

ALLERGY TESTING USING IN VIVO AND IN VITRO TECHNIQUES

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Allergic diseases, such as allergic rhinitis and atopic dermatitis, are caused by abnormal responses to substances such as pollens and foods. These substances, when recognized by the cells and antibodies that cause an allergic response, are called *allergens*. It is essential to the care of allergic patients to determine which allergens may be inciting their disease because this information is used to direct allergy prevention and treatment. This determination starts with a good history and physical examination, which provide important clues. For example, a patient who awakens every day with itchy, watery eyes and a stuffy nose but who otherwise feels well likely has an allergy to the dust mite allergens that are present in his or her bedding; however, a patient with similar symptoms that vary with the seasons likely has a pollen allergy. Another patient, for example, who experiences shortness of breath, mouth and tongue swelling, and an urticarial rash after eating shellfish very likely has a food allergy to shellfish. With the information provided by a good history and physical, it is possible to identify specific allergies that a patient is likely to have and, subsequently, to order specific, cost-effective allergy testing.

The allergist has a variety of tests to identify the allergens that may be responsible for an individual's allergic disease. These tests include in vivo skin tests, such as prick and intradermal skin tests, and in vitro

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tests, such as radioallergosorbent tests (RAST). The choice of which appropriate tests to order and perform requires a fundamental understanding of the allergic response, the mechanism of action of in vivo and in vitro testing, and the relative risks and benefits of the different tests available.

THE IMMEDIATE HYPERSENSITIVITY RESPONSE

To understand allergy testing, it is essential to understand the function of IgE, mast cells, and other elements of the immediate hypersensitivity response. The pathway to the immediate hypersensitivity response starts with the allergen, which is recognized by a specialized group of white blood cells called antigen-presenting cells (APCs), which include macrophages, dendritic cells, and Langerhans' cells (Fig. 1).¹⁴ These APCs absorb, repackage, and then present the antigen to T lymphocytes. T lymphocytes, in turn, secrete proteins, called cytokines, such as interleukin-4 (IL-4), which, in conjunction with second signals such as the physical contact of T lymphocytes with B lymphocytes, stimulate B lymphocytes to create IgE specific to the allergen.¹⁴ This IgE is secreted

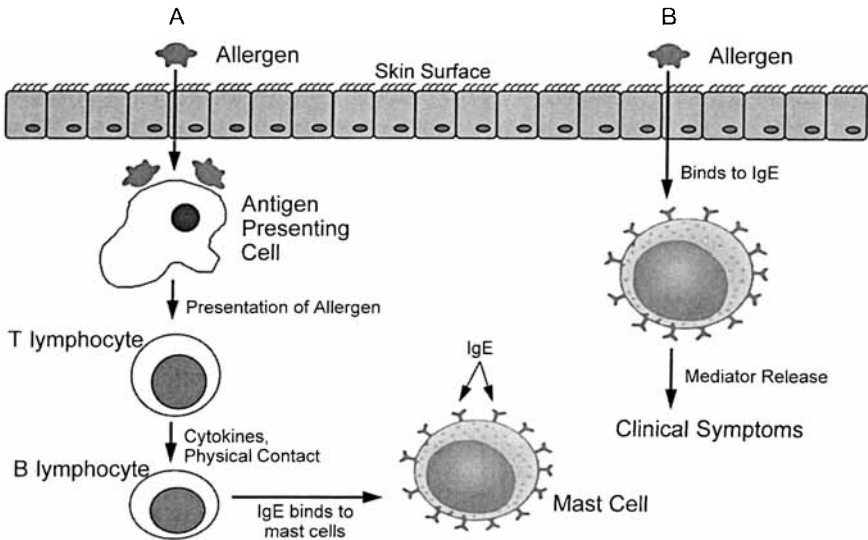


Figure 1. The allergic cascade. Sensitization (A) begins with the allergen, which is recognized by antigen-presenting cells (APCs), which absorb, repackage, and present the antigen to T lymphocytes. T lymphocytes then secrete cytokines and produce second signals, such as physical contact, that stimulate B lymphocytes to create IgE specific to the allergen. This IgE is secreted into the blood and binds to mast cells. In provocation (B) an individual is re-exposed to an allergen that binds to the specific IgE on the surface of mast cells, thus triggering degranulation and the release of the mediators of the immediate hypersensitivity response from mast cells.

