

Overview of Serological-Specific IgE Antibody Testing in Children

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Abstract Allergic diseases are among the most common chronic conditions in the pediatric population. Allergy diagnostic testing is an important part of the evaluation/management of allergic patients because the history may not be precise enough to identify the specific allergen sensitivity. In addition to providing information about specific sensitivities, allergy diagnostic tests have some predictive value in terms of future risk of developing an allergic condition and the severity/persistence of the allergic disease. The two most commonly used methods of confirming allergen sensitization are skin testing and measurement of serum-specific IgE. Both methods have similar diagnostic value in terms of sensitivity and specificity, with both parameters varying with the clinical scenario and allergen tested. Currently, there are three US Food and Drug Administration–cleared, serum-specific IgE assays used in the United States. The three assays report comparable analytic sensitivity, with the coefficients of variation of the precision, reproducibility, and linearity being less than 15%. However, comparative studies have demonstrated significant inter-assay variability, suggesting that they detect different populations of IgE antibody in human sera or do not measure the same antibodies with the same efficiency. Current specific IgE assays utilize allergen extract reagents. Testing with these reagents may identify sensitivity to clinically irrelevant allergens. This diagnostic limitation has spurred the development of molecular diagnostic tests, also referred to as *component-resolved*

diagnostics, which utilize purified native or recombinant allergens to detect IgE sensitivity to individual allergen molecules. These advancements in serum IgE testing may enhance the precision of allergy diagnostic testing, which may decrease the need for oral food challenges and improve the specificity of allergen immunotherapy.

Keywords Allergen-specific IgE · Percutaneous skin test · Component-resolved diagnostics · Molecular diagnostics · Serum IgE · Allergy test

Introduction

Allergic conditions represent some of the most common chronic and costly diseases seen in the pediatric population in Westernized societies. It is estimated that 60 million US residents have some allergic condition [1]. Approximately 40% of children have allergic rhinitis [2], whereas atopic dermatitis and asthma affect 15% to 30% [3] and 9% [4], respectively, of children younger than 18 years of age. It is estimated that 4% of children have IgE-mediated food allergy [5]. In the Third National Health and Nutrition Examination Survey (NHANES III), conducted from 1988 to 1994, 54% of 10,508 participants in the US population had one or more positive skin prick tests (SPTs) to a panel of 10 allergens [6]. In this survey, children 4 to 17 years of age exhibited a positive SPT to at least one of four aeroallergens [7]. Compared with the NHANES II survey conducted between 1976 and 1980, the prevalence to the six common allergens tested in both surveys was 2.1 to 5.5 times higher in NHANES III [6]. These high rates of aeroallergen sensitization have been accompanied by an estimated doubling in the incidence of allergic respiratory diseases in the past two decades [7].

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From 2000 to 2005, the estimated direct medical cost of allergic rhinitis rose from 6.1 to 11.2 billion dollars (all in 2005 US dollars) [8]. The estimated 2010 total US direct medical care costs of asthma were estimated at 15.6 to 18 billion dollars [9]. These estimates do not incorporate the considerable indirect costs, which include over-the-counter medications, comorbidities, and lost productivity.

Allergy testing is an important component of the diagnostic evaluation of these conditions for several reasons. Although clinical history plays a paramount role in establishing the allergy diagnosis, it may not be precise enough to identify the specific allergen sensitivity [10]. Allergy diagnostic testing, in the context of the clinical history, will help identify the specific allergen triggers. This will in turn help direct appropriate treatment recommendations, which may include elimination diets, environmental control measures, and specific allergen immunotherapy. In addition to confirming present allergy, allergic diagnostic tests may have some predictive value in terms of future risk [11–14] and the severity/persistence of the allergic disease [15–17]. The two most commonly used methods of confirming allergen sensitization are skin testing (in vivo) and measurement of serum-specific IgE (in vitro). Both methods have distinct advantages and limitations in the diagnostic process, with one method being preferred over the other in certain clinical scenarios (eg, serum-specific IgE in severe atopic dermatitis). The focus of this review is on serum allergen-specific IgE testing (s-IgE) in the diagnosis and management of pediatric allergy. Topics covered in this review include the utility of s-IgE in food and inhalant allergy diagnosis, s-IgE assays currently in use in the United States, and the recently introduced component-resolved diagnostic (CRD) assays. Although the primary focus of the review is s-IgE, the discussion includes some comparisons with allergy skin tests in terms of diagnostic value. Regardless of which diagnostic test method is used, it is important to use the clinical history to guide which allergens are selected for testing and to interpret the test results because a positive blood or skin allergy test yields information on sensitization, which is not always equivalent to clinical allergy (ie, sensitivity).

