An erythemic peripheral halo or flare secondly appears as a result of arteriole dilatation. Finally, a circular urticarial papule or wheal is observed, as a result of extravasation of plasma into the skin, which is generally maximal by 15 to 20 minutes. The immediate wheal and flare reaction can be followed by a late-phase reaction in five to six hours, which appears as a poorly defined edema-like reaction that usually disappears by 24 hours.

The administration of an allergen extract can be accomplished by either a prick/puncture or intradermal injection. Puncture skin testing involves placing a drop of allergen extract on the skin of the forearm or back and the introduction of allergen into the epidermis with a needle puncture. A variety of single-point, multipoint, and bifurcated needles have been used. After the prick or puncture, the excess allergen is removed by blotting with tissue paper or gauze. An immediate reaction (wheal and erythema) is read at 15 to 20 minutes as it reaches its maximum size. Care is taken to space individual tests at a sufficient distance from each other so as not to produce overlapping erythema. Because of the direct skin irritation, false-positive results can occur.

The intradermal skin (ID) test is estimated to be 1000 to 10 000 times more sensitive by concentration than is the puncture test. A 0.01 mL to 0.05 mL volume (optimally 0.02 mL) of diluted allergen extract in a 0.5 mL to 1.0 mL syringe is injected intracutaneously through a 26 to 27 gauge needle. A 0.02 mL injection will initially produce a superficial 2 mm to 3 mm diameter bleb. Like the puncture test, the ID skin test is read at 15 to 20 minutes, when the reaction is maximal. Dilutions of extract of more than 1:1000 w/v are commonly used to minimize false-positive irritation and the potential for systemic reactions. Subcutaneous administration of the allergen may lead to a false-negative result. The volume of allergen extract that is injected only slightly influences the size of the wheal and flare reaction, whereas its concentration is the most important determinant of final ID skin test results. Intradermal testing allows an investigator to perform a skin test titration to determine quantitatively the patient's skin sensitivity. For serial titration, the same volume (eg, 0.02 mL) of 3 to 10-fold serial dilutions of allergen extract is injected into different sites in the skin, and the concentration of allergen required to produce a wheal or erythema of a defined mean diameter (eg, 8 mm wheal) is interpolated. The higher the concentration of allergen required to induce the defined wheal or erythema size, the less sensitive the patient is to that

allergen preparation. Skin testing or other forms of challenge testing should be used with caution on patients on active medication with drugs that may affect the results (eg, antihistamines, steroids, antidepressants).

Although skin testing is widely used to identify sensitized individuals and relevant allergens, some cautionary comments on its use as a standard upon which *in vitro* tests are judged should be advanced. These include the occurrence of large coefficients of variation; the fact that very few allergen extracts are characterized or standardized; operator and interpretive variables exist; and patient variables such as age, placement of skin tests, sex, time of day, and extract stability issues exist. In addition, skin tests do not seem to reflect quantitative levels of specific IgE, even applying the identical allergen (ie, rBet v 1) in a titrated manner to achieve individual skin sensitivities.⁶⁰ False-positive skin tests are also possible and it is very difficult to provide proof of specificity of skin tests without further information.

5.2.3.2 *In Vivo* Provocation Tests

Bronchial and nasal provocation challenges are performed primarily as research procedures to identify a relationship between an inhaled substance and a change in the patient's bronchial or nasal physiology. Bronchoprovocation studies with methacholine can also be useful in the identification of hyperreactive airways. The bronchoprovocation procedure involves the administration of either methacholine or histamine via a calibrated nebulizer, starting at doses of 0.05 mg/mL to 0.1 mg/mL and doubling the concentration up to 10 mg/mL to 25 mg/mL. Alternatively, allergen extracts can be administered in increasing doses. Pulmonary function is monitored after each dose. A positive response is usually defined as the concentration of agonist that results in a drop in the forced expiratory volume per second (FEV1) of 20% or more from the baseline (which must be >70% of that predicted for valid interpretation). In contrast, nasal provocation in its simplest form involves the controlled administration of buffer (human serum albumin-saline) or increasing concentrations of allergen into a washed nasal passage. The symptoms (eg, number of sneezes) induced and/or mast cell mediators released into nasal lavage fluids (eg, TAME esterase and histamine) after each concentration of allergen indicates the relative level of sensitivity to that allergen. Nasal airway resistance is a less satisfactory end point because of high intrinsic variations.

5.2.3.3 Double-Blind Placebo-Controlled Food Challenge (DBPCFC)

Food-induced gastrointestinal reactions (eg, nausea, colic, vomiting, urticaria, and diarrhea) may occur within minutes to hours following the consumption of food allergens by a sensitized individual. Commonly eaten foods that are known to contain potent allergens include cow milk (caseins, β -lactoglobulin, α -lactalbumin); chicken egg white (ovalbumin, ovomucoid, ovotransferrin); cereal grains (wheat, rye, barley, oats); legumes (peanut, soybean, white bean); fish; and seafood (shrimp, crabs, lobsters, oysters). The DBPCFC is considered the definitive diagnostic test for food allergy.⁶¹ This normally begins with strict elimination of the suspect foods for 7 to 14 days before the challenge. An equal number of randomly alternating food allergen and placebo challenges, starting with 125 mg to 500 mg of lyophilized food, are then administered to the patient in a fasting state, doubling the dose every 15 to 60 minutes. Clinical reactivity can be ruled out once 10 g of lyophilized food blinded in liquid or capsules is tolerated. Negative DBPCFCs must then be confirmed with an open feeding challenge under observation to rule out possible false-negative challenges. Although considered a standard procedure, the diagnostic or clinical sensitivity and specificity of the DBPCFC is not known.

5.2.4 *In Vitro* Diagnostic Tests

Historically, the first assays for total and allergen-specific IgE assays were reported in 1967.¹⁰ Total IgE levels were used in the early years as a tool for identifying atopic individuals⁶²⁻⁶⁴; however, it became clear that total serum IgE levels were nonspecific for diagnosing allergic disease. Individuals with no apparent allergies could have total IgE levels in high percentiles above the age-adjusted nonatopic norm

based on skin testing studies.⁶⁴ Moreover, allergic individuals could have low total serum IgE levels in the low nonatopic percentiles. Thus, there was a period when total serum IgE was infrequently quantified in the diagnostic work-up of an allergic patient, except possibly in cases of allergic bronchopulmonary aspergillosis (ABPA) or suspected atopic asthma, where total serum IgE appeared to correlate with disease severity.^{65,66} The allergen-specific IgE measurement thus became the primary *in vitro* analysis used as an alternative diagnostic confirmatory test to *in vivo* methods to both identify the “sensitized state” (IgE antibody positivity) of the individual and to identify the allergen specificity or specificities to which the individual had become sensitized. In recent years, the total serum IgE measurement has regained an important place in allergy management because of the introduction of antihuman IgE treatment (eg, omalizumab) that is used to treat allergic asthma.⁶⁷ For this reason, the background, current state of the technical art and clinical utility of both total and allergen-specific IgE assays are discussed in detail.

5.3 Total Serum IgE

The concentration of IgE in the serum is highly age dependent. IgE concentrations in cord serum are low, usually < 2 IU/mL (< 4.8 ng/mL), because it does not cross the placental barrier in significant amounts. Mean serum IgE levels progressively increase in healthy children up to the age of 10 to 15 years. The rate of this rise in serum IgE toward adult levels is slower than that of IgG, but comparable to the rise of IgA. Atopic infants have an earlier and steeper rise in serum IgE levels during their early years of life as compared with nonatopic controls. An age-dependent decline in total serum IgE may occur from the second through eighth decades of life. The percentile of a patient’s serum IgE level should be compared to reference intervals established with sera from an age-stratified, healthy (nonatopic) population.⁶⁴ Serum IgE levels that are significantly higher than the appropriate, age-adjusted, nonatopic reference range are considered abnormally elevated and are strongly associated with atopic disorders such as allergic rhinitis, extrinsic asthma, and atopic dermatitis. Extreme elevations in serum IgE are observed commonly in parasite infections, rare cases of cancer that selectively produce IgE myeloma protein, and are necessary for the diagnosis of hypergamma-globulinemia E (Job’s Syndrome). Normal or low total IgE levels in some persons with asthma suggest that IgE-mediated mechanisms play only a minor or insignificant role in the pathogenesis of their condition. Low total IgE levels can thus support the diagnosis of nonallergic (intrinsic) asthma, and they can help to exclude allergic bronchopulmonary aspergillosis.⁶⁵ As mentioned above, the reported overlap between IgE levels in atopic and nonatopic populations is considerable, which minimizes its general clinical utility in the diagnostic work-up of an allergy patient. Thus, while an elevated serum IgE can be useful indicating the clinical diagnosis of allergic respiratory or skin diseases, a low or normal value does not eliminate the possibility of IgE-mediated mechanisms. Therefore, the total serum IgE must be interpreted carefully within the relevant clinical context for each patient.

In 2003, omalizumab was licensed for use in the treatment of allergic asthma. Omalizumab is a humanized IgG1 antihuman IgE Fc that binds to an epitope on the epsilon-heavy chain, which itself binds to the alpha chain of the high affinity IgE receptor (α -Fc ϵ R1). Administration of omalizumab blocks the binding of IgE to Fc ϵ R1 and down regulates the number of Fc ϵ R1s on effector cells (mast cells and basophils). The consequence is a reduction in allergic symptoms in most individuals following allergen exposure.⁶⁸ Individuals with total serum IgE levels between 30 IU/mL to 700 IU/mL are candidates for omalizumab therapy. A total serum IgE is required to ensure that the patient is a candidate for omalizumab and for determination of the proper dose to ensure optimal treatment efficacy.^{68,69} A research assay that employs a biotin-labeled or insolubilized form of α -Fc ϵ R1 has been developed to quantify the percentage of the total “free” IgE.¹⁷ Free IgE refers to those IgE molecules in the serum of patients receiving omalizumab that remain unbound with omalizumab and able to bind Fc ϵ R1.

5.4 Allergen-Specific IgE

In contrast to total serum IgE, the presence of allergen-specific IgE antibody in serum identifies an individual as *sensitized*, and it provides a strong but not perfect indication that the individual has a

propensity to exhibit an allergic reaction upon reexposure to the allergen.^{40,41} For several decades, the diagnostic sensitivity and specificity of allergen-specific IgE assays have been defined in relationship to the patient's clinical history and/or their skin test results using cross-sectional populations. These studies have led to the general conclusion that the diagnostic sensitivity of most serum IgE antibody measurements is comparable to that of prick puncture skin testing for respiratory and food allergy, and complementary to the intradermal skin test for venom and drug allergy. Modern autoanalyzers now provide accurate, reproducible, and quantitative measures of IgE antibody specific for hundreds of clinically relevant allergen specificities. With these quantitative estimates of IgE antibody, investigators have begun to document the relationship between a given level of allergen-specific IgE antibody in serum and the probability that the patient will manifest an allergic reaction following allergen exposure.

The most significant application of *probability-based risk assessment* has been in the area of food allergy.⁷⁰⁻⁷⁷ Children with defined levels of specific IgE antibody in their serum to peanut, egg white, cow's milk, and fish have been shown to have a defineable probability of clinical sensitivity to their respective allergens as assessed by DBPCFC. In one illustrative study in 2001, prospectively collected sera from 100 children and adolescents who had been previously evaluated by a skin test and DBPCFC were analyzed for food-specific IgE antibody.⁷⁰ The investigators were able to identify a diagnostic level of IgE antibody for egg (7 kIUa/L), milk (17 kIUa/L), peanut (14 kIUa/L) and fish (20 kIUa/L), above which they could predict clinical reactivity in this group with more than 95% certainty. They concluded that by measuring the concentration of food-specific IgE antibody with a particular assay system, it was possible to identify a subset of children who were highly likely (>95%) to experience a clinical reaction to egg, milk, peanut, or fish. Quantitative serological measurements for food-specific IgE antibody may therefore be able to eliminate the need for time-consuming DBPCFCs in a significant number of children who are suspected of having food allergy. Analogous work was initiated by other investigators to reproduce these findings. Interestingly, they were able to establish such cutoff values only for egg (13 kIUa/L) on a 95% probability level and milk (89 kIUa/L) on a 90% probability level, but not for wheat and soy.⁷¹ The authors concluded that such probability data need to be ascertained for each allergen separately, and the age of the patient population under investigation must also be taken into account. Further explanation for these conflicting results are qualitative differences in the clinical phenotype of the patient populations studied and quantitative differences in prevalence of the disease in the population, if applied outside tertiary referral centers. Therefore, predictive values for a certain group are not readily applied to other populations; corrections are needed or diagnostic levels should be defined for each specific cohort separately.^{72,77} In addition, published probability-based risk curves that have been defined using data from one IgE antibody assay should not be used when food-specific IgE levels have been measured with other assays until further clinical validation studies have been done, since different assays measure different populations of food-specific IgE antibodies.⁷³ Quantitative allergen-specific IgE antibody data may in the future also be applicable in other areas than food allergy. However, this will require additional standardization and control studies in different populations with clinically well-defined phenotypes.^{74,75}

5.4.1 Interpretation of Allergen-Specific IgE Antibody Results

A positive allergen-specific IgE result indicates an allergic sensitization and it is considered clinically relevant and confirming when the individual has allergic symptoms following exposure to that allergen. This is the general interpretation that applies for all diagnostic *in vivo* and *in vitro* allergy tests that are based on the presence of allergen-specific IgE antibody. Each positive diagnostic allergy test result should therefore be carefully evaluated in the context of the patient's clinical history and physical examination. In cases of a mismatch between the clinical history and result of the IgE antibody test, a provocation test involving an allergen challenge or results of a cellular-based mediator release assay⁷⁸ may be useful to clarify this discrepancy.

Several conditions can lead to false-negative, false-positive, or clinically irrelevant IgE antibody test results. Assignment of "clinically irrelevant" to an IgE antibody test result does not necessarily mean the

analytical measurement was performed in a technically incorrect manner. The existence of allergen-specific IgE in the skin or blood does not “prove” a clinically manifested allergy by itself. A positive clinical history must confirm this relationship. A number of conditions that can lead to false-negative or false-positive IgE antibody results are highlighted in the subsequent section.

5.4.1.1 Possible Causes of False-Negative Serum IgE Antibody Results

Local IgE antibody production and/or low total serum IgE

One infrequent cause of negative IgE antibody results, despite a convincing clinical history (eg, symptoms of allergic rhinoconjunctivitis with typical seasonal pattern) and a positive (concordant) challenge test (eg, nasal provocation test), could be an exclusively local production of IgE without detectable elevations of antibody released systemically into the serum. Another rare condition is undetectable allergen-specific IgE levels in serum to allergen sources such as hymenoptera venom in nonatopic individuals with extremely low total IgE levels (eg, <5 IU/mL), despite a convincing history of an anaphylactic reaction following an allergen exposure (eg, insect sting). These cases can be shown often to have IgE antibody on effector cells that are detectable with functional cellular assays that demonstrate crosslinking of IgE. This is a special circumstance where the basophil activation or mediator release tests with their extraordinarily high analytical sensitivity may be particularly useful.⁷⁹

Low abundance of the responsible IgE-binding allergen in an extract

False-negative allergen-specific IgE results can occur when allergenic extracts used to prepare allergen-containing reagents (eg, allergosorbents) have a low abundance of the principal allergen. Underrepresentation of the sensitizing protein due to natural low abundance, poor extraction, or low stability can lead to nondetectable allergen-specific IgE. An illustration of this problem is the labile nature of *Hevea brasiliensis* (Hev b) group 5 allergen that presents in a low abundance in extracts used to prepare commercial allergosorbents. This led to the low of detection of IgE anti-Hev b 5, which in some cases lead to false-negative results in individuals known to have a positive history of natural rubber latex allergy.⁸⁰ This problem was corrected by supplementing the *Hevea brasiliensis* extract with recombinant Hev b 5 prior to manufacturing the allergosorbent. Caution, however, must be exercised by IgE antibody assay manufacturers when an allergen extract reagent is supplemented with a purified native or recombinant allergen molecule that is naturally in low abundance or missing. This was illustrated by a problem that occurred with the supplementation of recombinant Cor a 1 into a hazelnut extract that was Cor a 1 deficient. The Cor a 1-supplemented hazelnut reagent caused laboratories to detect IgE anti-Bet v 1 in the sera of individuals who had birch pollen, but not hazelnut-induced allergic disease as a result of the extensive cross-reactivity between Cor a 1 and Bet v 1.⁸¹

5.4.1.2 Possible Causes of False-Positive Results

False-positive allergen-specific IgE antibody results are considered rare; however, there are a number of conditions where they may occur. Among the possible causes are extremely high total serum IgE levels (typically >20 000 kIU/L), unusual extracts that possess a high nonspecific IgE binding capacity, and laboratory-based errors. Alternatively, there are a number of situations where IgE antibodies may be detected; however, they do not correlate with a positive clinical history.

The presence of IgE antibody in the absence of clinically evident allergic disease emphasizes the important point that the allergen-specific IgE measurement is designed to provide confirmation of “sensitization” (IgE antibody positivity) and not clinical disease. A concurrent clinical history is required to translate a positive IgE antibody measurement into a diagnosis of allergic disease. The IgE antibody measurement also aids the clinician in verifying the allergen specificity that led to the patient’s sensitization. When the IgE antibody result is negative, it can support the clinician’s decision that the allergen specificity is not involved in the patient’s allergic symptoms.

The following are possible causes of false-positive IgE antibody measurements.

Monovalent allergens that evade IgE antibody crosslinking

If an allergen contains only a single epitope, “monomeric” allergen-specific IgE binding can occur without the ability to crosslink IgE on the surface of effector cells. The absence of IgE crosslinking can lead to a negative *in vivo* provocation (skin test) or *in vitro* basophil functional assay result. This, however, remains more of a theoretical concern, since most of the allergens of a sufficient molecular weight possess multiple IgE-binding epitopes. Moreover, small monovalent allergens (eg, drugs such as penicillin) may haptenize onto larger carrier proteins and thus become functionally multivalent.

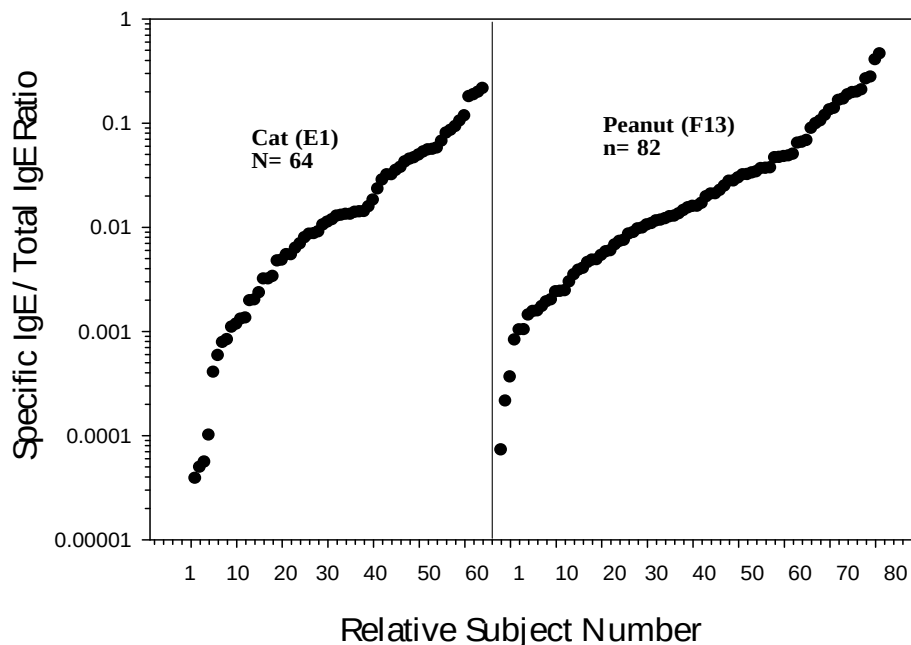


Figure 1. *Left Panel:* Cat allergen-specific IgE to total IgE ratios in serum that range from 0.004% to 21.4% for 64 randomly selected individuals who have a history of cat allergy. *Right Panel:* Peanut allergen-specific IgE to total IgE ratios that range from 0.007% to 45.9% for 82 randomly selected individuals who have a positive history of food allergy associated with peanut ingestion. Data were provided by the Johns Hopkins University DACI Reference Laboratory.

Low allergen-specific IgE to total IgE ratio

The presence of specific IgE antibody in an individual with no apparent clinical allergy to that allergen (so-called clinically irrelevant IgE antibody) has been theoretically linked to a low specific to total IgE ratio in serum. Figure 1 illustrates the range of specific to total IgE ratios for randomly selected individuals with serological sensitivity (IgE antibody positivity) to cats (N=64) and peanuts (n=82). The specific to total IgE ratios range from < 0.005% to as high as 46%. Individuals with a low specific to total IgE ratio (eg, < 0.005%) will have effector cell receptors, which are saturated with antibodies of many different specificities. As such, when these individuals are exposed to a relevant allergen, the allergen would not be expected to as effectively cross-link IgE and trigger the mast cell or basophil to produce and release mediators as might be expected in an individual with a high specific/total IgE ratio of 46%. In the latter case, almost one out of every two IgE molecules bound to the effector cell will have the same allergen specificity and thus the probability of triggering the cell with that allergen will be higher. This is only one of the many variables together with FcεR1 receptor number and the effector cell's releasability that can determine the extent of mediator release following an allergen exposure.

Cross-reactivity as underlying cause for false-positive results

Cross-reactivity is the ability of an (IgE)-antibody to react with or bind an antigen (allergen) that did not initially stimulate its production. Proteins from heterogeneous plant and animal families that do not induce the primary sensitization can subsequently bind IgE due to phylogenetic and/or structural similarities. This “serological cross-reactivity” can be shown to be clinically relevant and occurs only when it leads to allergic symptoms (ie, pollen-related food allergen sensitization involving proteins such as Bet v 1) and is often low (ie, sensitizations to panallergens like profilin). The potential for cross-reactivity causing positive IgE antibody results therefore makes the case for performing allergen-specific IgE measurements only when there is clinical hint based on a history for allergen-driven symptoms.

Cross-reactive carbohydrate determinants

Allergen-specific IgE can bind to certain carbohydrate structures on plant and insect glycoproteins as well as peptide epitopes. Thus, in contrast to peptide epitopes, “cross-reactive carbohydrate determinants” (CCD) can display structural homology across many allergen groups and thus lead to extensive cross-reactivity.⁸² Unrelated glycoproteins may display identical CCDs, which cause most plant-derived allergens to display IgE anti-CCD reactivity. For this reason, serologic IgE antibody positivity to pollen-related food allergens can result from binding to CCDs.⁸³ Even diagnostic assays that involve the use of stable food allergens such as wheat and peanut can also be hampered by CCD interference, since even these foods may contain CCDs as a result of numerous glycosylated proteins.

The clinical relevance of IgE antibodies specific for CCD has been an issue of intense debate. CCD reactive IgE antibody is generally considered specific and a valid technical finding. Thus, it is considered a true-positive IgE measurement. However, its presence in serum does not necessarily correlate with clinically manifested allergic symptoms due to presumably lower affinity and an inferior biological activity of IgE anti-CCD.⁸⁴⁻⁹⁴ Therefore, IgE antibody specific for food allergens can be present in the sera from pollen-allergic individuals without any food-related clinical symptoms after ingestion of the offending culprit. As an example, IgE anti-CCD to peanut was demonstrated in grass pollen-allergic individuals without clinical relevance, since they could tolerate consumption of peanuts.⁸⁸

In contrast, some patients can suffer from the presence of IgE anti-CCD that is considered “clinically relevant.” CCD-containing Lyc e 2, but not the deglycosylated molecule from tomatoes, may induce mediator release from basophils obtained from tomato-sensitive individuals.⁹² This same observation was made involving the celery allergen Api g 5 using IgE antibody from a celery-allergic patient.⁹⁵ Subjects displaying glycan-specific IgE from celery and zucchini developed allergic symptoms to CCD-containing foods during a DBPCFC.^{94,95} Finally, IgE antibodies specific for the oligosaccharide, galactose- α -1,3-galactose, can be involved in inducing anaphylaxis in individuals receiving cetuximab.⁹⁶ Cetuximab is a chimeric mouse-human monoclonal antibody specific for epidermal growth factor receptor that contains galactose- α -1,3-galactose moieties on the Fab portion of its heavy chain.

Thus, IgE antibodies specific for CCDs should be considered in the case where there are discrepant skin test and serological results. They are noted to be the cause of frequent positive IgE antibody results to a large panel of diverse plant-derived allergens. IgE-binding to horseradish peroxidase, bromelain and ascorbat oxidase, and glycoproteins with extensive glycosylation should be considered an indication for IgE anti-CCD related positivity. Specific assay reagents using MUXF, which is an isolated CCD without a protein backbone, can be used in the detection of CCD-specific IgE reactivity to confirm IgE antibody specificity.

5.5 Therapeutic Interventions With Potential to Interfere in IgE Antibody Assays

A number of therapeutic interventions may cause interference in serological total and allergen-specific IgE immunoassays. Any intervention that significantly changes the composition or volume of the patient's blood compartment (eg, blood transfusion with autologous, heterologous, or animal protein [bovine hemoglobin], chemotherapy, fluid administration, or a recent systemic anaphylaxis) has the potential to cause inaccuracy in reported levels of total and allergen-specific IgE antibody. The administration of omalizumab (antihuman IgE) has been shown to interfere with some of the clinically used total and allergen-specific IgE antibody assays.²⁸ Select drugs such as steroids or antihistamines do not generally interfere in total or allergen-specific IgE antibody measurements unless in the case of steroids, there is significant immunosuppression with long-time usage. Traditional immunotherapy may cause problems for certain assay designs due to a potential increase of allergen-specific immunoglobulins of isotypes other than IgE, such as IgG and IgA.

6 Specimens

6.1 Patient Material

6.1.1 Specimen Type

Serum and plasma (serum + fibrinogen) are considered the working specimens for the total and allergen-specific IgE antibody assays discussed in this document. Alternative specimen types such as tear fluid, bronchial or nasal lavage fluid (BAL), sputum, cerebral spinal fluid, and cell supernatants are primarily used in research, and few manufacturers have indicated that their assay can analyze these specimens. The problems of nonparallelism or protein matrix-related effects can become exacerbated if less blood than is required for the collection device is drawn from the patient. Grossly hemolyzed, icteric, or lipemic serum/plasma specimens should not be used in the analysis of IgE antibodies due to possible interference. Potential interfering substances, for example, high levels of hemoglobin, bile, and lipids, should be identified by the manufacturer and listed in the product literature.

6.1.2 Storage Conditions

Based on empirical work, the following general recommendations can be made about the stability of the blood specimen under various storage conditions. Even though serum shipped in styrofoam serum mailers may remain at room temperature for up to one week without apparent degradation of IgE (as measured in immunochemical assays), it is recommended that separated serum/plasma remain at room temperature (eg, 18 °C to 25 °C) for no longer than eight hours. Actual storage requirements should be studied by those who work in the clinical laboratory before final recommendations are given to clients of their laboratory services.

If assays cannot be completed within the eight-hour period, then it is recommended that the serum/plasma specimen should be frozen at -20 °C. Freezing at lower temperatures (eg, -70 °C to -120 °C) is not necessary, even though it is not detrimental to the immunoreactivity of IgE antibodies over time. Ideally, serum/plasma specimens should not be repeatedly frozen and thawed because this can cause deterioration of many proteins, including IgE. Frost-free freezers are not recommended for long-term storage because the freeze/thaw cycles can cause radical temperature shifts, which can be detrimental to proteins in the specimen. Upon long-term storage, frozen specimens begin to show phase separations in which proteins collect at the bottom of the tube. These specimens that demonstrate two phases should be mixed carefully before use. If a serum/plasma separator device is used, the directions of the manufacturer are to be followed and total volumes designed for the device should be collected.

6.2 Quality Control of IgE Assays

The intra- and interlaboratory variation and daily interassay validation of total serum IgE and allergen-specific IgE immunoassays are commonly evaluated by manufacturers and clinical laboratory end users. Repetitive analysis of human serum pools that contain varying amounts of IgE antibody can ensure quality of results reported over time. While strategies have been proposed for the construction of IgE antibody-containing quality control serum pools, practical difficulties in obtaining large (multiliter) quantities of serum from clinically well-characterized noninfectious, atopic subjects have limited the utility of these theoretical protocols. The most practical strategy has been the collection of as many units of plasma as possible (at least 10) from highly sensitized and otherwise healthy persons who have a positive history, a positive confirmatory test result (skin test and/or serological test), and who have not received allergen immunotherapy. Individuals on active allergen immunotherapy may develop microgram per milliliter quantities of specific IgG antibodies that can interfere in some assays that measure nanogram per milliliter levels of IgE antibody. These serum pools, when “blended” in equal volumes, contain heterogeneous mixtures of antibody of multiple isotypes and allergen specificities. While the theoretical goal of this process is to maximize the IgE antibody heterogeneity so the serum pool mimics the humoral immune response of the general population of allergic subjects, in fact, it has the opposite effect in practice. The mixing of many sera tends to concentrate the most common IgE antibody specificities (allergen 2, Figure 2) and dilute out the minor IgE antibody specificities (allergens 1 and 3, Figure 2). Because the use of a single individual serum may over-represent idiosyncratic minor reactivities, and a mixture of different sera dilutes out of these same minor specificities, both individual sera and serum pools should be used, as a compromise, in documenting the validity of specific IgE antibody assays.

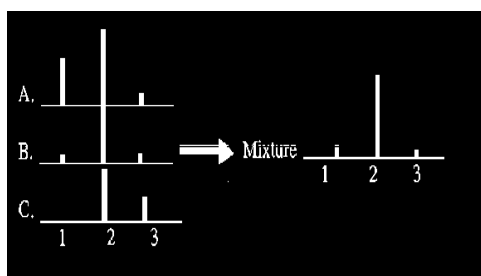


Figure 2. In this schematic, the relative concentration of IgE antibody from three subjects (A, B, and C) for three allergen specificities (1, 2, and 3) is depicted by the relative height of the bar. When these sera are mixed, the concentration of the IgE antibodies with specificities for minor (less allergenic) allergens is diluted, often beyond the detection of IgE antibody assays. Human serum pools thus concentrate major IgE antibody specificities while diluting out minor IgE antibody specificities.

