

# Task Force on Allergy Skin Testing

## In Vivo Diagnostic Tests of Immediate Hypersensitivity Reactions

9/04/2008

PHILIPPINE SOCIETY OF ALLERGY, ASTHMA AND IMMUNOLOGY, INC

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The main objective of the Task Force on Allergy Skin Testing was to formulate a local standard for performing, reporting and interpreting allergy skin tests that could be adopted by the society.

The Allergy Diagnostic Testing: An Updated Practice Parameter, representing an evidenced-based, peer reviewed, broadly accepted consensus opinion and published in the Annals of Allergy, Asthma and Immunology in March 2008, served as the main resource of this report. The GLORIA module on Diagnosis of IgE Sensitization and the medical literature in both foreign and local journals were also used as references.

The Task Force members were divided into 2 groups. One group handled the Prick/Puncture Test while the other group tackled the Intracutaneous Tests. The following issues contained in the summary statements of the practice parameter that can potentially impact the local allergy practice were discussed: technique, devices, area of testing, concurrent drugs, age, extracts, controls used, number of tests, reading and interpretation of test results. Clinical significance, sensitivity, specificity, negative and positive predictive value, limitations and safety issues were also dealt with. Recommendations for further studies and research were made. If applicable, summary statements were to be adopted to the report.

## PRICK/PUNCTURE TESTS

***Summary Statement 1: Skin tests have evolved as cost-effective and reliable methods of diagnosing clinical immediate hypersensitivity. They are used to confirm clinical sensitivity to inhaled and food allergens. Under carefully defined circumstances, these tests are also useful in the diagnosis of drug and chemical hypersensitivity reactions. (B)***

Background:

Over the last 3 decades, the prevalence of allergic diseases has markedly increased in developed countries and all over the world.<sup>1,2</sup> Accordingly, the need of allergy testing has increased as well.<sup>3</sup>

Skin testing represents the primary diagnostic tool in allergy that is used to confirm that a specific allergen suggested by medical history has induced an Immunoglobulin E (IgE) antibody response.<sup>4</sup> When properly performed, this test is generally considered to be the most convenient, most effective and least expensive screening tool for determining the presence of IgE mediated sensitivity. It gives the clinician and the patient immediate information on reactivity to individual allergens.<sup>5</sup>

All persons presenting with severe, persistent or recurring allergic symptoms (asthma, allergic rhinitis, atopic dermatitis, urticaria, food allergy, insect sting reactions, latex allergy) should undergo prick/puncture tests in order to identify the specific allergen that may be causing their symptoms.<sup>3</sup>

## TECHNIQUE

***Summary Statement 2: Several devices can be used to perform the prick/puncture tests. No single device has been objectively shown to be superior to the others. Proper training with a chosen instrument is necessary to obtain optimal results. (C)***

Although numerous comparative studies have been done to determine which of these instruments is superior, no clear cut advantage for any single or multi-test device has been demonstrated. Furthermore, there is a significant interdevice wheal size variability in these studies for both the positive and negative controls test sites.<sup>6</sup>

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**Table 1. Size of Wheals That Are Larger Than 99% of the Wheals With Saline, Using the Same Device on Subject's Back by the Same Operator<sup>a 6</sup>**

Devices 1	0.99 Quintile of reactions at the negative control sites, mm	Devices 2	0.99 Quintile of reactions at the negative control sites, mm
Quintest (HS) puncture	0	Duo Tip (Lincoln) twist	3.5
Smallpox needle (HS) prick	0	Bifurcated needle (ALO) prick	4.0
Duo Tip (Lincoln) prick	1.5	Multi Test (Lincoln) puncture	4.0
Lancet (HS)	2.0	Bifurcated needle (ALO) puncture	4.5
Lancet (ALK)	3.0	Quick Test (Pantrex)	4.0
DermaPICK II	0	Greer Track (Greer)	3.5
<sup>b</sup> Blood lancets	?		
<sup>b</sup> Hypodermic needle	?		

Abbreviations: HS, Hollister Steir; Greer, Greer Laboratories; ALO, Allergy Labs of Ohio; Lincoln, Lincoln Diagnostics; ALK, ALK America.

<sup>a</sup> Devices 1 are those for which a 3-mm wheal would be significant. Devices 2 are those for which a more than 3-mm wheal should be used as significant.

<sup>b</sup> Blood lancets and hypodermic needles are used locally. However, there are no studies available that compare their performance with the aforementioned foreign instruments.

Therefore, in order to obtain optimal results, more important than the device, a clinician (and/or technician) should be properly trained in the use of a particular instrument. Proficiency testing protocols may also help assure the aptitude of those administering the skin test.<sup>6</sup>

### Method of Application:

#### Procedure:

##### 1. Prick Test

A sharp instrument (hypodermic needle, solid bore needle, blood lancet) is passed through a drop of extract or control solutions (histamine, saline) at a 45° to 60° angle to the skin. The skin is then gently lifted, creating a small break in the epidermis through which the suspected allergen solution penetrates.

