

Practice Parameters for Allergy Diagnostic Testing

INTRODUCTION

The purpose of the Workgroup on Practice Parameters for Allergy Diagnostic Testing was to formulate an outline of the rational basis for proper use and interpretation of the most important clinical and laboratory tests used by allergists. This should serve not only to confirm the presence of allergic factors but also guide the proper choice of therapy. The American Academy of Allergy, Asthma, & Immunology (AAAAI) and the American College of Allergy, Asthma, and Immunology (ACAAI) have jointly accepted the responsibility for establishing allergy diagnostic parameters in order to promote advancement and improvements in care for 35 million allergic patients and facilitate the education of physicians who care for them.

Similar to the organizational process used in developing the published Practice Parameters for the Diagnosis and Treatment of Asthma, more than 30 experts in the field of allergy and immunology prepared critical reviews which were coordinated and stylized into a uniform monograph by the coeditors. A substantial portion of background research for this joint endeavor has been gleaned from an expert consensus statement concerning diagnostic technology sponsored by the National Institute of Allergy and Infectious Diseases and published in the Journal of Allergy and Clinical Immunology supplement, Volume 82: September, 1988. One of the major conclusions of that workshop was that periodic reassessment of diagnostic techniques should be mandatory in view of the rapidity of technology transfer to clinical practice. To accomplish such an up-to-date revision of diagnostic methods, the Joint Task Force commissioned an expanded national expert panel with a wide range of basic research and clinical practice experiences in allergy and immunology. After completion of the preliminary draft manuscript, all Joint Task Force members critically reviewed, revised and in some instances, contributed additional information to the final product. Additional critiques were obtained from members of the practicing allergy community and other experts and appropriate corrections or omissions were incorporated. From previous experience with similar review procedures, it is anticipated that suggestions will continue to be received from other allergy specialists. These will be noted and addressed wherever applicable and documentable in future updates of these guidelines. Since these documents incorporated the efforts of many participants, no single individual, including those who served on the committee or the Joint Task Force, is authorized to provide an official AAAAI interpretation of these Practice Parameters. Any request for information about or an interpretation of these Practice Parameters by the AAAAI or the ACAAI should be directed to the Executive Offices of the AAAAI, the ACAAI and the Joint Council of Allergy Asthma and Immunology.

In accordance with the guidelines suggested by the American Medical Association, a complete and well documented version was prepared to serve as a reference source for current utility and validity of diagnostic tests. This is available as a separate publication dealing with the following procedures:

- diagnostic tests of IgE-dependent reactions,
- diagnostic tests of cell-mediated immune reactions,
- allergens,
- organ challenge tests, and
- controversial and unproven tests.

It was also recognized that clinical practitioners would be more likely to benefit by the availability of an abbreviated epitome of the complete guide-lines document. Therefore, those allergy testing principles that were considered to have the most relevance in the day-to-day practice of clinical allergy and immunology were extracted from the original manuscript as a readily available summary of practice guidelines for allergy diagnostic testing. This summary was also organized to facilitate ready reference to the unabridged version of the guidelines monograph should more detailed information and/or bibliography be required about a specific test.

DIAGNOSTIC TESTS OF IgE DEPENDENT REACTIONS (IMMEDIATE HYPERSENSITIVITY)

Percutaneous and Intracutaneous In Vivo Diagnostic Skin Tests

Skin tests for IgE-mediated disease are acknowledged to be the most clinically applicable techniques in the assessment of allergic patients. Although skin scratch tests were among the earliest to be used for this purpose, the vast majority of allergists now use prick/puncture and/or intracutaneous skin tests, since the amount of allergen delivered by these methods is better controlled than by scratch tests. The summary statements in this section concern general principles of testing, specific applications of both prick/puncture and intracutaneous tests, allergen standardization and significance of the late phase cutaneous skin reaction.

A. General Principles of Testing for Immediate Hypersensitivity

- The hazards of blood contamination with the use of all instruments must be given appropriate attention and all testers need to observe appropriate bonier techniques.

