

Allergic Rhinitis—Current Approaches to Skin and In Vitro Testing

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It generally is accepted that the pathophysiology and symptoms of allergic rhinitis are based on an IgE-mediated type I Gell and Coombs reaction. During the management of patients suspected of having allergic rhinitis, there are various points at which it is appropriate to confirm this mechanism. Doing so opens the door for treatment options not otherwise available to the patient and bolsters diagnostic confidence for the patient and the physician. In many circumstances this confirmation will set the stage for more effective, timely, and efficient pharmacologic, environmental, and immunotherapeutic management. Confirming the mechanism and the offending triggers responsible for symptoms and measuring antigen sensitivity objectively sets the stage for a more tailored approach to symptom control and improved outcomes.

Box 1 summarizes the currently acceptable tests that can confirm IgE-mediated respiratory allergen sensitivity. This article focuses on skin and in vitro testing techniques.

Skin testing

Using the skin as a test organ to identify aeroallergen sensitivity was first reported in 1873 [1]. Skin testing remains the most widely practiced method for several good reasons. Mast cells are the key effector cells in the type I reaction and reside in the subepithelial layer of nearly all epithelial organs, including the respiratory tract and the skin. Obviously, the skin is visible, accessible, and measurable. The abundance of skin affords the clinician

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Box 1. Tests to confirm IgE-mediated respiratory allergen sensitivity**Skin tests**

- Intracutaneous (intradermal)
- Intradermal single-dilution test
- Intradermal dilutional test

Epicutaneous tests

- Prick/puncture tests
- Multiple-antigen
- Single-antigen

End-organ provocation tests

- Nasal provocation
- Bronchial provocation
- Conjunctival provocation

In vitro serologic tests to measure aeroallergen-specific IgE

- Radioisotope labeling
- Enzyme-linked labeling
- Fluorescence labeling

the option of tailoring strategies by being able to challenge the patient with multiple antigen strengths, thereby improving test sensitivity and leading to fewer false-negative results. A skin test challenges the patient with the same antigen extracts that will be used in immunotherapy. In effect, the patient's response to specific antigens is being assessed by bioassay [2]. Thus, skin testing should assist in a safe and seamless transition to immunotherapy for the patient who is contemplating immunotherapy.

Intradermal testing

Intracutaneous (intradermal) testing by injection of appropriately diluted liquid allergen extract into the dermal layer of the skin is one of the most reliable and proven skin-testing techniques. Akin to a tuberculin test, a 4-mm intracutaneous wheal is created by injecting a small amount of antigen (usually 0.01–0.02 mL) using a one-piece syringe and a 27-gauge needle. This technique allows contact of antigen with subepithelial mast cells. If IgE-mediated sensitivity is present, and the patient has been previously sensitized to the test allergen, the allergen-specific IgE proteins coating the mast cells will become cross-linked with the antigen, causing mast cell degranulation and eventual growth/expansion of the wheal. The wheal is remeasured within 10 to 20 minutes; if the wheal has grown to 7 mm or greater, the test is positive. An accompanying erythematous reaction surrounding the positive wheal, termed the “flare reaction,” offers further confirmation.

Unfortunately there are factors besides allergy that can affect skin whealing responses, making interpretation sometimes challenging. Medications that inhibit the whealing response include antihistamines, tricyclic antidepressants, and systemic beta agonists. Also, pediatric and geriatric patients can be less reactive. Factors that enhance the whealing response include dermatopathologies (dermatographism, eczema, urticaria), and beta antagonists (beta blockers). Because almost all antigen extracts contain glycerin, sensitivity to glycerin also can enhance the whealing response. Enhancement also can occur if the patient recently ingested a food (eg, cantaloupe) that contains surface proteins that are similar enough to the antigenic determinants (epitopes) of an allergen (eg, ragweed) used in a skin test. This phenomenon, referred to as “food cross-reactivity,” effectively augments the immune response. Finally, intradermal wheal tests that are placed too close together (<2 cm) that cause positive wheal and flare reactions may initiate axonal reflexes that can further enhance other proximate whealing responses [3]. Under many circumstances these factors must be minimized, if not eliminated, before performing skin tests, or at least must be accounted for in interpreting the results of skin tests.