Myths Regarding Age and Allergy Diagnostic Testing

Certain misperceptions regarding the appropriate age to perform allergy testing have been propagated throughout the general medical and allergy/immunology community. Confusion exists regarding the youngest age at which skin testing that provides meaningful results can be performed. One study that evaluated 78 infants from birth to age 24 months demonstrated hyporeactivity to histamine, particularly in infants younger than 6 months of age [18].

The allergen SPT produced similarly reduced wheal sizes ranging from 2 to 5 mm, but the results correlated with the clinical history and s-IgE. This suggests that allergen skin testing can produce valid results even in young infants, albeit with a smaller wheal size for the histamine and allergens.

Another misconception about age and allergy testing is the thought that aeroallergen blood or skin testing should not be performed in young children because they have not had enough exposure to be sensitized or are too young to be considered for allergen immunotherapy. The perceived lower age limit for allergy testing may depend on the basis of the misperception (eg, <2 years of age for sensitization exposure or ≥5 years of age to start allergen immunotherapy). In the Cincinnati Childhood Allergy and Air Pollution Study, a birth cohort study designed to determine the prevalence of aeroallergen sensitization in the offspring of atopic parents, aeroallergen sensitivity was found in a significant percentage of children at age 1 year [19]. High-risk infants, who were enrolled if one of their parents reported allergy respiratory symptoms and had a positive SPT to a common aeroallergen, were evaluated with a SPT panel that included milk, egg, and 15 aeroallergens at age 1 and 2 years. At age 1 year, of the 680 enrolled infants, 18.0% were positive to one or more aeroallergens. By category of allergens, 9.7% were sensitized to pollens, 7.5% to molds, 4.3% to house dust mite and/or cockroach, and 3.4% to dog and/or cat. The sensitization persisted in 65.7% of the children tested at age 2 years. It was subsequently determined that the infants with positive SPT responses to tree pollen during the first year of life had a fivefold increased risk of allergic rhinitis at age 3 years [20•]. These studies suggest that the current clinical practice of avoiding aeroallergen testing before age 2 years should be reassessed in high-risk children.

Allergy Laboratory Tests

Clinically Used s-IgE Assays

Allergen-specific IgE antibody assays are designed to detect and measure circulating IgE antibodies that can bind to specific allergens. The ability of the assay to detect allergen-specific IgE is dependent on several factors, including the quality of the allergen component, its ability to quantify the s-IgE directed at the allergen component, and the degree of interference from the binding of non-IgE isotypes [21••]. The first commercial s-IgE assay, the radioallergosorbent test (RAST), was introduced in 1972. It utilized a paper disc allergosorbent, on which various allergens were covalently coupled [21••]. After a buffer wash, bound IgE was detected with a radioisotopically

labeled anti-IgE in a gamma counter. The quantity of counts per minute bound was proportional to the amount of bound allergen-specific IgE antibody, but the assay was not quantitative. Subsequent generations of s-IgE assays utilizing enzyme-linked immunoassays have been able to yield quantitative IgE results [22•]. Current assays no longer utilize radioactivity, making the term RAST inappropriate for describing current serological-specific IgE assays.