- First generation antihistamines should be discontinued for 24 to 72 hours prior to testing. Hydroxyzine may affect the skin test if taken within up to 96 hours before the test. Astemizole may suppress skin tests for as long as 2 to 3 months or more. Other non-sedating antihistamines may need to be withheld for up to one week. Tricyclic antidepressants may require 7 to 14 days for recovery of skin test reactivity. H₂ antagonists may cause mild suppression and should be discontinued for 24 hours prior to testing.
- Although short-term systemic corticosteroids (30 mg daily for 1 week) do not suppress skin tests, chronic and relatively high dose corticosteroids (>20 mg/day) can partially suppress skin test reactions. Also, regular administration of potent topical corticosteroids for a period of weeks may suppress immediate skin tests over areas where they have been applied and therefore should be discontinued over these sites for 2 to 3 weeks prior to testing.
- Skin tests should not be performed at skin sites with active dermatitis. If they are done in the presence of dermatographism, they must be interpreted with caution.
- There are virtually no age limitations for performance of skin tests. However, skin test reactivity may be diminished in infants and the elderly.
- The proper interpretation of skin tests requires that allergen extracts be of known composition and potency (see "Allergen Standardization"). Unfortunately, these data are not available for all manufactured allergens currently being used by clinicians.
- The potency of allergen extracts deteriorates with time, dilution and exposure to increasing temperatures. Skin test reagents should be properly stored in a temperature-monitored refrigerated space. Expiration dates should be checked on a regular basis. Precautions to prevent cross contamination or bacterial contamination should be in place.
- To properly interpret allergy skin tests that detect immediate hyper-sensitivity, both positive (histamine) and negative (diluent) controls need to be performed.
- The proficiency of the skin tester should be validated by intra-patient and interpatient test results. The testing techniques used for these validation tests will vary depending on the device being used. Ongoing staff inservice programs are important to ensure quality.
- Histamine control tests should be read 15 minutes after application for determination of their peak reactivity.
- Although allergy skin testing is considered to be a safe procedure, adverse events, such as large local reactions and systemic symptoms, may occur in extremely sensitive individuals. Deaths from anaphylaxis after skin testing have been reported. These extremely rare systemic symptoms are less likely with prick/puncture than intracutaneous tests. However, it is recommended that full emergency equipment and drugs should be on hand for treatment of a potentially life-threatening event. Special precautions should be considered in patients receiving beta-adrenergic blocking agents or monoaminoxidase inhibitors.
- Since patients who are pregnant may rarely have uterine contractions associated with systemic reactions, they should be skin tested only if the results are contemplated to have substantial and immediate therapeutic implications.
- The appropriate clinical indications for retesting may include changing symptoms, new exposures, 3 to 5 years of venom immunotherapy or evaluation of newly discovered, purified or standardized allergens.
- Avoidance measures and extract formulations for immunotherapy should be based on the skin tests coupled with adequate clinical correlation, i.e., integrating with the history and physical findings obtained by face to face contact with the patient.

B. Prick/Puncture Tests

- Prick/puncture tests are widely used for confirmation of clinical immediate hypersensitivity induced by a wide variety of naturally occurring inhalant and food allergens. On a per test basis they are generally considered to be the most convenient, least expensive and most specific screening method for detecting the presence of IgE antibodies in patients with appropriate exposure histories. Under carefully defined circumstances, these tests are also useful in the diagnosis of drug and chemical hypersensitivity (eg, chloroplatinate salts, sulfone-chloramides, acid anhydrides, etc.) reactions.
- Since it is impossible to quantify the exact amount of injected material by prick/puncture tests, allergic skin responses are dependent upon the skill of the individual tester, the reliability of the device, the color of the skin, the status of skin reactivity on the day of the test, the potency and stability of test extracts (especially optimum concentrations), the depth of the puncture needle and the force, duration and the angle of the application device. If these quality controls are not assiduously applied, interpretation of the tests could vary from one technician to another.
- Prick/puncture tests are usually performed on the upper back or volar surface of the forearm.
- Prick/puncture tests with allergens should be read at the peak of the reaction, usually 15 to 20 minutes after application. Both erythema and wheal should be measured in a standardized manner and the method(s) of measuring should be recorded.
- A prick/puncture skin test wheal response of at least 3 mm (with equivalent erythema) > than the diluent control done at the same time is required as proof of the presence of allergen specific IgE.
- The larger the prick/puncture skin test reaction, the more likely it is to be of clinical significance. However, the presence of a positive prick/puncture skin test per se does not establish whether clinical sensitivity currently is present.
- Prick/puncture tests are generally less sensitive than intracutaneous tests. For inhalant allergens, prick/puncture tests are generally felt to correlate better with the presence of clinical allergy. However, intracutaneous testing may detect relevant

sensitivity and should be considered when the prick/puncture test is negative or equivocal to allergens strongly suggested by the patient's history or exposure, or when skin sensitivity may be decreased such as in infants or older patients.

- When using specific purified allergens, "false-positive" (non IgE-mediated) irritant reactions are less likely, and true sensitization can be more easily established with prick/ puncture than with intracutaneous tests, with certain important exceptions (i.e., Hymenoptera venom and penicillin).
- Prick/puncture tests are generally considered to be more specific than the usual test strength of intracutaneous tests when both are compared with inhalation or ingestion challenges.
- Generally, fewer prick tests need to be performed in infants and very young children because these age groups are not likely to be sensitized to as many allergens as older children and adults. In younger patients, sensitization is more apt to reflect intense, prolonged exposure to allergens encountered earliest in life (i.e., foods, house dust mites, indoor molds, indoor insects and animal danders) rather than pollen.
- Recognition of a positive skin test by the patient may be useful in gaining cooperation for allergy avoidance measures.

C. Intracutaneous Tests

- Intracutaneous tests are generally used when increased sensitivity is the main goal of testing (i.e., when prick/puncture tests are negative despite a compatible history of exposure). They permit identification of a larger number of clinically reactive patients, especially those with lower skin test sensitivity. In addition, sensitivity to low potency al-lergenic extracts may best be evaluated by this method.
- As a general rule, the starting test dose of intracutaneous extract solu-tions in patients with a preceding negative prick/puncture test should range between 100 and 1,000 fold dilutions of the prick/puncture test solution.
- The reproducibility of intracutaneous tests is affected by the same variables as those described for prick/puncture tests.
- Both erythema and wheal diameters should be measured and recorded.
- Any reaction larger than the negative control may indicate the presence of specific IgE antibody. However, given the lower specificity of intracutaneous testing, small positive reactions may not be clinically relevant.

