

Allergy Review Series X: Progress in diagnosis of allergy *in vitro*

IgE antibody in the serum – detection and diagnostic significance

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Accepted for publication 30 April 2003

Normally, immunoglobulin E (IgE) is a tissue-bound molecule. It is present in the serum in only nanogram amounts, in equilibrium with that bound to mast cells, basophils, and other cells. The subject of study for many years before its description, IgE was termed a 'reagin' because it shared the heat-lability of the complement proteins. Johansson's discovery of an untypable myeloma protein, initially termed IgND, in the serum of a Swedish farmer permitted separation of relatively large amounts of the protein and preparation of rabbit antisera for use in immunoassay (1). Confirmation that IgND was identical to the γ E discovered by Ishizaka and Ishizaka (2) and that both were the elusive reagin of immediate hypersensitivity reactions quickly resulted in design and commercialization of immunoassays for allergen-specific IgE. The early Phadebas RAST (RadioAllergoSorbent Test, Pharmacia, Uppsala, Sweden) has been made obsolete by advances in assay technology. While the term 'RAST' is often used colloquially to describe any immunoassay for allergen-specific IgE, it is best avoided because it describes a detection system now rarely used, and it is a trademark.

A typical modern immunoassay uses allergenic source materials bound to a matrix. Patient serum is added. Following incubation and washing, an enzyme-labeled anti-human IgE antibody is added. After a second incubation and washing, enzyme substrate is added, response is measured, and results are calculated and reported. Most assays report quantitative results.

Reagents and equipment for testing

Allergenic materials

Because they are biologic products, allergen extracts have substantial, inherent lot-to-lot and manufacturer-

to-manufacturer variability. Allergenic extracts and other materials used for immunosorbent matrix preparation should be procured from a source that can ensure the highest quality and, ideally, ensure some type of standardization in order to minimize lot-to-lot variation. For clinical purposes, immunoassay manufacturers normally use materials prepared by commercial suppliers. For investigational use, detailed directions for preparation of allergenic extracts are available (3). Before following these extraction methods, it would be wise to consult with the laboratory director of a commercial allergen extract manufacturing firm regarding updated methods. In addition, optimal coupling to a solid phase might require special extraction procedures, and different source materials may need different coupling methods.

Because the human IgE response is polyclonal, a patient who is allergic to timothy grass pollen could have multiple specificities to epitopes on different timothy grass allergens. Accordingly, extracts should ideally be well characterized to verify that all major and minor allergens are present in the extract, and that all bind to the matrix. The source material may contain crossreactive carbohydrate determinants (CCDs) with epitopes recognized by human IgE (4). Because CCDs may produce clinically irrelevant false positive results, studies are needed to determine how to account for this factor in matrix preparation. Pure, recombinant allergens are currently available for use in immunoassays (5). Because each allergen-matrix system has its own individual performance characteristics, specific IgE immunoassay is not one test, but several hundred.

Immunsorbent matrix

Plastic, cellulose, and agarose are the materials most commonly used for the immunsorbent matrix. To avoid assay interference from high levels of other allergen-specific isotypes (such as IgG) or very high levels of nonspecific IgE, matrices with very high binding capacity should be employed in quantitative testing. Agarose beads, the ImmunoCAP (Pharmacia Diagnostics, Uppsala, Sweden) solid phase, and the AlaSTAT (Diagnostic Products Corporation, Los Angeles, CA) liquid matrix have high binding capacities. Plastic tubes, plastic microtiter plates, and paper disks have relatively low binding capacities and are better suited for qualitative testing or specific investigational purposes. The saturation point of a conventional paper disk is less than 10 kU/l IgE (6).

Recognition antibody

The anti-human IgE antibody used to recognize human IgE captured by allergen should have essentially no crossreactivity with other immunoglobulin classes. It must also recognize all IgE allotypes. Various preparations with monoclonal or polyclonal antibodies, or mixtures thereof, are available. Polyclonal antibodies, most commonly prepared in rabbits, give optimal analytic sensitivity.

Signal detection

Early immunoassays used radioiodinated (^{125}I) recognition antibodies. Most modern methods employ enzyme labeled antibodies. The enzyme used depends on the chosen enzyme substrate and the system to be used (fluorometry, colorimetry, spectrophotometry or chemiluminescence) for signal detection.

