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Allergy Diagnostic Testing: An Updated Practice Parameter

Practice Parameter

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The American Academy of Allergy, Asthma and Immunology (AAAAI) and the American College of Allergy, Asthma and Immunology (ACAAI) have jointly accepted responsibility for establishing the Allergy Diagnostic Testing: An Updated Practice Parameter. This is a complete and comprehensive document at the current time. The medical environment is a changing environment and not all recommendations will be appropriate for all patients. Because this document incorporated the efforts of many participants, no single individual, including those who served on the Joint Task Force, is authorized to provide an official AAAAI or ACAAI interpretation of these practice parameters. Any request for information about or an interpretation of these practice parameters by the AAAAI or ACAAI should be directed to the Executive Offices of the AAAAI, the ACAAI, and the Joint Council of Allergy, Asthma and Immunology. These parameters are not designed for use by pharmaceutical companies in drug promotion.

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PREFACE

The major emphasis of this updated version of Practice Parameters for Allergy Diagnostic Testing is focused on how technological refinements and their validations during the past decade are being incorporated into the diagnostic armamentarium of allergists/clinical immunologists and how their optimal use enables confirmation of human clinical sensitivity. The term *allergy* in this Practice Parameter denotes major categories of human hypersensitivity. Pertinent clinical immunologic techniques are oriented to this category of adaptive immunity but not to infection, cancer, or transplantation immunology.

The impetus for Practice Parameters for Allergy Diagnostic Testing originally stemmed from a consensus conference sponsored by the National Institute of Allergy and Infectious Diseases and published as a supplement to the *Journal of Allergy and Clinical Immunology* in September 1988. One of the major conclusions of that workshop was that periodic reassessment of diagnostic techniques should be mandatory, and in keeping with that recommendation, the 1995 Practice Parameters for Allergy Diagnostic Tests further reviewed and considered new developments up to that time. In the 13-year interval since that publication, there has been an exponential progression of basic and translational immunologic research, some of which produced novel and practical diagnostic possibilities. Obviously, these advancements necessitated an overhaul of the 1995 Allergy Diagnostic Parameter commensurate with the extensive database currently available. The ultimate goals were to formulate recommendations based on evidence-based literature and to achieve balanced use of classic and new diagnostic methods.

The working draft of the Parameter on Allergy Diagnostic Tests update was based on an outline jointly conceived by James T. Li and I. Leonard Bernstein and realized by a work group (Robert Hamilton, Sheldon Spector, Ricardo Tan, David I. Bernstein, Scott Sicherer, David B. K. Golden, and David Khan) chaired by I. Leonard Bernstein. As with previous parameters, the draft was based on a review of the medical literature using a variety of search engines, such as PubMed. Published clinical and basic studies were rated by categories of evidence and used to establish the strength of recommendations (Table 1). The initial draft was then reviewed by all members of the Joint Task Force and subsequently by the American Academy of Allergy, Asthma and Immunology (AAAAI), the American College of Allergy, Asthma and Immunology (ACAAI), and the Joint Council of Allergy, Asthma and Immunology and a number of experts on in vivo and in vitro diagnostic immunology selected by the supporting organizations. Comments were also solicited from the general membership of these societies via their Web sites. This document therefore represents an evidence-based, broadly accepted consensus opinion. The peer review process and general format of the Practice Parameter are consistent with recommendations of the American College of Medical Quality, which defines practice guidelines. As such, it is anticipated to serve as a reference source for current utility and validity of allergy diagnostic tests.

The organization of Practice Parameters on Allergy Diagnostic Tests is similar to previous Joint Task Force parameters except that a single algorithm with annotations was not appropriate to the mission of the parameter. The broad range of diagnostic techniques for varying purposes could not possibly be stratified into a uniform paradigm encompassing diverse clinical sensitivity disorders that require objective confirmatory tests. An Executive Summary is followed by a collation of Summary Statements, which also precede referenced narrative discussions on each subject. The Practice Parameter is divided into 2 parts: part 1 is a detailed description of diagnostic modalities currently available to allergists/clinical immunologists. It encompasses both IgE and cell-mediated in vivo (skin and patch) and in vitro tests for a wide spectrum of inhalant, food, and contactant allergens. Organ challenge tests are discussed in greater detail in this revised Practice Parameter because controlled challenges or supervised exposure ultimately serve as the appropriate gold standard for assessing whether clinical sensitivity is present. Consonant with their recent emergence as diagnostic adjuncts, the section concerning current status of cytokines and chemokines has been expanded. A new section on "Other Immunologic Tests" has been added in recognition that many allergists/clinical immunologists have considerable interests and expertise in a variety of laboratory immunologic techniques commonly used to corroborate the diagnosis of non-IgE, non–cell-mediated clinical immunologic diseases. A discussion about unproven techniques is relevant because these methods still have advocates who promote them to patients desperately seeking alternative approaches for their particular problems.

Part 2 considers optimal utilization and integration of evidence-based diagnostic methods for various clinical situations, which include inhalant, food, insect venom, drug and contact sensitivities. Practice parameters of diagnosis and management for each of these clinical entities have been previously published with algorithms tailored to fit the specific clinical situation. Many of the diagnostic recommendations of part 2 were extracted or in some cases quoted verbatim from each of these published guidelines.

The Joint Task Force acknowledges that rapid advancements in diagnostic technology could render specific past and current recommendations obsolete at any time and that attempts to revise will have to be undertaken at appropriate intervals. Nevertheless, whatever the update interim period may be, the allergy/clinical immunology community should be prepared to accept novel new diagnostic techniques, provided that they are validated by scientifically accepted approaches.

The overall objectives of this Parameter on Allergy Diagnostic Tests are tripartite: (1) to develop a reliable reference resource for selecting appropriate diagnostic tests; (2) to provide guidelines and support for the practicing physician on how diagnostic tests should be used in an appropriate and

cost-effective manner; and (3) to improve the quality of care of patients by facilitating prompt and accurate diagnosis of their hypersensitivity disorders.

EXECUTIVE SUMMARY

There is a wide array of diagnostic modalities for human hypersensitivity diseases. Among these, skin tests for immediate hypersensitivity and delayed hypersensitivity are of paramount importance. As immunologic diagnostic technology advances, in vitro tests for both IgE- and cell-mediated immunity have also assumed greater significance. In some instances, lymphocyte functional assays may be applicable for confirmation of humoral or cell-mediated immunity cytotoxicity syndromes, as well as classic delayed hypersensitivity reactions.

Specific cellular components of both immediate hypersensitivity– and cell-mediated immunity induced inflammation can be identified by their unique transcription markers, protein products, or cell surface differentiation markers. An increase in eosinophils and their products often occurs in both immediate- and late-phase responses of IgE-mediated reactions. The role of the basophil in such reactions can also be evaluated by basophil histamine release tests and, more recently, the basophil activation test. When tests for IgE-mediated immunity are equivocal, organ challenge testing is the most direct way of ascertaining whether bona fide clinical sensitivity exists.

Mononuclear cells (monocytes, macrophages, and lymphocytes) are essential constituents of adaptive immunity. In particular, their role in cell-mediated immunity has long been recognized. Lymphocyte subsets, their cytokines, and their chemokines may be readily identified and measurable in body fluids and tissue sites. Several applications of this technology have become standard clinical tests (eg, $CD4^+$ cells in acquired immunodeficiency); others are being vigorously pursued (eg, interleukin [IL] 6, IL-8, IL-10, and transforming growth factor β). Increases in specific cytokines such as macrophage inhibitory factor (MIF) and IL-16 are associated with active cell-mediated immunity processes.

Well-established techniques to detect IgG/IgG subclass antibodies by enzyme-linked immunosorbent assay (ELISA), immunodiffusion, and immunoprecipitation are available for specific antigens and autoantibodies. Antigen antibody complexes may be associated with increased C1q binding and cryoglobulins.