Therefore, before proceeding with skin testing, the clinician needs to determine that the skin responds normally to mast cell provocation by exhibiting the usual effects of mast cell degranulation and mediator release that lead to normal wheal growth. Likewise, it also is necessary to make sure that the skin does not respond abnormally by exhibiting unsuspected degranulation and thus abnormal or unusual wheal growth to stimuli that ordinarily should not cause degranulation [3]. These goals should be easy to accomplish by taking a good history and by performing positive- and negative-control tests. If these control tests do not yield appropriate results, skin testing should be suspended, because the results of further tests probably will not be reliable.

The positive-control intradermal test will determine the capability of the patient's skin to mount a histamine-mediated wheal and flare reaction. This test is administered by creating a 4-mm wheal using histamine at a strength of approximately 0.004 mg/mL (Box 2) and should yield a 7-mm or larger wheal after 10 minutes (a positive response). If this test fails to produce a positive response, false-negative responses should be expected if allergenic extracts are also applied; thus, testing should be suspended for that day, and a search for wheal-inhibition factors should be performed. In vitro tests may be indicated in this situation [3].

The negative-control intradermal test, akin to a placebo control, will determine the capability of the patient's skin to resist a false-positive wheal or flare reaction to what should be an innocuous substance. This test is accomplished by placing a 4-mm intradermal wheal using a diluent such as phenolated saline or human serum albumin. The 4-mm negative-control wheal should not enlarge beyond 5 mm after 10 minutes. If further enlargement does occur, such “positive” wheal growth would be assumed to occur

Box 2. Mixing directions for a histamine positive-control test*Method 1*

Mix 2 mL of aqueous (not glycerinated) histamine phosphate (0.275 mg/mL) with 3 mL of phenolated saline. Then make a #2 dilution by titrating two times (1:5 dilutions).

Method 2

Use aqueous (not glycerinated) histamine phosphate (2.75 mg/5 mL). Then make a #3 dilution by titrating three times (1:5 dilutions).

also when antigens are applied, yielding false-positive test results. If a false-positive response occurs, further skin testing should be suspended, and a search for nonallergic wheal-enhancement factors should be sought. In vitro tests may be indicated in this situation.

Glycerin is the preservative used in almost all manufactured allergenic extracts. Because glycerin sensitivity can cause wheal enhancement and thus false-positive results, the glycerin-control test, which is another variation of a negative-control test, becomes necessary when glycerin-containing extracts are used.

Most extract concentrates used by the clinician contain 50% glycerin. Testing should not be performed with intradermal tests using extract concentrates, but there may be instances in which weaker dilutions prepared from these concentrates still contain high concentrations of glycerin. For instance, preparation of #1, #2, and #3 dilutions using the fivefold concept will yield concentrations of 10%, 2%, and 0.04% glycerin, respectively. Many allergists use a 10-fold dilution system, in which 1:100, 1:1000, and 1:10,000 dilutions yield glycerin concentrations of 5%, 1%, and 0.05%, respectively. To eliminate the possibility of false positives from glycerin sensitivity, these glycerin concentrations need to be tested in the absence of antigen if they are to be used in antigen testing.

For the fivefold system, the glycerin-control test is accomplished by creating fivefold dilutions #1, #2, and #3 from a vial containing 50% glycerin. One then creates 4-mm wheals from the strongest dilution anticipated during antigen testing and reads the test after 10 minutes. If the test is negative (little or no wheal growth), there is no need to test other glycerin dilutions. If the test is positive (≥ 7 mm), weaker dilutions should be tested up to a #3 dilution if the use of comparable dilutions is anticipated during antigen testing. If responses to any of the first three dilutions are positive (≥ 7 mm), wheals from antigen dilutions of comparable glycerin strength must be compared with these control wheal sizes and interpreted with caution. If the wheal growth from an antigen test is 2 mm larger than a glycerin-control test at the same dilution, the antigen test is considered positive and valid.

Therefore, a glycerin-control test that yields positive wheal growth (≥ 7 mm) does not necessarily contraindicate further skin testing, but caution should be exercised.