##### 2. Puncture Test

The skin test device is instead passed through the drop at a 90° angle to the skin. Devices used in this manner generally are designed with a sharp point and a shoulder (0.9 or 1 mm) to prevent excess penetration into the dermis. With some devices, the technique can be modified with a slight rotating twist after the puncture is made.

##### 3. Other devices are used with no need to place a drop of the allergen extract on the skin beforehand. The best possible results are obtained by using the manufacturer's recommended skin testing technique.

- a. Some devices are submerged in a well containing the allergen extract (GreerPick, HS Quintest, Prilotest allergen-adsorbed plastic skin needle).<sup>7,8</sup>
- b. Devices with multiple heads have also been developed to apply several skin tests at the same time.<sup>7,9</sup>

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- c. Another device involves needles coated with standardized allergens (Phazet).  
10,11,12,13
  - d. It has been previously mentioned that blood lancets are used locally for skin prick tests. These lancets are commonly submerged in a well containing the allergen extracts and are administered as described above. Allergists and/or technicians trained with the use of this device and method of administration report that they have been able to obtain reproducible results. However, there is still a need for studies comparing the efficacy and performance of blood lancets submerged in allergen extracts vis à vis the other devices.
4. Each device should be discarded after use. In 1995, the United States Occupational Safety and Health Administration (OSHA) called attention to the possible safety and health risks to blood-borne pathogens that may arise with the practice of using a single device for multiple applications and wiping the device between tests. OSHA also opined that the technician could unintentionally be pricked with the device when wiping it between tests.

***Summary Statement 3: To ensure proper interpretation, positive and negative controls should be performed at the same time as allergen tests. (B)***

Currently, the preferred positive control for prick/puncture tests is a 10-mg/mL histamine dehydrochloride solution. The negative control may either be saline, glycerinated phosphate buffer solution or 50% glycerinated (Human Serum Albumin) HSA–saline if concentrated extracts are used.

***Summary Statement 4: To achieve quality assurance among technicians, consistency in skin test performance should be demonstrated by skin testing proficiency protocols. (C)***

Considerable care should be given to proper training of skin test technicians. To confirm such standards, it is recommended that all technicians performing skin testing undergo evaluation of their technique. The procedure below outlines a suggested proficiency testing protocol.

Quality Assurance Protocol:

- Using desired skin test device, perform skin testing with positive (histamine  $1^{-10}$ ) and negative controls (saline  $1^{-10}$ ) in an alternate pattern on a subject's back.
- Record histamine results at 8 minutes by outlining wheals with a felt tip pen and transferring results with transparent tape to a blank sheet of paper.
- Record saline results at 15 minutes by outlining wheal and flares with a felt tip pen and transferring results with transparent tape to a blank sheet of paper.
- Calculate the mean diameter as  $(D + d)/2$ ; D = largest diameter and d = perpendicular diameter at the largest width of D.
- Histamine
  - Calculate the mean and SDs (see Appendix for formula) of each mean wheal diameter
  - Determine coefficient of variation (CV) = SD/mean

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- Quality standard should be CV less than 30%
- Saline
  - All negative controls should be < 3-mm wheals and < 10-mm flares.

### READING THE TEST RESULTS:

***Summary Statement 5: The peak reactivity of prick/puncture tests is 15 to 20 minutes at which time both wheal and erythema diameters (or areas) should be recorded in millimeters and compared with positive and negative controls. (B)***

A standardized approach to reading the tests has not yet been achieved. For example, some clinicians advocate immediate blotting of the allergen after the prick/puncture test to reduce the risk of an adverse reaction, whereas others leave the allergen in place for 20 minutes. No essential difference has been found between these techniques.

A prick/puncture test with a response of at least 3-mm diameter (with equivalent erythema) more than diluent control done at the same time is required as proof of the presence of cutaneous allergen specific IgE. The size of the reaction will be recorded as:

- mean wheal diameter,  $D + d/2$  (with D indicating the largest diameter of the wheal and d indicating the largest diameter perpendicular to D),

Since trauma may affect wheal size, an allergen response less than 3 mm generally should not be regarded as positive. Devices that produce wheals that exceed 3 mm at negative control sites should be avoided (See Table 1).

The significance of a large flare or erythema with a wheal size of less than 3mm cannot be ascertained at the present time.

***Summary Statement 6: Qualitative scoring (0 to 4+) is no longer used by many clinicians because of marked inter-physician variability in scoring and interpretation of this method. (B)***

***Summary Statement 7: Generally, skin tests/prick/puncture tests are age, and gender (and race) independent. (C)***

Skin tests may be performed in infants as young as 1 month of age.<sup>6</sup>

Skin reactions vary with age. Infants react predominantly with a large flare but a small wheal. The criteria for positivity in this age group should always compare the size of the wheal induced by allergen extracts with that elicited by the positive control solutions.<sup>5</sup> An allergen induced wheal can be considered positive if it is similar in size to that induced by histamine, even though the actual wheal size is less than 3mm. A local study done by Cua et al documented that skin test reactivity in infancy was best demonstrated at the age of 7 months.<sup>14</sup>