Prick/puncture tests or intracutaneous tests are the preferred techniques for IgE-mediated hypersensitivity. It is advisable to use prick/puncture devices, which are relatively nontraumatic and elicit reproducible results when placed on specific areas of the body (ie, arms or back). Optimal results depend on use of potent test extracts and proficiency of the skin tester (ie, demonstration of coefficient of variation $<30\%$ at different periods). It is essential that objective wheal-and-flare responses be recorded in millimeters (diameter or area) because cutoff levels (in millimeters) may obviate the necessity for confirmatory respiratory and food allergen challenge tests. This interpretation system also enables easier comparison among physicians. Intracutaneous tests are generally used for specific allergens (ie, Hymenoptera venoms and penicillin), but they may also be applied if prick/puncture test results are negative and there is a strong historical likelihood of clinical allergy to specific allergens. Some clinicians prefer intracutaneous tests without preceding prick/puncture tests, but when this alternative is elected, special care must be taken to ensure that intracutaneous allergen concentrations are nonirritant and correlative with end organ sensitivity. However, there are safety concerns when intracutaneous tests are performed without preceding prick/puncture tests. A suggested way of determining appropriate intracutaneous test concentrations is a serial end point titration regimen, one of which reported that intracutaneous dilutions between 1:12,500 and 1:312,000 (wt/vol) were nonirritant. Late-phase cutaneous responses, which reflect the persistent IgE allergic inflammatory milieu, may occur after either prick/puncture or intracutaneous tests but are more likely to do so after the latter. Preliminary data suggest that decrease of late-phase cutaneous response may occur after successful allergen immunotherapy.

The prototypic skin test for delayed hypersensitivity is the tuberculin skin test, which is evaluated by degree of induration in millimeters 48 hours after application. Similar tests are no longer commercially available for pathogenic fungi (eg, *Histoplasma capsulatum*). A positive tuberculin reading varies from 10 to 15 mm in induration, depending on the incidence of active tuberculosis within the indigenous population of the patient. Decreased cell-mediated immunity response or anergy may be evaluated by delayed hypersensitivity antigens (ie, tetanus toxoid, *Candida,* and *Trichophyton*) to which most members of a population have been exposed. Formerly the validity of anergy testing was compared with the mean number of positive reactions elicited by 4 to 5 delayed hypersensitivity antigens in a large normal control population. Absence of reactivity to all or all except 1 was equated with complete or relative anergy, respectively. Currently, there are only 3 delayed hypersensitivity antigens for testing (tetanus toxoid, *Candida,* and *Trichophyton*), and these have not been evaluated in a large population as described above. Therefore, interpretation of anergy using these 3 antigens is circumspect. Concurrent anergy and tuberculin skin testing is no longer recommended in patients with human immunodeficiency virus (HIV) suspected of having mycobacterial infections.

Allergic contact dermatitis (ACD) is a special form of delayed hypersensitivity evaluated by epicutaneous or patch tests. More than 3,700 substances have been reported to induce contactant sensitivity. Direct irritants may cause irritant contact dermatitis (ICD), which often is morphologically indistinguishable from ACD. The irritancy threshold of each test agent must be predetermined to exclude the possibility of ICD. Patch testing should be considered for any dermatitis for which contactant exposure, either natural or secondary to topical agents, might be implicated. Most ACD can be detected by 65 substances recommended by the North American

Contact Dermatitis Research Group. The only available Food and Drug Administration (FDA)–cleared patch test kit is the T.R.U.E. test, which covers a range of approximately 25% to 30% of the most common ACD contactant allergens. Therefore, customized patch testing is often necessitated. Patch tests are read at least twice (48 and 72 to 96 hours after application) and occasionally 7 days later in the case of weak ACD allergens. Such allergens can also be detected by a repeat open application test protocol. Atopy patch tests to foods and drugs are being investigated as a complementary aid in the diagnosis of food and drug allergies. These tests have not yet been validated by a sufficient number of controlled studies.

Laboratory tests may also provide useful information to evaluate either immediate hypersensitivity or cell-mediated immune reactions. Currently, commercial availability considerations are such that specific IgE tests are used more frequently than is the case for functional in vitro cell-mediated immunity assays. Within the past decade, however, immunoassays of certain cell-mediated immunity products (ie, cytokines or chemokines) may be demonstrating sufficient predictability to be considered as surrogates of cell-mediated immunity.

The discovery of IgE and availability of IgE myelomas enabled the production of large quantities of IgE. This permitted the production of highly specific anti-human IgE antibodies, which led to immunoassays capable of measuring both total IgE and allergen specific IgE concentrations in serum and body fluids. A succession of modified assays ensued. Subsequent modifications are calibrated using heterologous interpolation against the World Health Organization (WHO) 75/502 international human serum IgE reference preparation, thereby establishing a uniform system of specific IgE antibody in quantitative kilo international units (kIU) per liter (ie, 1 kIU = 2.4 ng IgE). The method of total and specific IgE assays are discussed in detail, including the indications, advantages, and limitations of these assays. The FDA guideline regulations now stipulate guidance regulations for all IgE methods, including semiautomatic, automatic, and multiplexed systems. According to these quality assurance suggestions, each allergen assay should include its specific homologous reference serum (ragweed vs ragweed reference serum) as an additional internal control whenever sufficient quantities of specific reference sera can be obtained. It is anticipated that multiplexed arrays for assays of IgE will soon be generally available. Secondary antibody detector systems for these modified techniques include chemiluminescence and fluorescence. Allergen specificity and cross-allergenicity may be determined by an inhibition technique. Although correlation of higher kIU levels of specific IgE to clinical sensitivity for some allergens is equivalent to prick/puncture tests, skin prick/puncture tests generally have better overall predictability and are the preferred initial diagnostic approach.

Interpretation of both skin and serum specific IgE tests is highly dependent on the constitutive allergenicity, potency, and stability of the allergen extract being used. For these reasons, sensitivity tends to be higher among pollens, certain foods, dust mite, fungi, and certain epidermals compared with venoms, drugs, and chemicals. Recommendations for allergen immunotherapy based solely on results of skin or specific IgE tests without appropriate clinical correlation are not appropriate.

IgG and IgG subclasses can be measured using immunoassays similar to those used for allergen specific IgE. Controversy exists regarding whether increases of IgG4 are valid harbingers of either diagnosis or clinical efficacy after immunotherapy. Specific IgG/IgG4 results do not correlate with oral food challenges and are not recommended for the diagnosis of food allergy.

Other less frequently used assays for IgE-mediated reactions include histamine release from basophils and plasma tryptase secondary to mast cell degranulation. The latter test may be useful in the detection of anaphylaxis and mastocytosis.

Eosinophils and their generated products, such as eosinophilic cationic protein (ECP), are key cells in allergic inflammation, particularly late-phase responses. Increased numbers of these cells in nasal smears and induced sputum may be useful indicators of the existence and extent of allergic inflammation. In the case of sputum, they may also be indicative of asthma exacerbation or the presence of chronic eosinophilic bronchitis or esophagogastritis.

The basophil activation test, as detected by the expression of CD63 and/or CD203C surface markers by flow cytometry, is being vigorously investigated for both diagnosis and serial monitoring of therapeutic efficacy. This test has not yet been cleared in the United States by the FDA.

Cell types that contribute to cell-mediated immunity reactions include lymphocytes, monocytes, macrophages, dendritic cells, Langerhans cells, and granulocytes. Most laboratory tests of cell-mediated immunity quantify lymphocyte function with respect to (1) proliferation; (2) production of inflammatory mediators, cytokines, and chemokines; (3) monitoring of cytotoxic reactions; and (4) regulation of immune responses. Techniques to measure each of these functions are discussed in the context of advantages and disadvantages of each method. Several nonradioactive assays of lymphocyte proliferation and cytotoxicity are now available. Although a functional assay of macrophage inhibition is not commercially available, the cytokine responsible for this test, MIF, can be measured by immunoassay. Other cytokines or chemokines of special importance to cell-mediated immunity, such as IL-12, IL-16, and monocyte chemoattractant proteins (MCPs) 1, 2, and 3, can also be measured by ELISA immunoassays.

Evaluation of non-IgE and non–cell-mediated immunity clinical immunologic diseases may include laboratory screening for (1) primary and acquired immunodeficiency, (2) immune-mediated gammopathies, (3) complement activation disorders, and (4) a diverse spectrum of autoimmune and vasculitic diseases. Brief summaries of diagnostic techniques

available for these entities are discussed in part 1. Many of them have evolved to ELISA and Western and immunoblot assays, although indirect immunofluorescence tests are still required for confirmation in certain autoimmune diseases. Tests of complement activation are especially important in patients who present with signs of leukocytoclastic vasculitides.

