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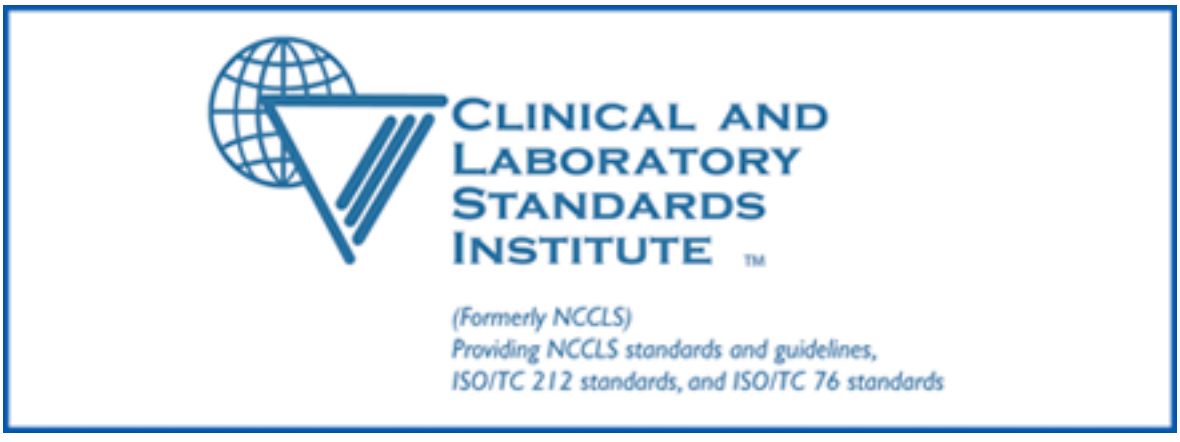
Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities; Approved Guideline



This document provides guidance for the design, analytical performance, standardization, and quality assurance of laboratory assays used in the measurement of total serum IgE and IgE antibodies of defined allergen specificity.

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I/LA20-A
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EFFECTIVE SEPTEMBER 2001

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Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities; Approved Guideline

Abstract

Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities; *Approved Guideline* (NCCLS document I/LA20-A) is written for both laboratorians (users) and manufacturers (producers) of IgE antibody assay reagents and kits. The guideline summarizes the current state of technology by describing the immunoassay configurations of currently used clinical IgE antibody assays, biological specimens that are routinely tested, and practical methods for the evaluation of component reagents. Emphasis is placed on nomenclature and methods for evaluating the specificity of allergen-containing assay reagents and human, IgE-specific, immunologic reagents. Strategies for a unified calibration scheme among IgE antibody assays are discussed with a focus on the pros and cons of the homologous and heterologous interpolation methods. Procedures are included for evaluating assay precision, analytical accuracy, sensitivity, parallelism, and interference. Guidelines for supplier validation and quality assurance, intra laboratory quality control practices, and interlaboratory proficiency testing are discussed within the context of practical procedures. Finally, performance targets are outlined with recommendations for both the manufacturer and diagnostic allergy laboratory.

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Contents

Foreword

Analytical methods employed in the diagnostic allergy laboratory are evolving as a result of improvements in reagents and advances in technology. New developments in immunological assays for IgE antibodies of defined specificity have been occurring, however, without the benefit of a consensus document prepared by the user, manufacturer, and regulator that defines both the current status of the technology and target goals for future reagent validation and quality control, assay calibration, and overall quality assurance. To date, the absence of such a consensus guideline has resulted in the production of assays that generate IgE antibody results of possibly unclear or misleading specificities and in nonstandardized units that cannot be compared among the available commercial methods. This can cause difficulty for the laboratory worker who must select among reagents, perform the assay, and demonstrate proficiency in intra laboratory and interlaboratory quality assurance programs. Moreover, the diagnostic kit manufacturers have no official benchmark targets for the validation and improvement of existing assays and development of future methods. This document examines issues that relate to the specimen, reagent validation and quality control testing, calibration systems, intra- and interlaboratory quality assurance, and areas for future development by manufacturers. While this document focuses on assays that assist in the diagnosis of type I hypersensitivity reactions in humans, the overall approach used in this document is intended to form the basis for guidelines that relate to the design, calibration, evaluation, and quality assurance of diagnostic antibody assays used in other subspecialities of clinical immunology.

Universal Precautions

Because it is often impossible to know which might be infectious, all patient blood specimens are to be treated with universal precautions. Guidelines for specimen handling are available from the U. S. Centers for Disease Control and Prevention [*MMWR* 1987;36(suppl 2S):2S–18S]. NCCLS document M29, *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, deals specifically with this issue.

Key Words

Allergen, allergy, assay methods, IgE antibody of defined specificity, performance evaluation, quality assurance, type I hypersensitivity.

Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobin E (IgE) Antibodies of Defined Allergen Specificities; Approved Guideline

1 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 they injected serum from an allergic person intradermally into the skin of an unsensitized (nonallergic) person, followed 24 hours later by injection of specific antigen into the same skin site.[16] This passively transferred allergic or *PK reaction* reached a maximum within 10 minutes, persisted for about 20 minutes, and then gradually disappeared. The antibody responsible for this reaction was shown to be thermolabile, losing its sensitizing activity after heating serum at 56 °C for several hours. In 1967, this antibody was identified as a fifth human immunoglobulin isotype and designated IgE.[17, 18]

The diagnosis of human allergic diseases involves the combined use of a careful clinical history, physical examination, and in vivo and in vitro assay methods for the detection of IgE antibodies of defined allergen specificities in tissue or serum, respectively. Over nearly a quarter century, the clinical allergy laboratory has played a key role in facilitating the diagnosis of human allergic diseases by using commercially available kits for total IgE and allergen-specific IgE antibody. Commercially available assays have continued to improve in analytical sensitivity and reproducibility through automation and improved reagent quality. These technical advances have permitted IgE antibody assays to approach a degree of quantitation and interlaboratory standardization comparable to the best clinical assays for human antibodies of other isotypes and specificities. With new IgE antibody assays and calibration schemes, however, have come antibody results that are reported in different units that have little in common with each other. Moreover, 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 has increased confusion as to actual allergen specificities that are available (see Appendix A). Increased regulations have also placed new demands on manufacturers, regulators and users of total and allergen-specific IgE antibody kits to document the quality of their assays more effectively. This guideline was written with the goal of coming to a consensus on these issues and creating two sets of operationally achievable procedures, one that can be used by all 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 maximize the quality of reported IgE antibody results.

NOTE: In the United States, the Clinical Laboratory Improvement Act of 1988 (CLIA '88) also sets the stage for reclassification of diagnostically useful antibodies into the group of regulated analytes. Due to their advanced degree of development and the undisputed utility of IgE antibody measurements as an aid to the diagnosis of allergic diseases, assays for IgE antibodies of defined allergen specificities are poised to be among the first group of antibody assays that will be rigorously scrutinized when human antibodies reach a regulated analyte status.

2 Scope

This proposed guideline provides a basis for defining the achievable state of serological assay technology that will be used by clinical laboratory workers to measure total serum IgE and future IgE antibodies of defined allergen

specificities. The focus of this document is limited to reagent technology, assay design, and methods used in the validation and quality assurance of component anti-IgE and allergen reagents and the finally configured assays. Attention is given to allergen nomenclature and methods used in qualifying allergenic protein mixtures, which are used as primary reagents to determine the specificities of IgE antibodies, which are detected in each assay.

While the needs of the clinical laboratory worker (user) and manufacturer (producer of products) are only partly overlapping, this guideline is designed to serve both groups. The primary working thesis is that the user need not repeat extensive quality assurance of assay components once they pass qualification procedures at the manufacturer level as outlined in this guideline. Therefore, this guideline emphasizes operationally achievable qualification practices that can be employed by the manufacturer to validate the quality and test the performance of component reagents and configured assay systems. I/LA20 also outlines alternative tests that can be used by clinical laboratory workers to supplement manufacturer testing and quality control practices with the ultimate goal of optimizing overall quality assurance.

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 measure total human serum IgE and human IgE antibody of defined allergen specificities.

- To define the current state of allergen-specific IgE assay calibration and outline a calibration strategy that all manufacturers can adopt to offer the extrapolation of their assay results in a common unitage.

- To define the limits to which results from different IgE antibody assays may be cross-compared.

- To outline practical manufacturer, intra-laboratory, and interlaboratory quality control measures that can be incorporated into an overall quality assurance scheme to ensure valid and reproducible total serum IgE and IgE antibody measurements.

Total serum IgE assays are used 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 serum 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 antibodies is effectively demonstrated by multiple early research and clinical studies.[2–5] For this reason, *a review of the clinical utility of serum IgE antibody measurements in the diagnosis and management of human allergic diseases is excluded from this guideline*. The primary goal of this document is to provide the reader with unambiguous guidelines as to how component reagents are validated and configured into optimized, clinically useful 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.

3 Definitions

The definitions used in this document were obtained from dictionaries, NCCLS guidelines, and other standardization documents as specified in this section. Many of the generic definitions are modified to relate to assays of IgE.

General medical terms described in the glossary have been obtained from *Dorland's Illustrated Medical Dictionary*. The majority of the terms in this glossary have been extracted from NCCLS document NRSCL8—*Terminology and Definitions For Use in NCCLS Documents.* Comments linking each definition to IgE antibody assays are provided when appropriate. Although NCCLS documents generally use units that are fully acceptable within the Système International d'Unités (SI), these do not always coincide with the units recommended by the International Union of Pure and Applied Chemistry (IUPAC) and by the International Federation of Clinical Chemistry (IFCC) for reporting results of clinical laboratory measurements. NCCLS documents also include the IUPAC/IFCC-recommended units of volume (L) and substance (molecular) concentration (mol/L) in parentheses, where appropriate.

Accuracy, Measurement accuracy// Result accuracy**,** *n* - Closeness of the agreement between the result of a measurement and a true value of the measurand ***(VIM93-3.5).*** **NOTE**: In the case of total serum IgE assays, the WHO IgE Standard 75/502[6,7] and the United States IgE Standard have been used as a primary reference material to promote 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. Several research-based secondary IgE antibody standards are available that have been calibrated in mass per volume units using antibody adsorption or depletion methods.[8]

Affinity Constant, K_a, n - **Immunology**, The equilibrium constant for the receptor + ligand reaction. **NOTES:** a) Strictly, the term only applies to homogeneous receptors and their ligands. Typically, however, polyclonal antibody preparations, which are heterogeneous in their affinity for homogeneous ligands, are used; b) the term also expresses the intrinsic binding strength of a receptor-ligand pair.

Affinity, n - **Immunology**, A measure of the attraction or force of association between a single antigenic site and a single antibody to that site.

Allergen, n - An immunogen that when introduced into an immunocompetent and predisposed host elicits the formation of IgE antibodies. Appendix A contains a list of allergens.

Allergen extract, n - A mixture of glycoproteins, lipoproteins, or protein-conjugated chemicals/drugs that have been solubilized from a defined (usually biological) source and that can elicit an IgE antibody response in exposed persons.

Allergen potency, n -The composite concentration of all of the allergenic epitopes within an allergen that together produce a defined biological (type I hypersensitivity) response in predisposed allergic persons.

Allergen source (raw) material, n - The starting raw material from which allergenic extracts are obtained. 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, and food stuffs.

Allergen reagent, n - The component of the IgE antibody assay that contains allergen either bound to a solid phase (immobilized allergen) or in solution phase (liquid allergen). These reagents, like the allergen extracts from which they are derived, are composed of heterogeneous mixtures of proteins or chemicals/drugs that are conjugated on carrier proteins. Ideally, this allergen reagent contains all of the allergenic epitopes that are recognized by persons who are predisposed to developing a type I hypersensitivity to the allergen of interest.

Allergosorbent, n - A solid phase material to which allergenic molecules are attached by covalent coupling methods or adsorption.

Analyte, n - The constituent or characteristic of the sample to be measured. **NOTE:** 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**: For the diagnostic allergy

laboratory, the IgE molecule may be considered the primary analyte of interest.

Antibody, n - **1)** Any of numerous Y-shaped protein molecules produced by B cells as a primary immune defense, each molecule and its clones having a unique binding site that can combine with the complementary site of a foreign antigen, as on a virus or bacterium, thereby disabling the antigen and signaling other immune defenses ***(RHUD2CD-)***; **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. **NOTES:** a) An antibody molecule is, by definition, monospecific, but it might also be *idiospecific, heterospecific, polyspecific*, or of *unwanted specificity*. It cannot be non-specific, except in the sense of nonimmuno-chemical binding; b) these proteins are immunoglobulins and bind by means of specific binding sites to a specific antigenic determinant.

Antigen, n - **Immunology,** Any substance that can stimulate the production of antibodies by an organism and combine specifically with them.

Atopy, n - The inherited tendency to develop immediate-type hypersensitivity to common and generally harmless substances.[9]

Avidity, n - The combined intensity of attraction of all antigenic sites on an antigen molecule and all antibodies to those sites. Avidity depends on the antibody isotype (valence), the size and conformation of the antigen molecules, and the number of antigenic epitopes.

Binding capacity, n - Within the context of this document, the binding capacity refers to the number of human IgE antibody molecules that an allergen-containing reagent (e.g., allergosorbent, liquid-phase allergen) can bind reproducibly under standardized assay conditions (pH, ionic strength, protein matrix, time, temperature). 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 reproducible manner.

Calibration material/calibrator, n - A material (e.g., solution) or device of known quantitative/qualitative characteristics (e.g., 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. **NOTES:** a) 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; b) the calibration material must be traceable to a national or international reference preparation or reference material when these are available; c) calibration materials with different amounts of analytes may be used to establish a calibration or response "curve" over a range of interest; d) the term “standard,” commonly used in clinical laboratory protocols and having there a meaning as described here, specific and different from use in the Federal Register, February 28, 1992 published in the United States); e) the terms “primary” and “secondary standard” are used by WHO and ISO to refer to calibration materials.

Calibrated assays, n - Three levels of calibrated IgE antibody assays are proposed. They are 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 (see semiquantitative assay, Section 6.2.2.2); **2)** calibrated titration assays, such as assays calibrated to a total serum IgE dose–response curve, which generate reliable, reproducible results, and behave consistently with respect to parallelism (see quantitative assays, Section 6.2.2.3); **3)** quantitative assays, which are not commercially available that are calibrated, homologous assays. They produce reproducible and accurate results, and behave appropriately with respect to parallelism and recovery (see quantitative assay, Section 6.2.2.3). 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.

Conjugate, n - 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 more recent fluid-phase assays, the conjugate may be a biolabeled allergen reagent.

Control/control material, *n* - 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 ten individual assays. A range of values (2 SD, 95% confidence interval) are computed for the control specimen and used in Levy–Jennings quality control charts to identify assays that are out of control.

Cross-Reactivity, n – ***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 (Cf. LA1-A, IL18-A, DI1-A). **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, n -A mathematical process that converts assay-response data [e.g., counts per minute (CPM)-bound, absorbance units] into interpolated dose results. The dose–response relationship in the assay is defined by the standard, reference, or calibration curve.

Depletion analysis, n - Depletion analysis is a procedure that is used to estimate the quantity of IgE antibody in the calibration process of a prospective reference serum. 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.[10] 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 pre-absorbed 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 IgE that was depleted or removed from the serum by the solid-phase allergen.[8] 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.[11]

Detection limit//limit of detection, n - 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 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:** Assuming 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.

Dynamic range, n - See Linearity.

Efficiency, n - 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, n - // determinant// (determinant), n - **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 (Cf. ILA15, ILA18, DI1-A). **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 (e.g., *Der p* 1 and *Der f* 1 in house dust) and (2) demonstrate identity and qualify extracts before the manufacturing of allergen-containing reagents.

Heterologous interpolation, n - This is a term that defines 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 interest.[10] 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 (e.g., *Ambrosia artemisfolia,* 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 IU/mL (or mass units) 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, n - Antibodies in test sera that can bind to immunoglobulins from other species (e.g., human antimouse immuno-globulins). 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 (e.g., rheumatoid factor). In either case, these heterophile antibodies can induce false-positive or false-negative clinical test results depending on the assay design.

Homologous interpolation, n - This is a term that defines 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 interest.[10] In this assay, both the calibrator and test specimens are analyzed using the identical assay reagents. **NOTE:** In an assay that measures IgE anticommon ragweed, 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 a (homologous) in the IgE antishort ragweed calibration curve that has been precalibrated in arbitrary units or mass units per volume (e.g., 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.[8,11] **NOTE:** 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.

IgE, n - 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. It binds with high affinity to Fc-epsilon RI receptors on mast cells and basophils and 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, n - 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 total IgE and allergen-specific IgE assays are noncompetitive immunometric assays.

Immunoglobulin, n - A glycoprotein composed of two heavy and two light chains that functions as an antibody. Human immunoglobulins have been subdivided into different isotypes (IgM, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE), each of which possess 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.)

Immunodiffusion, n - An immunological method for measuring antigens (e.g., serum proteins) in which a small amount of serum (e.g., 5 μL) is pipetted into a well cut into a porous agarose gel. 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:** 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.