into the blood and binds to mast cells.¹⁴ The IgE remains on the surface of these mast cells and, when an individual is re-exposed to the antigen, binds to the allergen and triggers degranulation and then release of the mediators of the immediate hypersensitivity response, such as histamine.¹⁴ Histamine and other mediators of the immediate hypersensitivity response released by mast cells lead to a wide variety of local and systemic changes, which results in such typical clinical findings as urticaria, angioedema, and bronchoconstriction.¹⁴

IN VIVO ALLERGY TESTING

In vivo (or skin) testing for immediate hypersensitivity, for the purposes of this review, is defined as the exposure, via percutaneous or intradermal routes, of a small group of mast cells to allergen.^{1, 3} If these mast cells have, on their surfaces, the IgE specific for the allergen in question, they will degranulate, leading to the wheal (localized swelling) and flare (localized redness). Skin testing has been a key diagnostic tool for many years and remains useful for many reasons.

Before skin testing is performed, however, patients must be carefully screened by medical personnel. For example, patients should be screened for risk of possible adverse reaction to the skin testing, resistance to the medical treatment of adverse reactions, unacceptable risk of injury if an adverse reaction should occur, or high likelihood that skin test results will not be useful. Such patients include those who have a history of exceptionally severe allergic reactions, pregnant patients (because of the risk of fetal injury or premature uterine contractions should an allergic reaction occur), and patients with chronic medical conditions, such as unstable angina.⁷ In addition, patients taking β blockers should have this class of drugs discontinued or replaced for at least three days before skin testing, because β blockers inhibit the normal response to epinephrine, a key resuscitative drug in the management of allergic reactions.⁷ Another consideration before skin testing is performed is the anticipated value of skin test results. For example, patients should not take antihistamines for at least 48 hours before testing because these drugs inhibit the very reactions that skin tests are attempting to provoke and create a risk for false-negative results.⁷ Also, the use of chronic and high-dose oral or parenteral corticosteroids can reduce skin test reactivity, as can the long-term application of potent topical steroids to areas being tested.⁷ In addition, patients with dermatographism or severe dermatitis may be unsuitable for skin testing because of their heightened level of skin reactivity and the potential for false-positive results. After careful screening has been performed, patients may be skin tested.

There are two commonly used types of skin tests for immediate hypersensitivity.¹⁰ The first is the epicutaneous (prick or scratch) test. In this test, a small amount of antigen is placed on the skin surface and a sharp point from a needle or another device is used to minimally puncture or scratch the skin (Fig. 2). After 15 minutes, the test sites are



Figure 2. Skin prick test for allergy. A gloved practitioner, after having applied drops of diluted allergen to the skin surface, pricks through the drops using a puncture device.

examined (Fig. 3), and the results are interpreted with a scoring system such as the one illustrated in Table 1. Most allergists consider a positive skin test result to be a wheal 3 to 5 mm larger in size than the negative control and assign such a result the score of 3+.¹⁶ The scoring of skin test results, however, requires a high degree of experience, and the somewhat subjective adjustments made in scoring by the experienced practitioner, based upon such considerations as the skin test technique that had been used and reactivity to positive and negative controls, are as important as the actual parameters used for scoring. One consideration, for example, is that skin test reactivity varies somewhat with age.¹⁹ Using a scoring system is important, however, because there is a correlation between reactivity and clinical allergy.¹ The second type of test is the intradermal (ID) test (which is actually intracutaneous). In this test, a volume of antigen extract is injected just under the skin surface, in a manner similar to the Mantoux test for tuberculosis, to create a distinct 2 to 3 mm diameter bleb. After 15 minutes, the puncture sites are examined and are scored based on another scoring system (Table 2). The same considerations described previously for prick test scoring also apply to ID test scoring. Both of these types of tests may be