D. Number of Skin Tests

- The evaluation of inhalant allergy may require up to 70 prick-puncture tests followed by up to 40 intracutaneous tests, which are ordinarily performed when prick/puncture tests are negative. Under special circumstances and in certain geographic areas, a greater number of prick/puncture and/or intracutaneous tests may be appropriate. However, in many parts of the country and probably in most cases, fewer tests are required.
- The number of prick/puncture tests performed for suspected food hypersensitivity may vary from less than 20 to as many as 80 tests, depending on the clinical situation.

E. Late Phase Cutaneous Reactions

- The late phase cutaneous reaction is characterized by erythema, induration, edema and dysesthesia that develops progressively at sites of immediate wheals and flares. It becomes apparent 1 to 2 hours after application, peaks at 6 to 12 hours and usually disappears after 24 to 48 hours. The significance of this interesting response is not well defined but late phase reactions generally are not of additive value in the diagnosis of clinical allergy.
- The propensity to develop the late phase cutaneous response is dependent on the type of antigen, host sensitivity and the concentration of injected allergen.
- Since the clinical relevance of the late phase cutaneous response is uncertain at this time, it is not recommended that therapeutic interventions be based only on this test.
- In Vitro Techniques Although skin tests are presently the preferred method of testing in making the diagnosis of allergy, in vitro tests may also be useful.

F. IgE Antibodies

- For most allergens, in vitro allergen specific immunoassays detect IgE antibody in the serum of most but not all patients who respond clinically to those allergens. The precise sensitivity of these immunoassays compared with prick/puncture skin tests has been reported to range from 50% to 90% with the average being about 70 to 75% for most studies. Therefore, skin tests are presently the preferred tests for the diagnosis of IgE-mediated sensitivity.
- Despite a number of recent improvements in in-vitro assay methods, recommendations accompanying available allergen specific IgE assays still lack agreement in determining the point at which a result is positive.

- As is the case with skin tests, a direct correlation cannot be assumed between evidence of allergen-specific IgE antibody and clinical disease. Therefore, the interpretation of these results by the physician requires correlation with the history and physical "examination obtained by face-to-face contact with the patient.
- Currently available assays for allergen-specific IgE are all based on the principle of immunoabsorption. The three most common methods of expressing results are:
 1. levels >2 or >3 standard deviations from a mean of pooled normal control sera,
 2. comparison to a calibration curve from a known allergic serum, and
 3. a modified radioimmunosorption method in which the number of radioactive counts in each unknown are standardized and converted to class scores. The latter method increases sensitivity at the expense of specificity and, therefore, should be interpreted with care.
- Interpretation of specific IgE immunoassays may not always be clear-cut because of accuracy limitations of individual methods. Although most methods compare reasonably well with respect to positive or negative sera, problems arise in the interpretation of the indeterminate lower ranges of the respective methods.
- To facilitate accurate interpretation, all assays for specific IgE should report results on known positive and negative sera run with each lot of reagents. In addition, a total serum IgE should also be performed on all samples assayed for specific IgE in order to eliminate the confounding variable of nonspecific binding caused by high total IgE. Patients receiving allergen immunotherapy have elevated IgG antibodies that may interfere with specific IgE assays.
- Other confounding variables may also affect the reliability of specific IgE immunoassays. These include inadequate presentation of the allergen on the test substrate, the presence of cross-reactive allergenic determinants, and the purity and standardization of the allergens used in these in vitro tests. If the recommended technique is altered (e.g., cutting the allergen coated discs in half, the validity of the test can be questioned.
- In vitro IgE-specific antibody tests performed with mixtures of allergens on the same disc may be misleading and should not be used.
- Generally, specific IgE immunoassays utilizing extracts with potent allergenic components correlate better with clinical sensitivity while tests of substances with weaker allergenic epitopes demonstrate substantially less correlation with various indices of clinical sensitization.
- A useful property of the specific IgE immunoassay is its ability to determine specificity of a reaction by means of inhibition of specific IgE antibody binding. This technique also can be used to standardize allergenic extracts, estimate the quantities of allergens, and evaluate cross-reactivity between allergens.
- Because of the inherent pitfalls in the sensitivity and reliability of IgE specific immunoassays, clinical applications are not completely defined and are still evolving.
- Specific IgE immunoassays are not recommended as a definitive answer for several specific clinical situations. They provide neither diagnostic nor prognostic information when measured in the cord blood of newborn infants. They do not have sufficient sensitivity for absolute positive prediction of anaphylactic sensitization to venoms, penicillin and other anaphylactogens (ie, chymopapain). If specific IgE tests are negative in these situations, skin tests are mandatory.
- Specific IgE immunoassays may be preferable to skin testing under special clinical situations:
 1. testing of patients with severe dermatographism, ichthyosis, or generalized eczema;
 2. testing in patients who have been receiving long-acting antihistamines, tricyclic antidepressants, or medications that may put the patient at undue risk if they are discontinued;
 3. testing of uncooperative patients with mental or physical impairments;
 4. the evaluation of cross-reactivity between insect venoms;
 5. postmortem examination for IgE antibodies to identify allergens responsible for lethal anaphylaxis;
 6. as adjunctive laboratory tests for disease activity of allergic bronchopulmonary aspergillosis and certain parasitic diseases; and
 7. when clinical history suggests an unusually greater risk of anaphylaxis from skin testing than usual (e.g., when an unusual allergen is not available as a licensed skin test extract).
- Given the many constraints about the technologic and interpretative problems of specific IgE tests, formulation of allergen treatment extracts on the basis of specific IgE in vitro tests without adequate clinical correlation-i.e., integration with the history and physical findings obtained by face-to-face contact with the patient is unreliable and is not recommended.