Specific organ challenge tests may facilitate or confirm clinical diagnosis under certain circumstances: (1) investigation of potential "new" allergens, (2) confirmation of clinical diagnosis when the history is suggestive but skin and/or in vitro test results are negative, (3) confirming food allergy, (4) monitoring of therapy, and (5) substantiating occupational sensitivity. This section has been expanded substantially to include detailed descriptions of the indications and objective techniques for evaluating allergen-specific conjunctival, nasal, and bronchial challenges. Protocols for food challenges are discussed in the part 2 section on "Evaluation of Food Allergy." Details of laboratory supervised and workplace challenges for confirmation of occupational asthma (OA) are also included.

A new section, "Inflammatory Biomarkers of Upper and Lower Airway Fluids," has been added because such techniques often provide confirmatory evidence of suspected clinical diseases (eg, eosinophilic vs neutrophilic asthma; bronchoalveolar lavage (BAL) $CD8^+$ lymphocytic alveolitis as an indicator of hypersensitivity pneumonitis). In addition, current diagnostic roles of 2 new noninvasive methods (exhaled nitric oxide and exhaled breath condensate) are summarized.

A brief review of unproven tests is included near the end of part 1. The unproven nature of these tests is supported by placebo-controlled studies in some instances. In other situations, clinical samples submitted for diagnostic evaluation yielded completely false results.

The section on allergens has been retained because it is one of the most reliable sources of plant, animal, and chemicals to which North American patients are exposed. As cited previously, the number of positive allergenic contactants exceeds 3,700. A reliable reference source for such contact substances may be found in the patch test discussion of part 1. The allergens section also reviews essential information about cross-allergenicity, which should aid the clinician in specific decisions about skin tests and allergen immunotherapy. (Also see Allergome – a database of allergenic molecules – http://www.allergome.org.)

Part 2 of this parameter provides evidence-based likelihood decisions on selecting confirmatory laboratory diagnostic tests for inhalant, food, insect venom, drug, and contactant allergies. When the data are not sufficiently evidence based for such choices, alternative pathways are suggested. In each of these clinical subsections, discussions about use of in vivo vs in vitro tests are commensurate with Category I evidence criteria. All clinical topics in part 2 provide a basis for integrating historical features, physical signs, and diagnostic recommendations of previously published Practice Parameters (Disease Management of Drug Hypersensitivity: A Practice Parameter; Allergen Immunotherapy: A Practice Parameter; Stinging Insect Hypersensitivity: A Practice Parameter; Food Allergy: A Practice Parameter; and Contact Dermatitis: A Practice Parameter), with the current updated diagnostic techniques presented in part 1.

Table 1. Classification of Recommendations and Evidence Category

Category of Evidence
Ia Evidence from meta-analysis of randomized controlled trials.
Ib Evidence from at least 1 randomized controlled trial.
IIa Evidence from at least 1 controlled study without randomization.
IIb Evidence from at least 1 other type of quasi-experimental study.
III Evidence from nonexperimental descriptive studies, such as comparative studies, correlation studies, and case-controlled studies.
IV Evidence from expert committee reports, the opinion or clinical experience of respected authorities, or both.
LB Evidence from laboratory-based studies.
Strength of Recommendation
A Directly based on category I evidence.
B Directly based on category II evidence or extrapolated from category I evidence.
C Directly based on category III evidence or extrapolated from category I or II evidence.
D Directly based on category IV evidence or extrapolated from category I, II, or II evidence
E Directly based on category LB evidence.
F Based on consensus of the Joint Task Force on Practice Parameters.
NR Not rated

COLLATION OF SUMMARY STATEMENTS

Summary Statement 1. First described in 1867 by Dr Charles Blackley, skin tests (prick/puncture and intracutaneous) have evolved as reliable, cost effective techniques for the diagnosis of IgE-mediated diseases. (B)

Summary Statement 2. Prick/puncture tests are used to confirm clinical sensitivity induced by aeroallergens, foods, some drugs, and a few chemicals. (B)

Summary Statement 3. A number of sharp instruments (hypodermic needle, solid bore needle, lancet with or without bifurcated tip, and multiple-head devices) may be used for prick/puncture tests. (C)

Summary Statement 4. Although a number of individual prick/puncture comparative studies have championed a particular instrument, an objective comparison has not shown a clear-cut advantage for any single or multitest device. Furthermore, interdevice wheal size variability at both positive and negative sites is highly significant. (C)

Summary Statement 5. Optimal results can be expected by choosing a single prick/puncture device and properly training skin technicians in its use. (C)

Summary Statement 6. Although prick/puncture tests are generally age, sex, and race independent, certain age (children younger than 2 years and adults older than 65 years) and racial (African American children) factors may affect their interpretation. (C)

Summary Statement 7. Skin test allergens used for prick/puncture tests should be potent and stable. (B)

Summary Statement 8. To ensure proper interpretation, positive (histamine) and negative (saline or 50% glycerinated human serum albumin [HSA]–saline) should be performed at the same time as allergen tests. (B)

Summary Statement 9. 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)

Summary Statement 10. Qualitative scoring (0 to 4+; positive or negative) is no longer used by many clinicians because of interphysician variability in this method of scoring and interpretation. (B)

Summary Statement 11. The diagnostic validity of prick/puncture tests has been confirmed not only in patients exposed to allergens under natural conditions but also in patients undergoing controlled organ challenge tests. (B)

Summary Statement 12. Although prick/puncture testing often correlates with exposure history, there are significant exceptions to this observation. (B)

Summary Statement 13. 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)

Summary Statement 14. 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)

Summary Statement 15. The reliability of prick/puncture tests depends on the skill of the tester, the test instrument, color of the skin, skin reactivity on the day of the test, age, and potency and stability of test reagents. (C)

Summary Statement 16. False-positive prick/puncture test results may occur (1) to tree pollens in honey bee–sensitive patients due to cross-reactive carbohydrate determinants present in honey bee venom and (2) in tree-sensitive patients being tested to tree pollens no longer indigenous to the area. (C)

Summary Statement 17. The rare occurrence of specific positive organ challenge test results in patients with both negative prick/puncture and intracutaneous test results suggests that alternative pathways, including locally secreted IgE, IgE-independent, or nonimmune stimuli may activate mediator release in the end organ. (C)

Summary Statement 18. Life-threatening generalized systemic reactions are rarely caused by prick/puncture tests. In a recent retrospective survey, 1 death was reported in a patient who received 90 food prick/puncture tests at one time. (C)

Summary Statement 19. Intracutaneous tests will identify a larger number of patients with lower skin test sensitivity and are used when increased sensitivity is the main goal of testing. (B)

Summary Statement 20. Intracutaneous tests are useful for evaluation of anaphylaxis, particularly drug (ie, penicillin) and *Hymenoptera* venom anaphylaxis. (A)

Summary Statement 21. When compared with specific nasal challenge, skin end point titration (SET) is equivalent to prick/puncture skin tests. (B)

Summary Statement 22. Intracutaneous tests should be performed with small volumes (approximately 0.02 to 0.05 mL) of allergens injected intracutaneously with a disposable 0.5- or 1.0-mL syringe. (C)

Summary Statement 23. As a general rule, the starting dose of an intracutaneous allergen test ranges from 100- to 1,000-fold more dilute than the allergen concentration used for prick/puncture tests. (C)

Summary Statement 24. Intracutaneous tests are read 10 to 15 minutes after injection and both wheal and erythema (in millimeters) should be recorded. (B)

Summary Statement 25. The diagnostic sensitivity of intracutaneous tests is probably greater than prick/puncture tests when testing for penicillin, insect venom, or certain drug class (eg, insulin, heparin, muscle relaxants) hypersensitivity. (C)

Summary Statement 26. The greater sensitivity of titrated intracutaneous tests, especially in the erythema component, is an advantage for determining biologic potency of allergen extracts and biologic allergy units (BAU) as based on intracutaneous erythema assays in sensitive human volunteers. (B)

Summary Statement 27. At dilutions between 10^{-2} and 10^{-3} (wt/vol), intracutaneous tests for most allergens exhibit poor efficiency in predicting organ challenge responses and correlating with the presence of detectable serum specific IgE. (C)

Summary Statement 28. There are limited data about equivalency of sensitivity, specificity, and predictive indices between intracutaneous and prick/puncture tests when compared with organ challenge tests. One study demonstrated that more dilute intracutaneous concentrations were comparable to prick/puncture tests in predicting positive nasal challenges. (C)