Data reduction and analysis

Using detection limit theory (7), one can determine an assay cutoff and report simple qualitative results. All modern methods report semiquantitative results using a 'class system', often seven classes with increasing numbers reflecting greater amounts of specific IgE. A class arbitrarily termed '1/0' is sometimes used to designate equivocally positive results. Unfortunately, the class systems differ substantially from assay method to assay method, and are not at all interchangeable. Modern methods also report quantitative results related to World Health Organization (WHO) standard 75/102 for IgE. Doing so requires the use of a multi-point calibration curve, using sera with known amounts of allergen-specific or total IgE. The mass law predicts that such a curve, in a log-log plot, should be linear in its mid-portion. The curve will flatten at higher levels due to the assay's having reached its saturation point,

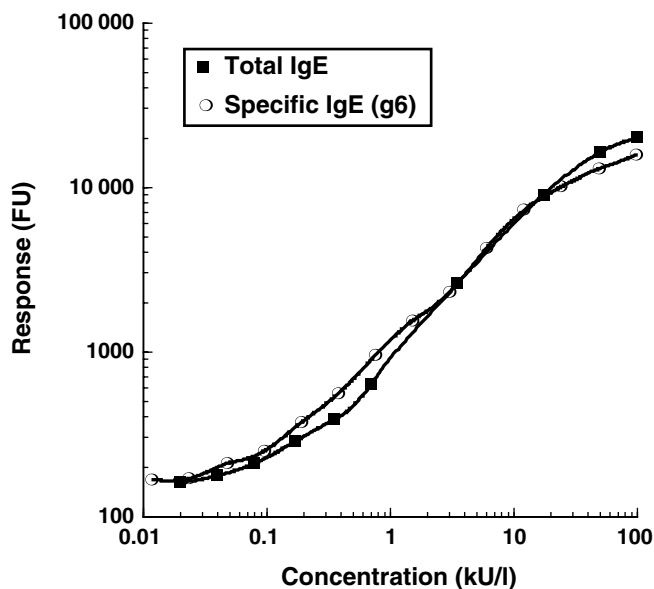


Figure 1. Dilution curves of a total IgE reference standard (black boxes, thicker line) and a high-titer patient sample containing a high titer of IgE antibody to timothy grass pollen (g6; open circles, thinner line). In this log-log plot, the mid-portion of each curve is essentially linear. The flattening at higher levels is due to solid phase saturation. At lower levels, flattening reflects the increasing contribution of assay background to the total response. Assays were performed using a Pharmacia CAP System. Total IgE was measured using anti-IgE bound to a solid phase; a timothy grass extract was coupled to the solid phase. The similarity of the two dilution curves reflects 'parallelism' throughout the assay range. Additionally, the pollen dilution curve appears to be linear to a value of 0.1 kU/l, well below the manufacturer's clinically based 0.35 kU/l cutoff.

and will flatten at lower levels due to the increasing influence of assay background (Fig. 1). At both extremes, interassay and intraassay coefficient of variation will be high. Data reduction in its simplest form employs the manual use of graph paper (either linear-linear, log-log or logit-log). Various data analysis methods are appropriate for computer analysis of standard curves; these include the Rodbard logistic models and cubic spline interpolation.

Equipment

Most manufacturers of commercial diagnostic test methods supply necessary reagents and instrumentation as a test 'system'. Many such systems are computer controlled and some are fully automated, requiring little if any operator intervention once samples and reagents are loaded. Others require pipetting and operation by a skilled individual. Mixing and matching of reagents and equipment from different manufacturers and custom designed systems would only be appropriate for an expert laboratory with excellent quality controls.

Quantitative testing

Quantitative testing requires several assumptions. First, all allergens should be bound to the immunosorbent matrix, and all epitopes should be exposed and available for binding by specific IgE. The binding capacity of the allergen-matrix must be high, so that allergen epitopes are in excess relative to antibody, and so that binding of allergen epitopes by antibodies of other classes (such as IgG) does not interfere with IgE binding. Each allergenic extract contains multiple allergens and each allergen contains several epitopes that can be recognized by human IgE. Thus, there are multiple mass law interactions to consider. All clinically important IgE antibodies must have sufficient affinity to bind allergen epitopes and be detected. As the assay reaches the saturation point and fewer binding sites are available, the mass law favors binding of high affinity antibodies. The clinical relevance of specific IgE antibody affinity for allergen is unknown; affinity studies using human IgE are difficult (8). The reagents used for the reference curve should be calibrated in mass units related to an international standard. Ideally, there should be an allergen specific reference curve for each unknown. However, since doing so is not practical, verification of parallelism of allergen-specific IgE dilution curves to a reference dilution curve is necessary (Fig. 1).