- Total quantitative serum IgE measurements have been standardized by national and international reference nomenclature. High IgE values in particular may result from technical errors, and it is essential that these possible sources of error be monitored closely by individual laboratories.
- The clinical applications and interpretation of total serum IgE concentrations are of modest value. This test is useful as a predictor of asthma in epidemiologic studies but is less useful in assessing the risk of allergic disease on an individual basis. Total IgE is elevated in many specific disease syndromes including
 1. allergic bronchopulmonary aspergillosis,
 2. eczema and the hyper-IgE syndrome (dermatitis and recurrent pyogenic infections),
 3. certain stages of HIV infection,
 4. IgE myeloma,
 5. drug-induced interstitial nephritis,
 6. graft versus host disease,
 7. several parasitic diseases, and
 8. several immune deficiency diseases.

G. IgG Antibodies

- Allergen-specific IgG and IgG subclasses may also be measured by using immunoabsorption assays similar to those used to measure allergen specific IgE. As yet, there are no blinded proficiency surveys for evaluating the reliability of allergen specific IgG assays, and they should not be used routinely for diagnostic testing.
- Although it is recognized that food allergen-specific IgG may be found in many normal and allergic individuals, there is insufficient evidence that the presence or quantity of food allergen-specific IgG produced as a result of natural exposure is related to allergic disease. Further, the measurement of subclass specific IgG, antibodies to foods has been inconsistent between various studies. Thus, food-specific IgG or IgG, antibodies have no recognized diagnostic value.
- There is a modest association between the quantity of venom-specific IgG produced in response to immunotherapy and protection from allergic reactions induced by an insect sting but the value of measuring IgG to other allergens during or after immunotherapy has not been demonstrated. The predictive value of subclass specific IgGs, particularly IgG₄, for successful immuno-therapy is not proven at present.

H. Allergen Standardization

- Standardization of extracts is an important issue with respect to potency and reliability of extracts used for skin testing. Current methods include bioequivalency techniques and/or in vitro comparisons with major allergenic epitopes. Most allergens, however, still are identified and labeled by weight/volume or protein nitrogen units.
- Bioequivalent standardization of allergenic extracts can be accomplished by comparative prick/puncture or intracutaneous skin tests in relevant allergic populations. In the United States, the FDA determined that the intracutaneous technique should be used for assigning standardized unitage (i.e., bioequivalency allergy units [BAU]). In other countries, several other functional bioassay standards have been proposed.
- Immunoassays utilizing both immunodiffusion and immune-absorption technology have been applied as in vitro methods of allergen standardization. Although these techniques are highly reproducible in the case of allergens containing specific purified components, their future usefulness for this purpose is limited because of the great variability of allergenic epitopes in extracts employed in clinical practice.

I. Measurement of Allergic Mediators

- Histamine release from human basophils is a valuable research tool for in vitro investigations of allergy. In ragweed-allergic persons there is a good correlation between the severity of the clinical symptoms and the extent of in vitro histamine release. Histamine release is also an essential method for assessing the roles of various histamine releasing factors which may be either IgE dependent or independent.
- The recent availability of several sensitive immunoassays for histamine is a significant technological advance for measuring histamine in various biologic fluids, or release from whole blood, isolated basophils, mast cells or histamine-containing cultured cells. The improved detection of lower levels of histamine by these immunoassays is at least equal to the sensitivity of the standard fluorescence method.

- The measurement of tryptase in serum may be a useful diagnostic tool in allergic diseases. In contrast to histamine, which is elevated only transiently in the serum, the increased tryptase serum concentrations can persist two to four hours after a systemic reaction and are useful in:
 1. the diagnosis of anaphylaxis,
 2. monitoring of allergen challenge, and
 3. activity status of mastocytosis.
- Clinical research is currently concerned with the role of cytosolic components (eosinophilic cationic protein and major basic protein) and activation markers of eosinophils. Although some of these preliminary results appear promising in specific clinical situations, these tests cannot be recommended yet for routine use.

DIAGNOSTIC TESTS OF CELL MEDIATED IMMUNE REACTIONS (DELAYED HYPERSENSITIVITY)

Whereas immediate skin testing is important in the diagnosis of IgE-mediated inhalant, food, venom, and penicillin allergies, delayed hypersensitivity testing (patch tests, delayed hypersensitivity or tuberculin-like intracutaneous tests as well as in vitro techniques) is often helpful in the diagnosis of contact dermatitis and the clinical evaluation of cell-mediated immunity.

