

Human IgE antibody serology: A primer for the practicing North American allergist/immunologist

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Abstract

The allergist/immunologist judiciously diagnoses allergic disease by using confirmatory IgE antibody data from in vivo and in vitro assays after the collection of a clinical history. After an overview of historical events, clinically available allergen-specific IgE assays from Phadia, Siemens, and Hycor are contrasted by their design and performance characteristics. The assays share comparable working ranges, analytical sensitivities, and excellent precision, reproducibility, and linearity to a performance standard of <15% coefficients of variation. However, multiple interlaboratory studies have confirmed that the 3 IgE antibody assays either detect different populations of IgE antibody or do not measure the same antibodies with comparable efficiencies. The clinical consequence is that IgE antibody results from the 3 assays are not interchangeable or equivalent. Data generated with one assay cannot be directly extrapolated to published predictive outcomes based on IgE antibody levels from a different assay. The transition from allergen extract-based to allergenic components reagents is discussed, emphasizing the chip-based microarray's strength in identifying IgE antibody cross-reactivity. US Food and Drug Administration-cleared point-of-care IgE antibody lateral flow cassettes are overviewed. Finally, IgE antibody concentration, affinity, clonality (epitope specificity), and specific activity (specific/total IgE ratio) are examined as humoral immune response parameters measured by serologic assays that affect effector cell degranulation and ultimately allergic disease expression.

INTRODUCTION

The diagnosis of human allergic disease begins and ends with the patient's clinical history and a physical examination.¹When the clinical history identifies allergic symptoms in the patient that have a temporal relationship to a definable and relevant allergen exposure, IgE antibody sensitization is then confirmed using either in vivo skin test challenges (puncture/intradermal) or in vitro blood tests (allergen-specific IgE antibody serologic assays). If there is a mismatch between the history and the result of 1 or several of these primary diagnostic tests for sensitization, then a secondary provocation test may be used to adjudicate the veracity of the history-driven diagnosis. Such secondary level provocation tests can include a placebo-controlled food challenge, nasal challenge, bronchial challenge, and in vitro assays evaluating mediator release or surface marker expression involving the patient's basophils after incubation with allergen.²

This article summarizes fundamental principles and performance characteristics of current serologic allergen-specific IgE antibody assays available to the practicing allergist from clinical laboratories. It provides an overview of the current status and future trends associated with the

serologic measurement of allergen-specific IgE antibodies. It is written for the allergist/immunologist in practice in North America, and thus it assumes a fundamental knowledge of the pathophysiology associated with allergic disease. It does not discuss the detection of IgE antibody by skin test methods, secondary level provocation tests, or non—US Food and Drug Administration (FDA)—cleared serologic assays that are used outside North America. It does not discuss other serologic measurements performed by the diagnostic allergy laboratory, which include the quantification of total serum IgE, serum tryptase, serum eosinophil cationic protein, serum immunoglobulin and complement levels, or the presence of IgG precipitins for the assessment of hypersensitivity pneumonitis.

IgE was initially identified in 1967 as the reagent that mediates type I hypersensitivity.^{3,4} By 1972, the first commercial assay for allergen-specific IgE had been developed,⁵ called the RAST. The original RAST was configured as a paper disc allergosorbent on which many different allergens of varying specificities were covalently coupled. Serum was incubated with this solid-phase allergen, permitting antibodies of all isotypes to bind. After a buffer wash to remove unbound proteins, bound IgE antibody was detected with radioiodinated polyclonal antihuman IgE. After this second incubation and a second buffer wash, bound radioactivity was quantified in a gamma counter. The quantity of counts per minute bound was proportional to the amount of IgE antibody specifically bound to the immobilized allergen. The assay was initially calibrated with a birch-specific IgE reference serum that was bound in increasing quantities to a birch allergen—paper disc solid phase and graded in classes patterned after skin testing grades. Understanding of this original basic RAST assay design allows the reader to understand immediately the design of the 3 current IgE antibody assays that are clinically used in North America (discussed below). The allergist should, however, cease using the term “RAST” because it is outdated. The term “in vitro IgE antibody assay” appropriately contrasts serologic assays with in vivo skin testing methods, but some IgE assays are no longer performed in test tubes. Current immunoassays for allergen-specific IgE antibody in serum are best referred to as “serologic IgE antibody assays” or ideally by the actual name of the specific assay.