In selected cases, the serum pool can be further characterized for its heterogeneity by a variety of immunological methods, such as cross-radio immunoelectrophoresis or Western blot analysis with soluble antigen inhibition. Potentially competing antibody isotypes (IgG, IgA) may also be directly measured by immunoassay to examine the possibility of competitive inhibition or blocking of IgE antibody binding to minor allergen determinants. For internal manufacture quality control purposes, manufacturers of allergen-containing reagents should develop a collection of individual sera and a bank of reference serum pools that have been prepared with sera from untreated patients who are allergic to each of the allergen specificities in their menu. Theoretically, the serum pool should have IgE specificities directed against all potential allergen activities.

7 Immunochemical Methods

Immunoglobulin E, as an antigen or an antibody, is currently assayed in serum using a variety of commercially available analytical methods. This guideline includes a section on total serum IgE

procedures because they are among the most well-standardized and best-performing immunoassays available in the clinical immunology laboratory today. Moreover, total serum IgE assays are presently used to evaluate patients who are candidates for the receipt of anti-IgE therapy (omalizumab) (see Section 5.3). The primary focus of this section, however, is on assays of IgE antibody that employ a variety of reagents and assay designs.

7.1 Total Serum IgE Assays

Historically, IgE has been measured using a number of competitive and noncompetitive, solid and liquid phase, isotopic and nonisotopic immunoassays that use antibodies specific for human IgE as either capture and/or detection reagents. In most assays, these antibodies are insolubilized on a solid phase (capture antibody) and/or directly conjugated with a label (radio-nuclide, enzyme, or fluorophor). Most currently used total IgE assays are performed on automated platforms that have improved precision and reproducibility, while ensuring maximal sensitivity and specificity. Despite their use of different solid-phase and detection immunological reagents, commercially available assays have been cross-standardized to a common primary human IgE standard (WHO 75/502)³⁶ and most are cross-checked with a common secondary IgE reference preparation.^{35,37} Results are uniformly reported in International Units of IgE per volume (IU/mL). Some laboratories convert the IU/mL to mass units per volume using 2.4 to 2.42 as a conversion factor (1 IU = 2.42 ng). These developments have led to highly reproducible total IgE assays, which produce results that agree well with each other. Illustration of the excellent performance of total IgE immunoassays can be seen in data obtained from interlaboratory proficiency surveys such as the Diagnostic Allergy SE survey conducted by the College of American Pathologists. The SE survey involves analysis of five or six sera every 17 weeks by over 150 clinical laboratories worldwide that use a variety of commercially available total serum IgE assays. Public domain survey data confirm that these assays display excellent interassay precision and analytical accuracy with acceptable parallelism and a dynamic range, typically from ~1 to 1000 IU/mL. An illustration of the excellent agreement with intermethod coefficients of variation < 10% for the most widely used commercially available total serum IgE assays is presented in Table 2.

Table 2. Intermethod Agreement of Widely Used Total Serum IgE Immunoassays*

System	N	SE11	SE12	SE13	SE14	SE15	SE99
A	14	634	233	410	454	1421	454
B	93	602	252	399	445	1375	444
C	39	534	238	372	405	1167	398
D	21	616	268	417	453	1423	451
Mean		597	248	400	439	1347	437
1 SD		44	16	20	23	122	26
Intermethod coefficient of variation		7.3%	6.3%	4.9%	5.3%	9.0%	6.0%

*IgE results in this table are presented in kIU/L for six challenge specimens (SE11 to SE15 and SE99) from 167 laboratories with permission from the 2006 SE Survey (Cycle C) of the College of American Pathologists, Northfield, IL. Presented in this table are total serum IgE data generated with the following methods (not in this order in table): Siemens (formerly Dade-Behring) (Nephelometer System), Phadia (CAP System), Siemens (Advia Centaur and Immulite System), where more than 10 individual measurements for each method were reported to the College of American Pathologists.

A research assay has been reported for the quantitation of total free IgE²¹ (see Section 5.3). In this assay, biotin- α -Fc ϵ R1 is added to serum from a patient receiving omalizumab. Typically, specimens are tested at one to three months of omalizumab treatment. Free IgE (free of omalizumab binding) binds the biotin-

labeled- α -Fc ϵ R1. The serum mixture is then added to solid phase anti-IgE. Bound biotin-labeled- α -Fc ϵ R1 is detected with avidin-enzyme and developed with substrate. The amount of bound biotinylated alpha chain following the final buffer wash is proportional to the amount of free IgE in the original serum specimen. A reference curve is constructed using a commercially available total serum IgE calibrator that is calibrated in IU/mL of total IgE and contains no omalizumab. It is anticipated that clinical assays such as this will become available to monitor patients receiving anti-IgE therapy.

7.2 Allergen-Specific IgE Assays

7.2.1 Components of Allergen-Specific IgE Assays

As discussed in Section 5.2.4, the RAST was initially introduced for the detection of IgE antibodies with a defined allergen specificity.¹⁰ This noncompetitive, heterogeneous, immunoradiometric assay employed allergen insolubilized on paper discs (allergosorbent) to bind specific antibodies of all isotypes from serum, a separation step of free and bound human antibody, and the use of radiolabeled antihuman IgE to detect bound IgE antibodies. Subsequently, many commercial variants on this theme were developed⁹⁷⁻⁹⁹ and many of these have subsequently been discontinued. A reverse or capture format of this assay uses a liquid-phase allergen. In this assay, IgE is initially captured from serum with a solid-phase anti-IgE, and then specific IgE antibody is detected with labeled allergen. These reverse assays are rarely used clinically. Their cited advantage over assays that use allergosorbents is that they do not measure low affinity IgE antibody that may have minor clinical consequence. They have remained primarily research tools and are not discussed further in this document. All other assays for IgE antibody have the following commonalities:

- Reaction vessel: plastic (polyethylene) or glass tube, plastic microtiter plate well, plastic stick, polyethylene cap with an internal sponge matrix, and carbohydrate filament-coated silicone chip.
- *Allergen-containing reagent*: solid-phase allergosorbent or liquid-phase labeled allergen.

Generally, the allergen-containing reagent is a separate or individual reagent for each allergen specificity. Sometimes, a defined combination of allergens is coupled or assembled into one reagent. These are used in multiallergen screens and can either contain select allergens from many specificity groups (eg, weed, grass, tree, epidermal, mite, mold in one reagent) or include many specificities from a single allergen group (pollen from birch, oak, maple, willow, and mesquite trees). Moreover, some allergosorbents contain recombinant allergens to supplement native allergens that are present in low concentration (eg, latex with rHev b 5).⁸⁰ In one clinically used assay, liquid phase allergen is biotinylated and it is subsequently bound during the analysis to a solid phase using an insolubilized avidin. This assay uses solution phase chemistry to bind antibody and subsequent insolubilization to facilitate the separation of the free from allergen-bound antibody.

- *Antihuman IgE Fc detection reagent* (epsilon heavy-chain specific)
- *Calibration system* (eg, reference serum containing a defined amount of IgE to construct a total IgE calibration curve)
- Reaction buffer medium (salts, proteins) to maintain pH and provide a protein matrix for the analyte of interest to ensure minimum nonspecific binding
- Specimens: human serum presumably containing IgE antibody and negative serum controls not containing allergen-specific IgE antibody
- Data-processing system (data-processing software, algorithm)

Of these, the *allergen-containing reagent* is considered the most complex assay component in terms of its preparation from raw biological material and its interbatch quality control and validation. Allergen preparations are inherently complex mixtures of proteins (see Section 8) that vary widely in their size and net charge distributions. Each component protein is also known to differentially elicit a heterogeneous humoral immune response that varies in terms of the isotype (IgE, IgG, IgA, IgM) and antibody concentration.

The second most important reagent is the *antihuman IgE detection reagent* that confers the IgE specificity on the assay. This reagent can be either polyclonal in nature and produced in other species (eg, rabbit, goat, sheep, horse) or murine monoclonal antibodies that bind to defined epitopes, generally on the Fc region of the human IgE molecule. Mixtures of monoclonal and polyclonal antibodies specific for IgE have also been used by different manufacturers to obtain parallelism and linearity over a wide working range. A labeled form of human α -Fc ϵ R1 has also been used as a detection protein for human IgE; however, its use has been limited to the total free IgE assay and the detection of IgE antibodies from horses, dogs, and cats in an allergosorbent-based, noncompetitive immunometric assay.^{21,100} Manufacturers use various procedures and qualifying reagents (IgE myeloma, polyclonal human IgE, chimeric IgE antibodies) to document specificity and certify the antihuman IgE detection reagent's performance within the context of their respective assay system.

The third component of these assays, which has historically varied extensively among commercial assays, is the *calibration system* that was used to define the level of IgE antibody that is measured by the assay. Since the first edition of this document, there has been a single, accepted, industry-wide, uniform strategy for calibrating allergen-specific IgE antibody assays. A "heterologous" total serum IgE calibration curve is used to convert response units generated by the assay into quantitative allergen-specific IgE antibody dose estimates in IUa/mL. The "a" indicates "allergen-specific" and it distinguishes the unit from the IU/mL unitage used in the total IgE assay. The historical methods of reporting results commonly used the measured response signal, such as counts or normalized counts. This is no longer considered clinically useful. Other methods involve interpolating the measured response into arbitrary units per unit volume of serum using a homologous calibration curve. This is commonly used in research assays.

The first three of the seven technical variables of the IgE antibody assay listed previously (allergosorbent, anti-IgE reagent, and calibration system) and alternative assay formats are subsequently discussed in reverse order in this guideline.

7.2.2 Alternative Assay Formats

Point-of-care tests (POCTs) are diagnostic assays that are performed at the bedside, outpatient clinic, or community pharmacy. These tests are often miniaturized, hand-held devices with a simplified chemistry that involves the addition of a whole blood drop to a plastic cassette window. Lateral flow migration carries the serum proteins up a membrane, mixing them with various reagents as they migrate. The assay is designed to produce a rapid analytical result with a qualitative (positive/negative) or semiquantitative estimate of IgE antibody to one or multiple allergen specificities.

A multiplex assay is a procedure by which analytes of multiple specificities are detected and in some cases, quantified in a single serum specimen using a single reaction mixture of reagents. In the context of this document, a multiplex assay would be one that measures IgE antibody to multiple allergen specificities using a single reaction step. One illustration of a multiplex IgE assay is the chip-based microarrayed allergosorbent test in which individual purified allergens (often recombinant in nature) are adsorbed onto spots in triplicate on a silicon microchip. Incubation of a small quantity of serum with the microchip exposes a patient's serum to many different allergen specificities at one time. Following a buffer wash to remove unbound serum proteins, bound IgE is then detected with antihuman IgE conjugate and a subsequent addition of substrate.

Laboratory developed tests (LDTs) are customized assays that are occasionally developed by specialized clinical laboratories to detect the presence of IgE antibodies to antigens, which may either be biochemically and/or medically uncharacterized or which are not registered or cleared by regulatory agencies. Establishment of analytical validation is especially important in such assays, since clinical performance parameters may not be available to judge clinical relevance. Competitive inhibition and specificity studies may be helpful in establishing the analytical performance of this assay. In some cases, the clinical relevance of this analytical measurement may be documented in the literature, so the laboratory that develops this test should compile the available literature regarding the clinical interpretation of the results. The absence or paucity of clinical performance parameters, however, requires particular cautious interpretation of assay results.

7.2.3 IgE Antibody Immunoassay Classification Scheme

Commercially available IgE antibody immunoassays can be classified into a qualitative, semiquantitative, or quantitative assay, depending on the degree to which the assay result accurately reflects the quantity of IgE antibody in the test specimen and the assay's precision requirements.¹⁷ Each assay category has its utility and limitations as an aid in the diagnosis and research of human allergic disease. While all three of these assay categories are defined in Section 4.2, illustration of the type of result presentation, standardization method, standards/reference calibrators, and precision requirements are summarized in Table 3 and discussed in this section.

Table 3. General Classification Scheme for Allergen-Specific IgE Assays

Classification	Result Presentation	Standardization Method	References Calibrators and Controls
Qualitative	• Reactive or nonreactive • Positive or negative An “indeterminate” zone may be included	• Single or dual calibrators to normalize assay	• One reference sample • Negative and positive control
Semiquantitative	• Arbitrary units or classes	• Single or dual calibrators to normalize assay	• Negative and bilevel positive controls (occasionally trilevel controls) • References unique for the test system made from pooled patient sera
Quantitative	• Units related to a generally recognized reference preparation (eg, WHO IgE standard 75/702)	• Multipoint standard curve used for interpolation	• Trilevel positive controls • References calibrated towards a qualified reference preparation

7.2.3.1 Qualitative Assay

A qualitative (screening) assay produces one of two results: either the analyte is (1) nonreactive, negative, nondetectable, or absent; or (2) reactive, positive, detectable, or present.¹⁷ It is not intended to provide a precise estimate of concentration of that analyte. These assays almost always have a single reference specimen, which defines the positive threshold level of the assay.

Results close to this predefined “cutoff” point or positive threshold are classified as borderline or indeterminate. A positive test result implies only that the assay signal exceeds the analytical threshold or positive cutoff point that has been set to obtain an arbitrary combination of detection limits and specificity. Ideally, a positive assay signal indicates the presence of specific IgE antibody in the specimen.

One illustration of a qualitative assay is the multiallergen screening test in which five or more different allergen specificities are present in a single allergen reagent, and the presence of IgE antibody to these allergen specificities is evaluated in a single analytical measurement. Due to the large number of allergenic proteins, this multiallergen-containing reagent is more difficult to quality control for reproducibility of the allergen composition and quantity than if there was only one allergen specificity. Moreover, allergic patient sera containing differing amounts of IgE antibody to the spectrum of allergens generate widely varying response levels in different multiallergen screening assays as a function of the different quantity and relative proportions of the allergens represented on the allergosorbent.

Additional illustrations include the dipstick assay and the POCT. In the dipstick assay, IgE antibody to a single allergen is determined to be either present or absent based on a visual evaluation of the color and intensity. Likewise, the POCT is designed to provide a positive or negative result using the presence or absence of a visual band or dot as indication of the presence of IgE antibody.

7.2.3.2 Semiquantitative Assay

A semiquantitative (titration) assay system provides an additional option over the qualitative assay in terms of defining the magnitude of the response. The variations in a positive signal detected by the assayist are commonly presented in terms of a series of increasing grades or classes (eg, I to VI; low to high); in arbitrarily defined units per milliliter (AU/mL) determined relative to a supplier-specific, heterologous dose-response curve; an end-point dilution to which the signal becomes negative (eg, titer); or in comparison to a qualitative grading scheme (eg, color chart). A semiquantitative assay may be functionally defined as an assay that employs a single or multipoint calibration curve.¹⁷ It might not be able to identify accurately a two-fold change in IgE antibody level when a test serum is diluted two-fold. The semiquantitative assay produces antibody estimates in units that are relative and not traceable to any common international reference material. Finally, these assays are often unable to consistently achieve one or more of the criteria of linearity, dilution recovery, and parallelism that are typical of quantitative assays.

7.2.3.3 Quantitative Assay

A quantitative assay produces an accurate and reproducible estimate of the concentration of an analyte, such as IgE antibody, in the test specimen based on homologous or heterologous interpolation from a calibration curve.^{17,97,101} It fulfills the analytical criteria for quantitation, including parallelism (linearity), recovery, and precision across the assay's working range. In the absence of any absolute IgE antibody standard of a defined allergen specificity, a quantitative assay may be functionally defined as one that can accurately detect a two-fold reduction in IgE antibody when the serum specimen is diluted two-fold across the working range of the assay. Quantitative assays employ a multipoint standard or calibration curve from which unknown data are interpolated. A reference curve can be constructed using heterologous or homologous interpolation methods. While it is desirable to have truly quantitative assays in which results are reported in units traceable to an internationally recognized standard (eg, WHO 75/502), this is not possible using the homologous interpolation scheme, which requires individual homologous calibrated IgE antibodies, one for each of the hundreds of allergen specificities. Thus, the calibrated titration assay that uses heterologous interpolation of allergen-specific IgE antibody results from a total IgE calibration curve is an acceptable alternative. It is the calibration scheme that has been generally adopted for commercial IgE antibody assays. In this calibration system, IgE antibody specific for each allergen specificity is interpolated from a single heterologous or different calibration curve, such as a total serum IgE reference curve. In doing so, the assay can, in theory, be traceable to an internationally recognized total serum IgE standard. Quantitative assays tend to be among the most complex tests (eg, immunoassays) and their results are reported in gravimetric ($\mu\text{g/L}$ [total serum IgE assays]) or international units (kIU/L [total serum IgE assays] or kIUa/L [allergen-specific IgE antibody assays]).

8 Qualification of Assay Reagents

8.1 Antihuman IgE Antibodies

Antibodies specific for human IgE have been used both as capture and detection reagents in total and allergen-specific IgE antibody assays, respectively. Once purified, the antihuman IgE reagent antibodies are either used directly as a solution phase antibody or subjected to chemical modification in the form of radio-labeling, enzyme-labeling, or chemical and physical immobilization on solid-phase matrices.

These key reagents confer the specificity on the assays discussed in this guideline for IgE, and thus they must be highly specific for unique determinants on epsilon-heavy chains. The earliest polyclonal reagents were prepared by immunizing animals with purified IgE myeloma proteins. Monoclonal antibodies specific for human IgE¹⁰² are commercially available. Some manufacturers now employ mixtures of monoclonal and polyclonal antihuman IgE both in the capture of IgE from serum or in the detection of previously bound IgE antibody.

Table 4 summarizes the information that may be provided on specification sheets for antihuman IgE reagents. By definition, these antibodies should have an undisputed and exclusive reactivity to human IgE. They should demonstrate the lowest achievable cross-reactivity (eg, < 0.001%) to human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, and IgD. The procedure and general materials that are needed to demonstrate the “specificity” of the reagent using dilutional analysis are summarized for clarity in Appendix C. Specificity of the final antihuman IgE reagent may be documented by two assay methods: (1) direct binding (dilutional analysis) using insolubilized purified human immunoglobulins of all available isotypes and known allotypes; and (2) competitive inhibition by addition of non-IgE immunoglobulins into the assay system where they will ultimately be used, in concentrations exceeding those expected in human serum.

Table 4. Qualification of Antihuman IgE Reagents

Parameter	Illustration
Antibody source	Mouse, goat, sheep, rabbit, horse
Antibody type	Polymonoclonal, polyclonal, monoclonal, or monoclonal/polyclonal mixture
pH range for storage	7.0–7.5
Preservatives	0.05% thimerosal or azide after sterile filtration
Label type	Enzyme, ¹²⁵ I or fluorophor colloidal gold conjugates, luminescence
Source of human IgE	IgE myeloma protein or affinity purified polyclonal IgE
% Cross-reactivity with human IgG	Target ≤ 0.001%*
% Cross-reactivity with human IgA	Target ≤ 0.001%*
% Cross-reactivity with human IgM	Target ≤ 0.001%*
% Cross-reactivity with human IgD	Target ≤ 0.001%*
Source of non-IgE immunoglobulins	List individual company names, lot numbers, purity
Method of cross-reactivity analysis	Dilutional analysis (Appendix C)

*Specificities shown by direct-binding studies with characterized, purified human immunoglobulins.

8.2 Allergen-Containing Reagents

The allergen-containing reagent is the critical component that confers the allergen specificity on the IgE antibody immunoassay. Generally, it is the most complex and highly variable reagent in the IgE antibody assay. There are many possible causes for heterogeneity of allergen-containing reagents, even when a single manufacturer uses the same extract source and chemicals and the same manufacturing procedure. Some of these causes for heterogeneity are listed in Table 5.

Table 5. Potential Causes of Native and Recombinant Allergen-Containing Reagent Heterogeneity

• Native allergen source: Misidentification, contamination, and inherent biological variation
• Recombinant allergen source: Variability associated with expression vectors, minor differences in primary sequencing, variation in protein folding, and degree of glycosylation
• Classical extraction process: Interlot variation in allergen composition
• Variability in allergen-reagent manufacturing (coupling to solid support [solid phase matrix] or direct labeling [eg, with biotin]): Interlot variation
• Variable stability of allergen during storage
• Heterogeneous internal allergen reference standards used in quality control
• Heterogeneous IgE antibody specificities in human quality control serum pools
• Different assays and acceptance criteria used in validation of final reagents

Despite these potential causes of allergen-reagent heterogeneity, manufacturers generally minimize variability within their respective assays by recommending the use of component reagents by specific lot numbers. When used in the prescribed combinations, the assay should display intra- and interlot variation within predetermined, reproducible target ranges. For research purposes or when patients are transferred among clinicians using different assay systems, it might be desirable to compare the consistency and the magnitude of the interlot and interassay variability using common analytical methods. Toward this purpose, a set of proposed procedures for the testing of allergen-containing reagents are described in Appendix D.

8.2.1 Nomenclature of Allergen Source Groups

Several hundred substances in the indoor (home/work) and outdoor environments are associated with the induction of allergic responses in previously sensitized persons.

For simplicity in referring to *in vitro* allergen specificities, the different allergen groups have been classified using a unique single letter code: grass pollen (G), weed pollen (W), tree pollen (T), epidermal allergens (E), mold spores and mycelia (M), dust mites (D), insect venoms and excretions (I), foods (F), and occupational allergens (U or K). This coding scheme was developed by manufacturers using an arbitrary numbering system. Difficulty with this nomenclature system, however, has stemmed from the use of the same code to define allergen-containing assay reagents that have been prepared with extracts from widely different species of the same genus or different allergen sources altogether. Fortunately, the allergen's common name and scientific name (genus and species) are also used as illustrated in Appendix A (eg, W1 or weed 1 for common ragweed or *Ambrosia artemisiifolia*). While no significance has been attached to the code assigned to the allergen specificity, manufacturers have generally adopted this arbitrarily established system. The allergen nomenclature addendum in this guideline provides a harmonized listing of the allergen codes currently used by IgE antibody assay manufacturers along with their common name, Latin name, and allergen grouping. The company sources that provide particular allergen specificities are noted in this table with a single letter-number code. Those specificities offered by various manufacturers of IgE antibody assays, which have not been characterized as described in Sections 8.2.1.1 and 8.2.1.2 using immunochemical and serological methods, have been designated in Appendix A as esoteric, rare, or undocumented.

With current use of allergen extracts and both native and recombinant allergenic molecules in IgE antibody assays, the allergen code system discussed above that involves a letter and an arbitrarily assigned sequential number has become imprecise. As an alternative for defining allergenic molecules, users of IgE antibody assays may refer to the International Union of Immunological Societies (IUIS) Nomenclature Committee's website (www.allergen.org) to obtain an official allergenic molecule designation. These are listed in Appendix A. For instance, hevein, a potent allergen found in natural rubber latex (*Hevea brasiliensis*), is defined as "Hev b 6.02." For allergenic extracts, the user may refer to an alternative independent website (www.allergome.org) for information and references that are organized by a unique numerical code that has been assigned for each allergen specificity (see Appendix A).

8.2.1.1 Characterized Complex and Purified Allergen Preparations

To date, only five allergen extracts (*Dermatophagoides pteronyssinus* [dust mite], *Canis familiaris* [dog hair/dander], *Betula verrucosa* [birch tree], *Ambrosia artemisiifolia* [common ragweed], and *Phleum pratense* [Timothy grass]) of the many hundreds have been accepted by WHO for use as international standards. Each of the hundreds of clinically important allergen extracts described in the allergen nomenclature addendum are composed of many individual allergenic protein, glycoprotein, and lipoprotein molecules, some of which are considered major allergens (to which a large number of sensitized persons react); others are called minor allergens (to which a small number of sensitized persons react). The definition of major and minor allergens varies among reports in the literature. Often, 50% of individuals sensitized to a given allergen within those sensitized to the allergenic source is used as a criterion for discriminating between a minor (<50%) and major allergen. Note that this is arbitrarily defined and provides only a relative importance of the particular allergen, and is largely related to the selection criteria applied to the tested cohort and the size of the test population. A comprehensive list of all well-known purified allergens is available from the IUIS Nomenclature Committee's website (www.allergen.org).

The standardization of a given extract begins by identifying the individual allergenic proteins in a highly reactive allergen extract using IgE antibody-containing serum in combination with a number of immunochemical methods (isoelectric focusing, immunoblot, Western blot analysis, competitive

inhibition immunoassay). Differential solubility of the various proteins in an extract will dictate which ones remain in an immunoreactive form. Once the individual allergen molecules are defined by their isoelectric point, molecular weight, and frequency of positivity with IgE-containing sera from clinically characterized sensitized persons, they are assigned a major or minor allergen status by a consensus committee of scientists. The major allergens are often characterized further by isolation, amino acid sequencing, and cloning. Occasionally, the biological activity of the major allergens is determined.

8.2.1.2 Allergen Quality Assurance (General Aspects)

Ideally, a quality control program for allergen-containing reagents should evaluate the *presence, relative quantity, and immunoreactivity* of each allergen component in each of the hundreds of native allergen extracts, allergen isoforms, and recombinant allergens. Presently, this is accomplished to varying degrees using heterogeneous pools of serum from persons who are known to be allergic to the allergen of interest, and which (ideally) contain IgE antibody to every allergenic epitope produced by that allergen specificity. The difficulty of this task is augmented when the same serum pool is needed by all the manufacturers of an allergen extract for that specificity over extended periods to demonstrate long-term consistency of their allergenic components.

All proteins within a given allergen source must be considered potentially allergenic. Therefore, most agree that each protein in a particular extract should be represented in molar excess in or on an *in vitro* assay reagent so it is available to bind IgE antibodies from all potential B-cell clones. In this manner, all the IgE antibody of a particular specificity can be detected in the serum of any person in a quantitative manner. An alternative strategy proposes to examine the IgE antibody response to individual component allergens using purified native and recombinant allergenic proteins.

In cases where allergen reagents are sold with insufficient evidence for an association with a clear clinical end point, clearance from a governmental regulatory agency may not be possible, and it is obviously unrealistic to expect a laboratory to validate such a test. It is recommended that the manufacturer and user laboratories not provide these as clinical tests, but instead offer them as investigational use devices under IRB oversight, require informed consent, and gather data to establish whether they are clinically valid or not.

8.2.1.3 Allergen Quality Assurance (Reagent Selection Considerations)

Monoclonal antibodies that bind to selected major allergens in a particular allergen mixture can be used as one tool to quality control the final allergen-containing reagents. Their use minimizes dependency on expensive, variable, and rare heterogeneous IgE-containing human serum pools. A panel of such monoclonal antibodies that are known to bind to all the principal allergenic components for a given specificity could be reproducibly provided to all manufacturers for use over the years to quality control allergen-containing reagents, and “at least” demonstrate that the major allergens are present and immunoreactive. This issue has sparked debate and disagreement because of the following inherent assumptions.

- A single epitope on a major allergenic molecule (marker protein) may be used as an indicator of the presence and relative amount of that allergenic molecule in an IgE antibody assay allergen-reagent.
- The presence and relative amount of the major allergen *marker protein* epitope to which the monoclonal antibody binds may be used to predict the presence and relative amount of other epitopes, on the *same marker protein* or other (either major or minor) allergenic molecules from a heterogeneous allergen extract source.

Since individual monoclonal antibodies bind to a single epitope on an allergenic molecule, their binding does not predict the presence or relative amount of the other allergenic molecules for that specificity. This

limitation can be partially addressed by using a panel of monoclonal antibodies or a well-characterized polyclonal antibody to monitor the potency and immunoreactivity of allergen-containing reagents through their various stages of production.

A panel of purified recombinant allergenic molecules is used to detect primary IgE antibody responses to the clinically important and highly immunogenic environmental allergens. While this may be an attractive prospect because it would simplify reagent preparation and promote reproducibility and standardization, it raises concerns. Allergic patients are known to respond immunologically to different combinations of isoallergens. Thus, a single recombinant allergen that does not necessarily represent all isoallergen forms of a major allergen may not be *globally diagnostic* or able to detect IgE antibody specific for all forms of an allergen. While such a reagent is not optimal, is an *isoallergen-limited* reagent that detects IgE antibody to at least one isoallergen useful diagnostically? The debate continues over the use of recombinant allergens in the manufacturing and quality control of *globally* vs *isoallergen-limited* reagents. Future clinical studies that assess the diagnostic sensitivity, specificity, and the positive and negative predictive values of assays using panels of recombinant allergens will clarify the question of their clinical utility in the diagnosis of allergic disease.

Thus, despite the questions that remain about monoclonal antibodies and recombinant allergens, the fact is the protein distribution on final allergen-containing reagents produced by different manufacturers varies in its protein composition, allergenic potency, and immunoreactivity. For this reason, it must be assumed that allergen-containing reagents from different manufacturers bind different specificities of IgE antibody until it is shown otherwise.⁷³

Theoretical consideration of the importance of linear vs conformational epitopes in inducing prolonged vs transient IgE antibody responses is an additional issue that suggests the use of portions or epitope segments of allergenic molecules to better understand the humoral immune response of allergy patients.¹⁰³ However, further study of the utility of linear vs conformational forms of epitopes is needed to clarify their diagnostic utility.

Summarizing the issues in this section, each allergen-containing reagent is destined to detect a slightly different population of IgE antibodies and it will not be interchangeable with reagents from other manufacturers. This allergen-associated variability is difficult to separate from other assay performance issues when comparing different assay configurations to each other.

8.2.2 Procedures for Qualifying Allergen-Containing Reagents

Extensive quality control testing is performed by the allergen producer/supplier to validate the allergen source material and document the potency of the allergen extract.^{104,105} Each supplier uses a different set of testing methods, some of which are listed in Table 6. Compounding conditions (binding to solid phase, labeling) and prequalification of the allergen source material before manufacturing are highly manufacturer dependent.

Before releasing the finished allergen-containing reagent for sale, final qualification should be performed in an assay that resembles the final assay in which the reagent is to be used. While a number of qualifying parameters need to be documented on the finalized reagent (see Table 7), this might not be possible with the majority of extracts. Most of these tests require the use of human serum pools and humanized monoclonal antibodies (chimeric IgE molecules with defined specificity), but they might exclude the use of murine monoclonal antibodies or animal antisera, which are useful in earlier stages of reagent qualification. The allergen-containing reagent's binding capacity, immunoreactivity, specificity, interlot reproducibility, and real-time stability are all important parameters that may be evaluated during final reagent testing. Appendix D provides a description of some of the methods used to qualify allergen-containing reagents.