Table 1. PRICK SKIN TEST SCORING

Reaction	Score
Flare without wheal	1+
Wheal $\leq$ 2 mm	2+
Wheal $\leq$ 3 mm	3+
Wheal $\leq$ 4 mm or with pseudopodia	4+

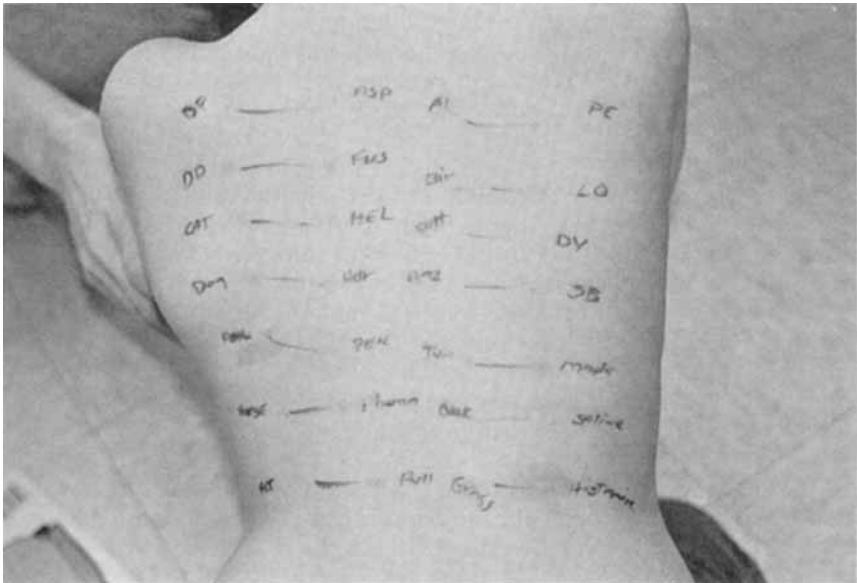


Figure 3. Skin prick test results. This typical panel of Pacific Northwest aeroallergens only shows reactivity to the histamine control (*lower right*), which would be scored as 4+ based on the size of the wheal (the pale, raised area in the larger flat flare). The dark marking under “feather” (*fourth from top*) is skin hyperpigmentation and is not the location of a prick.

performed on the volar surface of the arm or on the back (which is slightly more reactive than the arm). Prick tests should be placed at least 3 cm apart, and ID tests should be placed at least 5 cm apart to reduce the potential for false-positive results.¹ In addition, a positive control, such as histamine, and a negative control, such as the diluent used for preparation of the allergen solution, are essential to the proper interpretation of both types of test results.¹ Both tests are frequently used, and they provide separate and complementary information.

In practice, the prick test is used before the ID test for a number of reasons. The prick test exposes the patient to a much smaller amount of antigen and is, therefore, less likely to lead to severe allergic reactions (such as anaphylaxis).¹ Also, the prick test is less painful and is easier and faster to perform than the ID test.¹ Unlike ID testing, prick testing

Table 2. ID SKIN TEST SCORING

Reaction	Score
Wheal ≤ 3 mm	1+
Wheal ≤ 4 mm	2+
Wheal ≤ 5 mm	3+
Wheal ≤ 6 mm or with pseudopodia	4+

can be performed using glycerinated allergen solutions, which are more stable.¹ Finally, prick test results generally correlate better with clinical allergy history than ID testing.^{1, 8} Because it is designed to expose the patient to a minimal amount of allergen, however, the prick test is limited somewhat in its ability to identify all patients who are allergic to a given substance. For example, skin prick tests have been demonstrated to detect between 73% and 100% of sensitive patients, relative to oral food challenges in patients with food allergies or to nasal and bronchial provocation tests in patients with inhalant allergies.¹⁶ In contrast, the ID test, because it exposes the patient to a larger dose of antigen, is believed to be more sensitive (able to identify more patients who have a specific allergy) but less specific (more false positives), in part because of the nonspecific irritant effect of the test.^{7, 8, 16} For this reason, the ID test is commonly used in practice after a patient who is believed to be likely to be sensitive to a given allergen has demonstrated a negative or equivocal result to a prick test to that allergen.¹ The ID test may be especially useful in the evaluation of agents responsible for anaphylaxis, such as penicillin and insect venom, because of the test's increased sensitivity and because of the danger to a patient who has demonstrated anaphylaxis of having any specific IgE to these allergens.³ As mentioned previously, ID testing also may give more false-positives than prick testing. For this reason, ID tests, like all allergy tests, should be performed and interpreted by qualified allergy specialists.