Summary Statement 29. Similar comparative equivalency studies based on history and symptoms alone revealed that intracutaneous tests were comparable to prick/puncture tests only at intracutaneous titration end points between 10^{-5} and 10^{-6} g/mL (wt/vol). (B)

Summary Statement 30. Because clinical use of intracutaneous tests is usually restricted to a single dose (ie, 1:1,000 wt/vol), which may be irritant, predictive accuracy of these tests at this concentration is often confounded by false-positive results. (C)

Summary Statement 31. For most allergens, a fixed dilution (1:1,000 wt/vol) of intracutaneous tests has poor efficiency in predicting organ challenge responses. (A)

Summary Statement 32. Intracutaneous tests are occasionally negative in venom-sensitive patients who experience life-threatening reactions. (C)

Summary Statement 33. Repetitive ($\geq$2) intracutaneous penicillin testing may sensitize a small number of individuals to penicillin. (C)

Summary Statement 34. Immediate systemic reactions are more common with intracutaneous tests; 6 fatalities were reported in a recent retrospective survey. (C)

Summary Statement 35. Prescreening with prick/puncture tests is a practical way to avoid life-threatening reactions to intracutaneous tests. (C)

Summary Statement 36. If prick/puncture prescreening is not used, preliminary intracutaneous serial threshold titrations should be considered, starting at high dilutions (eg, 10^{-5} to 10^{-8} g/mL [wt/vol]). This is of particular importance if exquisite sensitivity (eg, anaphylaxis to foods and drugs) is suspected. (D)

Summary Statement 37. The late-phase cutaneous response is a continuation of either prick/puncture or intracutaneous testing, generally the latter, and is characterized by erythema, induration or edema, and dysesthesia. (B)

Summary Statement 38. The late-phase cutaneous response may occur after both immune and nonimmune activation. Many allergens have been implicated. (B)

Summary Statement 39. The late-phase cutaneous response should be read between the 6th and 12th hours after the skin tests are applied; measurements of mean diameter and/or area of induration or edema should be recorded. (B)

Summary Statement 40. Although the clinical relevance of late-phase cutaneous response is not as yet fully established, several randomized, controlled studies suggest that reduction in sizes of late-phase cutaneous response may parallel clinical response to immunotherapy. (B)

Summary Statement 41. The same principles that pertain to safety of skin tests apply to late-phase cutaneous responses. (C)

Summary Statement 42. Preadministration of drugs, such as calcineurin inhibitors, misoprostol, prednisone, and azelastine, before application of skin tests partially or completely inhibit the late-phase cutaneous response. (B)

Summary Statement 43. 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 (eg, region of the country), occupation, and activities. Routine use of a large number of skin tests or routine annual tests without a definite clinical indication are clearly not justified. (D)

Summary Statement 44. Respiratory challenge tests are used when an objective gold standard for establishing clinical sensitivity is indicated. (B)

Summary Statement 45. Conjunctival challenge tests are usually conducted for suspected localized eye allergy but in some cases they may also be helpful in investigating nasal allergy. (B)

Summary Statement 46. Conjunctival challenge tests are evaluated by symptoms of itching and objective indices, including tear volume, amount of mucus, and palpebral or bulbar erythema. (B)

Summary Statement 47. Nasal challenges provide objective evidence of clinical sensitivity when the diagnosis is in question or in situations when it is desirable to evaluate efficacy of therapeutic management. (B)

Summary Statement 48. Nasal challenge responses are evaluated by subjective symptoms and objective measurements of nasal airway resistance, the number of sneezes, and the measurement of inflammatory mediators in nasal secretions. (B)

Summary Statement 49. Specific (allergic) bronchial challenge provides a measure of lower airway clinical sensitivity when there is uncertainty or dispute. (B)

Summary Statement 50. Guidelines for the performance of specific bronchial challenge include factors such as withholding certain medications before the test, determining the initial allergen dose by preliminary skin or methacholine challenge testing, a beginning forced expiratory volume in 1 second (FEV_1) baseline of 70% or better, the amount or duration of exposure to allergen, measurement of FEV_1 at intervals after the exposure, careful observation for late-phase responses, comparison to a placebo-controlled challenge usually performed the day before the specific challenge, and, optionally, repetition of methacholine challenge 24 to 48 hours after specific challenge for evaluation of induced bronchial hyperresponsiveness. (B)

Summary Statement 51. Occupational challenge testing requires special precautions with respect to the innate toxicity of the suspected allergen and special apparatuses used to measure and control the quantity of challenge substances, such as potentially irritating volatile agents and dust. (B)

Summary Statement 52. A practical clinical method of assessing OA is prospective monitoring of the worker at and away from work by serial peak expiratory flow rates (PEFRs) or FEV_1 values if this can be arranged by mutual agreement of employee and employer. (B)

Summary Statement 53. Many inflammatory correlates can be evaluated and studied serially in respiratory and other body fluids, such as nasal smears or lavage, induced sputum, or BAL. These may define specific phenotypes or in some cases predict severity. (B)

Summary Statement 54. Exhaled nitric oxide is a noninvasive measure of airway inflammation and is useful for monitoring objective responses to topically administered corticosteroids. (B)

Summary Statement 55. Although breath condensate analysis is an evolving noninvasive method for evaluation of asthma, results are still variable and further refinements are required before it can be accepted as a valid diagnostic method. (C)

Summary Statement 56. Bronchoalveolar lavage obtained through flexible bronchoscopy is useful in phenotyping asthma. The finding of lymphocytic alveolitis may suggest a diagnosis of hypersensitivity pneumonitis. (B)

Summary Statement 57. Cystic fibrosis may not only be confused with asthma but certain genetic variants may be associated with increased asthma risks. (B)

Summary Statement 58. Although major phenotypes of α_1-antitrypsin deficiency do not occur in asthma, recent surveys demonstrated a high prevalence of asthma in young ZZ homozygous antitrypsin deficiency patients. (B)

Summary Statement 59. Purified protein derivative (PPD) of tuberculin is the prototype antigen recall test and provides direct evidence that hypersensitivity, as opposed to toxicity, is elicited by the antigens in *Mycobacterium hominis* or related mycobacterial species. (B)

Summary Statement 60. The tuberculin skin test is elicited by the intracutaneous injection of 0.1 mL of standardized PPD starting with the intermediate strength of 5 tuberculin units. (C)

Summary Statement 61. Recall antigen skin tests are used to evaluate cellular immunity in patients with infection (eg, life-threatening sepsis), cancer, pretransplantation screening, endstage debilitating diseases, and the effect of aging. (C)

Summary Statement 62. Reduced or absent recall antigen tests are termed *anergy,* which develops frequently in certain diseases, such as hematogenous tuberculosis, sarcoidosis, and atopic dermatitis. (C)

Summary Statement 63. Candida albicans, *Trichophyton mentagrophytes,* and *Tetanus* toxoid, the currently available recall antigens, are injected intracutaneously in the same way as the PPD test. (C)

Summary Statement 64. The size of the delayed skin test reaction is measured 48 hours after antigen challenge, and the largest diameter of the palpable firm area that outlines the induration response should be measured to the nearest millimeter. (C)

Summary Statement 65. When a single intracutaneous antigen (other than PPD) is used to evaluate prior sensitization to a potential pathogen, a reaction of 5 mm or greater may suffice as the cutoff point for positive tests, but smaller reactions (2 to 4 mm) may be clinically important. (C)

Summary Statement 66. The absence of delayed-type hypersensitivity to all the test antigens would suggest an anergic state. (C)

Summary Statement 67. The most important use of delayed-type hypersensitivity skin testing is epidemiologic screening of susceptible populations exposed to bacterial and fungal pathogens. (C)

Summary Statement 68. The widest application of recall antigen testing is the detection of anergy and as an in vivo clinical correlate of cell-mediated immunoincompetency. (C)

Summary Statement 69. Although anergy testing was formerly conducted frequently in HIV patients to determine whether a concurrent negative tuberculin skin test result rules out active tuberculosis, recent evidence mitigates against this approach. Recall antigen anergy in HIV patients has also been investigated as an indicator of staging, progression of disease, and response to therapy. (C)

