

Laboratory Analyses in the Diagnosis of Human Allergic Disease

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The clinical immunology laboratory provides support to the allergist in the diagnosis and management of human allergic diseases. Following a clinical history, the detection of allergen-specific immunoglobulin E (IgE) in serum can be useful in the definitive diagnosis of an IgE antibody-mediated hypersensitivity. Total serum IgE, the multiallergen screen, and mast cell tryptase are less commonly measured analytes in the work-up of the allergic patient. More recently, the clinical laboratory has employed monoclonal antibody based-enzyme immunoassays to quantify dust mite, cat, dog, and cockroach indicator aeroallergen levels in indoor home and work environments. Levels of allergenic proteins in environmental and medical/consumer products are measured by other assays such as the competitive inhibition radioallergosorbent test. Methods of quantification, internal quality control, and external proficiency testing programs are emphasized in this report. © 1997 Academic Press

The licensed clinical laboratory plays a significant role in the diagnosis and management of human allergic diseases. The diagnosis of an immediate-type (IgE-antibody mediated) hypersensitivity begins with a comprehensive clinical history and is subsequently supported by confirmatory tests in which allergen-specific IgE antibody is detected in the subjects' blood or skin. If there is a mismatch between the clinical history and the diagnostic skin test or serological test, a nasal, bronchial, or skin contact provocation may be

performed with a characterized allergen reagent to clarify the true allergic status of the individual.

This article examines the current status of laboratory-based (*in vitro*) methods for the detection and quantification of allergen-specific IgE in blood. Results from IgE antibody assays are contrasted with those obtained with *in vivo* diagnostic procedures as discussed by Peebles and Hartert in this issue (1). Once the patient has been diagnosed, their allergy is often managed using a combination of treatments. Allergen-specific IgG antibody measurements can be used to assess changes in humoral immunity that occur as a result of allergen avoidance or administration of allergen during immunotherapy. This topic is discussed elsewhere in detail (2) and is not examined in this report. Another report in this issue examines the laboratory role in the quantitation of allergen in environmental specimens such as settled dust to identify problem areas and facilitate allergen avoidance (3). Since mast cell tryptase is an excellent marker of mast cell degranulation, Hogan and Schwartz (4) address reagent development and the assay methods employed in the measurement of tryptase in serum. Quantitation of allergen in consumer and medical products using competitive inhibition assays that employ human IgE antibody as a reagent is discussed in this report.

ALLERGEN-SPECIFIC IgE AS A DIAGNOSTIC ANALYTE

IgE antibody-mediated (type 1) hypersensitivity or allergic disease develops in two discrete stages.

The initial sensitization phase occurs following the exposure of a predisposed but IgE antibody-negative individual to an allergenic substance. IgE circulates in the blood and binds to high-affinity epsilon Fc receptors on basophils and mast cells. The degree of sensitization observed in an individual depends in part on the concentration of allergen, the route and duration of allergen exposure, and the genetic predisposition of the individual. In a second phase, the sensitized but often symptom-free individual may experience any of a spectrum of allergy symptoms on reexposure to the same allergen. These symptoms can range from respiratory responses (asthma or rhinitis), skin reactions (urticaria, dermatitis), and gastrointestinal symptoms to anaphylactic shock. As discussed elsewhere in this issue (5), allergens are ubiquitous proteins released from pollens of grasses, weeds, and trees and epidermal antigens from domestic animals, insect venoms, drugs, mold spores, and some generally well-tolerated foods. Historically, γ -globulin E (IgE), named for its ability to mediate the erythema reaction, was first identified in the mid-1960s independently by the Ishizakas and Bennich and Johansson (6, 7). Since then, a variety of immunoassays have been developed to detect and quantify total serum IgE and, more importantly, IgE antibodies of defined allergen specificities in human biological fluids.