Isoallergens, n - Proteins (allergens) that are essentially identical except for minor differences in their primary amino acid composition or substituted side chains.[13]

Linearity, n - 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)*** [Modified]. **NOTE:** Linearity typically refers to overall system response (i.e., the final analytical answer rather than the raw instrument output (Cf. EP6, EP10). See "parallelism" and Section 9.2.

Matrix, n - All of the components of a material system, except the analyte. **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 with a low total serum IgE is considered an ideal diluent or protein matrix for IgE antibody assays.

Nonspecific binding (NSB), n - Attachment not attributable to specific binding to a receptor (Cf. LA1-A). **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 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 can also associate nonspecifically with reactants, giving a falsely high measure of specific IgE antibody levels.

Parallelism, n - Extent to which the dose–response relationship between two materials (i.e., calibrator versus unknown specimens) is constant for the examined range of concentrations (Cf. LA1-A). **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."

Precision, n - **1)** The closeness of agreement between independent test results obtained under prescribed conditions ***(ISO Guide 30)***; **2)** closeness of agreement between a series of measurements, under specified conditions, of a substance or biological product ***(WHO-BS/95.1793)***; **3)** the closeness of agreement between independent test results obtained under stipulated conditions ***(ISO3534-1-3.14)***; **4)** agreement between replicate measurements. **NOTE:** 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 (Cf. H26). **NOTE:** Intraassay precision refers to agreement within an individual assay run. Interassay precision refers to results obtained from different sets of assays.

Precision profile, n - The precision of an assay across the analyte concentration range of interest. 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.[14] The precision profile is also referred to as the "imprecision profile" by some investigators. See Section 9.1 for further clarification.

Predicate device, n - In the United States, a "predicate device" for previously marketed allergens is an assay or device for the in vitro measurement of an allergen-specific IgE antibody that has been cleared for marketing by the Food and Drug Administration (FDA) or that was in commercial distribution before 1976. 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.

Qualitative assay, n - 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 the presence of IgE antibody specific to the allergen tested in the subject's blood (definition modified from Section 5.1.1.1).[15]

Quantitative assay, n - 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 [e.g., 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 (e.g., immunoassays) and their results can be reported in gravimetric (ng/mL [total serum IgE assays]) or international units (IU/mL [allergen-specific IgE antibody assays]).

Radioallergosorbent test (RAST), n - The RAST was the first immunoassay system available for the measurement of human IgE antibodies to defined allergen specificities in serum.[1] In its original form, the RAST employed a paper disc solid phase to which allergen is 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 I^{125}- labeled polyclonal antihuman IgE. Results were reported in classes or in arbitrary units by interpolation from a heterologous IgE anti-Birch pollen reference curve. Many minor modifications of this original assay format have been commercialized and they are discussed in the text.

Recombinant allergens, n - Allergenic molecules that have been produced in vitro by recombinant deoxyribonucleic acid (DNA) techniques. **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. No clinically used IgE antibody assays are available currently that use recombinant allergens in the manufacturing of their allergen-containing reagents.

Recovery, n - The measurable increase in analyte concentration in a sample after adding a known amount of an identical substance (Cf. LA1-A). **NOTE:** Unspiked and spiked serum specimens are then analyzed in the assay and the proportion of the analyte that is detected over pre-existing 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, n - 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 (e.g., I to VI, low to high); in arbitrarily defined units per milliliter determined relative to a supplier-specific heterologous dose-response curve or an endpoint dilution at which the assay signal becomes negative (e.g., titer); or in comparison to a qualitative grading scheme (e.g., 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. Ideally, but not exclusively, response signal units generated by semiquantitative assays correlate with the level of IgE antibody in the blood and the patient's sensitivity to the test allergen specificity (definition modified from Section 5.1.1.2).[15]

Sensitivity (analytical), n - **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 [35,36] **2)** change in the response of the measuring system divided by the corresponding change in the quantity or property of the analyte.[37] **NOTE:** This term should not be used synonymously with detection limit.

Specificity (analytical), n - 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:** Within the context of this guideline, specificity refers to an assay which is specific for IgE and shows no cross-reactivity of the antihuman IgE reagent with other classes of human antibodies (e.g., 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.

4 IgE: Properties and Effects

Table 1 contrasts established 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_e1, C_e2, C_e3, and C_e4), that possess its unique antigenic attributes, which confer its special biological properties. IgE has 12% carbohydrate and a reported molecular weight of 190,000 daltons. Serum IgE levels 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 due primarily to a relatively high fractional catabolic rate (71% intravascular pool catabolized/day). 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_eRI), which resides on the membrane surface of basophils and tissue mast cells.

4.1 Biological Activity of IgE

Development of the IgE antibody mediated "allergic" state involves two discrete phases: the sensitization phase and a subsequent challenge.[19] One of hundreds of foreign substances (allergens) initially enters the blood, the respiratory tract, or the gastrointestinal system. Macrophages and T-cell lymphocytes interact with the allergen and present it to B-cell lymphocytes, which subsequently differentiate into plasma cells. In predisposed persons, some activated plasma cells produce IgE antibodies, which bind to 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. These events comprise the *sensitization phase*.

Upon a second exposure of the sensitized person to an allergen (*challenge),* allergen cross-links IgE antibodies attached to basophil and/or mast cell surface receptors, causing 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 D_2. 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 activities. All these events may ultimately lead to an immediate type I hypersensitivity response, which manifests in a spectrum of reactions ranging from localized swelling and erythema in the skin (wheal and flare) to more systemic bronchoconstriction, urticaria (hives), angioedema, and, in severe cases, anaphylaxis and death.

4.2 Evaluation of the Human Allergic State

During the evaluation of a person suspected of having an allergic disease, total serum IgE and allergen-specific IgE are the primary analytes that are commonly monitored.

4.2.1 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.[20] An age dependent decline in total serum IgE may occur from the second through eighth decades of life. A patient's serum IgE level should be compared with reference intervals established with sera from an age-stratified, healthy, (nonatopic) population. Serum IgE levels that are significantly higher than the appropriate, age-adjusted, nonatopic reference ranges 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 and are necessary for the diagnosis of hypergamma-globulinemia E 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 IgE levels can thus support the diagnosis of nonallergic (intrinsic) asthma and they can help to exclude bronchopulmonary aspergillosis. The reported overlap between IgE levels in atopic and nonatopic populations, however, is considerable.

Thus, while an elevated serum IgE can be useful in confirming 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.

4.2.2 Allergen-Specific IgE

In contrast to total serum IgE, the presence of allergen-specific IgE antibody in the serum of a subject is highly predictive of an individual's propensity to exhibit an allergic reaction upon re-exposure to the allergen. Before its identification as a novel immunoglobulin, IgE could only be detected by in vivo bioassays (skin test, bronchial or nasal provocation tests). Purification of IgE myeloma protein and the subsequent production of polyclonal antisera specific for IgE led to the development of the first in vitro assays (radioallergosorbent test or RAST) for the detection of allergen-specific IgE antibody in serum. Since then, many commercial variants based on the original RAST design have been developed for the measurement of IgE antibodies of defined allergen specificities (see Section 5.2). In the 1970s, early cross-comparison studies of assay methods evaluated the clinical sensitivity and specificity of the different in vivo and in vitro assays in the diagnosis of human allergic disease. Such intermethod comparisons demonstrate, in general, that the results of serological immunoassay methods for IgE antibody usually agree well with leukocyte and mast cell histamine release assays,[21–23] and provocation tests, such as the skin test and inhalation provocation test.[3, 21, 24] Generally, differences in the results obtained with provocation and serological tests can be traced to either the heterogeneity of the allergen preparation used and/or the inherent sensitivity and specificity of the respective assays.

Table 1. Physical and Chemical Properties of IgE and IgG Antibodies

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 ave %	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*	190000	150000	150000	160000	150000
H chain domain #	5	4	4	4	4
Hinge (amino acids)	none	15	12	62	12
Tail piece	NO	NO	NO	NO	NO
Property	**IgE**	**IgG1**	**IgG2**	**IgG3**	**IgG4**
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
Synthetic rate (mg/kg/day)	0.002	33	33	33	33
% 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	0–0.0001 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

Table compiled from general reference: Hamilton RG. Human immunoglobulins. N. Rose, ed. In *Handbook of Human Immunology*. Boca Raton, FL: CRC Press, 1996.

5 Specimens

5.1 Patient Material

5.1.1 Specimen Type

Serum and plasma (serum + fibrinogen) are considered the working specimens for total and allergen-specific IgE antibody assays discussed in this document. As indicated in Section 2, all human blood products are to be treated with "universal precautions" because it is impossible to know which specimens might be infectious (see CDC publication, Update, 1988). Specific suggestions for specimen handling are also provided in NCCLS document M29, *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids and Tissue*. Currently, all total serum IgE and IgE antibody assays are qualified for use with serum. The addition of anticoagulants used in the collection of plasma may interfere with the accurate quantitation of the levels of total IgE in blood.

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. A detailed description of the procedures that are recommended for specimen collection and handling is presented in NCCLS document H18— *Procedures for Handling and Processing of Blood Specimens;* and M26— *Methods for Determining Bactericidal Activity of Antimicrobial Agents.*[25, 26] 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.

5.1.2 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 (e.g., 21 to 23 °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 (e.g., –70 °C to –120 °C) is not necessary, even though it is not detrimental to the immuno-reactivity 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.

5.2 Quality Control of Human Serum Pools

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 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 QC serum pools, practical difficulties in obtaining large (multiliter) quantities of serum from clinically well-characterized 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 ten) from highly sensitized persons who have a positive history, a positive confirmatory test result (skin test and/or serological test), and who have not received allergen immunotherapy. 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 that the serum pool mimics the 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 in Figure 1) and dilute out the minor IgE antibody specificities (allergens 1 and 3). 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 testing assay quality.

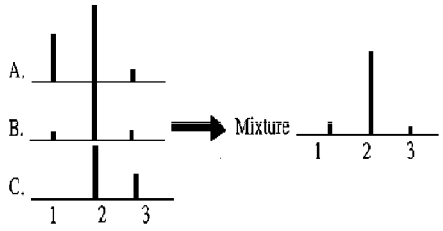

Figure 1. In this schematic, the relative concentration of IgE antibody from three subjects (A, B, and C) for three allergen specificities (1, 2, and 3) are depicted by the relative height of the bar. When these sera are mixed, the concentration of the IgE antibodies with specificities for minor (less allergenic) allergens are 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 cross-radio immunoelectrophoresis or Western blot analysis. Potentially competing antibody isotypes (IgG, IgA) may also be measured by immunoassay to examine the possibility of competitive inhibition or blocking of IgE antibody binding to minor allergen determinants. For internal manufacture QC purposes, manufacturers of allergen-containing reagents should develop 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.

6 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 laboratory today. The primary focus of this section, however, is on assays of IgE antibody that employ a wide variety of reagents, calibration systems, and assay designs.

6.1 Total Serum IgE Assays

Historically, IgE has been measured by a spectrum 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). Despite the use of widely different solid-phase and detection immunological reagents, the commercially available assays are cross-standardized to a common primary human IgE standard (WHO 75/502) and most are cross-checked with a common secondary IgE reference preparation (US Standard for Human Serum IgE, NIAID-NIH/BOB-FDA, Cat A-699-001-500).

Results from currently used assays 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 2.44 as a conversion factor (1 IU/mL = 2.44 ng/mL).

Based on the College of American Pathologist's SE Diagnostic Allergy (SE) survey, the commercially available total serum IgE assays appear to display good interassay precision and analytical accuracy with good parallelism and a dynamic range, typically from 1–1000 IU/mL. Excellent interassay agreement of ten commercially available total serum IgE assays with intermethod CVS <15% is achievable as shown by representative results from the College of American Pathologists' 1993 SE-C Survey (Table 2).

6.2 Allergen-Specific IgE Assays

6.2.1 Components of Allergen-Specific IgE Assays

As discussed in Section 4.4.2, the radioallergo-sorbent assay, or RAST, was initially introduced in 1968 for the detection of IgE antibodies with a defined allergen specificity. This noncompetitive, heterogeneous, immunoradiometric assay employed an allergen insolubilized on

Table 2. Intermethod Agreement of Commercially Available Total Serum IgE Immunoassays*

System	Assay	N	SE15	SE14	SE13	SE12	SE11
A	FIA	42	16.7	44.4	85.4	166.9	625
B	FIA	11	14.2	45.5	95.7	199.9	788
C	FIA	38	10.9	29.4	62.6	143.2	542
D	EIA	17	16.7	45.2	87.9	176.6	604
E	RIA	30	13.2	41.0	84.9	173.3	629
F	EIA	45	15.0	43.8	88.6	173.6	667
G	EIA	20	11.9	36.3	78.1	165.7	546
H	RIA	10	14.6	36.7	72.5	165.0	590
I	EIA	15	17.8	53.1	100.8	198.1	762
J	RIA	20	14.2	44.2	86.9	167.4	669
Mean		248	14.5	42.0	84.3	173.0	642.2
1 SD			2.12	6.5	11.0	16.5	82.3
Intermethod coefficient of variation			15.0%	15.5%	13.1%	9.5%	12.8%

* All IgE results in this table are presented in IU/mL with permission from the 1993 SE Survey (Cycle C) of the College of American Pathologists, Northfield, IL.

paper discs (allergo-sorbent) 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 have been developed. More recently, liquid-phase allergen has been used in "reverse" assays in which IgE is initially captured from serum with a solid-phase, anti-IgE, and then specific IgE antibody is detected with labeled allergen. Because these reverse assays are rarely used clinically and they are primarily research tools, they are not discussed extensively herein. All other assays for IgE antibody have the following commonalities:

- Reaction vessel: plastic or glass tube, plastic microtiter plate well, plastic stick
- *Allergen-containing reagent*: solid-phase allergosorbent or liquid-phase labeled allergen (a separate reagent for each allergen specificity or defined combination of allergens are both available)
- Reaction buffer medium (salts, proteins) to maintain pH and provide a protein matrix for the analyte of interest
- Specimens: human serum (presumably containing IgE antibody) and a negative serum control containing no allergen-specific IgE antibody
- *Antihuman IgE Fc detection reagent* (epsilon heavy-chain specific)
- *Calibration system* (e.g., reference serum, total IgE calibration curve)
- Data-processing system (data-processing software, algorithm)

Of these, the *allergen-containing reagents* are considered the most complex assay components in terms of their preparation from raw biological material and their interbatch quality control and validation. Allergen preparations are inherently complex mixtures of proteins (Section 7) that vary widely in their size and net charge distributions. Each component protein is also known to differentially elicit a heterogeneous humoral immune response in predisposed humans that varies in terms of the isotype (IgE, IgG, IgA, IgM) and concentration of antibody.

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 (e.g., rabbit, goat, sheep, horse) or murine monoclonal antibodies that bind to defined epitopes generally on the Fc region of the human IgE molecule. Manufacturers use various procedures and qualifying reagents (IgE myeloma, polyclonal human IgE, chimeric IgE antibodies) to document its specificity and certify the antihuman IgE detection reagent's performance within the context of their respective assay system.

The third component of these assays that varies widely among commercial assays is the *calibration system* that is used to define the level of IgE antibody that is generated by the assay. At present, there is no single, accepted, industry wide, uniform method for interpolating or converting response units generated by the assay into dose estimates based on common units. Some methods report out a measured response signal, such as counts or normalized counts. Other methods interpolate their measured responses into arbitrary units per unit volume of serum using a calibration curve. For purposes of this guideline, these methods have been classified as quantitative procedures as defined in Section 3.

These three aspects of the seven technical variables of the IgE antibody assay listed previously are the primary focus of the qualification procedures discussed subsequently in this guideline.

6.2.2 IgE Antibody Immunoassay Classification Scheme

Commercially available IgE antibody immuno-assays 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. Each assay category has its utility and limitations as an aid in the diagnosis and research of human allergic disease, which are not discussed here because it is beyond the scope of this guideline. While all three of these assay categories are defined in Section 3, 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.

6.2.2.1 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. 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 indeterminent. 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 IgE antibody in the subjects.

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, reagents in this assay are more difficult to quality control for the reproducibility of the allergen composition and quantity. Moreover, allergic patient sera containing differing amounts of IgE antibody to the spectrum of allergens generates widely varying response levels in different screening assays as a function of the different quantity and relative proportions of the allergens represented in the assay. A second illustration is the dipstick assay in which IgE antibody to a single allergen is determined to be either present or absent based on a visual evaluation of the color and intensity.