Summary Statement 70. Although the standardized PPD antigen has been used for many years as a predictor of active or latent tuberculosis infection, confounders, such as susceptible populations, BCG vaccination, and cross-sensitization with other atypical mycobacterial species have all affected the diagnostic accuracy of the tuberculin skin test and, by extrapolation, other delayed-type hypersensitivity tests. (C)

Summary Statement 71. The gross appearance of a late-phase cutaneous response and delayed-type hypersensitivity reactions may not be completely distinguishable except that the latter are more characterized by prolonged induration. (B)

Summary Statement 72. Although systemic corticosteroids will render delayed-type hypersensitivity skin test results uninterpretable, 28 days of treatment with high-dose inhaled fluticasone (220 μg, 2 puffs twice a day) did not suppress delayed-type hypersensitivity to PPD in healthy volunteers. (B)

Summary Statement 73. Neither anergy nor tuberculin testing obviates the need for microbiologic evaluation when there is a suspicion of active tuberculosis or fungal infections. (F)

Summary Statement 74. Several new in vitro assays (ie, interferon-γ and polymerase chain reaction) appear to be more reliable in predicting active tuberculosis in BCG-vaccinated persons or when cross-sensitivity to atypical mycobacteria may coexist. (C)

Summary Statement 75. Immediate hypersensitivity reactions, including anaphylaxis, have been reported after tuberculin skin tests. (D)

Summary Statement 76. The number of skin tests for delayed, cell-mediated hypersensitivity reactions is limited. (C)

Summary Statement 77. First introduced by Jadassohn in 1896, the epicutaneous patch test has evolved as the definitive diagnostic technique for the diagnosis of allergic contact dermatitis (ACD). (A)

Summary Statement 78. When clinical evaluations suggest that exposure to a specific contactant has occurred either in an occupational or nonoccupational clinical setting, patch testing can be used to confirm the diagnosis. (C)

Summary Statement 79. From a public health perspective, patch testing is useful to identify potential health hazards of unknown and newly introduced contact allergens for the medical community and industrial hygienists. (C)

Summary Statement 80. The most common patch test techniques are the individual Finn Chamber and the T.R.U.E. TEST, an FDA-approved screening method for screening contactant allergens. The T.R.U.E. TEST is preloaded with 23 common contactants and vehicle control that have been previously incorporated into a dried-in-gel delivery system, which is coated onto a polyester backing to form a patch template. (B)

Summary Statement 81. If photocontact sensitivity is suspected, the appropriate allergens should be subjected to pho-

topatch tests primarily in the UV-A range of 320 to 400 nm. (C)

Summary Statement 82. Traditionally, patch tests remain in place for 48 hours. After the 48-hour patch test reading, additional readings at 3 to 4 days and in some cases 7 days after the original application of the patch yield the best overall reading reliability. (C)

Summary Statement 83. A descriptive reading scale developed by 2 major international ACD research groups is the current standard for interpreting patch test results. (C)

Summary Statement 84. Although patch tests are indicated in any patient with a chronic eczematous dermatitis if ACD is suspected, patch tests are especially important in identifying both ICD and ACD in the occupational setting. (C)

Summary Statement 85. Other important exposures associated with ACD include the use of topical medication, including corticosteroids, plant-induced ACD, and dermatitis occurring after the use of cosmetics and personal hygiene products. (C)

Summary Statement 86. Unprotected work and repetitive exposure to surfactants may predispose patients to occupational dermatitis, including ICD and ACD. (C)

Summary Statement 87. Certain contactant allergens in the T.R.U.E. TEST panel, such as nickel and some rubber chemicals, have a high degree of relevant (approximately 75%) correlation with clinical sensitivity but others do not (eg, hydroxycitronellal, thimerosal). (B)

Summary Statement 88. Patch tests are most effective when the patients are selected on the basis of a clear-cut clinical suspicion of contact allergy and they are tested with the chemicals relevant to the problem; these conditions satisfy the prerequisites of high pretest probability. (C)

Summary Statement 89. Although the diagnostic accuracy of contactants cannot be compared with other in vivo or in vitro tests, diagnostic concordance between patch test sensitivity and the outcomes of repeated open provocation tests has been demonstrated for some contactants. (B)

Summary Statement 90. The chief limitation to traditional patch testing for the diagnosis of ACD is the lack of a suitable gold standard by which it can be evaluated in terms of diagnostic accuracy predictors and likelihood ratios. (C)

Summary Statement 91. Other technical limitations of patch tests include the inclusion of relevant contact allergens, use of the proper vehicle, application to the proper skin area, proper reading and interpretation, and the ability to correlate the tests with the patient's specific exposure. (A)

Summary Statement 92. Other limiting factors concern reproducibility, lack of information about irritant thresholds, and minimal elicitation concentrations (MECs) for many common chemicals in the human environment. (C)

Summary Statement 93. The inability to separate irritants from allergic responses is often encountered in the angry back syndrome, which occurs in approximately 6% of cases and is likely to develop in patients with a longer duration of the primary dermatitis. (C)

Summary Statement 94. Negative patch test reactions may occur even when the tests are performed with the correct sensitizing materials because the test fails to duplicate the conditions under which the dermatitis developed (eg, abrasions, frequent use of irritating soaps, washing the hands with solvents). (C)

Summary Statement 95. Systemic ACD after patch testing is rare, as is reactivation of patch test reactions after oral ingestion of related allergens or even by inhalation of budesonide in patients with sensitization to topical corticosteroids. (B)

Summary Statement 96. It is possible to sensitize a patient who had not been previously sensitized to the allergen being tested. This is particularly true of plant contactants, such as poison ivy or oak and aniline dyes. (B)

Summary Statement 97. Two major variants of traditional patch tests are available: the atopy patch test (ATP) and repeated use test (RUT). (B)

Summary Statement 98. Atopy patch tests have been evaluated in patients with atopic dermatitis and eosinophilic esophagitis as an adjunct for the diagnosis of inhalant and food allergy. (B)

Summary Statement 99. Atopy patch tests for foods are prepared with dried or desiccated foods mixed into an aqueous solution and placed in 12-mm Finn Chambers before positioning on the patient's back. (B)

Summary Statement 100. Atopy patch tests for the diagnosis of drug allergy are performed by incorporating liquid or powdered drugs into petrolatum or aqueous solvents, which are added to 12-mm Finn Chambers and placed on the back. (B)

Summary Statement 101. Use tests have been developed for weak sensitizers (repeated open application test [ROAT]), substances with poor percutaneous absorption (strip patch test), and several premarketing dose response provocation tests for determining the minimal sensitizing dose of potential contactants in human volunteers. (B)

Summary Statement 102. In the strip patch test penetration of substances is enhanced by repeated adhesive tape stripping before application of the contactant patch to the stripped area. (B)

Summary Statement 103. The ROAT is an exaggerated use test designed to determine a patient's biologic threshold or response to a suspected contactant, especially if this has not been achieved with prior open or closed patch testing. (B)

Summary Statement 104. Although clinical relevance is still evolving with regard to the APT, several investigative groups have reported that this test may be an adjunct in detection of specific allergens in atopic dermatitis and eosinophilic esophagitis. (B)

Summary Statement 105. The role of the atopy patch in determining clinical allergy to food is indeterminate. (B)

Summary Statement 106. The lack of standardization of APTs for diagnosis of both food and drug allergy is the chief limitation. (C)

Summary Statement 107. Although the purpose of APTs is to test for food and drug nonimmediate reactions, the possibility of anaphylaxis must be considered because there could be significant percutaneous absorption of proteins and/or simple chemicals with high anaphylactogenic potential. (B)

Summary Statement 108. The appropriate number of atopic patch tests is indeterminate because they are not routinely performed. (D)

Summary Statement 109. Because ACD is frequently caused by unsuspected substances, up to 65 patch tests may be required for diagnosis. (B)

Summary Statement 110. Total serum IgE concentrations are reported in international units or nanograms per milliliter (1 IU/mL = 2.44 ng/mL). (A)

Summary Statement 111. Total IgE is cross-standardized with the WHO 75/502 human reference IgE serum verified by periodic proficiency surveys. (B)

Summary Statement 112. The clinical applications of total serum IgE are of modest value. High serum IgE concentrations occur in allergic bronchopulmonary *Aspergillosis* (ABPA), the therapeutic response of which is evaluated by serial IgE values. (B)

Summary Statement 113. Total serum IgE is required for assessing the suitability of a patient for omalizumab therapy and determining the initial dose. (B)