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Skin test wheals increase in size from infancy, peaks in adulthood (third decade) and then often decline after age 50. But significant skin test responses may still be demonstrated in patients well over 65 years.<sup>6</sup>

**Summary Statement 8: The use of certain drugs, some physiologic factors as well the area of the body where the procedure is done may affect the validity of prick/puncture tests. (B)**

A. The use of concurrent drugs (Table 2)

The concurrent usage of certain drugs which have the potential to suppress the release of histamine in the early and late phase reactions of the allergic inflammatory response can affect the validity of skin prick tests or intracutaneous tests.<sup>6</sup>

1. Antihistamines (H1 or H2 blockers)

Antihistamines or H1 blockers vary considerably in their ability to suppress the wheal and flare response induced by histamine. The duration of the inhibitory effect appears to be linked to the pharmacokinetics of the drug and its metabolites. Most studies show that first and second generation antihistamines should be discontinued 2 to 3 days before skin tests.<sup>6</sup> However, some antihistamines must be discontinued for a longer period of time such as cetirizine,<sup>6</sup> hydroxyzine<sup>6</sup>, levocetirizine<sup>15</sup>, clemastine,<sup>6</sup> loratadine,<sup>6</sup> desloratadine,<sup>15</sup> and ketotifen<sup>16</sup>. Some tricyclic antidepressants also have antihistaminic action and must be discontinued also before skin testing.<sup>6,16</sup>

Tachyphylaxis, as defined by the reduction of the inhibitory effects of prolonged used of the antihistamine on skin tests, has been reported for chlorpheniramine but not with second generation antihistamines.<sup>16</sup>

Topical H1 antihistamines such as levocabastine may also have inhibitory effects on skin testing.<sup>16</sup>

Histamine 2 antagonists may also cause mild suppressions, and their use must be discontinued for 24 hours before testing.<sup>6</sup>

2. Corticosteroids

Short-term oral corticosteroids (30 mg of prednisone daily for 1 week) do not suppress skin tests. Opinions vary regarding the effect of long-term or high dose (>20 mg/day) oral corticosteroids on suppression.<sup>6,16</sup>

However, prolonged and repetitive application of potent topical steroids for more than 3 weeks may suppress skin test reactions over areas of application.<sup>6</sup>

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Inhaled corticosteroids have not been tested for suppressive effects of skin tests, because therapeutic doses produce fewer systemic effects than oral steroids, it may be predicted that they should not modify skin tests.<sup>16</sup>

### 3. Other anti-inflammatory drugs

Oral prostaglandin D2 inhibitors (indomethacin) given several hours before testing may increase wheal response. However, cysteinyl leukotriene antagonists have negligible effects on skin testing.<sup>6</sup>

Theophylline slightly reduces skin test reactions but does not need to be stopped before skin testing.<sup>16</sup>

Local treatment with a topical immunomodulator may decrease wheal and flare reactions in skin prick testing.<sup>17,18,19</sup>

### 4. Beta agonists and antagonists

Short acting Beta-2 agonists in the usual doses used for the treatment of asthma do not usually inhibit allergen-induced skin tests. Long-acting inhaled Beta-2 agonists such as formoterol and salmeterol may significantly decrease skin test reactivity, but definitive results are lacking. Conversely, Beta-blocking agents such as propranolol can significantly increase skin reactivity.<sup>16</sup>

### 5. Other drugs

Inhaled cromones and nifedipine do not alter skin test responses; however, other drugs such as dopamine, and clonidine may decrease reactivity of the skin to histamine.<sup>16</sup>

Ace inhibitors increase reactivity of the skin to allergen, histamine, codeine and bradykinin.<sup>16</sup>

**Table 2. Inhibitory Effect of Various Treatments on IgE Mediated Skin Tests**

Drug	Degree	Duration(days)	Significant
<b>H1 antihistamines</b>			
Azelastine, nasal	++++	3-10	Yes
Cetirizine	++++	3-10	Yes
Chlorpheniramine	++	1-3	Yes
Clemastine	+++	1-10	Yes

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Desloratadine <sup>15</sup>	++++	3-10	Yes
Diphenhydramine	0 to +	1-3	Yes
Doxepin	++	3-11	Yes
Ebastine	++++	3-10	Yes
Fexofenadine	+++	2	
Hydroxyzine	+++	1-10	Yes
Levocabastine	Possible		Yes
Levocetirizine <sup>15</sup>	++++	3-12	Yes
Loratadine	++++	3-10	Yes
Mequitazine	++++	3-10	Yes
Promethazine	++	1-3	Yes
<b>H2 antihistamines</b>			
Cimetidine	0 to +		No
Ranitidine	+		No
<b>Corticosteroids</b>			
Systemic, short term	0		
Systemic, long-term	Possible		Yes
Inhaled	0		
Topical	0 to +		Yes
<b>Beta-2 adrenergic agonists</b>			
Inhaled	0 to ++		No
Oral, injection	0 to ++		No
Formoterol	Unknown		
Salmeterol	Unknown		
<b>Others</b>			
Ketotifen	++++	>5	Yes
Impiramines	++++	>10	Yes