Currently, there are three US Food and Drug Administration (FDA)-cleared clinically used s-IgE assays: HYTEC-288 (Hycor Biomedical, Garden Grove, CA), Immulite (Siemens Healthcare Diagnostics, Los Angeles, CA), and ImmunoCAP (Phadia AB, Uppsala, Sweden) (Table 1). There are also some in-house tests available to physicians in the United States that have not been well-studied and/or have not received clearance from the FDA. FDA clearance indicates that the assay demonstrated equivalence to an existing predicate device. For an assay to be FDA cleared, manufacturers must provide data per the Clinical Laboratory Standards Institute guidelines on 35 or more “clinically defined” positive sera, along with 100 nonatopic, healthy donors showing that there is no nonspecific binding to the allergen/solid phase. Lack of FDA clearance indicates that the assay’s performance has not been independently verified. Assays that have not been FDA cleared cannot be sold across state lines. In addition to a requirement that the general assay is FDA cleared, each allergen-containing reagent must also be separately FDA cleared. Allergen-containing reagents that have not been individually FDA cleared are offered as research reagents and are currently labeled as ASRs (analyte-specific reagents). During the next few years, ASRs will be phased

out, and all allergen components used in the three assays will be required to be FDA cleared.

The basis of the three FDA-cleared, clinically used s-IgE assays rests on the binding of the allergen-specific IgE in the patient’s serum to whole allergen extracts immobilized to a solid phase. After a washout phase aimed at removing extraneous materials (eg, nonbinding antibodies), a labeled anti-IgE antibody is added. The label may be an enzyme that reacts with a substrate (eg, fluorescein). The amount of bound allergen-specific IgE is calculated via interpolation from a standard calibration curve, which is linked to the World Health Organization IgE standard and reported in arbitrary mass units (kilo international units of allergen-specific antibody per unit volume of sample [kUa/L]). In the ImmunoCAP system, which is the most extensively studied assay, 1 international unit is equal to 2.42 ng of s-IgE [22•]. Conversion ratios have not been established with other systems. Based on the World Health Organization IgE standard, the three assays report comparable analytic sensitivity, with the coefficients of variation of the precision, reproducibility, and linearity being less than 15%, which is considered excellent for a clinical assay [21••].

In addition to these three FDA-cleared assays, there are multiallergen IgE screening assays designed to detect IgE antibodies binding to a panel of common aeroallergens or food allergens in a single test. A positive test does not identify the specific allergen sensitivity, but it has the highest predictive value for atopic disease of any single laboratory test currently available [21••, 23]. One study of 239 children observed prospectively from birth to 2 years of age aimed to assess the diagnostic efficacy of a

Table 1 Fundamental features of clinically used allergen-specific IgE antibody assays

Name	Company ^a	Solid phase matrix	Enzyme-labeled detection antibody	Substrate ^b	Calibration system	Analytical sensitivity ^c
HYTEC-288	Hycor-Agilent	Paper disc	Alkaline phosphatase labeled anti-IgE	p-nitrophenyl phosphate	Total IgE system	0.1 kUa/L
ImmunoCAP	Phadia	Cellulose sponge	B-galactosidase labeled anti-IgE	4-methyl-umbelliferyl β-D galactoside	Total IgE system	0.1 kUa/L
Immulite	Siemens	Biotinylated-allergen and avidin particle	Alkaline phosphatase labeled anti-IgE	4-methoxy-4-(3-phosphatephenyl)-spiro-1, 2 dioxetane-3,2'-admantine	Total IgE system	0.1 kUa/L
Immuno solid phase Allergen Chip (ISAC) ^d	Phadia	Biochip	B-galactosidase labeled anti-IgE	4-methyl-umbelliferyl β-D galactoside	Positive controls	Semiquantitative

^a Alphabetic listing

^b The HYTEC-288 is a colorimetric assay; the ImmunoCAP is a fluoroimmunoassay; the Immulite is a chemiluminescent assay

^c Manufacturer-reported analytical sensitivity has been cleared by the US Food and Drug Administration

^d This assay is widely available in Europe; however, in the United States, it has not yet been cleared by the US Food and Drug Administration and is available only as a research tool

