

VARIABLES IN ALLERGY SKIN TESTING

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Skin testing is usually the preferred method for the diagnosis of immunoglobulin E (IgE)-mediated hypersensitivity because it is simple, inexpensive, and the results are immediately available. Like any laboratory test, however, a number of variables can affect the outcome, such as:

Variations with age:

Reactivity to histamine

IgE-mediated sensitivity

Chronobiologic variation:

Diurnal

Seasonal

Variations with the menstrual cycle

Variable reactivity of different parts of the body:

Variations on the back

Variations between the back and forearm

Some of these variables, such as differences in reactivity with age and sites on the body, are characteristics of the patient and are beyond the control of the operator. Other variables, such as previous use of medication, quality of the extracts, and techniques employed for performing the test, are potentially controllable by the operator, who must make informed decisions in regard to these aspects. This article reviews the evidence for these variables in the skin test response.

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VARIABLES BEYOND THE CONTROL OF THE OPERATOR

Variations with Age

The prevalence and overall magnitude of positive allergy skin tests vary with patients' age.^{3, 4} The number and size of the prick skin reactions in an unselected population was shown to increase throughout childhood to about age 20 and then gradually decline until age 50, after which the decline was more rapid.³ The prevalence of positive skin reactions to allergen seemed to be a result of the interplay of cutaneous reactivity to histamine and the underlying IgE-mediated sensitivity.⁴ IgE-mediated sensitivity increases throughout childhood. The maximum number of RAST reactions to grass and dust mite was encountered between ages 10 and 15 years.¹⁰ Thereafter, specific IgE levels began to decline. The diameter of the skin test reaction to 27 mg/mL histamine hydrochloride assessed in patients of all ages was found to increase from 3.8 mm in the 0- to 3-year-olds, peak at 6.2 mm at age 21 to 30 years, and then decline progressively with each decade to 4.5 mm in those over age 60 years.²⁵ The important effect that histamine reactivity can have on the prevalence of positive skin tests is suggested by a study comparing the threshold of skin reactivity to histamine with the number of positive skin tests to allergen in a general population study.²⁷ Fifty-six percent of those subjects least reactive to histamine had all negative skin tests and only 15% had six or more positive reactions. In the group most reactive to histamine, only 11% of subjects had all negative skin tests and 60% had six or more positive reactions.

Chronobiologic Variation

It has been reported that there is a marked variation of skin test reactivity over the course of the 24-hour day; however, recent studies failed to confirm this finding as an important variable in allergy skin testing.³⁰ Twenty children and 20 adults all sensitive to ragweed underwent titrated prick skin testing to ragweed and histamine at 8 AM and 4 PM. The reactions were significantly larger at 8 AM by statistical analysis, but the difference was not of a degree to effect clinical interpretation.³⁰ Variation of skin tests with the season of the year also have been examined.²² Subjects periodically underwent titrated prick skin tests to a tree pollen extract, ragweed extract, perennial allergen extract, and histamine. Testing was performed in July before the ragweed season, in late August at the peak of the ragweed season, in October about 1 month after the end of the ragweed season, and in February, well after the pollen season. Subjects were most reactive in October and February and least reactive in July and August. The circannual variation was observed for histamine and allergen extracts, indicating that this variation was unrelated to allergen exposure.

Variations with the Menstrual Cycle

Skin test reactions to histamine and allergen vary in women with their menstrual cycle.¹¹ Reactions to both are larger in midcycle (days 12–16) than during the menses (day 1–4) or the late progesterone phase (days 24–28).

Variable Reactivity of Different Parts of the Body

It has been long recognized that the skin of the forearm is less reactive on allergy skin testing than skin on the back.¹⁸ A study found the diameter of the wheals to be 27% smaller and flares to be 14% smaller on the forearm than on the back with prick skin testing with allergen. Both differences were highly significant ($P < 0.001$) but would be clinically important only for marginal reactions. Similar but smaller differences were noted for histamine skin tests in the two regions of the body. In 76 patients who had skin testing to the same allergens performed on the back and the forearm, there were 2.3% more positive reactions on the back (173 on the back versus 164 on the forearm).¹⁸

It has been noted that there is a gradient of reactivity of the skin of the back.¹⁶ The lower back is more reactive to histamine and allergen compared with the upper back. The wheal diameters were 30% less with allergen and 19% less with histamine on the upper compared with the lower back.