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Phenothiazines	++		Yes
Theophylline	0 to +		No
Cromolyn	0		
Dopamine	+		
Clonidine	++		
Local anesthetic, EMLA cream	0		
Montelukast	0		
Pimecrolimus, topical	++		Yes
Specific Immunotherapy	0 to ++		No

Adapted from Bernstein IL et al Allergy Diagnostic Testing: An Updated Practice Parameter. Ann Allergy Asthma Immunol March 2008;100 (s18)and Demoly P et al in Adkinson NF et al Middleton's Allergy: Principles and Practices 6<sup>th</sup> edition. Mosby Inc@2003( p637).

### B. Other Physiologic Factors

1. Suppression of endogenous cortisol –suppresses late phase<sup>6</sup>
2. Menstrual cycle –increased reactivity in midcycle <sup>6</sup>
3. Skin color – larger reaction in dark skin<sup>6</sup>
4. Racial differences – variability seen<sup>6,16</sup>
5. Short term UVB radiation – decrease reactivity by 50%<sup>6</sup>
6. Circadian rhythms – there is a minimal circadian variation but this does not affect the clinical interpretation of the skin test.<sup>16</sup>
7. Seasonal variations- skin test reactivity has been demonstrated to be accentuated after pollen seasons and then declines before the next season.<sup>16</sup>
8. Pathologic conditions<sup>16</sup>
  - Decreased reactivity – atopic dermatitis, chronic renal failure, cancer, diabetic neuropathy, spinal cord injuries, anaphylaxis ( less than 1 week)
9. Specific Immunotherapy- a decreased wheal and flare reaction has been noticed in patients undergoing specific immunotherapy with inhalant allergens and Hymenoptera venoms.<sup>16</sup>

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### C. Area of Body

The site of skin testing may affect the results. The midback and upper back are more reactive than the lower back. The back as a whole is more reactive than the forearm. The antecubital fossa is the most reactive portion of the arm, whereas the wrist is the least reactive. The ulnar side of the arm is more reactive than the radial side. It is recommended that tests should not be placed in areas 5 cm from the wrist or 3 cm from the antecubital fossae.<sup>6,16</sup>

Regardless of location, it is recommended that there should be sufficient space (approximately 2 to 2.5 cm) between each applied allergen.<sup>6</sup>

***Summary Statement 9: The number of skin tests and the allergens selected for skin testing should be determined based on the patient's age, history, environment and living conditions, occupation and activities. Routine use of large numbers of skin tests or routine annual tests without a definite clinical indication are clearly not justified. (D)***

The scope and number of skin tests for allergy diagnosis reflect the clinician's scientific knowledge and clinical experience.

Special clinical situations and exposures must be kept in mind when selecting skin test reagents. Infants and very young children may require fewer prick/puncture tests, as their exposure to allergens is of a lesser magnitude compared to older children or adults. For toddlers, sensitization may be limited to frequently encountered food and indoor allergens rather than pollens. If inhalation allergy is narrowly confined to certain seasons, a limited number of relevant skin tests would suffice for confirmation of the clinical diagnosis. By contrast, perennial symptoms would require a more extended skin test panel of both indigenous outdoor and indoor inhalants. For both scenarios, testing to food allergens would be inappropriate unless a history of food allergy happened to be a concurrent problem of the patient.

The Joint Task Force on Practice Parameters after considering all variables and confounders suggests a core panel of indoor and outdoor inhalant allergens which includes representative species of the major classes of trees, grasses, and weeds, commonly sampled species of fungi, and a group of well-recognized indoor allergens (e.g.  $\leq 70$  prick/puncture tests) is justified as an initial diagnostic evaluation.

There is general agreement that significant indoor allergens such as house dust mite, prevailing indoor fungal allergens (*Penicillium* species, *Aspergillus* species, *Alternaria alternata*), cockroach, and epidermals (cat, dog, feathers), should be tested in patients with perennial respiratory symptoms.

However, the geographic variability of airborne-pollinating plants raises an important concern about how to select the number of skin tests and treatment reagents for this class of allergens. Certain key botanical and aerobiologic considerations are applicable to the selection process. A clinically significant pollen allergen should satisfy the 5 Thommen postulates:

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(1) the pollen is constitutively allergenic, as determined by symptoms occurring during its exposure in addition to the presence of a positive skin test result; (2) the pollen is anemophilous (wind-dispersed pollen); (3) the pollen is produced in sufficiently large quantities; (4) the pollen is sufficiently buoyant to be carried considerable distances; and (5) the plant producing the pollen is widely and abundantly distributed.

Because of the lack of recent aerobiological studies, no recommendations as to the content of the core panel of inhalant allergens to be used locally can be made at the present time.