Table 6. Examples of Biochemical Methods for Qualifying Allergen Extracts/Components

Nonimmunological methods	
1.	Total protein (Nitrogen or Lowry): defines amount of allergenic and nonallergenic protein in extracts, and can quantify these molecules in terms of weight.
2.	Enzymatic analysis: In cases where the allergenic component is an enzyme, it may be useful to measure the specific enzymatic activity, especially if immunochemical detection reagents are not available. For example, the measurement of hyaluronidase and phospholipase activities in hymenoptera venoms can be used to define their allergenic activity.
3.	Amino acid analyses: not applicable to extracts, defines amino acid composition in percentage of each, but not the primary sequence.
4.	SDS-polyacrylamide gel electrophoresis (SDS-PAGE): discriminates native allergenic and nonallergenic components in an extract on the basis of their molecular weight. The estimated molecular weight determined by SDS-PAGE can differ from that calculated from the amino acid sequence as posttranslational factors influence the final molecular weight. SDS-PAGE can be applied to allergen components to detect purity, the presence of dimers and trimers, and sometimes it can show the presence of isoforms.
5.	Isoelectric focusing (IEF): discriminates allergenic components in an extract on the basis of their isoelectric point. IEF can often discriminate isoforms.
6.	2D-electrophoresis: combines the two previous methods discriminating components in an extract or isoforms in a native component preparation by both protein MW and isoelectric point.
7.	Sequencing: defines the primary amino acid sequence and composition of a given component.
8.	Mass spectrometry: applicable to allergenic components including recombinant proteins; defines purity of the preparation; discriminates among isoforms, defines the molecular weight of the component.
Immunochemical Methods	
1.	<i>RAST or ELISA inhibition</i> : As applied to allergenic extracts or allergen components, it defines the reciprocal relationship between allergen concentration and the percentage of inhibition of IgE antibody binding. This relationship strongly depends on allergen extract composition, number of homologous components or epitopes, and the specificities of the IgE antibody in the serum or serum pool used. Testing an allergenic extract should involve using it both on solid phase as an allergosorbent and in solution phase as a soluble antibody inhibitor. Testing performed with only one of these configurations does not necessarily provide comprehensive information, since the confirmation of particular epitopes can be differentially presented on molecules from two source extracts.
2.	<i>Specific Allergen Content</i> : Quantitative immunoelectrophoresis, such as cross (radio)-immunoelectrophoresis and rocket immunoelectrophoresis; radial immunodiffusion; two-site (sandwich) ELISA; or competitive ELISA inhibition assay methods may be validated to measure specific allergen concentrations in extracts. The performance characteristics of these quality control assays will depend mainly on the characteristics of the specific detection antibody employed (eg, monoclonal or polyclonal).
3.	<i>Western blot analysis</i> : Solid phase immunoassay on paper following the separation of allergen components by SDS-PAGE, isoelectrofocusing, or 2D-electrophoresis. Once proteins are separated by one of these methods, specific IgE containing sera are applied. IgE in the serum binds to allergenic components insolubilized on nitrocellulose paper immunoblot of the gel, and bound antibody is detected with a labeled secondary anti-IgE antibody.

Table 7. Idealized Qualification of Finalized Allergen-Containing Reagents *

Illustration 1

Qualifying Parameter	Description, Method, or Source Information
Allergen extract name (genus, species)	Natural rubber latex (<i>Hevea brasiliensis</i>)
Allergen extract source	<i>H. brasiliensis</i> rubber tree sap (nonammoniated); confirmed by supplier
Binding capacity	10 ng IgE antibody/tube based on plateau of dilution recovery analysis
Parallelism	< 15% intradilutional coefficient of variation based on dilution-recovery analysis with 10 different sera
Specificity	< 1% cross-reactivity based on inhibition with heterologous allergens in same allergen group, alternatively, comparison of regression slopes of dose-response curves, which depends on the IgE antibody specificities in the sera used
Interlot reproducibility	IgE binding capacity range from 5–15 ng IgE antibody per tube based on testing of quality control serum pool with three lots of latex allergen-containing reagent
Stability (document expiration date)	1 year based on real-time repetitive testing of quality control serum
Nonspecific binding (NSB) analysis	Evaluation with two or more sera containing high levels of irrelevant IgE [†]
Known purified allergens	Hev b 1, 2, 3, 4, 5, 6.02, 7, 8, 9, 10, 11, and 12, and Hev b 13

* Illustrated by a model latex allergen reagent. This type of datasheet is not available for many allergen specificities.

[†]The level of irrelevant IgE in serum used to test for nonspecific binding is selected depending on the characteristic of the allergen being tested. For example, when evaluating a single sensitivity to a discrete allergen (eg, penicillin), a lower level of nonspecific IgE in the NSB control is appropriate. In contrast, when testing of multiple allergen-specificities such as to food allergens, a higher level of irrelevant IgE in the NSB control would be more appropriate.^{104,105}

Illustration 2*

Qualifying Parameter	Description, Method, or Source Information
Allergen extract name (genus, species)	Common Ragweed (<i>Ambrosia artemisiifolia</i>)
Allergen extract source	<i>A. artemisiifolia</i> pollen; purity confirmed by supplier
Binding capacity	10 ng IgE antibody/tube based on plateau of dilution recovery analysis
Parallelism	< 15% intradilutional coefficient of variation based on dilution-recovery analysis with 10 different sera
Specificity	< 1% cross-reactivity based on inhibition with heterologous allergens in the same allergen group, alternatively, comparison of regression slopes of dose-response curves, which depends on the IgE antibody specificities in the sera used
Interlot reproducibility	IgE binding capacity range from 5–15 ng IgE antibody per tube based on testing of quality control serum pool with three lots of ragweed allergen-containing reagent
Stability (document expiration date)	1 year based on real-time repetitive testing of quality control serum
Nonspecific binding (NSB) analysis	Evaluation with two or more sera containing high levels of irrelevant IgE [†]
Known purified allergens	Amb a 1, 2, 3, 5, 6, 7, 8, 9, 10

*Illustrated by a model latex allergen reagent. This type of datasheet is not available for many allergen specificities.

[†]The level of irrelevant IgE in serum used to test for nonspecific binding is selected depending on the characteristic of the allergen being tested. For example, when evaluating a single sensitivity to a discrete allergen (eg, penicillin), a lower level of nonspecific IgE in the NSB control is appropriate. In contrast, when testing of multiple allergen-specificities such as to food allergens, a higher level of irrelevant IgE in the NSB control would be more appropriate.^{104,105}

9 Calibration Systems for Assays of IgE Antibodies

In the late 1960s, the simple presence of IgE antibody in the patient's serum that was able to bind to a defined allergen specificity was used as a diagnostic indicator of allergic disease.⁹⁹ Currently, the probability of a patient experiencing severe allergic symptoms following exposure to an allergen source has been shown to be related (among other biological factors) to the quantity of IgE antibody specific for that allergen specificity that can be measured in the patient's serum.^{70-72,74,75,97,99} This identified relationship between IgE antibody levels and symptoms has motivated manufacturers to develop quantitative assays for determining the amount of IgE antibody in the patient's serum that is specific for a defined allergen specificity.

Classically, IgE antibody levels were reported using a number of arbitrary units, depending on the degree of quantitation afforded by the assay system (see Table 8). In *qualitative assays*, IgE antibody in the serum specimen is reported as positive or negative based on the level of the response signal measured, as compared with a preassigned positive threshold level. In a number of *semiquantitative assays*, both a

positive/negative result, the magnitude of the signal measured (eg, luminescent units [LU], millivolts [mVolts]), the class score, the adjusted or normalized counts (from modified scoring systems), or the percent of the lowest control (alternative scoring system) are reported. The magnitude of the signal is related in terms of rank order to (but not consistently directly proportional to) the quantity of IgE antibody present in the test serum. Most of these arbitrary units and artificial calibration schemes are presented here for historic reasons and are no longer in clinical use.

Quantitative IgE antibody assays employ the most advanced methods of assay calibration. The purpose of the calibration portion of the quantitative assay is to define the dose-response relationship of the assay so response results obtained by testing patients' sera can be interpolated in dose units that relate to the relative quantity of IgE antibody in the serum. Both *homologous* and *heterologous interpolation* methods have been successfully used in commercially available IgE antibody assays.¹⁷ The homologous interpolation procedure promotes overall assay parallelism and maximizes the assay's working range by using the same solid-phase allergen throughout the assay, and constructing a calibration curve with human IgE antibody of the same allergen specificity as is to be detected in the test sera. In general, the IgE antibody-containing reference serum pool dilutes out in the same manner as the test serum IgE, thus ensuring assay parallelism. While there are advantages in terms of defining the working range and limits of detection of the assay for each allergen specificity, the primary limitation of this approach is the requirement for liter quantities of human serum pools that contain IgE antibody specific for each allergen specificity to be tested. It is difficult to maintain a serum bank that can supply these large quantities of human serum in a reproducible manner between lots, especially for the less common allergen specificities.

Table 8. Methods of Assay Calibration and IgE Antibody Units

Qualitative assays (no calibration curve)
Positive/negative based on preassigned positive threshold
Semiquantitative assays
(1) Single-point calibration (negative, low moderate, high levels)
(2) Multipoint calibration curve: arbitrarily derived class scoring system (0–5 classes)
Quantitative assays (multipoint calibration curve)
(1) Homologous interpolation
(2) Heterologous interpolation (calibrated titration assays)
• Representative allergen (birch)
• Anti-IgE (total IgE) calibration component of assay

Because of constraints placed on assays using the homologous interpolation calibration as a result of limited IgE antibody-containing human serum pools, heterologous interpolation from a total IgE calibration curve has been adopted as the calibration strategy for present day quantitative IgE antibody assays that involve hundreds of different allergen specificities. The heterologous interpolation system has become the industry standard, and the homologous interpolation has so far only been used in assays that are no longer commercially available. In the heterologous interpolation system, a total serum IgE calibration curve is run simultaneously with the allergen-specific IgE portion of the assay, using an IgE calibrator that is traceable to the WHO 75/502. The attractive feature is the availability of this calibration strategy to all manufacturers of IgE antibody assays. Linearity has been demonstrated by showing that the calibration (total IgE) dose-response curve portion of the assay dilutes out in parallel with IgE antibody levels as measured in patient sera. This system has been adopted as the most generic solution to the problem of interassay cross-standardization.

Automation and reagent stability have permitted the development of assay systems that store a multipoint reference curve in computer memory that can be repetitively used for up to 30 days. Control specimens are analyzed to validate the reference curve stored in memory for that assay run up to a 30-day period. At present, it is not clear what IgE antibody levels between 0.1 kIU/L and 0.35 kIU/L mean clinically.

Possibly, these low levels of IgE antibody will have value for allergen specificities such as peanut, where very low levels of IgE antibody in blood can indicate a patient's sensitization status to an important food allergen. In addition, pediatric samples with often very low but relevant IgE antibody levels may be analyzed with an expanded dynamic range and increased assay sensitivity. Further studies are needed to clarify this issue and variability associated with this lower analytical sensitivity.

The manufacturer has the responsibility to ensure the calibration portion of the assay dilutes out in parallel with patient test sera. One procedure for demonstrating parallelism is the dilution recovery analysis, which is discussed in detail in Appendix B. The user of an assay has the responsibility of analyzing a sufficient number of internal quality control sera in each analysis to validate the assay's calibration curve.

10 Assay Performance Evaluation

Analytical performance of the total serum IgE and IgE antibody assays can be subdivided into precision and reproducibility (variation), dynamic range, detection limits, analytical accuracy, and linearity. Methods that are used by manufacturers to document the performance of their assays before they are released for use are summarized in the following text and described in detail in the appendixes of this document.

10.1 Analytical Accuracy

The term "accuracy" refers to the nearness of a single measurement result to its accepted or true value. Moreover, an accurate measurement is one characterized by a small error that is unbiased by either systematic or random errors. In the case of total serum IgE assays, the WHO IgE Serum Standard 75/502 and the US IgE Standard have been used as primary reference materials to assess accuracy. For IgE antibody assays of defined allergen specificity, there are currently no universally accepted IgE antibody standards with calibrated levels of allergen-specific IgE.¹⁰⁶⁻¹⁰⁸ Therefore, it has been difficult to demonstrate the accuracy of any IgE antibody assay with recovery studies that examine how well the assay can detect the known amounts of analyte that have been added to a protein matrix. Several research-based secondary IgE antibody standards prepared using antibody adsorption or depletion methods and humanized IgE chimeric antibodies have been used to calibrate IgE antibody assays in mass per volume units and study the quantitative nature of the assays. Chimeric IgE antibodies have been used to demonstrate that 1 kIUa/L of specific IgE antibody may, under certain conditions, be as measured in one commercially available IgE antibody assay equivalent to 1 kIU/L of total IgE.¹⁰⁹ Dilution recovery plots have been used to examine what the assay measures with what is expected from an ideal immunoassay. Actual values given by different assays with the same diluted samples were compared by regression and least squares analysis to the expected ideal assay, which has a dilution recovery slope of 1 on a semilogarithmic plot.⁷³ This procedure can be effective in evaluating accuracy and quantitative nature of IgE antibody assays across their reported working ranges.

10.2 Assay Interference

There are several factors in human serum that have the potential for interfering with the performance of the total serum IgE and IgE antibody immunoassays. The manufacturer should evaluate what effect human rheumatoid factor, naturally occurring and therapeutically administered antihuman IgE autoantibodies, and human IgG anti-allergen antibodies have on the accuracy of their assay. Human rheumatoid factors, which are autoantibodies that cross-link human immunoglobulins, are known to cross-link immunoglobulins from other species. As such, they can interfere in assays that use both polyclonal and monoclonal antisera produced in animals, and they might cause false-positive and false-negative results in immunoassays that measure human antibodies, depending on the particular assay design. Naturally occurring anti-IgE has been detected in many human sera and bound to basophils. Their clinical significance is presently unclear.¹¹⁰ Therapeutically administered antihuman IgE antibodies

(omalizumab) are known to interfere in some total and allergen-specific IgE assays.²⁸ The potential for their interference in human IgE antibody assays should be evaluated during the validation stage of assay development. IgG anti-allergen interference has been extensively documented for some assays where allergen-excess conditions are difficult to achieve. This issue should be examined for select allergen systems where specific IgG antibodies are known to reach $\mu\text{g/mL}$ levels in untreated subjects (eg, hymenoptera venom immunotherapy). Finally, cross-reactive carbohydrate determinants may produce positive IgE antibody results, the clinical significance of which is not clear.⁸³⁻⁹⁵ The use of a nonspecific carbohydrate matrix (eg, bromelain or peroxidase) to assess this potential cross-reactivity has been reported and needs further investigation.¹¹¹

11 Quality Assurance

Quality assurance of patient testing systems, such as those used by clinical laboratories to measure total serum IgE and IgE antibody of defined specificities, remains vital to ensuring the validity of reported results. Three areas of quality assurance should be considered for total and allergen-specific IgE antibody assays. The first is performed by the manufacturer at the time of reagent and assay production. If performed effectively, the manufacturer quality assurance testing has the effect of minimizing subsequent internal intralaboratory quality control testing that must be conducted in the clinical laboratory. This second level of quality assurance by the clinical laboratory user is intended to demonstrate that each assay is “in control.” The third level of quality assurance involves participation of the laboratory staff in an external quality assurance survey.

11.1 Quality Assurance in Manufacturing

The type of quality assurance testing that is performed on an assay during manufacturing depends on its complexity and expected degree of quantitation. Once the component reagents have passed their performance evaluation, then the assembled assay should be tested with sera from clinically documented nonallergic (IgE antibody-negative) and allergic (IgE antibody-positive) persons. A structured quality assurance program with “multirule” procedures is extensively described by Westgard and Klee.¹¹²

11.1.1 Total Serum IgE

For total serum IgE assays, sera containing IgE levels that span the reported dynamic range of the assay (typically ~ 1 to ~ 2000 kIU/L) should be shown to be within 95% confidence limits of a range that has been established by at least 10 previous measurements. With this approach, 2.5% of the assay lots would be expected to fail, whether there is something wrong with the reagents or assay run or not. Westgard’s Rules¹¹² are useful in interpreting quality control data to minimize false rejection and to maximize the detection of random and systematic errors. The primary goal of this testing should be to document the reproducibility of the assay with a precision profile (plot of CV_{dose} vs Dose) and the detection limits (minimum detectable dose) and linearity (interdilutional %CV) with a dilution-recovery analysis. All these analyses must demonstrate that the assay is correctly calibrated to an internationally recognized human IgE standard.

11.1.2 IgE Antibody Assays

For qualitative IgE antibody assays, testing of a panel of allergen-specific IgE antibody positive and negative control sera should generate results that identify their correct IgE antibody assignment. The goal of these quality assurance analyses is to document the appropriateness of the positive threshold and validate the allergen specificity of the assay. Additionally, for semiquantitative and quantitative assays, the calibration curve should be validated with at least three quality control sera containing levels of IgE antibody that cover two to three times the detection limits, the assay range midpoint, and within 10% of the upper extreme plateau level. For quantitative assays only, reproducibility of the assay should be

documented with a precision profile and verification of the assay's limits of detection and linearity using a dilution-recovery analysis.

11.2 “Internal” Intralaboratory Quality Control

The establishment of an internal quality control program is a requirement of regulations governing clinical laboratories. Refer to CLSI document C24¹¹³ for suggestions on developing an internal quality control program. Individual strategies can vary depending on the assay design and equipment used. This section provides one strategy for the internal quality control of total IgE and allergen-specific IgE antibody assays. Alternative strategies may be suitable if they can be validated by the laboratory.

11.2.1 Total Serum IgE

A daily intralaboratory quality control program for total serum IgE assays commonly involves the analysis of three control sera for which there are well-documented IgE ranges. The control sera should be selected so they cover the low (1 kIU/L to 10 kIU/L), medium (50 kIU/L to 100 kIU/L), and high (1000 kIU/L to 2000 kIU/L) regions of a total serum IgE assay's calibration curve. A problem can be identified with the particular assay if one of the internal control specimens produces a result that is repetitively out of the expected control range (>2 SD from mean defined by 10 to 15 previous assays). For some automated assays, singlicate measurements have been shown by the manufacturer and the laboratory to be acceptable because of highly reproducible assay results. For those assays in which replicate measurements are run, high interduplicate %CVs should be repeated for confirmation of accuracy.

11.2.2 IgE Antibody Assays

Several IgE antibody assay formats are commercially available, each of which may require a slightly different quality control strategy. All the assay formats, however, share the use of different allergen-containing reagent components. This special aspect of IgE antibody assays has created two schools of thought on what constitutes a proper internal quality control program for human IgE antibody assays.

One approach suggests that each allergen-containing reagent (with a different specificity) is a separate unique test that should have its own specific IgE quality control serum, which should be tested in each assay when it is used. This strategy does not recognize the extensive stability and quality control testing that is conducted on each allergen-containing reagent by the manufacturer before it is released for use. Moreover, this strategy is impractical and costly in that it would require each laboratory to maintain a vast serum bank of IgE-positive sera for every allergen specificity. The alternative approach views the IgE antibody assay as a system that employs several hundred different allergen-containing reagent “components.” As such, each allergen-containing reagent is considered already quality controlled by the manufacturer, and the user must 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 can be readily accomplished with the analysis of three sera containing high, medium, and low levels of IgE antibody, in a manner similar to those used in the total serum IgE assay.

For laboratories that run semiquantitative or quantitative assays, minimally a single IgE antibody positive control serum is generally analyzed in each assay to provide a second level of quality assurance. To accomplish this, some laboratories identify a group of 5 to 15 common allergen specificities (eg, common ragweed, oak tree, *Alternaria tenuis*, dog dander, and *Dermatophagoides farinae*) for which IgE antibody containing human sera are plentiful. A large serum pool is then prepared that contains IgE antibody to all 5 to 15 specificities and analyzed against all 5 to 15 allergen-containing reagents separated throughout each assay run. This specific IgE antibody testing program provides confirmation of the calibration portion of the assay, and it validates the other common reagents that may be exclusively used in the allergen-specific IgE portion of the assay. A more detailed discussion of internal quality control testing is presented in CLSI document C24.¹¹³

11.3 “External” Interlaboratory Proficiency Testing

All laboratories that perform total serum IgE measurements are required to demonstrate satisfactory performance in one of several external interlaboratory proficiency testing surveys. Similar expectations are present for laboratories that measure allergen-specific IgE antibodies. To meet these requirements, it is recommended that a survey challenge the laboratory at a minimum three times a year, with at least five sera per cycle. One well-subscribed program available internationally is the SE survey conducted by the College of American Pathologists.^{99,108} In addition to providing a challenge for total serum IgE, it also provides five allergen-specific IgE antibody challenges per serum. In this survey, there is a sixth wild card specimen that is analyzed in the first and third cycles to allow specific IgE antibody measurements to rarer allergen specificities to be tested. Examples of other international proficiency surveys include the UKNEQAS survey conducted in Europe, the Australian RCPA Quality Assurance Program in Immunology (allergy specialty), and the Instand e V Ringversuche program in Germany. In addition, manufacturers of specific IgE assay reagents may offer their own quality assurance program for individuals who purchase their reagents. The latter clubs are useful, but not viewed as sufficiently independent to serve as an impartial measure of laboratory performance for laboratory inspectors. The primary goal of these proficiency testing programs is to identify laboratories that accurately measure total serum IgE and correctly identify sera that contain IgE antibody of differing allergen specificities. Total serum IgE results in kIU/L are compared to peer group means and ranges, and outlier laboratories are identified that exceed the 99% confidence limit for their peer group. More recently, peer group data for many of the quantitative assays have been useful in assessing individual laboratory performance.

Table 9. Illustrative External Proficiency Surveys for Diagnostic Allergy Laboratories

Variable	Survey (CAP-SE)*
Cycles per year	3 (every 17 weeks)
Sera per cycle	5 (individual or pools)
Multiallergen IgE antibody screen per serum	1
IgE antibody measurements per serum	5 (mixed positive/negative)
Allergen-specific IgE analyses per year per laboratory	75
Total IgE analyses per year	15
Definition of outliers	Method consensus
Reporting units	kIUa/L

*CAP SE refers to the College of American Pathologists’ Survey.

Total serum IgE results are viewed within the context of the peer group 95% confidence interval for any given method. Moreover, bias in a particular total serum can be identified by noting consistent trends (values always higher or lower than the mean + 1 SD) and exceeding the intermethod 95% confidence interval.

When evaluating the allergen-specific IgE antibody results, surveys collate two types of allergen-specific IgE antibody data: (1) qualitative results (positive/negative) that reflect the presence, and in some cases, the relative level of IgE antibodies from semiquantitative assays such as in multiallergen screen; and (2) unit results (eg, kIUa/L) that are produced by quantitative IgE antibody assays. Qualitative results are in error when a laboratory obtains a positive or negative result, which is inconsistent with peer laboratories performing the same method. Moreover, differences in the quantitative unit results produced by a single laboratory can be compared to a peer group range of unit results produced by peer participants that perform the same method. On a fundamental level, outlier laboratories can be identified when a laboratory detects IgE antibody in a serum from a nonatopic (clinical history negative, negative skin test result) person. Likewise, an outlier laboratory can be identified when an undetectable IgE antibody result

is reported for a serum from an atopic individual (positive clinical history and a positive skin test and or provocation test). Moreover, with sufficient numbers of laboratories ($n > 10$) providing quantitative measurements of allergen-specific IgE antibody, it is possible to determine if any single laboratory result is within or outside the 95% peer group range.

12 Assay Performance Targets

Evaluation of the performance of a commercial total serum IgE or IgE antibody assay begins with a *careful review* of the assay procedure and reagent product inserts that supply detailed descriptions of the assay design, component reagents, and manufacturer-derived quality assurance testing. Performance results supplied by the manufacturer in the product insert and associated published literature should include documentation of the assay's dynamic range, intra- and interassay precision profile analyses, analytical detection limits, dilution-recovery results, nonspecific binding levels for representative allergen-containing reagents using sera from nonatopic persons and high IgE containing sera (IgE myelomas), and results of tests on the allergen and IgE specificity of the assay. Table 10 summarizes the subcommittee's recommendations for evaluation criteria and illustrative minimal performance targets for total serum IgE and allergen-specific IgE antibody assays.

12.1 Specific Recommendations for Manufacturers of Allergen-Specific IgE Antibody Assay Kits and Reagents

Two types of manufacturer's product literature are recommended for use with total serum IgE and allergen-specific IgE antibody assays. The first is a *general product information brochure*, which covers issues common for the assay method, and the second is a *specific product information sheet* that is unique for each allergen-containing reagent and assay method combination. Both are supplied by the manufacturer to the user.

12.1.1 General Product Information Brochure

The manufacturer's general product literature that accompanies each assay should cover the following issues. This information should be written in a manner that is consistent with applicable regulations as set forth in the user's country.

- A description of the *intended use* of the kit.
- A statement on the *intended category* of the assay (qualitative, semiquantitative, or quantitative). In some cases, several categories apply. For instance, a quantitative assay may also employ a semiquantitative assay format such as an allergen-specific IgE assay where a multiallergen screen is also analyzed.
- A description of the *common assay reagents* (buffers, antihuman IgE conjugate) and *required general laboratory supplies*.
- The detailed description of the *general assay procedure* with a step-by-step use of any *interpolation schemes* if there is an internal calibration curve.
- Appropriate *specimens and dilutions* to be analyzed in the assay.
- *Expiration date* of common assay reagents.
- Expected and achievable *intra-assay, interassay precision* across the linear range of the assay (precision profile: Plot of CV_{dose} vs Dose).

- Results of *dilution-recovery analyses* for assessment of assay linearity, primarily for quantitative assays.
- Recommendations for daily laboratory quality control and external proficiency testing programs.
- Test *limitations, known interfering substances, and cautions regarding assay performance and interpretation of data (see Section 12.1.3).*

Table 10. Evaluation Criteria and Minimal Performance Targets for IgE Antibody Assays

Assay Evaluation Criteria		
Analytical Evaluation Criteria	Performance Target	
Linear range (sensitivity limit-highest calibrator) [“units”]	M*	Assay and unit dependent
Precision within an assay (intra-assay) [%CV]		
Low (sample closest to lowest calibrator)	M/U†	< 10%
Medium (sample between high and low calibrator)	M/U	< 10%
High (sample closest to highest calibrator)	M/U	< 10%
Precision total imprecision (interassay) [%CV]		
Low (sample closest to lowest calibrator)	M/U	< 20%
Medium (sample between high and low calibrator)	M/U	< 15%
High (sample closest to highest calibrator)	M/U	< 15%
Detection limit (lowest level distinguishable from a “zero” sample)	M/U	Assay and unit dependent
Dilution recovery (indicates capacity of solid phase)	M	Ideal = 100%
Nonspecific binding of IgE (for allergens only; high IgE sample [IU/mL or ng/mL] normally > 1000 IU/mL)	M	Ideal < 10% low standard
Correlation to other technologies (define predicate device)		
Slope (analytical sensitivity)	M	Ideal = 1.0
Correlation coefficient [r=1]	M	Ideal r = 1.0
Stability of assay (calibration curve) % change over time [%]	M	< 10%
Interferences and cross-reactivity		
IgG, IgM, IgA, IgD [%]	M	< 0.001%
Other interference, eg, hemolytic, lipemic samples, high allergen-specific IgG antibody following immunotherapy	M	None
Other Assay Characteristics to Consider		
Serum requirements (microliter/test)	M	<100 uL/test
Unit definition and standardization	M	Assay dependent
Data reduction algorithm	M	Assay dependent

*M = Manufacturer

†U = User

12.1.2 Separate Product Information Sheet for Allergen-Containing Reagents

Individualized product information leaflets may be supplied with the reagent or in a website, which provides a description of the common and scientific name of each of the allergen-containing reagents provided by the company. Each allergen's summary describes:

- Allergen code and the general geographic distribution of the allergen within the areas where the product is sold.
- Current knowledge about the allergen (eg, degree of standardization by WHO and characterization of individual allergen components).
- A summary of specificity (cross-reactivity) studies with other allergenic extracts from the same allergen source and different sources (eg, latex vs kiwi or avocado). The same applies to allergenic molecules where indication about allergen groups should be provided (eg, Bet v 2: belongs to the plant profilin group).
- Expiration information.
- Interlot variability results using three lots of product with three quality control sera.
- References relating to immunochemical characterization and quality control of allergen. Where possible, manufacturers should use common allergen nomenclature defined by the IUIS Nomenclature Committee (www.allergen.org) to minimize confusion among users and promote maximum clarity in interlaboratory proficiency surveys.

12.1.3 Assay Limitations

Laboratory tests for allergen-specific IgE antibodies have been established as being of value in the clinical evaluation of allergic disease. However, no specific IgE test result provides absolute evidence of the presence or absence of allergic disease. Information obtained from any test method about serum levels of specific IgE antibodies can indicate sensitization to test allergens. The presence of IgE antibodies of a defined allergen specificity must be considered in conjunction with information obtained from the clinical evaluation of the patient and other diagnostic procedures in making a diagnosis of allergic disease. The levels of specific IgE antibodies measured in any specimen by assays from different manufacturers can vary due to differences in assay methods and reagent specificity, especially associated with the allergen-containing reagent. Thus, results reported in terms of specific IgE levels from different assays cannot be used interchangeably. This applies also to allergenic molecules, although differences to a lesser extent are expected. This aspect deserves further study.

12.2 Recommendations for the Diagnostic Allergy Laboratory

Laboratory personnel may refer to comparative assay data and critical reviews published in refereed journals, public domain performance data from interlaboratory proficiency studies (eg, CAP SE Survey), and data generated by validation studies conducted in-house to make decisions to aid in their selection of total serum IgE and allergen-specific IgE antibody assays and reagents.

The user of a commercial assay that fulfills the target performance characteristics defined in Table 10 should not need to repeat extensive quality assurance tests that have been performed on the individual component reagents and finalized assay by the manufacturer, often using sera that are difficult to obtain. Rather, the daily quality control program conducted by the laboratory should be designed to identify random and systematic errors caused by the laboratory professional, equipment, and internal laboratory-related problems. The type and number of quality control sera run in each assay should be appropriate for

the assay's degree of quantitation. Quality control sera should be selected that permit validation of the assay's calibration curve, which is an indication that the assay is in control. Daily testing of each allergen-containing reagent for performance is not recommended if it is used according to the manufacturer's procedure and the reagent lot has not expired. The laboratory should define unambiguous tolerance limits for positive and negative controls based on the manufacturer's recommendations and in-house validation studies. Daily performance of quantitative assays is best evaluated by plotting unit data from each control in Levey-Jennings quality control charts.