A wide variety of allergens for prick and ID testing is currently commercially available. A typical prick test order sheet, which includes common food allergens and aeroallergens such as dust mites, house pets, molds, weeds, grasses, and trees, is illustrated in Figure 4. It is accepted practice to obtain 70 or more different prick tests, followed by up to 40 different ID tests for inhalant allergens with lower numbers for food allergens.⁷ Generally, however, only 30 or 40 prick tests are required for most patients. In infants and young children, fewer tests are generally ordered because they are generally sensitive to a smaller variety of allergens. It is also common practice to perform prick (but not ID) tests using locally prepared allergens, such as latex, fresh fruits, and fresh vegetables. ID tests are usually not performed with these allergens because they are not standardized and the risk is greater for severe allergic reactions, such as anaphylaxis, and false positives because of contaminants.

Quality control is an important issue for allergy skin testing on many levels. First, the quality of allergen preparations used is critical to the validity of skin tests. It is best to use potent, standardized, allergen extracts from commercial manufacturers.⁷ There is, however, variability in allergen preparations even among the large manufacturers. There are also a number of quality control issues to address within the skin testing procedure: The person performing the tests needs to have a fundamental understanding of allergy mechanisms and skin testing, testing needs to be reproducible, and allergen extract quality needs to be maintained by proper storage and replacement.⁷ Also, because of demonstrated vari-

FOODS	PP	ID	FOODS	PP	ID	FOODS	PP	ID	INHALANT'S	PP	ID	INHALANT'S	PP	ID
CEREAL MIX			MEATS			SEEDS			HOUSEHOLD			TREES		
1 Corn			9 Beef			Cottonseed			1 D. Farnae			15 Alder		
2 Rice			10 Chicken			Flaxseed			2 D. Perron			16 Birch		
3 Wheat			11 Pork			Safflower						17 Cotton Poplar		
Barley			Lamb			Sesame						18 Hazelnut		
Oat			Turkey			Sunflower						19 Juniper		
Rye									ANIMALS			20 Maple		
			FISH MIX			VEGETABLES			3 Cat			21 Oak		
4 EGG WHITE			12 Codfish			24 Peanut			4 Dog			Ash		
5 COW MILK			13 Halibut			25 Potato, white			5 Feathers			Russian Olive		
Goat milk			14 Salmon			26 Soy Bean			6 Horse			Sika Spruce		
			15 Snapper			27 Tomato			Cattle			Walnut		
FRUITS			16 Tuna			Asparagus			Gerbil			Willow		
6 Apple			Mackerel			Bean, string			Goat					
7 Banana			Perch, Lake			Broccoli			Guinea Pig			22 GRASS MIX		
8 Orange			Trout			Carrots			Hamster			Bermuda		
Apricot						Cauliflower			Mouse					
Avocado			SHELL MIX			Celery			Parakeet			WEEDS		
Blackberry			17 Clam			Garlic			Rabbit			23 English Plantain		
Blueberry			18 Crab mix			Lettuce			Rat			24 Lamb's Quarters		
Cantaloupe			19 Oyster			Lima Bean						25 Scotch Broom		
Cherry			20 Shrimp			Mushroom						26 Yellow Dock		
Citrus Mix			Lobster									27 Kodia		
Cranberry			Scallops			Onion			MOLDS			28 Pigweed		
Grape						Peas			7 Alternaria			29 Russian Thistle		
Mango			NUTS			Potato, yam			8 Aspergillus			30 Sagebrush		
Peach			21 Almond			Squash			9 Fusarium			31 Ragweed mix		
Pear			22 Hazelnut						10 Helminth			Fireweed		
Pineapple			23 Walnut			MISC.			11 Hormodend					
Plum			Brazil Nut			28 Chocolate			12 Penicillium			H HISTAMINE		
Raspberry			Cashew			Baking yeast			13 Phoma			C SALINE		
Strawberry			Pecan			Gum acacia			14 Pullularia					
Watermelon			SPICE MIX			Karaya gum						Staff		
Cinnamon			Ginger			Oregano						Date		
Clove			Nutmeg			Sage								
H Histamine			C Saline											