Only prick/puncture tests should be performed to define food sensitivity. Based on recent extensive food allergy research, relatively few foods are responsible for most clinical food allergy suggested by the patient's history and pretest probability. In many instances, the history suggests the appropriate allergens for testing; in other situations, a preliminary diet history and diaries provide additional clues. This is useful for the diagnosis of IgE-mediated clinical entities. Food tests are inappropriate for investigation of chronic idiopathic urticaria (CIU) or angioedema.

Additional test allergens may be required for exposures to occupational allergens, in patients with unusual hobbies or personal contact with less common pets (e.g. rodents) or livestock. There is insufficient evidence, however, to justify tests for non-proven agents, such as newsprint, sugar, cornstarch, tobacco smoke, cotton, formaldehyde, and smog.<sup>6</sup>

### ***Summary Statement 10: The reliability of prick/puncture tests also depends on the stability and potency of the allergenic extracts. (B)***

Allergen extracts are subject to deterioration with storage, and this deterioration is accelerated by dilution, mixing with other extracts and high storage temperatures. The addition of 50% glycerin by the manufacturers helps retard this process.<sup>6,20</sup>

Loss of potency in dilute extracts may be due to adsorption of proteins onto the surface of the vial. Adding HSA may decrease this loss.<sup>20</sup>

It is important to follow the manufacturer's recommendations on how to properly store allergen extracts. They should be kept in cold storage (4°C) to ensure stability. In doing so, pure and undiluted extracts may keep for around 6-12 months.

The March 2008 Practice Parameters on Allergy Diagnostic Testing recommends the use of HSA (0.03%)-saline as a diluent (for intracutaneous testing and for skin test threshold testing). Human serum albumin-saline with 50% glycerine is the suggested negative control solution. In the Philippines, **glycerinated PBS** (phosphate buffer solution) is probably the most widely used diluent as it is readily available and economical.

It has been stated that incorporating glycerine or HSA to allergen extracts can retard their deterioration and loss of potency. Although **glycerinated PBS** is widely used locally as a



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diluent, its efficacy in keeping the allergens stable and potent have not been studied. There is also a paucity of researches comparing it with other diluents (such as glycerin and HSA).

***Summary Statement 11: Many studies have verified the sensitivity and specificity of prick/puncture tests for both inhalant and food allergens when correlated with nasal and oral challenge tests. (B)***

It is generally accepted that prick/puncture tests are less sensitive than intracutaneous tests. This is partially explained by the larger volumes of test solutions administered by the intracutaneous route. To compensate for this, positive prick/puncture tests require that the test extracts be 50 to 100 times more concentrated than intracutaneous test solutions. This relative lack of sensitivity to prick/puncture tests can be partially compensated for by avoidance of glycerinated extracts. On the other hand, prick tests are more specific than intracutaneous tests because the increased sensitivity at a fixed concentration of the intracutaneous test (1 in 1,000 wt/vol) may be responsible for a small but reproducible number of false-positive reactions, presumably because of an irritant effect.

Comparative investigations have been conducted to establish cutoff values, sensitivity, specificity and predictive indices of these tests with respect to inhalants and selected food allergens. Interpretation of these results varies, depending on whether the comparative gold standard is clinical history or controlled provocation challenges.

- With respect to inhalant allergens, several investigations have demonstrated that using positive nasal provocation challenges as a standard, the sensitivity of prick/puncture tests ranges from 85% to 87%, whereas the specificity of these tests is between 79% and 86%. A metaanalysis comparing prick/puncture tests to nasal challenge revealed positive likelihood ratios and negative likelihood ratios to the following allergens: cat = 4.93 and 0.08; tree pollen = 16.17 and 0.03; grass pollen = 3.23 and 0.04; house dust allergen = 4.06 and 0.05.
- A comparative study of allergic asthmatic patients undergoing nonspecific methacholine challenge causing a 20% fall in FEV1 using either  $\leq 4$  mg/mL (wt/vol) or  $\leq 8$  mg/mL (wt/vol) methacholine concentration computed for sensitivity, specificity and negative predictive value of prick/puncture tests. The study suggested that positive prick/puncture skin test results are more likely to be associated with asthma of greater severity. A negative prick/puncture test result decreased the probability of having asthma by 10- to 20-fold in subjects whose pretest probability was low to moderate.  
A local study done on younger children ( $\leq 5$  yrs) with physician diagnosed asthma showed no significant association between skin test positivity and asthma severity. They note though that the study had majority of subjects classified as having intermittent and mild persistent asthma.<sup>21</sup>
- The diagnostic accuracy of prick/puncture tests in food allergy has been compared with patients (mostly children) who have positive open or double-blinded controlled positive reactions to specific foods. In several of these studies, it was possible to determine cutoff levels of skin prick/puncture tests wheal diameters that were 100% diagnostic for several foods (eg,  $\geq 8$  mm for milk;  $\geq 7$  mm for egg;  $\geq 8$  mm for peanuts). These specific food cutoff values also indicate the probability of more severe food allergy because the

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controlled oral food challenges to which these were compared reproduced clinical anaphylactic events, which could be carefully monitored and treated. However, cutoff wheal sizes associated with high likelihood of allergy are variable, depending on the age (older children and infants), device, and reagents. Therefore, unless cutoff values are ascertained and validated, the need for confirmation by provocation challenges cannot be eliminated yet.

