

Skin Testing and Immunoassays for Allergen-Specific IgE

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Introduction

The clinical evaluation of a patient with a suspected allergic disease begins with a detailed history, physical examination, and selected ancillary laboratory tests (such as pulmonary function testing or radiographs) intended to define and characterize the patient's disease state. Allergy testing helps define triggers of allergic disease (such as rhinitis, asthma, or atopic dermatitis), or another condition in which allergy may play a role (such as urticaria, anaphylaxis, adverse food reactions, or adverse drug reactions). These tests are intended to detect Type I (IgE-mediated hypersensitivity) sensitization, and results should be interpreted and acted on by the clinician who has examined the patient. Allergy tests, strictly speaking, are not tests for the diagnosis of allergic disease; they simply report the presence of detectable amounts of allergen-specific IgE that might in part be responsible for triggering disease symptoms.

Allergy testing employs carefully prepared extracts of pollens, fungi, animal products, foods, and certain other materials such as latex. More purified products are available for most insect venoms and drugs. The use of highly purified allergens (from plant or animal sources) or pure recombinant allergens in allergy testing is currently considered investigational. Extracts are complex mixtures of allergenic proteins and other substances. Each extract may contain several allergens. Those that are recognized by a majority of sensitized patients are termed "major" allergens, and those that are less prevalent in the patient population are termed "minor" allergens. For successful testing of an individual patient, an extract must contain all relevant major and minor allergens. The worldwide process of extract standardization (proceed-

ing at a glacially slow pace in the United States) has produced well-characterized extracts with steady improvements in consistency of potency, although products are in no way interchangeable. The plant and animal allergen extracts used for skin testing and immunotherapy are the same or similar to those used for specific IgE immunoassays.

Skin Testing

Vaughan credited a Dr. Kirkman with the invention of skin testing in 1835, also noting that Salter reported contact urticaria from a cat in 1850 (1). To establish an etiologic role for grass pollen in allergic rhinitis, Blackley tested himself in 1865 (2). The modern era of skin testing began when Rufus I. Cole performed a scarification test in a buckwheat-allergic patient (3) and Oscar Schloss did a scratch test in an egg-allergic child (4). Within a few years, skin testing was widely used in allergy clinics worldwide. For the purpose of study, skin test methods can be described as epicutaneous or intracutaneous. Each method has certain advantages and limitations.

Epicutaneous Skin Testing

The epicutaneous methods in general use are prick testing, puncture testing, and scarification. The term "prick/puncture" is a misnomer because the prick and puncture methods differ significantly. Because scratch testing, in which a linear abrasion is made with a knife or needle prior to application of extract, is uncomfortable and traumatic, it is less widely used.

Prick Testing

Pepys has described the modified prick test that is in general use worldwide (5). In this method, a drop of extract is placed on the skin. A sharp needle is introduced into the skin at an angle, and the most superficial layers of the skin are lifted up with no downward pressure on the skin, introducing a minute amount of extract (6). This method is well-suited for general use, and is the technique of choice for dermatographic patients. A variety of testing devices is available (7).

Puncture Testing

In puncture testing, a drop of extract is placed on the skin and the testing device, often a modified blood lancet, is placed perpendicular to the skin. When a lancet is used, it is placed gently on the skin and tapped gently with a finger. This method, recommended by the European Academy of Allergy and Clinical Immunology, is considered highly reproducible (8). A bifurcated needle, originally designed for smallpox vaccinations, may also be used. This device is placed on the skin with enough downward pressure to form a small indentation in

the skin and rocked forward and backward, and from side to side. This method has been adopted by the Office of Biologics of the United States Food and Drug Administration (FDA). Yet other devices are placed on the skin with enough downward pressure to create an indentation in the skin. Because puncture methods to some extent involve downward pressure on the skin, they are less appropriate for dermatographic individuals than modified prick testing, although they are easier to learn and use. Some devices can be dipped in the extract before skin application.

Scarification

True scarification, in which a knife blade abrades an area of skin before the extract is applied, is no longer commonly used. Some devices, however, are designed to be dipped in extract, applied to the skin with downward pressure, and twisted, producing a small abrasion.

Intracutaneous Skin Testing

Intracutaneous, or intradermal, skin testing was popularized by Robert Cooke in the early 20th century. Until recently, it was the primary method used by allergists trained in many of the Eastern allergy centers. Intradermal testing remains the method of choice for testing with low-potency extracts. Testing is performed with a disposable tuberculin syringe and small-gauge needle. A small amount (about 0.02 mL) of dilute extract is injected into the superficial layers of skin, taking care to make wheals approximately the same size (6). Intradermal testing is more difficult and time-consuming than the epicutaneous methods, and is more painful for the patient. Irritant reactions occur commonly, and can be difficult to distinguish from truly positive reactions, particularly when any reaction greater than that of the saline control is considered positive.