If control tests have yielded the appropriate results, the clinician can proceed with either single-dilution or multiple-dilution intradermal testing. Various dilutions for use in testing should be prepared ahead of time in individual glass vials and should be refrigerated when not in use. These vials should be arranged in a logical sequence in a test tray/board. Dilutions in common use by otolaryngologists are based on a fivefold system. These dilutions are created by withdrawing exactly 1 mL of antigen from the stock bottle with a one-piece sterile needle syringe and injecting it into a 5-mL vial containing 4 mL of diluent. This vial is labeled the #1 dilution and now contains antigen that is five times less concentrated than the concentrate in the stock bottle. The #2 dilution, which will contain antigen that is five times less concentrated than the #1 dilution, is created by withdrawing exactly 1 mL from the #1 vial and injecting it into another 5-mL vial containing 4 mL of diluent. This process usually is repeated until dilutions #1 through #6 have been created. This sequence is repeated for each antigen anticipated for testing. In clinical practice it almost never is necessary to make or test with dilutions weaker than the #6 dilution.

Diluents are relatively inert liquids that are used to dilute the strengths of allergenic extracts to create various dilutions. The most popular diluent in use is phenolated normal saline, because it is relatively inexpensive, nonirritating, and sterile because of the 0.4% phenol that is added. Some clinicians use phenolated human serum albumin, which reduces adherence to the walls of a glass vial ("walling effect"), but it is more expensive and does have the theoretical risk of promoting human viral transmission.

Because of protein degradation, the potency of allergenic extracts can decrease over time, but glycerin is an effective preserver of potency and sterility at strengths of 50% for up to 3 years. Glycerin also prevents "walling" in glass vials. For these reasons, extract concentrates used by most clinicians are placed by the manufacturer in vials containing 50% glycerin.

As one prepares dilutions #1 through 6, however, both glycerin concentrations and antigen concentrations become weaker and weaker. Thus, the potency-preserving properties of the glycerin dissipate, and the potency of most dilutions prepared on a test board becomes unpredictable after 6 to 8 weeks, if properly refrigerated, and even earlier if not properly refrigerated. Therefore it is necessary to replace all dilutions with newly prepared dilutions for intradermal testing every 6 to 8 weeks, and these dilutions should be refrigerated as much as possible [4].

Single-dilution intradermal testing

Intracutaneous (intradermal) testing using a single dilution of an antigen has been practiced for many decades. Following appropriate responses from

the placement of intradermal positive and negative controls, a 4-mm intracutaneous wheal is created by injecting a small amount of antigen (usually 0.01–0.02 mL) using a one-piece 27-gauge syringe. The wheal is remeasured within 10 to 20 minutes. If the wheal has grown to 7 mm or greater, the test is positive. The dilution used depends on variable factors; but commonly used single dilutions include 1:500 (the #2 dilution in a 5-fold system), 1:1000 (the #2 dilution in a 10-fold system), 1:10,000 (the #3 dilution in a 10-fold system), and 1:12,500 (the #4 dilution in a fivefold system) (Table 1).

Testing with a single dilution is quick and efficient; however, testing with one dilution by itself does not give the quantitative or relational information that would be provided by testing responses to additional dilutions of different strengths. In addition, the use of a single-dilution intradermal test with concentrated antigen can be accompanied by an increased risk of significant adverse systemic or local responses. To that end, some clinicians couple a single-dilution test with either another intradermal test using a weaker dilution or with a prick/puncture test. Many clinicians, however, use only one dilution to qualify the presence of IgE-mediated allergy; others also may quantitate sensitivity roughly based on the size of the wheal growth from that one single dilution.

Multiple-dilution intradermal testing using serial dilutions

Intracutaneous (intradermal) testing using multiple serial dilutions of the same antigen has been practiced for many decades. This method of intradermal testing was introduced in the 1930s but probably was most popularized in the 1950s by Herbert Rinkel [5–8].

The historical terms for intradermal testing using multiple serial dilutions were “serial dilution testing” [9] and “skin endpoint titration.” These names have been replaced by the term “intradermal dilutional testing.”

Table 1
Dilution comparisons in a 10-fold versus fivefold system

10-fold system		5-fold system	
Concentrate	1:100 w/v ^a	Concentrate	1:20 w/v ^a
#1 Dilution	1:100 w/v	#1 Dilution	1:100 w/v
#2 Dilution	1:1,00 w/v	#2 Dilution	1:500 w/v
#3 Dilution	1:10,000 w/v	#3 Dilution	1:2,500 w/v
#4 Dilution	1:100,000 w/v	#4 Dilution	1:12,500 w/v
#5 Dilution	1:1,000,000 w/v	#5 Dilution	1:62,500 w/v
		#6 Dilution	1:312,500 w/v

Abbreviation: w/v, weight per volume

^a Concentrate w/v is commonly 1:10, 1:20, or 1:100. 1:10 represents 1 gram extracted in 10 mL of solution [33].