Figure 4. Typical skin test order sheet for common foods and aeroallergens, and includes columns for both prick (PP) and intradermal (ID) testing. The selection of aeroallergens has been tailored to the Pacific Northwest region. Numbered items = most commonly tested foods and aeroallergens.

ability between devices and techniques, it is important to carefully select skin test devices and techniques and to consistently use them.^{6,9} In addition, because there is some variability in the scoring systems and allergen extracts used in different clinics for testing, it is useful to record on the test sheet the scoring system used and the source of the allergen extracts.¹ Finally, the office in which testing is performed should be well prepared for adverse reactions, with such supplies as injectable epinephrine; injectable antihistamines and steroids; stethoscopes and sphygmomanometer; parenteral medication delivery equipment (intravenous, subcutaneous, and intramuscular); and oral airways.² Intubation and cardioversion equipment may also be useful, but the risk of the use of these items in suboptimal circumstances may, in many cases, outweigh their benefits.² Of course, office staff should be familiar with the proper use of the above medications and equipment and should have well established emergency protocols.² A final quality control issue is the outdated practice of reusing syringes for antigen injections for different patients, which is clearly inappropriate because of the risk for transmission of blood-borne diseases.¹

IN VITRO ALLERGY TESTING

The principal feature of in vitro allergy testing is the attempt to find serum IgE specific for a given allergen. Most of this type of testing is performed with immunoabsorption "sandwich" techniques. In these techniques, the allergen in question is bound to an insoluble support. This bound allergen is then incubated for a period of time with the patient's serum while the patient's specific IgE antibodies, if they exist, bind to the antigen. Next, a labeled anti-human antibody is added, and the level of this label is measured. A very commonly used example of this in vitro testing is RAST testing.^{4, 11, 15, 18} Results are commonly quantified using standard deviations from normal sera, comparison to calibration curves, or other methods and are commonly expressed in class scores (e.g., 0+ to 4+), similar to skin tests.⁷ In many selected situations, in vitro allergy testing can be very useful.

There are a number of clinical indications for in vitro testing.^{4,7} For example, when standardized prick and ID testing is not available, such as in the case of some foods, in vitro testing may provide superior diagnostic information. Furthermore, both standardized and nonstandardized antigens, when used for skin testing, always place patients at some small risk for severe allergic reactions, such as anaphylaxis, whereas there is no such patient risk associated with in vitro testing.¹⁷ Also, patients with dermatographism or severe dermatitis may get better results from in vitro testing because their skin test results may be difficult to interpret.¹⁷ In addition, mentally impaired patients who may not be able to cope with the patience and mild discomfort needed for skin testing may be more suitable for in vitro testing.⁷ Finally, those patients who are unable to stop antihistamine use long enough for skin

testing may be candidates for in vitro testing, which is not influenced by medication. There are disadvantages to in vitro testing, however. For example, results typically take several days, whereas skin testing takes about 20 minutes.³ Also, there is a difference in cost. A recent report by the AAAAI states that in vitro testing typically costs at least twice as much as skin testing.⁴ Finally, there are questions about the value of in vitro test results relative to skin testing. Because skin testing results reflect an allergic function, whereas in vitro testing assesses the potential for allergic function, skin testing is more clearly reflective of the clinically relevant allergic status of a patient. For example, the sensitivity of in vitro tests relative to prick skin tests has been reported to range from 50% to 90%.^{7, 17} Furthermore, because in vitro testing is less sensitive than skin testing, in no situations may in vitro tests be used to rule out risk for anaphylaxis to allergens, such as insect venom and penicillin, in patients who have a clinical history that suggests a risk for anaphylaxis.^{3, 17} Therefore, in vitro allergy testing, although valuable, needs to be used in appropriate settings and carefully interpreted.