Low level assays

Early and modern immunoassays lack sensitivity when compared to results of conservatively and carefully performed and interpreted skin testing. Assay sensitivity, relative to skin testing or another comparison standard, can be improved by lowering the assay cutoff, but doing so can result in a loss of specificity. Test manufacturers differ in how they recommend an assay cutoff. Some include a 'true zero' point in the calibration curve, and others permit extrapolation lower than a certain reference point on the calibration curve. In such cases, the assay's lower limit of detection must be known and taken into consideration. Other manufacturers have set a clinical cutoff by analysis of serum from nonallergic blood donors. The latter approach is limited by the fact that normal individuals have measurable levels of allergen-specific IgE. An examination of the 0.35 kU/l global cutoff used in the Pharmacia CAP System suggested that this cutoff is several standard deviations above assay background for most, but not all, allergen ImmunoCAPs (9). This observation was confirmed in the newer UniCAP System (10), and is illustrated in Fig. 1.

Because some clinicians in the United States wanted low level assay results reported in a class system comparable to that of Modified RAST, Pharmacia devised an Alternative Scoring Method (ASM) available as part of the CAP System. For some allergen ImmunoCAPs, the ASM cutoff is very close to background (9).

However, in a study comparing results of testing with a custom-made fire ant venom ImmunoCAP with that of titrated intradermal skin testing, Ford and coworkers reported 100% sensitivity and 100% specificity when results reported by the ASM system were compared to skin test results (11).

Assay cutoffs, whether analytically or clinically based, are arbitrary. An analytical cutoff should reflect optimal assay sensitivity and specificity related to background. This laboratory cutoff for an assay should be the lowest value at which it is still possible to distinguish signal from noise at a certain statistical level of confidence. Increasing worldwide clinical experience with quantitative assays suggests that clinical cutoffs will vary according to both the allergen in consideration and the patient's clinical circumstances. The interpretation of results is a matter for the clinician, not a laboratory director.

Standardization and quality control

In general, specific IgE immunoassays are notable for a deplorable lack of standardization (12, 13), even though there are published guidelines for assay design, performance, standardization, and quality assurance (14). Certain methods deliver consistent results from assay run to assay run, both within and between laboratories. True standardization is probably not possible because of varying sources for raw allergenic materials (15), differing methods for binding allergen to a detection matrix, and different detection systems. The multiple class systems used to report semiquantitative results should be abandoned in favor of the reporting of quantitative results.

For quality control purposes, each assay run should include at least one known negative and at least one known positive sample. Ideally, this should be done for each allergen matrix tested because each allergen matrix represents an individual test with its own performance characteristics. The large range of allergen matrices available precludes doing so in routine clinical testing. Positive quantitative quality control results can be plotted and analyzed by standard methods, such as Levy-Jennings plots. Commercial clinical laboratories should participate in a proficiency testing program in which serum samples are sent blindly for analysis. Many manufacturers offer testing programs, and the College of American Pathologists offers such a program in its Diagnostic Allergy Survey (CAP SE).

In the CAP SE survey, five coded serum samples are sent to participating laboratories each year. Each is analyzed in routine fashion for total IgE level, if the laboratory performs that test, multiallergen screening (if the laboratory performs that test) and also for specific IgE to several different allergen solid phases, as specified by survey instructions. Participants receive test summaries that allow them to compare their performance to that of other laboratories. Total IgE levels are formally

evaluated by the CAP SE survey; each sample should be within 3 SDs of the peer group mean, and deficiencies are reported. Test results may be sent to appropriate state and federal regulatory authorities. The CAP survey does not presently set performance standards for specific IgE testing, but it is nonetheless a useful way for laboratories to compare their performance to that of others that use the same method and scoring system.

Test methods

Some of the test methods available in the United States have been described elsewhere (16). In the modern era, no one assay method has been officially designated as a universal standard. The Pharmacia CAP System (Pharmacia, Uppsala Sweden) is in worldwide use, and is a *de facto* standard to which other methods are compared (12, 13, 17, 18).