TRANSITION TO CURRENT IgE ANTIBODY TECHNOLOGY

Since the emergence of the original 1972 RAST, well known companies have come into the market with modifications to circumvent the original RAST patents that protected covalent coupling of proteins to paper discs, binding of antibodies to solid-phase antigens, and the use of tracer-labeled anti-IgE to detect bound IgE. Despite initial successful introductions of their assays, such as the Array (Abbott Laboratories, Abbott Park, Ill) and Magic-Lite (Ciba Corning Diagnostics, currently Chiron Diagnostics, Walpole, Mass) to name only a few, these companies left the diagnostic allergy field for a variety of reasons. The most common reason related to inherent challenges associated with the complexity of the allergen component of these assays. Those companies who have remained with FDA-cleared IgE antibody assays include Phadia (formally Pharmacia, Kalamazoo, Mich), Siemens Healthcare Diagnostics (Deerfield, Ill), and Hycor Biomedical (Garden Grove, Calif). They have understood that the ability of an assay to detect allergen-specific IgE antibody is only as good as the quality of the allergen component in their assay and thus its ability to quantify specific IgE antibody directed against its allergen-containing reagent while avoiding interference from non-IgE isotypes of allergen-binding antibody. These assays are designed to minimize the loss of allergenic epitopes in the preparation

of their extract-derived allergen-containing reagents and insure that allergens in the solid phase are in molar excess to the concentration of circulating antibody so that potential interference of IgE binding by non-IgE binding antibodies is minimized.

CURRENT IgE ANTIBODY ASSAY TECHNOLOGY

The basic chemistry of the allergen-specific IgE assay has remained essentially unchanged since 1972. A solid-phase allergen binds antibody, and bound IgE antibody is detected with a labeled anti-IgE reagent. These 2 reactions are separated by incubation periods and buffer washes. The Clinical and Laboratory Standards Institute Guideline⁶ examines the analytical performance characteristics and clinical utility of immunologic assays for human IgE antibodies of defined allergen specificities. It provides the most comprehensive discussion of the common and unique features for each of the 3 clinically used FDA cleared assays. Salient features of the 3 clinically used assays are summarized in Table I. All assays report comparable analytical sensitivity and calibration schemes traceable to the World Health Organization IgE standard (75/502). Automation has optimized each of the 3 assays' precision, reproducibility, and linearity to a performance standard of <15% coefficients of variation, which is considered a mark of excellence for clinical assays. However, a 2007 report⁷ and a comprehensive 2010 re-examination⁸ of their analytical performance (Fig 1) confirm that the 3 assays either detect different populations of IgE antibody in human sera or do not measure the same antibodies with comparable efficiencies. Interassay differences were confirmed by using masked specimen-based proficiency survey data involving 12 coded human sera that were analyzed for IgE antibody to 16 allergen specificities in; 200 federally certified clinical diagnostic allergy laboratories in North America. Interassay differences have been attributed to the heterogeneity in the patients' IgE antibody responses, differences in the composition of the allergen extract– based reagents used, and particulars of the assays' calibration system. Because the allergen extracts used in the 3 assays are all from different sources, it has not been possible to define why the populations of IgE antibodies that are detected by the 3 assay systems differ. To date, there has been no systematic study using the identical allergen source in all 3 assays to investigate why they measure different levels of specific IgE antibody to a given allergen specificity in any given serum. However, it is known that within a manufacturer's assay, new lots of allergen-reagent of the same specificity generally agree within 15% variance as a result of cross-calibration against the previous lot. There are the occasional exceptions, especially when a new lot of allergen reagent is supplemented with a recombinant component that is known to be labile in the original extract to improve its performance.

TABLE I. Fundamental features of clinically used allergen-specific IgE antibody assays

Name	Company*	Solid-phase matrix	Enzyme-labeled detection antibody	Substrate†	Calibration system	Analytical sensitivity‡
HYTEC-288	Hycor-Agilent	Paper disc	Alkaline phosphatase–labeled anti-IgE	p-Nitrophenyl phosphate	Total IgE System	0.1 kU _A /L
ImmunoCAP	Phadia	Cellulose sponge	β-Galactosidase-labeled anti-IgE	4-Methyl-umbelliferyl β-D galactoside	Total IgE System	0.1 kU _A /L
Immulite	Siemens	Biotinylated-allergen and avidin particle	Alkaline phosphatase–labeled anti-IgE	4-Methoxy-4-(3-phosphatephenyl)-spiro-(1,2 dioxetane-3, 2'-adamantane	Total IgE System	0.1 kU _A /L

*Alphabetical listing.