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multiallergen screening test, Phadiatop Infant (Uppsala, Sweden), in detecting IgE sensitization and the development of atopic manifestations [24]. Specific IgE sensitization was determined by SPT and s-IgE to food and inhalant allergens. The study found that children with clinical symptoms of atopic diseases had significantly increased levels for Phadiatop Infant ($P < 0.01$), with the results corresponding to positive and negative predictive values of 89% and 99%, respectively. At the 2010 National Institutes of Health (NIH)-sponsored Asthma Outcomes Workshop on the specific clinical and laboratory measures to be used in all NIH-sponsored asthma studies, Phadiatop was the only biomarker for allergy/atopy recommended for inclusion in the final report (Busse, personal communication).

Performance Patterns of Clinically Used s-IgE Assays

Although the three assays report comparable analytic sensitivity, studies have suggested significant differences in their performance patterns of the clinically used s-IgE assays such that the results from different assays (eg, Immulite, ImmunoCAP, HYTEC-288) are not generally comparable even if they are reported in the same units. Analysis of their performance indicated that the three assays either detect different populations of IgE antibody in human sera or do not measure the same antibodies with comparable efficiencies [25, 26, 27]. Proficiency survey data involving coded human sera analyzed for IgE antibody to 16 different allergens in 200 federally certified clinical diagnostic allergy laboratories discovered significant inter-assay differences [26]. These differences may be due to several factors, such as heterogeneity in the patients' IgE antibody responses and compositional differences in the allergen extract-based reagents used.

In a study designed to analyze the accuracy and precision of different s-IgE assays, 12,708 blinded serum samples were sent to 6 major US laboratories that utilized 5 different assays. The study found that only one laboratory assay (ImmunoCAP) performed nearly as well as the ideal standard in terms of the slope and coefficient of variation but noted extensive variability in the other four laboratory assay systems with respect to both these parameters [28]. One study that compared the accuracy of Turbo RAST (Agilent Technologies Co., Santa Clara, CA), Immulite, and ImmunoCAP in serum samples and mouse-human IgE chimeric antibodies with known specificity and quantity found poor agreement in the qualitative (presence or absence of s-IgE) and quantitative (amount of s-IgE) test results [25]. Similar discrepancies were found in another study that evaluated the performance patterns of these three assays in measuring s-IgE to milk, egg, peanut, cat, birch, and *Dermatophagoides farinae* [27]. Combined, these

studies suggest that the three assays differ in their assessment of allergen-specific IgE antibodies, possibly because they measure different IgE antibody populations. Because the results from the different assays are not interchangeable or equivalent, data generated with one assay cannot be extrapolated to predict outcomes with another assay. In other words, if a peanut s-IgE level of 14 kUa/L measured with an ImmunoCAP assay predicted a 95% or greater risk of a positive oral peanut challenge in one clinical trial [29], it cannot be assumed that the same s-IgE peanut level confers a similar risk if the measurement was obtained on a different system (eg, HYTEC-288).

The Next Generation of s-IgE: Component-Resolved Diagnostics

One of the limitations of current allergy diagnostic skin and s-IgE tests is that crude allergen extracts are used to test specific allergen sensitivity. Allergen extracts are complex heterogeneous mixtures of allergenic and nonallergenic proteins, glycoproteins, and polysaccharides. Diagnostic tests utilizing these complex mixtures may identify specific IgE to cross-reactive but clinically irrelevant allergens such as profilins. Profilins are highly conserved actin-binding proteins found in most fruits and vegetables, as well as many pollens, that may account for cross-reactions between botanically unrelated species. An individual with a profilin-specific IgE may appear to have sensitivity to multiple pollens to which they have no clinical allergy symptoms during seasonal exposure. Although specific IgE sensitivity to the profilin Bet v 2 may account for oral allergy symptoms in birch-allergic individuals when they eat celery or apples, it would not likely produce birch pollinosis. In contrast, individuals with specific IgE to the pathogenesis-related protein (PR-10) Bet v 1, which is found in 95% of birch pollen-sensitized individuals, may experience allergic rhinitis and/or asthma symptoms during birch season [30]. Skin or s-IgE test with crude allergen extract would not be able to distinguish between these different specific IgE sensitivities (Bet v 1 and Bet v 2). Similar problems exist with venom allergy diagnosis, in which cross-reactive carbohydrate epitopes found in most venom extracts and some unrelated pollen and plant-derived materials (eg, latex) can make identification of the sensitizing venom difficult [31]. This lack of precision could lead to inappropriate decisions regarding allergen immunotherapy content and other management issues. This diagnostic limitation has spurred the development of molecular diagnostic tests (CRD) that utilize purified native or recombinant allergens to detect IgE sensitivity to the individual allergen molecules. Two non-FDA-cleared CRD assays are currently available in the United States. One utilizes the ImmunoCAP technology to assess