Proponents of this technique point out that patients vary in sensitivity to individual antigens and therefore vary in tolerance to initial antigen doses when starting immunotherapy [10]. Assessing test accuracy over a range of antigen strengths serves both to confirm allergy and to quantify the intensity of the response. The latter information allows more precise tailoring of beginning immunotherapy doses, which in theory will bring relief in a more efficient and timely manner without compromising safety. In addition, the premise that some patients' allergies are less sensitive than others suggests that some patients may not always test positive to single-dilution tests containing weaker dilutions or to prick/puncture tests; thus they falsely will be labeled negative.

Whether or not one chooses actually to perform intradermal dilutional testing on patients, an understanding of its principles facilitates a better understanding of all other forms of testing. The clinician who understands the relationship of intradermal dilutional testing to other testing can move with facility between these methods and thus diversify diagnostic options [4,11].

In intradermal dilutional testing, consecutive dilutions of antigenic extracts are applied in a sequential manner from weakest to strongest. Positive whealing demonstrates the presence of allergy (qualitative test), and comparison of whealing responses at different dilutions demonstrates the degree of sensitivity (quantitative test). Quantitation of sensitivity allows the safe initial starting dose for desensitization immunotherapy to be determined.

The first technical step in intradermal dilutional testing is the application of positive, negative, and glycerin controls. If these control tests do not yield appropriate results, skin testing should be suspended, because the results of further tests probably will not be reliable. The second step is to create skin wheals containing test antigens for titration testing. All wheals produced should be 4 mm in diameter and should be read 10 to 20 minutes after the wheal is created. Begin with a test dilution that is anticipated to be weak enough (usually #6 dilution) to produce a negative response. Then apply progressively stronger dilutions to the point at which either a positively reacting strength is confirmed with the next strongest dilution or all wheals are negative down to a #2 dilution. If the patient is allergic to the test antigen, there will be a series of wheals that progresses from negative to positive and shows increasingly larger wheals with stronger dilutions [4,11].

For positive responses, the end point of titration is the first or weakest antigen dilution that produces a positive wheal (Fig. 1). It is the intradermal dilution at which the patient's response turns from negative to positive. This end point represents the level (ie, the intensity) of the patient's sensitivity to that particular antigen. The end point represents the antigen dilution that may be used safely to begin immunotherapy. To confirm that the end point is authentic, Rinkel [12] advocated the placement of a "confirming" skin

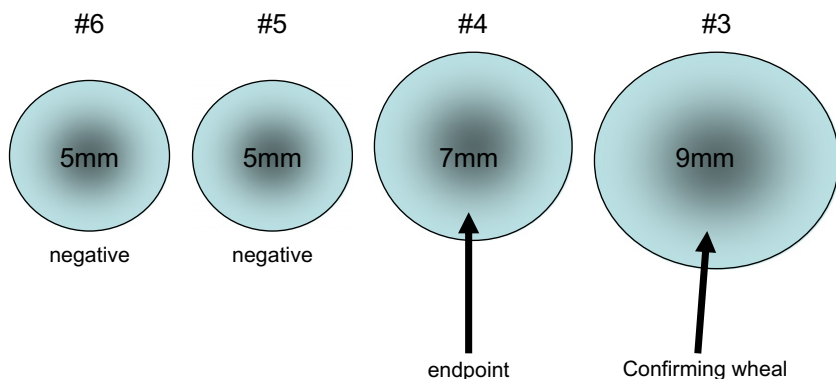


Fig. 1. Multiple-dilution intradermal testing using serial dilutions.

wheel using an antigen strength one dilution stronger than the one that produced the end point wheal. Because this confirming dilution is five times stronger than the end point dilution, it should produce a wheal that is at least 2 mm greater in diameter than the preceding wheal. This confirming wheal validates the end point (see Fig. 1).