Finally, it is imperative that the assay method used in measuring total serum IgE or allergen-specific IgE antibody in human serum be listed on each page of the laboratory report so the user can determine which published predictive data can be used for interpretation of the reported data. It is the responsibility of the diagnostic allergy laboratory staff to make sure the assay method that is used to measure total IgE and/or allergen-specific IgE antibody levels in human serum is clearly listed on the final report. The listing of the assay method on the report provides critical information on this formal document to the medical professional who will make clinical decisions based on the result.

12.3 Recommendations for the Medical Professional

The clinical history drives the diagnosis of human allergic disease. Once the history has provided a strong suspicion of IgE mediated disease, *in vivo* and *in vitro* methods both can be used to confirm the presence of allergen-specific IgE antibody in the skin and blood, respectively. Detection of IgE antibody of a defined allergen specificity in the skin and blood simply provide confirmation for sensitization (IgE antibody positivity), not allergic disease. The positive clinical history makes the critical link with these confirmatory test results to identify clinically evident human allergic disease.

There are circumstances where the skin test and the serology have their distinct advantages and limitations in the diagnostic process. In general, good concordance has been identified on a dichotomous basis between a positive skin test and a positive serology for most potent aeroallergens from trees, grasses, weeds, epidermal allergens (eg, cat and dog), and dust mite allergens. Difficulties with the direct comparison of the skin test and serology results in any given study often can be isolated to differences in the technique or assay (methodology) and quality/stability of the extract or allergosorbent (biological reagent). Exceptions to the general rule of good intermethod concordance (eg, a negative skin test or IgE serology with a positive clinical history) require repeat analysis or use of an alternative method. After repeat analysis, if the IgE antibody test results (either skin test or serological IgE antibody analysis) remain inconsistent with the patient's clinical history, the overriding criteria in making the final diagnosis should be the clinical history and physical examination combined with clarity from a challenge test.

When selecting IgE antibody detection methods in the diagnostic process, *in vivo* skin testing has been generally favored with drug and insect skin allergy, while IgE-mediated allergic disease involving mold and food allergens are areas where serological measurement of IgE antibody have become increasingly favored. This is especially true with children where serological methods have generated quantitative IgE antibody results specific for milk, egg, peanut, soy, wheat, and other foods known to be allergenic.

When sending a serum to a clinical laboratory, the referring medical professional should follow several simple steps to make sure that they are obtaining the highest quality serological results.¹¹⁴ First, the medical professional should ensure that the laboratory is accredited by the appropriate accrediting body. This accreditation ensures that the laboratory personnel who are performing and overseeing IgE antibody testing are qualified, proper records are being kept, and the laboratory has documented, validated, and written procedures in place to ensure quality of reported IgE antibody results.

Second, the referring medical professional should become informed about what assay method is used in the laboratory to measure total and allergen-specific IgE antibody. Not all methods perform equally well or possibly measure the same population of IgE antibodies or not all allergen-specific IgE antibody data

can be used with published disease prediction algorithms. To this end, the medical professional should require that the assay method used to measure the total and allergen-specific IgE antibody be written clearly on the laboratory report. Ask for an example of a laboratory report to verify this. There are occasions in some countries where medical professionals are required to send their patient's serum to a capitated or so-called in-network laboratory that is dictated by the patient's medical insurance policy. Capitated laboratories establish a contract with the insurance company to receive part of or all blood work for claims that are reimbursed by the insurance company. In doing so, the laboratory provides a favorable fee structure and it is assured of receiving a certain amount of work. In these cases, which commonly involve patients who belong to health maintenance organizations (HMOs), the medical professional will not know what assay method was used to measure total and allergen-specific IgE. This is why the medical professional should require that the assay method is written on the laboratory report, and the laboratory verifies in writing that it does not deviate from using the indicated assay. Moreover, this should address the concern that a clinical laboratory may manufacture some of its own allergen-containing reagents and not properly validate or quality control them. When the physician is familiar with a particular assay method, he/she can specify that the method be used.

Third, the clinical laboratory is required to have established quality assurance practices in place to ensure high quality of its reported results. Medical professionals should request a year's worth (typically three challenge cycles) of external proficiency data to verify that they are performing their total and allergen-specific IgE measurements in a manner consistent with peer laboratories. By reviewing the proficiency survey data in the form of a participant summary, the medical professional can quickly determine how well the assay method performs in general and the laboratory performed in particular. The laboratory staff will need to identify what result the laboratory reported, since most participant summary reports are coded so laboratories are not identified except to the organization conducting the survey.

Fourth, allergen-specific IgE antibody measurements performed using a defined instrument or assay platform need to meet the appropriate regional and/or national requirements for *in vitro* diagnostic testing.

12.4 Recommendations for Regulators and Laboratory Inspectors

Measurands that are quantified by assays used in clinical laboratories are divided into regulated and unregulated analytes. Total serum IgE is an example of a regulated analyte. The IgE molecule is well characterized (see Table 1), and an internationally accepted IgE standard has been widely available with assigned mass per volume unitage (1 Unit of IgE = 2.42 ng of IgE). Thus, all manufacturers of total serum IgE assays can calibrate to the same reference preparation and the result is excellent intermethod agreement between the results of total serum IgE assays from different manufacturers (see Table 2). A laboratory that performs poorly on an external proficiency survey and consistently reports total serum IgE results in the >3 SD or <3 SD range for its peer group with the same method will lose the privilege of measuring total serum IgE and reporting it to its clients.

In contrast to total serum IgE, allergen-specific IgE has remained a nonregulated analyte. This means there are no rigorous criteria for inspectors to identify a laboratory that performs poorly and remove its privilege to perform allergen-specific IgE antibody measurements. In the early years of allergen-specific IgE assay development, no uniform standardization scheme existed. This meant different units reflecting relative levels of IgE antibody remained totally arbitrary. The clinically important information at that time was in the absence or presence of IgE antibody as a marker for "sensitization." Gradually, the total IgE heterologous calibration system was adopted across methods for detecting allergen-specific IgE antibody and assays became traceable to a human IgE international standard. Furthermore, the total IgE calibration curve was shown for most assays to be linear or parallel to dilutions of patient specimens when corrected for dilution. This has led to the generation of quantitative assays, where at least in one case, 1 IUa/L of allergen-specific IgE antibody has been proven to be equivalent to 1 IU/L of total serum IgE.¹⁰⁹ Moreover, interdilutional coefficients of variation of $< 20\%$ can typically be achieved across the working range of the assay with most of the automated IgE antibody platforms.

The European experience has been somewhat different and the concept of regulated and nonregulated analytes does not exist. The responsibility for the selection and validation of the laboratory methods employed falls on the laboratory director and the medical director.

Laboratory inspectors should expect to see a listing of the assay method used to measure total and allergen-specific IgE antibody on all laboratory reports. Moreover, there should be clear notation of which allergen-containing reagents are used by the clinical laboratory.

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Appendix A. Allergen Nomenclature Addendum (Scientific Names and Codes of Allergens)

The compendium that begins on the following page provides the user (laboratorian, clinician), regulator-inspector, and manufacturer with the following allergen nomenclature information:

Column	Information Provided
1	Master list of current allergen codes used by companies
2	Assay Descriptor (thread-based system, disc-based system, sponge-based system, biotin-allergen-based system, chip-based system)
3	Product's common name
4	Source taxonomical name
5	Strength of documentation of the substance as an allergen (1 = well documented in literature; 2 = moderate documentation; 3 = weak documentation)
6	International Union of Immunological Societies (IUIS) Nomenclature Code (www.allergen.org) for allergenic molecules
7	Numerical Allergome code (www.allergome.org) for extracts and allergenic molecules
8	Nature of the allergenic molecule: NN = native; RR = recombinant

The primary goal of this appendix is to harmonize the allergen code list that is used by all manufacturers to refer to a particular allergen specificity.

To ensure its accuracy and maximize its utility, CLSI plans to update this addendum on a regular basis after consultation with manufacturers and scientists.

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
g						Grass pollens					
g1	x		x	x	x	Sweet vernal grass	<i>Anthoxanthum odoratum</i>	1		1718	
g2	x		x	x	x	Bermuda grass	<i>Cynodon dactylon</i>	1		1796	
g3	x		x	x	x	Cocksfoot grass, Orchard grass	<i>Dactylis glomerata</i>	1		1798	
g4	x		x	x	x	Meadow fescue	<i>Festuca elatior</i>	1		1820	
g5	x		x	x	x	Rye-grass (perennial rye grass)	<i>Lolium perenne</i>	1		1868	
g6	x		x	x	x	Timothy grass	<i>Phleum pratense</i>	1		1924	
g7	x		x	x	x	Common reed (common reed grass)	<i>Phragmites communis</i>	1		1927	
g8	x		x	x	x	Meadow grass, Kentucky blue	<i>Poa pratensis</i>	1		1935	
g9			x	x		Redtop, Bentgrass	<i>Agrostis stolonifera</i>	1		1337	
g9	x					Redtop, Bentgrass	<i>Agrostis gigantea</i> (<i>Agrostis alba</i>)	2		1701	
g10	x		x	x	x	Johnson grass	<i>Sorghum halepense</i>	1		1979	
g11	x		x	x		Brome grass	<i>Bromus inermis</i>	1		1754	
g12	x		x	x	x	Cultivated rye (cultivated rye grass)	<i>Secale cereale</i>	1		1969	
g13	x		x	x	x	Velvet grass	<i>Holcus lanatus</i>	1		1842	
g14	x		x	x	x	Cultivated oat (cultivated oat grass)	<i>Avena sativa</i>	1		1736	
g15	x		x	x	x	Cultivated wheat (cultivated wheat grass)	<i>Triticum aestivum</i> (<i>Triticum</i> spp.)	1		2090	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
g16	x		x	x		Meadow foxtail (meadow foxtail grass)	<i>Alopecurus pratensis</i>	1		1338	
g17	x		x	x	x	Bahia grass	<i>Paspalum notatum</i>	1		1908	
g24			x			Wheat grass, Western	<i>Agropyron smithii</i> (<i>Elymus smithii</i>)	3		2160	
g30			x			Bluegrass, annual	<i>Poa annua</i>	2		3685	
g70			x	x		Wild rye grass	<i>Elymus triticoides</i>	1		2243	
g70	x					Wild rye grass	<i>Elymus condensatus</i>	1		2241	
g71	x		x	x		Canary grass	<i>Phalaris arundinacea</i>	1		2302	
g201			x	x		Barley, cultivated	<i>Hordeum vulgare</i>	1		1846	
g202	x		x	x		Maize, corn (cultivated corn)	<i>Zea mays</i>	1		2014	
g203	x		x	x		Salt grass	<i>Distichlis spicata</i>	1		1805	
g204			x	x		False oat-grass	<i>Arrhenatherum elatius</i>	1		1336	
g205				x		Phl p 1	<i>Phleum pratense</i>	1	Phl p 1	549	RR
a551		x				Phl p 1.0102	<i>Phleum pratense</i>	1	Phl p 1.0102	551	RR
g206				x		Phl p 2	<i>Phleum pratense</i>	1	Phl p 2	555	RR
a3419		x				Phl p 2.0101	<i>Phleum pratense</i>	1	Phl p 2.0101	3419	RR
a559		x				Phl p 5.0101	<i>Phleum pratense</i>	1	Phl p 5.0101	559	RR
g208				x		Phl p 4	<i>Phleum pratense</i>	1	Phl p 4	557	NN
g209				x		Phl p 6	<i>Phleum pratense</i>	1	Phl p 6	569	RR

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a3420		x				Phl p 6.0101	<i>Phleum pratense</i>	1	Phl p 6.0101	3420	RR
g210				x		Phl p 7	<i>Phleum pratense</i>	1	Phl p 7	570	RR
a3422		x				Phl p 7.0101	<i>Phleum pratense</i>	1	Phl p 7.0101	3422	RR
g211				x		Phl p 11	<i>Phleum pratense</i>	1	Phl p 11	552	RR
g212				x		Phl p 12, Profilin	<i>Phleum pratense</i>	1	Phl p 12, Profilin	553	RR
a3416		x				Phl p 12.0101	<i>Phleum pratense</i>	1	Phl p 12.0101	3416	RR
g213				x		Phl p 1, Phl p 5b	<i>Phleum pratense</i>	1	Phl p 1, Phl p 5b		RR
g214				x		Phl p 7, Phl p 12	<i>Phleum pratense</i>	1	Phl p 7, Phl p 12		RR
g215				x		Phl p 5b	<i>Phleum pratense</i>	1	Phl p 5b	558	RR
g216				x		Cyn d 1	<i>Cynodon dactylon</i>	1	Cyn d 1	266	NN
a450		x				Lol p 1	<i>Lolium perenne</i>	1	Lol p 1	450	NN
a3233		x				Cyn d 12.0101	<i>Cynodon dactylon</i>	1	Cyn d 12.0101	3233	RR
g701			x			Phl p 1.0102, Phl p 5.0101	<i>Phleum pratense</i>	1	Phl p 1.0102, Phl p 5.0101		RR
g702			x			Phl p 7.0101	<i>Phleum pratense</i>	1	Phl p 7.0101	3422	RR
g703			x			Phl p 12.0101	<i>Phleum pratense</i>	1	Phl p 12.0101	3416	RR

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
w						Weed pollens					
w1	x		x	x	x	Common ragweed	<i>Ambrosia artemisiifolia</i> (<i>Ambrosia elatior</i>)	1		1710	
w2	x		x	x	x	Western ragweed	<i>Ambrosia psilostachya</i>	1		1711	
w3	x		x	x	x	Giant ragweed	<i>Ambrosia trifida</i>	1		1712	
w4	x		x	x	x	False ragweed	<i>Ambrosia acanthicarpa</i> (<i>Franseria acanthicarpa</i>)	1		2169	
w5			x	x	x	Wormwood	<i>Artemisia absinthium</i>	1		2178	
w5	x					Wormwood	<i>Artemisia annua</i>	1		2179	
w6	x		x	x	x	Mugwort	<i>Artemisia vulgaris</i>	1		1728	
w7	x		x	x		Marguerite, ox-eye daisy	<i>Chrysanthemum leucanthemum</i>	1		1567	
w8	x		x	x	x	Dandelion	<i>Taraxacum vulgare</i>	1		1985	
w9	x		x	x	x	Plantain (English), Ribwort	<i>Plantago lanceolata</i>	1		1933	
w10	x		x	x	x	Goosefoot, lamb's quarters	<i>Chenopodium album</i>	1		1768	
w11	x		x	x	x	Saltwort (prickly), Russian thistle	<i>Salsola kali</i> (<i>Salsola pestifer</i>)	1		1961	
w12	x		x	x	x	Goldenrod	<i>Solidago virgaurea</i> (<i>Solidago</i> spp.)	1		3623	
w13	x		x	x	x	Cocklebur, common	<i>Xanthium commune</i>	1		2401	
w14	x		x	x	x	Common pigweed (rough pigweed)	<i>Amaranthus retroflexus</i>	1		1537	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
w15	x		x	x		Scale, Lenscale	<i>Atriplex lentiformis</i>	1		2193	
w16			x	x	x	Rough marsh elder	<i>Iva ciliata</i>	1		2269	
w16	x					Rough marsh elder	<i>Iva annua</i>	1		2267	
w17	x		x	x	x	Firebush (Kochia)	<i>Kochia scoparia</i>	1		1538	
w18	x		x	x	x	Sheep sorrel	<i>Rumex acetosella</i>	1		2353	
w19	x		x	x		Wall pellitory	<i>Parietaria officinalis</i>	1		1906	
w20	x		x	x	x	Nettle	<i>Urtica dioica</i>	1		2390	
w21	x		x	x	x	Wall pellitory	<i>Parietaria judaica</i>	1		1904	
w22				x		Japanese hop	<i>Humulus japonicus</i> (<i>Humulus scandens</i>)	1		1847	
w23	x		x	x		Yellow dock	<i>Rumex crispus</i>	1		2354	
w24	x		x			Spiny pigweed	<i>Amaranthus spinosus</i>	2		923	
w27			x			Carnation	<i>Dianthus</i> spp.	2		1168	
w28			x			Rose	<i>Rosa rugosa</i>	2		850	
w33			x			Clover	<i>Trifolium pratense</i>	3		2382	
w35			x			Mexican tea	<i>Chenopodium ambrosioides</i>	2		2221	
w36	x		x			Rabbit bush	<i>Ambrosia deltoidea</i> (<i>Franseria deltoidea</i>)	3		2172	
w37	x		x			Salt bush, annual	<i>Atriplex wrightii</i>	3		2196	
w39			x		x	Water hemp, Western	<i>Amaranthus rudis</i> (<i>Acnida tamariscina</i>)	3		3688	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
w41			x			Burrobrush	<i>Hymenoclea salsola</i>	3		2264	
w43	x		x			Common sagebrush	<i>Artemisia tridentata</i>	2		2184	
w45			x	x		Alfalfa	<i>Medicago sativa</i>	1		2280	
w46	x		x	x		Dog fennel	<i>Eupatorium capillifolium</i>	2		2247	
w50			x			French marigold	<i>Tagetes patula</i>	3		3691	
w53			x			Geranium	<i>Geranium</i> spp.	3		3693	
w54			x			Aloe	<i>Aloe vera</i>	3		3694	
w67			x			Groundsel bush	<i>Baccharis halimifolia</i>	3		2199	
w69	x		x			Iodine bush	<i>Allenrolfea occidentalis</i>	3		2162	
w70			x			Ragweed, slender	<i>Ambrosia confertiflora</i>	3		2171	
w75	x		x			Wing scale (wingscale)	<i>Atriplex canescens</i>	3		2191	
w82			x	x		Careless weed	<i>Amaranthus palmeri</i>	1		2134	
w90			x			Japanese hop	<i>Humulus japonicus</i> (<i>Humulus scandens</i>)	1		1847	
w202			x			Azalea	<i>Rhododendron</i> spp.	3		3699	
w203	x		x	x	x	Rape (rape pollen)	<i>Brassica napus</i>	1		1750	
w204			x	x		Sunflower	<i>Helianthus annuus</i>	1		1838	
w206			x	x		Camomile	<i>Matricaria chamomilla</i>	1		1875	
w207			x	x		Lupin	<i>Lupinus</i> spp.	1		2045	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
w209	x					Ragweed mix	<i>Ambrosia</i> spp.	2			
w210				x		Sugar-beet	<i>Beta vulgaris</i>	1		2200	
w211				x		Par j 2	<i>Parietaria judaica</i>	1	Par j 2	507	RR
w213			x			Camellia	<i>Camellia japonica</i>	3		3700	
w220			x			Knotgrass	<i>Polygonum</i> spp. (<i>Bistorta</i> spp.)	3		3773	
w230				x		Amb a 1	<i>Ambrosia artemisiifolia</i> (<i>Ambrosia elatior</i>)	1	Amb a 1	24	NN
w231				x		Art v 1	<i>Artemisia vulgaris</i>	1	Art v 1	753	NN
w232				x		Sal k 1	<i>Salsola kali</i>	1	Sal k 1	3348	NN
a3554		x				Par j 1.0103	<i>Parietaria judaica</i>	1	Par j 1.0103	3554	RR
a508		x				Par j 2.0101	<i>Parietaria judaica</i>	1	Par j 2.0101	508	RR
a512		x				Par j 3.0102	<i>Parietaria judaica</i>	1	Par j 3.0102	512	RR
a24		x				Amb a 1	<i>Ambrosia artemisiifolia</i> (<i>Ambrosia elatior</i>)	1	Amb a 1	24	NN
a789		x				Amb a 1.3	<i>Ambrosia artemisiifolia</i> (<i>Ambrosia elatior</i>)	1	Amb a 1.3	789	RR
a3375		x				Mer a 1.0101	<i>Mercurialis annua</i>	1	Mer a 1.0101	3375	RR
a3307		x				Hel a 2.0101	<i>Helianthus annuus</i>	1	Hel a 2.0101	3307	RR
a753		x				Art v 1	<i>Artemisia vulgaris</i>	1	Art v 1	753	NN
t						Tree pollens					
t1			x	x	x	Box-elder	<i>Acer negundo</i>	1		2136	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t1	x					Maple	<i>Acer saccharum</i>	2		2155	
t2	x		x	x		Gray alder, speckled alder (alder)	<i>Alnus incana</i>	1		2163	
t3			x	x	x	Common silver birch	<i>Betula verrucosa</i>	1		1741	
t3	x					Black birch (birch)	<i>Betula nigra</i>	1		2202	
t4			x	x	x	Hazel, hazelnut	<i>Corylus avellana</i>	1		1778	
t4	x					American hazel (hazelnut)	<i>Corylus americana</i>	1		2232	
t5	x		x	x	x	American beech (beech)	<i>Fagus grandifolia</i> (<i>Fagus americana</i>)	1		2249	
t6	x		x	x	x	Mountain juniper, Mountain cedar	<i>Juniperus ashei</i> (<i>Juniperus sabinoides</i>)	1		1851	
t7	x		x	x	x	Oak	<i>Quercus alba</i>	1		1955	
t8	x		x	x	x	Elm	<i>Ulmus americana</i>	1		2385	
t9	x		x	x	x	Olive	<i>Olea europaea</i>	1		1888	
t10			x	x		Walnut	<i>Juglans californica</i>	1		2125	
t10	x					Walnut	<i>Juglans nigra</i>	1		2044	
t11			x	x	x	Maple leaf sycamore, London plane, Plane tree	<i>Platanus acerifolia</i>	1		1932	
t11	x					Sycamore	<i>Platanus occidentalis</i>	1		2317	
t12			x	x		Willow	<i>Salix caprea</i>	1		2355	
t12	x					Willow	<i>Salix nigra</i>	2		2358	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t13			x			Jasmine	<i>Jasminum</i> spp.	3		3702	
t14	x		x	x	x	Cottonwood	<i>Populus deltoides</i>	1		2324	
t15	x		x	x	x	White ash	<i>Fraxinus americana</i>	1		2253	
t16	x		x	x		White pine	<i>Pinus strobus</i>	1		2312	
t17	x		x	x	x	Japanese cedar	<i>Cryptomeria japonica</i>	1		1784	
t18	x		x	x	x	Eucalyptus, gum-tree	<i>Eucalyptus globulus</i> (<i>Eucalyptus</i> spp.)	1		716	
t19	x		x	x	x	Acacia	<i>Acacia longifolia</i> (<i>Acacia</i> spp.)	1		2110	
t20	x					Mesquite	<i>Prosopis glandulosa</i>	2		2330	
t21	x		x	x	x	Melaleuca, cajeput tree	<i>Melaleuca quinquenervia</i> (<i>Melaleuca leucadendron</i>)	1		1565	
t22	x		x	x		Pecan, hickory	<i>Carya illinoensis</i> (<i>Carya pecan</i>)	1		2024	
t23	x		x	x		Italian/Mediterranean/ funeral cypress	<i>Cupressus sempervirens</i>	1		1794	
t24			x	x	x	Japanese cypress	<i>Chamaecyparis obtusa</i> (<i>Chamaecyparis</i> spp.)	1		1767	
t25				x	x	European ash	<i>Fraxinus excelsior</i>	1		1827	
t25			x			Russian olive	<i>Elaeagnus angustifolia</i>	2		1596	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t26			x			Pine, scotch	<i>Pinus sylvestris</i>	3		2313	
t27	x		x			Maple, red	<i>Acer rubrum</i>	3		2153	
t29			x			Acacia	<i>Acacia</i> spp.	2		2110	
t30			x			Birch, white	<i>Betula populifolia</i>	3		2204	
t32			x		x	Willow, black	<i>Salix nigra</i>	3		2358	
t33	x		x			Ash, Arizona	<i>Fraxinus velutina</i>	3		2256	
t35			x			Cedar, salt	<i>Tamarix gallica</i>	2		719	
t37	x		x	x		Bald cypress (white bald cypress)	<i>Taxodium distichum</i>	1		1671	
t38			x			Elm, Chinese/Siberian	<i>Ulmus pumila</i>	3		2388	
t40			x			Hazelnut tree	<i>Corylus americana</i>	3		2232	
t41	x		x	x		White hickory	<i>Carya alba</i> (<i>Carya tomentosa</i>)	2		2214	
t42	x		x			Oak, red	<i>Quercus rubra</i>	3		2342	
t43	x		x			Loblolly pine	<i>Pinus taeda</i>	3		2314	
t44	x		x	x		Hackberry	<i>Celtis occidentalis</i>	2		2217	
t45			x	x		Cedar elm	<i>Ulmus crassifolia</i>	2		2387	
t47			x			Juniper, one seed	<i>Juniperus monosperma</i>	3		2271	
t48			x			Pine, lodgepole	<i>Pinus contorta</i>	3		3704	
t49			x			Pine, ponderosa	<i>Pinus ponderosa</i>	2		1589	
t51			x			Tree of Heaven	<i>Ailanthus altissima</i>	2		693	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t52			x			Western white pine	<i>Pinus monticola</i>	3		2309	
t54				x		Russian olive	<i>Elaeagnus angustifolia</i>	2		1596	
t55				x		Scotch broom	<i>Cytisus scoparius</i>	2		3642	
t56				x		Bayberry	<i>Myrica cerifera</i>	2		1601	
t57				x		Red cedar	<i>Juniperus virginiana</i>	2		1855	
t60	x					Western juniper	<i>Juniperus occidentalis</i>	2		2272	
t61			x			Sycamore	<i>Platanus occidentalis</i>	2		2317	
t70	x		x	x		Mulberry (white mulberry)	<i>Morus alba</i>	1		1247	
t71	x		x	x	x	Red mulberry	<i>Morus rubra</i>	1		2288	
t72	x		x	x		Queen palm	<i>Arecastrum romanzoffianum</i>	1		2176	
t73	x		x	x		Australian pine	<i>Casuarina equisetifolia</i>	1		1126	
t74			x			Hibiscus (marshmallow)	<i>Hibiscus moscheutos</i>	3		3705	
t75			x			Broom	<i>Genista anglica</i>	3		3706	
t77	x					Oak mix (red, white, black)	<i>Quercus</i> spp.	2			
t80	x					Japanese cypress	<i>Chamaecyparis obtusa</i>	1		1767	
t81	x					Japanese alder	<i>Alnus japonica</i>	3		2164	
t83	x		x			Mango tree	<i>Mangifera indica</i>	3		2048	
t86			x			Mistletoe	<i>Viscum album</i>	3		2412	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t88			x			Oleander	<i>Nerium oleander</i>	3		3707	
t90			x			Walnut, black	<i>Juglans nigra</i>	2		2043	
t92			x			Agave	<i>Agave lechuguilla</i>	3		3708	
t96	x		x			Poplar, white (poplar)	<i>Populus alba</i>	2		715	
t103	x		x			Virginia live oak (live oak)	<i>Quercus virginiana</i>	2		2345	
t105			x			Pepper tree	<i>Schinus molle</i>	2		1007	
t110			x			Orange tree	<i>Citrus sinensis</i>	2		2027	
t201	x		x	x		Spruce, Norway spruce	<i>Picea abies</i> (<i>Picea excelsa</i>)	3		2306	
t202			x			Alder, smooth	<i>Alnus incana</i> ssp. <i>Rugosa</i> (<i>Alnus rugosa</i>)	2		2163	
t203			x	x		Horse chestnut	<i>Aesculus hippocastanum</i>	2		1595	
t205				x		Elder	<i>Sambucus nigra</i>	1		1963	
t206			x	x		Chestnut	<i>Castanea sativa</i>	1		1764	
t207			x	x		Douglas fir	<i>Pseudotsuga menziesii</i> (<i>Pseudotsuga taxifolia</i>)	2		3644	
t208	x		x	x		Linden	<i>Tilia cordata</i>	1		712	
t209	x		x	x		Horn beam	<i>Carpinus betulus</i>	1		1761	
t210	x		x	x	x	Privet	<i>Ligustrum vulgare</i>	1		1862	
t211	x		x	x		Sweet gum	<i>Liquidambar styraciflua</i>	2		2278	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t212				x		Cedar	<i>Libocedrus decurrens</i>	2		1206	
t213				x		Pine	<i>Pinus radiata</i>	1		1116	
t214				x		Date palm	<i>Phoenix canariensis</i>	2		718	
t215				x		Bet v 1	<i>Betula verrucosa</i>	1	Bet v 1	89	RR
t701			x			Bet v 1.0101	<i>Betula verrucosa</i>	1	Bet v 1.0101	90	RR
t215			x			Lilac	<i>Syringa vulgaris</i>	1		1984	
t216				x		Bet v 2, Profilin	<i>Betula verrucosa</i>	1	Bet v 2	127	RR
t702			x			Bet v 2.0101	<i>Betula verrucosa</i>	1	Bet v 2.0101	3136	RR
t217				x		Pepper tree	<i>Schinus molle</i>	1		1007	
t217			x			Red alder	<i>Alnus rubra</i>	3		2166	
t218				x		Virginia live oak	<i>Quercus virginiana</i>	1		2345	
t218	x		x			Bayberry (bayberry/sweet gale)	<i>Myrica gale</i>	3		3643	
t219				x		Palo verde	<i>Cercidium floridum</i>	3		2219	
t219	x		x			Red cedar	<i>Juniperus virginiana</i>	1		1855	
t220				x		Bet v 4	<i>Betula verrucosa</i>	1	Bet v 4	129	RR
t221				x		Bet v 2, Bet v 4	<i>Betula verrucosa</i>	1	Bet v 2, Bet v 4		RR
t221			x			Brazilian peppertree	<i>Schinus terebinthifolius</i>	3		2208	
t222	x			x		Cypress (Arizona cypress)	<i>Cupressus arizonica</i>	1		1793	
t223				x		Oil palm	<i>Elaeis guineensis</i>	1		1809	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
t224				x		Ole e 1	<i>Olea europaea</i>	1	Ole e 1	482	NN
t225				x		Bet v 6	<i>Betula verrucosa</i>	1	Bet v 6	131	RR
t226				x		Cup a 1	<i>Cupressus arizonica</i>	1	Cup a 1	256	NN
t228			x			Aspen, quaking	<i>Populus tremuloides</i>	3		2328	
t229			x			Eastern hemlock	<i>Tsuga canadensis</i>	3		3710	
t230			x			Redwood (sequoia)	<i>Sequoia sempervirens</i>	2		3711	
t232			x			Pussy willow	<i>Salix discolor</i>	3		2356	
t250			x			Golden chain	<i>Laburnum anagyroides</i> (<i>Laburnum vulgare</i>)	3		3712	
t280	x		x			Locust tree	<i>Robinia pseudoacacia</i>	1		717	
t401	x					Brazilian peppertree	<i>Schinus terebinthifolius</i>	3		2208	
t402	x					Mastic tree	<i>Pistacia lentiscus</i>	2		1009	
t404	x					Tree of heaven	<i>Ailanthus altissima</i>	2		693	
t406	x					Date palm	<i>Phoenix dactylifera</i>	1		1925	
a235		x				Cor a 1.0103	<i>Corylus avellana</i>	1	Cor a 1.0103	235	RR
a3055		x				Aln g 1.0101	<i>Alnus glutinosa</i>	1	Aln g 1.0101	3055	RR
a90		x				Bet v 1.0101	<i>Betula verrucosa</i>	1	Bet v 1.0101	90	RR
a95		x				Bet v 1.0401	<i>Betula verrucosa</i>	1	Bet v 1.0401	95	RR
a3136		x				Bet v 2.0101	<i>Betula verrucosa</i>	1	Bet v 2.0101	3136	RR
a3425		x				Pla a 1.0101	<i>Platanus acerifolia</i>	1	Pla a 1.0101	3425	RR