Summary Statement 114. As with total IgE, commercial specific IgE antibody assays are calibrated using heterologous interpolation against the WHO 75/502 human IgE reference serum, thereby enabling a uniform system of reporting. (E)

Summary Statement 115. In addition to WHO 75/502 calibration, an earlier specific IgE classification system was based on internal positive calibration curves from a positive control heterologous serum containing specific IgE antibodies, which in the original RAST was white birch specific. However, FDA clearance for modified specific IgE tests requires use of homologous internal control allergic sera whenever this is possible to obtain. (E)

Summary Statement 116. The precise sensitivity of these immunoassays compared with prick/puncture skin tests has been reported to range from less than 50% to more than 90%, with the average being approximately 70% to 75% for most studies; similar sensitivity ranges pertain when immunoassays are compared with symptoms induced after natural or controlled organ challenge tests. (C)

Summary Statement 117. As with skin tests, the interpretation of specific IgE results requires correlation with the history, physical examination, and, in some cases, symptoms directly observed after natural or laboratory exposure to allergens. This cannot be accomplished by commercial remote practice laboratories, which base recommendations for immunotherapy on a history form submitted by the patient and specific IgE results. (B)

Summary Statement 118. Because the constitutive allergenicity, potency, and stability are variable among commercial allergen extract reagents, sensitivity and the positive predictive value of both prick/puncture and specific IgE tests generally tend to be higher among pollens, stable anaphylactogenic foods, house dust mite, certain epidermals, and fungi compared with venoms, drugs, and chemicals. (C)

Summary Statement 119. Proper interpretation of specific IgE test results needs to take into consideration variables such as the binding affinity or avidity of allergens, solid-phase systems, cross-reactive proteins and glycoepitopes, specific IgG antibodies in the test system, and high total serum IgE (>20,000 IU). (E)

Summary Statement 120. A multiallergen (up to 15 allergens bound to a linear solid-phase system) test can screen for atopic status, following which allergen specific tests are required for more definitive evaluation. (C)

Summary Statement 121. Specific IgE immunoassays are not recommended as a definitive confirmatory test for several specific clinical conditions. They provide neither diagnostic nor prognostic information when measured in the cord blood of newborn infants. They do not have sufficient sensitivity for foolproof prediction of anaphylactic sensitivity to venoms or penicillins. (B)

Summary Statement 122. Specific IgE immunoassays may be preferable to skin testing under special clinical conditions, such as widespread skin disease, patients receiving skin test suppressive therapy, uncooperative patients, or when the history suggests an unusually greater risk of anaphylaxis from skin testing. (B)

Summary Statement 123. Determination of allergen specificity by inhibition of specific IgE binding is a unique attribute of specific IgE testing. (E)

Summary Statement 124. Automated systems using multiplexed allergen assays are being rapidly developed. One of these is cleared by the FDA for the simultaneous measurement of 10 allergens. (E)

Summary Statement 125. Allergen specific IgG may be measured by immunodiffusion or immunoabsorption. (E)

Summary Statement 126. Immunodiffusion antibodies to cow's milk are associated with Heiner's disease, a non-IgE disorder that presents in infants with pulmonary infiltrates. (B)

Summary Statement 127. IgG and IgG subclass antibody tests for food allergy do not have clinical relevance, are not validated, lack sufficient quality control, and should not be performed. (B)

Summary Statement 128. Although a number of investigators have reported modest increases of IgG4 during venom immunotherapy, confirmation and validation of the predictive value of IgG4 for therapeutic efficacy of venom immunotherapy are not yet proven. (C)

Summary Statement 129. The probability distribution of specific IgE for several anaphylactogenic foods (peanuts, egg white, cow's milk, and codfish) can define clinical sensitivity as verified by double-blind oral challenge tests; similar relationships have been defined for several respiratory allergens. (A)

Summary Statement 130. Although allergens can be standardized either by radioimmunodiffusion or immunoassay inhibition based on major allergenic epitopes, the FDA selected BAU instead because in vitro analytic techniques would have been variable from allergen to allergen and would have caused great confusion. (C)

Summary Statement 131. Histamine and leukotriene release measurements from human basophils after incubation with allergen are valuable research tools for in vitro investigations of allergy. (B)

Summary Statement 132. The recent availability of several sensitive immunoassays for histamine and leukotriene C4 is a significant technological advance for measuring these mediators in various biologic fluids or release from whole blood, isolated basophils, mast cells, or other cultured cells. (B)

Summary Statement 133. Histamine and its N-methyl histamine metabolite may be measured in 24-hour urine samples after suspected anaphylactic episodes. (B)

Summary Statement 134. Plasma tryptase, particularly the β form, should be obtained within 4 hours after an anaphylactic episode. (B)

Summary Statement 135. Combined α and β species of plasma tryptase are elevated in patients with systemic mastocytosis. (A)

Summary Statement 136. Eosinophils in body fluids correlate highly with the diagnosis of allergic rhinitis, allergic asthma, and eosinophilic bronchitis. (B)

Summary Statement 137. Elevated eosinophil derived substances (ie, ECP) and chemoattractants (ie, eotaxin) in body fluids are indicators of allergic inflammatory disease. (B)

Summary Statement 138. A basophil activation test measured by expression of CD63 and CD203c and detected by flow cytometry is being evaluated for many IgE-mediated disorders. (C)

Summary Statement 139. Tests that quantify lymphocyte function measure the ability of lymphocytes to (1) proliferate, (2) produce inflammatory mediators and cytokines or chemokines, (3) mount cytotoxic responses, and (4) regulate immune responses. (B)

Summary Statement 140. Lymphocyte proliferative responses may be evaluated by either nonspecific mitogens (eg, phytohemagglutinin, concanavalin A, or pokeweed) or specific soluble and cell-bound antigens. (B)

Summary Statement 141. 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. (B)

Summary Statement 142. Cytokines (IL-1 through IL-33) and growth factors are glycoproteins produced by a variety of cells that are capable of altering activities of other cells through interaction with specific surface receptors. (E)

Summary Statement 143. Chemokines are small (8 to 10 kDa) proteins secreted by many immune and nonimmune cells with essential roles in inflammatory and immune reactions, including the late-phase cutaneous response. (E)

Summary Statement 144. Cytokine and chemokine profiles play essential roles in allergic inflammation and are being increasingly evaluated as phenotypic markers and in the differential diagnosis of human hypersensitivity disorders. (B)

Summary Statement 145. Other bioactive indices of cell-mediated immunity include cytotoxic assays, cultures of mixed lymphocytes, and macrophage inhibition. (E)

Summary Statement 146. Most cytokines and chemokines can be measured by commercial ELISA and ELISpot immunoassays. (E)

Summary Statement 147. Proinflammatory cytokines or chemokines, which are particularly associated with cell-mediated immunity, include interferon-γ, IL-12, tumor necrosis factor α (TNF-α), IL-16, MIF, macrophage inflammatory protein 1 (MIP-1), and MCP 1, 2, and 3. (B)

Summary Statement 148. Simple, cost-effective tests include (1) an absolute lymphocyte count, (2) the absolute number of $CD4^+$ T cells, and (3) the $CD4^+/CD8^+$ ratio. (B)

Summary Statement 149. Investigation of non-IgE and non–cell-mediated clinical immunologic disorders may require tests that indicate abnormal adaptive and innate immune reactions. (B)

Summary Statement 150. Abnormal serum and urine proteins, including cryoglobulins, may be associated with several abnormal immune syndromes. (B)

Summary Statement 151. The inflammatory consequences induced by immune functions may be detected by nonspecific tests, such as a complete blood cell count with differential, sedimentation rate, C-reactive protein, and other acute-phase reactants. In some instances, functional assays of neutrophils and macrophages may be necessary to pinpoint inflammatory responses. (B)

Summary Statement 152. Evaluation of complement activation with a decrease of C3 and C4 may indicate complement deficiency, drug reactions, or the presence of immune complexes, which often are associated with increases in serum cryoglobulins and C1q binding. (B)

Summary Statement 153. Autoantibody profiles offer important diagnostic adjuncts in the diagnosis of collagen vascular diseases, vasculitides, and cytotoxicity disorders. (B)

Summary Statement 154. Procedures for which there is no evidence of diagnostic validity include cytotoxic tests, provocation-neutralization, electrodermal testing, applied kinesiology, iridology, hair analysis, or food specific IgG, IgG4, and IgG/IgG4 antibody tests. (B)