sensitivity to the individual allergen components. The other is a microarray-based assay (Immuno solid phase Allergen Chip, Phadia AB, Uppsala, Sweden) that can contain 103 individual allergens from 47 species (Table 1) [32•]. A microarray contains hundreds or thousands of molecules or molecular fragments attached to a surface in a highly ordered pattern [33•]. Allergen microarray assays generally use chemically modified glass as the substrate, whereas conventional s-IgE assays use cellulose capsules or discs to fix the allergens [33•]. Similar to other s-IgE assays, allergen microarrays utilize fluorescently labeled anti-IgE antibodies to detect the allergen-specific IgE, but the results are only semiquantitative. The small amount of patient serum (20 µL) required is a clear advantage of the allergen microarray assay, particularly in young populations, for whom cooperation with skin testing or venipuncture may pose some significant challenges. This amount can be obtained through capillary blood. In contrast, conventional s-IgE assays typically require 10 to 100 mL per allergen test [32•].

Specific Allergens

Food

Serum-specific IgE testing and SPT have a similar diagnostic utility in evaluating children presenting with possible food-related allergic conditions. Both carry potential for false-negative and false-positive results and need to be interpreted in the context of the patient's history. There may be instances in which one is positive and the other negative, and in these cases, the clinical history would be the overriding criterion for establishing the final diagnosis. Limitations of SPT include lack of standardization of the commercially available food extracts, which may result in considerable variation in the extract's composition and relevant allergenic protein content. Labile food proteins may also be destroyed or altered during the extraction process. SPT to the fresh food may address this limitation.

The double-blind, placebo-controlled oral food challenge (OFC) is ultimately the gold standard for confirming or excluding the diagnosis of food allergy. The decision on which type of allergy test to use will depend on several factors, including the child's ability to cooperate with venipuncture or the skin test procedure. However, certain clinical situations may favor s-IgE over skin testing (eg, widespread atopic dermatitis or severe anaphylaxis).

Studies have demonstrated a direct correlation with the magnitude of the allergen-specific IgE concentration or SPT response and the likelihood that clinical allergy will be present [13, 34]. Studies have attempted to correlate allergen-specific IgE levels with the results of OFC to

establish cutoff values for which the likelihood of a positive challenge is a certain percent (eg, 95% will react on OFC) [12]. Important variables that may affect this correlation include the patient's age, disease, and the assay used. To date, the studies have not been able to provide consistent data such that a specific cutoff value can be assigned that will provide optimal sensitivity and specificity across all populations [35]. Although many studies have demonstrated a direct correlation between increasing allergen-specific IgE levels and positive challenge, the relationship between the allergen-specific IgE level and the severity of the reaction is less clear. In a study of 55 peanut-allergic children who underwent an open OFC, reaction severity correlated with the peanut-specific IgE level and SPT wheal size [36]. Another study investigating the clinical characteristics of 870 patients with persistent peanut allergy found a significant correlation with lower respiratory symptoms after accidental peanut exposure and peanut s-IgE levels [17].