VARIABLES SUBJECT TO CONTROL BY THE OPERATOR

Variables subject to control by the operator include:

- Medication
- Extract quality
- Relation to adjacent positive reactions
- Percutaneous versus intradermal testing
- Devices employed for percutaneous testing

Medication

A number of medications can suppress skin reaction to histamine and allergen. The suppressive effect of the histamine H_1 antagonists is well recognized.⁷ A 3-day washout of antihistamines is generally advised and is usually sufficient. Histamine H_2 antagonists also suppress the skin reaction to histamine, though the magnitude is much less.¹⁴ It may be advisable to omit this class of medication for 24 hours before to testing.

Skin test suppression by medication used for the treatment of depression is probably common, but largely unstudied. The tricyclic antidepressants have well-recognized antihistaminic properties. The effect of a single dose of 25 mg of desipramine and doxepin was compared.²⁴ The former produced significant suppression of the wheal for 2 days and the flare for 1 day, whereas the latter caused suppression of the wheal for 4 days and the flare for 6 days. The effect of regular ingestion would last longer.

A 1-week course of daily glucocorticosteroids was reported to have no effect on immediate skin tests.²⁶ Contradictory results have been reported with long-term oral steroid administration. A retrospective study suggested suppression of codeine, and by inference allergen, skin test reactions occurred in patients receiving daily oral corticosteroids for months to years.²¹ A prospective study, however, in which 33 subjects received a median daily dose of 20 mg prednisone for a median period of 2 years failed to demonstrate skin test suppression.⁹

It has been recognized for many years that high dose allergy immunotherapy results in a reduction in skin test reaction to the allergen administered which is detectable by the time the subject reaches his or her maintenance level.^{5, 8} This finding is true even when maintenance has been achieved employing a rush protocol.¹²

Extract Quality

The reaction on allergy skin testing is effected by the quality of the allergy extract employed. It has been recognized for some time that certain foods seem to contain allergens that are inherently labile or broken down by enzymes in the extracts.² This phenomenon particularly is encountered with certain fruits.²³

Even extracts that are of good potency when received from the manufacturer can deteriorate with storage.¹⁵ This is primarily a problem with the dilute extracts employed for intradermal testing, because they cannot be prepared in 50% glycerin as can the extracts used for prick skin testing. The rate of loss of potency varies with extracts; ragweed was reported to very stable, whereas grasses, cat, and *Dermatophagoides farinae* all suffered a significant loss of potency at a 1:10 dilution of the concentrate.¹⁷ This finding suggests that extracts for intradermal testing should be replaced every month or, at the most, every 3 months.

Adjacent Positive Reactions

It often has been recommended that allergy skin tests not be performed closer than 5 cm apart. A prospective study, however, found that when performing skin testing with the solid bore needle on the back, there was not a significant difference in false-positive reactions at control sites placed 2.0 to 7.5 cm distant from a strongly positive test.¹⁸ False-

positive skin tests have been reported adjacent to histamine control tests with intradermal skin testing²⁸; however, the tests were intradermal and the negative sites had been injected with dilute hymenoptera venom extract which may have some activity on the blood vessels or mast cells, favoring a whealing reaction.

Percutaneous versus Intradermal

Allergy skin testing can be performed by intradermal injection of the allergen extract or by the percutaneous route either by prick or puncture. Each method has certain advantages. Percutaneous testing advantages include the following:

- Safer
- Technically less demanding
- Rapid
- Little discomfort
- Many allergens in one session
- Positive and negative easily distinguished (if low trauma device employed)
- Positive correlates better with clinical symptoms
- Steeper dose response curve

Intradermal testing advantages include the following:

- More sensitive (1000 to 100,000 fold).
- More reproducible

The prick or percutaneous method is generally favored, at least for the initial screening.