Clinical indications for testing

The primary purpose for testing is to determine whether a patient presenting to a clinician for evaluation and management of a disorder of ‘allergic hypersensitivity’ (19) has demonstrable allergen-specific IgE. Testing is also employed in population based epidemiologic studies of allergic sensitization.

In the United States and in many parts of the world, allergy-immunology clinicians utilize skin testing as the primary method for detection of specific IgE. The tests are usually applied and the results are usually recorded by a trained technician. Skin testing is a difficult art and a science that requires training and experience to perform with both accuracy and consistency (20), and for interpretation of results (21). In skilled hands, it is quick and easy, and is considered the most sensitive way to detect allergen-specific IgE in a clinical setting. Nearly 100 years of clinical use have made it the standard to which other methods for detection of allergen-specific IgE are compared.

In allergy-immunology practice, specific IgE immunoassays are most useful as an alternative when skin testing is for some reason not practical or possible (Table 1). Reasons for this are both scientific and nonscientific; issues of relative diagnostic sensitivity and specificity, turnaround time, proficiency and competency, and cost and insurance reimbursement are important factors. A multiperspective comparison of skin testing and *in vitro* testing is presented in Table 2. Results of conservatively performed and interpreted skin testing, done under optimal conditions, usually agree with those of modern specific IgE immunoassays, but there is expert consensus that the two methods are not interchangeable (22). When there are discrepancies in the results, the skin test is usually positive and the immunoassay is negative, a

Table 1. Selected clinical situations in which specific IgE immunoassay is appropriate either as a substitute for, or in addition to, skin testing. Adapted from Ref. (16)

<i>Strong indications</i>
Clinical suspicion for allergic sensitization is high, but results of epicutaneous and intradermal skin testing are negative or equivocal
Testing indicated for an allergenic substance (e.g. latex, industrial chemicals) not available as a licensed extract for skin testing
Patient taking a medication that would preclude skin testing (e.g. antihistamines or related drugs; beta blockers) but cannot be stopped
Part of routine evaluation for allergic bronchopulmonary aspergillosis
<i>Relative indications</i>
Limited allergy testing in a patient with asthma (in accordance with published guidelines), rhinitis, atopic dermatitis, or other conditions of suspected allergic hypersensitivity
Testing indicated for an allergenic substance not stocked in the allergy office for skin testing
Positive skin test controls are negative
Very young children and the elderly
Extremely severe dermographism; extensive skin disease
Increased risk for systemic reaction from skin testing (uncontrolled asthma, anaphylaxis, etc.)
Pregnancy
Quantitative results may be clinically useful in evaluation of atopic dermatitis and other conditions of allergic hypersensitivity

situation thought more due to limitations of the immunoassay than to problems with skin testing (23). Although results of comparison studies differ, most modern studies show sensitivity of about 70–90% when immunoassay is compared to epicutaneous skin testing (17, 24), a finding that is not surprising when one considers that IgE is a homocytotropic antibody.

Commercially available immunoassays should be able to detect moderately high circulating levels of specific IgE (>1 µg/l); in such situations, the finding of a positive immunoassay with a negative skin test would lead one to question whether CCDs are present or whether the skin test had been performed correctly. It appears that the failing of immunoassay is in the detection of lower IgE levels. The issue of test sensitivity in the evaluation of anaphylaxis (e.g. systemic reactions to latex, penicillin, or Hymenoptera venom) is particularly important. In such situations, a negative immunoassay warrants confirmation by skin testing when appropriate testing materials are available. The clinical relevance of clearly positive skin tests when the specific IgE immunoassay is clearly negative warrants detailed study.

Skin testing is inappropriate for use by clinicians not trained in its use. Additionally, in general practice the cost of keeping allergen extracts in stock is prohibitive when test volume is low. There is some consensus that specific IgE immunoassays are useful tools when used in defined circumstances by primary care physicians and others who evaluate and manage patients with asthma and the other diseases of allergic hypersensitivity (25). Guidelines for evaluation and management of asthma

Table 2. Similarities and differences between skin testing and specific IgE immunoassays. Adapted from Ref. (16)