Quality control is an important issue for in vitro allergy testing. The primary quality control issue is that this type of testing is commonly performed in a wide variety of settings, from small private offices to large commercial laboratories, with antigens prepared by a variety of methods, which makes the standardization of reagents and techniques difficult.^{4, 18} In fact, a large amount of variability in allergen standards used between different laboratories performing in vitro testing has recently been reported.⁵ Therefore, test results between different laboratories may not be comparable. Furthermore, it is essential that laboratory personnel are well trained in the performance and interpretation of in vitro allergy testing, as well as the maintenance and troubleshooting of equipment and allergen standards.¹⁵ Positive and negative controls should always be used for in vitro allergy testing.¹⁵ A laboratory manual with information about the reconstitution and proper use of each allergen standard is essential.¹⁵ Excellent record keeping is also needed for appropriate performance of in vitro allergy testing.¹⁵ To circumvent many of the quality control issues of in vitro allergy testing, many allergists send their patient samples to commercial laboratories, where results are generally more reproducible, which helps to create greater comparability between different labs.

TOTAL SERUM IgE TESTING

Sometimes, in the evaluation of the potentially atopic patient, the patient's total IgE is measured. This test is commonly performed using the "sandwich" technique similar to the technique described previously for in vitro specific allergy testing.¹¹ This technique is a much less specific and sensitive assay than skin and in vitro tests for allergy.⁷ For example, it has been demonstrated that patients with levels of IgE within the "normal range" may be allergic, and patients with high levels of IgE

may not be allergic.¹³ Nonetheless, some investigators have sampled cord blood for total IgE levels to screen for later development of allergic disease.^{12, 17} Current data is unclear about the correlation between cord blood IgE levels and the development of allergic disease¹⁷; therefore, the use of cord blood total IgE levels to predict later allergic disease is not an accepted practice at this time.^{4, 7} There are situations, however, when a total IgE level may be helpful.^{7, 17} For example, there are rare patients for whom specific allergens to which they are sensitized cannot be detected by commonly used tests, but they have clinical presentations, such as anaphylaxis, that strongly suggest allergic causes. In these patients, an IgE level may be helpful in guiding the health care provider to obtain or to not obtain further allergy testing. There are also some rare conditions, such as the hyperimmunoglobulinemia E syndrome, that present with, among other features, very elevated IgE levels and eczema.¹⁷ In those patients who resemble the clinical descriptions of such syndromes, a total IgE level may be very helpful to make a diagnosis. In general, however, total IgE levels contribute much less to the evaluation and care of the potentially allergic patient than a well-performed history and physical examination, complemented by specific allergy testing.

CONCLUSION

Allergy skin and in vitro testing, when used by trained professionals in conjunction with good history taking and physical examination techniques, is an essential component of the evaluation of the potentially allergic patient. An understanding of the basic physiology of the allergic response is also essential to obtain the appropriate tests and to correctly interpret the results. For example, most allergists chiefly rely on skin testing, as opposed to in vitro testing, because, among other reasons, the biochemical reaction that leads to interpretable results is closer to the true physiology of the allergic response and, therefore, is more likely to reflect the true allergic status of an individual patient. As a corollary to this statement, it is clear that the simple presence of allergen-specific IgE molecules in the serum is not always reflective or predictive of true allergy, which, by definition, involves a clinical response to allergen. In vitro allergy testing, however, is very useful in conjunction with and, sometimes, in place of, skin testing for allergy. Furthermore, both skin and in vitro testing are improving significantly as more allergens are being standardized. Total IgE levels are occasionally a useful adjunct to the complete allergy evaluation but cannot replace the thoughtful use of the history, physical examination, and specific allergy testing.

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