Percutaneous testing is particularly favored because of its safety, ease, speed, and relative comfort. In one private practice, 10,400 patients were prick skin tested without a systemic reaction, and two patients reacted after intradermal testing.¹³ At the Mayo Clinic, 16,505 patients underwent prick skin testing with a systemic reaction rate of 0.3 per 1000. Intradermal skin tests were performed following negative prick skin tests with a systemic reaction rate of 0.7 per 1000.²⁹

Given that good quality allergy extracts are generally available and evoke positive reactions in many asymptomatic individuals, it has been questioned whether there is a need for the increased sensitivity of the intradermal test.¹ When 150 subjects who denied either rhinitis or asthma were skin tested with 13 standardized allergy extracts by the prick method, 29% of those who denied either personal or family history of atopy and 42% of those with no personal history but a positive family history of atopy had at least one significant positive prick skin test.¹ In an epidemiologic survey, intradermal skin testing was performed in subjects with a negative prick skin test.⁶ There was only a small, although significantly greater, number of subjects with positive intradermal skin tests in the allergic compared with the nonallergic group.⁶ For

individual allergens there was no difference in the percent with positive intradermal tests between the allergic and nonallergic groups for Bermuda grass, cat, *Dermatophagoides farinae*, olive tree, or rabbit bush. For careless weed, there were significantly more positive intradermal reactions in the allergic group. In a prospective study, patients who gave a history of seasonal allergic rhinitis were studied for the clinical relevance of a positive intradermal skin test to grass when they had a negative prick skin test to the same antigen.¹⁹ Patients' clinical sensitivity to grass was assessed by nasal challenge and the correlation between symptoms and pollen counts during the grass pollen season. Though approximately 50% of patients with positive prick skin tests to grass reacted positively to these two tests, none of the patients who only had positive intradermal skin test reacted positively to both. In their response to the two tests of clinical sensitivity, patients sensitive only to grass on intradermal skin test did not differ in their percent of positive responses to either test from those patients with negative intradermal skin tests to grass pollen extract (Table 1).¹⁹ Under the conditions of this study, it seemed that the prick skin test to grass was sufficiently sensitive to identify all those subjects who were clinically sensitive. The clinical relevance of a positive intradermal skin test to cat, in the presence of a negative prick test, also has been assessed.³² One hundred twenty patients with asthma, with or without clinical cat allergy, were exposed to a room contaminated with cat dander. Both positive prick skin tests and in vitro tests for cat sensitivity were predictive of positive reactions to exposure (Table 2).² The rate of reactions to cat exposure was the same in the prick skin test negative subjects, whether or not they had a positive intradermal skin test to cat. The authors concluded that prick skin tests and in vitro tests, but not intradermal tests, were efficient in the diagnosis of cat allergy.³²

The relative sensitivity of the intradermal to the prick skin test has been variously reported. Because the slope of the wheal is steeper with prick than with intradermal testing, it is clear that the relative sensitivity varies with the size of the reaction. Relative sensitivities of 1:1000 may exist with small reactions, and ratios of 1:100,000 may exist with large

Table 1. ASSESSMENT OF THE CLINICAL RELEVANCE OF POSITIVE INTRADERMAL SKIN TESTS TO GRASS IN PATIENTS WITH NEGATIVE PRICK SKIN TESTS

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Table 2. PREDICTIVE EFFICACY OF PRICK AND INTRADERMAL SKIN TESTS AND RASTs IN PREDICTING REACTIONS TO CAT EXPOSURE

Category	Positive Cat Challenge
PST +	38/41
PST -	10/39
RAST +	27/27
RAST -	12/44
PST - ID +	6/26
PST - ID -	4/13

From Wood RA, Phipatanakul W, Hamilton RG, et al: A comparison of skin prick tests, intradermal skin tests, and RASTs in the diagnosis of cat allergy. *J Allergy Clin Immunol* 103:773-779, 1999; with permission.