Summary Statement 155. Although North American inhalant allergens are botanically and ecologically diverse, several expert committees consisting of members with botanic and mycologic expertise have compiled and selected 36 key allergens in North America, based on Thommen's postulates. (D)

Summary Statement 156. For individual patients, the choice of test allergens is guided by the history and physical

examination and the physician's knowledge, training, and experience. (B)

Summary Statement 157. A well-designed skin test and laboratory ordering form should provide useful information to the ordering physician, his/her staff, health care providers, and other physicians who may be consulted in the future. (B)

Summary Statement 158. The best indicators in the selection of appropriate pollens for clinical use are extensive prevalence in the air and concurrent allergy symptoms during annually recurrent seasons when such pollens are expected to be present in the ambient air. (B)

Summary Statement 159. The clinical significance of a single fungus test reagent may be difficult to ascertain because of important confounders, such as sampling method, culture conditions, nonculturable species, allergenic differences between spores, and hyphae and preferential ecologic niches. (A)

Summary Statement 160. For clinical purposes, molds are often characterized as outdoor (*Alternaria* and *Cladosporium* species), indoor (*Aspergillus* and *Penicillium* species), or both (*Alternaria, Aspergillus,* and *Penicillium* species). (B)

Summary Statement 161. Five *Hymenoptera* venom extracts are available for evaluation of anaphylactic reactions to honeybee, yellow jacket, yellow hornet, white faced hornet, and *Polistes* wasp. A whole-body extract is the only currently available diagnostic reagent for fire ant sting allergy. (A)

Summary Statement 162. Major inhalant acarid and insect allergens include several species of house dust mite and cockroach. (A)

Summary Statement 163. Animal clinical sensitivity is most often associated with domestic pets (cats, dogs, birds) and laboratory animals (rodents, rabbits). Specific testing is guided by history of appropriate animal exposure. (A)

Summary Statement 164. Selection of food tests for IgE-mediated clinical sensitivity is usually tailored to the patient's temporal history, which may be supplemented by a food diary. (A)

Summary Statement 165. Although commercial skin tests for drugs, biologics, and chemicals are not available, specialized medical centers prepare and use such tests under appropriate clinical situations. The validity of such tests is adjudged on a case by case basis. (C)

Summary Statement 166. More than 300 low- and high-molecular-weight occupational allergens have been identified. Test reagents for these agents are generally available in specialized occupational allergy centers. (A)

Summary Statement 167. A variety of plant or plant-derived proteins or glycoproteins may be associated with systemic allergic symptoms. (A)

Summary Statement 168. Chemicals, plant resins, and lipid constituents are the chief causes of ACD, which requires patch testing for confirmation. (A)

Summary Statement 169. As previously emphasized, knowledge of specific patterns of cross-reactivity among tree, grass, and weed pollens is essential in preparing an efficient panel of test reagents. (A)

Summary Statement 170. Although cross-reactivity among related pollen families can usually be ascribed to specific epitopic determinants, more diffuse cross-reactivity due to plant profilins and cross-reactive carbohydrate determinants may also be present. (A)

Summary Statement 171. Cross-reactivity data on fungi are extremely sparse. (C)

Summary Statement 172. The skin prick/puncture test is superior to intracutaneous testing for predicting nasal allergic symptoms triggered by exposure to pollen. (B)

Summary Statement 173. A skin prick/puncture test is superior to intracutaneous testing for predicting allergic rhinitis and allergic asthma triggered by cat allergen exposure. (B)

Summary Statement 174. The skin prick/puncture can be used to rule out allergic rhinitis and allergic asthma triggered by cat allergen exposure. (B)

Summary Statement 175. Knowledge of allergen cross-reactivity and local aerobiology is important in selecting appropriate allergens and in minimizing the number of allergens required for skin and specific IgE tests. (D)

Summary Statement 176. In general, skin prick/puncture testing is more sensitive for identifying sensitization to inhalant allergens and confirming clinical allergy. However, specific IgE assays with defined quantifiable threshold levels can also predict positive respiratory responses after allergen exposure. (B)

Summary Statement 177. Demonstration of sensitization to an occupational agent by specific IgE and/or skin testing alone is insufficient to establish a diagnosis of OA. (B)

Summary Statement 178. Skin prick testing with certain well-characterized occupational protein allergens possesses adequate sensitivity such that a negative skin test result ($<$3-mm wheal diameter) can be used to rule out clinical allergy. (B)

Summary Statement 179. Test performance characteristics of specific IgE assays and skin testing for detection of chemical IgE-mediated sensitization must undergo validation and reproducibility in controlled studies using standardized antigens and assay protocols before these can be considered reliable for routine evaluation of workers suspected of OA. (B)

Summary Statement 180. In patients undergoing evaluation for suspected work-related natural rubber latex (NRL) allergy, a positive skin prick test result with a NRL extract (if available) is preferred to demonstration of elevated specific IgE with an FDA-cleared assay due to higher sensitivity of the former. Current IgE-mediated allergy and asthma caused by NRL allergens is highly unlikely in the presence of a negative skin prick test result with a reliable crude NRL allergen extract. Elevated in vitro specific IgE levels can be used to confirm NRL allergy, but a negative result does not exclude NRL allergen sensitization. (B)

Summary Statement 181. The primary tools available to evaluate patients' adverse reactions to foods include history (including diet records), physical examination, prick/puncture

skin tests, serum tests for food specific IgE antibodies, trial elimination diets, and oral food challenges. (B)

Summary Statement 182. A detailed dietary history, at times augmented with written diet records, is necessary to determine the likelihood that food is causing the disorder, identify the specific food, and determine the potential immunopathophysiology. (D)

Summary Statement 183. With regard to evaluations for IgE antibody–associated food allergies, tests for food specific IgE antibody include percutaneous skin tests (prick/puncture tests) and serum assays. In general, these tests are highly sensitive (generally >85%) but only modestly specific (approximately 40% to 80%) and therefore are well suited for use when suspicion of a particular food or foods is high. They are not effective for indiscriminate screening (eg, using panels of tests without consideration of likely causes) and therefore generally should not be used for that purpose. (B)

Summary Statement 184. Intracutaneous (intradermal) skin tests for foods are potentially dangerous, are overly sensitive, increase the chance of a false-positive test result, and are not recommended. (D)

Summary Statement 185. Based on studies in infants and children, increasingly higher concentrations of food specific IgE antibodies (reflected by increasingly larger percutaneous skin test size and/or higher concentrations of food specific serum IgE antibody) correlate with an increasing risk for a clinical reaction. (B)

Summary Statement 186. A trial elimination diet may be helpful to determine if a disorder with frequent or chronic symptoms is responsive to dietary manipulation. (D)

Summary Statement 187. Graded oral food challenge is a useful means to diagnose an adverse reaction to food. (B)

Summary Statement 188. A number of additional diagnostic tests are under investigation, including APTs and tests for IgE binding to specific epitopes. (B)

Summary Statement 189. The rational selection, application, and interpretation of tests for food specific IgE antibodies require consideration of the epidemiology and underlying immunopathophysiology of the disorder under investigation, estimation of prior probability that a disorder or reaction is attributable to particular foods, and an understanding of the test utility and limitations. (D)

Summary Statement 190. Diagnostic skin and/or specific IgE tests are used to confirm clinical sensitivity to venoms in a patient with a history of a prior systemic reaction. (B)

Summary Statement 191. Although diagnostic tests identify species specificity of venom sensitization, they do not reliably predict severity of the sting reaction. (B)

Summary Statement 192. Standardized honeybee, *Polistes,* and *Vespula* antigens are commercially available as skin test reagents. (A)

Summary Statement 193. The skin test reagent available for evaluation of imported fire sting allergy is a nonstandardized whole-body extract. (C)

Summary Statement 194. In the case of a history of anaphylaxis to *Hymenoptera* venoms, intracutaneous skin tests are generally performed to 5 of the available venoms in a dose response protocol (up to 1 μg/mL [wt/vol]) when preliminary prick/puncture test results are negative. (B)

Summary Statement 195. The FDA-cleared specific IgE assays have comparable specificity but decreased sensitivity compared with venom skin tests. (B)