6.2.2.2 Semiquantitative Assay

A semiquantitative (titration) assay system provides an additional option over the qualitative assay in terms of defining the

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

Classification	Result Presentation	Standardization Method	References Calibrators and Controls
Qualitative	-Reactive or negative -Nonreactive or positive An "indeterminant" 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 (e.g., WHO IgE standard 75/702)	-Multipoint standard curve used for interpolation	-Trilevel positive controls (see Section 10.1) -References calibrated towards a qualified reference preparation

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 (e.g., I to VI; low to high); in arbitrarily defined units per milliliter (U/mL) determined relative to a supplier-specific, heterologous dose–response curve; an endpoint dilution to which the signal becomes negative (e.g., titer); or in comparison to a qualitative grading scheme (e.g., color chart). A semiquantitative assay may be functionally defined as an assay that employs a single or multipoint calibration curve. It might not be able to identify accurately a twofold change in IgE antibody level when a test serum is diluted twofold. The semiquantitative assay produces antibody estimates in units that are relative and not traceable to any common reference material. Finally, these assays are unable to meet consistently the criteria of linearity, dilution recovery, and parallelism that are achieved typically by quantitative assays.

6.2.2.3 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. It fulfills the analytical criteria for quantitation, including parallelism, recovery, precision, and linearity 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 twofold reduction in IgE antibody when the serum specimen is diluted twofold. 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 (see Section 8). While it is desirable to have truly quantitative assays in which results are reported in units traceable to an internationally recognized standard (e.g., WHO 75/502), this is not possible using the homologous interpolation scheme, which requires individual homologous calibrated IgE antibodies, one for each allergen specificity. Thus, the calibrated titration assay that uses heterologous interpolation is an acceptable alternative because IgE antibody specific for each allergen specificity is interpolated from a 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 (e.g., immunoassays) and their results are reported in gravimetric (μg/L [total serum IgE assays]) or international units (kIU/L [allergen-specific IgE antibody assays]).

7 Qualification of Assay Reagents

7.1 Antihuman IgE Antibodies

Antibodies specific for human IgE have been used as both 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. More recently, monoclonal antibodies specific for human IgE have been prepared and some are used in research[12] and commercial immunoassays. 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 available on the 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 (e.g., <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; (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.

7.2 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.

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.

7.2.1 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.

These are classified, for convenience, into different allergen groups, each using a single letter code: grass pollen (G),weed pollen (W), tree pollen (T), epidermal allergens (E), mold spores and mycelia (M), dust mite (D), insect venoms and excretions (I), foods (F), and occupational allergens (U or K). The common name and scientific name (genus and species) of each individual allergen source is identifiable by a number following the group code (e.g., W1 or weed 1 for common ragweed or *Ambrosia artemisiifolia*). While no significance is attached to the actual number

Table 4. Qualification of Antihuman IgE Reagents

Parameter	Illustration
Antibody source	mouse, rabbit, horse
Antibody type	polymonoclonal, polyclonal, or monoclonal
pH range for storage	7.0–7.5
Preservatives	0.05 % Thimerosal or Azide
Label type (e.g., ^{125}I-enzyme)	enzyme, ^{125}I or fluorophor
Source of human IgE	IgE myeloma protein or affinity purified polyclonal IgE
% Crossreactivity with human IgG	Target = <0.001%*
% Crossreactivity with human IgA	Target = <0.001%*
% Crossreactivity with human IgM	Target = <0.001%*
Parameter	**Illustration**
% Crossreactivity 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.

Table 5. Potential Causes of Allergen-Containing Reagent Heterogeneity

- Allergen source: Misidentification, contamination, and inherent biological variation
- Extraction process: Interlot variation in allergen composition
- Allergen-reagent manufacturing: Interlot variation
- Variable binding of allergen to labels or solid supports
- Variable stability of allergen during storage
- Heterogeneous internal allergen reference standards used in quality control
- Heterogeneous IgE antibody specificities in human serum QC pools
- Different assays and acceptance criteria used in validation of final reagents

assigned to the allergen specificity, manufacturers have generally adopted the code system that was used in the first patented IgE antibody immunoassay, the paper disc-based Phadebas RAST. The allergen nomenclature addendum in this guideline provides a summary of the currently used allergen codes along with their common name, Latin name, allergen grouping, and known cross-reactivity. The company sources that provide particular allergen specificities are noted in this table with a single letter code. Difficulty with this nomenclature system 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 all together.

For instance, T12 has been used to describe an extract of the willow tree that has been prepared from either *Platnus acerifolia* or *occidentalis* organism. While there is often extensive cross-reactivity among allergenic proteins extracted from source material obtained from different species of a particular genus, the fact that source materials using the same code differ widely among allergen manufacturers is cause for confusion. Coding variations should be avoided as much as possible or clarification should be made available on request to the manufacturer.

7.2.1.1 Characterized Complex and Purified Allergen Preparations

To date, only five allergen extract preparations of the many hundreds have been approved by the World Health Organization for use as international standards. Approval for three others is pending (see Table 6). Ipsen et al.[27] and King et al.[13] provide a detailed overview

Table 6. Illustrative Characterized Allergen Extracts

Common Name	Genus/Species	Approved by WHO	Reference
Bermuda grass	*Cynodon dactylon*	Pending	Baer et al, 1986
Rye grass	*Lolium perenne*	Pending	Stewart et al, 1988
Timothy grass	*Phleum pratense*	Yes	Gjesing et al, 1985
Common ragweed	*Ambrosia artemisiifolia*	Yes	Helm et al, 1984
Birch tree	*Betula verrucosa*	Yes	Arntzen et al, 1989
Dog hair/dander	*Canis familiaris*	Yes	Larsen et al, 1988
Alternaria extract	*Alternaria alternata*	Pending	Helm et al, 1987
House dust mite	*Dermatophagoides pteronyssinus*	Yes	Ford et al, 1985

for the primary allergen sources and allergen nomenclature with a description of the characteristics of their primary purified allergen molecules.

Each of the hundreds of allergen extracts described in the allergen nomenclature addendum are composed of many individual allergenic 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 standardization of a given extract begins with a painstaking process of 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). Once the individual allergen molecules are reproducibly 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.

Table 7 illustrates a representative set of purified allergen molecules from each allergen group. A detailed list of the characterized purified allergens that are recognized by the WHO/IUIS Allergen Nomenclature Subcommittee is presented in the Allergen Nomenclature Addendum (Appendix C).

7.2.1.2 Allergen Quality Assurance (Theoretical Considerations)

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 allergen extracts. 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 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 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. However, one provocative question queries whether monoclonal antibodies that bind to restricted allergenic epitopes on selected major allergens in a particular allergen mixture might be used to quality control the final allergen-containing reagents in an attempt to minimize dependency on expensive, variable, and rare heterogeneous IgE-containing human serum pools. A panel of such monoclonal

Table 7. Illustrative Purified Allergens

Allergen Source	Genus /Species	Purified Allergen
Common ragweed	*Ambrosia artemisiifolia*	Amb a 1, Amb a 2, Amb a 3, Amb a 5, Amb a 6, Amb a 7
Birch tree	*Betula verrucosa*	Bet v 1, Bet v 2
Dog hair dander	*Canis familiaris*	Can f 1
House dust mite	*Dermatophagoides pteronyssinus*	Der p 1, Der p 2, Der p 3, Der p 4, Der p 5, Der p 6, Der p 7
Alternaria	*Alternaria alternata*	Alt a 1

antibodies 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 allergen epitopes are present and immunoreactive. This proposal has sparked intense 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 MAb 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 the heterogeneous allergen extract source.

Critics of the use of Mab quality control reagents contend that one epitope or allergen molecule cannot represent the presence and quantity of a heterogeneous mixture of allergenic molecules from a single source. Thus, one cannot use the binding of individual MAbs to make any judgement (good or bad) about the diagnostic quality of the allergen-containing reagent based on the knowledge that a marker protein is present and that an MAb can bind to it. While an allergen-containing reagent that does not contain a particular major allergen might not be considered optimal, can it still be useful diagnostically in detecting IgE antibody specific for other major and/or minor determinants in the same allergen source material? What role should monoclonal antibodies that react to a limited number of "marker" IgE binding epitopes play in the quality control of allergen-containing reagents? Heterogeneous mixtures of monoclonal antibodies and polyclonal IgE antibody present in human serum pools are used presently in monitoring the potency and immunoreactivity of some allergen-containing reagents through their various stages of production.

Other investigators have proposed that future IgE antibody assays might use a panel of purified (recombinant) allergenic molecules 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 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 over the use of recombinant allergens in the manufacturing and quality control of *globally* versus *isoallergen-limited* reagents is a topic for future consideration. Despite the questions that remain about recombinant allergens and monoclonal antibodies, the fact is that the protein distribution on final allergen-containing reagents produced by different manufacturers vary in their protein composition, allergenic potency, and immunoreactivity.

Thus, each allergen reagent is destined to detect a slightly different population of IgE antibodies and they will not be interchangeable between manufacturers kits.

Because the allergen-containing reagent's specificity is qualified by well-characterized reference serum pools, possibly the most

achievable goal in the process of allergen-reagent standardization is the preparation of a common reference serum pool that contains IgE antibodies specific for an ideal or maximal number of allergen epitopes to which all in vitro assay allergen reagents can be compared.

7.2.2 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. Each supplier uses a different set of testing methods, someof which are listed in Table 8 on p. 22. The results of this testing should be collated into a *Certificate of Assurance* by the allergen supplier that accompanies the allergen source material to the reagent manufacturer. The details of these analyses are summarized elsewhere[13] and are beyond the scope of this guideline. Compounding conditions (binding to solid phase, labeling) and prequalification of the allergen source material before manufacturing are also beyond the scope of this guideline. 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 9 on page 22), this might not be possible with the majority of extracts. Most of these tests require the use of human serum pools (Section 5.2); thus, 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 should be evaluated during final reagent testing. Once collected, this information should be prepared in a concise data sheet that can be provided to the user as a reference guide. Appendix D presents methods for qualifying allergen-containing reagents.

Table 8. Immunochemical Methods for Qualifying Allergen Extracts

Nonimmunological methods
Total protein (Ninhydrin, modified Bradford or Lowry)
Amino acid analyses
Isoelectric focusing (IEF)
SDS-polyacrylamide gel electrophoresis
Immunochemical methods
RAST inhibition
Quantitative immunoelectrophoresis, such as cross-(radio)-immunoelectrophoresis and rocket immunoelectrophoresis
Western blot analysis
IEF immunoblot analysis
Single radial immunodiffusion
Cellular and in vivo assays
Basophil histamine release
Skin test titration

Table 9. Idealized Qualification of Finalized Allergen-Containing Reagents*

Qualifying Parameter	Description, Method, or Source Information
Allergen extract name (genus, species)	Natural rubber latex (*Hevea brasiliensis*)
Allergen extract source	*H. brasiliensis* rubber tree sap (non-Ammoniated); 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 sera
Specificity	< 1 % cross-reactivity based on inhibition with heterologous allergens in same allergen group, alternatively, comparison of regression slopes of dose–response curves
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–Hev b 4 references
Source of human antisera used in qualification of allergen reagent	CBER-FDA IgE anti latex serum pool (not available for many allergen specificities)
Reference allergen used for validation of allergen preparation	E5–nonammoniated latex CBER-FDA (not available for many allergen specificities)

*Illustrated by a model latex allergen reagent (K82, Appendix A).

†The level of irrelevant IgE in serum used to test for NSB is to be selected depending on the characteristic of the allergen being tested. For illustration, for the evaluation of a single sensitivity to a discrete allergen (e.g., penicillin), a lower level of NSB control is appropriate, whereas for testing of plural sensitivities to food allergens, a higher NSB control is more appropriate.

8 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. More recently, it has been suggested that the probability of a patient experiencing severe allergic symptoms following exposure to an allergen source is 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.[28] This proposed relationship between IgE antibody levels and symptoms needs further substantiation and it has motivated investigators to quantify the amount of IgE antibody in the patient's serum that is specific for a defined allergen specificity.

Classically, IgE antibody levels are reported in a number of units depending on the degree of quantitation afforded by the assay system (Table 10). 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, and the magnitude of the signal measured [e.g., 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.

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 that 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 (see definitions in Section 3) have been successfully used in commercially available IgE

Table 10. 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)
- Modified scoring system
- Alternative scoring system (ASM)

(2) Multipoint calibration curve
- 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 calibration component of assay

antibody assays. The homologous interpolation procedure promotes overall assay parallelism and maximizes the assay's working range by (1) using the same solid-phase allergen throughout the assay and (2) 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 insuring 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 less common allergen specificities.

Because of constraints placed on assays using the homologous interpolation calibration as a result of limited IgE antibody-containing human serum pools, the heterologous interpolation procedures have been viewed as the most achievable calibration strategy for present day quantitative IgE antibody assays that involve hundreds of antigen specificities. The earliest Phadebas RAST, in essence, used a heterologous interpolation scheme by analyzing a multipoint IgE anti-Birch reference curve from which all other IgE antibody results were interpolated in Phadebas RAST units per milliliter (PRU/mL). The PRU is an arbitrary unit that was originally traceable to a primary IgE standard but was not widely adopted. More recently, a total serum IgE calibration curve has been run simultaneously with the allergen-specific IgE portion of the assay. This heterologous interpolation scheme uses an IgE calibrator that is traceable to a primary IgE standard, such as the WHO 75/502. As such, this calibration strategy is available to all manufacturers of IgE antibody assays. Providing that the calibration (total IgE) portion of the curve dilutes out in parallel with IgE antibody levels as measured in patient sera, this system may be the most generic solution to the problem of interlaboratory cross-standardization. Presently, several quantitative IgE antibody assays have successfully employed this strategy.

Finally, automation and reagent stability have permitted the development of assay systems that store a multipoint reference curve in computer memory for a number of days. Control specimens are then analyzed to validate the reference curve stored in memory for that assay run.

The manufacturer has the responsibility to ensure that 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 Section 9 and Appendix B. The user of the assay is responsible for analyzing a sufficient number of internal quality control sera in each analysis to validate the assay's calibration curve.

9 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 parallelism. Methods that are used by manufacturers to document the performance of their assays before they are released for use are summarized in the text or described in detail in the appendices.

9.1 Analytical Accuracy

The term “accuracy” refers to the nearness of a measurement 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[6,7] and the United States IgE Standard have been used as a primary reference materials to promote accuracy. For IgE antibody assays of defined allergen specificity, there currently are no universally accepted IgE antibody standards with calibrated levels of allergen-specific IgE. 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 are available that have been calibrated in mass per volume units using antibody adsorption or depletion methods.[8] Reference sera should be used to determine the degree of analytical accuracy of total serum IgE and IgE antibody assays.

9.2 Assay Interference

There are several factors in human serum that have the potential of interfering with the performance of the total serum IgE and IgE antibody immunoassays. The manufacturer should evaluate what effect human rheumatoid factor, antihuman IgE autoantibodies, and human IgG antiallergen have on the results of their assay. Human rheumatoid factors, which are autoantibodies that cross-link human immunoglobulins, are also 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.[12] Antihuman IgE auto-antibodies have been detected in most human sera[31] and their clinical significance is presently unclear. The potential for their interference in human IgE antibody assays should be considered during the validation stage of assay development. Finally, IgG antiallergen interference has been extensively documented for some assays where antigen-excess conditions are difficult to achieve. This issue should be examined for select allergen systems where IgG antibodies are known to reach μg/mL levels in untreated subjects (e.g., Hymenoptera venom).

10 Quality Assurance (QA)

In recent years there has been increased emphasis and regulatory focus on the quality of patient testing systems, such as those used by clinical laboratories to measure total serum IgE and IgE antibody of defined specificities. There are three areas of quality assurance that should be considered for IgE assays. The first is performed by the manufacturer at the time of assay production. If performed effectively, the manufacturer quality assurance testing can minimize subsequent internal intra laboratory quality control testing that must be conducted by the clinical laboratory user as a secondary level of QA to demonstrate that the assay is “in control.” Participation in external quality assessment surveys that evaluate IgE antibody assay performance is encouraged and, in some regions, is required.

NOTE: Because in the United States, human IgE is classified by CLIA ‘88 as a regulated analyte and IgE antibodies

are currently not, laboratories that measure total serum IgE should demonstrate satisfactory performance in a suitable external interlaboratory proficiency program as a third level of QA.

10.1 Quality Assurance During 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. Statistical process control methodology, as described by Kiemele and Schmidt,[32] provides a structured approach to ensuring the quality of the assay components and the ultimate assembled assay. An alternative structured quality assurance program with "multirule" procedures is extensively described by Westgard and Klee.[33]

10.1.1 Total Serum IgE

For total serum IgE assays, sera containing IgE levels that span the reported dynamic range of the assay should be shown to be within 95% confidence limits of a range that has been established by at least ten 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. Run's Rules[32] or Westgard's Rules[34] are useful in interpreting quality control data to minimize false rejection and to maximize the detection of random systematic errors. The primary goal of this testing should be to document the reproducibility of the assay with a precision profile and the detection limits, linearity, and parallelism with a dilution-recovery analysis. All these analyses must demonstrate that the assay is correctly calibrated to an internationally recognized human IgE standard.

10.1.2 IgE antibody Assays

For qualitative IgE antibody assays, testing of a panel of IgE antibody positive and negative control sera should generate results that identify their correct IgE antibody assignment. The goal of these QA analyses should be to document 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 QC sera with levels of IgE antibody that cover to two 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 the limits of detection, linearity, and parallelism of the assay demonstrated with a dilution-recovery analysis.