†The HYTEC-288 is a colorimetric assay; the ImmunoCAP is a fluorimmunoassay; the Immulite is a chemiluminescent assay.

‡Manufacturer reported analytical sensitivity has cleared by the FDA.

Allergen-Specific IgE Assay Performance CAP SE Survey 2009

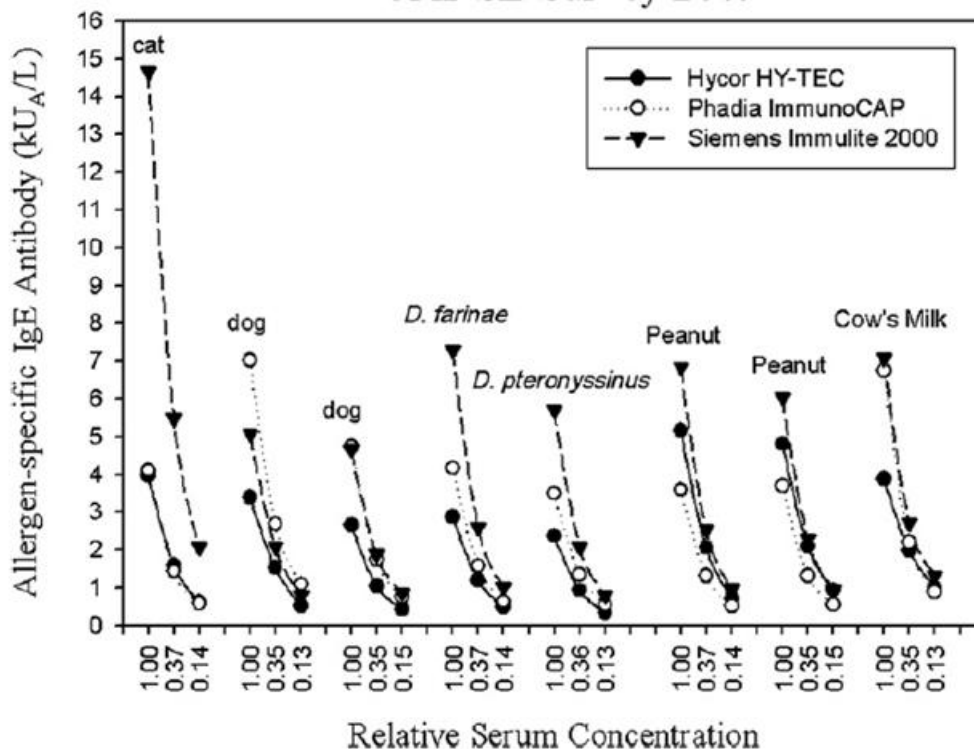


FIG 1. Indoor and food allergen-specific IgE levels are presented in kU_A/L as measured by 3 FDA-cleared autoanalyzers in the C-2008 and A-2009 cycles of the Diagnostic Allergy SE Survey conducted by the College of American Pathologists. Each specimen was twice diluted ~ 2.75 -fold from the previous dilution. All 3 methods displayed good interlaboratory within-assay agreement and linearity with interdilution coefficient of variation $<15\%$. However, differences in the absolute levels of IgE antibody measured among the 3 assays are shown, with desired intermethod coefficients of variation $<20\%$ observed in only $\sim 20\%$ of the measurements performed. Two different sera were analyzed for IgE antidog, and they show clear IgE heterogeneity-related intermethod differences. The same serum specimen was analyzed by participating laboratories in 2 separate survey cycles for IgE antipeanut, and these confirmed intermethod differences. Reproduced with permission from Hamilton RG. Proficiency survey based evaluation of clinical total and allergen-specific IgE assay performance. Arch Pathol Lab Med 2010;134.²¹