wheals. An additional advantage of intradermal testing is greater reproducibility; however, with good technique the prick test can be reproducible.³¹ If greater accuracy is required, it may be simpler to perform the prick skin testing in duplicate rather than resort to intradermal testing. One indication for intradermal testing may be in situations in which the quality of the extract is poor, which is a problem primarily with dog and mold extracts. For the patient with in-home dog exposure, it may be of value to perform an intradermal test if the prick skin test is negative before reassuring the patient that the pet is not contributing to their symptoms. The situation is not analogous with mold extracts: positive skin tests are not required to institute environmental controls, and the relevance of a positive intradermal test as an indication for allergen immunotherapy is questionable.

Devices

The multiple devices available for percutaneous skin testing are described elsewhere in this issue. Several factors should be considered in device selection, including the following:

- One device per patient or one device per skin test

- Sensitivity to operator technique

- Separate or simultaneous application and introduction of allergen extract

If one device is used per patient, it should be one from which residual extract can be wiped easily. This is accomplished most easily with a solid bore needle in which a wet cotton ball adequately removes the remaining extract. Alternatively it is possible to remove residual extract from the bifurcated needle but only by both swishing in liquid and wiping with a cotton ball. The same swish-and-wipe procedure may suffice for single pronged lancets but has not been studied. Several devices have been designed to produce more consistent results in the hands of different technicians. Three commercially available devices (Morrow-Brown needle, ALK Lancet, and Hollister-Steir Prick Lancetter)

all have a 1-mm point with a widening above the point to limit penetration and ensure similar penetration with varying pressure. Application of the extract and puncture in one operation is another attribute. This is possible with the Multi Test (Lincoln Diagnostics, Decatur, IL), DermaPIK (Greer Laboratories, Lenoir, NC), Duotip (Lincoln Diagnostics, Decatur, IL), and Quintest (Bayer Allergen Products, Spokane, WA).

Several available devices can be employed by the prick method, including the solid bore needle, bifurcated needle, Duotip, and DermaPIK. In addition to good reproducibility with experienced technicians, prick testing allows reliable testing in patients with dermatographism.

The various devices currently available vary in the results obtained. It may be most convenient to consider the devices as variable in their degree of trauma to the skin.¹⁶ As the degree of trauma induced by a device increases, larger amounts of extract penetrate the epidermis, producing larger reactions. At the same time there is generally a parallel increase in patient discomfort and the likelihood of a nonspecific reaction at the site of saline controls or what should be negative tests with allergen extract. The occurrence of a reaction at what should be a negative site is not even consistent in the same subject during the same testing session.^{16, 20} It is important to establish the potential range of nonspecific reactions with each device and each technician. If these reactions exceed the customary lower limit for a significant test of 3-mm wheal or a 10-mm erythema, it is necessary to calculate new thresholds that can be the 95 or 99 quartiles of the reactions to saline, depending on the degree of certainty desired.¹⁶

RECORDING SKIN TEST RESULTS

The results of allergy skin testing, like any laboratory test, should be recorded in a manner to provide clear, easily interpretable information to other physicians and interested parties. Two types of information should be provided on the report: the results of the skin tests and the conditions under which they were conducted. This information should include the following:

Conditions of testing:

Percutaneous or intradermal

Concentration of extract employed

For percutaneous testing, the device employed

Location of testing (back or arm)

Size of reaction to negative (saline) and positive (histamine) controls

Grading of skin test reactions:

Measure of perpendicular diameters of the wheal and flare in millimeters (preferred)

Grading reactions 0 to 4+ with a key to the interpretation of these semiquantitative terms

Anything less than this is unacceptable; this includes grading 0 to 4+ without a description of their meaning and grading plus and minus.

Given the variability introduced by the factors described in this article, not providing such information as the site on the body and device used for percutaneous testing clearly limits the interpretability of the data. The use of arbitrary grading systems without a description of their meaning to report results also leads to a lack of clarity.

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