10.2 "Internal" Intralaboratory Quality Control

The establishment of internal quality control programs may be required by local or national regulations. Refer to NCCLS document C24—*Internal Quality Control Testing: Principles and Definitions*, for suggestions on developing an internal QC program. Individual strategies can vary depending on the assay design and equipment used. This section recommends one useful strategy for the general internal quality control of total IgE and IgE antibody assays.

10.2.1 Total serum IgE

A daily intra laboratory QC program for total serum IgE assays commonly involves the analysis of three control sera for which there is a well-documented IgE range. Control sera should be selected so that they cover the low (1–10 IU/mL), medium (50–100 IU/mL), and high (1000–2000 IU/mL) regions of a total serum IgE assay's dynamic range. 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 control range (>2SD from mean defined by 10–15 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 replicates measurements are run, high interduplicate %CVs should be considered candidates for a repeat analysis.

10.2.2 IgE Antibody Assays

There are many different IgE antibody assay formats commercially available, each of which may require a slightly different QC strategy. All the assays, 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 QC program for human IgE antibody assays. One extreme expresses the view that each allergen-containing reagent (with a different specificity) is a separate test that should have its own specific IgE QC serum, which should be tested in each assay when it is used. This strategy does not recognize the extensive stability and QC testing that is conducted on each allergen-containing reagent by the manufacturer before it is released for use. It would also require that each laboratory maintain a vast serum bank of IgE-positive sera for every allergen specificity, which is impractical and costly. The more achievable extreme is to view 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 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 discussed earlier.

For laboratories that run semiquantitative or quantitative assays, a single IgE antibody positive control serum may be run additionally in each assay to provide a second level of quality assurance. To accomplish this, some laboratories identify a group of five common allergen specificities (e.g., common ragweed, oak tree, *Alternaria tenuis,* dog dander, and *Dermatophagoides farinae*) for which IgE antibody containing human sera are plentiful.

They then prepare a large serum pool that contains IgE antibody to all five specificities and analyze it against one allergen-containing reagent in each assay that can be rotated among the five allergen specificities during the course of five runs. This specific IgE antibody testing provides an additional check on the calibration portion of the assay and validates other common reagents that may be exclusively used in the allergen-specific IgE portion of the assay. A more detailed discussion of internal QC testing is presented in NCCLS document C24.

10.3 "External" Interlaboratory Proficiency Testing

All laboratories that perform total serum IgE measurements can be required by local or national regulations to obtain a satisfactory performance in one of several possible external interlaboratory proficiency testing surveys. To meet these requirements, it is recommended that a survey challenge the laboratory three times a year with at least five sera during each cycle. One well-subscribed program is the SE survey conducted by the College of American Pathologists (CAP; Northfield, IL [see Table 11]). In addition to providing a challenge for total serum IgE, it also provides challenges for allergen-specific IgE antibody. The primary goal of this and similar 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 IU/mL are compared to peer group means and ranges, and outlier laboratories are identified that exceed the 99% confidence limit for their peer group.

When evaluating the IgE antibody results, the survey should collate two types of allergen-specific IgE antibody data: (1) qualitative grade or class results that reflect the presence and the relative level of IgE antibodies and (2) unit results that are produced by the quantitative IgE antibody assays. Differences in the semiquantitative class results produced by a single laboratory can be compared to a peer group range of classes produced by participants that perform the same method. Outlier laboratories can be identified when a laboratory detects IgE antibody in a serum from a nonatopic (clinical history negative,

Table 11. External Proficiency Survey for Diagnostic Allergy Laboratories

Variable	CAP-SE
Cycles per year	3 (every 17 weeks)
Sera per cycle	5 (individual or pools)
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	Classes and units

*Printed with permission from the College of American Pathologists.

negative skin test result) person by reporting a class result other than "0." Likewise, an outlier laboratory can be identified when a class 0 is reported for an IgE antibody containing serum that is collected from an atopic individual (positive clinical history, positive skin test). It has been difficult to score the class 1 results that are considered negative by some laboratories and borderline positive by others. Because the CAP survey operates on the basis of peer group consensus, if >90% of laboratories performing a method do not agree on the result, then that measurement is not graded in that cycle of the survey. The unit results have only recently been collected; thus, most performance decisions to date have been based on the class results supplied by participating laboratories.

11 Assay Performance Targets

Evaluation of the performance of a commercial total serum IgE or IgE antibody assay begins with *a careful review* of the product insert that supplies a detailed description of the assay design, component reagents, and manufacturer-derived QA testing. Performance results supplied by the manufacturer in the product insert should include the assay's dynamic range, precision profile analyses, detection limits, dilution-recovery results, nonspecific binding levels with sera from nonatopic persons, and results of tests on the allergen and IgE specificity of the assay. Table 12 summarizes the subcommittee's recommendations for evaluation criteria and illustrative minimal performance targets for IgE antibody.

11.1 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 IgE antibody assays. The first is *general product information,* 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 may be supplied for that assay method by the manufacturer.

11.1.1 General Product Information

The general manufacturer's product literature that accompanies each assay kit should cover the following issues. This information should be written in a way that is consistent with applicable regulations.

- A description of the *intended use* of the kit.
- A statement on the *intended category* of the assay (qualitative, semiquantitative, or quantitative).
- A description of the *common assay reagents* (buffers, antihuman IgE) and *required general supplies*.
- The detailed description of the *general assay procedure* with a step-by-step use of any *interpolation schemes* from any supplied internal calibration curve.
- Appropriate *specimens and dilutions* to be analyzed in the assay.

Table 12. 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 (inter assay) [%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 =
Nonspecific binding of IgE (for allergens only; high IgE sample [IU/mL or ng/mL] normally > 1000 IU/mL)	M/U	ideal < 50% low standard
Correlation to other technologies (define predicate device)		
Slope (analytical sensitivity)	M	ideal = 1.0
Correlation coeff [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, e.g., hemolytic, lipemic samples	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.

- *Expiration date* of common assay reagents.
- Expected and achievable *intra-assay, inter-assay precision* across the linear range of the assay (precision profile).
- Results of *dilution-recovery analyses* for parallelism assessment, especially for quantitative assays.
- Recommendations for daily laboratory quality control.
- Test *limitations and cautions (see Section 11.1.3).*

11.1.2 Separate Product Information Sheet for Allergen-Containing Reagents

A compendium of individualized product inserts or information sheets may be supplied, which provides a description of the common and scientific names of the allergen-containing reagents provided by the company. Each allergen's summary describes:

- Its general geographic distribution in the areas where the product is sold.
- Current knowledge about the allergen (e.g., degree of standardization by WHO and characterization of individual allergen components).
- A summary of specificity (cross-reactivity) studies with other allergens from the same allergen group and different groups (e.g., latex versus kiwi or avocado).
- Expiration information.
- Interlot variability results using three lots of product with three QC sera.
- References relating to immunochemical characterization and QC of allergen. Where possible, manufacturers should use common allergen nomenclature defined by the WHO-IUIS Allergen Nomenclature Subcommittee[13] and common product codes (e.g., W1 for common ragweed, see Appendix A) to minimize confusion among users and promote maximum clarity in interlaboratory proficiency surveys.

11.1.3 Limitations and Cautions Statement

The following statements relating to clinical relevance and interpretation of the results should be included in bold letters on the front page of all product inserts relating to allergen-specific IgE assays.

"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."

11.2 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 (e.g., 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 IgE antibody assays and reagents.

The user of a product that fulfills the target performance characteristics defined in Table 12 should not need to repeat extensive QA tests that have been performed on the individual 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 technician, equipment, and internal laboratory-related problems. The type and number of QC sera run in each assay should be appropriate for the assay's degree of quantitation (see section 6.2.2, Table 3). 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 manufacturers recommendations and in-house validation studies. Daily performance of quantitative assays can be best evaluated by plotting unit data from each control in Levey–Jennings QC charts.

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

Introduction

The objectives of this addendum are threefold. First, this compendium which begins on the following page, provides the user, regulator, and manufacturer with a master list of abbreviated codes, common names, company sources, and Latin names of all known allergen specificities. The overall goal of this compendium is to create a common allergen code list that is used by all manufacturers to refer to a particular allergen specificity. The third column lists those companies that have manufactured allergen-containing reagents. For allergens with no manufacturer source code, the allergen specificity is to be considered strictly investigational (for research use only). Second, this list provides a quick reference of primary cross-reactivities among the established and research allergens. Known purified allergens are noted below their major grouping, systematic as well as original names (in parentheses) and reported molecular weight. Finally, the allergens designated in the right hand column of Table A-1 as "Class A" are well established in the literature. All other allergens not labeled as Class A have limited characterization and therefore are not presently considered well established. To ensure its accuracy and maximize its utility, NCCLS plans to update this addendum on a regular basis after consultation with manufacturers to ensure its accuracy and maximize its utility.

Company Codes

A = Abbott Diagnostics (responded, R. Lindberg, Ph.D.)
B = BioWhittaker (responded, B. Pasciak)
C = Ciba Corning Diagnostics - ALK A/S (responded, H. Lowenstein, Ph.D.)
D = Diagnostic Products, Inc. (responded, J. Weiss, Ph.D.)
H = Hycor Biomedical Inc. (responded, T. Li, Ph.D.)
M = Mast Immunosystems, Inc. (responded, R. Driver)
P = Pharmacia Diagnostics (responded, L. Yman, Ph.D.)
Q = Quidel (responded, S. Miller, Ph.D.)
S = Sanofi-Diagnostics Pasteur (responded, S. Mertens)

Shaded areas denote allergens prepared from a second species of the same genus, often with the same allergen code.

Table A-I. Grass Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
G1	Sweet Vernal Grass	*BCDHMPQS*	*Anthoxanthum odoratum*	G3, G5, G6, G8	grass-A
G2	Bermuda Grass	*ABCDHMPQS*	*Cynodon dactylon*	G10, G17	grass-A
G2-I	Cyn d 1		*32 kD*	NA	grass
G3	Orchard Grass or Cocksfoot	*BCDHMPQS*	*Dactylis glomerata*	G6	grass-A
G3-I	Dac g 1(AgDg1)		*32 kD*	NA	grass
	Dac g 2		*G3-II*	NA	grass
			G3-III	Dac g 5	grass
G4	Meadow Fescue	*BCDHMPS*	*Festuca elatior*	G3, G5, G6, G8, G13	grass-A
G5	Rye Grass	*BCDHMPQS*	*Lolium perenne*	Genus Lolium, G3, G6, G8, G13	grass-A
G5-I	Lol p 1 (group I)		*27 kD*	NA	grass
G5-II	Lol p 2 (group II)		*11 kD*	NA	grass
G5-III	Lol p 3 (group III)		*11 kD*	NA	grass
G5-V	Lol p 5		*31 kD*	NA	grass
G5-IX	Lol p 9 (Lol p Ib)		*31/35 kD*	NA	grass
G6	Timothy Grass	*ABCEHMPQS*	*Phleum pratense*	G3, G4, G5, G8, G13	grass-A
G6-I	Phl p 1		*27 kD*	NA	grass
G6-V	Phl p 5 (Ag25)		*32 kD*	NA	grass
G7	Common Reed Grass	*BCDHPS*	*Phragmites communis*	NA	grass-A
G8	Meadow, Kentucky Blue, June Grass	*BCDHMPQS*	*Poa pratensis*	other species of Poa	grass-A
G8-I	Poa p 1 (group I)		*33 kD*	NA	grass
G8-V	Poa p 5		*31 kD*	NA	grass
G8-IX	Poa p 9		*32/34 kD*	NA	grass
G9	Redtop or Bentgrass	*CHP*	*Agrostis stolonifera*	G3, G5, G6, G8,	grass-A
G9	Bentgrass	*B*	*Agrostis maritima*	G3, G5, G6, G8,	grass
G19	Redtop	*BDMS*	*Agrostis alba*	G3, G5, G6, G8,	grass-A
G29	Redtop	*H*	*Agrostis alba*		grass
G10	Johnson Grass	*BCDHMPS*	*Sorghum halepense*	G17, maize	grass-A
G10-I	Sor h 1		*NA*	NA	grass
G11	Brome Grass	*BCDHPS*	*Bromus inermis*	G12	grass-A
G12	Cultivated Rye Grass	*BCDHPQS*	*Secale cereale*	G11, G15	grass-A

Table A-I. Grass Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
G15	Cultivated Wheat Grass	*BCDHP*	*Triticum sativum*	G5, G6. G12	grass-A
G101	Cultivated Wheat Grass	*S*	*Triticum sativum*	G5, G6. G12	grass
G16	Meadow Foxtail Grass	*CDHPS*	*Alopecurus pratensis*	G3, G4, G5, G6, G8	grass-A
G17	Bahia Grass	*BCDHMPQS*	*Paspalum notatum*	G10	grass-A
G18	Corn Grass	*BC*	*Zea mays*	NA	grass
G18	Barley Grass	*S*	*Hordeum vulgare*	NA	grass-A
G19	Dog's Tail Grass	*H*	*Cynosurus cristatus*		grass
G20	Corn Grass	*CS*	*Zea mays*	NA	grass
G202	Corn, Cultivated	*H*	*Zea mays*		grass
G24	Wheat Grass, Western	*H*	*Agropyron smithii*		grass
G25	Cultivated Wheat Pollen	*H*	*Triticum aestivum*		grass
G27	Oat Grass Tail	*H*	*Arrenathermum elatius*		grass
G28	Cultivated Barley Pollen	*B*	*Hordeum vulgare*	NA	grass
G201	Cultivated Barley	*H*	*Hordeum vulgare*		grass
G31	Wheat Grass	*H*	*Agropyron repens*		grass
G70	Wild Rye Grass	*DP*	*Elymus triticoides*	NA	grass
G71	Canary Grass	*DP*	*Phalaris arundinacea*	NA	grass
G100	Salt Grass	*S*	*Distichlis spicata*	NA	grass
G203	Salt Grass	*H*	*Distichlis spicata*		grass

Table A-II. Weed Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-Reactivity	Allergen Group and Class
W1	Common ragweed	*ACDHMPQS*	*Ambrosia artemisiifolia*	W2-W4, W13, W16	weed-A
W1-I	Amb a 1 (antigen E)		*38 kD*	NA	weed
W1-II	Amb a 2 (antigen K)		*38 kD*	NA	weed
W1-III	Amb a 3 (Ra3)		*11 kD*	NA	weed
W1-V	Amb a 5 (Ra5)		*5 kD*	NA	weed
W1-VI	Amb a 6 (Ra6)		*10 kD*	NA	weed
W1-VII	Amg a 7 (Ra7)		*12 kD*	NA	weed
W1	Short ragweed	*B*	*Ambrosia artemisiifolia*	W2-W4, W13, W16	weed-A
W2	Western ragweed	*BCDHPS*	*Ambrosia psilostachya*	W1, W3, W4	weed-A
W3	Giant ragweed	*ABCDHMPS*	*Ambrosia trifida*	W1-W4 W13, W16	weed-A
W3-V	Amb t 5 (Ra5G)		*4.4 kD*	NA	weed
W4	False ragweed	*BCDHMPS*	*Franseria acanthicarpa*	W1-W3	weed-A
W5	Wormwood	*CHMPS*	*Artemisia absinthium*	W7	weed-A
W5	Wormwood	*D*	*Artemisia annua*	W7	weed
W5	Sagebrush (common)	*B*	*Artemisia tridentata*		weed
W6	Mugwort	*ABCDHMPQS*	*Artemisia vulgaris*	W7	weed-A
W6 II	Art v 2		*35 kD*	NA	weed
W7	Margueite, Ox-eye daisy	*BCDHPS*	*Chrysanthemum leucanthemum*	W5, W6	weed-A
W8	Dandelion	*BCMP*	*Taraxacum vulgare*	W6	weed-A
W8	Dandelion	*S*	*Taraxacum officinalis*	W6	weed
W80	Dandelion	*H*	*Taraxacum officinalis*		weed
W8	Dandelion	*D*	*Taraxacum spp.*	W6	weed
W9	English plantain, Ribwort	*ABCDHMPQS*	*Plantago lanceolata*	NA	weed-A
W10	Lambs quarters Goosefoot	*BCDHMPS*	*Chenopodium album*	W15, W17	weed-A
W11	Russian thistle, Saltwort (prickly)	*BCHMPQ*	*Salsola kali*	W15, W17	weed
W11	Russian thistle	*DS*	*Salsola pestifer*	W15, W17	weed-A
W12	Golden rod	*CHP*	*Solidago virgaurea*	NA	weed-A
W12	Golden rod	*S*	*Solidago candensis*	NA	weed
W12	Golden rod	*BD*	*Solidago spp.*	NA	weed
W72	Golden rod	*H*	*Solidago spp.*		weed
W13	Cocklebur	*CDHMPS*	*Xanthium commune*	W1-W3, W16	weed-A
W14	Common pigweed, Rough pigweed	*BCDHMPS*	*Amaranthus retroflexus*	W10	weed-A
W15	Scale, Lenscale	*CHPS*	*Atriplex lentiformis*	W10, W17	weed-A