CLINICAL IMPLICATION

Historically, accurate detection of the presence of IgE antibody to a particular allergen specificity was sufficient for the allergist to confirm sensitization and support a suspicion of an allergic response that was associated with a particular allergen exposure.⁶ As serologic assays evolved from dichotomous qualitative measures of IgE antibody (eg, positive vs negative) to a more quantitative status, clinical research has shown that in certain cases, the level of allergen-specific IgE antibody can be predictive of a positive allergen challenge test such as relates to food allergy and the presence of more severe disease (eg, wheeze) in children with asthma (see illustrative references⁹⁻¹²). However, the fact that the 3 clinically used IgE antibody assays differ in

their assessment for specific IgE for different allergens (Fig 1) has important implications. It indicates that IgE antibody data generated with one assay cannot be directly extrapolated to published predictive out-comes that are based on IgE antibody levels from a different assay method.⁶⁻⁸In other words, specific IgE antibody levels measured in different commercial assays are not interchangeable or equivalent. The allergist using serologically derived IgE antibody data should, however, note that different IgE antibody predictive values have emerged in the literature beyond the initial proof of concept studies as a result of differences in the allergic populations studied (mode and level of allergen exposure, demographics [especially age], disease states (eg, presence or absence of atopic dermatitis)), the IgE antibody assay used, and the challenge protocols used.^{7,10}

Thus, currently, the most prudent use of a positive specific IgE antibody result is as a risk factor for allergic disease and not as a definitive indicator of the presence of allergic disease.

MULTIALLERGEN IgE ANTIBODY SCREENING ASSAYS

Patients who provide an equivocal or negative history for allergic disease may be best evaluated serologically with a multiallergen screen. This analysis is designed to measure IgE antibody to a panel of aeroallergens or food allergens in a single analysis,¹³ and it displays the highest negative predictive value for atopic disease of any single laboratory test currently available. A negative multiallergen screen reduces the probability that an IgE-dependent process is the cause of reported clinical symptoms.

EVOLUTION FROM ALLERGEN EXTRACT-BASED TO COMPONENT-BASED ASSAYS

The allergist in North America currently receives measurements from IgE antibody assays that use biological extract-based allergen reagents. Select allergen components are currently available as allergen reagents in FDA-cleared IgE antibody assays; however, most of these can be used only for research purposes because they themselves are not yet FDA-cleared. There are several exceptions to this rule. These include a number of purified drugs (eg, penicillin, insulin); single well characterized allergens such as casein, a-lactalbumin, b-lactoglobulin, and BSA from cow's milk that are available commercially in pure form; and isolated cases in which a component allergen, generally in a recombinant form, has been added to an extract during manufacturing to address the labile nature of one of the major allergens that is poorly represented on the extract-based allergosorbent. Two representative illustrations of this latter trend involving Phadia reagents are provided with a positive and negative consequence. Labile Hev b 5, when supplemented into *Hevea brasiliensis* natural rubber latex extract-based allergosorbent, has led to a 10% increase in diagnostic sensitivity with no apparent loss of diagnostic specificity.¹⁴In contrast, labile Cor a 1, which was added to the hazelnut (*Corylus avellana*) extract-based allergosorbent, has caused the unanticipated negative consequence of decreased specificity, secondary to detecting cross-reactive IgE anti-Bet v 1 in patients with birch pollen allergy.¹⁵

This has largely been the extent of introduction of component allergen-based IgE antibody diagnostics to the North American allergist. However, the allergen component-based IgE antibody microarray that has been available in Europe for a number of years¹⁶has been introduced to North

America. The Immuno-CAP–Immuno Solid phase Allergen Chip, which was originally developed by VBC Genomics and optimized by Phadia, currently has >100 native purified/recombinant expressed component allergens from 43 allergen sources that are spotted in triplicate onto glass slides in a microarray. It is attractive for its use of 20µL serum and its ability to identify cross-reactive IgE antibody patterns among allergen families with known structural homology, including the Bet v 1 homologs, profilins, lipid transfer proteins, tropomyosins, and albumin allergen families in the sera of individuals with food allergy.¹⁷In addition, a number of studies have indicated that sensitization to a particular allergenic epitope (as opposed to others from a particular allergen extract) can be predictive of resultant allergic symptoms after allergen exposure.¹⁸⁻²⁰The microarray chip design is powerful in its ability to accommodate any number of allergenic components as they become available and clinically documented. Although not yet FDA-cleared, ImmunoCAP–Immuno Solid phase Allergen Chip data can be obtained on a research basis from select sites in North America. The relative analytical sensitivity of the microarray compared with available extract-based assays and the potential interference of IgE binding to component allergen microspots by allergen-binding IgG and possibly IgA antibodies²¹are analytical issues still to be clarified.