TOTAL SERUM IgE

Total serum IgE is currently measured in international units (IU) per ml with 1 IU equivalent to 2.4 ng of IgE protein. Levels of serum IgE in the healthy population are age and sex dependent. IgE begins at <2 IU/ml in cord blood, increases to 12 to 618 IU/ml (95% confidence limit) by 10 to 20 years, and declines thereafter to 10 to 506 IU/ml (8, 9). A statistically higher serum IgE has been reported in males compared to females (10), but this difference is unlikely to be of any clinical significance. A moderately elevated total serum IgE may reinforce a clinical diagnosis of allergic rhinitis, allergic asthma, and atopic dermatitis. However, extremely high levels of serum IgE are observed in individuals with parasitic infections and are necessary in the diagnosis of the hypergammaglobulinemia E syndrome. Normal or low total serum IgE levels in individuals with asthma suggest that IgE-mediated mechanisms may

play only a minor role in the pathogenesis of their condition. Normal serum IgE levels can also be useful in excluding the diagnosis of bronchopulmonary aspergillosis. Serial total serum IgE levels can be useful in confirming an expected seasonal boost in IgE that is commonly seen after environmental exposure to an allergen to which the patient has become sensitized. In terms of clinical utility, however, there is a wide overlap in serum IgE levels among atopic and nonatopic individuals. Therefore, the clinician must refrain both from discounting allergy when the IgE is low and from automatically inferring an allergic etiology when the total serum IgE is high.

A variety of competitive and noncompetitive solid-phase immunometric assays have been successfully employed over the years for the measurement of the total level of IgE in human serum. The most widely employed procedure has been the two-site immunometric assay that involves quantitatively binding IgE antibody from serum to an insolubilized polyclonal or monoclonal anti-human IgE and detecting bound human IgE with labeled anti-human IgE (11). The amount of labeled anti-human IgE detected following the final reaction step is proportional to the level of IgE present in the initial test serum. The solid phases employed in these assays have included, among others, cellulose paper disks, agarose, microcrystalline cellulose, cellulose threads, microtiter plates, and most recently a high binding capacity sponge material. The total IgE assays generally exhibit excellent sensitivity (as low as 0.2 ng of IgE per ml), precision [coefficients of variation (CV) $\leq$ 15%], and good interassay agreement due to well-characterized international serum IgE standards. One advantage of the assay is the removal of non-IgE serum factors with a washing step that otherwise may confound low serum IgE level measurements. The only technical disadvantage of this assay design is the requirement for a second incubation, which lengthens the time required to complete the test and increases the number of technical manipulations required. By using reagents in molar excess to serum IgE levels, most clinically used assays have achieved adequate sensitivity (e.g., 1 ng/ml) while also permitting a 1-day assay turn-around time.

One useful research assay for measuring total serum IgE is the immunoenzymetric assay (IEMA), which employs a commercially available capture monoclonal anti-human IgE (HP6061) on a microtiter plate to bind IgE from serum and a different biotinylated or enzyme-conjugated anti-human IgE

(HP6029) to detect bound IgE (12). This IEMA is ideally designed to measure the level of IgE protein in human serum, heparinized or cation-chelated plasma, and research specimens such as cell culture medium and respiratory tract secretions (saliva, nasal washings, and bronchial secretions) (13). Use of the commercially available monoclonal antibodies in tandem maximizes diagnostic sensitivity and specificity and reproducibility while making this assay available to a broad spectrum of researchers.

The high degree of interassay agreement among the existing commercial and research total serum IgE assays is a direct result of the availability of two widely used IgE reference preparations: the World Health Organization International Reference Standard for human IgE (No. 68/341) and the US IgE Reference Serum Preparation. Both have been useful as primary standards from which secondary standard serum pools have been cross-calibrated for IgE content using the value transfer technique (e.g., three to five dilutions in 10 assays). Once the total serum IgE in a secondary or working standard has been accurately defined, it can be employed for daily laboratory use with periodic reevaluations against the primary reference preparation to ensure quality control. In most laboratories, a high IgE reference serum pool is prepared by combining equal amounts of serum from 50 to 100 individuals who have had previously documented total IgE levels in the range 200 to 800 IU/ml. The problem of nonparallelism has been occasionally observed in the high-end range of total serum IgE IEMA as a result of atypical nonspecific binding when sera containing >2000 IU/ml are used as reference.