Epicutaneous vs Intracutaneous Skin Testing

Many of the clinicians who employ intradermal tests for inhalant allergy testing use a 50- to 100-fold dilution (1:500 [w/v] or 1:1000 [w/v]) of the stock extract (1:10 [w/v] or 1:20 [w/v])(6) when an epicutaneous test with the stock extract is negative or equivocal, yet clinical suspicion of allergic sensitization is high (7). Although this approach does increase the clinical sensitivity of skin testing, it does so with a loss of clinical specificity. The clinical relevance of a positive intradermal test for inhalants (e.g., pollens, fungi, animal danders) has not been clearly established. In one study of grass sensitization in Colorado, a positive intradermal test result in the face of a negative epicutaneous test did not predict occurrence of symptoms either in an ensuing grass-

pollen season or results of a nasal grass-pollen challenge (9). In testing for drug or venom hypersensitivity, most clinicians perform a preliminary epicutaneous test followed either by a single intradermal test or titrated intradermal tests, because these materials are less potent compared to timothy pollen. There is a general consensus that intradermal testing for food hypersensitivity is rarely, if ever, indicated (7).

Most allergist-immunologists also agree that the use of titrated intradermal skin tests by the skin endpoint-titration method, as proposed by Rinkel and modernized by Willoughby (10), to determine an immunotherapy starting dose is not necessary. Some practitioners of this method employ extract concentrations at concentrations greater than 1:500 (w/v). The clinical relevance of positive test results at these high concentrations has not been established.

In Vitro Immunoassays for Specific IgE

IgE is a homocytotropic molecule present in the serum in nanogram amounts, in an equilibrium with that bound to cells in tissues and in the circulation. Initially termed "reagin," it was easy to demonstrate by skin testing but difficult to identify in the serum. The discovery of an untypable myeloma protein, initially termed IgND, in the serum of a Swedish farmer permitted preparation of rabbit antisera for use in immunoassay (11). Confirmation that IgND was identical to the γ E discovered by the Ishizakas (12) and that both were the elusive reagin of allergy quickly resulted in design and commercialization of immunoassays for allergen-specific IgE. These early assays did not have the sensitivity and specificity of skin testing, and they were not widely adopted by allergist-immunologists. They were welcomed by other practitioners as an alternative to the complex skin endpoint-titration method. Modifications of the early Phadebas RAST (radioallergosorbent test, Pharmacia, Uppsala Sweden) were proposed by Fadal and Nalebuff in order to improve sensitivity relative to skin testing and to provide immunotherapy starting doses. Although classic modified RAST provides increased sensitivity, compared to skin testing, there is also a loss of specificity (13). Currently, the modified RAST is not always performed in accordance with its original description. Some commercial methods use substantially different reagents and incubation protocols, and convert optical density (OD) or other units to "counts per minute."

A typical modern immunoassay uses well-characterized allergenic source materials bound to a solid-phase matrix. This incubates with patient serum. Following 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.

The allergenic extracts and other materials used for immunosorbent-matrix preparation should be procured from a source that can ensure the highest quality. For clinical use, immunoassay manufacturers usually use materials prepared by commercial suppliers. As is the case with skin testing, extracts should 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 cross-reactive carbohydrate determinants (CCDs) with epitopes recognized by human IgE (14). 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 investigational use in immunoassays (15). The difficult procedures for successful cyanogen bromide (CNBr) coupling of allergen to solid phases vary from allergen to allergen. Thus, specific IgE immunoassay is not one test, but many, because each allergic-matrix system has its own individual performance characteristics.

Solid-phase materials of plastic, cellulose, or agarose are most commonly used for the immunosorbent 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. Plastic tubes and 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 (13).

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

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

All modern methods report semiquantitative results using a "class system," often 7 classes with increasing numbers reflecting greater amounts of specific IgE. A class of "1/0" is sometimes used to designate equivocal results. Unfortunately, the class systems differ substantially from assay to assay method, and are not at all interchangeable. Modern methods also report quantitative results related to WHO standard 75/102 for IgE. Doing so requires use of a multi-point calibration curve, using sera with known amounts of allergen-specific or total IgE.

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 pipeting and operation by a more skilled individual.