POINT-OF-CARE IgE ANTIBODY ANALYSIS

A point-of-care lateral flow assay cassette called the ImmunoCAP Rapid (Phadia)²² has recently been cleared by the FDA. This credit card–size device is composed of 2 wells, one for whole blood and the other for developing solution. There are 2 rectangular windows, each with a separate nitrocellulose membrane strip containing the allergen zone on which allergen extracts (5 per strip) have been individually coated, forming a total of 10 parallel lines in the cassette. A drop of blood from a finger prick is used to provide a 20-minute semiquantitative estimate of IgE antibody to 10 allergen specificities, currently configured with cat dander, *Dermatophagoides farinae* and *pteronysinus*, Bermuda grass, short ragweed, oak tree, *Alternaria alternata*, timothy grass, elm tree, and dog dander. This device is intended for use by primary care physicians, who would then refer IgE-positive patients to an allergist for a more comprehensive diagnostic work-up.

HUMORAL IMMUNE RESPONSE PARAMETERS RELEVANT TO EFFECTOR CELL RELEASE

Recombinant IgE antibodies and Der p 2 allergens were recently used to investigate how IgE antibody concentration, affinity, clonality (epitope specificity), and specific activity (specific/total IgE ratio) each affect effector cell degranulation.²³Higher IgE antibody concentrations and affinity, greater heterogeneity or diversity in the IgE antibody repertoire (broader clonality), and higher specific IgE to total IgE ratios each contribute to greater effector cell degranulation. This observation led to a critique of current IgE antibody assays that provides only an estimate of concentration and essentially no information on these other parameters involving affinity, clonality, or specific activity.²⁴Because clinically used assays (Table I) are solid-phase, allergen extract–based, and noncompetitive, their design favors the detection of minimally relevant (low affinity) as well as the high-affinity IgE antibody. They also provide no information about clonality other than the detection of antibody specific for a mixture of allergenic components insolubilized from an extract. Moreover, the total serum IgE concentration is rarely measured

clinically, partially because of cost and its wide overlap among atopic and non-atopic populations.²⁵ Thus, the specific IgE to total IgE ratio is almost never computed, and yet it has particular clinical importance, because any defined level of IgE antibody will have a different significance if produced in the midst of a high total serum IgE (eg, atopic dermatitis) as opposed to a low total serum IgE. A future goal of assay design is to configure IgE antibody assays so they directly provide more information about the clinically relevant IgE antibody response parameters including affinity, clonality, and specific activity.

MONITORING PATIENTS RECEIVING ANTI-IgE THERAPY

Since 2003, when omalizumab was licensed for use in patients with severe atopic asthma, total and allergen-specific IgE measurements have been used to determine appropriate anti-IgE dosing schedules. The omalizumab package insert indicates that IgE levels cannot be accurately measured after omalizumab is administered because of interference of assays by anti-IgE. However, in a 2006 survey-based study investigating this issue,²⁶ the ImmunoCAP was shown to be the only serologic IgE assay to exhibit no significant interference when therapeutic levels (50-fold molar excess) of anti-IgE were present in the blood. Thus, contrary to the manufacturers' suggestion, total and allergen-specific IgE antibody can be detected in the presence of omalizumab.

Building on the importance of the IgE specific activity, patients with cat allergy who received anti-IgE therapy in a clinical study with cat-specific to total IgE ratios <1% were more likely to have a reduction in their basophil allergen threshold sensitivity than patients with IgE-specific activities > 4%.²⁷ Conceptually, the lower the IgE anticat in relation to the total IgE in serum, the lower the probability that several cat-specific IgE antibodies will bind in proximity to Fcε receptors on the basophil (or mast cell) surface. Thus, this lower specific activity reportedly translates into a more effective reduction in overall effector cell degranulation when the circulating free IgE is reduced by 90% after omalizumab administration.