Most total serum IgE assays that are considered "clinically useful" have a working range from 1 to 200 IU/ml. An analytical sensitivity of 0.5 IU/ml (1.2 mg/liter) can be measured with statistical accuracy by the monoclonal antibody-based immunometric assay. Agreement among replicates in these assays is usually excellent and an interassay reproducibility of <15% CV can be readily achieved. Parallelism between the reference and test serum dilutions curves is an indication of good recovery and overall assay quality. By analysis of each serum specimen at two dilutions, an interdilutional %CV can be computed after the interpolated results are corrected for serum dilution. Interdilutional CVs of <20% are achievable with most test sera and they demonstrate good assay parallelism, minimum nonspecific binding, and an absence of serum matrix interference

problems. Quality control of the total serum IgE assay is routinely accomplished by inclusion of two or three well-studied sera in each assay. The positive control sera should span the clinically useful ranges of the dose-response curve (<10 IU/ml, 100 to 250 IU/ml, and >1000). The reproducibility of the known IgE content of these sera together with good agreement between results obtained at two dilutions demonstrates reliable assay results that fulfill Federal and State laboratory licensing requirements.

Total serum IgE has been classified as a regulated analyte by the amended Clinical Laboratory Improvement Act (CLIA) of 1988. This means that CLIA-licensed clinical laboratories that measure IgE in serum must show acceptable proficiency in one of several available external proficiency surveys. One such survey that is conducted by the College of American Pathologists (SE survey) sends 5 challenge sera to each participating laboratory during each of three cycles per year (15 challenges per year) for assessing total serum IgE (see below).

ALLERGEN-SPECIFIC IgE ANTIBODY ASSAYS

A diagnosis of IgE-mediated allergic disease requires the unequivocal detection of allergen-specific IgE antibody in the skin or serum. As discussed by Peebles and Hartert (1), the skin test has been widely used by the allergist as a primary diagnostic test that provokes IgE-sensitized skin mast cells to release mediators such as histamine and induce a swelling (wheal) and erythema (flare). A number of skin conditions such as eczema, dermographism, psoriasis, and urticaria make skin testing difficult in both adults and children. Children, especially under 10 years of age, do not readily cooperate for skin testing. Moreover, skin reactivity can be suppressed by prior use of antihistamines and other medications that cannot always be stopped prior to skin testing. In these cases, analysis of a serum from the patient for IgE antibody is often preferred over skin testing in the diagnostic work-up for allergic disease. Detection of allergen-specific IgE antibody in the serum indicates that the individual has become sensitized, even though the individual may not experience allergy symptoms. However, they have an increased probability of having allergy symptoms associated with IgE-mediated disease upon reexposure to the

same allergen. The quantity of IgE antibody in serum may but does not always correlate with the risk of severity of these symptoms. The association between allergic symptoms and antibody quantity is often confounded by other factors such as the IgE antibody affinity, the presence of IgG blocking antibodies, and the relative biochemical sensitivity or "releasability" of the subject's IgE-laden effector cells (14). IgE antibody is therefore necessary but not sufficient for expression of allergic disease. For many food, drug, and insect allergies, only about half of those individuals with IgE antibody will react clinically when challenged with the appropriate allergen. IgE results must therefore be carefully integrated with other clinical information for proper diagnostic interpretation.

The radioallergosorbent test (RAST) was the first reported serological immunoassay for allergen-specific IgE antibody in human serum. Allergen-coated paper disks were incubated with the reference, control, and patient's serum, allowing specific antibody of all immunoglobulin classes to bind. The solid phase was then washed, and bound IgE antibody was detected with radiolabeled, polyclonal anti-human IgE detection antibody. The level of radioactivity bound was directly related to the specific IgE antibody content of the original serum. For those investigators who compared their IgE antibody results to a standard reference serum, insolubilized allergen and radiolabeled anti-IgE antibody were used in molar excess to maximize assay performance.