Table A-II. Weed Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-Reactivity	Allergen Group and Class
W16	Rough marshelder	*BCDHMPS*	*Iva ciliata*	W1, W4, W13	weed-A
W17	Firebush, Kochia	*BCDHPS*	*Kochia scoparia*	W10	weed-A
W18	Sheep sorrel	*CDHMP*	*Rumex acetosella*	NA	weed-A
W22	Sheep sorrel	*B*	*Rumex acetosella*	NA	weed-A
W100	Sheep sorrel	*S*	*Rumex acetosella*	NA	weed-A
W19	Wall pellitory, Parietaria officinalis	*ABCDHPQS*	*Parietaria officinale*	W20, W21	weed-A
W20	Common stinging nettle	*BCDHP*	*Urtica dioica*	W19, W21	weed-A
W103	Nettle	*S*	*Urtica lyalli*	NA	weed
W21	Wall pellitory, Parletaria judaica	*CDHPS*	*Parietaria judaica*	W19, W20	weed-A
W21	Dock (yellow)	*B*	*Rumex crispus*	NA	weed
W23	Dockweed (yellow)	*H*	*Rumex crispus*		weed
W22	Hop, Japanese	*P*	*Humulus scandens*	NA	weed
W90	Hop, Japanese	*D*	*Humulus japonicus*	NA	weed
W22	Chrysanthemum	*H*	*Chrysanthemum spp.*		weed
W23	Cocklebur (common)	*MB*	*Xanthium strumarium*	NA	weed
W73	Cocklebur (common)	*H*	*Xanthium strumarium*		weed
W23	Dahlia	*C*	*Dahlia spp.*	NA	weed-A
W25	Dahlia	*H*	*Dahlia variabilis*		weed
W24	Wormwood	*B*	*Artemisia absinthium*		weed
W24	Lucerne	*C*	*Medicago sativa*		weed
W24	Spiney Pigweed	*H*	*Amaranthus spinosus*		weed
W101	Spiney Pigweed	*S*	*Amaranthus spinosus*	NA	weed
W25	Geranium	*S*	*Pelargonium spp.*	NA	weed
W53	Geranium	*H*	*Geranium spp.*		weed
W26	Narcissus	*C*	*Narcissus pseudonarcissus*		weed
W26	Narcissus	*H*	*Narcissus spp.*		weed
W27	Carnation	*H*	*Dianthus spp.*		weed
W28	Rose	*C*	*Rosa rugosa*		weed
W28	Rose	*HS*	*Rosa spp.*	NA	weed
W29	Sunflower pollen	*B*	*Helianthus spp.*	NA	weed
W204	Sunflower, Common	*H*	*Helianthus annuus*		weed
W30	Tulip	*HS*	*Tulipa gesneriana*	NA	weed
W30	Tulip	*C*	*Tulipa spp.*		weed
W32	Tulip	*H*	*Tulipa spp.*		weed
W29	Colza	*H*	*Brassica rapa*		weed

Table A-II. Weed Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-Reactivity	Allergen Group and Class
W32	Chamomile	*CS*	*Matricaria chamomilla*	NA	weed
W206	Chamomile	*H*	*Matricaria chamomilla*		weed
W33	Primrose	*S*	*Primula spp.*	NA	weed
W63	Primrose	*H*	*Primula variabilis*		weed
W33	Clover, sweet	*H*	*Trifolium pratense*		weed
W34	Ironwood	*H*	*Ostrya virginiana*		weed
W35	Mexican tea	*H*	*Chenopodium ambrosoides*		weed
W35	Cannabis	*C*	*Cannabis sativa*		weed
W36	Bush, rabbit	*H*	*Franseria deltoides*		weed
W37	Saltbush	*H*	*Atriplex spp.*		weed
W39	Water hemp, Western	*H*	*Acnida tamariscina*		weed
W40	Hyacinth	*H*	*Hyacinthiodes spp.*		weed
W40	Bellis	*C*	*Bellis perennis*		weed
W41	Burrobush	*H*	*Hymenoclea salsola*		weed
W42	Poverty weed	*H*	*Iva axillaris*		weed
W43	Sagebrush, common	*H*	*Artemisia tridentata*		weed
W44	Forsythe	*H*	*Forsythe suspensa*		weed
W45	Alfalfa	*H*	*Medicago sativa*		weed
W46	Sugar beet	*H*	*Beta vulgaris*		weed
W76	Willow herb	*H*	*Epilobium angustifolium*		weed
W77	Aster, China	*H*	*Callistephus chinensi*		weed
W82	Careless Weed	*H*	*Amaranthus palmeri*		weed
W102	Careless Weed	*S*	*Amaranthus palmeri*	NA	weed
W100	Coltsfoot	*H*	*Tussilago farfara*		weed
W203	Rape pollen	*D*	*Brassica napus*	NA	weed

Table A-III. Tree Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
T1	Box Elder (Maple, Sycamore Genus)	*BCHMPQ*	*Acer negundo*	NA	tree-A
T1	Maple	*D*	*Acer saccharum*	NA	tree
T2	Alder	*C*	*Alnus glutinosa*	NA	tree-A
T2	Alder (grey)	*HMPQS*	*Alnus incana*	T3, T7	tree
T2	Alder	*B*	*Alnus rhombifolia*	NA	tree
T2	Alder	*D*	*Alnus rugosa*	NA	tree
T202	Alder (smooth)	*H*	*Alnus rugosa*		tree
T217	Alder (red)	*H*	*Alnus rubra*		tree
T2-I	Aln g 1		*Alnus glutinosa, 17 kD*	NA	tree
T3	Common Silver Birch	*CHMPQS*	*Betula verrucosa*	T2, T7	tree-A
T215	Bet v I		*17 kD*	NA	tree
T216	Bet v II (profilin)		*15 kD*	NA	tree
T3	Birch	*BD*	*Betula nigra*	NA	tree
T30	White Birch	*H*	*Betula populifolia*		tree
T4	Hazel	*CHPQS*	*Corylus avellana*	T2, T3, T5, T7, F17	tree-A
T4-I	Cor a 1		*17 kD*	NA	tree
T4	Hazelnut	*B*	*Corylus spp.*		tree
T4	Hazelnut	*D*	*Corylus americana*	T2, T3, T5, T7, F17	tree
T40	Hazelnut	*H*	*Corylus americana*		tree
T5	American Beech	*BHMPS*	*Fagus grandifolia*	T7	tree
T5	Beech	*D*	*Fagus americana*	T7	tree
T5	Beech	*C*	*Fagus silvatica*	NA	tree-A
T50	European Beech	*H*	*Fagus silvatica*		tree
T6	Mountain Juniper, Mountain Cedar	*BCDHMPQS*	*Juniperus sabinoides*	NA	tree-A
T6-I	Jun s 1		*50 kD*	NA	tree
T?-I	Jun v 1		*Juniper virginiana, 45–50 kD*	NA	tree
T216	Cedar, red	*H*	*Juniperus virginiana*		tree
T7	White Oak	*BCDHMPQS*	*Quercus alba*	T5	tree-A
T7-I	Que a 1		*17 kD*	NA	tree
T103	Live Oak	*HS*	*Quercus virginiana*	T7	tree
T42	Red Oak	*H*	*Quercus rubra*		tree
T8	American Elm, White Elm	*BCDHMPS*	*Ulmus americana*	NA	tree-A
T38	Chinese/Siberian Elm	*H*	*Ulmus pumilla*		tree
T45	Fall Blooming/Scrub Elm	*H*	*Ulmus crassifolia*		tree
T9	Olive (European)	*ABCDHMPQS*	*Olea europea*	T15	tree-A

Table A-III. Tree Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-Reactivity	Allergen Group and Class
T10	Black Walnut	*BD*	*Juglans nigra*	NA	tree
T90	Black Walnut	*H*	*Juglans nigra*		tree
T101	Black Walnut	*S*	*Juglans nigra*	NA	tree
T91	Walnut	*H*	*Juglans regia*		tree
T11	London Plane, Maple Leaf Sycamore	*CHMPS*	*Platanus acerifolia*	NA	tree-A
T11	Sycamore	*BD*	*Platanus occidentalis*	NA	tree
T61	Sycamore	*H*	*Platanus occidentalis*		tree
T12	Willow	*CMP*	*Salix caprea*	T14	tree-A
T12	Willow	*BDS*	*Salix nigra*	NA	tree
T32	Black Willow	*H*	*Salix nigra*		tree
T62	Willow	*H*	*Salix spp.*		tree
T13	Jasmine	*H*	*Jasminum spp.*		tree
T14	Cottonwood, Poplar	*CDHMPS*	*Populus deltoides*	T12	tree-A
T14	Cottonwood	*B*	*Populus trichocarpa*	NA	tree
T15	White Ash	*BCDHMPS*	*Fraxinus americana*	T9	tree-A
T33	Arizona Ash	*H*	*Fraxinus velutina*		tree
T16	White Pine	*CDHPS*	*Pinus strobus*	none known	tree-A
T16	White Pine	*B*	*Pinus monticola*	NA	tree
T26	White Pine	*H*	*Pinus silvestris*		tree
T43	Loblolly Pine	*H*	*Pinus taeda*		tree
T17	Japanese Cedar (Sugi)	*BCDMQ*	*Cryptomeria japonica*	rare	tree-A
T17-I	Cry j 1		*41-45 kD*	NA	tree
T17-II	Cry j 2		*NA*	NA	tree
T17	Chestnut	*S*	*Castanea sativa*	NA	tree
T203	Horse Chestnut	*H*	*Aesculus hippocastanum*		tree
T117	Japanese Cedar (Sugi)	*S*	*Cryptomeria japonica*	rare	tree
T18	Eucalyptus, Gum Tree	*BCHMPS*	*Eucalyptus spp.*	T21	tree-A
T18	Eucalyptus, Gum Tree	*D*	*Eucalyptus globulus*	T21	tree
T19	Acacia, White Sallow, Wattle, Sydney Golden	*CHMPS*	*Acacia longifolia*	T20	tree-A
T19	Acacia	*B*	*Acacia baileyana*	NA	tree
T19	Acacia	*D*	*Acacia spp.*	NA	tree
T29	Acacia	*H*	*Acacia spp.*		tree
T20	Mesquite, Honey Locust	*BDMP*	*Prosopis juliflora*	T19	tree-A
T20	Mesquite	*H*	*Prosopis spp.*		tree
T20	Privet	*S*	*Ligustrum vulgare*	NA	tree
T102	Mesquite, Honey Locust	*S*	*Prosopis juliflora*	T19	tree
T21	Melaleuca, Cajeput-Tree	*DHMP*	*Melaleuca leucadendron*	T18	tree-A

Table A-III. Tree Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-Reactivity	Allergen Group and Class
T21	Aspen	*B*	*Populus tremuloides*	NA	tree
T21	Lilac	*S*	*Syringa vulgaris*	NA	tree
T212	Lilac	*H*	*Syringa vulgaris*		tree
T22	Pecan, Hickory	*MP*	*Carya illinoensis*	T10	tree-A
T100	Pecan, Hickory	*S*	*Carya illinoensis*	T10	tree-A
T22	Pecan	*D*	*Carya illinoensis*	T10	tree
T221	Pecan	*H*	*Carya illinoensis*		tree
T22	Arizona Cypress	*B*	*Cupressus arizonica*	NA	tree
T23	Italian, Funeral Cypress	*CDHPS*	*Cupressus sempervirens*	T6	tree-A
T23	Privet	*MB*	*Ligustrum ovalifolium*	NA	tree
T210	Common Privet	*H*	*Ligustrum vulgare*		tree
T24	Japanese Cypress	*P*	*Chamaecyparis spp.*	NA	tree
T24	Lime Tree	*S*	*Tilia spp.*	NA	tree
T270	Lime Tree	*H*	*Tilia cordata*		tree
T24	Melaleuca (Punk tree)	*B*	*Melaleuca leucadendron*		tree
T25	Australian Pine	*B*	*Casuarina spp.*	NA	tree
T25	Elder	*S*	*Sambucus spp.*	NA	tree
T25	Elder	*C*	*Sambucus nigra*		tree
T260	European Elder	*H*	*Sambucas nigra*		tree
T26	Pecan	*MB*	*Carya illinoensis*	T18	tree
T30	Sorbus	*C*	*Sorbus aucuparia*		tree
T31	Picea	*C*	*Picea abies*		tree
T33	Aesculus	*C*	*Aesculus hippocastanum*		tree
T35	Cedar, salt	*H*	*Tamarix gallica*		tree
T37	Cypress, white bald	*H*	*Taxodium distichum*		tree
T41	Hickory, white	*H*	*Carya alba*		tree
T44	Hackberry	*H*	*Celtus occidentalis*		tree
T47	Juniper, one seed	*H*	*Juniperis monosperma*		tree
T51	Tree of Heaven	*H*	*Ailanthus altissima*		tree
T60	Savin tree	*H*	*Juniperus sabina*		tree
T70	Mulberry	*DHMP*	*Morus alba*	NA	tree
T72	Queen palm	*DP*	*Cocos plumosa*	NA	tree
T106	Queen palm	*S*	*Cocos plumosa*	NA	tree
T272	Queen Palm	*H*	*Cocos plumosa*		tree
T73	Australian Pine	*DH*	*Casuarina equisetifolia*	NA	tree
T104	Australian Pine	*S*	*Casuarina equisetifolia*	NA	tree
?	River Birch	*A*	*Betula nigra*	NA	tree

Table A-III. Tree Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-Reactivity	Allergen Group and Class
T18	Hinoki	*S*	*Chamaecyparis obtusa*	NA	tree
T?	Car b 1 Hornbeam		*Carpinus betulus, 17 kD*	NA	tree
T110	Orange tree	*H*	*Citrus spp.*		tree
T203	Chestnut	*C*	*Castanea sativa*	NA	tree-A
T208	Lime Tree	*C*	*Tilia cordata*		tree
T211	Sweet gum	*H*	*Liquidambar styrcailflua*		tree
T218	Bayberry/sweet gale	*H*	*Myrica gale*		tree
T222	Hawthorn	*H*	*Crataegus spp.*		tree
T250	Golden chain	*H*	*Laburnum spp.*		tree
T280	Locust tree	*H*	*Robinia pseudoacacia*		tree

Table A-IV. Epidermal Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
E1	Cat dander-epithelium	*ABCDHMPQS*	*Felis domesticus*		epidermal-A
E1-I	Fel d 1 (cat 1)-cat saliva		*38 kD*	NA	epidermal
E2	Dog epithelium	*ABDHPQS*	*Canis familaris*	E5	epidermal
E3	Horse dander	*BCDHMPS*			epidermal-A
E4	Cow dander	*BCDHPS*			epidermal-A
E5	Dog Dander (Alsatian/poodle)	*CDHMPQS*	*Canis familaris*	E2	epidermal-A
E5-I	Can f 1		*25 kD*	NA	epidermal
E5-II	Can f 2		*27 kD*	NA	epidermal
E6	Guniea pig epithelium	*BCDHPS*			epidermal-A
E7	Pigeon droppings	*BCDHPS*			epidermal
E11	Pigeon feathers	*C*			epidermal
E215	Pigeon feathers	*H*			epidermal
E21	Bovine serum	*H*			epidermal
E22	Mink hair	*H*			epidermal
E23	Pigeon serum	*H*			epidermal
E24	Canary serum	*H*			epidermal
E25	Chicken serum	*H*			epidermal
E25	Feather mix (chicken, duck, goose)	*B*			epidermal
E26	Parrot serum	*H*			epidermal
E27	Lovebird feathers	*H*			epidermal
E50	Finch feathers	*H*			epidermal
E51	Finch droppings	*H*			epidermal
E52	Fox	*H*			epidermal
E62	Camel	*H*			epidermal
E70	Goose feathers	*CDHS*			epidermal-A
E71	Mouse epithelium	*BCDHS*	*Mus musculus*	E72, E76	epidermal-A
E72	Mouse urine proteins	*BCDHS*	*Mus musculus*	E71, E76	epidermal
E72-I	Mus m 1 (MUP)		*19 kD*	NA	epidermal
E73	Rat epithelium	*BCDHS*	*Rattus norvegius*	E74, E75	epidermal-A
E74	Rat urine proteins	*CDHS*	*Rattus norvegius*	E73, E75	epidermal
E74-I	Rat n 1		*17 kD*	NA	epidermal
E75	Rat serum proteins	*DHS*	*Rattus norvegius*	E73,E74	epidermal
E76	Mouse serum proteins	*DHS*	*Mus musculus*	E71, E72	epidermal
E77	Budgerigar droppings	*BCDHPS*			epidermal
E78	Budgerigar feathers	*BCDHPS*			epidermal-A
E79	Budgerigar serum proteins	*CHPS*			epidermal

Table A-IV. Epidermal Allergens (Continued)