Interestingly, a correlation was reported between the serum allergen-specific IgE to total IgE ratio and a questionnaire-derived clinical response to allergen immunotherapy in patients reported to be monosensitized to grass, *Parietaria judaica*, *Olea europaea*, and house dust mite. Individuals with a higher specific IgE to total IgE ratio in serum were reported to have a more effective response to allergen-specific immunotherapy.²⁸

With increased emphasis on the potential clinical importance in the IgE specific activity (specific/total IgE ratio) in anti-IgE therapy and allergen-specific immunotherapy, a population study was performed to examine the distribution of specific to total IgE ratios among atopic American patients.²⁹ IgE specific activity distributions were plotted against age, allergen specificity, and total serum IgE by using 18,950 paired total and specific IgE antibody data obtained from the analysis of sera from 3614 subjects with allergy and covering 182 allergen specificities. The fraction of specific IgE antibody of the total serum IgE was shown to be dependent on age, epitope specificity (see data²⁹), and the total serum IgE level (Fig 2). The group of individuals with allergy (typically the youngest) with the lowest total serum IgE levels tended to have the highest allergen-specific IgE to total IgE ratios. The specific activity thus tends to be an evolving humoral immune response parameter within an atopic individual until the age of 10 to 15 years, when adult levels of IgE antibody are typically achieved. Hymenoptera venom (54%), peanut (33%), and milk (27%) were the 3 allergen specificities that elicited the highest percentage of sensitized individuals with IgE specific activities >4%. These are also among the allergen

specificities that elicit the most severe allergic reactions worldwide. Practically, the specific IgE to total IgE ratio may have its greatest clinical importance in patients with a low total serum IgE level and in mono-sensitized patients.