Standardization and Quality Control

Some specific IgE immunoassay methods are notable for a deplorable lack of standardization (16), even though there are published guidelines for assay design, performance, standardization, and quality assurance (17). Certain methods, however, 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 (18), 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.

Importance of Quantitation

The fundamental clinical question to be addressed by specific IgE immunoassay is qualitative: whether or not specific IgE is detectable in a serum sample. The question of how much is present is secondary, but recent 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. 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 by as much as 50% (19).

Skin Testing vs Immunoassay for Specific IgE

In the United States, allergy-immunology clinicians utilize skin testing as the primary method for detection of specific IgE (7). Skin testing is both an art and a science that requires training and experience to perform with accuracy and consistency (20). 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 must be compared.

Allergist-immunologists use specific IgE immunoassays as an alternative when skin testing is for some reason not practical or pos-

Table 1
Selected Clinical Situations in Which In Vitro Testing Is Appropriate
Either as a Substitute for, or in Addition to, Skin Testing

Absolute indications

- Clinical suspicion for allergic sensitization is high, but 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 but cannot be stopped
- Part of routine evaluation for allergic bronchopulmonary mycosis

Relative indications

- Limited allergy testing in a patient with asthma (in accordance with NAEPP guidelines), rhinitis, or other allergic diseases
 - Testing indicated for an allergenic substance not stocked in the allergy office for skin testing
 - Patients who can not stop antihistamines or related drugs; beta blockers
 - Positive ST controls are negative
 - Very young children and the elderly
 - Extremely severe dermatographism; 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 atopic diseases
-

sible (*see* Table 1). Reasons for this are both scientific (*see* Table 2) and nonscientific (*see* Table 3). Issues of relative diagnostic sensitivity and specificity, turnaround time, proficiency and competency, and cost and insurance reimbursement are particularly important (21). Results of conservatively performed and interpreted skin testing, done under optimal conditions, usually agree with those of modern specific IgE immunoassays, but the two methods are not interchangeable (22). When results are discrepant, the skin test is usually positive and the immunoassay is negative, a situation thought more owing 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 (13,24), 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 levels of specific IgE ($> 1 \mu\text{g/L}$); 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

Table 2
Similarities and Differences Between Skin Testing and
In Vitro Testing: Technical Factors

Skin testing	In vitro testing
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	Some methods require operator assistance and are considered highly complex; others are automated and are considered moderately complex. All methods require a sophisticated operator with training and experience in laboratory medicine
Various systems used to report results. Results from one allergy practice not interchangeable with results from another allergy practice.	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

of lower IgE levels. The issue of test sensitivity in the evaluation of anaphylaxis (e.g., systemic reactions to latex (24), penicillin (25), or Hymenoptera venom) is particularly important. In such situations, a negative immunoassay warrants confirmation by skin testing when appropriate testing materials are available.

Skin testing is inappropriate for use by clinicians not trained in allergy. Additionally, the cost of keeping allergen extracts in stock is

Table 3
Similarities and Differences Between Skin Testing and
In Vitro Testing: Other Issues

Skin testing	In vitro testing
Less cost on a test-per-test basis than in vitro testing	Can cost 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

prohibitive when test volume is low. 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 other allergic diseases (26). Guidelines for evaluation and management of asthma published by the National Asthma Education and Prevention Program (NAEPP) specifically recommend that asthma patients requiring regular symptomatic treatment should be evaluated for allergic sensitization (27). A similar recommendation is appropriate for patients with chronic rhinitis and atopic dermatitis. In vitro testing 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. Testing, however, should not be considered a substitute for a detailed allergy history.

Screening for allergic sensitization has also been promoted as a means of identifying a need for allergy consultation. In multi-allergen screening, a matrix is loaded with mixtures of allergenic extracts and results are reported as positive or negative. Screening panels comprised of individual common allergenic substances are also used. Many practicing allergists believe that allergy consultation should be based on a clinician's history and examination, the severity of symptoms, and the patient's response to management. Screening tests only look for common allergenic substances and will miss the more unusual items that might be identified in a detailed allergy history. Additionally, allergist-

immunologists are also experts in the management of nonallergic rhinitis, asthma and related disease.

Allergy Diagnosis Revisited

Despite the fact that they are often promoted as tests for “allergy diagnosis,” skin tests and 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 (28,29). Additionally, some patients with the classic atopic diseases (among them, rhinitis, asthma, and atopic dermatitis) are allergic and have easily demonstrable allergic sensitization, and other patients with these diseases are nonallergic. Even in a symptomatic allergic individual, a positive test 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 allergic disease upon allergen exposure, and in and of itself determine treatment.

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