A. Epicutaneous and Intracutaneous In Vivo Diagnostic Skin Tests

- Allergic contact dermatitis is an immunologically mediated event that is typically an edematous (histologically, spongiotic) dermatitis. The inflammatory component of allergic contact dermatitis is primarily a cell-mediated, immunologic reaction but it may also contain an IgE component.
- Patch testing is used to determine the causative agent in contact eczematous dermatitis. The usefulness of patch testing to identify allergic reactions other than those originating on cutaneous surfaces has not been determined.
- It is estimated that the 20 to 30 antigens used in the usual routine screening panel of patch tests will identify between 50% and 70% of the clinically relevant causes of allergic contact dermatitis.
- Patch tests are generally placed on the upper back, avoiding the paraspinal skin at least 2.5 cm from the midspinal area. They should be removed and read at 48 hours and again at 72 and/or 96 hours, or earlier if a reaction is occurring.
- A positive patch test requires correlation with likely sources of an exposure to the substance in the patient's environment including hobbies, habits, occupation, cosmetics, lotions, and ointments.
- Skin tests for delayed-type allergic reactivity are used to evaluate cellular immunity in infection, cancer, suspected immune disorders, pre-transplantation screening, aging, and altered nutrition. Recall antigen skin tests can be used to predict survival of immunocompromised patients, detect disease-related changes in immunity, and follow the outcomes of therapy.
- Intact delayed type hypersensitivity provides evidence of cell-mediated immunity and predicts the host's ability to eliminate obligate intracellular pathogens and parasites. In contrast, anergy provides evidence of impaired cellular immunity and/or absence of prior sensitization.
- The purified protein derivative (PPD) of tuberculin is the prototype of a recall skin test antigen. The intracutaneous type skin test first described by Mantoux is the preferred method for documenting cell-mediated immunity to PPD. Although the tuberculin tine test and a "Multi-test" tine panel are widely used as surrogates of the tuberculin-type intracutaneous test, they are not universally acceptable because of a high prevalence of false negative reactions and the fact that the cellular infiltrate associated with the tine reaction does not become comparable to the classic tuberculin 48-hour reaction until the 96-hour reading.
- The reproducibility of delayed skin tests, including tuberculin and other recall antigens, is not known.
- Recall skin tests are usually done with antigens developed for other purposes. These antigens include mumps, trichophyton, *Candida albicans*, tetanus toxoid and streptokinase-dornase. None of these preparations are at present standardized for the purpose of detecting recall immunity.
- Both immediate and delayed hypersensitivity reactions can be manifested after tuberculin-like skin tests. Early and late phase reactions of the IgE-mediated response are measured at 15 to 20 minutes and at six hours, respectively. The reaction after 24 hours is a mixture of the IgE-mediated late phase reaction and the beginning of delayed hyper-sensitivity. The 48 and 72 hour delayed cutaneous reactions are almost entirely reflections of delayed hypersensitivity. Thus, depending on the time of the observation, a positive reaction to a test antigen indicates that either immediate or delayed cutaneous hypersensitivity is intact.
- The size of the delayed skin test reaction is measured at 48 hours after antigen challenge. The diameter of a palpable, firm and indurated area of >2 mm is considered to be the threshold for a clinically measurable reaction and evidence of immunity.
- When tests with multiple antigens are collectively interpreted, the identification of two or more >2 mm diameter reactions can be interpreted as reliable evidence of intact delayed cutaneous hypersensitivity. When a single intracutaneous antigen is used to

evaluate prior sensitization to a potential pathogen, a 2:5 mm reaction is required as a more conservative threshold for a positive reaction but smaller reactions (2 to 4 mm) should be correlated with the clinical situation. The use of test panels is recommended in order to avoid costly in vitro studies.

- The absence of hypersensitivity to all test antigens suggests an anergic state. The diagnosis of anergy, however, can be established with recall antigen skin tests only when at least 95% of comparably exposed normal people demonstrate positive reactions to at least one of the test antigens.