Although OFC is the gold standard for diagnosing food allergy, there are several limitations, including the need for trained personnel, appropriate resuscitation equipment, and the patient time involved, as well as the risk of producing a severe reaction. CRD may provide greater diagnostic and prognostic proficiencies that may reduce the need for OFC. A population cohort study of 933 children investigated whether CRD could distinguish between peanut allergy and tolerance in peanut-sensitized individuals as defined by a positive SPT or allergen-specific IgE [37•]. Marked differences were found in the pattern of component recognition between children who did and did not tolerate the peanut OFC, with the peanut component Ara h 2 identified as the most important predictor of clinical allergy. Another birth cohort study of 4,089 children that assessed peanut allergy through questionnaires found that 87% of the peanut-allergic patients had IgE reactivity to Ara h 1, 2, or 3, but not Ara h 8, which is the Bet v 1 homologue [38]. More reactions with respiratory symptoms were reported in children sensitized to Ara h 2 plus Ara h 1 or 3 versus Ara h 2 alone. In addition to predicting presence and possibly the severity of the clinical allergy, CRD may predict persistence of clinical allergy. Persistent milk allergy was seen in individuals with greater IgE epitope diversity compared with those who had outgrown their milk allergy in a study that compared IgE and IgG₄ binding patterns using a peptide microarray [39•]. Likewise, persistent egg allergy was seen in children with serum IgE antibodies to specific sequential epitopes of ovomucoid [40].

Aeroallergens

As with food allergy diagnostic testing, s-IgE and SPT have similar performance patterns in terms of sensitivity, specificity, and predictive values. There may be clinical

situations in which one test may be more sensitive or specific than the other, and the performance of both tests may vary with the allergen tested. Unfortunately, there is no single gold standard for confirming clinical aeroallergen allergy. Using a variety of “gold standards” for defining true clinical allergy (eg, nasal or bronchial challenge), some studies have found SPT to be more sensitive than s-IgE (lower false-negative rate) [41–43] and s-IgE to be more specific (fewer false positives) [44]. Although collectively these studies do not suggest that one method is superior to the other, SPT continues to be the recommended diagnostic tool for aeroallergen sensitivity in the most recently updated Allergen Immunotherapy Practice Parameter [45]. In addition to the potential greater sensitivity, the Allergen Immunotherapy Practice Parameter states several reasons for this recommendation, including being able to use the skin test results to determine a starting dose for allergen immunotherapy. Other advantages to skin testing that are often cited include the immediate availability of the test results and potentially lower costs. Skin test results may correlate better with aeroallergen disease severity than s-IgE. In a study designed to compare the relative ability of SPT, nasal provocation, and s-IgE levels to reflect symptom severity in grass pollen- or birch-allergic patients, a good correlation was found between nasal provocation and SPT results, whereas the intensity of the biologic reactions did not correlate with the allergen-specific IgE levels [46]. As discussed previously, CRD may be useful in allergen immunotherapy by distinguishing sensitizations to major allergens from potentially clinically irrelevant cross-reacting allergens, and this may make specific allergen immunotherapy more specific.

Conclusions

Allergic diseases represent a significant percentage of chronic pediatric illnesses. There are several reasons why it is important to identify the specific allergen sensitivity with the appropriate allergy diagnostic tests. Clinical history may not be sufficient to correctly determine the specific allergen(s) causing the symptoms, and this may lead to inappropriate treatment recommendations such as unnecessary elimination diets or allergen avoidance measures. There are distinct limitations and advantages of the two most commonly used allergy diagnostic methods, SPT and s-IgE, that may vary depending on the testing circumstances (eg, allergic condition for which the testing is being performed, availability and quality of allergen extract). Patient preference and ability to cooperate with the testing procedure likely will be more important in the pediatric population and a determinant of which method is chosen. Multiple studies have demonstrated that the likelihood of true clinical allergy directly correlates

with the SPT and allergen-specific IgE results. Some studies suggest a similar relationship with SPT and s-IgE levels and allergic reaction severity. Advances in allergy laboratory testing through CRD should improve the prognostic and diagnostic accuracy of food evaluations, which may eliminate or significantly decrease the need for OFC in the future. Similar advances may improve the accuracy of aeroallergen diagnosis, which in turn may guide clinicians to prescribe more specific allergen immunotherapy.

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