Despite the application of appropriately responding controls, unusual whealing responses that make interpretation challenging still may occur. Occasionally, the next stronger wheal following the first positive wheal grows only to the same size as the first positive wheal. This phenomenon is called a “plateau” response because there is no progression of wheal growth, as should occur with the confirming wheal. Ideally one then would apply the next stronger dilution in hopes of identifying a confirming wheal. If a confirming wheal results, the positive wheal that immediately preceded the confirming wheal is the end point because it is the dilution that initiated progressive whealing. Occasionally a “flash” whealing response will occur, which is a negative wheal followed by a very large wheal (usually ≥ 10 –12 mm) in response to the next stronger dilution. The cause of the “flash response” is unknown but is postulated to be the ingestion of a cross-reacting food (eg, melons in patients allergic to ragweed) or the age/potency of the antigen being tested. Repeating the test in a few days generally yields a more conventional response [3].

In a series of negative responses for one antigen, how far does one titrate intradermal dilutional tests to look for a positive result? As long as titrations are negative, antigens are tested down to the #2 dilution. If no positive wheal occurs by that point, most clinicians end the test for that antigen and consider the patient to be negative to that antigen. Clinicians should not test with a #1 dilution if a #2 test was negative. The only time to place a #1 dilution for inhalant testing is to confirm a #2 end point. There are no objective data supporting the clinical significance of a positive #1 dilution if

that dilution is the only positive dilution. Furthermore, performing intradermal tests with full-strength (undiluted) extract concentrates to confirm a positive #1 dilution poses unacceptable risk of anaphylaxis. Therefore, one should not perform intradermal testing with full-strength (undiluted) extract concentrates [11].

There is no definite, universally followed pattern in the number of dilutions applied at one time for each antigen or which dilution strength is placed first. There are, however, some general guidelines that should be followed. First, one should start with relatively weak dilutions and work toward stronger dilutions. As a general practice, it is safe to begin testing most patients at a #4 dilution to limit the number of needle sticks necessary for testing. Because of the potential for increased sensitivity, however, weaker dilutions (#6 dilutions) should be used when testing (1) patients who have asthma (especially steroid-dependent asthma), (2) patients receiving beta-antagonist medication, (3) patients who have a history of previous significant reactions to skin testing or immunotherapy, (4) patients who have had a recent large exposure to suspect antigens, and (5) for antigens that are in season [13].

Intradermal dilutional testing can be time consuming, painful, labor intensive, and expensive. In an effort to save time, needle sticks, and costs, some shortcuts that are acceptable. One can save a small but significant amount of time and number of steps by using "simultaneous dual consecutive dilutions." This testing is accomplished by first applying a #6 dilution of the antigen in question. If the result is negative, place the next two stronger dilutions (#5 and #4). If these results are negative, place the next two stronger dilutions (#3 and #2). If any dilution is positive, allow pattern and size to determine the next step in an effort to identify the end point [14]. Another time- and cost-saving variation on classical intradermal dilutional testing is using extrapolation. Begin at either a #4 or #6 dilution (in low-risk or high-risk patients, respectively) as a screen and then, depending on positive or negative wheal responses, test only even-numbered dilutions (#4 and #2) and extrapolate the end point.

There are several benefits of intradermal titration testing. It is both qualitative and quantitative. Because allergy sensitivities change with antigen exposure [2], multiple samplings over a continuum of dilution strengths beginning with weak dilutions give an accurate and safe [15] appraisal of allergic sensitivity, regardless of whether the allergen is in season. The test is highly sensitive, because patients who have relatively low sensitivity to certain antigens and who may react only to stronger dilutions are identified as the skin titration progresses. It also provides a methodical approach to determining a safe starting dose for immunotherapy, regardless of the patient's current ambient exposure to those antigens [16]. Although circumstances may make it impractical to perform a full titration on every patient, the principles of quantitative testing and development of a treatment regimen tailored to each individual patient remain valid.

Epicutaneous testing

Epicutaneous tests also were one of the earliest methods used for the diagnosis of inhalant allergy. Scratch tests were probably the earliest and involved the placement of antigen onto the abraded surface of the superficial epidermis. Scratch tests possessed multiple disadvantages, however, and have been largely replaced by prick/puncture techniques. In another technique, patch testing, which is used primarily for chemical contact and drug sensitivity, allergen is applied to the surface of the skin under occlusion. Skin responses are noted 48 hours after the application of the patch.

Prick/puncture testing

Prick/puncture testing has become the most popular skin test for the diagnosis of respiratory IgE-dependent allergy. It was first described in the 1920s [17] but was not in clinical use until the 1950s [18]. The prick/puncture technique involves the introduction of concentrate antigen extract into the dermis through a superficial prick/puncture with a sharp instrument (eg, scalpel, needle, or plastic needle). The penetrating end of the instrument may be single, bifurcated, or multipronged. Prick/puncture testing is quick, easy, and safe, even for highly sensitive patients. It commonly is used without other supplemental testing for the diagnosis of allergy and the provision of immunotherapy.