B. In Vitro Tests of Cell-Mediated Immunity

- In vitro tests of cell-mediated immunity are used to assess
 1. cellular function in patients who may have recurrent types of infections (e.g., fungal, mycobacterial, and protozoan);
 2. depressed cellular immunity (e.g., acquired immune deficiency syndrome, sarcoidosis, and cancer);
 3. certain cases of drug hypersensitivity;
 4. chemical hypersensitivity (toluene diisocyanate, beryllium); and
 5. autoimmune disease (e.g., Guillain Barre' syndrome, chronic hepatitis, and thyroiditis).
- Tests that quantify lymphocyte function measure the ability of lymphocytes to:
 1. proliferate,
 2. produce inflammatory mediators and cytokines,
 3. mount cytotoxic responses, and
 4. regulate immune responses.
- Lymphocyte proliferative responses may be evaluated by either nonspecific mitogens (e.g., phytohemagglutinin, concanavalin A or pokeweed) or specific soluble and cell-bound antigens.
- In vitro proliferative responses to some soluble antigens, but not mitogens, have been shown to correlate with in vivo delayed hypersensitivity. The role, however, of lymphocyte proliferation as measured in vitro in the pathogenesis of the delayed-type hypersensitivity tissue reaction is unclear.
- Several chemotactic and migration inhibitory factors produced by lymphocytes more closely parallel the results of delayed skin tests than does lymphocyte proliferation.
- Although results of chemotactic and migratory inhibitory assays are not diagnostic in an individual patient, they have adjunctive clinical value for identifying certain pathogenic factors, monitoring the results of therapy, and the clinical course of patients with depressed cellular function.
- Several simple cost-effective screening tests are available for the evaluation of competency of cell-mediated immunity. These include:
 1. an absolute lymphocyte count (a count under 1200/mm³ suggests an abnormal immune response),
 2. number of total T-cells as measured by anti-CD3 surface markers,
 3. estimation of CD4⁺ helper cell populations and CD8⁺ suppresser/ cytotoxic populations by immuno-fluorescent staining with appropriate phenotypic cell markers,
 4. measurement of T-cell lymphocyte activation by IL-2 secretion or fluorescent anti-CD25 and/or anti-HLA-DR monoclonal antibodies, and
 5. determination of the relative percentage of CD45RO⁺ CD29⁺ T-cell lymphocytes as an indication of the number of memory T-cells.
- Laboratory assessment of cytokines, monokines, chemokines, and adhesion molecules has limited clinical utility at the present time. Sufficiently sensitive immunoassays, however, are available for the presence of several key cytokines in body fluids and in situ hybridization techniques make it possible to determine the relative percentage of cells expressing mRNA message for specific cytokines. In particular, the recent separation of CD4⁺ helper cells into subsets of TH0, TH1 and TH2 sub-populations is under intensive investigation at the present time.

ALLERGENS

Accurate allergy diagnosis depends upon the correct choice of allergens for testing. This section describes differences in allergens and their use in diagnostic testing. From time to time, patients may present with symptoms caused by previously unidentified substances that potentially could denote new allergens. There is a role for testing these patients to identify newly suspected allergens,

Nevertheless, for such agents as newsprint, formaldehyde, tobacco smoke, smog, cotton, sugar and human dander, there is insufficient evidence to justify their use as allergen test reagents.

A. Inhalant Allergens

- The number and variety of environmental allergens responsible for allergic disease is extremely large. Because aerobiologic data necessary for identification of allergens are at times limited, the number of allergens identified to date is far from complete.
- Aeroallergens that are well established as causes of IgE-mediated disease include pollens, fungi, algae, animal emanations, insects, acarides (mites), food proteins, additives, antibiotics, drugs, and occupational allergens. There may well be instances in which specific allergens appropriate for diagnostic testing of suspected disease have not been identified.
- Selection of the number and type of allergens appropriate for testing depends upon their relevance to the patient's history including significant exposure, objective proof of the allergenicity of the substances in question, and the availability of suitable allergenic material for testing.
- When a cause and effect relationship between exposure and IgE-mediated disease is not immediately apparent, the selection of test substances depends upon scientific evidence and experience concerning allergenicity and clinical importance of the substances in question.
- Some pollens have cross-allergenicity and the clinician must be aware of which ones are clinically relevant in order to avoid unnecessary duplication. When common airborne pollens are not substantially cross-reactive, however, testing and treatment with multiple locally prevalent pollens may be necessary to avoid significant omissions.
- A well designed allergy skin testing form should reflect clinically relevant aeroallergens, foods and other substances that may be implicated in allergic reactions. The skin testing form should include documentation of the methods of testing, the concentration of allergens and the measurement (in mm) of reaction sizes of both wheals and flares.
- Allergic disease caused by insects and acarides can be due to stings, bites, direct contact, or inhalation of allergenic material associated with these organisms. Relevant allergens may derive from a variety of sources including venoms, saliva, hemoglobin, intestinal proteins contained in fecal particles, and exterior body parts of the organism.
- Skin testing with specific venom is the recommended method of confirming anaphylactic sensitivity caused by stings of Hymenoptera insects. Since fire ant venom is not available, testing with whole-body fire ant extracts is used to demonstrate anaphylactic sensitivity to this insect. Whole-body insect extracts are also used for detection of respiratory hypersensitivity to cock-roaches or other household and swarming insects.
- Allergens derived from many animals cause IgE-mediated allergic disease. Cat allergens have been well characterized and are derived from sebaceous glandular protein in skin. To a lesser extent, allergens can also be identified in cat saliva. Allergens identified from other animal species have not been as well studied but may originate from various sources including urinary protein.
- Extracts of house dust contain various allergens. Variability in composition and potency of allergens in commercial crude house dust extract and the availability of more selective allergen extracts for testing individual components (e.g., house dust mites, cockroach, epidermals) of house dust makes the use of commercial house dust extract less important.
- Fungi (molds) can contribute to symptoms of respiratory allergy. The technical difficulty of isolating specific fungal antigens from either spores or hyphae has made it particularly difficult to characterize clinically significant allergens for some of these species. For clinical purposes, fungi may be classified as outdoor (e.g., *Alternaria* sp and *Cladosporium* sp) and/or indoor (e.g., *Aspergillus* sp, *Penicillium* sp).
- Since there is minimum cross reactivity among fungal species and genera, clinical tests with fungal mixtures may often fail to detect specific sensitivity to an individual fungal organism.