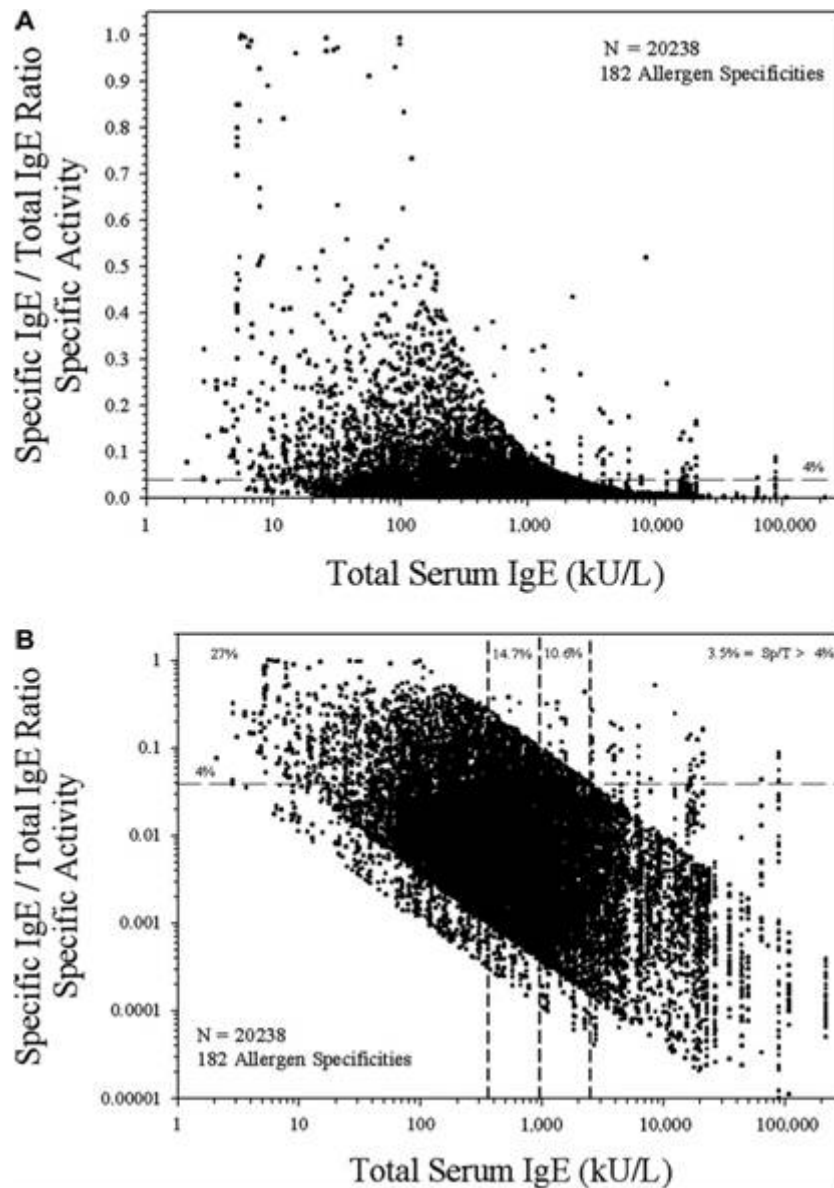


FIG 2. IgE specific activity (allergen-specific IgE to total IgE ratio) for 20,238 total IgE: allergen-specific IgE pairs involving 182 allergen specificities. A, The ordinate at a linear variable. B, The same data as the common log of the IgE specific activity with the vertical dashed lines indicating the first, second, third, and fourth quartiles of the population. A shows that the predominance of high specific activities above an arbitrarily selected threshold level of 4% (whose clinical relevance is defined²⁷) in the low total serum IgE range from 20 to 5000 kU/L. B shows in a different format that the probability of obtaining a high IgE specific activity²⁶(eg, >4%) increases progressively with decreasing total serum IgE concentration. Reproduced with permission from Hamilton RG, McGlashan DW, Saini SS. IgE antibody specific activity in human allergic disease. *Immunol Res* Jan 12, 2010[e pub].²⁹

EXPECTATIONS OF THE DIAGNOSTIC LABORATORY

The allergist/immunologist should expect the allergen-specific IgE tests to be performed with sufficient quality control to insure accuracy.^{30,31} For some judicious laboratories, this involves interspersing up to 15 IgE antibody controls of differing allergen specificities throughout an assay run and expecting a 15% or lower between-assay coefficient of variance for 95% of them.⁶ The laboratory should be federally licensed under the Clinical Laboratory Improvement Act of 1988 and should successfully participate in a masked external proficiency program such as the SE survey conducted by the College of American Pathologists.^{6,8,26} Because allergen-specific IgE results obtained with different assays are not interchangeable, the method used to measure the allergen-specific IgE must be printed on the final laboratory report. It is the responsibility of the allergist to insure that these expectations are met.³² Finally, allergen-specific IgE results reported by a diagnostic allergy laboratory are analytical measurements that are subject to systematic and random errors. If the reported IgE antibody result is inconsistent with the patient's history, the allergist should have the laboratory repeat the analysis for verification, possibly with a new blood specimen or by an alternative IgE antibody detection method with established performance characteristics.³³