Compared with clinical history alone, the diagnostic accuracy of prick/puncture tests showed more limited capacity to predict clinical sensitivity for both inhalant and food allergens. (C)

- Sensitivity, specificity, and the predictive indices have also been compared with clinical history, both for inhalant and food allergens. Analysis of these studies revealed no unifying principle about the accuracy of prick/puncture skin tests as predictors for allergic rhinitis and asthma. The limited capacity of skin prick/puncture tests for predicting clinical symptoms was also tested by structured interviews with patients undergoing aeroallergen skin tests. Patients were found to have limited ability to correctly predict positive skin test results to aeroallergens based on their own clinical symptom experiences.

### **CLINICAL RELEVANCE AND CORRELATION WITH OTHER ALLERGY TESTS**

The proper interpretation of the prick puncture test requires a thorough knowledge of the medical history and physical examination findings. A positive skin test alone in an asymptomatic patient does not automatically mean that the individual is allergic. (Though it may predict subsequent clinical allergy especially if the wheal size is  $\geq 4\text{mm}$ )<sup>6</sup> However, a positive skin test that correlates with a history suggestive of clinical sensitivity strongly indicates the allergen as the cause of the disease. Conversely, a negative skin test with a negative clinical history makes an allergic condition unlikely.<sup>5</sup>

The diagnostic accuracy of the prick/puncture tests have also been correlated with organ challenge tests (bronchial, nasal, oral challenge). Given a patient with a strong allergic history and a positive skin test, the correlation between a positive skin test and bronchial or nasal challenges is highly significant.<sup>5</sup> Nonetheless, there are some exceptions to this observation (when it comes to certain foods). Not all patients with a positive (food) skin test will react during an organ (food) challenge. Likewise, there are patients who test negative on the prick/puncture test (or in vitro IgE assays) but may respond during an organ challenge test.<sup>5,6</sup> These may be due to the inherent limitations of these procedures. Also, it reiterates the point that a positive skin test does not necessarily translate to clinical allergy.<sup>6</sup> By the same token, correlations vary when unstandardized extracts are used and when there is a discrepancy between the history and the skin tests.<sup>5</sup>

## LIMITATIONS

**Summary Statement 12: The reliability and interpretation of the prick/puncture test is heavily dependent on the skill and interpretation of the individual tester, the reliability of the test instrument, the color of the skin, the status of skin reactivity on the day of the test (see above), and experimental differences between duplicate prick tests.(C)**

Medications previously mentioned can suppress skin reactivity (concurrent drugs sections).

Some patients with severe dermatographism, ichthyosis, or generalized eczema, or extensive scarring will not be ideal candidates for in vivo allergy tests.<sup>22</sup>

Prick skin tests will be quite difficult to perform in uncooperative patients with mental or physical impairments.<sup>21</sup>

Prick skin tests are discouraged when clinical history suggests an unusually greater risk of anaphylaxis from skin testing than usual.<sup>21</sup>

The hazards of blood contamination with the use of all instruments must be given appropriate attention. Allergists and technicians alike must always make use of adequate and appropriate barrier techniques and avoid accidental needle punctures.<sup>6</sup>

Whenever possible, extracts with known biologic potency should be used. For example, freshly made food extracts or the prick-prick method where the tester first pricks the fresh food and then the skin may be used because commercial extracts of fruits and vegetables lose potency over relatively short periods.<sup>6</sup>

Rarely, patients with both negative prick/puncture and intracutaneous tests will have positive organ challenge tests. This suggests that alternative pathways, including locally secreted IgE, IgE-independent, or non-immune stimuli may activate mediator release in the end organ.<sup>6</sup>

## SAFETY

**Summary Statement 13: Life-threatening generalized systemic reactions are rarely caused by prick/puncture tests.**

The overall rate of generalized reactions was 521 per 100,000 tested children. One fatality was confirmed in a 12-year survey of fatal reactions to allergen injections and skin testing with food allergens – a patient with moderately persistent asthma on whom 90 food prick tests were applied at one time.<sup>6</sup>

Local prevalence studies of adverse reactions to prick/puncture skin tests are not available.

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In our setting the concurrent use of  $\beta$ -blockers and angiotensin-converting enzyme inhibitors is a contraindication to skin testing because the necessary drugs that can override the effects of the beta blockers during an anaphylactic reaction are not readily available.<sup>6</sup>

### INTRACUTANEOUS TESTS<sup>6</sup>

**Summary Statement 14:** *Intracutaneous tests (ICT) will identify a larger number of patients with lower skin test sensitivity when increased sensitivity is needed., ie when the prick/puncture test results are negative despite a convincing history of symptoms upon exposure. Sensitivity to low potency allergen extracts may also be best evaluated by this method. (B)*

#### TECHNIQUE

**Summary Statement 15:** *Intracutaneous test should be performed with small volumes (0.02-0.05 ml) of allergens injected intracutaneously using a disposable 1 ml (tuberculin) syringe with an attached gauge 26-30 needle. (C)*

**Summary Statement 16:** *The starting dose of an intracutaneous allergen test should be 100-1000 fold more dilute than the allergen concentration used in the prick test. (C)*

The positive histamine control solution should be 100-fold dilution of the concentration used in the skin prick/puncture test (0.01mg/ml) A phosphate buffer solution or any other diluent used in the extract solution will serve as the negative control.