B. Foods

- Immediate or IgE-mediated hypersensitivity to foods is particularly important in young children and adolescents but should be considered at any age if the history is suggestive.
- The prevalence of immediate hypersensitivity reactions to foods appears to decline with age based on evidence that the number of positive skin reactions confirmed by double-blind oral food challenge is less than 3% in the general adult population.
- Nearly any food can be allergenic. Since allergenicity tends to be highly specific to a given food, the absence of test reactivity to one member of a group of related foods cannot be taken as evidence for the lack of sensitivity to other foods in the group. Although many food allergens have been well characterized, standardized food extracts are not currently available. Further, relevant allergens present in the native food responsible for a reaction may not be present in the extracted testing material. Many foods contain antigens which are rapidly lost, perhaps by proteolytic digestion during storage; therefore, testing wild fresh food juices may be useful, especially in suspected allergic reactions to fruits and berries.

- The patient's history is paramount in the selection of foods for testing, and a patient's spontaneous history may need to be supplemented by a meticulous diary of foods eaten and symptoms observed. This approach is indicated primarily in patients with the potential for food-induced anaphylaxis.
- Exhaustive prick/puncture skin or in vitro testing to all of the 200 or more currently available food extracts is rarely indicated. In general intracutaneous tests often give false positive results particularly if tested in concentrations stronger than 1:1,000. Intracutaneous skin tests for food allergy may be considered under special circumstances:
 - when the prick test is negative despite a very strong associative history and idiopathic anaphylaxis.
 - In the latter situation, proper precautions should be taken for treatment of possible severe systemic reactions.
 - In children less than 3 years of age, the most important food allergens include milk, egg white, peanuts, soybean, wheat, and citrus fruits.

C. Antibiotics, Other Drugs and Chemicals

- IgE-mediated mechanisms may be responsible for adverse reactions to many antibiotics, other pharmaceuticals and biologic proteins. For many of these agents the specific relevant allergens have either not been identified or are not commercially available.
- The major indication for penicillin skin testing is in history-positive patients who require treatment for a severe infection with an organism that will not respond as well to a structurally unrelated antibiotic. Skin tests may also be used to determine whether IgE-mediated mechanisms were involved in a reaction occurring in the recent past.
- Penicilloyl polylysine is the reagent used to detect IgE antibody to the major allergenic determinant of penicillin (i.e., penicilloyl) and is positive in about 80% of penicillin-sensitive patients.
- Sensitization to antigenic determinants of the minor metabolic pathway ("minor determinants") correlates best with risk for anaphylactic sensitivity to penicillin but these determinants are not commercially available.
- A negative skin test using fresh or old penicillin is unreliable for ruling out minor penicillin determinant allergy.
- In vitro tests for IgE antibodies to penicillin presently lack sufficient sensitivity and should not be used to determine whether penicillin allergy exists.
- Although most reactions to penicillin are due to sensitization to metabolic products derived from the beta-lactam ring, side chain specific determinants of different penicillins may be responsible for some instances of sensitivity to beta-lactam classes of antibiotics.
- Cross reactions to the cephalosporin family of drugs occur in 6% to 15% of patients who have both positive histories and positive skin tests to penicillin.
- Screening skin testing to the beta lactam class of antibiotics in a patient with a negative history for prior reactions to Penicillin is not recommended.
- The incidence of adverse reactions to sulfonamide-related drugs has increased since the advent of the acquired immune deficiency syndrome. About 30% of patients with histories of reactions to this group of drugs demonstrate immediate skin tests to polyamino acid reagents containing sulfa ligands.
- Skin and in vitro tests may be used to detect sensitivity to biologics (e.g., insulin, enzymes, protamine, heparin, intravenous immunoglobulin preparations, and other blood products) and certain vaccines containing trace amounts of large molecular weight substances. The predictive clinical value for most of these tests has not been well established.
- Many chemicals (e.g., sulfonechloramides, azo dyes, parabens, fragrances) used as additives in foods, drugs, and cosmetics may induce either IgE-mediated reactions or contact dermatitis, or both.

D. Occupational Allergens

- Over 200 large and small molecular weight substances used in industry have been shown to cause allergic symptoms by classical immunologic mechanisms.
- When exposure to these compounds is by the respiratory route, rhinitis, asthma, and/or hypersensitivity pneumonitis are the most common manifestations of sensitization.
- Many small molecular weight chemical substances in the work-place are known to induce the most common immunologic occupational disease, allergic contact dermatitis.

E. Miscellaneous Plant Products

- Many plant products such as chymopapain, pyrethrum, cottonseed, psyllium, and vegetable gums have been associated with allergic symptoms.

- The prevalence of sensitization and anaphylaxis to latex protein contained in hospital gloves, airborne sources, and medical appliances has greatly increased in both patients and hospital personnel. Sensitive individuals may react to latex in catheters, endotracheal tubes, intravenous injection parts or other hospital materials containing latex.
- Testing with latex proteins and/or other plant products may be indicated in selected patients but it should be emphasized that standardized and well characterized extracts are not currently available for most of these agents.

F. Contact Allergens

- Chemicals, plant resins, and lipid materials are the chief causes of allergic contact dermatitis.
- Patch testing with 20 to 30 allergens in a screening patch test panel will detect as many as 70% of all cases of allergic contact dermatitis.
- Detecting the cause of allergic contact dermatitis in individual cases initially may involve screening patch tests to suspected products, followed by tests to the specific components if they can be determined.