Code	Common Name 9Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
E82	Rabbit epithelium	*BCDHMPS*			epidermal-A
E83	Swine epithelium	*BCDHPS*			epidermal-A
E84	Hamster epithelium	*BCDHPS*			epidermal-A
E85	Chicken feathers	*CDHPS*			epidermal-A
E86	Duck feathers	*BCDHPS*			epidermal-A
E87	Rat Epithelium and Protein	*H*	*Rattus norvegius*		epidermal
E88	Mouse Epithelium and Protein	*H*	*Mus musculus*		epidermal
E89	Turkey feathers	*H*			epidermal
E89	Rat urine	*B*			epidermal
E90	Canary feathers	*CS*			epidermal
E90	Rat droppings	*H*			epidermal
E91	Parrot feathers	*CDS*			epidermal
E213	Parrot feathers	*H*			epidermal
E91	Lovebird droppings	*H*			epidermal
E92	Mouse droppings	*H*			epidermal
E93	Parakeet feathers	*H*			epidermal
E95	Duck down	*H*			epidermal
E96	Goose down	*H*			epidermal
E98	Parrot droppings	*H*			epidermal
E99	Canary feathers	*H*			epidermal
E201	Canary feathers	*H*			epidermal
E204	Bovine serum albumin	*H*			epidermal
E208	Chinchilla	*H*			epidermal
E209	Gerbil hair	*H*			epidermal
E216	Deer hair/dander	*H*			epidermal

Table A-V. Mold Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
M1	Penicillium (spores/mycelium)	*ABCDHMPQS*	*Penicillium notatum*	M9	mold-A
M2	Cladosporium (spores/mycelium)	*BCDHMPQS*	*Cladosporium herbarum*	NA	mold -A
M17	Cladosporium fulvum	*H*	*Cladosporium fulvum*		mold
M32	Cladosporium cladospor	*H*	*Cladosporium cladosporoides*		mold
M3	Aspergillus (spores/mycelium)	*ABCDHMPQS*	*Aspergillus fumigatus*	NA	mold-A
M3-I	Asp f 1		*18 kD*	NA	mold
M3-?	Asp f ?		*90 kD*	NA	mold
M4	Mucor (spores/mycelium)	*BCDHMPS*	*Mucor racemosus*	NA	mold-A
M20	Mucor mucedo	*H*	*Mucor mucedo*		mold
M22	Mucor spinosus	*H*	*Mucor spinosus*		mold
M5	Candida (disrupted cells)	*BCDHMPQS*	*Candida albicans*	NA	mold-A
M5-I	Cand a ?		*40 kD*	NA	mold
M6	Alternaria (spores/mycelium)	*ABCDHMPQS*	*Alternaria alternata/tenuis*	M10, M16	mold-A
M6-I	Alt a 1		*28 kD*	NA	mold
M7	Botrytis (spores/mycelium)	*CDHMPS*	*Botrytis cinerea*	NA	mold-A
M8	Helminthosporium (spores/mycelium)	*CDHMPS*	*Helminthosporium halodes*	NA	mold-A
M80	Helminthosporium interseminatum	*H*	*Helminthosporium interseminatum*		mold
M9	Fusarium (spores/mycelium)	*DHMPS*	*Fusarium moniliforme*	M1, M3, M6	mold-A
M9	Fusarium	*C*	*Fusarium spp.*		mold
M18	Fusarium colmorum	*H*	*Fusarium colmorum*		mold
M49	Fusarium oxysporium	*H*	*Fusarium oxysporium*		mold
M10	Stemphylium (spores/mycelium)	*CDHMPS*	*Stemphylium botryosum*	M6, M16	mold-A
M11	Rhizopus (spores/mycelium)	*CDHMPS*	*Rhizopus nigricans*	NA	mold-A
M12	Aureobasidium (spores/mycelium)	*CDHPS*	*Aureobasidium pullulans*	NA	mold-A
M13	Phoma (spores/mycelium)	*CDHMPS*	*Phoma betae*	NA	mold-A
M14	Epicoccum (spores/mycelium)	*CDHMPS*	*Epicoccum nigrum*	NA	mold-A
M15	Trichoderma (spores/mycelium)	*CDHMPS*	*Trichoderma viride*	NA	mold-A
M16	Curvularia (spores/mycelium)	*CDHMPS*	*Curvularia lunata*	M6, M10	mold-A
M46	Curvularia spicifera	*H*	*Curvularia spicifera*		mold
M17	Aspergillus	*S*	*Aspergillus amstelodami*	NA	mold

Table A-V. Mold Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
M36	Aspergillus	*H*	*Aspergillus terreus*		mold
M19	Aspergillus	*HS*	*Aspergillus versicolor*	NA	mold
M21	Aspergillus	*H*	*Aspergillus clavatus*		mold
M29	Aspergillus	*H*	*Aspergillus repens*		mold
M33	Aspergillus	*H*	*Aspergillus niger*		mold
M47	Aspergillus	*H*	*Aspergillus nidulans*		mold
M48	Aspergillus	*H*	*Aspergillus oryzae*		mold
M20	Neurospora	*CS*	*Neurospora intermedia (sitophila)*		mold
M23	Neurospora	*H*	*Neurospora intermedia (sitophila)*		mold
M21	Paecilomyces	*CS*	*Paecilomyces spp.*	NA	mold
M24	Paecilomyces	*H*	*Paecilomyces spp.*		mold
M22	Penicillium	*S*	*Penicillium brevicompactum*	NA	mold
M25	Penicillium	*H*	*Penicillium brevicompactum*		mold
M26	Penicillium	*H*	*Pencillium citrinum*		mold
M27	Penicillium	*H*	*Pencillium commune*		mold
M28	Penicillium	*H*	*Pencillium expansum*		mold
M30	Penicillium	*H*	*Pencillium roqueforti*		mold
M31	Penicillium	*H*	*Pencillium viridicatum*		mold
M23	Merulius	*S*	*Merulius lacrymans*	NA	mold
M34	Merulius	*H*	*Merulius lacrymans*		mold
M24	Sporobolomyces	*S*	*Sporoblolomyces roseus*	NA	mold
M35	Sporobolomyces	*H*	*Sporoblolmyces roseus*		mold
M25	Aspergillus	*S*	*Aspergillus flavus*	NA	mold
M29	Chaetomium	*C*	*Chaetomium globosum*		mold
M208	Chaetomium	*H*	*Chaetomium globosum*		mold
M35	Rhodotorula	*C*	*Rhodotorula rubra*		mold
M37	Sporobolomyces	*C*	*Sporobolomyces salmonicolor*		mold
M40	Malassezia furfur	*C*	*Pityrosporum ovale (Mal.f)*		mold
M42	Aureobasidium pullulans	*H*	*Aureobasidium pullulans*		mold
M43	Saccharomyces carlsbergensis	*H*	*Saccharomyces carlsbergensis*		mold
M44	Saccharomyces cerevisae	*H*	*Saccharomyces cercvisae*		mold

Table A-V. Mold Allergens (Continued)

Code	Common NamePurified Allergen Code	Sourc	Latin NameMolecular Weight	KnownCross-reactivity	AllergenGroup and Class
M202	Cephalosporium (spores/myelium)	*D*	*C. acremonium*	NA	mold
M203	Trichosporon		*Trichosporon pullulans*	NA	mold
M204	Ulocladium		*Ulocladium chartarum*	M6	mold
M205	Trichophyton	*HDM*	*Trichophyton rubrum*	NA	mold
M37	Trichophyton	*H*	*Trichophyton mentagrophytes*		mold
M39	Trichophyton	*H*	*Trichophyton verrucosum*		mold

Table A-VI. Mite Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
D1	House dust mite	*ABCDHMPQS*	*Dermatophagoides pteronyssinus*		mite-A
D1-I	Der p 1 (antigen P1)		*25 kD*		mite
D1-II	Der p 2		*14 kD*		mite
D1-III	Der p 3 (trypsin)		*28/30 kD*		mite
D1-IV	Der p 4 (amylase)		*60 kD*		mite
D1-V	Der p 5		*14 kD*		mite
D1-VI	Der p 6 (chymotrypsin)		*25 kD*		mite
D1-VII	Der p 7		*22-28 kD*		mite
D2	House dust mite	*ABCDHMPQS*	*Dermatophagoides farinae*		mite-A
D2-I	Der f 1		*25 kD*		mite
D2-II	Der f 2		*14 kD*		mite
D2-III	Der f 3		*30 kD*		mite
D3	House dust mite	*BCDHP*	*Dermatophagoides microceras*		mite-A
D3-I	Der m 1		*25 kD*		mite
D70	Storage mite	*BCDHPS*	*Acarus siro*		mite-A
D71	Storage mite	*BCDHPS*	*Lepidoglyphus destructor*		mite-A
D71-?	Lep d 1		*15 kD*		mite
D72	Storage mite	*BCDHPS*	*Tyrophagus putrescentiae*		mite-A
D73	Storage mite	*BCDHS*	*Glycyphagus domesticus*		mite-A
D74	Storage mite (house dust mite)	*BCDHS*	*Euroglyphus maynei*		mite-A

Table A-VII. Dust Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
H1	House dust (Greer Laboratories)	*BCDPQS*			dust-A
H2	House dust (Hollister-Stier Lab)	*BCDHPQS*			dust-A
H3	House dust (Bencard)	*C*			dust-A
H3	House dust (Biowhittaker)	*B*			dust
H4	House dust (Allergopharma)	*CDH*			dust-A
H5	House dust (HAL)	*H*			dust
H6	House dust (Japan)	*D*			dust

Table A-VIII. Venom Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
I1	Honeybee (venom—collected by electric stimulation)	*BCDHPQS*	*Apis mellifera*		venom-A
I1-I	Api m 1 (phospholipase A_2)		*16 kD*		venom
I1-II	Api m 2 (hyaluronidase)		*44 kD*		venom
I1-IV	Api m 4 (melittin)		*3 kD*		venom
I2	White-faced or bald-faced hornet American/European) or wasp (Great Britain) (venom from dissected sacs)	*BDHPQS*	*Dolichovespula maculata*		venom-A
I2-I	Dol m 1 (Phospholipase A_1)		*35 kD*		venom
I2-II	Dol m 2 (Hyaluronidase)		*44 kD*		venom
I2-V	Dol m 5 (antigen 5)		*23 kD*		venom
I3	Yellow jacket (American), Common wasp (European)-venom-dissected sacs	*BCDPS*	*Vespula spp., V. vulgaris, V. rufa, V. squamosa groups*		venom-A
I3-Vf	Ves f 5 (antigen 5)		*Vespula flavopilosa, 23 kD*		venom
I3-Vg	Ves g 5 (antigen 5)		*Vespula germanica, 23 kD*		venom
I3-Im	Ves m 1 (phospholipase A1)		*Vespula maculifrons, 33.5 kD*		venom
I3-IIm	Ves m 2 (hyaluronidase)		*Vespula macrulifrons, 44 kD*		venom
I3-Vm	Ves m 5 (antigen 5)		*Vespula maculifrons, 23 kD*		venom
I3-Vp	Ves p 5 (antigen 5)		*Vespula pensylvanica, 23 kD*		venom
I3-Vs	Ves s 5 (antigen 5)		*Vespula squamosa, 23 kD*		venom
I3-Vvi	Ves vi 5 Wasp		*Vespula vidua, 23 kD*		venom
I3-Iv	Ves v 1 (phospholipase A_1)		*Vespula vulgaris, 35 kD*		venom
I3-IIv	Ves v 2 (hyaluronidase)		*Vespula vulgaris, 44 kD*		venom
I3-Vv	Ves v 5 (antigen 5)		*Vespula vulgaris, 23 kD*		venom
I3-Ic	Ves c 1 (phospholipase) Hornet		*Vespa crabro, 34 kD*		venom
I3-Vc1	Ves c 5.0101 (antigen 5) Hornet		*Vespa crabro, 23 kD*		venom

Table A-VIII. Venom Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
I4-I	Pol a 1 (phospholipase A_1)		*Polistes annularis, 35 kD*		venom
I4-IIa	Pol a 2 (hyaluronidase)		*Polistes annularis, 44 kD*		venom
I4-Va	Pol a 5 (antigen 5)		*Polistes annularis, 23 kD*		venom
I4-Ie	Pol e 1 (hyaluronidase)		*Polistes exclamans, 35 kD*		venom
I4-Ve	Pol e 5 (antigen 5)		*Polistes exclamans, 23 kD*		venom
I4-Vf	Pol f 5 (antigen 5)		*Polistes fuscatus, 23 kD*		venom
I4-Vm	Pol m 5 (antigen 5)		*Polistes metricus, 23 kD*		venom
I5	Yellow-hornet, yellow-faced hornet (American) (venom from–dissected sacs)	*BDPS*	*Dolichovespula arenaria*		venom
I5-5	Dol a 5 (antigen 5)		*23 kD*		venom
I6	Cockroach (whole bodies)	*BCDHPS*	*Blattella germanica*		insect-A
I6-I	Bla g 1				insect
I6-II	Bla g2		*20 kD*		insect
I7	Midge	*P*	*Chironomus yoshimatsui*		insect
I7-I	Chi t 1 (hemoglobin)	*P*	*Chironomus thummi, 16 kD*		insect
I17	Midge	*H*	*Chironomus yoshimatsui*		insect
I7	Bumble bee	*S*	*Bombus spp.*	NA	venom
I7-I	Bom p 1 (phospholipase)		*Bombus pennsylvanicus, 16 kD*		venom
I7-IV	Bom p 4 (protease)		*Bombus pennsylvanicus*	NA	venom
I8	Moth		*Bombyx mori*		insect
I12	Ant	*S*	*Formica spp.*		insect
I14	Horse fly	*S*	*Tabanus spp.*		insect
I27	Sudan fly	*H*			insect
I37	Red midge larva	*H*			insect
I40	Gadfly	*H*			insect
I47	Waterflea	*H*			insect
I70	Fire ant (whole bodies-S invicta)	*BDHPS*	*Solenopsis invicta*		venom
I70-II	Sol i 2		*13 kD*		venom
I70-III	Sol i 3		*24 kD*		venom

Table A-VIII. Venom Allergens (Continued

Code	ommon Name Purified Allergen Code	ource	atin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
I72	Green nimitti midge	*S*	*Cladotanytarsus lewisi*		insect
I73	Blood worm (freeze-dried larvae)	*P*	*Chironomus riparius*		insect
I73	Blood worm (freeze-dried larvae)	*D*	*Chironomus plumosus*		insect
I73	Blood worm (freeze-dried larvae)	*CS*	*Chironomus thummi*		insect
I74	NA		*NA*		insect
I75	Hornet, European hornet (American)	*BCH*	*Vespa crabro*		venom-A
I76	Berlin beetle (whole larvae)		*Trogoderma angustum*		insect
I80	Bumblebee	*H*	*Bombus spp.*		insect
I100	American cockroach	*CS*	*Periplaneta americana*	NA	insect-A
I201	Horse bot fly (whole bodies)		*Gasterophilus intestinalis*		insect
I202	Grain weevil		*Sitophilus granarius*		insect
I203	Mediterranean flour moth		*Ephestia kuehniella*		insect

Table A-IX. Food Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F1	Egg white	*BCDHMPQS*			A
F2	Cow's milk (skimmed milk)	*BCDHPQS*	*NA*	F231	dairy-A
F231	Boiled cow's milk	*H*	*NA*	F2	dairy
F3	Codfish (fish muscle)	*BCDHMPQS*	*Gadus morhua*		fish-A
F3-I	Gad c 1 (allergen M)		*Gadus callarias, 12 kD*		fish
F4	Wheat (whole grain)	*BCDHMPQS*	*Triticum aestivum*		grain/grass -A
F5	Rye (whole grain)	*BCDHMPS*	*Secale cereale*		grain/grass -A
F6	Barley (whole grain) cereal flour	*BCDHMPS*	*Hordeum vulgare*	G5, F4, F90	grain/grass -A
F7	Oats (whole grain)	*BCDHMPS*	*Avena sativa*	F4, F5, F6	grain/grass -A
F8	Maize, corn, polenta (whole grain)	*BCDHMPQS*	*Zea mays*		grain/grass -A
F9	Rice (unpolished rice)	*BCDHMPQS*	*Oryza sativa*	F4, F5, F8	grain/grass -A
F10	Sesame seed (whole seed)	*CDHMPS*	*Sesamum indicum*		seed/nut-A
F11	Buckwheat (whole seed)	*BCDHMPS*	*Fagopyrum esculentum*		grain-A
F12	Pea (ripe dried seed)	*BCDHMPS*	*Pisum sativum*		legume-A
F13	Peanut (raw and shelled)	*BCDHMPQS*	*Arachis hypogaea*		legume-A
F14	Soybean (ripe dried seed)	*BCDHMPQS*	*Glycine max*		legume-A
F15	White bean (ripe dried seed)	*CDHMPS*	*Phaseolus vulgaris*		legume-A
F15	Navy bean	*B*	*NA*		legume
F16	Walnut	*BC*			seed/nut
F17	Hazel nut, filbert (shelled nuts)	*BCDHMPQS*	*Corylus avellana*		seed/nut-A
F18	Brazil nut (shelled nuts)	*CDHPS*	*Bertholletia excelsa*		seed/nut-A
F19	Edible chestnut	*B*	*NA*		seed/nut
F20	Almond (sweet almonds)	*BCDHMPS*	*Amygdalus communis*		seed/nut-A
F21	Herring	*C*			fish-A
F21	Cane sugar	*H*			food
F22	Trout	*CS*			fish
F204	Trout	*H*			fish
F23	Crab (boiled crab meat)	*BCDHMPQS*	*Cancer pagurus*		crustacean -A
F24	Shrimp (boiled Atlantic shrimp)	*CDHBS*	*Pandalus borealis*		crustacean -A