More recently, the RAST has been subjected to numerous technical variations by a large number of investigators and commercial manufacturers. These variations have chiefly involved the use of different polymers for allergen insolubilization and the use of nonisotopically (e.g., enzyme) labeled monoclonal and polyclonal anti-human IgE reagents. Carbohydrate matrices (Sephadex, agarose, and cellulose) paper disks, sponges, cellulose threads, polystyrene test tubes, and 95-well microtiter plates have all been used in the RAST-type assays. These polymers differ with respect to their allergen-binding capacity, nonspecific binding properties, long-term stability, and ease of washing. When maximum sensitivity is required, or if sera from hyperimmunized patients with high levels of allergen-specific IgG antibodies are to be studied, the use of a solid phase with the highest allergen-binding capacity is needed. For suitably equipped clinical laboratories with adequate facilities for centrifugation, aspiration, and pi-

petting, one of the carbohydrate particle matrices is preferred because of its high antigen binding capacity. Commercially available paper disk and sponge solid-phase antigens may be attractive to some laboratories that prefer not to prepare their own reagents. Polystyrene solid phases such as microtiter plates are satisfactory with some purified allergens or simple allergen mixtures where there is a predominant primary allergen. However, because of problems with their limited binding capacity and the pI dependency of allergen binding, their routine use in clinical and research assays with complex antigen mixtures is not recommended.

There are multiple methods of data analysis for allergen-specific IgE antibody assays. The simplest analysis has been to compute the ratio of the assay signal (e.g., counts per minute, optical density, fluorescence signal units) obtained with a patient's serum relative to a negative IgE antibody control serum from a nonatopic individual. Patient/negative serum ratios > 2.5 can be shown to be statistically positive above the negative serum binding, and thus they are considered by many investigators to be evidence for IgE antibody. A second approach has involved expression of IgE antibody results in arbitrary "classes" ranging from 0 (negative) to +6 (strongly positive) depending on the magnitude of the response relative to arbitrary divisions of a standard curve. This grading system may be misinterpreted to imply a severity of clinical disease with progressive increases in IgE antibody levels. Such an inference is not justified, however, since in certain allergen systems (e.g., molds), the most sensitive individuals may not exceed a class + 2 on a scale constructed from either a grass or birch pollen-specific IgE or total serum IgE (heterologous) dose-response curve. A third approach involves homologous interpolation in which IgE antibody levels are interpolated from a reference serum curve prepared with sera containing IgE antibody of the same allergen specificity. The most practical approach has been that of heterologous interpolation, in which IgE antibody results are reported in IU/ml from a total serum IgE reference curve. However, precise clinical interpretation of specific antibody content reported using any of these units will await clinical correlation studies that have not been easy to perform.

STANDARDIZATION

Since the presence of IgE antibody in the serum and skin has been highly associated with a predispo-

sition for allergic disease to that allergen specificity, IgE antibody is poised to be one of the first antibody assays to be regulated by CLIA licensing agencies. As such, a debate has arisen as to the best methods to perform quality control on an assay system that has over 200 individual reagent components with different allergen specificities. Moreover, there has been concern about the heterogeneity of the units of antibody reported from the commercially available assays. To begin the process of developing consensus, a group of academic, industrial, and government scientists under the auspices of the National Committee on Clinical Laboratory Standards has prepared a guideline that extensively examines the issue of standardization of IgE antibody assays (15).

To perform homologous interpolation methods and generate IgE antibody results in mass per volume units, a calibrated IgE antibody reference serum is needed. Two approaches have been proposed for use by researchers for absolute standardization of the IgE antibody-containing reference sera. The first approach involves elution of a portion of the specific IgE bound to the allergen solid phase followed by measurement of the quantity and proportion of IgE eluted. This approach allows standardization of reference and test sera in weight/volume terms, usually in nanograms per milliliter (16). A second approach involves the total depletion of specific antibody by immunoabsorption and subsequent measurement of the reduction of total IgE protein observed with a total serum IgE assay (17). This second approach is useful only when the IgE antibodies of interest constitute a significant fraction of the total serum IgE. The two techniques give comparable results, and in both cases it is necessary to standardize only the reference serum. The quantity of IgE antibody in the test sera may then be estimated by "homologous interpolation" from a standard curve that has been constructed with multiple dilutions of the reference serum that contains defined levels of IgE antibody of the same allergen specificity. This outcome allows comparison of IgE antibody results in different allergen systems, each with its own calibrated reference serum, and it facilitates interlaboratory standardization. The principal limitation to this system of standardization is the requirement for large amounts of serum containing specific IgE antibody to each of the clinically relevant allergen specificities.