The intracutaneous tests are placed on the upper arm or volar surface of the forearm rather than the back to allow for the application of a tourniquet should systemic symptoms occur. However, no information on the differences in skin test reactivity between these two areas is available.

**Summary Statement 17:** *Because it requires a shallower needle depth embedded in the dermis, the bevel down method rather than a bevel up needle entry during intracutaneous testing is recommended for the following reasons: (B)*

- takes less time<sup>23</sup>
- higher success rate than the bevel-up method<sup>22</sup>
- fewer instances of the solution being squirted into the air<sup>22</sup>
- less bleeding<sup>22</sup>
- fewer test failures<sup>22</sup>
- associated with less pain<sup>22</sup>

Technical training for precision and reproducibility of intracutaneous tests should be emphasized.

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## READING OF TEST RESULTS

**Summary Statement 18: Intracutaneous tests are read 10-15 minutes after injection, and both wheal and erythema in millimeters should be measured and recorded. (B)**

For histamine controls and allergen sites, the size of the reaction may be recorded as a mean wheal diameter,  $D + d/2$  (with  $D$  indicating the largest diameter of the wheal and  $d$  indicating the largest diameter perpendicular to  $D$ ). A test is considered positive if the wheal diameter is at least 3 mm above the negative control.<sup>24</sup>

As with the prick/puncture tests, the significance of a large flare or erythema with a wheal size of less than 3mm cannot be ascertained at the present time.

## CLINICAL RELEVANCE:

Intracutaneous skin testing is 300-1000 fold more sensitive than prick- puncture test. ICT should be interpreted cautiously because of the presence of false positive reactions and should always be correlated with history and exposure.<sup>25</sup>

## SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE INDICES AND LIMITATIONS

**Summary Statement 19: Intracutaneous tests for most allergens exhibit poor efficiency in predicting organ challenge responses and correlating with the presence of detectable serum specific IgE at dilutions between  $10^{-2}$  and  $10^{-3}$  (wt/vol). (C)**

- The predictive accuracy of intracutaneous tests is often confounded by false-positive results because of its irritant effect at concentrations 1:1,000 wt/vol.
- Quantitative estimates of sensitivity, specificity and the predictive indices are difficult to evaluate because most of the clinical experience with allergen intracutaneous testing has been performed at a single dilution (1:1,000 wt/vol). For most allergens, a fixed dilution (1:1,000 wt/vol) of intracutaneous tests has poor efficiency in predicting organ challenge responses, the most reliable predictor of clinical sensitivity. This limitation appeared to apply to most of the common indoor and outdoor allergens.
- Intracutaneous tests often do not correlate well with serum specific IgE levels. This is based on a recent study that showed binding of allergen specific IgE antibodies to the  $\alpha$  chain of the Fc $\epsilon$ RI receptor was suboptimal and did not correlate with either intracutaneous or specific basophil sensitivity

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**Summary Statement 20: There are limited data about equivalency of sensitivity, specificity and predictive indices between intracutaneous and prick/puncture tests when compared with organ challenge tests. (C)**

**Summary Statement 21: Based on history and symptoms alone, comparative equivalency studies revealed that intracutaneous tests were comparable to prick/puncture test only at intracutaneous titration end points between  $10^{-5}$  and  $10^{-6}$  g/ml. (B)**

- One study demonstrated that more dilute intracutaneous concentrations were comparable to prick/puncture tests in predicting positive nasal challenges.
- End point intracutaneous titrations of a single allergen (ragweed) were compared with history and specific in vitro IgE (RAST) in a group of patients being evaluated for possible clinical allergy. At intracutaneous titration end points between  $10^{-6}$  and  $10^{-8}$  g/ml (wt/vol), 70% of the patients had a positive history and 50% had a positive RAST result. At intracutaneous end points between  $10^{-5}$  and  $10^{-3}$  g/ml (wt/vol), only 60% of patients gave positive histories and 15% exhibited specific IgE. This indicates that more dilute end point titration threshold levels of intracutaneous tests could approach the diagnostic accuracy of prick/puncture tests.
- Comparing intracutaneous end point titration, skin prick/puncture tests and nasal provocation determined by acousting rhinometry, the investigation revealed that skin prick/puncture tests were more sensitive (85.3% vs 79.4%) and more specific (78.6% and 67.9%) than intracutaneous end point titration as a screening procedure
- A study comparing intracutaneous tests to skin prick/puncture tests at 30 and 3,000 biologic units/ml, respectively, showed positive predictive values of 87.1% and 79.1% for intracutaneous and prick/puncture test, respectively
- Compared with clinical history, the positive predictive value for detection of allergic sensitization was 77% for intracutaneous tests and 86% for prick/puncture tests