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F25	Tomato (frozen juice)	*BCDHMPQS*	*Lycopersicon lycopersicum*		vegetable-A
F26	Pork	*BCHMPS*	*Sus* spp.		meat-A
F27	Beef	*BCDHMPS*	*Bos* spp.		meat-A
F29	Watermelon	*H*			fruit
F30	Grapefruit	*C*			fruit
F30	Aubergine	*H*			vegtable
F31	Carrot (fresh frozen)	*BCDHMPS*	*Daucus carota*		vegetable-A
F32	Lemon	*C*			fruit
F32	Oyster mushroom	*H*			fungi
F33	Orange (fresh frozen juice)	*BCDHMPQS*	*Citrus sinensis*		fruit-A
F35	Potato (fresh)	*BCDHMPS*	*Solanum tuberosum*		fruit-A
F36	Coconut (dry coconut)	*CDHPS*	*Cocos nucifera*		seed/nuts-A
F37	Blue mussel (canned mussel)	*CDHPS*	*Mytilus edulis*		mollusk-A
F38	Spinach	*B*			vegetable
F40	Tuna, Yellow Fin (fish muscle)	*BCDHMPS*	*Thunnus albacares*		fish-A
F41	Salmon (Atlantic) (fish muscle)	*BCDHMPS*	*Salmo salar*		fish-A
F42	Haddock	*H*			fish
F43	Mother's milk	*H*			food
F44	Strawberry (frozen berry)	*BCDHMPS*	*Fragaria vesca*		fruit-A
F45	Yeast (brewer's yeast)	*BCDHMPS*	*Saccharomyces cerevisiae*		fungi-A
F155	Brewer's yeast	*H*	*Saccharomyces cerevisiae*		fungi
F45	Baker's yeast	*H*			fungi
F337	Yeast	*H*			fungi
F46	Cabbage	*B*			vegetable
F47	Garlic (garlic powder)	*CDHMPS*	*Allium sativum*	F48, F261	vegetable-A
F47	Lettuce	*B*			vegetable
F48	Onion (freeze-dried)	*CDHP*	*Allium cepa*		vegetable
F108	Onion (freeze-dried)	*S*	*Allium cepa*		vegetable
F48	Pepper (bell)	*B*			vegetable
F152	Green bell pepper	*H*			vegetable
F46	Red pepper	*H*			vegetable
F49	Apple (whole, ripe, green apple)	*CBDHPS*	*Malus sylvestris (Pyrus malus)*	F17, F35, F31, T3	fruit-A

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F51	Grape	*B*			fruit
F52	Chocolate	*BHM*			food
F54	Sweet potato (R) (fresh tuber)		*Ipomea batatas*		vegetable
F122	Sweet potato	*H*	*Ipomea batatas*		vegetable
F55	Common millet (R), proso	*HS*	*Panicum milliaceum*		grain-grass
F56	Italian Foxtail millet (R)		*Setaria italica*	F9	grain-grass
F56	Sea perch	*S*		NA	fish
F57	Japanese millet, cockspur, Barnyard grass		*Echinochloa crus-galli*		grain-grass
F57	Goose	*S*		NA	fowl
F158	Goose	*H*			fowl
F58	Pacific squid/flying squid (meat)		*Todarodes pacificus*	F258	mollusk
F58	Duck	*CS*		NA	fowl
F157	Duck	*H*			fowl
F59	Octopus (fresh frozen muscle)		*Octopus vulgaris*		mollusk
F60	Jack mackerel/scad (fish muscle)		*Trachurus japonicus*		fish
F61	Sardine, Japanese Pilchard	*H*	*Sardinops melanosticta*	F308	fish
F63	Kefir	*H*			dairy
F65	Perch	*H*			fish
F65	Lentil	*B*		NA	legume
F235	Lentil	*H*			legume
F67	Cheese, parmesan	*H*			dairy
F68	Ewe's cheese	*H*			dairy
F72	Pineapple	*B*			fruit
F72	Coriander	*H*			food
F75	Egg yolk (freeze-dried)	*BCDHMPS*			dairy-A
F245	Whole egg	*H*			dairy
F76	Alpha lactalbumin (cow's milk, major cow milk allergen)	*BCDHPS*		F2	dairy
F77	Beta-lactoglobulin (cow's milk, major whey milk protein)	*BCDHPS*		F2	dairy
F78	Casein, (added to infant formula)	*BCDHMPS*		F2	dairy-A

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F81	Cheese, cheddar type	*BCDHMPS*	*NA*		dairy-A
F82	Cheese, mold type	*BCDHPS*	*NA*		dairy-A
F83	Chicken meat	*BCDHMPS*	*Gallus* spp.		fowl-A
F233	Gal d 1Ovomucoid hens egg white	*H*	*Gallus domesticus,* *28 kD*		dairy
F232	Gal d 2 Ovalbumin hens egg white	*H*	*Gallus domesticus, 44 kD*		dairy
F83-III	Gal d 3-conalbumin (Ag22)		*Gallus domesticus,* *78 kD*		dairy
F83-IV	Gal d 4-lysozyme		*Gallus domesticus,* *14 kD*		dairy
F84	Kiwi fruit, Chinese gooseberry	*BCDHPS*	*Actinidia chinensis*		fruit-A
F85	Celery (fresh roots/stalks)	*BCDHMPS*	*Apium graveolens*		vegetable-A
F86	Parsley (fresh leaves)	*BCHPS*	*Petroselinum crispum*		spice
F87	Melon (honeydew melon)	*BCDHPS*	*Cucumis melo*		fruit
F88	Mutton, lamb	*CDHMPS*	*Ovis* spp.		meat
F89	Mustard (black/white seeds)	*CDHPS*	*Brassica/Sinapis*		spice-A
F89-Ib	Bra j 1 (25 albumin) oriental mustard		*Brassica juncea, 14 kD*		spice
F89-Is	Sin a 1 (25 albumin) yellow mustard		*Sinapis alba, 14 kD*		spice
F90	Malt-barley	*CDHMP*	*Hordeum vulgaris*	F6	from grains
F90-I	Hor v 1 (BMAI-1)		*14 kD*		from grains
F102	Malt-barley or sorghum	*S*	*Hordeum vulgare*	F6	from grains
F91	Mango (whole fruit)	*BCDHP*	*Mangifera indica*		fruit
F93	Mango (whole fruit)	*P*	*Mangifera indica*		fruit
F92	Banana (ripe, fresh fruit)	*BCDH*	*Musa spp.*	F87, W1, K82	fruit
F29	Banana (ripe, fresh fruit)	*S*	*Musa spp.*	F87, W1, K82	fruit
F93	Cacao (cocoa powder)	*BCH*	*Theobroma cacao*		stimulant
F73	Cacao (cocoa powder)	*S*	*Theobroma cacao*		stimulant
F94	Pear (fresh fruit)	*BCHS*	*Pyrus communis*		fruit
F95	Peach (fresh fruit)	*BCDHS*	*Prunus persica*		fruit
F96	Avocado	*CDHS*	*Persea americana*	F84	fruit
F96	Avocado, Alligator pear, Prickly pear (fresh, ripe pulp)	*M*	*Persea americana*	F84	fruit
F97	Cherry	*C*			fruit
F98	Millet	*C*			grain
F99	Sunflower seeds	*C*			seed/nut

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F104	Onion	*B*			vegetable
F104	Laurel	*H*			food
F105	Chocolate	*DS*			stimulant
F105	Garlic	*B*			vegetable
F106	Paprika	*B*	*NA*		spice
F249	Paprika	*H*			spice
F109	Cottonseed	*S*		NA	seed/nut
F110	Giant radish	*H*			vegetable
F115	Vinegar plant	*H*			food
F117	Savoy	*H*			food
F118	Zucchini	*H*			food
F119	Radish	*H*			vegetable
F120	Venison	*H*			meat
F121	Pinto bean	*H*			legume
F124	Plaice	*C*			fish
F124	Spelt	*H*			food
F126	Peppermint herbs	*H*			spice
F129	Sole	*H*			fish
F131	Black olive	*H*			vegtable
F132	Wild boar	*H*			meat
F132	Green bean	*C*			vegetable
F135	Summer squash	*H*			vegtable
F136	Red beet	*H*			vegetable
F139	Goat's cheese	*H*			dairy
F140	Bran	*H*			grain
F141	Corn flour	*H*			grain
F142	Flounder	*H*			fish
F142	Veal	*C*			meat
F143	Roquefort cheese	*H*			dairy
F148	Casto bean oil	*H*			
F149	Sago	*H*			food
F150	Edam cheese	*H*			dairy
F151	Soybean (bruised grain)	*H*			grain
F156	Potato powder	*H*			vegetable
F156	Raspberry	*C*			fruit
F22	Raspberry	*H*			fruit
F159	Chickpea	*B*	*NA*		legume

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F162	Nectarine	*H*			fruit
F163	Turnip-cabbage	*C*			vegetable
F163	Kohlrabi	*H*			food
F164	Cress	*H*			food
F166	Leek	*H*			food
F170	Cheese (Switzerland)	*H*			dairy
F171	Black currant	*C*			food
F172	Flaxseed	*C*			food
F174	Fig	*H*			fruit
F176	Red currant	*H*			food
F177	Cranberry	*H*			fruit
F178	Rhubarb	*H*			fruit
F179	Raisin	*H*			fruit
F180	Morello cherry	*H*			fruit
F181	Gooseberry	*H*			fruit
F183	Sunflower seed	*H*			seed/nut
F188	Radish	*C*			vegetable
F189	Red cabbage	*C*			vegetable
F198	Flaxseed (bruised grain)	*H*			grain
F201	Pecan (peeled nut)	*D*	*Carya illinoensis*		seed/nut
F103	Pecan (peeled nut)	*HS*	*Carya illinoensis*		seed/nut
F133	Cashew	*H*	*Anacardium occidentale*		seed/nut
F202	Cashew nut (shelled nuts)	*H*	*Anacardium occidentale*		seed/nut
F203	Pistachio nut (fresh, mashed nuts)	*H*	*Pistacia vera*		seed nut
F204	Rainbow trout (fish muscle)		*Oncorhynchus mykiss*		fish
F205	Herring (fish muscle)	*H*	*Clupea harengus*		fish
F21	Herring (fish muscle)	*S*	*Clupea harengus*		fish
F206	Mackerel (fish muscle)	*C*	*Scomber scombrus*		fish-A
F207	Clam	*H*			mollusk
F208	Lemon (whole fruit)	*H*	*Citrus limon*		fruit
F32	Lemon (whole fruit)	*S*	*Citrus limon*		fruit
F209	Grapefruit (whole fruit)	*H*	*Citrus paradisi*		fruit
F30	Grapefruit (whole fruit)	*S*	*Citrus paradisi*		fruit
F210	Pineapple (fresh fruit)	*H*	*Ananas comosus*		fruit
F72	Pineapple (fresh fruit)	*S*	*Ananas comosus*		fruit
F211	Blackberry (frozen berry)	*H*	*Rubus fruticosus*		fruit
F212	Mushroom, champignon (whole)	*H*	*Agaricus hortensis*		fungi

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F215	Gooseberry	*C*			
F215	Lettuce (fresh lettuce)	*DH*	*Lactuca sativa*		vegetable
F100	Lettuce (fresh lettuce)	*S*	*Lactuca sativa*		vegetable
F216	Cabbage (whole head)	*CHM*	*Brassica oleracera var capitata*		vegetable
F112	Red cabbage	*H*	*Brassica oleracera var capitata*		vegetable
F113	Chinese cabbage	*H*			vegetable
F115	Pickled cabbage	*H*			vegetable
F217	Brussel sprouts (fresh frozen)	*H*	*Brassica oleracea var gemmifera*		vegetable
F218	Sweet pepper (powder)		*Capsicum annuum*		vegetable
F218	Red pepper	*H*			spice
F219	Fennel seed (seeds)		*Foeniculum vulgare*	F276	spice
F116	Fennel	*H*	*Foeniculum vulgare*		spice
F220	Cinnamon (dried bark)	*CH*	*Cinnamonum* spp.		spice
F221	Coffee (roasted coffee)	*H*	*Coffea* spp.		stimulant
F74	Coffee (roasted coffee)	*S*	*Coffea* spp.		stimulant
F222	Tea (Earl Grey–Twinings)	*H*	*Camellia sinensis*		stimulant
F113	Tea (Earl Grey–Twinings)	*S*	*Camellia sinensis*		stimulant
F97	Camomile tea	*H*			stimulant
F125	Peppermint tea	*H*			stimulant
F223	Green olive	*H*			vegtable
F224	Poppy seed (dried seeds)	*CDH*	*Papaver somniferum*		seed/nut
F225	Pumpkin (fresh fruit)		*Cucurbita pepo*		vegetable
F226	Pumpkin seeds (whole seeds)		*Cucurbita pepo*		seed nut
F227	Sugar beet seed (whole seed)		*Beta vulgaris*		seed/nut
F234	Vanilla (dried vanilla bean)	*CH*	*Vanilla planifolia*		spice
F236	Whey (powder)-byproduct of cheese making	*C*			dairy
F237	Apricot	*H*			fruit
F238	Oregano (dried leaves)	*H*	*Origanum vulgare*		spice
F239	Cuttle fish	*F*			fish
F241	Gouda cheese	*H*			dairy
F242	Cherry (frozen berry)		*Prunus avium*		fruit
F242	Bing cherry	*H*			fruit
F244	Cucumber (fresh fruit)	*CH*	*Cucumis sativus*		vegetable
F246	Flour of “Guar” kernel	*H*			grain

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F252	Groats	*H*			food
F253	Pine nut, pignoles (seeds)		*Pinus edulis*		seed/nut
F254	Plaice (fish muscle)	*H*	*Pleuronectes platessa*		fish
F255	Plum (pitted fruit)	*CH*	*Prunus domestica*		fruit
F256	Walnut (shelled nuts)	*HM*	*Juglans* spp.		seed/nut
F16	Walnut (shelled nuts)	*S*	*Juglans* spp.		seed/nut
F257	Coconut	*H*			fruit
F258	Squid (squid meat)		*Loligo* spp.	F58	mollusk
F259	Grape (fresh fruit)	*CH*	*Vitis vinifera*		fruit
F260	Broccoli (fresh frozen florets)	*H*	*Brassica oleracea var italica*		vegetable
F261	Asparagus (fresh green shoots)	*H*	*Asparagus officinalis*		vegetable
F262	Aubergine, eggplant (unpeeled)		*Solanum melongena*		vegetable
F263	Pepper, green (dried, unripe seeds)		*Piper nigrum*		spice
F264	Eel (fish muscle)	*H*	*Anguilla anguilla*		fish
F55	Eel (fish muscle)	*CS*	*Anguilla anguilla*		fish
F265	Caraway (seeds)	*C*	*Carum carvi*		spice
F266	Masce (dried arrillus)		*Myristica fragrans*		spice
F267	Cardamom (cardemon) (seeds)		*Elettaria cardamomum*		spice
F268	Clove (dried flower)	*CH*	*Syzygium aromaticum*		spice
F269	Basil (dried leaves)	*H*	*Ocimum basilicum*		spice
F270	Ginger (dried root)	*CH*	*Zingiber officinale*		spice
F271	Anise (herb of carrot family) seeds	*CH*	*Pimpinella anisum*		spice
F272	Tarragon (dried leaves)		*Artemisia dracunculus*		spice
F273	Thyme (dried leaves)	*H*	*Thymus vulgaris*		spice
F274	Marjoram (dried leaves)	*CH*	*Origanum majorana*		spice
F275	Lovage (dried leaves)		*Levisticum officinale*		spice
F276	Fennel (fresh leaves, stalks)		*Foeniculum vulgare*	F219	spice
F277	Dill (fresh leaves/crowns)	*CH*	*Anethum graveolens*		spice
F278	Bay leaf (dried leaves)		*Laurus nobilis*		spice
F279	Pepper, chili, cayenne (dried fruit)	*H*	*Capsicum frutescens*		spice
F19	Pepper, cayenne	*H*	*Capsicum frutescens*		spice