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a573		x				Pla a 2	<i>Platanus acerifolia</i>	1	Pla a 2	573	NN
a256		x				Cup a 1	<i>Cupressus arizonica</i>	1	Cup a 1	256	NN
a482		x				Ole e 1	<i>Olea europaea</i>	1	Ole e 1	482	NN
a490		x				Ole e 2	<i>Olea europaea</i>	1	Ole e 2	490	NN
a3423		x				Pho d 2.0101	<i>Phoenix dactylifera</i>	1	Pho d 2.0101	3423	RR
d						Mites					
d1	x		x	x	x	House dust mite	<i>Dermatophagoides pteronyssinus</i>	1		1803	
d2	x		x	x	x	House dust mite	<i>Dermatophagoides farinae</i>	1		1801	
d3	x		x	x		House dust mite	<i>Dermatophagoides microceras</i>	1		1802	
d70	x		x	x		Storage mite	<i>Acarus siro</i>	1		1696	
d71	x		x	x		Storage mite	<i>Lepidoglyphus destructor</i>	1		1859	
d72	x		x	x		Storage mite	<i>Tyrophagus putrescentiae</i>	1		2001	
d73	x		x	x		Storage mite	<i>Glycyphagus domesticus</i>	1		1833	
d74	x		x	x		House dust mite	<i>Euroglyphus maynei</i>	1		1815	
d75			x			Storage mite	<i>Chortoglyphus arcuatus</i>	2		1177	
d76			x			Storage mite	<i>Gohieria fusca</i>	2		1163	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
d201	x		x	x		House dust mite	<i>Blomia tropicalis</i>	1		1744	
d202				x		Der p 1	<i>Dermatophagoides pteronyssinus</i>	1	Der p 1	310	NN
d203				x		Der p 2	<i>Dermatophagoides pteronyssinus</i>	1	Der p 2	316	NN
d204				x		Der f 2	<i>Dermatophagoides farinae</i>	1	Der f 2	302	NN
a310	x	x				Der p 1	<i>Dermatophagoides pteronyssinus</i>	1	Der p 1	310	NN
a316	x	x				Der p 2	<i>Dermatophagoides pteronyssinus</i>	1	Der p 2	316	NN
a295	x	x				Der f 1	<i>Dermatophagoides farinae</i>	1	Der f 1	295	NN
a302	x	x				Der f 2	<i>Dermatophagoides farinae</i>	1	Der f 2	302	NN
a3258		x				Der p 10.0101	<i>Dermatophagoides pteronyssinus</i>	1	Der p 10.0101	3258	RR
m						Microorganisms					
m1	x		x	x	x	<i>Penicillium chrysogenum</i> (<i>Penicillium notatum</i>)	<i>Penicillium chrysogenum</i> (<i>Penicillium notatum</i>)	1		1912	
m2	x		x	x	x	<i>Cladosporium herbarum</i> (<i>Hormodendrum</i>)	<i>Cladosporium herbarum</i> (<i>Hormodendrum</i>)	1		1775	
m3	x		x	x	x	<i>Aspergillus fumigatus</i>	<i>Aspergillus fumigatus</i>	1		1730	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m4	x		x	x	x	<i>Mucor racemosus</i>	<i>Mucor racemosus</i>	1		2291	
m5	x		x	x	x	<i>Candida albicans</i>	<i>Candida albicans</i>	1		1757	
m6	x		x	x	x	<i>Alternaria alternata</i> (<i>Alternaria tenuis</i>)	<i>Alternaria alternata</i> (<i>Alternaria tenuis</i>)	1		1708	
m7	x		x	x	x	<i>Botrytis cinerea</i>	<i>Botrytis cinerea</i>	1		1630	
m8	x		x	x	x	<i>Drechslera halodes</i> (<i>Setomelanomma rostrata</i> , <i>Helminthosporium halodes</i>)	<i>Drechslera halodes</i> (<i>Setomelanomma rostrata</i> , <i>Helminthosporium halodes</i>)	1		3645	
m9	x		x	x		<i>Fusarium moniliforme</i> (<i>Fusarium proliferatum</i>)	<i>Fusarium moniliforme</i> (<i>Fusarium proliferatum</i>)	1		1554	
m10	x		x	x	x	<i>Stemphylium botryosum</i>	<i>Stemphylium herbarum</i> (<i>Stemphylium botryosum</i>)	1		2637	
m11	x		x	x	x	<i>Rhizopus nigricans</i>	<i>Rhizopus nigricans</i>	1		1622	
m12	x		x	x		<i>Aureobasidium pullulans</i>	<i>Aureobasidium pullulans</i>	1		2197	
m13	x		x	x		<i>Phoma betae</i>	<i>Phoma betae</i>	1		2303	
m14	x		x	x	x	<i>Epicoccum purpurascens</i>	<i>Epicoccum purpurascens</i> (<i>Epicoccum nigrum</i>)	1		1810	
m15	x		x	x		<i>Trichoderma viride</i>	<i>Trichoderma viride</i>	1		2379	
m16	x		x	x		<i>Curvularia lunata</i>	<i>Curvularia lunata</i>	1		1795	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m17			x			<i>Cladosporium fulvum</i>	<i>Cladosporium fulvum</i>	3		3646	
m18			x			<i>Fusarium culmorum</i>	<i>Fusarium culmorum</i>	1		1828	
m19			x			<i>Aspergillus versicolor</i>	<i>Aspergillus versicolor</i>	2		1300	
m20			x			<i>Mucor mucedo</i>	<i>Mucor mucedo</i>	2		2289	
m21			x			<i>Aspergillus clavatus</i>	<i>Aspergillus clavatus</i>	2		2186	
m22				x		<i>Micropolyspora faeni</i>	<i>Saccharopolyspora rectivirgula</i> (<i>Micropolyspora faeni</i>)	1		3713	
m23				x		<i>Thermoactinomyces vulgaris</i>	<i>Thermoactinomyces vulgaris</i>	1		3647	
m24				x		<i>Stachybotrys atra</i>	<i>Stachybotrys chartarum</i> (<i>Stachybotrys atra</i>)	1		1981	
m24			x			<i>Paecilomyces</i> spp.	<i>Paecilomyces</i> spp.	2		2410	
m25				x		<i>Aspergillus versicolor</i>	<i>Aspergillus versicolor</i>	1		1300	
m25			x			<i>Penicillium brevicompactum</i>	<i>Penicillium brevicompactum</i>	1		1910	
m26				x		<i>Cladosporium cladosporioides</i>	<i>Cladosporium cladosporioides</i>	1		2230	
m26			x			<i>Penicillium citrinum</i>	<i>Penicillium citrinum</i>	1		1911	
m27				x		<i>Penicillium</i> spp.	<i>Penicillium</i> spp.	1			
m29			x			<i>Aspergillus repens</i>	<i>Aspergillus repens</i>	3		3714	
m30			x			<i>Penicillium roqueforti</i>	<i>Penicillium roqueforti</i>	3		2301	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m32			x			<i>Cladosporium cladosporioides</i>	<i>Cladosporium cladosporioides</i>	1		2230	
m34			x			<i>Serpula lacrymans</i>	<i>Serpula lacrymans</i>	2		2367	
m36			x	x		<i>Aspergillus terreus</i>	<i>Aspergillus terreus</i>	1		2187	
m37			x			<i>Trichophyton mentagrophytes</i>	<i>Trichophyton mentagrophytes</i>	1		2653	
m40			x			<i>Aspergillus amstelodami</i>	<i>Aspergillus amstelodami</i>	3		2185	
m43			x			<i>Saccharomyces carlsberg</i>	<i>Saccharomyces carlsbergensis</i>	3		3716	
m44			x			<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	1		1960	
m45	x		x			<i>Hormodendrum hordei</i>	<i>Hormodendrum hordei</i>	3		3717	
m46			x	x		<i>Bipolaris spicifera</i>	<i>Bipolaris spicifera</i>	1		2235	
m47			x			<i>Aspergillus nidulans</i>	<i>Aspergillus nidulans</i>	1		1735	
m48			x			<i>Aspergillus oryzae</i>	<i>Aspergillus oryzae</i>	1		1733	
m49			x		x	<i>Fusarium oxysporum</i>	<i>Fusarium oxysporum</i>	3		3649	
m50			x			<i>Micropolyspora faeni</i>	<i>Saccharopolyspora rectivirgula</i> (<i>Micropolyspora faeni</i>)	1		3713	
m51			x			<i>Thermoactinomyces vulgaris</i>	<i>Thermoactinomyces vulgaris</i>	2		3647	
m53			x			<i>Microspora canis</i>	<i>Microsporum canis</i> (<i>Microspora canis</i>)	3		2286	

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m54			x			<i>Aspergillus flavus</i>	<i>Aspergillus flavus</i>	1		1731	
m63			x			<i>Helminthosporium intersemin</i>	<i>Helminthosporium intersemin</i>	3			
m66			x			<i>Mucor plumbeus</i>	<i>Mucor plumbeus</i>	3			
m67			x			<i>Mycogone</i>	<i>Mycogone perniciosa</i>	3		2292	
m68			x			<i>Nigrospora oryzae</i>	<i>Nigrospora oryzae</i>	2		2295	
m69			x			<i>Rhodotorula</i>	<i>Rhodotorula rubra</i> (<i>Rhodotorula mucilaginosa</i>)	1		1958	
m70	x		x	x		<i>Malassezia furfur</i> (<i>Pityrosporum orbiculare</i>)	<i>Malassezia furfur</i> (<i>Pityrosporum orbiculare</i>)	1		1872	
m71			x			<i>Spondylocladium</i>	<i>Spondylocladium</i> spp.	3		3718	
m72			x			<i>Epidermophyton</i>	<i>Epidermophyton floccosum</i>	2		2245	
m73			x			<i>Epicoccum nigrum</i>	<i>Epicoccum purpurascens</i> (<i>Epicoccum nigrum</i>)	1		1810	
m80				x		Staphylococcal enterotoxin A (Sta a SEA)	<i>Staphylococcus aureus</i>	1		2139	NN
m80			x			<i>Helminthosporium</i> spp.	<i>Helminthosporium</i> spp.	2		1125	
m81				x		Staphylococcal enterotoxin B (Sta a SEB)	<i>Staphylococcus aureus</i>	1		2140	NN

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m88			x			<i>Stemphylium solani</i>	<i>Stemphylium solani</i>	3		3719	
m93			x			<i>Gliocladium fimbriatum</i>	<i>Gliocladium fimbriatum</i>	3		3720	
m94			x			<i>Phycomyces blakesleeanus</i>	<i>Phycomyces blakesleeanus</i>	3		3721	
m201	x		x	x		<i>Tilletia tritici</i> (<i>Ustilago nuda</i> , <i>Ustilago tritici</i>) (Barley smut)	<i>Tilletia tritici</i> (<i>Ustilago nuda</i> , <i>Ustilago tritici</i>)	1		2395	
m202	x		x	x		<i>Acremonium kiliense</i> (<i>Cephalosporium acremonium</i>)	<i>Acremonium kiliense</i> (<i>Cephalosporium acremonium</i>)	1		2218	
m203	x			x		<i>Trichosporon pullulans</i>	<i>Trichosporon pullulans</i>	2		2380	
m204	x			x		<i>Ulocladium chartarum</i>	<i>Ulocladium chartarum</i>	1		2389	
m205			x	x		<i>Trichophyton rubrum</i>	<i>Trichophyton rubrum</i>	1		1995	
m207	x		x	x		<i>Aspergillus niger</i>	<i>Aspergillus niger</i>	1		1732	
m208	x		x	x		<i>Chaetomium globosum</i>	<i>Chaetomium globosum</i>	2		2220	
m209				x		<i>Penicillium frequentans</i>	<i>Penicillium glabrum</i> (<i>Penicillium frequentans</i>)	1		2726	
m209			x			<i>Stachybotrys chartarum</i>	<i>Stachybotrys chartarum</i> (<i>Stachybotrys atra</i>)	1		1981	
m210				x		<i>Trichophyton mentagrophytes</i> var. <i>goetzii</i>	<i>Trichophyton mentagrophytes</i> var. <i>goetzii</i>	1		2653	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m211				x		<i>Trichophyton mentagrophytes</i> var. <i>interdigitale</i>	<i>Trichophyton mentagrophytes</i> var. <i>interdigitale</i>	1		2653	
m211			x			Oat smut	<i>Ustilago avenae</i>	3		2391	
m212	x					<i>Micropolyspora faeni</i>	<i>Saccharopolyspora rectivirgula</i> (<i>Micropolyspora faeni</i>)	3		3713	
m212			x			<i>Geotrichum candidum</i>	<i>Geotrichum candidum</i>	2		2258	
m213			x			Bermuda grass smut	<i>Ustilago cynodontis</i>	3		2392	
m214			x			Johnson grass smut	<i>Sphacelotheca cruenta</i>	3		3722	
m214	x					Johnson grass smut	<i>Sporisorium cruentum</i>	3		2370	
m215			x			Corn smut	<i>Ustilago maydis</i>	3		2393	
m218				x		Asp f 1	<i>Aspergillus fumigatus</i>	1	Asp f 1	62	RR
a3050	x	x				Asp r 1	<i>Aspergillus restrictus</i>	1	Asp r 1	3050	NN
m219				x		Asp f 2	<i>Aspergillus fumigatus</i>	1	Asp f 2	71	RR
m220				x		Asp f 3	<i>Aspergillus fumigatus</i>	1	Asp f 3	73	RR
m221				x		Asp f 4	<i>Aspergillus fumigatus</i>	1	Asp f 4	74	RR
m222				x		Asp f 6	<i>Aspergillus fumigatus</i>	1	Asp f 6	76	RR
m223				x		Staphylococcal enterotoxin C (Sta a SEC)	<i>Staphylococcus aureus</i>	1		2141	NN

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
m224				x		Staphylococcal enterotoxin D (Sta a SED)	<i>Staphylococcus aureus</i>	1		2142	NN
m226				x		Staphylococcal enterotoxin TSST (Sta a TSST)	<i>Staphylococcus aureus</i>	1		2143	NN
m227				x		<i>Malassezia</i> spp. (<i>Pityrosporum</i> spp.)	<i>Malassezia</i> spp. (<i>Pityrosporum</i> spp.)	1			
m228				x		<i>Aspergillus flavus</i>		1		1731	
m229				x		Alt a 1	<i>Alternaria alternata</i> (<i>Alternaria tenuis</i>)	1	Alt a 1	722	RR
m300	x					<i>Eurotium</i> spp.	<i>Eurotium</i> spp.	3		2248	
m304	x					<i>Aspergillus oryzae</i>	<i>Aspergillus oryzae</i>	1		1733	
m305	x					<i>Penicillium brevicompactum</i>	<i>Penicillium brevicompactum</i>	1		1910	
m309	x					<i>Aspergillus terreus</i>	<i>Aspergillus terreus</i>	2		2187	
m310	x					<i>Aspergillus nidulans</i>	<i>Aspergillus nidulans</i>	1		1735	
m311	x					<i>Aspergillus flavus</i>	<i>Aspergillus flavus</i>	1		1731	
m312	x					<i>Aspergillus clavatus</i>	<i>Aspergillus clavatus</i>	2		2186	
a11		x				Alt a 1.0101	<i>Alternaria alternata</i> (<i>Alternaria tenuis</i>)	1	Alt a 1.0101	11	RR
a3063		x				Alt a 6.0101	<i>Alternaria alternata</i> (<i>Alternaria tenuis</i>)	1	Alt a 6.0101	3063	RR

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a3207		x				Cla h 8.0101	<i>Cladosporium herbarum</i> (<i>Hormodendrum</i>)	1	Cla h 8.0101	3207	RR
e						Epidermals and animal proteins					
e1	x		x	x	x	Cat epithelium and dander	<i>Felis domesticus</i>	1		1819	
e2	x		x	x		Dog epithelium	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1		1756	
e3	x		x	x	x	Horse dander	<i>Equus caballus</i> (<i>Equus spp.</i>)	1		1813	
e4	x		x	x	x	Cow dander	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos spp.</i>)	1		2020	
e5	x		x	x	x	Dog dander	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1		1756	
e6	x		x	x	x	Guinea pig epithelium	<i>Cavia porcellus</i>	1		1765	
e7	x		x			Pigeon droppings	<i>Columba palumbus</i>	3			
e7				x		Pigeon droppings	<i>Columba livia</i>	1			
e23			x			Pigeon serum		3			
e24			x			Canary serum	<i>Serinus canarius</i>	3			
e25			x			Chicken serum	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus spp.</i>)	2		2038	
e26			x			Parrot serum		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
e62			x			Camel		3			
e70	x		x	x		Goose feathers	<i>Anser anser</i>	1			
e71	x		x	x	x	Mouse epithelium	<i>Mus musculus</i> (<i>Mus</i> spp.)	1		2051	
e72	x		x	x		Mouse urine proteins (Mouse urine)	<i>Mus musculus</i> (<i>Mus</i> spp.)	1		1881	
e73			x	x		Rat epithelium	<i>Rattus norvegicus</i>	1		2072	
e73	x					Rat epithelium	<i>Rattus rattus</i>	1		3650	
e74			x	x		Rat urine proteins	<i>Rattus norvegicus</i>	1		1957	
e74	x					Rat urine proteins (rat urine)	<i>Rattus rattus</i>	1		3652	
e75			x	x		Rat serum proteins	<i>Rattus norvegicus</i>	1		2071	
e75	x					Rat serum proteins	<i>Rattus rattus</i>	1		3651	
e76	x		x	x		Mouse serum proteins	<i>Mus musculus</i> (<i>Mus</i> spp.)	1		2052	
e77	x		x	x		Budgerigar droppings	<i>Melopsittacus undulatus</i>	1			
e78	x		x	x		Budgerigar feathers	<i>Melopsittacus undulatus</i>	1			
e79			x	x		Budgerigar serum proteins	<i>Melopsittacus undulatus</i>	1			
e80	x		x	x		Goat epithelium	<i>Capra hircus</i>	1		2023	
e81	x		x	x		Sheep epithelium	<i>Ovis aries</i> (<i>Ovis</i> spp.)	1		2060	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
e82	x		x	x	x	Rabbit epithelium	<i>Oryctolagus cuniculus</i> (<i>Oryctolagus</i> spp.)	1		2054	
e83	x		x	x		Swine epithelium	<i>Sus scrofa</i> (<i>Sus scrofa domestica</i> ; <i>Sus</i> spp.)	1		2089	
e84	x		x		x	Hamster epithelium	<i>Cricetus cricetus</i>	1		1782	
e84				x		Hamster epithelium	<i>Cricetus cricetus</i> , <i>Mesocricetus auratus</i> , and <i>Phodopus sungorus</i>	1			
e85	x		x	x		Chicken feathers	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	3			
e86	x		x	x		Duck feathers	<i>Anas platyrhynchos</i>	3			
e87			x	x		Rat epithelium, serum proteins, and urine proteins	<i>Rattus norvegicus</i>	1		2072	
e87	x					Rat epithelium, serum proteins, and urine proteins (rat)	<i>Rattus rattus</i>	1		3650	
e88	x		x	x		Mouse epithelium, serum proteins, and urine proteins (mouse)	<i>Mus musculus</i> (<i>Mus</i> spp.)	1		2051	
e89	x		x	x		Turkey feathers	<i>Meleagris gallopavo</i>	1			
e90				x		Budgerigar serum proteins, feathers, and droppings	<i>Melopsittacus undulatus</i>	1			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
e91				x		Pigeon serum proteins, feathers, and droppings	<i>Streptopelia roseogrisea</i>	1			
e91	x					Parrot feathers	<i>Psittacidae</i> spp.	3			
e92				x		Parrot serum proteins, feathers, and droppings	<i>Ara</i> spp.	1			
e93				x		Pigeon serum proteins	<i>Streptopelia roseogrisea</i>	1			
e94				x		Fel d 1	<i>Felis domesticus</i>	1	Fel d 1	345	RR
a345	x	x				Fel d 1	<i>Felis domesticus</i>	1	Fel d 1	345	NN
e95			x			Duck down	<i>Anas domesticus</i>	3			
e98			x			Parrot droppings		3			
e101				x		Can f 1	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1	Can f 1	174	RR
a174	x	x				Can f 1	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1	Can f 1	174	NN
e102				x		Can f 2	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1	Can f 2	175	RR
e196				x		Parakeet feathers	<i>Nymphicus hollandicus</i>	1			
e197				x		Parakeet droppings	<i>Nymphicus hollandicus</i>	1			
e198				x		Parakeet serum	<i>Nymphicus hollandicus</i>	1			
e199				x		Canary bird serum	<i>Serinus canarius</i>	1			
e200				x		Canary bird droppings	<i>Serinus canarius</i>	1			
e201	x		x	x		Canary bird feathers (Canary feathers)	<i>Serinus canarius</i>	1			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
e202				x		Reindeer epithelium	<i>Rangifer tarandus</i>	1		1594	
e203			x	x		Mink epithelium	<i>Mustela</i> spp.	1		1379	
e204				x		Bos d 6, BSA, bovine serum albumin	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1	Bos d 6	165	NN
a165		x				Bos d 6, BSA, bovine serum albumin	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1	Bos d 6	165	NN
a166		x				Bos d 7	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1	Bos d 7	166	NN
e205			x	x		Horse, serum proteins	<i>Equus caballus</i> (<i>Equus</i> spp.)	1		2030	
e206				x		Rabbit, serum proteins	<i>Oryctolagus cuniculus</i> (<i>Oryctolagus</i> spp.)	1		2056	
e208			x	x		Chinchilla epithelium	<i>Chinchilla laniger</i>	1		2224	
e209	x		x	x	x	Gerbil epithelium	<i>Meriones unguiculatus</i>	1		2283	
e210			x	x		Fox epithelium	<i>Vulpes vulpes</i>	1		1380	
e211				x		Rabbit, urine proteins	<i>Oryctolagus cuniculus</i> (<i>Oryctolagus</i> spp.)	1		1889	
e212				x		Swine, urine proteins	<i>Sus scrofa</i> (<i>Sus scrofa domesticus</i> ; <i>Sus</i> spp.)	1		2086	
e213			x	x		Parrot feathers	<i>Ara</i> spp.	1			
e214			x	x		Finch feathers	<i>Lonchura domestica</i>	1			
e215			x	x		Pigeon feathers	<i>Streptopelia roseogrisea</i> (<i>Streptopelia</i> spp.)	1			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
e216			x	x		Deer epithelium	<i>Dama dama</i>	1		1152	
e217			x	x		Ferret epithelium	<i>Mustela putorius</i>	2		939	
e218				x		Chicken droppings	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1			
e219	x			x		Chicken, serum proteins	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1		2038	
e220				x		Fel d 2, Cat serum albumin	<i>Felis domesticus</i>	1	Fel d 2	346	NN
e221				x		Can f 3, Dog serum albumin	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1	Can f 3	176	NN
a176		x				Can f 3, Dog serum albumin	<i>Canis familiaris</i> (<i>Canis domesticus</i>)	1	Can f 3	176	NN
e222				x		Swine serum albumin (Sus s PSA)	<i>Sus scrofa</i> (<i>Sus scrofa domesticus</i> ; <i>Sus</i> spp.)	1		757	NN
e223			x			Llama		3			
e224			x			Angora		3			
e225			x			Lovebird feathers		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f						Foods					
f1	x		x	x	x	Egg white	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1		1832	
f2	x		x	x	x	Milk	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1		1747	
f3	x		x	x	x	Fish (cod) (codfish)	<i>Gadus morhua</i>	1		1831	
f4	x		x	x	x	Wheat	<i>Triticum aestivum</i> (<i>Triticum</i> spp.)	1		1993	
f5	x		x	x	x	Rye	<i>Secale cereale</i>	1		2076	
f6	x		x	x	x	Barley	<i>Hordeum vulgare</i>	1		2040	
f7	x		x	x	x	Oat	<i>Avena sativa</i>	1		2018	
f8	x		x	x	x	Maize, corn	<i>Zea mays</i>	1		2092	
f9	x		x	x	x	Rice	<i>Oryza sativa</i>	1		2058	
f10	x		x	x	x	Sesame seed	<i>Sesamum indicum</i> (<i>Sesamum orientale</i>)	1		1971	
f11	x		x	x		Buckwheat	<i>Fagopyrum esculentum</i> (<i>Fagopyrum</i> spp.)	1		1816	
f12	x		x	x	x	Pea (green pea)	<i>Pisum sativum</i>	1		1931	
f13	x		x	x	x	Peanut	<i>Arachis hypogaea</i>	1		1723	
f14	x		x	x	x	Soybean	<i>Glycine max</i>	1		1834	
f15	x		x	x	x	White bean	<i>Phaseolus vulgaris</i>	1		1923	
f17	x		x	x	x	Hazel nut	<i>Corylus avellana</i>	1		2028	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f18	x		x	x		Brazil nut	<i>Bertholletia excelsa</i>	1		1738	
f19	x		x			Cayenne pepper	<i>Capsicum frutescens</i>	2		2213	
f20	x		x	x	x	Almond	<i>Amygdalus communis</i> (<i>Prunus dulcis</i>)	1		1948	
f21	x		x			Sugar cane	<i>Saccharum officinarum</i>	2		1514	
f22			x			Raspberry	<i>Rubus idaeus</i>	2		1093	
f23			x	x	x	Crab	<i>Cancer pagurus</i>	1		2129	
f23	x					Crab	<i>Callinectes sapidus</i>	2		2209	
f24			x	x	x	Shrimp	<i>Pandalus borealis</i> , <i>Penaeus monodon</i> , <i>Metapenaeopsis barbata</i> , <i>Metapenaeus joyneri</i>	1		1893	
f24	x					Shrimp	<i>Penaeus</i> spp.	1			
f25	x		x	x	x	Tomato	<i>Lycopersicon esculentum</i> (<i>Lycopersicon lycopersicum</i>)	1		1870	
f26	x		x	x	x	Pork	<i>Sus scrofa</i> (<i>Sus scrofa domesticus</i> ; <i>Sus</i> spp.)	1		2088	
f27	x		x	x	x	Beef	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1		2019	
f29			x			Watermelon	<i>Citrullus lanatus</i> (<i>Citrullus vulgaris</i>)	2		1773	
f31	x		x	x	x	Carrot	<i>Daucus carota</i>	1		1799	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f32			x			Oyster mushroom	<i>Pleurotus ostreatus</i>	2		1148	
a290		x				Dau c 1.0103	<i>Daucus carota</i>	1	Dau c 1.0103	290	RR
f33	x		x	x	x	Orange	<i>Citrus sinensis</i>	1		1774	
f35	x		x	x	x	Potato	<i>Solanum tuberosum</i>	1		1977	
f36	x		x	x		Coconut	<i>Cocos nucifera</i>	1		3559	
f37	x		x	x		Blue mussel	<i>Mytilus edulis</i> (<i>Mytilus</i> spp.)	1		1413	
f40	x		x	x	x	Tuna	<i>Thunnus albacares</i> (<i>Thunnus</i> spp.)	1		2375	
f41	x		x	x	x	Salmon	<i>Salmo salar</i>	1		1962	
f42	x		x	x		Haddock	<i>Melanogrammus aeglefinus</i>	1		1482	
f43			x			Mother's milk	<i>Homo sapiens</i>	1		3750	
f44	x		x	x	x	Strawberry	<i>Fragaria vesca</i> (<i>Fragaria</i> spp.)	1		2251	
f45	x		x	x	x	Yeast, baker's	<i>Saccharomyces cerevisiae</i>	1		1960	
f47	x		x	x	x	Garlic	<i>Allium sativum</i>	1		1706	
f48	x		x	x	x	Onion	<i>Allium cepa</i>	1		1704	
f49	x		x	x	x	Apple	<i>Malus x domestica</i> (<i>Malus</i> spp.)	1		1871	
f50	x		x	x		Chub mackerel	<i>Scomber japonicus</i>	1		1966	
f51			x	x		Bamboo shoot	<i>Phyllostachys pubescens</i>	1		2304	