A drop of antigen extract concentrate can be placed on the skin and then delivered to the epidermis by the puncture device. Alternatively, the antigen extract, if glycerinated, can be carried on the sharp end of the device by dipping it into a well containing the extract. Glycerinated solutions are necessary for material to adhere to the prick device when dipped. Single-antigen testing devices are useful when isolated tests are desired. Multiple-antigen testing devices enable simultaneous testing for multiple antigens and controls.

The technique of single-antigen testing with a single-tip hypodermic or plastic needle begins with the placement of a small drop of antigen concentrate (1:20 weight per volume [w/v] or standardized antigen concentrate) onto the skin. The needle then is used to elevate or “tent” the skin through the drop of antigen, taking care not to penetrate into the dermis or to cause bleeding. The technique of single-antigen testing using a bifurcated needle begins with dipping the tip into a testing well containing antigen concentrate. The bifurcated needle allows a small amount of antigen to be held by capillary action within its two tines. The needle then is placed vertically on the skin, and the skin is slightly indented, with uniform pressure, as the device is rotated rapidly through 360° without causing bleeding. The skin responses are read (compared with positive and negative controls) for each individual antigen at 20 minutes.

Plastic devices that contain multiple puncture heads mounted on a handle allow several antigens to be tested simultaneously. The device is dipped into

trays containing multiple antigen concentrates and controls. The puncture heads then are placed on a relatively level skin surface, and the skin is punctured with a slight rocking motion that provides uniform pressure on all heads.

Prick/puncture tests may be performed on the upper back, volar surface of the forearms, upper outer aspects of both arms, and the anterior thighs. Antigens in concentrate with 50% glycerin should be used for testing when the dip technique is used, because glycerin is oily and thus helps antigen adhere to the sharp tip. Begin by cleaning the skin with alcohol. Avoid placing tests closer together than 2 cm. Ideally, the controls should be applied and read before the placement of antigen extracts; however, with multiple-antigen testing, controls are placed at the same time as antigens. Therefore, if the skin responses for the controls are not appropriate, the antigen responses will not be reliable. The positive control should be glycerinated (not aqueous) histamine, 2.75 mg/mL, for the reasons mentioned previously. The negative control should be 50% glycerin because test antigens contain 50% glycerin. Apply the prick punctures without causing bleeding, but make sure that the skin is sufficiently penetrated to avoid false-negative responses. As the device is taken off the skin, small droplets of antigens will remain at the individual testing sites and should not be wiped clean for at least 5 minutes. The skin area tested should be kept relatively immobile during this first 5 minutes to prevent cross-contamination of antigens. The wheal responses should be read after 20 minutes. Flare reactions are not used to judge the significance of the response.

The clinician interprets the prick/puncture tests by measuring wheal responses in comparison to positive and negative controls after 20 minutes. A wheal from a test antigen that grows to a diameter of 3 mm or more than a negative control is judged positive. A strongly and unequivocally positive wheal has been shown to be roughly equivalent to a #3 or #4 dilution intradermal dilutional testing end point [11].

Prick/puncture tests are useful because they are quick, easy, safe, relatively inexpensive, and are easy to teach and administer. Their specificity and sensitivity are comparable to (and perhaps better than) other forms of skin testing [19].

Screens

Initial allergy testing nearly always should begin with a screening battery of 8 to 14 antigens (and controls) in the categories of seasonal pollens, dust mites, molds, and cat, using either prick/puncture, single-dilution intradermal (eg, 1:12,500, which is a #4 dilution in a fivefold system), or in vitro methods. If these tests are negative, the likelihood of clinically significant IgE allergy to the antigens tested and to other antigens not tested is low [20,21].

Using prick/puncture and intradermal techniques in combination

Combining prick with single-dilution intradermal testing, referred to as “modified quantitative testing,” can provide qualitative and quantitative information in a cost-effective way. The prick/puncture test is performed first to estimate the presence and level of sensitivity. In most instances, this test is followed by an intradermal single-dilution test in an effort to estimate the end point that would have been identified by intradermal dilutional testing.