G. General Principles of Cross-Reactivity Among Plant-Derived Allergens

- Botanical taxonomy has been used to infer various degrees of cross-reactivity among plants; however, such data are limited for pollens and sparse for fungi. Even among closely related species, unique allergenic epitopes may exist and have clinical relevance for patients exposed to that species.
- There is marked allergenic diversity among tree pollens. Exceptions occur in some conifers and members of the birch and beech families (oak, elder, and hazel).
- Shared antigens are the rule for the large fescue grass subfamily or the "northern pasture grasses"; however, complete cross-reactivity rarely occurs. Timothy grass and Johnson grass, in particular, have additional unique antigens of possible clinical importance. Bermuda grass does not cross-react with northern grasses but does exhibit minor cross-reactivity with western prairie grasses.
- Weeds of the composite family including ragweed of the genus *Ambrosia*, are potent sensitizers and extensively cross-react. The sages and mugworts (genus *Artemisia*) also cross-react strongly within the genus. Other cross-reacting weeds are chenopods, amaranths, and *Atriplex* weeds. Russian thistle and burning bush show only partial cross reactivity.
- The number of pollen extracts used in allergy diagnosis and therapy must be determined by allergen prevalence, geographic distribution, and allergenic relationships. A precise basis for establishing guidelines for choice of allergen extracts is not presently available. Sampling for allergens that show clinical relevance on a regional or local basis should provide evidence needed for testing to less common sensitizing airborne substances.

ORGAN CHALLENGE TESTS

Provocative tests have been broadly applied under research conditions for many years, but there also may be clinical situations in which they can be useful for confirmation of clinical disease. Space limitations permit only a cursory consideration of these diagnostic techniques. Detailed information can be obtained from the references cited in the unabridged version of these parameters and the published Asthma Practice Parameters document.

- Organ challenge test materials may be applied to the mucosae of the conjunctivae, nares, GI tract, or bronchi. Considerable experience with these methods is required for proper interpretation and analysis.
- Nasal challenge tests may be informative provided that the patient's nasal mucosa does not manifest nonspecific irritative responses and the results can be interpreted by objective measurements.
- Bronchial challenge tests are often used to evaluate new allergens and may be used to substantiate the role of allergens in patients with significant symptoms. Results of these tests are ordinarily evaluated by objective measures of pulmonary function and occasionally by characterization of bronchoalveolar lavage samples.
- All organ challenge tests should be preceded by a control test with di-luent and if possible, the procedure should be performed on a double-blind or at least single-blind basis.
- Bronchial challenge tests should be performed as dose-response assays wherein provocation concentration thresholds can be determined on the basis of the allergen concentration required to cause a 20% decrease (PC 20) of the FEV₁.
- Bronchial challenge tests with occupational allergens need to be carefully controlled with respect to dose and duration of exposure. When industrial small molecular weight agents are assessed, tests should be performed under conditions of continuous monitoring of the specific chemical being assessed so as not to exceed the threshold limit level permitted in the workplace.
- Double-blind, placebo-controlled, oral challenge tests are required to confirm suspected gastrointestinal or systemic symptoms occurring after ingestion of a food, food additive, and some drugs.

CONTROVERSIAL AND UNPROVEN TESTS

- As technologic advances occur in medicine, attempts are sometimes made to apply them to clinical situations in which they are not appropriate. To prevent unnecessary proliferation of such tests and restrain the already high cost of medical care, it is essential that scientific justification for these tests can be demonstrated rigorously.
- Unproven diagnostic techniques can be classified as:
 1. procedures that are invalid for any purpose (allergy diagnosis or other);
 2. procedures that are intrinsically capable of a valid measurement but not appropriate for diagnostic use in IgE-mediated allergy; and
 3. procedures that theoretically could be used in allergy diagnosis but are not appropriate for clinical use because of expense, problems with sensitivity, specificity or general availability.
- Procedures for which there is limited or no evidence of validity include the cytotoxic test, the provocation-neutralization procedure, electrodermal diagnosis, applied kinesiology, the "reaginic" pulse test, and chemical analysis of body tissues. Controlled studies for the cytotoxic and provocation-neutralization tests demonstrated that the results are not reproducible and do not correlate with clinical evidence of allergy. Electrodermal diagnosis and applied kinesiology have not been evaluated for efficacy. Similarly, the "reaginic" pulse test and chemical analysis of body tissues for various exogenous chemicals have not been substantiated as valid tests for allergy.
- Several valid test procedures including measurements of circulating IgG antibodies, immune complexes, serum immunoglobulin concentrations, complement components, and lymphocyte subsets have been reported to show abnormalities in some allergic patients. Although these tests are not useful for the diagnosis of IgE-mediated allergic diseases, they may at times have value in the differential diagnosis of proven immunologic problems, the manifestations of which may mimic allergic disease.
- Some laboratory procedures may be used, as diagnostic adjuncts under certain circumstances. These include in vitro histamine release, in vitro lymphocyte proliferation, and the serial endpoint titration technique. The first two tests are used chiefly for research purposes while the last is applicable in the determination of the bioequivalency of allergic extracts or for safety purposes in extremely sensitive individuals.