Since the preparation of separate calibrated reference sera for each of the hundreds of allergen speci-

ficiencies of clinical importance is not practical, an alternative generic strategy for calibrating IgE antibody assays has been adopted by most commercial assays. This method involves "heterologous interpolation" of response data from a total serum IgE reference curve that is run in parallel with the IgE antibody assay. It requires that the total serum IgE (heterologous) curve dilutes out in parallel with both the IgE antibody-positive control and patient test sera that are analyzed in the IgE antibody portion of the assay. Parallelism ensures reproducible estimates of IgE antibody when sera are analyzed at different dilutions. Using this approach, IgE antibody from any of hundreds of different allergen specificities can be interpolated from a single total serum IgE dilution curve, which is traceable to defined IU/ml levels of IgE in a primary WHO or US total serum IgE standard.

QUALITY ASSURANCE

Current IgE antibody assays, in their optimized form, possess an analytical sensitivity that approaches that obtained by prick puncture skin tests (0.2 to 0.5 ng/ml). The upper working range of the IgE antibody assays approaches 100 ng of specific IgE per ml. The sensitivity and working range of IgE antibody assays are influenced by the relative amount of specific antibody of other isotypes (e.g., IgG, IgA, or IgM) and the allergen quality and epitope density of the allergen-containing reagent. As with the total serum IgE assays, allergen-specific IgE assays can exhibit excellent agreement among replicates. Interexperimental reproducibility of <20% CV can also be achieved. Finally, parallelism between the reference and test serum dilution curves is highly dependent on the type of reference serum and calibration curve that is used in the assay. Assays that employ homologous interpolation methods with a reference serum that contains IgE antibody of the same allergen specificity as that being measured in the test assay in general exhibit better parallelism than those using heterologous interpolation methods. However, several new clinical assays that use heterologous interpolation from a total serum IgE reference curve have also been able to achieve good parallelism.

The most important region of the IgE antibody assay dose-response curve from a clinical point of

view is the minimal detectable dose. Positive thresholds can differ according to the blank subtraction and reporting methods used. Therefore, each allergen-specific IgE antibody assay should contain one or more positive and negative serum controls for select allergosorbents in addition to a reference serum dilution curve. All samples should be analyzed in duplicate to identify and minimize procedural errors except where semiautomated assays have been extensively validated for single measurements. Since the quality of each antibody measurement depends on parallelism between the dilution curves of the reference serum and test sera under study, two or three dilutions of select IgE antibody-positive test sera should be analyzed to confirm the assay's parallelism.

Clinical laboratories that perform total serum IgE and allergen-specific IgE antibody measurements are required to participate in an external proficiency testing program. The College of American Pathologists conducts a Diagnostic Allergy (SE) Survey that parallels the European external proficiency testing program discussed by Fifield in this issue (18). In the SE survey, five sera are sent in each of three cycles per year to participating diagnostic allergy laboratories throughout North America. Each serum is analyzed for IgE antibody to five predetermined allergen specificities that cycle among the 20 primary indoor and outdoor aeroallergens that reportedly produce most of the aeroallergen-related allergic disease (e.g., weeds, grasses, trees, pet epidermals, molds, occupational allergens, and food allergens). The first and third cycles of each year also include a sixth wild-card serum specimen that has been collected from a patient who exhibits Hymenoptera venom sensitivity, latex allergy, or allergies to less-common foods. The participating laboratory is asked to measure total serum IgE and allergen specific IgE to five allergen specificities in each of the SE survey's challenge specimens as if they were any other unknown patient specimens. Completed IgE antibody results are submitted to the survey's coordinating center in classes and units interpolated from the assay's reference curve. They are collated into a participant summary and the performance of any participating laboratory can be determined in relationship to its peers that use the same assay method. Since IgE antibody is still classified as a nonregulated analyte by CLIA, individualized laboratory performance is not formally graded. The results of the College of American Pathologists SE

survey have, however, become useful to the allergist who needs independent and unbiased verification of the quality of the laboratory that they use for their IgE antibody serology.

ALLERGIC DISEASE MANAGEMENT

Once the diagnosis has been made, the allergic patient may elect to be treated with pharmacotherapy, allergen-directed immunotherapy, and/or allergen avoidance therapy. Of these three modalities, allergen avoidance is particularly attractive because it involves the separation of the patient from the allergen, which tends to be less expensive and highly effective when the allergen sources are known.