Skin testing	Specific IgE immunoassay
<i>Technical factors</i>	
Requires use of excellent, well characterized allergen extracts or other source materials	Same
Extract quality can degrade over time, particularly for certain allergens	CNBr solid phase coupling appears to prevent loss of allergen recognition
Multiple methods, devices with different performance characteristics	Same
Lack of universal definition of a positive test result	Same
Undefined clinical relevance of a positive intradermal test result; suspected low specificity of intradermal testing for certain agents, particularly when high extract concentrations are used	Undefined clinical relevance of low range positive results; low specificity of low range results for certain methods
A manual technique that requires training, skill and expertise	All methods require a sophisticated operator with training and experience in laboratory medicine
Various systems used to report results. Results from one allergist not interchangeable with results from another allergist.	Different test methods use different systems to report results. Results from one method not interchangeable with those from another method. Certain assay methods are highly reproducible from lab to lab
No standards set for testing, personnel, quality control, accreditation	Complex federal standards defined by Clinical Laboratory Improvement Act of 1988
<i>Other factors</i>	
Extract can be from same manufacturer and lot as that used for immunotherapy prescription	Not generally possible
Less cost on a test-per-test basis than <i>in vitro</i> testing	Costs less than a combination of epicutaneous and intradermal skin testing
Results available in a few minutes	Results available in several hours to several days
Interpretation of test results requires appropriate clinical training and experience	Same
Primarily, a tool for specialists	Can be ordered by any clinician
Physician in direct control of testing and reporting of results	Ordering physician must trust an outside laboratory to report appropriate results
Risk (small) of systemic reaction to testing	Risk (small) of vasovagal syncope from phlebotomy

published by the National Asthma Education and Prevention Program specifically recommend that asthma patients requiring regular symptomatic treatment should be evaluated for allergic sensitization (26). A similar recommendation is appropriate for patients with chronic rhinitis and atopic dermatitis. Specific IgE immunoassay would be a suitable method for doing this, particularly in situations where specialist consultation is difficult to obtain, such as in geographically remote areas or certain managed care situations.

Test interpretation

The clinical importance of a specific IgE test result (whether the result is positive or negative) is a matter to be determined by the clinician who has interviewed and examined the patient. This requirement poses a dilemma for the laboratory director who is not an allergy clinician.

Despite the fact that they are often promoted as tests for 'allergy diagnosis', specific IgE immunoassays are best regarded as tests for the presence or absence of detectable specific IgE. IgE is normally present in the serum, and specific IgE can be found in patients with allergic diseases as well as in about 15% of asymptomatic normal individuals (27, 28). Additionally, some patients with the classic diseases of 'IgE-mediated allergic hypersensi-

tivity' (19) have easily demonstrable specific IgE antibody, and other patients with these diseases do not. Even in a symptomatic individual, a positive test result in and of itself is not *necessarily* clinically relevant. For example, the finding of honeybee specific IgE in a child who has had large local reactions from bee stings does not have the same clinical meaning as the same level of honeybee specific IgE found in a patient who has had anaphylaxis from a bee sting. Thus, it has traditionally been taught that the result of any test for specific IgE – immunoassay or skin test – will not in and of itself determine whether the patient has symptoms of IgE-mediated allergic hypersensitivity upon allergen exposure, and in and of itself determine treatment. For these reasons, it is not possible to define a normal range for specific IgE immunoassays. It is thus the primary responsibility of the laboratory director to ensure that analytical false positive and false negative reports are minimized to the extent practical.

The fundamental clinical question to be addressed by specific IgE immunoassay is qualitative – whether specific IgE is or is not detectable in a serum sample. The question of *how much* is present is secondary, but increasing experience with modern quantitative assay technology suggests that in at least some clinical situations, higher levels of specific IgE are more likely to be clinically relevant. Lower levels may or may not be

relevant. For example, in children with atopic dermatitis high levels of food-specific IgE are so strongly associated with a positive double-blind, placebo-controlled food challenge that the use of quantitative specific IgE immunoassays could in some cases decrease the need for food challenges (29).

Summary

Technology for detection and measurement of allergen-specific IgE has helped bring the field of allergy into the

modern era of medicine. This review has attempted to summarize technical and scientific issues in specific IgE immunoassay in a manner that is directly relevant to the care of patients. Challenges facing this technology include improving quality of the source materials used for testing, the rational incorporation of recombinant allergens in diagnostic testing, the universal reporting of quantitative results, and critical evaluation of the clinical significance of both low and high specific IgE levels.

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