### SAFETY

**Summary Statement 22: Immediate systemic reactions are more common with intracutaneous tests. Prescreening with prick/puncture tests is a practical way to avoid life threatening reactions to intracutaneous tests. (C)**

**Summary Statement 23: If prick/puncture tests are not performed routinely, preliminary threshold intracutaneous testing beginning at higher dilution (ie,  $10^{-5}$  to  $10^{-8}$  g/ml[wt/vol]) should be done.(D)**

- Although rare, adverse events after intracutaneous test can occur. Large local reactions, both immediate and late, may cause discomfort and occasionally mild, non progressive systemic reactions maybe associated with the latter. Immediate systemic reactions are more common with intracutaneous tests because larger volumes are injected.

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- Greater precaution should be observed if patients are suspected of having exquisite sensitivity, i.e. anaphylaxis to certain foods/drugs. In such cases, even prick/ puncture tests should be initiated with several serial 10-fold dilutions of the usual test concentration.
- Patients on  $\beta$ -adrenergic blocking agents and monoamine oxidase inhibitors may present special risk-benefit problems. If a systemic reaction should occur, epinephrine may not be totally effective in patients taking  $\beta$ -blockers, and epinephrine may adversely affect patients taking monoamine oxidase inhibitors.

### LATE PHASE CUTANEOUS REACTIONS (LPCR)

**Summary Statement 24:** *This response is a continuation of prick/puncture or intracutaneous testing characterized by erythema, induration, edema and dysesthesia becoming apparent 1 to 2 hours after the test, peaks at 6 to 12 hours and usually disappears after 24 to 48 hours. (B)*

Reactions develop progressively at sites of immediate wheal and flare reactions although, it may occur in the absence of an immediate skin test response.

Possible severe immediate reactions would occur only during the initial immediate phase and NOT during the late-phase cutaneous reactions. In cases when mediator release is intense enough, systemic reactions that occur during the reading period (6<sup>th</sup>-12<sup>th</sup> hr after the application) could possibly persist or worsen.

The use of antihistamines may offer symptomatic relief for persistent erythema and pruritus.

Preadministration of calcineurin inhibitors and misoprostol results in complete inhibition of LPCR, whereas prednisone and azelastine are partial inhibitors.

**Summary Statement 25:** *The late-phase cutaneous response may occur after both immune and non-immune activation. (B)*

Agents that cause immunologic activation of mast cells include anti-IgE antibodies and aeroallergens. The propensity to develop this response is dependent on the type of antigen, host sensitivity and concentration of the allergen

**Summary Statement 26:** *The mean diameter of induration and edema should be measured (in mm), recorded and compared with both the negative and positive controls. The minimum size of induration or erythema is not yet standardized. (B)*

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**Summary Statement 27: The clinical significance of this reaction is as yet known, although several randomized, controlled studies suggest that reduction in sizes of late-phase cutaneous response may parallel clinical response to immunotherapy. (B)**

It is not recommended that therapeutic interventions be based on this response.

### NUMBER OF TESTS

**Summary Statement 28: Although the Practice Parameters recommended 40 intracutaneous test as initial diagnostic evaluation, no definite number of intracutaneous tests can be recommended in our setting. ICT tests is done only if the prick/puncture test is negative inspite of significant exposure to an allergen by history. (D)**

### RECOMMENDATIONS

- Produce a practice guideline/procedural (step-by-step) manual demonstrating prick/puncture and intracutaneous bevel up skin testing.
- Create a registry for adverse reactions to skin prick tests and intracutaneous tests
- Do more studies on local pollen characterization such as which pollens are most reactive and which exhibit cross-reactivity so as to reduce the number of pollens being tested.
- Regularly update the list of the most common skin sensitizers (food and inhalant allergens).
- Update pollen calendar. Procure or improvise the production of a pollen collector.
- Perform potency studies on extracts over time

### SUGGESTIONS FOR FUTURE RESEARCHES:

- Compare the performance of the blood lancets and hypodermic needles versus the prick/puncture test devices used in other countries
- Compare the performance of blood lancets versus hypodermic needles
- Compare the performance and efficacy of using lancets submerged in wells with allergen extracts against other devices that make use of the same principle
- Compare the skin test reactivity to prick/puncture test of other sites than the upper back and volar forearm.

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- Investigate the reliability of using PBS as a diluent compared to HSA and/or glycerin
- Determine the effectivity of using the insulin syringe for intracutaneous skin testing
- Evaluate the skin test reactivity of the upper arm versus that of the volar forearm for intracutaneous skin testing
- Determine the appropriate volume for intracutaneous skin testing

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## APPENDIX

### Formula for Standard Deviation

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$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

$\sigma$  = standard deviation

$\Sigma$  = sum of

$x$  = each value in the data set

$\bar{x}$  = mean of all values in the data set

$n$  = number of value in the data set