Table A-IX. Food Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
F90	Pepper, black (dried seeds)	*S*	*Piper nigrum*		spice
F281	Curry (powder-Santa Maria)	*CH*	*NA*		mix of spices
F282	Nutmeg (seed)	*CH*	*Myristica fragrans*		spice
F92	Nutmeg (seed)	*S*	*Myristica fragrans*		spice
F284	Turkey (raw meat)	*HM*	*Meleagris* spp.		fowl
F285	Elk, moose		*Alces* spp.		meat
F286	Mare's milk			F78	dairy
F287	Red kidney bean (ripe, dried seed)	*H*	*Phaseolus vulgaris*		legume
F288	Blueberry (frozen berry)	*H*	*Vaccinium myrtillis*		fruit
F289	Date (whole dates)		*Phoenix dactylifera*		fruit
F290	Oyster (fresh whole oyster)	*H*	*Ostrea edulis*		mollusk
F291	Cauliflower (whole head)	*CH*	*Brassica oleracea var botrytis*		vegetable
F161	Cauliflower (boiled)	*H*	*Brassica oleracea var botrytis*		vegetable
F292	Guava, guayaba (whole fruit)		*Psidium guajava*		fruit
F293	Papaya (fresh fruit)		*Carica papaya*		fruit
F294	Passion fruit (fresh fruit)		*Passiflora edulis*		fruit
F295	Star fruit, carambola, karambol, tree cherry (fresh fruit)		*Averrhoa carambola*		fruit
F296	Carob-bean	*H*			legume
F299	Sweet chestnut (whole nut)	*DH*	*Castanea sativa*		seed/nut
F19	Sweet chestnut (whole nut)	*S*	*Castanea sativa*		seed/nut
F300	Goat's milk				dairy
F300	Pinto bean	*D*			seed/nut
F301	Persimmon, kaki (whole fresh fruit)		*Diospyros kaki*		fruit
F301	Fishmeal	*C*			
F302	Mandarin, clementine, satsumas, tangerine (peeled whole fruit)		*Citrus reticulata*		fruit
F302	Tangerine	*H*	*Citrus reticulata*		fruit
F302	Bolted flour	*C*			
F303	Halibut (fish muscle)		*Hippoglossus hippoglossus*		fish
F304	Spiny lobster/langust (meat)	*H*	*Palinurus spp.*		crustacean
F71	Spiny lobster/langust (meat)	*CS*	*Palinurus spp.*		crustacean

Table A-IX. Food Allergens (Continued)

Code	Common NamePurified Allergen Code	ourc	Latin NameMolecular Weight	nownCross-reactivity	AllergenGrupandClass
F306	Lime (peeled whole fruit)		*Citrus aurantifolia*		fruit
F307	Hake (fish muscle)		*Merluccius merluccius*		fish
F308	Sardine/pilchard (fish muscle)		*Sardina pilchardus*	F61	fish
F308	Borecole	*H*			food
F310	Allspice	*H*			spice
F311	Megrim/wiff (fish muscle)		*Lepidorhombus whiffiagonis*		fish
F312	Swordfish (fish muscle)		*Xiphias gladius*		fish
F313	Anchovy	*H*	*Engraulis encrasicolus*		fish
F314	Snail, escargot (uncooked snail)	*H*	*Helix aspersa*		mollusk
F315	Green bean	*H*			vegtable
F316	Comfrey	*H*			food
F322	Pike	*H*			fish
F324	Crayfish	*H*			fish
F326	Cumin	*H*			spice
F332	Baking aid	*H*			food
F333	Carp	*H*			fish
F334	Hops	*H*			grain
F334	Horseradish	*C*			
F336	Wine vinegar	*H*			food
F339	Chives	*C*			vegetable

Table A-X. Nematode/Parasite Allergen					
Code	**ommon Name Purified Allergen Code**	**ource**	**atin Name Molecular Weight**	**Known Cross-reactivity**	**Allergen Group and Class**
P1	Ascaris (adult worm)	*BCPS*	*Ascaris suum*		nematode
P1-I	Asc s 1		*10 kD*		nematode
P2	Echinococcus (eggs)	*BCPS*	*Echinococcus granulosus*		parasite
P3	Schistosomes	*P*	*Schistosoma mansoni*		parasite
P4	Anisakis (larvae in fish)	*D*	*Anisakis spp.*		nematode

Table A-XI. Drug/Hormone Allergen					
Code	**Common Name Purified Allergen Code**	**Source**	**Latin Name Molecular Weight**	**Known Cross-reactivity**	**Allergen Group and Class**
C1	Penicilloyl G	*CHPS*			drug-A
C2	Penicilloyl V	*CHPS*			drug-A
C3	ACTH	*S*		NA	hormone
C72	ACTH	*C*			
C206	ACTH	*H*			drug
C3	Chymopapain	*H*			drug
C4	Chymopapain	*S*		NA	drug
C101	Chymopapain (Sigma)	*C*			drug
C50	Ampicillin	*C*			drug
C70	Insulin, porcine	*CHS*			hormone
C71	Insulin, bovine	*CHS*			hormone
C72	Insulin, recombinant human	*HS*			hormone
C73	Insulin human	*C*			hormone
C99	Polylysine	*H*			drug
C201	Cephalosporin	*H*			drug
C202	Trimethoprim	*H*			drug
C203	Ampicillin	*H*			drug
C204	Amoxicillin	*H*			drug
C208	Pyrazolon	*H*			drug
C209	Phenacetin	*H*			drug
C210	Furosemide	*H*			drug
C211	Tetracycline	*H*			drug
C212	Erythromycine	*H*			drug
C213	Gentamycin	*H*			drug
C214	Gold-chloride	*H*			drug
C216	Doxycycline	*H*			drug
C217	Acetyl salicylic acid	*H*			drug
C223	Sulfamethoxazole	*H*			drug

Table A-XII. Occupational Allergens

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	Allergen Group and Class
K5	Flax	*C*			occupational
K8	Humulus, Hops	*C*			occupational
K13	Jute	*C*			occupational
K70	Green coffee bean	*P*	*Coffea arabica*		occupational
U70	Green coffee bean	*S*	*Coffea arabica*		occupational
K71	Castor bean	*P*	*Ricinus communis*		occupational
U71	Castor bean	*S*	*Ricinus communis*		occupational
K72	Ispaghula (psyllium)	*CP*	*Plantago ovata*		occupational
U72	Ispaghula (psyllium)	*S*	*Plantago ovata*		occupational
K73	Silk waste	*CP*	*Antheraea spp.*		occupational
U73	Silk waste	*S*			occupational
K74	Silk (cultured)	*CH*	*Bombyx mori*		occupational
U74	Silk	*S*	*Bombyx mori*		occupational
K75	Toluene diisocyanate-HSA (paints)	*CHP*			occupational -A
U75	Toluene diisocyanate-HSA (paints)	*S*			occupational
K76	4,4 diphenyl-Methane diisocyanate-HSA	*CHP*			occupational -A
U76	4,4 diphenyl-Methane diisocyanate-HSA	*S*			occupational
K77	1,6 Hexa-methylene diisocyanate	*CHP*			occupational -A
U77	1,6 Hexa-methylene diisocyanate	*S*			occupational
K78	Ethylene oxide-HSA	*CHP*			occupational
U78	Ethylene oxide-HSA	*S*			occupational
K79	Phthalic anhydride-HSA	*CHP*			occupational
U79	Phthalic anhydride-HSA	*S*			occupational
K80	Formaldehyde-HSA	*CHP*			occupational
U83	Formaldehyde-HSA	*S*			occupational
K81	Weeping Fig Tree	*CH*	*Ficus spp.*		occupational
U84	Ficus	*S*			occupational
U81	Cotton, cultivated	*S*			occupational
K82	Natural rubber latex	*CDHP*	*Hevea brasiliensis*		occupational
K85	Latex	*S*	*Hevea brasiliensis*		occupational
	Hev b1 (elongation factor)		*58kD*		
U82	Sheep's wool	*S*			occupational

Table A-XII. Occupational Allergens (Continued)

Code	Common Name Purified Allergen Code	Source	Latin Name Molecular Weight	Known Cross-reactivity	AllergenGroup and Class
U87	Sunflower seed	*S*			occupational
K85	Chloramin T	*CH*			occupational
U88	chloramine T	*S*			occupational
K86	Trimellitic anhydride-HSA (TMA)	*CH*			occupational
U86	Trimellitic anhydride-HSA (TMA)	*S*			occupational
K87	Alpha-amylase	*C*			occupational
K90	Isocyanate-PI	*C*			occupational
K91	Isocyanate-TMI	*C*			occupational
K100	Benzoate	*C*			occupational
K101	Salicylate	*C*			occupational
K201	Papain	*C*			occupational
K202	Bromelin	*C*			occupational
K208	lysozyme (egg white)				occupational

Table A-XIII. Miscellaneous Allergens

Code	**Common Name** purified allergen code	***Source***	***Latin Name*** mol weight	**Known Crossreact-ivity**	**Allergen Group and Class**
O1	Cotton linters	*H*			misc.
O2	Escherichia coli	*H*	*Escherichia coli*		misc
O3	Cotton (treated)	*H*			misc.
O7	Seminal plasma, human	*H*			misc.
O51	Streptococcus viridan	*H*	*Streptococcus viridan*		misc.
O70	Seminal fluid, human	*HS*			misc.
O71	Staphylococcus aureus	*H*	*Staphylococcus aureus*		misc.
O72	Sperm-sediment	*H*			misc.
O101	Micropolyspora	*C*	*Micropolyspora faeni*		misc.
O102	Thermoactinomycetes	*C*	*Thermoactinomycetes vulgaris*		misc.
O201	Tobacco	*HC*			misc.

misc. = miscellaneous

Appendix B. Dilution–Recovery Analysis (Parallelism)

Parallelism 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.

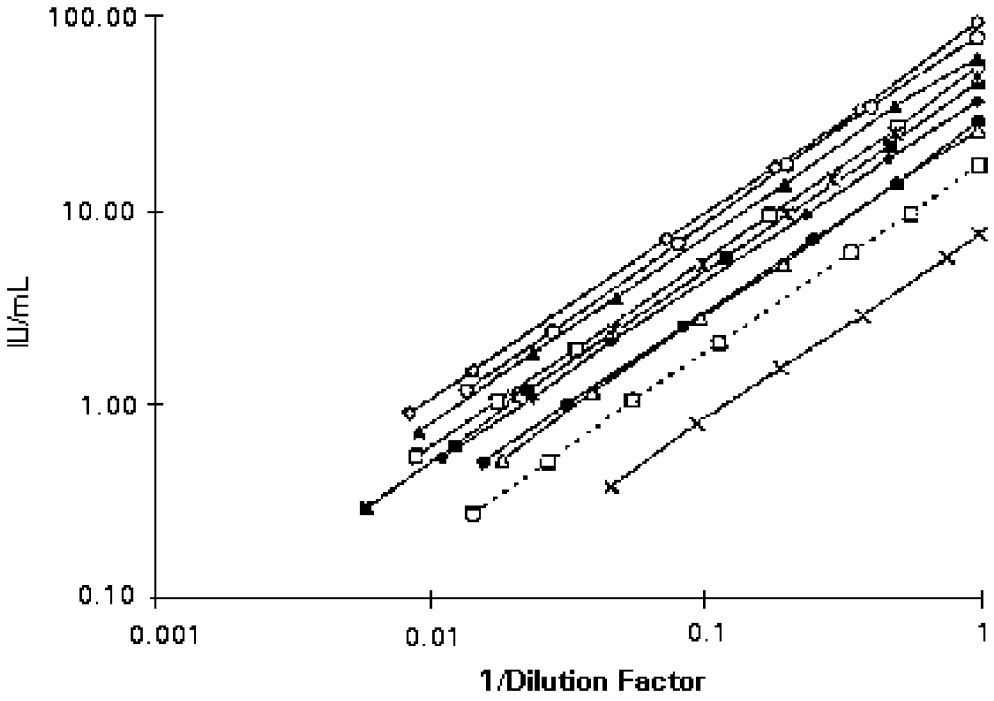


Figure B1. Illustrative dilution-recovery analysis of an IgE antibody immunoassay.

In this analysis, duplicate measurements were performed within one assay run for each of ten 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.

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 demonstrates interdilutional CVs < 15% over the linear range of the assay from 1 to 80 IU/ml of IgE antibody.

This procedure demonstrates parallelism and confirms the absence of matrix effects in total serum IgE and IgE antibody assays. 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 pipets. 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 then 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 2. 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) versus 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 (e.g., <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 endpoint of the analysis.

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 pipets
- 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 (1) has a restricted specificity for human IgE and (2) 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. The 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 (e.g., 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 (e.g., 50% binding or 25% binding) used to determine the cross-reactivity due to possible nonparallelism of the binding curves.

In the direct binding studies, ten 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 (e.g., 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:

$$\% \textit{ Cross-Reactivity} = [1 - (\underline{\textit{D-IgE} - \textit{D-nonIgE}})] \times 100$$
$$\textit{D-IgE}$$

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.

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 nonIgE 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 nonIgE proteins to competitively inhibit the binding of either unlabeled or labeled antihuman IgE to solid-phase human IgE.

In the competitive inhibition analyses, ten dilutions of each soluble inhibitor protein, including IgE and nonIgE 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 (e.g., substrate and stopping reagents for enzymes) in a way that reflects its projected use in the finalized assay. The response signal detected in the nonIgE 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:

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

where:

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

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 are 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 (ref.). 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 (see Section 9.6.

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 diluent
- 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).

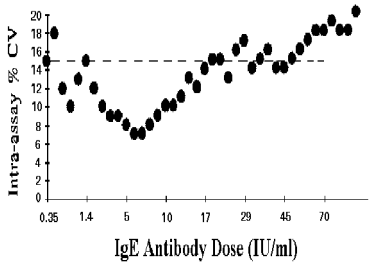
Alternative Qualifying Tests for Liquid Phase Allergen-Containing Reagents

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.

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 10–20 assays, which employ several lots of assay reagents. Replicate measurements can be used to compute the intra-assay coefficient of variation (e.g., 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 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.[14] Separate precision profiles can be generated using replicate analysis of sera within an assay (intra-assay; see Figure 3), 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 NCCLS guidelines EP5— *Evaluation of Precision Performance of Clinical Chemistry Devices*, and LA1—*Assessing the Quality of Radioimmunoassay Systems.*



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 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 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 suppress 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. 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 3 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 versus 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 (e.g., 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, ten sera, from clinically documented persons, that 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.

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.

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 ten determinations in at least four assays run on different days. Sokal and Rohlf[29] provide a more precise designation of the upper limit of the 95% confidence interval as the mean. 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 2 negative serum controls or negative serum pools in 5 assays (100 measurements). Determine the mean, standard deviation, and define the point on the calibration curve that is 2 standard deviations above the mean. This can be defined as the limit of detection of the assay, providing that the precision at this concentration is acceptable (e.g., <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).

Summary of Comments and Subcommittee Responses

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

Appendix B

1. Page 73. Section 3. Interpretation. Sentences 1, 2 and 3. Unclear since suggest better CVs for IgE antibody than for total serum IgE. Possible error in sentence two.

- **Sentences two through four in Section 3 of Appendix B have been revised for clarification.**

Table 1, Page 11

2. Physical and Chemical Properties of IgE and IgG Antibodies. Property Distribution: % intravascular. Column 2. Written as 5%. Page 10 Section 4 IgE: Properties and Effects suggests should be 50%.

- **In Table 1, % intravascular for IgE has been changed from 5 to 50%.**

General Comment

3. I suggest to extend the qualification scheme of allergen specific IgE assay reagents for reagent manufacturers: if several allergens are incorporated into one reaction, define the sensitivity of the combined assay in detecting IgE antibodies to each of its allergen components, as compared to e.g. the assay of the same manufacturer involving individually the allergens of that particular group.

- **It is not clear to the subcommittee what the commenter is requesting. Since multiallergen screening assay reagents are covered under the discussion of reagent quality assurance, no change has been made.**

Related NCCLS Publications*

C24-A **Internal Quality Control Testing: Principles and Definitions; Approved Guideline (1991)**. This document discusses the purpose of internal quality control; defines various analytical intervals such as "analytical run"; and addresses the use of quality control material and control data, including the use of data in quality assurance and interpretation.

C28-A **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline (1995)**. This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.

EP5-T2 **Evaluation of Precision Performance of Clinical Chemistry Devices—Second Edition; Tentative Guideline (1992)**. This document offers guidelines for designing an experiment to evaluate the precision performance of clinical chemistry devices; recommendations on comparing the resulting precision estimates with manufacturer's precision performance claims and determining when such comparisons are valid; and manufacturer's guidelines for establishing claims.

EP9-A **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (1995)**. EP9 addresses procedures for determining the bias between two clinical methods or devices and design of a method-comparison experiment using split patient samples and data analysis.

I/LA18-A **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline (1994)**. This guideline outlines specimen requirements, performance criteria, algorithms for the potential use of sequential or duplicate testing, recommendations for intermethod comparisons of immunological test kits for detecting infectious diseases, and specifications for development of reference materials.

LA1-A2 **Assessing the Quality of Radioimmunoassay Systems—Second Edition; Approved Guideline (1994)**. LA1-A2 provides definitions and procedures for properly assessing radioimmunoassay systems.

NRSCL8-P3 **Nomenclature and Definitions for Use in NRSCL and Other NCCLS Documents—Third Edition; Proposed Guideline (1996)**. This document contains proposed definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

NRSCL13-P **The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results; Proposed Guideline (1995)**. NRSCL13-P provides procedures for developing and evaluating definitive methods, reference methods, designated comparison methods, and reference materials to provide a harmonized clinical measurement system.

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*Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.