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f52			x			Cacao/chocolate	<i>Theobroma cacao</i>	2		1369	
f53			x			Scallop	<i>Argopecten irradians</i>	3		3723	
f54	x		x	x		Sweet potato	<i>Ipomoea batatas</i>	1		2265	
f55	x		x	x		Common millet	<i>Panicum miliaceum</i>	1		1062	
f56	x			x		Foxtail millet	<i>Setaria italica</i>	1		1055	
f57				x		Japanese millet	<i>Echinochloa crus-galli</i>	1		1056	
f58				x		Pacific squid	<i>Todarodes pacificus</i>	1		1992	
f59	x		x	x		Octopus	<i>Octopus vulgaris</i> (<i>Octopus</i> spp.)	1		2132	
f60			x	x		Jack mackerel, scad	<i>Trachurus japonicus</i>	1		2378	
f61	x			x		Sardine, pilchard	<i>Sardinops melanostictus</i> (<i>Sardinops</i> spp.)	1		2363	
f63			x			Kefir		3			
f65	x					Perch	<i>Sebastes marinus</i> (<i>Sebastes</i> spp.)	3		2366	
f65			x			Perch (rose fish)	<i>Sebastes alutus</i>	3		3724	
f67	x		x			Parmesan cheese		3			
f68			x			Ewe's cheese	<i>Ovis aries</i> (<i>Ovis</i> spp.)	3			
f75	x		x	x	x	Egg yolk	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1		2036	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f76	x		x	x		Bos d 4, alpha-lactalbumin	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 4	163	NN
a163		x				Bos d 4, alpha-lactalbumin	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 4	163	NN
f77	x		x	x		Bos d 5, beta-lactoglobulin	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 5	164	NN
a2738		x				Bos d 5.0102	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 5.0102	2738	NN
a2739		x				Bos d 5.0101	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 5.0101	2739	NN
f78	x		x	x	x	Bos d 8, Casein	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 8	167	NN
a167		x				Bos d 8, Casein	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 8	167	NN
a2734		x				Bos d 8 alphaS1-Casein	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 8	2734	NN
a2736		x				Bos d 8 beta-Casein	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 8	2736	NN
a2737		x				Bos d 8 kappa-Casein	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1	Bos d 8	2737	NN
f79	x		x	x		Gluten	<i>Triticum aestivum (Triticum spp.)</i>	1		651	NN
f80			x	x		Lobster	<i>Homarus gammarus</i>	1		1844	
f80	x					Lobster	<i>Homarus americanus</i>	1		1843	
f81	x		x	x	x	Cheese, cheddar type		1			
f82	x		x	x		Cheese, mold type		1			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f83	x		x	x	x	Chicken	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1		2037	
f84	x		x	x		Kiwi, green	<i>Actinidia deliciosa</i>	1		1697	
f85	x		x	x	x	Celery	<i>Apium graveolens</i>	1		1721	
f86	x		x	x		Parsley	<i>Petroselinum crispum</i>	1		1921	
f87			x	x		Melon	<i>Cucumis melo</i>	1		1786	
f87	x					Melon	<i>Cucumis melo</i> + <i>Citrullus lanatus</i>	1			
f88	x		x	x	x	Mutton (lamb)	<i>Ovis aries</i> (<i>Ovis</i> spp.)	1		2061	
f89	x		x	x		Mustard	<i>Brassica/Sinapis</i> spp.	1		1972	
f90	x		x	x	x	Malt	<i>Hordeum vulgare</i>	1		2040	
f91	x		x	x		Mango	<i>Mangifera indica</i>	1		1874	
f92	x		x	x	x	Banana	<i>Musa</i> spp.	1		1882	
f93	x			x	x	Cacao	<i>Theobroma cacao</i>	1		1369	
f94	x		x	x		Pear	<i>Pyrus communis</i> (<i>Pyrus</i> spp.)	1		1954	
f95	x		x	x	x	Peach	<i>Prunus persica</i>	1		1949	
f96	x		x	x	x	Avocado	<i>Persea americana</i>	1		1920	
f97				x		Yam	<i>Dioscorea</i> spp.	1		968	
f97			x			Chamomile tea	<i>Matricaria chamomilla</i>	2		1875	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f98				x		Gliadin	<i>Triticum aestivum</i> (<i>Triticum</i> spp.)	1		651	
f101			x			Bean herb	<i>Satureja hortensis</i>	3		3749	
f102			x			Cantaloupe	<i>Cucumis melo</i> var. <i>cantalupensis</i>	1		1786	
f105	x					Chocolate	<i>Theobroma cacao</i>	2		1369	
f109			x			Cottonseed	<i>Gossypium hirsutum</i>	2		2261	
f110			x			Giant radish	<i>Raphanus sativus</i>	2		2347	
f118			x			Zucchini	<i>Cucurbita pepo</i>	2		1788	
f119	x		x			Radish	<i>Raphanus sativus</i>	2		2347	
f120			x			Venison		3			
f121			x			Pinto bean	<i>Phaseolus vulgaris</i>	1		1923	
f122			x			Cheese, American		3			
f124			x	x		Spelt wheat	<i>Triticum spelta</i>	1		1996	
f125			x			Peppermint tea	<i>Mentha piperita</i>	3		2281	
f127			x			Black-eyed pea	<i>Vigna unguiculata</i>	2		1165	
f129			x			Sole	<i>Parophrys vetulus</i>	2		1484	
f131			x			Olive black	<i>Olea europaea</i>	2		2053	
f136			x			Red beet	<i>Beta vulgaris</i> var. <i>conditiva</i>	2		2200	
f140			x			Bran		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
fl141			x			Corn (vegetables)	<i>Zea mays</i>	1		2092	
fl143			x			Roquefort cheese		3			
fl147	x		x	x		Gulf flounder (flounder)	<i>Paralichthys albigutta</i> (<i>Paralichthys</i> spp.)	1		3748	
fl149			x			Sago		3			
fl152			x			Green bell pepper	<i>Capsicum annuum</i>	1		1759	
fl155			x		x	Brewer's yeast	<i>Saccharomyces carlsbergensis</i>	3		3716	
fl157			x			Duck	<i>Anas domesticus</i>	2		2016	
fl158			x			Goose	<i>Anser anser</i>	2		2017	
fl160			x			Camembert cheese		3			
fl162			x			Nectarine	<i>Prunus persica</i> var. <i>nucipersica</i>	1		1949	
fl163			x			Kohlrabi	<i>Brassica oleracea</i> var. <i>gongylodes</i>	2		1751	
fl166			x			Leek	<i>Allium porrum</i>	1		1705	
fl170	x		x			Cheese (Switzerland) (Swiss cheese)		3			
fl174			x			Fig	<i>Ficus carica</i>	1		1823	
fl177			x			Cranberry	<i>Vaccinium macrocarpon</i>	3		2398	
fl179			x			Raisin	<i>Vitis</i> spp.	1		2012	
fl182	x		x	x		Lima bean	<i>Phaseolus lunatus</i>	1		3656	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f183			x			Sunflower seed	<i>Helianthus annuus</i>	1		2039	
f198			x			Flaxseed (bruised grain)		3			
f199			x			Untreated native milk	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1		1747	
f200			x			Soy milk	<i>Glycine max</i>	1		1834	
f201	x		x	x		Pecan nut	<i>Carya illinoensis</i>	1		1762	
f202	x		x	x		Cashew nut (cashew)	<i>Anacardium occidentale</i>	1		1714	
f203	x		x	x		Pistachio	<i>Pistacia vera</i>	1		1008	
f204	x		x	x		Trout	<i>Oncorhynchus mykiss (Salmo gairdneri)</i>	1		2994	
f205	x		x	x		Herring	<i>Clupea harengus</i>	1		1370	
f206				x		Mackerel	<i>Scomber scombrus</i>	1		1967	
f207	x		x	x		Clam	<i>Ruditapes spp.</i>	1		2352	
f207					x	Clam	<i>Mercenaria mercenaria</i>	3		2282	
f208	x		x	x		Lemon	<i>Citrus limon</i>	1		1772	
f209	x		x	x		Grapefruit	<i>Citrus paradisi</i>	1		2228	
f210	x		x	x		Pineapple	<i>Ananas comosus</i>	1		1713	
f211			x	x		Blackberry	<i>Rubus fruticosus</i>	2		2351	
f212	x		x	x		Mushroom (champignon)	<i>Agaricus hortensis (Agaricus spp.)</i>	1		891	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f213	x		x	x	x	Rabbit	<i>Oryctolagus cuniculus</i> (<i>Oryctolagus</i> spp.)	1		2057	
f214	x		x	x		Spinach	<i>Spinacia oleracea</i>	1		1980	
f215	x		x	x		Lettuce	<i>Lactuca sativa</i>	1		1856	
f216	x		x	x		Cabbage	<i>Brassica oleracea</i> var. <i>capitata</i>	1		1751	
f217	x		x	x		Brussels sprouts	<i>Brassica oleracea</i> var. <i>gem</i>	1		1751	
f218	x		x	x	x	Paprika, sweet pepper	<i>Capsicum annuum</i>	1		1759	
f219	x			x		Fennel seed	<i>Foeniculum vulgare</i>	1		1824	
f219			x			Sage	<i>Salvia officinalis</i>	3		2361	
f220	x		x	x		Cinnamon	<i>Cinnamomum</i> spp.	1		2226	
f221	x		x	x		Coffee	<i>Coffea</i> spp.	1		1503	
f222	x		x	x		Tea	<i>Camellia sinensis</i>	3		2210	
f223	x		x			Green olive	<i>Olea europaea</i>	2		2053	
f224	x		x	x		Poppy seed	<i>Papaver somniferum</i>	1		1901	
f225				x		Summer squash, pumpkin	<i>Cucurbita pepo</i>	1		1788	
f225	x					Pumpkin	<i>Cucurbita maxima</i>	1		1787	
f226				x		Pumpkin seed	<i>Cucurbita pepo</i>	1		2029	
f226			x			Deer	<i>Dama dama</i>	3			
f227				x		Sugar-beet seed	<i>Beta vulgaris</i>	1		2200	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f227			x			Swordfish	<i>Xiphias gladius</i>	1		2013	
f231	x		x	x		Milk, boiled	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos spp.</i>)	1		1747	
f232	x		x	x		Gal d 2, Ovalbumin	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus spp.</i>)	1	Gal d 2	360	NN
f233	x		x	x		Gal d 1, Ovomucoid	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus spp.</i>)	1	Gal d 1	359	NN
a359		x				Gal d 1, Ovomucoid	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus spp.</i>)	1	Gal d 1	359	NN
a360		x				Gal d 2, Ovalbumin	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus spp.</i>)	1	Gal d 2	360	NN
a363		x				Gal d 5	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus spp.</i>)	1	Gal d 5	363	NN
f234	x		x	x		Vanilla	<i>Vanilla planifolia</i>	2		2399	
f235	x		x	x		Lentil	<i>Lens culinaris</i> (<i>Lens esculenta</i>)	1		1857	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f236	x		x	x		Cow's whey	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1			
f237	x		x	x		Apricot	<i>Prunus armeniaca</i>	1		1945	
f241			x			Gouda cheese		3			
f242	x		x	x		Cherry	<i>Prunus avium</i>	1		1946	
f244	x		x	x		Cucumber	<i>Cucumis sativus</i>	1		1789	
f245	x		x	x	x	Egg	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1		2035	
f246			x	x		Guar, guar gum	<i>Cyamopsis tetragonoloba</i>	1		2236	
f247			x	x		Honey		1			
f248			x			Rosemary	<i>Rosmarinus officinalis</i>	2		1438	
f253			x	x		Pine nut, pignoles	<i>Pinus edulis</i>	1		1588	
f253	x					Pine nut	<i>Pinus pinea</i>	2		714	
f254	x		x	x		Plaice	<i>Pleuronectes platessa</i>	1		1371	
f255			x	x		Plum	<i>Prunus domestica</i>	1		1947	
f255	x					Plum	<i>Prunus americana</i>	3		3622	
f256	x		x	x	x	Walnut	<i>Juglans</i> spp.	1		1850	
f258			x	x		Squid	<i>Loligo</i> spp.	1		1414	
f259	x		x	x		Grape	<i>Vitis vinifera</i> (<i>Vitis</i> spp.)	1		2012	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f260	x		x	x	x	Broccoli	<i>Brassica oleracea</i> var. <i>italica</i> (<i>Brassica oleracea</i> var. <i>cultivar</i>)	1		1751	
f261	x		x	x		Asparagus	<i>Asparagus officinalis</i>	1		1734	
f262	x		x	x		Aubergine, eggplant	<i>Solanum melongena</i>	1		875	
f263				x		Green pepper (unripe seed)	<i>Piper nigrum</i>	1		1930	
f263	x					Green pepper	<i>Capsicum annuum</i>	1		1759	
f264			x	x		Eel	<i>Anguilla anguilla</i>	1		1715	
f265	x			x		Caraway	<i>Carum carvi</i>	1		1489	
f265			x			Cumin	<i>Cuminum cyminum</i>	1		1792	
f266				x		Mace	<i>Myristica fragrans</i>	1		1121	
f267			x	x		Cardamon	<i>Elettaria cardamomum</i>	1		1287	
f268	x		x	x		Clove	<i>Syzygium aromaticum</i>	1		2372	
f269	x		x	x		Basil	<i>Ocimum basilicum</i>	1		2296	
f270	x		x	x		Ginger	<i>Zingiber officinale</i>	1		942	
f271	x		x	x		Anise	<i>Pimpinella anisum</i>	1		1928	
f272	x		x	x		Tarragon	<i>Artemisia dracunculus</i>	1		2181	
f273	x		x	x		Thyme	<i>Thymus vulgaris</i>	1		1437	
f274	x		x	x		Marjoram	<i>Origanum majorana</i>	1		3653	
f275			x	x		Lovage	<i>Levisticum officinale</i>	1		2277	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f276			x	x		Fennel, fresh	<i>Foeniculum vulgare</i>	1		2034	
f277	x		x	x		Dill	<i>Anethum graveolens</i>	1		1153	
f278	x		x	x		Bay leaf	<i>Laurus nobilis</i>	1		1439	
f279	x		x	x		Chili pepper	<i>Capsicum frutescens</i>	1		2213	
f280	x		x	x		Black pepper	<i>Piper nigrum</i>	1		1930	
f281	x		x	x		Curry (Santa Maria)		1			
f282	x		x	x		Nutmeg	<i>Myristica fragrans</i>	1		1121	
f283	x		x	x		Oregano	<i>Origanum vulgare</i>	1		1478	
f284	x		x	x	x	Turkey meat	<i>Meleagris gallopavo</i>	1		2049	
f285				x		Elk/moose meat	<i>Alces</i> spp.	1		2137	
f286				x		Mare's milk	<i>Equus caballus</i> (<i>Equus</i> spp.)	1		2032	
f287	x		x	x		Red kidney bean	<i>Phaseolus vulgaris</i>	1		1923	
f288	x		x	x		Blueberry	<i>Vaccinium myrtillus</i> (<i>Vaccinium</i> spp.)	1		2397	
f289	x		x	x		Date	<i>Phoenix dactylifera</i>	1		2064	
f290	x		x	x		Oyster	<i>Ostrea edulis</i> (<i>Ostrea</i> spp.)	1		2409	
f291	x		x	x		Cauliflower	<i>Brassica oleracea</i> var. <i>botrytis</i>	1		1751	
f292			x	x		Guava	<i>Psidium guajava</i>	1		2331	
f293	x		x	x		Papaya	<i>Carica papaya</i>	1		1763	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f294	x		x	x		Passion fruit, Maracuja	<i>Passiflora edulis</i> (<i>Passiflora</i> spp.)	1		1907	
f295				x		Carambola	<i>Averrhoa carambola</i>	3		2198	
f296			x	x		Carob	<i>Ceratonia siliqua</i>	1		1609	
f297			x	x		Gum arabic	<i>Acacia senegal</i> (<i>Acacia</i> spp.)	1		2135	
f298			x	x		Tragacanth	<i>Astragalus</i> spp.	1		2190	
f299	x		x	x		Sweet chestnut (chestnut)	<i>Castanea sativa</i>	1		2025	
f300			x	x		Goat milk	<i>Capra hircus</i>	1		1760	
f300	x					Pinto bean	<i>Phaseolus</i> spp.	1		1923	
f301	x			x		Persimmon (kaki fruit, sharon)	<i>Diospyros kaki</i>	1		932	
f302	x		x	x		Mandarin (tangerine, clementine, satsumas)	<i>Citrus reticulata</i>	1		2229	
f303			x	x		Halibut	<i>Hippoglossus hippoglossus</i>	1		1481	
f303	x					Halibut	<i>Hippoglossus stenolepis</i>	2		2988	
f304			x	x		Langust (spiny lobster)	<i>Palinurus</i> spp.	1		1898	
f305				x		Fenugreek	<i>Trigonella foenum-graecum</i>	1		1288	
f306	x		x	x		Lime	<i>Citrus aurantifolia</i>	2		2227	
f307				x		Hake	<i>Merluccius merluccius</i>	1		2285	
f308			x	x		Sardine (pilchard)	<i>Sardina pilchardus</i>	1		2362	

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f309	x		x	x		Chick pea	<i>Cicer arietinum</i>	1		1771	
f310				x		Blue vetch	<i>Lathyrus sativus</i>	1		1016	
f311				x		Megrim	<i>Lepidorhombus whiffiagonis</i>	1		2276	
f312	x			x		Swordfish	<i>Xiphias gladius</i>	1		2013	
f313	x		x	x		Anchovy	<i>Engraulis encrasicolus</i>	1		1480	
f313	x					Swordfish	<i>Xiphias gladius</i>	1		2013	
f314	x		x	x		Snail	<i>Helix aspersa</i>	1		1840	
f315	x		x	x		Green bean	<i>Phaseolus vulgaris</i>	1		1923	
f316				x		Rape seed	<i>Brassica napus</i>	1		1750	
f317	x		x	x		Coriander	<i>Coriandrum sativum</i>	1		1779	
f318				x		Jack fruit	<i>Artocarpus heterophyllus</i>	1		1727	
f319			x	x		Beetroot	<i>Beta vulgaris</i>	1		2200	
f320	x		x	x		Crayfish	<i>Astacus astacus</i>	1		2188	
f321			x	x		Horse meat	<i>Equus caballus</i> (<i>Equus</i> spp.)	1		2031	
f322				x		Red currant	<i>Ribes sylvestre</i>	1		1151	
f323				x		Gal d 3, Conalbumin	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1	Gal d 3	361	NN

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a361		x				Gal d 3, Conalbumin	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1	Gal d 3	361	NN
f324			x	x		Hop (fruit cone)	<i>Humulus lupulus</i>	1		2042	
f325				x		Sheep milk	<i>Ovis aries</i> (<i>Ovis</i> spp.)	1		1892	
f325			x			Saffron	<i>Colchicum autumnale</i>	2		3742	
f326				x		Sheep whey	<i>Ovis aries</i> (<i>Ovis</i> spp.)	2			
f327			x			Chervil	<i>Chaerophyllum tainturieri</i>	3		3741	
f328				x		Fig	<i>Ficus carica</i>	1		1823	
f329	x			x		Watermelon	<i>Citrullus lanatus</i>	1		1773	
f330				x		Rose hip	<i>Rosa</i> spp.	3		2074	
f330			x			Juniper berry	<i>Juniperus communis</i>	3		3740	
f331				x		Saffron	<i>Crocus sativus</i>	1		1783	
f332			x	x		Mint	<i>Mentha piperita</i>	1		2281	
f333				x		Linseed	<i>Linum usitatissimum</i>	1		905	
f333			x			Carp	<i>Cyprinus carpio</i>	1		1797	
f334				x		Bos d lactoferrin	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1		1065	NN
a1065		x				Bos d Lactoferrin	<i>Bos domesticus</i> (<i>Bos taurus</i> ; <i>Bos</i> spp.)	1		1065	NN

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f335				x		Lupin seed	<i>Lupinus albus</i>	1		2045	
f335			x			Pepper, green	<i>Piper nigrum</i>	1		1930	
f336				x		Jujube	<i>Ziziphus jujuba</i>	1		855	
f336			x			Wine vinegar	<i>Vitis vinifera</i> (Vitis spp.)	1		2012	
f337	x			x		Sole	<i>Solea solea</i>	1		2368	
f337	x					English sole	<i>Parophrys vetulus</i>	2		1484	
f338	x			x		Scallop	<i>Pecten</i> spp.	1		2837	
f338			x			Wine, white	<i>Vitis vinifera</i> (Vitis spp.)	1		2012	
f339				x		Allspice	<i>Pimenta dioica</i>	1		2305	
f339			x			Wine, red	<i>Vitis vinifera</i> (Vitis spp.)	1		2012	
f340				x		Cochineal extract (Carmine red)	<i>Dactylopius coccus</i>	1		938	
f340			x			Gelatin		1			
f341				x		Cranberry	<i>Vaccinium oxycoccus</i>	3		2398	
f341	x					Cranberry	<i>Vaccinium macrocarpon</i>	3		2396	
f342				x	x	Olive (black, fresh)	<i>Olea europaea</i>	1		2053	
f343	x			x		Raspberry	<i>Rubus idaeus</i>	1		1093	
f344	x			x		Sage	<i>Salvia officinalis</i>	1		2361	
f345	x		x	x		Macadamia nut	<i>Macadamia</i> spp.	1		1156	
f346				x		Abalone	<i>Haliotis</i> spp.	1		1836	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f346			x			Chives	<i>Allium schoenoprasum</i>	3		3737	
f347				x		Quinoa	<i>Chenopodium quinoa</i>	1		2223	
f348				x		Litchi	<i>Litchi chinensis</i>	1		1865	
f349				x		Chum salmon roe	<i>Oncorhynchus keta</i>	1		2689	
f350				x		Alaska pollock roe	<i>Theragra chalcogramma</i>	1		2692	
f351				x		Pen a 1 (Tropomyosin)	<i>Penaeus aztecus</i>	1	Pen a 1	515	RR
f351			x			Butter		3			
f352				x		Ara h 8	<i>Arachis hypogaea</i>	1	Ara h 8	1215	RR
f352			x			Ewe's milk	<i>Ovis aries (Ovis spp.)</i>	1		1892	
f353				x		Gly m 4	<i>Glycine max</i>	1	Gly m 4	749	RR
f354				x		Ber e 1	<i>Bertholletia excelsa</i>	1	Ber e 1	88	RR
f354			x			Caviar		3			
f355				x		Cyp c 1	<i>Cyprinus carpio</i>	1	Cyp c 1	263	RR
f358	x		x			Artichoke	<i>Cynara scolymus</i>	2		1104	
f360	x		x			Yogurt		3			
f368			x			Black bass	<i>Micropterus dolomieu (Micropterus dolomieu)</i>	3		3735	
f369			x	x		Catfish	<i>Ictalurus punctatus</i>	1		1203	
f374			x			Karaya gum	<i>Sterculia urens</i>	3		2371	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f375			x			Horseradish	<i>Armoracia rusticana</i>	1		1726	
f377			x			Maple syrup		3			
f379			x			Okra	<i>Abelmoschus esculentus</i>	2		1592	
f382			x			Beet, sugar	<i>Beta vulgaris var. altissima</i>	2		2200	
f381	x		x	x		Red snapper	<i>Lutjanus campechanus</i>	1		3733	
f384			x	x		Whitefish	<i>Salanx cuvieri</i>	1		3732	
f401	x					Loquat	<i>Eriobotrya japonica</i>	3		2246	
f402	x					Fig	<i>Ficus carica</i>	1		1823	
f403	x					Brewer's yeast	<i>Saccharomyces cerevisiae</i>	1		1960	
f405	x					Mint	<i>Mentha</i> spp.	3		2281	
f406	x					Arugula	<i>Eruca vesicaria</i>	2		1340	
f408	x					Whiting	<i>Merlangius merlangus</i>	3		2284	
f409	x					Goat's milk	<i>Capra hircus</i>	1		1760	
f410				x		Grouper	<i>Epinephelus</i> spp.	3		3731	
f411				x		Mahi mahi	<i>Coryphaena</i> spp.	3		3730	
f412				x		Orange roughy	<i>Hoplostethus atlanticus</i>	3		3729	
f413				x		Pollock	<i>Pollachius virens</i>	3		3728	
f414				x		Tilapia	<i>Oreochromis</i> spp.	1		2948	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
f415				x		Walleye pike	<i>Sander vitreus</i> (<i>Stizostedion vitreum</i>)	3		3727	
f416				x		Tri a 19.0101, Omega 5 Gliadin	<i>Triticum aestivum</i> (<i>Triticum</i> spp.)	1	Tri a 19.0101	3677	RR
f417				x		Api g 1.01	<i>Apium graveolens</i>	1	Api g 1.01	41	RR
f418				x		Api g 1.02	<i>Apium graveolens</i>	1	Api g 1.02	42	RR
a41		x				Api g 1.0101	<i>Apium graveolens</i>	1	Api g 1.0101	41	RR
a1454		x				Mal d 1.0108	<i>Apium graveolens</i>	1	Mal d 1.0108	1454	RR
f419				x		Pru p 1	<i>Prunus persica</i>	1	Pru p 1	602	RR
f420				x		Pru p 3	<i>Prunus persica</i>	1	Pru p 3	603	RR
a603		x				Pru p 3	<i>Prunus persica</i>	1	Pru p 3	603	NN
f421				x		Pru p 4	<i>Prunus persica</i>	1	Pru p 4	736	RR
f422				x		Ara h 1	<i>Arachis hypogaea</i>	1	Ara h 1	50	RR
f423				x		Ara h 2	<i>Arachis hypogaea</i>	1	Ara h 2	51	RR
f424				x		Ara h 3	<i>Arachis hypogaea</i>	1	Ara h 3	52	RR
f425				x		Cor a 8	<i>Corylus avellana</i>	1	Cor a 8	245	RR
f426				x		Gad c 1	<i>Gadus morhua</i>	1	Gad c 1	357	RR
f427				x		Ara h 9	<i>Arachis hypogaea</i>	1	Ara h 9	742	RR
f428				x		Cor a 1	<i>Corylus avellana</i>	1	Cor a 1	232	RR
a972		x				Pen m 1	<i>Penaeus monodon</i>	1	Pen m 1	972	NN
F351	x					Pen m 1	<i>Penaeus monodon</i>	1	Pen m 1	972	NN

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a3309		x				Hel as 1.0101	<i>Helix aspersa</i>	1	Hel as 1.0101	3309	RR
a419		x				Hor v 16	<i>Hordeum vulgare</i>	1	Hor v 16	419	NN
a420		x				Hor v 17	<i>Hordeum vulgare</i>	1	Hor v 17	420	NN
a527		x				Pen i 1	<i>Penaeus indicus</i>	1	Pen i 1	527	NN
a650		x				Tri a 18	<i>Triticum aestivum</i> (<i>Triticum</i> spp.)	1	Tri a 18	650	NN
a651		x				Tri a Gluten	<i>Triticum aestivum</i> (<i>Triticum</i> spp.)	1		651	NN
a1		x				Act d 1	<i>Actinidia deliciosa</i>	1	Act d 1	1	NN
a747		x				Act d 2	<i>Actinidia deliciosa</i>	1	Act d 2	747	NN
a2821		x				Act d 5	<i>Actinidia deliciosa</i>	1	Act d 5	2821	NN
a245		x				Cor a 8	<i>Corylus avellana</i>	1	Cor a 8	245	NN
a3077		x				Ana o 2.0101	<i>Anacardium occidentale</i>	1	Ana o 2.0101	3077	RR
h						House dust					
h1	x		x	x		Greer Labs., Inc.		1			
h2	x		x	x	x	Hollister-Stier Labs.		1			
h3	x					Bencard		2			
h6	x					Japan		2			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
i						Venoms and insects					
i1	x		x	x		Honey bee	<i>Apis mellifera</i>	1		1722	
i2	x		x	x		White-faced hornet	<i>Dolichovespula maculata</i>	1		1807	
i3	x		x	x		Common wasp (yellow jacket)	<i>Vespula</i> spp.	1		2400	
i4	x		x	x		Paper wasp	<i>Polistes</i> spp.	1		2322	
i5	x		x	x		Yellow hornet	<i>Dolichovespula arenaria</i>	1		1806	
i6			x	x		Cockroach, German	<i>Blattella germanica</i>	1		1742	
i6	x					Cockroach	<i>Blattella germanica</i> and <i>Supella longipalpa</i>	1			
i7				x		Midge	<i>Chironomus yoshimatsui</i>	1		1354	
i8	x		x	x		Moth	<i>Bombyx mori</i>	1		1368	
i47			x			Water flea	<i>Daphnia</i> spp.	2		2238	
i49			x			Deer fly	<i>Chrysops</i> spp.	2		999	
i51			x			Black ant	<i>Camponotus pennsylvanicus</i>	2		2212	
i54			x			Flea mix (dog/cat), common flea	<i>Ctenocephalides</i> spp.	2			
i70	x		x	x		Fire ant	<i>Solenopsis invicta</i>	1		1974	
i71			x	x		Mosquito	<i>Aedes communis</i>	1		1279	
i71	x					Mosquito	<i>Aedes</i> spp. and <i>Culex</i> spp.	2			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
i72				x		Green nimitti	<i>Cladotanytarsus lewisi</i>	1		1625	
i73				x		Blood worm	<i>Chironomus thummi</i>	1		1770	
i73	x					Blood worm	<i>Chironomus</i> spp.	2		1355	
i75	x		x	x		European hornet	<i>Vespa crabro</i>	1		2010	
i76				x		Berlin beetle	<i>Trogoderma angustum</i>	1		3654	
i77				x		European paper wasp	<i>Polistes dominulus</i>	1		1937	
i78			x			Fly	<i>Musca domestica</i>	2		1074	
i80			x			Bumblebee	<i>Bombus pennsylvanicus</i>	1		1745	
i100			x			Cockroach, American	<i>Periplaneta americana</i>	1		1917	
i201			x	x		Horse bot fly	<i>Gasterophilus intestinalis</i>	1		2257	
i202				x		Grain weevil	<i>Sitophilus granarius</i>	1		1536	
i203				x		Mediterranean flour moth	<i>Ephestia kuehniella</i> (<i>Anagasta kuehniella</i>)	1		931	
i204	x			x		Horse fly	<i>Tabanus</i> spp.	1		1477	
i205				x		Bumblebee	<i>Bombus terrestris</i>	1		1746	
i206	x			x		Cockroach, American	<i>Periplaneta americana</i>	1		1917	
i207			x	x		Cockroach, Oriental	<i>Blatta orientalis</i>	1		1743	
a3140		x				Bla g 2.0101	<i>Blattella germanica</i>	1	Bla g 2.0101	3140	RR
a3142		x				Bla g 5.0101	<i>Blattella germanica</i>	1	Bla g 5.0101	3142	RR
a543		x				Per a 7.0101	<i>Periplaneta americana</i>	1	Per a 7.0101	543	RR