Allergen Levels in Indoor Environments

To successfully accomplish avoidance, the individual must be known to be sensitized to an indoor allergen as evidenced by the presence of IgE antibody in their skin or blood and the allergen source must be accurately identified, often by a quantitative measurement of an indicator aeroallergen. A full-service diagnostic allergy laboratory can provide quantitative measurements of dust mite, cat, dog, and cockroach aeroallergens to determine relative burden levels. An article elsewhere in this issue examines in detail the immunometric assays that are used to quantify the most widely used indicator dust mite (Der p 1 and Der f 1), cat (Fel d 1), dog (Can f 1), and cockroach (Bla g 1) aeroallergens in indoor environments (3). Mold assessment in private homes and workplaces has been problematic because purified allergenic proteins such as Alt a 1 and Asp f 1, which are the group 1 aeroallergens released from *Alternaria alternata* and *Aspergillus fumigatus*, are not consistently produced even though these molds grow in a particular indoor environment. Unfortunately, the production of these allergens is highly dependent on variable growth conditions (e.g., temperature, humidity). Therefore, mold assessment in indoor environments has remained at the level of microbiological measurements in culture plates for total viable mold spore counts. Individual mold colonies, once identified, can be expanded by the mycologist, at great expense and effort, to speciate each individual colony. However, this process is difficult and often only the genus can be identified. An alter-

native strategy for determining the mold burden of an environment has been to quantify the total (viable and nonviable) mold spore levels indoors using a rotarod impactor or the more efficient Burchard spore trap, both of which are used for outdoor air evaluation. This is a rather new approach and at present, there are no defined levels of mold above which an environment is considered a "risk" for a mold allergic individual.

Allergen Levels in Consumer and Medical Products

Recently, many predisposed individuals who have either undergone multiple surgeries (e.g., children with spina bifida) or been occupationally exposed (e.g., healthcare workers) to dipped natural rubber latex-containing products such as gloves, balloons, and condoms have become sensitized to a number of allergenic natural rubber proteins. On reexposure, these individuals can manifest a spectrum of clinical symptoms ranging from localized hives and rhinitis with ocular tearing and itching to the other extreme of systemic anaphylaxis. Sensitized individuals with a clinical history for latex allergy have a positive skin test and a positive serology for IgE anti-latex (19, 20). In rare cases where there is a mismatch between the clinical history and the confirmatory diagnostic skin test or serology, a two-stage glove provocation challenge, can be used to clarify the allergic status of the individual (21). For employers such as hospitals, dental offices, and nursing homes that attempt to create a latex-safe environment for their employees and patients, direct quantitation of latex allergen in gloves prior to purchase can be useful.

A competitive inhibition format of the allergen-specific IgE antibody immunoassay is used to determine the presence and relative natural rubber latex allergen content in natural rubber-containing products (22). The rubber product is first extracted in a phosphate-buffered saline containing a carrier protein such as bovine serum albumin (1 g/ml, extraction overnight at room temperature with rotation). Test extracts or a reference latex preparation (e.g., E5 or E8 nonammoniated latex, FDA) are preincubated in test tubes with an IgE anti-latex-positive serum. The latex-containing solid phase (allergosorbent) is then added and the mixture rotated. Following an incubation period, bound IgE anti-latex is separated from the unbound IgE antibody with a wash step. IgE antibody bound to the latex allergosorbent is finally

quantitatively detected with a radionuclide, enzyme, or fluorophor-labeled anti-human IgE Fc. The more latex allergen in the original test or reference extract, the less IgE antibody is bound to the latex allergosorbent and the lower the response signal that is measured in the final reaction mixture.

Using this assay, powdered latex gloves have been identified as the principal latex-containing product found in the hospital setting that places both latex allergic patients and healthcare workers at risk of having allergic reactions (23). Avoidance programs in hospitals have now begun focusing on the removal of powdered latex gloves and substitution of powder-free latex and synthetic gloves. The sensitized healthcare worker should be required to use synthetic latex gloves in their work to maximize avoidance of latex allergen exposure. For safe care of the latex allergic patient, a latex-safe protocol that involves substitution of natural rubber latex-free products for any product known to contain natural rubber latex can be established.

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