For example, when testing for a single antigen, one begins by placing a prick/puncture test to that antigen after or simultaneously with the placement of positive and negative controls (see Fig. 2). If the result is negative, a single-dilution #2 intradermal test is placed after or simultaneously with positive and negative intradermal controls. If the #2 dilution test is negative, the patient is negative to that antigen. If the #2 dilution test is positive, the patient is positive, and an end point of #3 is assigned. If the initial prick test is positive with a wheal growth greater than 8 mm in comparison with a negative control, the patient is considered positive, and an end point of #6 is assigned. If the initial prick test is positive with wheal growth in the range of 3 to 8 mm larger than the control, the patient is considered positive, and a #5 intradermal test is placed after or simultaneously with positive and negative intradermal controls. If the #5 test is negative, an end point of #4 is assigned. If the #5 wheal grows to 7 to 8 mm, then an end point of #5 is assigned, and if the #5 wheal grows to 9 mm or greater, an end point of #6 is assigned.

It is important to note that the modified quantitative testing method assumes that a positive response to a prick test suggests a level of reactivity

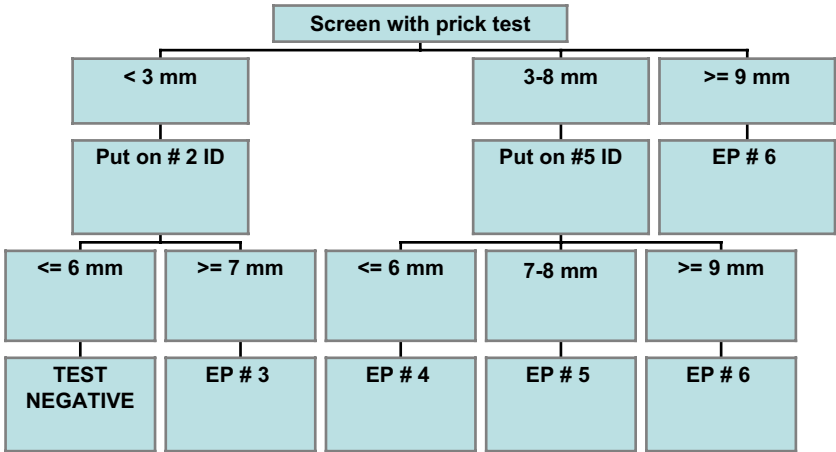


Fig. 2. Modified quantitative testing algorithm. EP, end point; ID, intradermal single-dilution test.

in the #3 to #4 range if tested with intradermal dilutional testing methods. This method also assumes that prick/puncture tests, if used alone, may be falsely negative in patients who have low sensitivity. In other words, the prick/puncture test, if performed alone, may not identify the patient who would test positive only to stronger dilutions in the #3 or #2 range [11].

Intradermal and prick/puncture testing compared

For several decades the utility of intradermal dilutional testing technique has been debated. Although intradermal dilutional testing offers a convincing method of qualifying allergy and accurately titrating and thus quantifying the intensity of allergy, it also is the most time-consuming, labor-intensive, and expensive approach to skin testing [22]. It also requires more supplies and technical expertise than other forms of skin testing. In fact, one recent study reported that the cost of performing a complete intradermal dilutional testing battery is approximately three times greater than the cost of prick testing for the same number of antigens [23].

Many, however, consider intradermal dilutional testing to be the most sensitive of all tests for allergy, in large part because of the provocation of skin responses to increasingly higher antigen concentrations. A remaining question is whether intradermal dilutional testing (or even single-dilution tests at high concentrations) successfully identifies patients who have allergy who would be missed (false negative) by a prick when used alone or by an *in vitro* test. A further question is whether the identification of and therapy for such culprit allergens (that otherwise might be missed) lead to better patient outcomes when these patients are treated. Or, to the contrary, testing patients at higher concentrations and volumes delivered to the dermis might increase the likelihood of false-positive responses leading to inappropriate diagnosis and therapy.