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a45		x				Api m 1	<i>Apis mellifera</i>	1	Api m 1	45	NN
a48		x				Api m 4	<i>Apis mellifera</i>	1	Api m 4	48	NN
c						Drugs					
c1	x		x	x		Penicilloyl G		1			
c2	x		x	x		Penicilloyl V		1			
c5				x		Ampicilloyl		1			
c6				x		Amoxicilloyl		1			
c7				x		Cefaclor		1			
c8				x		Chlorhexidin		1			
c9				x		Ceftriaxone		1			
c70	x		x	x		Insulin porcine (Sus s Insulin)	<i>Sus scrofa (Sus scrofa domesticus; Sus spp.)</i>	1		2122	NN
c71	x		x	x		Insulin bovine (Bos d Insulin)	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1		2118	NN
c73	x		x	x		Insulin human (Hom s Insulin)	<i>Homo sapiens</i>	1		2121	RR
c74	x			x		Gelatin bovine	<i>Bos domesticus (Bos taurus; Bos spp.)</i>	1			
c99			x			Polylysine		3			
c201			x			Cephalosporin mix		2			
c202			x	x		Suxamethonium (succinylcholine)		1			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
c203	x		x			Ampicillin		1			
c204	x		x			Amoxicillin		1			
c206			x	x		ACTH (Adrenocorticotrophic hormone)	<i>Homo sapiens</i>	1			NN
c207				x		Protamine (Onc m Protamine)	<i>Oncorhynchus mykiss (Salmo gairdneri)</i>	1		2145	NN
c208				x		Tetanus toxoid (Clo t Toxoid)	<i>Clostridium tetani</i>	1		2757	NN
c208			x			Pyrazolon derivatives		2			
c209				x		Chymopapain (Car p Chymopapain)	<i>Carica papaya</i>	1		1540	NN
c209			x			Paracetamol/acetaminophen		3			
c210			x			Furosemide		3			
c211			x			Tetracycline		3			
c212			x			Erythromycin		3			
c213			x			Gentamicin		3			
c214			x			Gold chloride		3			
c216			x			Doxycycline		3			
c217			x			Acetyl salicylic acid		3			
c222			x			Macrolid mix		3			
c223			x			Sulfamethoxazole		2			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
c225			x			Oxazepam		3			
c231			x			Articaine		3			
c232			x			Lidocaine		3			
c233			x			Mepivacaine		3			
c235			x			Tetracaine		3			
c242			x			Trimethoprim		2			
c250			x			Hyaluronan		3			
c255			x			Phenprocoumon		3			
c260				x		Quaternary ammonium, Morphine		1			
c261				x		Pholcodine		1			
c262				x		Rocuronium		1			
c280			x			Propionic acid		3			
c281			x			Diclofenac		3			
c282			x			Indomethacin		3			
c283			x			Naproxen		3			
c284			x			Procaine		3			
c285			x			Bupivacaine		3			
c286			x			Ibuprofen		3			
c288			x			Aminoglycoside mix		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
c294			x			Metamizole		3			
c300			x			Codeine		3			
c301			x			Rifampicin		3			
c304			x			Piroxicam		3			
c305			x			Clavulanic acid		3			
c306			x			Clindamycin		3			
c307			x			Ciprofloxacin		3			
c308			x			Cefuroxime		3			
c309			x			Cefalexin		3			
c311			x			Diazepam		3			
c312			x			Lactose		3			
c322			x			Ambroxol		3			
c327			x			Atropine sulfate		3			
c330			x			Benzalkonium chloride		3			
c343			x			Chloromezanone		3			
c350			x			Dexamethasone		3			
c360				x		Customer-specific substance		1			
c375			x			Fosfomycin		3			
c381			x			Hydrochlorothiazide		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
c386			x			Isosorbid dinitrate		3			
c388			x			Ketoprofen		3			
c391			x			Levothyroxine		3			
c392			x			Lisinopril		3			
c398			x			Metronidazole		3			
c409			x			Ofloxacin		3			
c417			x			Phenobarbital		3			
c424			x			Prednisolone		3			
c436			x			Spiramycin		3			
c470			x			Lecithin		3			
c602			x			Aluminum chloride		3			
c609			x			Chromium		3			
c613			x			Henna		3			
c615			x			Isopropyl alcohol (isopropanol)		3			
c616			x			Iodine (sodium iodide)		3			
c617			x			Cobalt		3			
c621			x			Lindane		3			
c623			x			Methacrylic acid ethoxy ethyl		3			
c625			x			Molybdic acid		3			

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Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
c628			x			Nickel chloride		3			
c629			x			Palladium (2-Acetate)		3			
c630			x			Pentachlorophenol (PCP)		3			
c632			x			Platanic chloride		3			
c635			x			Mercuric chloride		3			
c639			x			Tannic acid		3			
c640			x			Turpentine		3			
c641			x			Thimerosal		3			
c648			x			Zinc chloride		3			
c700			x			Formic acid		3			
c701			x			Aminobenzoic acid		3			
c702			x			Ascorbic acid		3			
c703			x			Benzoic acid		3			
c704			x			Quinoline yellow		3			
c705			x			Cyclamic acid		3			
c706			x			Yellow orange		3			
c707			x			Glutamic acid		3			
c708			x			Xanthan gum		3			
c710			x			Methylene blue		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
c712			x			Sodium sulfite		3			
c713			x			Methylparaben		3			
c714			x			Propylparaben		3			
c715			x			Saccharose		3			
c716			x			Sorbic acid		3			
c717			x			Tartrazine		3			
c725			x			Amaranth		3			
c726			x			Cochénille red		3			
c810			x			Tetanus	<i>Clostridium tetani</i>	1		2757	NN
o						Miscellaneous					
o1	x		x	x		Cotton, crude fibers	<i>Gossypium</i> spp.	1			
o3			x			Cotton (treated)	<i>Gossypium</i> spp.	3			
o70			x	x		Seminal fluid	<i>Homo sapiens</i>	1			
o71			x			<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	1		2138	
o72				x		Pichia pastoris crude extract customer specific	<i>Pichia pastoris</i>	3			
o72			x			Sperm-sediment	<i>Homo sapiens</i>	2			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
o73				x		Pichia pastoris crude extr. vector customer specific	<i>Pichia pastoris</i>	3			
o74				x		Pichia pastoris with vector customer specific	<i>Pichia pastoris</i>	3			
o201	x		x	x		Tobacco leaf, tobacco dust	<i>Nicotiana tabacum</i>	2			
o202				x		Artemia salina, fish feed	<i>Artemia salina</i>	2		2177	
o203				x		Tetramin, fish feed		2			
o206			x			Fish feed (vegetable)		3			
o207				x		Daphnia, fish feed	<i>Daphnia</i> spp.	1		2238	
o211				x		Mealworm	<i>Tenebrio molitor</i>	1		1021	
o212				x		Streptavidin	<i>Streptomyces avidini</i>	3			RR
o213				x		MBP (maltose binding protein)	<i>Escherichia coli</i>	3			RR
o214				x		CCD; MUXF3 from bromelain	<i>Ananas comosus</i>	1		3726	NN
o72	x					Enterotoxin A (Sta a SEA)	<i>Staphylococcus aureus</i>	1		2139	NN
o73	x					Enterotoxin B (Sta a SEB)	<i>Staphylococcus aureus</i>	1		2140	NN

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
p						Parasites					
p1	x		x	x		Ascaris	<i>Ascaris suum</i>	1		1729	
p2			x	x		Echinococcus	<i>Echinococcus granulosus</i>	1		2240	
p3			x	x		Schistosoma	<i>Schistosoma mansoni</i>	1		3725	
p4	x		x	x		Anisakis (Herring Worm)	<i>Anisakis simplex</i> (<i>Anisakis</i> spp.)	1		1716	
a3081		x				Ani s 3.0101	<i>Anisakis simplex</i> (<i>Anisakis</i> spp.)	1	Ani s 3.0101	3081	RR
a3079		x				Ani s 1.0101	<i>Anisakis simplex</i> (<i>Anisakis</i> spp.)	1	Ani s 1.0101	3079	RR
k						Occupational					
k4			x			Threshing dust		3			
k5			x			Flax		3			
k8	x		x			Hop (hops)	<i>Humulus lupulus</i>	2		2042	
k12			x			Grain mill dust		3			
k14			x			Kapok		3			
k16			x			Linen		3			
k20	x		x			Sheep's wool (treated) (wool)	<i>Ovis aries</i> (<i>Ovis</i> spp.)	3			
k21			x			Sheep's wool (Untreated)	<i>Ovis aries</i> (<i>Ovis</i> spp.)	3			
k33			x			Oak		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
k70	x		x	x		Green coffee bean	<i>Coffea</i> spp.	1		1503	
k71	x		x	x		Castor bean	<i>Ricinus communis</i>	1		1959	
k72	x		x			Ispaghula	<i>Plantago ovata</i>	2		988	
k72				x		Ispaghula	<i>Plantago psyllium/Plantago ovata</i>	1			
k73				x		Silk waste		1			
k74	x		x	x		Silk	<i>Bombyx mori</i>	1			
k75	x		x	x		Isocyanate TDI (Toluene diisocyanate)		1			
k76	x		x	x		Isocyanate MDI (Diphenylmethane diisocyanate)		1			
k77	x		x	x		Isocyanate HDI (Hexamethylene diisocyanate)		1			
k78			x	x		Ethylene oxide		1			
k79	x		x	x		Phthalic anhydride		1			
k80	x		x	x		Formaldehyde/Formalin		1			
k81	x		x	x		Ficus	<i>Ficus benjamina</i> (<i>Ficus</i> spp.)	1			
k82	x		x	x	x	Hevea latex	<i>Hevea brasiliensis</i>	1		1841	
k83	x		x	x		Cotton seed	<i>Gossypium hirsutum</i>	1		2261	
k84	x		x	x		Sunflower seed	<i>Helianthus annuus</i>	1		2039	

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
k85			x	x		Chloramin T		1			
k86	x		x	x		Trimellitic anhydride, TMA		1			
k87	x		x	x		Asp o 21, alpha-amylase	<i>Aspergillus oryzae</i>	1	Asp o 21	86	NN
k89			x			Orris root	<i>Iris florentina</i>	3			
k99			x			HSA (Human Serum Albumin) (Hom s HSA)	<i>Homo sapiens</i>	1		763	NN
k201			x	x		Car p 1, Papain	<i>Carica papaya</i>	1	Car p 1	709	NN
k202	x		x	x		Ana c 2, Bromelain	<i>Ananas comosus</i>	1	Ana c 2	694	NN
a694		x				Ana c 2, Bromelain	<i>Ananas comosus</i>	1	Ana c 2	694	NN
k203				x		Api m 1, Phospholipase A2	<i>Apis mellifera</i>	1	Api m 1	45	NN
k204				x		Maxatase	<i>Bacillus licheniformis</i>	1			NN
k205	x		x	x		Alcalase	<i>Bacillus</i> spp.	1			NN
k206			x	x		Savinase, Protease I (Bac l Subtilisin)	<i>Bacillus</i> spp.	1		903	NN
k208			x	x		Gal d 4, Lysozyme	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1	Gal d 4	362	NN

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a362		x				Gal d 4, Lysozyme	<i>Gallus domesticus</i> (<i>Gallus gallus domesticus</i> ; <i>Gallus</i> spp.)	1	Gal d 4	362	NN
k209				x		Hexahydrophthalic anhydrid		1			
k210	x			x		Maleic anhydrid		1			
k211				x		Methyltetrahydrophthalic anhydrid		1			
k212			x	x		Abachi wood dust	<i>Triplochiton scleroxylon</i>	1		1999	
k213				x		Pepsin (Sus s Pepsin)	<i>Sus scrofa</i> (<i>Sus scrofa domesticus</i> ; <i>Sus</i> spp.)	1		2924	
k213	x					TCPA		2			
k214				x		Bougainvillea	<i>Bougainvillea</i> spp.	3		2207	
k215				x		Hev b 1, Rubber elongation factor	<i>Hevea brasiliensis</i>	1	Hev b 1	379	RR
k216				x		Hev b 2, Beta 1,3 glucanase	<i>Hevea brasiliensis</i>	1	Hev b 2	386	RR
k217				x		Hev b 3, Prenyltransferase	<i>Hevea brasiliensis</i>	1	Hev b 3	387	RR
k218				x		Hev b 5, Acid protein	<i>Hevea brasiliensis</i>	1	Hev b 5	389	RR
k219				x		Hev b 6.01, Prohevein	<i>Hevea brasiliensis</i>	1	Hev b 6.01	391	RR
k220				x		Hev b 6.02, Hevein	<i>Hevea brasiliensis</i>	1	Hev b 6.02	392	RR
k220			x			Protease 2		3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
k221				x		Hev b 8, Hevea profilin	<i>Hevea brasiliensis</i>	1	Hev b 8	397	RR
k222				x		Hev b 9, Hevea enolase	<i>Hevea brasiliensis</i>	1	Hev b 9	404	RR
k222			x			Amylase		3			
k224				x		Hev b 11, Class 1 Chitinase	<i>Hevea brasiliensis</i>	1	Hev b 11	384	RR
k224			x			Lipase		3			
k225	x					Horse radish peroxidase (Arm r HRP)	<i>Armoracia rusticana</i>	1		862	NN
k225			x			Cellulase C		3			
k226	x					Ascorbate oxidase (Cuc p ascorbate oxidase)	<i>Cucurbita pepo</i>	1		695	NN
k226			x			Cellulase R		3			
K252			x			Cherry wood		3			
k301	x					Flour dust	<i>Triticum</i> spp.	3			
k501				x		Savinase customer specific	Proprietary knowledge of customer	3			
k502				x		Lipolase customer specific	Proprietary knowledge of customer	3			
k503				x		Termamyl customer specific	Proprietary knowledge of customer	3			
k504				x		Clazinas customer specific	Proprietary knowledge of customer	3			

Appendix A. (Continued)

Current Code	Biotin-Allergen-Based System	Chip-Based System	Disc-Based System	Sponge-Based System	Thread-Based System	Product	Source Taxonomical Name	Documentation category	IUIS Nomenclature Code	Allergome Code	Nature of the allergenic molecule (NN = Native; RR = Recombinant)
a3310		x				Hev b 1.0101	<i>Hevea brasiliensis</i>	1	Hev b 1.0101	3310	RR
a3314		x				Hev b 3.0101	<i>Hevea brasiliensis</i>	1	Hev b 3.0101	3314	RR
a3316		x				Hev b 5.0101	<i>Hevea brasiliensis</i>	1	Hev b 5.0101	3316	RR
a392		x				Hev b 6.02, Hevein	<i>Hevea brasiliensis</i>	1	Hev b 6.02	392	RR
a396		x				Hev b 7.02	<i>Hevea brasiliensis</i>	1	Hev b 7.02	396	RR
a403		x				Hev b 8.0204	<i>Hevea brasiliensis</i>	1	Hev b 8.0204	403	RR
a3317		x				Hev b 9.0101	<i>Hevea brasiliensis</i>	1	Hev b 9.0101	3317	RR
a382		x				Hev b 10.0102	<i>Hevea brasiliensis</i>	1	Hev b 10.0102	382	RR
a977		x				Hev b 11.0101	<i>Hevea brasiliensis</i>	1	Hev b 11.0101	977	RR

Appendix B. Dilution-Recovery Analysis (Parallelism-Linearity)

Parallelism (linearity) is a term that refers to an assay's ability to analyze patient samples in a manner that is identical to that which is used by the calibration system to convert the measured response signal into an analyte dose estimate. Parallelism can be effectively analyzed through a "dilution-recovery" analysis in which different sera are analyzed at multiple dilutions. The interpolated dose that is obtained for each serum at each dilution is then compared to the level that might be expected if the serum is diluted serially. Dilution-recovery studies provide essential information that can validate the calibration system selected for the assay. In addition to evaluating the degree of parallelism, dilution-recovery studies permit the manufacturer and user to evaluate the solid-phase, antibody-binding capacity, and the assay's limit of detection and dynamic range. Dilution-recovery experiments need to be designed to avoid pitfalls, such as the dilution-matrix effect and dilution-accuracy effects. A correctly designed experiment should use a diluent that mimics precisely a human serum specimen without IgE. For IgE antibody assays, accuracy of the serum dilution scheme can be evaluated by independently analyzing the same sera in the total serum IgE assay and validating the predicted net change in analyte. Interdilutional percentage coefficients of variation $< 15\%$ can be used as one benchmark for parallelism in assays that employ a suitable calibration system, linear range, and detection limit.

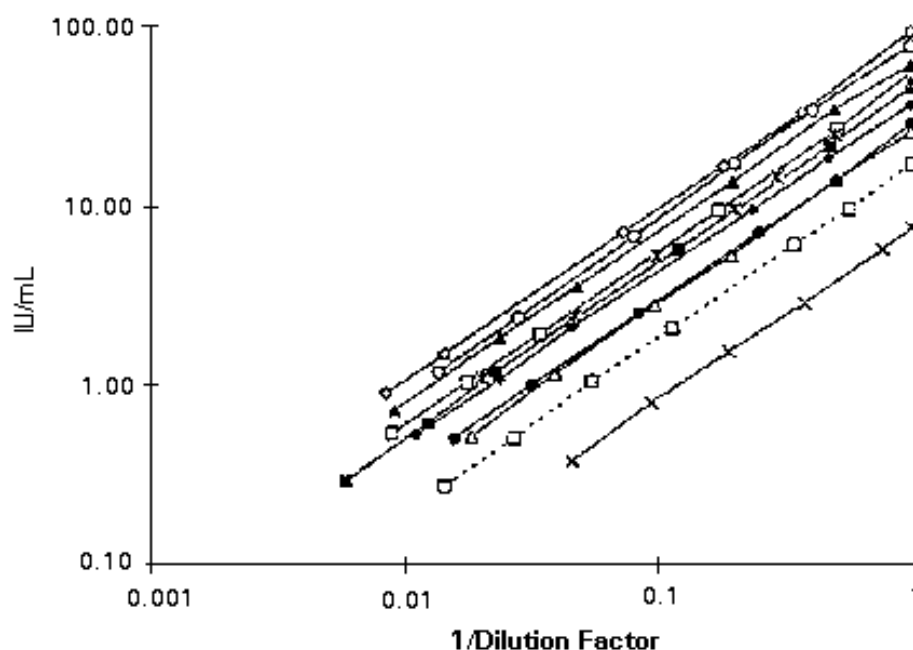


Figure B1. Illustrative Dilution-Recovery Analysis of an IgE Antibody Immunoassay. This figure does not illustrate the degree of parallelism achieved between 0.1 and 1 IU/mL, at the minimum detectable concentration of most IgE antibody assays.

In this analysis, duplicate measurements were performed within one assay run for each of 10 sera that were serially diluted in non-IgE-containing serum six to seven times. Specimens were selected that contain varying amounts of IgE antibody in undiluted serum, which spanned the linear range of the assay. Two concerns should be considered in light of recently commercialized calibration systems that decrease to 0.1 kIU/L. It may not be reasonable to expect allergen-specific IgE antibody assays to demonstrate linearity (with interdilutional coefficients of variation $< 20\%$) over a 1000-fold range in IgE antibody concentration (from 0.1 kIU/L to 100 kIU/L). Moreover, the investigator evaluating an assay may "rig" the dilution scheme with many closely spaced dilution intervals, especially in the mid-range of the assay,

Appendix B. (Continued)

to obtain evidence of ideal assay linearity. Dilution schemes should span the assay's working range from the analytical sensitivity to the maximum reported concentration of the assay.

In the parallelism-linearity study, the interpolated dose in IU/mL (Y-axis) is plotted as a function of the dilution factor (1/dilution factor; X-axis). The slope of each curve and the interdilutional coefficient of variation (SD/mean) of the interpolated doses at each serum dilution are computed as measures of assay parallelism. Each data point represents the mean IU/mL estimate of IgE antibody from duplicate measurements performed within the same assay for a single serum dilution. The illustrative assay in Figure B1 demonstrates interdilutional CVs < 15% over the linear range of the assay from 1 IU/mL to 80 IU/mL of IgE antibody. This assay demonstrates parallelism and confirms the absence of matrix effects in total serum IgE and IgE antibody components of the assay. This analysis can be performed on both semiquantitative and quantitative IgE antibody assays; however, it is specifically designed to demonstrate the ability of quantitative assays to generate the same interpolated result, irrespective of the dilution of the test sample analyzed or the region of the calibration curve used for interpolation.

Procedure

1. *Sample preparation:* A minimum of three test sera containing the analyte of interest are required for this analysis. Each test serum is first diluted at least four, and preferably five times (two- or threefold scheme) with an appropriate “zero” calibrator diluent using gravimetric or volumetric monitoring with calibrated pipettes. At least one of the dilutions should be in the “low” level range (2.5 to 5 times background). Each dilution should be prepared independently. In this procedure, serial dilutions should be prepared that are evenly spaced at twofold concentration differences, which cover the entire usable range of the calibration curve, with the lowest value being less than the lowest calibrator but higher than the minimum detectable dose as defined by methods described in Appendix E. IgE antibody concentrations that exceed the dynamic range of the calibration curve may also be included to examine the plateauing effects at high antibody levels.
2. *Analysis and Computations:* All undiluted and diluted test specimens are analyzed in the assay in random order, with the undiluted samples being run three times in different positions throughout the assay to ensure the accuracy of the results. All specimens should be assayed using a minimum of four replicates each. The interpolated results can be plotted as a function of the reciprocal of their dilution as demonstrated in Figure E1. Alternatively, expected results can be computed with the knowledge of the concentration in the undiluted specimen and the dilution factors. Once the interpolated results are obtained for the serum dilutions, the “percent recovery” can be calculated by dividing the observed mean result by the expected result for each dilution. Conversely, each observed mean can be multiplied by the appropriate dilution factor and an interdilutional %CV can be computed. A graph of the observed (Y-axis) vs expected (X-axis) results can be plotted, and linear regression analysis can then be used to determine the extent of linearity, parallelism, and high-end and low-end plateauing. Statistical analyses can be used to determine if the regression coefficient is within the 95% confidence limits expected based on chance alone; this confirms parallelism.
3. *Interpretation:* The most difficult issue associated with dilution-recovery studies is defining what performance is acceptable. For total serum IgE assays, a single analyte is measured, and thus expectations for recovery and linearity are high (eg, < 15% interdilutional %CVs). For allergen-specific IgE antibody assays that measure IgE antibody to multiple allergen epitopes, from a single allergen source material, the targets for recovery studies become less clear. Quantitative IgE antibody assay interdilutional %CVs below 20% can be considered acceptable. Many investigators question whether dilution-recovery studies are appropriate for evaluation of semiquantitative assays. Because the reference systems in these assays are not calibrated and they do not claim to be quantitative, nonlinearity is expected; reproducibility of the bias, rather than absolute parallelism, may be considered the most useful target end point of the analysis.

Appendix B. (Continued)*Minimally Required Reagents*

The following reagents are minimally required:

Finalized IgE antiallergen assay:

- Test sera containing IgE antibody specific for the allergen of interest (three minimum).
- Zero serum calibrator containing no allergen-specific IgE (sufficient for dilutions).
- Volumetric calibrated pipettes.
- Analytical balance to monitor dilutions gravimetrically.

Appendix C. Qualification of Antihuman IgE Reagents by Direct Binding Dilutional Analysis and Competitive Inhibition Immunoassay

This procedure analytically documents that an antihuman IgE reagent used in the detection of IgE antibodies of defined allergen specificity has a restricted specificity for human IgE, and exhibits no detectable cross-reactivity with human IgG, IgM, IgA, and IgD antibodies or other human or animal serum proteins that might be present in the sample or diluent used in the final analyses. There are two general approaches to the evaluation of the specificity of an antihuman IgE reagent: direct binding and competitive inhibition studies.

General Approaches

I. *Direct binding studies* test the ability of a prospective unlabeled antihuman IgE antiserum or a finalized labeled antihuman IgE reagent to bind to a solid phase to which potentially cross-reactive, purified analytes (eg, human IgG1-4, IgA1-2, IgD, and IgM) are bound. Each purified protein used in these analyses should be validated first using established immunochemical tests for its purity, the presence of the appropriate proteins of all relevant allotypes and subclasses, where appropriate, and the complete absence of human IgE. Each of these proteins is then individually insolubilized on one of several possible solid phases (microtiter plates, fine carbohydrate particles, sponge, cellulose thread, cellulose paper disks, or plastic beads). The chemistry used for coupling and the solid-phase material selected should reflect, as closely as possible, those used in the final design of the IgE assays. Results of direct-binding studies may vary as a function of the response level (eg, 50% binding or 25% binding) used to determine the cross-reactivity due to possible nonparallelism of the binding curves.

In the direct binding studies, 10 dilutions of the unlabeled or labeled antihuman IgE reagent (bracketing the proposed final working strength) are added to multiple tubes or wells in triplicate, each containing a predefined amount of either solid-phase human IgE or solid-phase non-IgE antigens. Minimally, these solid-phase non-IgE preparations should include human IgG (all four subclasses and a maximal number of Gm allotypes), human IgA (IgA1, IgA2m[1] and IgA2m[2]), human IgM, and human IgD. The amount of solid-phase antigen should be selected to ensure molar excess of the antigen to optimally test the binding of the antihuman IgE while also minimizing nonspecific binding to the solid phase. The reaction conditions (time, temperature, diluent, pH, agitation) should mimic, as closely as possible, the expected use of the labeled antihuman IgE in the finalized assay. Once the incubation period is complete and unbound anti-IgE is separated from bound anti-IgE with the appropriate number of buffer washes, then bound anti-IgE is detected. If unlabeled anti-IgE is used, it must be detected with an appropriately labeled second antibody specific for the primary antibody species. For labeled anti-IgE, the appropriate detection reagents are added (eg, substrate and stopping reagents for enzymes) in a manner that reflects its projected use in the finalized assay. The response signal detected in the non-IgE-solid-phase portion of the assay is then interpolated from the response signals measured with the IgE-solid-phase portion of the assay. A relative estimate of reactivity as a function of the highest concentration of the antihuman IgE binding to solid-phase human IgE is obtained. Percentage cross-reactivity is then computed using the following formula:

$$\% \text{ Cross-Reactivity} = \left[1 - \frac{(D\text{-IgE} - D\text{-nonIgE})}{D\text{-IgE}} \right] \times 100$$

where:

D-IgE = the dilution (relative concentration) of the anti-IgE that produces a 25% maximal response when binding to solid-phase IgE.

D-nonIgE = the dilution (relative concentration) of the anti-IgE that produces a 25% maximal response when binding to solid-phase non-IgE.

Appendix C. (Continued)

Minimally Required Reagents

- Antihuman IgE; either conjugated (I-125, enzyme or fluorophor labeled) or unlabeled
- Purified IgE solid-phase human
- Purified IgG solid-phase human (all four subclasses, all Gm allotypes possible)
- Purified IgA solid-phase human (both subclasses, both allotypic forms of IgA2)
- Purified IgM solid-phase human
- Purified IgD solid-phase human
- Soluble human IgE (chromatographically or affinity purified, myeloma or polyclonal, purity 99%)
- Basic assay reagents, supplies, and equipment.

II. *Competitive inhibition studies* involve the analysis of individual specimens, each containing high levels of separated, potentially cross-reactive analytes (human IgG, IgA, IgD, and IgM) and nondetectable levels of human IgE. In contrast to the direct binding studies, the non-IgE proteins are generally added to a human serum matrix that has been stripped of all immunoglobulins. The principle of this assay involves the evaluation of the ability of non-IgE proteins to competitively inhibit the binding of either unlabeled or labeled antihuman IgE to solid-phase human IgE.

In the competitive inhibition analyses, 10 dilutions of each soluble inhibitor protein, including human IgE and non-IgE proteins, are added to multiple tubes or wells in triplicate, each containing a predefined amount of antihuman IgE and solid-phase human IgE. Minimally, the soluble inhibitor proteins should include human IgG (all four subclasses and a maximal number of Gm allotypes), human IgA (IgA1, IgA2m[1] and IgA2m[2]), human IgM, and human IgD. As in the case of the direct binding studies, the amount of solid-phase IgE should be selected to ensure molar excess of the antigen to optimally test the binding of the antihuman IgE, while also minimizing nonspecific binding to the solid phase. The reaction conditions (time, temperature, diluent, pH, agitation) should mimic, as closely as possible, the expected use of the labeled antihuman IgE in the finalized assay.

(A) In a *sequential addition format*, the soluble proteins are preincubated with the antihuman IgE reagent before the mixture is added to the solid-phase IgE.

(B) In the *simultaneous addition format*, the soluble inhibitor and antihuman IgE are added simultaneously to the solid phase IgE.

Once the incubation period is complete and unbound antihuman IgE is separated from bound anti-IgE with the appropriate number of buffer washes, then bound anti-IgE is detected. Unlabeled bound anti-IgE should be detected with an appropriately labeled antibody specific for the species of the animal that produced the IgE. For labeled anti-IgE, the appropriate detection reagents are added (eg, substrate and stopping reagents for enzymes) in a way that reflects its projected use in the finalized assay. The response signal detected in the non-IgE inhibitor portion of the assay is then interpolated from the response signal curve measured with the human IgE inhibitor portion of the assay. A relative estimate of reactivity as a function of the concentration of soluble IgE required to produce 10% or 25% inhibition of the antihuman IgE binding to solid-phase human IgE is computed. Percentage cross-reactivity is then computed as follows:

$$\% \text{ Cross-Reactivity} = \frac{C\text{-IgE}}{C\text{-nonIgE}} \times 100$$

where:

C-IgE = the concentration of soluble IgE that produces a 25% inhibition of anti-IgE binding to solid-phase IgE.

Appendix C. (Continued)

C-nonIgE = the concentration of soluble non-IgE proteins that produce a 25% inhibition of anti-IgE binding to solid-phase IgE.

Minimally Required Reagents

- Antihuman IgE; either conjugate (I-125, enzyme or fluorophor labeled) or unlabeled
- Soluble human IgE (chromatographically or affinity purified, myeloma or polyclonal, purity 99%)
- Soluble human IgG, all four subclasses, all Gm allotypes possible (chromatographically or affinity purified, myeloma or polyclonal, purity 99%)
- Soluble human IgA that contains IgA1 and IgA2, and both allotypic forms of IgA2 (chromatographically or affinity purified, myeloma mixture or polyclonal, purity 99%)
- Soluble human IgM (chromatographically or affinity purified, myeloma or polyclonal, purity 99%)
- Soluble human IgD (chromatographically or affinity purified, myeloma or polyclonal, purity 99%)
- Purified solid-phase human IgE
- Basic assay reagents, supplies, and equipment.