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REFERENCES

1. Bernstein IL, Li JT, Bernstein DI, Hamilton RG, Spector SL, Tan R, et al. Allergy diagnostic testing: an updated practice parameter. *Ann Allergy Asthma Immunol* 2008;100:S1-148.
2. Hamilton RG. Methods in the diagnostic immunology laboratory. In: Adkinson NF Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER, editors. *Middleton's allergy: principles and practice*. 7th ed. St Louis: Mosby; 2008. p.1247-66.
3. Ishizaka K, Ishizaka T. Physicochemical properties of reaginic antibody. I. Association of reaginic activity with an immunoglobulin other than gamma A or gamma G globulin. *J Allergy* 1967;37:169-72.
4. Johansson SGO, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967;13:381-94.
5. Wide L, Bennich H, Johansson SGO. Diagnosis by anin vitrottest for allergen-specific IgE antibodies. *Lancet* 1967;2:1105-9.
6. Matsson PNJ, Hamilton RG, Esch RE, Halsey JF, Homburger HA, Kleine-Tebbe J, et al. Analytical performance characteristics and clinical utility of immunological assays for human IgE antibodies of defined allergen specificities. 2nd ed. Wayne (PA): Clinical Laboratory Standards Institute; 2008.
7. Wood RA, Segall N, Ahlstedt S, Williams PB. Accuracy of IgE antibody laboratory results. *Ann Allergy Asthma Immunol* 2007;99:34-41.
8. Hamilton RG. Proficiency survey based evaluation of clinical total and allergen-specific IgE assay performance. *Arch Pathol Lab Med* 2010;134.
9. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.
10. Eckman J, Saini SS, Hamilton RG. Diagnostic evaluation of food-related allergic diseases [review]. *Allergy Asthma Clin Immunol* 2009;5:2-8.
11. De Lovinfosse S, Charpin D, Dornelas A, Birnbaum J, Vervloet D. Can mite-specific IgE be used as a surrogate marker for mite exposure? *Allergy* 1994;49: 64-9.
12. Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children. *J Allergy Clin Immunol* 2005;116:744-9.
13. Williams PB, Siegel C, Portnoy J. Efficacy of a single diagnostic test for sensitization to common inhalant allergens. *Ann Allergy Asthma Immunol* 2001;86: 196-202.
14. Hamilton RG, Rossi CE, Yeang HY, Bernstein DI, Biagini R. Latex-specific IgE assay sensitivity enhanced using Hev-b 5 enriched latex allergosorbent. *J Allergy Clin Immunol* 2003;111:S174.
15. Sicherer SH, Dhillon G, Laughery KA, Hamilton RG, Wood RA. Caution: the Pha-dia hazelnut ImmunoCAP (F17) has been supplemented with recombinant Cor a 1 and now detects Bet v 1-specific IgE, which leads to elevated values for persons with birch pollen allergy. *J Allergy Clin Immunol* 2008;122:413-4.
16. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;16:414-6.
17. Alessandri C, Zennaro D, Zaffiro A, Mari A. Molecular allergology approach to allergic diseases in the pediatric age [review]. *Ital J Pediatr* 2009;35:29-41.
18. Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *J Allergy Clin Immunol* 2006;118:250-6.
19. Ott H, Folster-Holst R, Merk HF, Baron JM. Allergen microarrays: a novel tool for high-resolution IgE profiling in adults with atopic dermatitis. *Eur J Dermatol* 2010; 20:1-8.
20. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component resolved diagnostics. *J Allergy Clin Immunol* 2010;125:191-7.
21. Hamilton RG. IgG antibody interference diminishes accuracy of chip-based allergen-specific IgE assays. *J Allergy Clin Immunol* 2010;125:223.
22. Diaz-Vazquez C, Torregrosa-Bertet MJ, Carvajal-Urueña I, Cano-GarcinuñoA, Fos-Escriva`E, Garcı́a-Gallego A, et al. Accuracy of ImmunoCAP Rapid in the diagnosis of allergic sensitization in children between 1 and 14 years with recurrent wheezing: the IReNE study. *Pediatr Allergy Immunol* 2009;20:601-9.
23. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. *J Allergy Clin Immunol* 2008;122:298-304.
24. Hamilton RG, Saito H. IgE antibody concentration, specific activity, clonality, and affinity measures from future diagnostic confirmatory tests. *J Allergy Clin Immunol* 2008;122:305-6.
25. Barbee RA, Halomen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex and allergen skin tests reactivity. *J Allergy Clin Immunol* 1981;68:106-14.
26. Hamilton RG. Accuracy of Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab). *J Allergy Clin Immunol* 2006; 117:759-66.
27. Johansson SG, Nopp A, Oman H, Ankerst J, Cardell LO, Gr̃oñneberg R, et al. The size of the disease relevant IgE antibody fraction in relation to "total-IgE" predicts the efficacy of anti-IgE (Xolair) treatment. *Allergy* 2009;64:1472-7.

28. Di Lorenzo G, Mansueto P, Pacor ML, Rizzo M, Castello F, Martinelli N, et al. Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. *J Allergy Clin Immunol* 2009;123:1103-10.
29. Hamilton RG, McGlashan DW, Saini SS. IgE antibody specific activity in human allergic disease. *Immunol Res* 2010, Jan 12[e pub].
30. Yunginger JW, Ahlstedt S, Eggleston PA, Homburger HA, Nelson HS, Ownby DR, et al. Quantitative IgE antibody assays in allergic diseases. *J Allergy Clin Immunol* 2000;105:1077-84.
31. Johansson SG, Yman L. In vitro assays for immunoglobulin E: methodology, indications, and interpretation. *Clin Rev Allergy* 1988;6:93-139.
32. Hamilton RG. Responsibility for quality IgE antibody results rests ultimately with the referring physician [editorial]. *Ann Allergy Asthma Immunol* 2001;86:353-5.
33. Dolan WK. It is not yet time to stop skin testing, but.. *J Allergy Clin Immunol* 2000;105:1074-6.