These questions have not been settled finally, but mounting evidence indicates that intradermal testing may not be as accurate as once thought. For instance, intradermal testing with strong dilutions of antigen (1:500 to 1:1000 w:v), which are equivalent or close to a #2 intradermal dilution, have been associated with a significant number of false-positive results [11]. Also, positive intradermal tests with strong concentrations of antigens in the face of a negative prick test have not correlated with either end-organ antigen challenge or with symptoms during relevant pollen seasons. One study has concluded that intradermal testing, when coupled with prick tests, added little to the sensitivity of skin-prick testing and that combining the two has the potential of decreasing the specificity of prick tests [19,24,25]. Another study found that skin-prick testing correlated better with direct end organ challenge than did intradermal dilutional testing [26]. For the reasons described previously, the clinician should continue to search for and critically evaluate skin-testing techniques that are cost effective and accurate.

In vitro testing

In vitro testing of serum samples for the presence of antigen-specific IgE antibody has been available to many clinicians since the 1980s. In vitro testing is a passive test that does not provoke or challenge the patient with antigen as do skin and end-organ provocation tests. The validity of this test is based on the production of antigen-specific IgE antibodies in genetically predisposed patients who have been sensitized to the particular antigen. These tests are bioassays of antigen-specific and non-antigen-specific IgE antibodies in the serum. Because there is correlation between skin-test reactivity and the presence of measurable serum antigen-specific antibodies, the presence of these serum antibodies is assumed to correlate with the presence of these same antibodies when affixed to the surface of mast cells in the skin. There is both a qualitative relationship and a quantitative relationship between measured titers of antigen-specific antibodies and the intensity of skin reactivity. Thus, these tests can be used to tailor starting doses for immunotherapy for individual antigens.

The radioallergosorbent test was among the first available tests designed to measure immunoreactivity using bioassay of IgE antibodies. This technique became possible after the development of IgG antibodies to the antigen-specific IgE antibodies. The measurement of these anti-IgE IgG antibody complexes made it possible to determine both the presence and the amount of IgE in the serum. The measurement of these complexes was made possible initially by labeling the anti-IgE (IgG) antibodies with radioisotopes and measuring the radioactivity.

Thus, the earliest technology involved the incubation of a patient's serum containing antigen-specific IgE antibodies with antigen specific to that antibody mounted on paper discs. This interaction would result in an antigen-IgE antibody complex. Supernatant containing excess IgE specific to other antigens then was rinsed. These complexes then were incubated with radioisotope-labeled anti-IgE IgG antibodies. The supernatant containing the excess noncomplexed IgG then was rinsed, and the resultant radioactivity was measured, giving an indirect measure of the complexed antigen-specific IgE [27].

Because of problems of sensitivity and specificity with the original techniques, the process of isolating and measuring antigen-specific IgE in the serum has undergone many refinements. These refinements have included increasing the incubation times, improving rinse techniques, increasing serum samples, changing cut-off scores, and improving three-dimensional antigen-antibody contact during incubation. Also labeling techniques have diversified by using enzyme and fluorescein labels that can be measured using spectrophotometers and luminometers without the need for use of radioisotopes and Geiger counters [28–31].

The reporting of in vitro scores for antigen-specific IgE antibody is fairly straightforward because it reflects a fairly linear continuum from class 0 to class 5, based on the intensity of the measurements of the labeled anti-IgE

(IgG)–IgE antibody complexes in the patient's serum. Class 0 and class 1/0 are negative and equivocal, respectively, whereas classes 1 through 5 are associated to a linear increase in measurable antibody.

Interpretation of in vitro scores also is straightforward. A high class score is associated with the presence of higher antibody titers, which is an objective measure of the patient's allergic sensitivity to the particular antigen. The score does not necessarily reflect symptom severity, but it does correlate roughly with skin reactivity in skin tests with that antigen. This quantitative information is helpful in tailoring starting doses for individual antigens if immunotherapy is contemplated [32].

The benefits of in vitro testing include convenience (one venipuncture), the absence of effects caused by medications or skin conditions that contraindicate testing (as can occur with skin testing), the absence of risk of reactions from testing, and better patient cooperation and tolerance. Furthermore, if one accepts the premise that IgE-mediated hypersensitivity must be associated with the presence of significant elevations in serum IgE, in vitro testing can be used to diagnose such patients accurately and to identify or eliminate potential candidates for immunotherapy. Also, highly sensitive patients, as identified by high IgE titers, can be managed appropriately to minimize the risk of anaphylaxis associated with iatrogenic antigen exposure, as occurs with skin testing and immunotherapy. Not surprisingly, IgE levels are relied on to dose patients properly for insect venom immunotherapy and for anti-IgE monoclonal antibody therapy in asthmatics.

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