Additional Considerations for Specificity Analysis of the Antihuman IgE Reagent

- The within-run precision of the assay should be considered when determining the appropriate number of replicates for use with both immunoglobulin spiked and nonspiked specimens.
- In the case of two-site immunometric assays that use a capture and detection antibody pair to quantify total serum IgE, the specificity of both the capture and labeled antibodies should be evaluated together in the configuration of the actual assay using the soluble human IgE, IgG, IgM, IgA, and IgD in a serum matrix as unknowns. If a mixture of monoclonal and/or polyclonal antibodies is to be used in a final assay configuration, each mixture lot should be treated as a single reagent.
- Using hymenoptera venom as an example, venom immunotherapy can produce IgG antibody levels exceeding 50 µg/mL. Assuming a worst-case scenario of 50 µg/mL of IgG antibody, a 0.001% cross-reactivity of antihuman IgE with IgG would result in the detection of 0.5 ng of IgG antibody as IgE. In research assays, the analytical sensitivity of venom IgE assays has been identified as approximately 1 ng/mL. Thus, a 0.001% cross-reactivity of the antihuman IgE reagent with human IgG could result in a 33% overestimation in a worst-case scenario. This computation does not take into account possible additional underestimation of IgE antibody as a result of interference caused by competitive inhibition of IgG antibody in the IgE antibody assay.

Appendix D. Qualification of Allergen-Containing Reagents

These procedures validate the specificity of the allergen, and stability, nonspecific binding, and binding capacity of commercial allergen-containing reagents supplied for allergen-specific IgE measurements. The competitive inhibition assay design is most commonly used to evaluate the specificity of the allergen-containing reagents.

Specificity Analysis of Allergen-Containing Reagents (Identity Test)

In general, allergen extracts, or purified allergens, are diluted into allergic patient sera and then evaluated for their ability to “inhibit” the binding of highly positive, monospecific human IgE antibody to the allergen-containing reagent. In addition, documented monoclonal and polyclonal animal antisera with restricted specificity for potentially contaminative allergens may be used as the antibody source. At least five dilutions of the allergen extract are prepared at total protein concentrations typically ranging from 1 µg/mL to 100 µg/mL. A control diluent alone is used as the “0,” or uninhibited negative control, and three irrelevant allergen extracts are analyzed at the same protein concentration as “sham” controls. Each of these extract dilutions is mixed 1:1 with the human serum pool containing IgE antibody specific for the allergen of interest and the three irrelevant allergen extracts. Following a preincubation for four hours at room temperature (or one hour at 37 °C), the allergen-containing reagent is added and the remainder of the IgE assay is performed as per the manufacturer’s recommendations. The percentage inhibition is computed in quantitative assays for each extract condition as follows:

$$\frac{C - A}{C} \times 100$$

where:

C = mean IgE antibody concentration in units for the patient specimen mixed with diluent alone, and

A = mean IgE antibody concentration in units for the patient specimen mixed with each of the allergen extracts.

Alternatively, for semiquantitative assays, changes in the signal measured can be used to compute the percentage inhibition with increasing concentrations of extract added. Finally, the IgE antibody results may be plotted as percent inhibition on the Y-axis and allergen concentration on the X-axis to determine a reference point, such as 50% inhibition, which can be used to compare the specificity of each allergen-containing reagent as evaluated with the different extracts. Because the ultimate goal of this procedure is to monitor the specificity of multilots of an allergen-containing reagent of a particular specificity, the same lot of extract and IgE-containing serum pool should be used over time.

Minimally Required Reagents for Specificity Analysis

- Solid-phase allergen
- Individual human sera or a human serum pool containing IgE antibody with defined allergen specificity
- Monoclonal and polyclonal IgE antiallergen animal antisera
- Antihuman IgE conjugate (I-125, enzyme or fluorophor labeled)
- Serum diluents

Appendix D. (Continued)

- Soluble allergen extracts (allergen of interest, three irrelevant extracts from different allergen groups as sham controls).

Interlot Reproducibility, Nonspecific Binding, and Binding Capacity Testing of Allergen-Containing Reagents

The same competitive inhibition analysis can also be used to evaluate changes in potency of a single specificity of allergen-reagents between lots (batches) or as a function of storage under differing conditions to validate stability. In addition, the manufacturer, and the user to a more limited extent, can evaluate general performance (binding capacity, nonspecific binding, immunoreactivity, or potency) by analyzing a panel of control sera from clinically documented subjects with a defined allergy to the allergen in question. The level of binding or resultant IgE antibody estimates (in classes or units) with different sera run serially at five dilutions can provide a means of evaluating changes in the allergen-containing reagent's nonspecific binding and binding capacity (see Appendix B).

Alternative Qualifying Tests for Liquid Phase Allergen-Containing Reagents

With the newer, liquid-phase allergen reagents, other immunochemical qualification tests are possible. For instance, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can provide an indication of changes in the protein composition, even though this is an insensitive test for identifying changes in the allergen content of allergen-containing reagents. Alternatively, the quantity of allergenic components can be quantified by immunodiffusion methods or evaluated by size exclusion or ion exchange chromatography. These procedures may be considered supplemental and can usually be performed only by the manufacturer as additional quality control tests. These tests cannot be performed on insolubilized allergens that have been immobilized onto a solid phase.

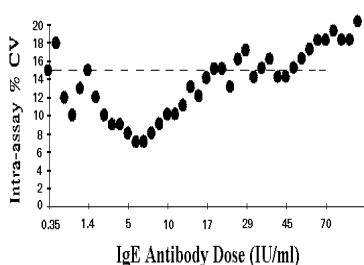
Stability Testing

As part of product labeling, the manufacturer should state the stability characteristics and the expiry date of allergen-containing reagents. Allergens vary in their inherent stability and allergen-containing reagents may be stored under inappropriate conditions, which may compromise the accuracy and consistency of the results being reported. The shelf-life, or the period of time during which the performance of the reagent is considered acceptable, should be based on accelerated, heat-stressed testing, and real-time testing of products in their final containers. Reconstituted stability testing should be applied to lyophilized reagents or reagents requiring dilution prior to use. In these cases, the stability characteristics of the reagent should be defined before and after reconstitution or dilution. For the determination of the stability of reagents after opening the primary container, stability should be defined using “on-board” conditions if used in conjunction with automated analyzers. Finally, stability studies should assess whether or not special shipping requirements are necessary because products may be exposed to elevated or sub-zero temperatures during transportation.

Appendix E. Precision Testing of Assays of IgE Antibodies

As part of the assay's final documentation, a manufacturer commonly analyzes up to 100 sera in 10 to 20 assays, which employ several lots of assay reagents. Replicate measurements can be used to compute the intra-assay coefficient of variation (eg, SD/mean) for a single assay run or the interassay variation among multiple assay runs. Assay variation observed for sera that are analyzed at different dilutions can provide an indication of parallelism in the assay. While the coefficient of variation in the assay's response variable (%CV_{response}) can be examined using a "response error relationship" analysis, the "*Precision Profile*" plot (see Figure E1) of the coefficient of variation of the reported dose estimate (%CV_{dose}), as a function of the dose, provides the best indication of systematic and random error variation that might be expected over the entire dynamic range of an assay. Separate precision profiles can be generated using replicate analysis of sera within an assay (intra-assay), between assay runs (interassay) that use different reagent lots (interreagent batch), and in assays that are run in different laboratories (interlaboratory). Precision profiles of two or more IgE antibody assays can be used to facilitate intermethod comparison.

Sera that are selected for use in precision profile analyses should contain IgE antibody levels that cover the entire reported dynamic range of the assay being evaluated. When precision profiles have sufficient data points at the proposed dynamic range extremes, a predefined interassay variation level can be used to define these linear range limits of the assay that minimize error associated with normal assay variation. A protocol in Appendix E provides a guide to the generation of these four precision profiles. The degree of assay variation (percentage coefficient of variation) is commonly used to judge assay performance at the assay's minimum detectable concentration, the midpoint of the linear range, and highest extreme of the dynamic working range (saturation point or plateau). A more detailed discussion of strategies for the assessment of assay precision is presented in CLSI/NCCLS guidelines EP05 and LA01.^{1,2}



IgE Antibody Dose (IU/mL)

Figure E1. Illustrative Precision Profile of an IgE Antibody Immunoassay. In this analysis, replicate measurements were performed within one assay run for each of 40 sera that contain IgE antibody levels, which span the linear range of the assay from 0.35 IU/mL to 80 IU/mL. The intra-assay coefficient of variation (SD/mean) of the interpolated dose as a percentage (Y-axis) is plotted as a function of the mean level of antibody in the specimen (X-axis). Each data point represents the mean CV of replicate measurements within the same assay for a single serum. The assay demonstrates intra-assay CVs < 15% from 1 IU/mL to 40 IU/mL of IgE antibody.

The linear range of an immunoassay is its functional range over which concentrations of an analyte can be accurately measured with a desired precision. The error envelope along the span of the calibrator's dose-response curve is generated during a precision profile analysis, and can be used to define statistically the limit of detection (lower limit) and saturation plateau (high limit) of the dynamic range of the assay. An assay's linear range reflects the range within which an assay can deliver accurate results. Analyte results outside the dynamic range of the assay are not analytically reliable. The dynamic range is dependent on an assay's capacity to present IgE binding epitopes in sufficient molar excess to available antibody and its ability to avoid nonspecific binding. Taken together with the assay precision, these variables define the applicable dynamic range that can be reproducibly demonstrated with multiple lots of reagents.

Appendix E. (Continued)

Determination of an assay's linearity from its precision profile analysis is illustrated in Appendix E for an allergen-specific IgE assay.

This procedure documents the intra-assay, interassay, and interreagent batch variation through repetitive analysis of quality control sera.

Intra- and Interassay Variation

Tri-Level Control Approach: Prepare three serum pools that contain low, moderate, and high levels of IgE antibody specific for the allergen specificities of interest. The levels of IgE antibody in these serum pools may be operationally defined as 2.5 to 5 times (low), 5 to 20 times (moderate), and greater than 20 times background (high). If the high serum pools are not available, testing should proceed with at least the low and moderate controls. Analyze them as routine unknowns in a total of 20 assays in triplicate over a period of at least 10 days. Perform an analysis of variance to compute the mean and standard deviation of the measurements for each of the triplicate measurements of each control (intra-assay) and for the three sera analyzed in the 20 sequential assays (interassay). Compute the coefficient of variation as $\%CV = (SD/mean) 100$. Finally, tabulate this information and present it as an indication of variation within the assay and between assays.

Imprecision Profile: For quantitative assays, a more rigorous approach called the imprecision profile may be used to evaluate the variation of the assay over small increments throughout the linear or working range of the assay. In this evaluation, 20 individual sera that incrementally span the limits of the reference curve are analyzed in 10 assays in triplicate over a period of 30 days using the same lot of reagents. From these data, the coefficient of variation is computed as the $\%CV = (SD/mean) 100$, and the percentage coefficient of variation is plotted on the Y-axis vs the patient sample IgE antibody concentration estimate in units on the X-axis. This plot is the imprecision profile and it allows the manufacturer to identify low and high extremes of the linear range to still permit the user to achieve a predefined target intra- and interassay variation (eg, 5, 10, or 15% CV). It also provides a clear indication of change in variation as a function of the concentration of the IgE antibody. Imprecision profile analysis can also be performed with semiquantitative assays, such as those that use a modified scoring system. In this case, signal measurements (normalized or adjusted counts) are used instead of antibody concentration units to compute assay coefficients of variation.

Interreagent Batch Variation

To determine the variance between different batches of assays, a minimum of three lots of assays are evaluated and a minimum of three assays per lot are analyzed, each in triplicate with at least three control sera that contain IgE antibody in low, moderate, and high levels (see trilevel control approach discussed previously for suggested levels of IgE antibody). To evaluate the overall allergen epitope retention between lots, 10 sera, from clinically documented persons, which contain the appropriate allergen-specific IgE, are evaluated in two runs for each assay being evaluated. While an interreagent batch imprecision profile can be computed, rarely are sufficient data points collected along the entire linear range of the assay to derive meaningful conclusions. Thus, the coefficients of variation are best reviewed in a tabular format for evidence of shifts and trends.

Appendix E. (Continued)

Minimally Required Reagents

The following reagents are minimally required:

- Total serum IgE assay or IgE antibody assay
- At least three human sera that contain IgE antibody to the defined allergen specificity
- For an imprecision profile, 20 sera from allergic patients with the desired IgE antibody specificity.
- For interbatch variation, at least three different lots of assays.

Interdilutional variation is best evaluated in dilution-recovery analyses as described in Appendix B.

References for Appendix E

¹ CLSI/NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition*. CLSI/NCCLS document EP05-A2. Wayne, PA: NCCLS; 2004.

² CLSI/NCCLS. *Assessing the Quality of Radioimmunoassay Systems—Second Edition; Approved Guideline*. CLSI/NCCLS document LA01-A2. Wayne, PA: NCCLS; 1994.

Appendix F. Determination of the Limit of Detection for an IgE Antibody Assay

The detection limit of an assay is the lowest limit of the dynamic range that can distinguish an IgE antibody-induced response signal from background noise produced in the absence of any analyte. More specifically, it is the smallest quantity of an analyte that can be reproducibly and statistically distinguished from the variance of the background, or a zero calibrator, in a given assay system. It is usually defined at the upper limit of the 95% confidence interval of the mean level produced by the 0 calibrator based on at least 10 determinations in at least four assays run on different days. The detection limit has also been called the positive threshold of the assay. A relative assessment of detection limits can be performed by analyzing increasing dilutions (preferably prepared gravimetrically) of a serum sample and then calculating how far a sample can be diluted, until the signal becomes statistically nondistinguishable from the 0 analyte control.

This procedure determines the minimal detectable analyte concentration that can be measured by an IgE antibody assay. This analysis can be performed with semiquantitative and quantitative assays.

Procedure

Analyze 20 replicates of at least two negative serum controls or negative serum pools in five assays (100 measurements). Determine the mean, standard deviation, and define the point on the calibration curve that is 3.3 SD above the mean. This can be defined as the limit of detection of the assay, providing that the precision at this concentration is acceptable (eg, < 10%CV, see precision profile analyses, Appendix E).

Minimally Required Reagents

The following reagents are minimally required:

- Total serum IgE assay or IgE antibody assay.
- Negative serum controls. These specimens are defined as sera from clinical history and skin-test-negative subjects. The binding produced by the sera should not be inhibitable by preincubation with soluble antigen.
- Precision profile assessment of the assay (see Appendix E).

Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Consensus Comments and Subcommittee Responses

I/LA20-A: Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities; Approved Guideline

Appendix A. Allergen Nomenclature Addendum (Scientific Names and Codes of Allergens)

1. Why aren't Latin names listed for all allergens?
 - **The final document includes Latin names for each of the allergens where it is available.**
2. We received information from an organization that the allergen E91 is the number for parrot feathers. They refer to CLSI document I/LA20-A. According to the International Allergen List version 6.0, E91 is the number of lovebird droppings and E213 for parrot feathers. Can you provide the correct number for parrot feathers?
 - **These codes were corrected and were extracted from the current, up-to-date database presented by www.allergome.org.**

Appendix A, Table A-XI. Drug/Hormone Allergen

3. In the CLSI/NCCLS document I/LA20-A published in 1997, codes for allergens are provided. One of our customers says that the code for ampicillin used by Pharmacia is c5, but it is not in I/LA20-A. Does CLSI have a newer version? Is CLSI planning to make an addendum? We use CLSI as the authority when we make our codes for allergen analyses.

To give you an idea of what we are dealing with, see:

http://www.labinfo.dk/English/ifcc_iupac_uk.asp
<http://www.labinfo.dk/English/Documents/allergology.htm>

- **The final document includes Latin names for each of the allergens where it is available.**

Appendix C. Qualification of Antihuman IgE Reagents by Direct Binding Dilutional Analysis and Competitive Inhibition Immunoassay

4. Additional Considerations for Specificity Analysis of the Antihuman IgE Reagent section: Using Hymenoptera venom as an example, venom immunotherapy can produce IgG antibody levels exceeding 50 µg/mL (ref.). Assuming a worst-case scenario of 10 µg/mL of IgG antibody, a 0.001% cross-reactivity of antihuman IgE with IgG would result in the detection of 0.5 ng of IgG antibody as IgE. In research assays, the analytical sensitivity of venom IgE assays is identified as approximately 1 ng/mL. Thus, a 0.001% cross-reactivity of the antihuman IgE reagent with human IgG could result in a 33% overestimation in a worst-case scenario. This computation does not take into account possible additional underestimation of IgE antibody, as a result of interference caused by competitive inhibition of IgG antibody in the IgE antibody assay.
 - **The suggested correction was made. The reference to Section 9.6 was deleted.**

Summary of Delegate Comments and Subcommittee Responses

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

General

1. Clarification around the quality control requirement for the individual allergen IgE would be good.
 - **The quality control requirement that is unique to IgE antibody assays was consensus derived from manufacturers, because there was no original reference that provided this information.**
2. The recommendation to put the assay method, manufacturer, on every patient report is interesting. I am unaware of this practice for any other testing.
 - **This is a critical issue for reports containing IgE antibody data, because all IgE antibody assays provided different estimates of IgE antibody from any given human serum specimen. It is important for the clinician to know the method so he/she can effectively interpret the validity and meaning of the IgE antibody result. Moreover, IgE data produced by certain methods such as the Phadia system and not other methods can be used to define clinically relevant food allergy based on published predictive theory curves using Phadia data.**

Foreword

3. Last sentence: “This is a living document ~~that~~ is intended...”
 - **The sentence was rewritten.**

Section 1, Scope

4. Second bullet: “To define ~~the~~ reasons why...”
 - **The sentence was revised as suggested.**

Section 2, Introduction

5. Second paragraph, first sentence: “The diagnosis of human allergic diseases involves the combined use of a careful recorded clinical history...”
 - **The sentence was revised as suggested.**
6. Second paragraph, first sentence: Positioning of specific IgE as “confirmatory” sounds too passive. Is that really the consensus intention?
 - **Yes, this is the consensus of the subcommittee. See the response to comment 1.**

Section 4.1, Note on Terminology

7. Second sentence: “Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve ~~worldwide~~ uniformity.”
 - **The sentence was revised as suggested.**

Section 4.2, Definitions

8. Definition of “analyte,” first NOTE: Insert a comma after “property.”
 - **A comma was inserted as suggested in NOTE 2.**
9. Definition of “control/control material,” last sentence: “Used in Levey-Jennings” “should be used in (eg, Levey-Jennings).”
 - **The current form of the statement provides better clarity to readers by specifically stating the Levey-Jennings quality control chart.**
10. Definition of “detection limit/limit of detection,” first sentence: Change “variance of the background” to “background (including variation in background).”
 - **The sentence was revised as suggested in NOTE 1.**
11. Definition of “epitope/determinant,” last sentence: Allergen designations Der p 1 and Der f 1 should not be in italics.
 - ***Der p 1* and *Der f 1* were italicized for better clarity.**
12. Definition of “recombinant allergens,” first sentence: They are certainly not produced “*in vitro*.” Change to “recombinant allergen – allergenic molecule produced in a heterologous cell system using a recombinant gene construct encoding the allergenic protein; **NOTE:** A description...” The word “liable” further down should probably be something else, so please check.
 - **The sentence was revised to clarify this point, and “liable” was corrected to “labile.”**

Section 5, Biochemical and Clinical Properties of IgE

13. First sentence, Table 1: The paragraph refers to Table 1. The paragraph is on page 13 while Table 1 is on page 22. Insert Table 1 in closer proximity to the reference in the article.
 - **Table 1 was moved to the beginning of Section 5.**

Section 5.2.2, Physical Examination (now Postphysical Examination)

14. The title of the section is “Physical Examination.” Physical examination is discussed in Section 5.2.1, yet Section 5.2.2 is labeled “Physical Examination.” Section 5.2.2 discusses steps after the physical examination. Change the title of Section 5.2.2 to “Postphysical Examination.”
 - **The title was revised as suggested.**

Section 5.4.1, Interpretation of Allergen-Specific IgE Antibody Results

15. In our opinion, the discussion on “false positive” misses the most important aspect, namely the relationship between sensitization (immunology) and allergy (defined by symptom), and that IgE tests are not tests for allergy but for sensitization. For this reason, “clinically irrelevant IgE” and similar expressions should be avoided, because they do not take into account the view of allergy as an evolving disease.

This section should also bring attention to the significance of the negative test result for exclusion of an allergen from suspicion. Provided the test has high sensitivity, not only a positive test result is useful in the diagnostic workup.

- **Section 5.4.1.2, *Possible Causes of False-Positive Results* was revised to address this concern. The following clarification was added:**

“The presence of IgE antibody in the absence of clinically evident allergic disease emphasizes the important point that the allergen-specific IgE measurement is designed to provide confirmation of ‘sensitization’ (IgE antibody positivity) and not clinical disease. A concurrent clinical history is required to translate a positive IgE antibody measurement into a diagnosis of allergic disease. The IgE antibody measurement also aids the clinician in verifying the allergen specificity that led to the patient’s sensitization. When the IgE antibody result is negative, it can support the clinician’s decision that the allergen specificity is not involved in the patient’s allergic symptoms.”

16. First paragraph, second sentence: “This is the general interpretation that applies for all diagnostic *in vivo* and *in vitro* allergy tests that are based on the presence of allergen-specific IgE antibody.”

- **The sentence was revised as suggested.**

Section 5.4.1.2, Possible Causes of False-Positive or Clinically Irrelevant Results (now Possible Causes of False-Positive Results)

17. Fifth paragraph, first sentence: “Allergen-specific IgE can bind to certain carbohydrate structures on plant and insect glycoproteins, as well as peptide epitopes.”

- **The sentence was revised as suggested.**

18. Under the heading on *Cross-reactive carbohydrate determinants as a cause for irrelevant results*, the recently reported findings on allergic reactions caused by alpha-gal (galactose-alpha-1,3-galactose) should be included (Chung et al. *N Engl J Med*. 2008;358:1109-1117).

- **This reference was added and a comment related to it was inserted in Section 5.4.1.2 under the section titled, “Cross-reactive carbohydrate determinants.”**

Section 6.1.2, Storage Conditions

19. First paragraph, second sentence: The room temperature example is 21 °C to 23 °C. This range is very narrow and perhaps unrealistic. Change the room temperature range to 18 °C to 25 °C.

- **The temperature was revised as suggested in Section 6.1.2.**

Section 7.2.2, Alternative Assay Formats

20. First paragraph, first sentence: “performed...or home by unskilled individuals.” Delete “home” and “unskilled individuals” as when performed at the bedside or in clinics, the staff are usually trained in the use of the instrument; or use the definition of POCT from CLSI document AST04.

- **The sentence was revised as suggested.**

Section 7.2.3, IgE Antibody Immunoassay Classification Scheme

21. Table 3, Result Presentation, first box: Change to, “Reactive or nonreactive” and “positive or negative.”

- **The column was revised as suggested.**

Sections 8.2.1, Nomenclature of Allergen Source Groups; Section 12.1.2, Separate Product Information Sheet for Allergen-Containing Reagents; and Appendix A. Allergen Nomenclature Addendum (Scientific Names and Codes of Allergens) (and possible other sections)

22. The www.allergome.org website is a nonpeer-reviewed private enterprise website and is not responsible for any officially accepted allergen nomenclature. It cannot be regarded and accepted as a “master allergen compendium” (see page 54).

The WHO/IUIS Allergen Nomenclature Subcommittee is responsible for the peer-reviewed process of defining and naming of allergen molecules and is the official body that should be referenced in this document, not the Allergome website.

- **The last paragraph in Section 8.2.1 was revised to read:**

“With current use of allergen extracts and both native and recombinant allergenic molecules in IgE antibody assays, the allergen code system discussed above that involves a letter and an arbitrarily assigned sequential number has become imprecise. As an alternative for defining allergenic molecules, users of IgE antibody assays may refer to the International Union of Immunological Societies (IUIS) Nomenclature Committee’s website (www.allergen.org) to obtain an official allergenic molecule designation. These are listed in Appendix A. For instance, hevein, a potent allergen found in natural rubber latex (*Hevea brasiliensis*) is defined as “Hev b 6.02.” For allergenic extracts, the user may refer to an alternative independent website (www.allergome.org) for information and references that are organized by a unique numerical code that has been assigned for each allergen specificity (see Appendix A).”

Section 12.1, Specific Recommendations for Manufacturers of Allergen-Specific IgE Antibody Assay Kits and Reagents

23. Individualized allergen-specific product inserts provided with all available allergen-specific reagents would not be practically manageable for the manufacturers and end users. However, it is recommended for the manufacturers to provide allergen-specific information via leaflets and/or websites.

- **The text in Section 12.1.2 was revised to read:**

“Individualized product information leaflets may be supplied with the reagent or in a website, which provides a description of the common and scientific name of each of the allergen-containing reagents provided by the company.”

Section 12.2, Recommendations for the Diagnostic Allergy Laboratory

24. Second paragraph, second sentence: “errors caused by the technician” – This also may be a technologist or biochemist. Therefore, change to “laboratory professional.”

- **“Technician” was replaced with “laboratory professional.”**

Section 12.3, Recommendations for the Medical Professional

25. Fifth paragraph, fourth sentence: “There are occasions in North America...” – Change “North America” to “the United States,” as this statement does not necessarily apply to Canada.

- **The sentence was revised to read: “There are occasions in some countries where medical professionals are required to send their patient’s serum to a capitated or so-called in-network laboratory that is dictated by the patient’s medical insurance policy.”**

Appendix A. Allergen Nomenclature Addendum (Scientific Names and Codes of Allergens)

26. See the comment above regarding the Allergome website; thus, the last sentence of the text on page 54, “This compendium represents a subset of the master allergen compendium available at www.allergome.org” should be deleted.

- **The sentence was deleted as suggested, and IUIS Nomenclature Codes were inserted in Appendix A.**

27. In Table A1, the specific names of the different manufacturers must be given as “assay descriptors” and not technology-based descriptions as biotin allergen-based, disc based, etc. Worldwide, there are several manufacturers using disc technology and biotin-based technologies and they do not necessarily use the same allergen codes as the companies actually contributing in Appendix A. If this list (Table A1) is able to fulfill its purpose to provide correct and useful information both to end users and manufacturers, the true source of

allergen codes/names must be given in the table. Thus, the names of the actual contributing manufacturers or the brand names of the products must be given, as was also done in the previous version of I/LA20.

- **To keep the document balanced in all aspects, CLSI policy strictly prohibits stating manufacturer/brand names of any products.**

28. Appendix A, allergen list, Table A1: Comments below relate to ImmunoCAP allergen designations (ie, in the present draft list under Sponge-Based Systems).

Page 60, a24: This is the designation for ISAC Amb a 1 (Chip-based technology), so move the X to the correct column.

- **Appendix A was updated to add the IUIS Nomenclature Codes and reflect all recommendations on a new allergen at the time of document development.**

29. Page 68 – Add new allergen:

d204 Der f 2 *Dermatophagoides farinae* Allergome code: 302 RR

- **See the response to comment 28.**

30. Page 80, f24: Change *Pandalus borealis* to *Pandalus borealis*, *Penaeus monodon*, *Metapenaeopsis barbata*, *Metapenaeus joyneri*.

- **See the response to comment 28.**

31. Page 82, f76: Change to Bos d 4, alpha-lactalbumin.

- **See the response to comment 28.**

32. Page 82, f77: Change to Bos d 5, beta-lactoglobulin.

- **See the response to comment 28.**

33. Page 83, f78: Change to Bos d 8, casein.

- **See the response to comment 28.**

34. Page 83, f79: Delete “(Tri a gluten).”

- **See the response to comment 28.**

35. Page 93, f323: Change to Gal d 3, conalbumin.

- **See the response to comment 28.**

36. Page 93, f334: Change to Bos d lactoferrin.

- **See the response to comment 28.**

37. Page 96, f416: Change to Tri a 19.01010, Omega-5 Gliadin.

- **See the response to comment 28.**

38. Page 102, c260: Correct the spelling of Quaternary ammonium, Morphine.

- **See the response to comment 28.**

39. Page 108, k87: Change to Asp o 21, alpha-amylase.

- **See the response to comment 28.**

40. Page 108, k201: Change to Car p 1, Papain.

- **See the response to comment 28.**

41. Page 108, k202: Change to Ana c 2, Bromelain.

- **See the response to comment 28.**

42. Page 108, k203: Change to Api m 1, Phospholipase A2.

- **See the response to comment 28.**

43. Page 109, k208: Change to Gal d 4, Lysozyme.

- **See the response to comment 28.**

NOTES

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 the most current edition of CLSI/NCCLS 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:

Documents & Records Organization Personnel	Equipment Purchasing & Inventory Process Control	Information Management Occurrence Management Assessments—External & Internal	Process Improvement Customer Service Facilities & Safety
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I/LA20-A2 addresses the QSEs 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 & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
					X C24 C28 EP05 EP06 EP10 EP17 H26 I/LA30 M29			EP10	H26		M29

Adapted from CLSI/NCCLS 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/NCCLS 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/LA20-A2 addresses the clinical laboratory path of workflow steps 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.

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
					X			

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

Related CLSI Reference Materials*

- C24-A3** **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.
- C28-A3** **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition (2008).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- EP05-A2** **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.
- EP06-A** **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (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.
- EP10-A3** **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition (2006).** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.
- 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.
- H26-A** **Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers; Approved Standard (1996).** This document addresses performance goals for analytical accuracy and precision for multichannel hematology analyzers; the relationship of these goals to quality control systems and medical decisions; and recommendations for minimum calibrator performance and the detection of measurement errors.
- I/LA30-A** **Immunoassay Interference by Endogenous Antibodies; Approved Guideline (2008).** 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 are addressed along with assessment of methods used to eliminate interference.
- LA01-A2** **Assessing the Quality of Radioimmunoassay Systems—Second Edition; Approved Guideline (1994).** This document contains definitions, procedures, and related information on receptors, radiolabeled analytes, separation reagents, and calibrators for properly assessing radioimmunoassay systems.
- 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.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

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Southern Health Care Network
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Touro Infirmary (LA)

Tri-Cities Laboratory (WA)

Tri-City Medical Center (CA)

Trident Medical Center (SC)

Trinity Medical Center (AL)

Tripler Army Medical Center (HI)

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