Summary Statement 196. Paradoxically, as many as 16% of insect-allergic patients with negative venom skin test results have positive results on currently available specific IgE in vitro tests. (B)

Summary Statement 197. A small percentage of patients (1%) with negative results to both skin and in vitro tests may experience anaphylaxis after a field sting. (B)

Summary Statement 198. A skin test refractory period lasting up to 6 weeks after a venom sting has been demonstrated by recent data. (B)

Summary Statement 199. Because of the predictive inconsistencies of both skin and serum specific IgE tests, patients with a convincing history of venom-induced systemic reactions should be evaluated by both methods. (D)

Summary Statement 200. Cross-allergenicity among insect venoms is (1) extensive among vespid venoms, (2) considerable between vespids and *Polistes*, (3) infrequent between bees and vespids, and (4) very limited between yellow jacket and imported fire ants. (B)

Summary Statement 201. If *Hymenoptera* venom sensitivity is suspected, initial prick/puncture tests followed by serial endpoint titration with intracutaneous tests may be required. (B)

Summary Statement 202. Venom skin test may be repeated once or twice at 3- to 6-month intervals to confirm the diagnosis in a patient who initially had negative test results. (D)

Summary Statement 203. When the diagnosis is highly suspected but not proved by skin and specific IgE tests, supervised live insect challenge sting may confirm clinical sensitivity. Nevertheless, most patients with suspected venom allergy do not require live stings. (D)

Summary Statement 204. Evaluation of drug-specific IgE antibodies induced by many high-molecular-weight and several low-molecular-weight agents is often highly useful for confirming the diagnosis and prediction of future IgE-mediated reactions, such as anaphylaxis and urticaria. (B)

Summary Statement 205. Neither immediate skin nor tests for specific IgE antibodies are diagnostic of cytotoxic, immune complex, or cell-mediated drug-induced allergic reactions. (B)

Summary Statement 206. The availability of specific laboratory tests for non–IgE-mediated drug allergies is limited. (C)

Summary Statement 207. Atopy patch tests, lymphocyte proliferation tests, and basophil activation tests are additional diagnostic tests for drug allergy. Further studies are required to confirm their clinical utility in the evaluation of drug allergic patients. (B)

Summary Statement 208. A graded challenge (test dose) is a procedure to determine if a drug is safe to administer and is intended for patients who are unlikely to be allergic to the given drug. In contrast to desensitization, a graded challenge does not modify the immune response to a drug. (B)

Summary Statement 209. Atopy patch tests, lymphocyte proliferation tests, and basophil activation tests are additional diagnostic tests for drug allergy. Further studies are required to confirm their clinical utility in the evaluation of drug allergic patients. (B)

Summary Statement 210. Penicillin skin testing is the most reliable method for evaluating IgE-mediated penicillin allergy provided that the necessary reagents are available. When performed with both major and minor determinants, the negative predictive value of penicillin skin testing for immediate reactions approaches 100%, whereas the positive predictive value is between 40% and 100%. (B)

Summary Statement 211. Skin testing with penicilloyl-polylysine and penicillin G appears to have adequate negative predictive value in the evaluation of penicillin allergy. (C)

Summary Statement 212. Penicillin skin test–negative patients (as determined by testing with major and minor determinants) may receive penicillin, and depending on which skin test reagents are used and the reaction history, the first dose may need to be given via a test challenge with a lower dose under observation. (D)

Summary Statement 213. In the absence of validated skin test reagents, the approach to patients with a history of penicillin allergy is similar to that of other antibiotics for which no validated in vivo or in vitro diagnostic tests are available. Therapeutic options include (1) prescribing an alternative antibiotic, (2) performing a graded challenge, and (3) performing penicillin desensitization. (D)

Summary Statement 214. In patients who have reacted to semisynthetic penicillins, consideration should be given to skin test the implicated antibiotic and penicillin determinants. (B)

Summary Statement 215. There are no validated diagnostic tests of sufficient sensitivity for evaluation of IgE-mediated allergy to antibiotics other than penicillin. (C)

Summary Statement 216. Skin testing with nonirritating concentrations of other antibiotics is not standardized. A negative skin test result does not rule out the possibility of an immediate-type allergy. A positive skin test result suggests the presence of drug-specific IgE antibodies, but the predictive value is unknown. (C)

Summary Statement 217. A presumptive diagnosis of aspirin-exacerbated respiratory disease (AERD) can often be made by history; however, in some cases, aspirin provocation tests might be considered for a definitive diagnosis. (B)

Summary Statement 218. Urticaria, angioedema, and anaphylactic reactions to nonsteroidal anti-inflammatory drugs (NSAIDs) are distinctly different drug reactions from AERD reactions. In contrast to AERD reactions, anaphylactic reactions to NSAIDs are usually drug specific, and patients typically tolerate other structurally dissimilar NSAIDs. (B)

Summary Statement 219. Skin testing is a useful diagnostic tool in cases of perioperative anaphylaxis, and when skin testing is used to guide subsequent anesthetic agents, the risk of recurrent anaphylaxis to anesthesia is low. (C)

Summary Statement 220. Skin testing is not helpful in cases of taxane-induced anaphylactoid reactions. (C)

Summary Statement 221. Skin testing to carboplatin yields favorable predictive values. (C)

Summary Statement 222. Skin testing with asparaginase before treatment is recommended but does not identify all patients at risk of reactions. (C)

Summary Statement 223. Skin testing for diagnosis of local anesthetic allergy is limited by false-positive reactions. The gold standard for establishing a diagnosis of local anesthetic allergy is the provocative challenge. (C)

Summary Statement 224. The specificity and sensitivity of skin tests for systemic corticosteroid allergy are unknown, and cases of corticosteroid allergy with negative skin test results to the implicated corticosteroid have been reported. (D)

Summary Statement 225. For most allergic reactions to additives, skin tests are of no diagnostic value, and placebo-controlled oral challenges are required. (D)

Summary Statement 226. Contact dermatitis is a common skin disorder seen by allergists and dermatologists and can present with a spectrum of morphologic cutaneous reactions. (C)

Summary Statement 227. The initial approach to clinical diagnosis of CD is to distinguish between ACD and ICD. (C)

Summary Statement 228. The inflammatory lesions of CD may result from either ACD or ICD mechanisms. Factors that affect response to the contact agent include the agent itself, the patient, the type and degree of exposure, and the environment. (A)

Summary Statement 229. Tissue reactions to contactants are attributable primarily to cellular immune mechanisms except for contact urticaria. (A)

Summary Statement 230. Irritant contact dermatitis is usually the result of nonimmunologic, direct tissue reaction and must be clearly differentiated from ACD. (A)

Summary Statement 231. The diagnosis of ACD is suspected from the clinical presentation of the rash, which then must be supported by a history of exposure to a putative agent and subsequently confirmed by patch testing whenever this is possible. (C)

Summary Statement 232. The skin site of the dermatitis is important in the diagnosis of ACD because the area of predominant involvement and the regional distribution of the lesions often reflect the area of contact with the allergen. (A)

Summary Statement 233. Epicutaneously applied patch tests are the standardized diagnostic procedures to confirm ACD. (A)

Summary Statement 234. Patch tests are indicated in any patient with a chronic, pruritic, eczematous, or lichenified dermatitis if underlying or secondary ACD is suspected. (C)

Summary Statement 235. Patch test results are affected by oral corticosteroids but not by antihistamines. (A)

Summary Statement 236. Reading and interpretation of patch test results should conform to principles developed by the International Contact Dermatitis Research Group and the North American Contact Dermatitis Research Group. (A)

Summary Statement 237. A 96-hour reading may be necessary because 30% of relevant allergens that are negative at the 48-hour reading become positive in 96 hours. (A)

Summary Statement 238. Nonstandardized and customized patch testing is often required, depending on the patient's exposure history. (C)

Summary Statement 239. A problem-oriented approach to diagnostic patch testing using evidence-based principles of likelihood ratios and posttest probability is more likely to confirm clinical ACD than a randomly selected patch test approach. (B)

Summary Statement 240. Several in vitro procedures are being investigated for the diagnosis of ACD. (A)

Summary Statement 241. The differential diagnosis for CD is influenced by many factors, such as the clinical appearance of the lesions, distribution of the dermatitis, and associated systemic manifestations. (B)

Summary Statement 242. Occupational contact dermatitis (OCD) is an inflammatory cutaneous disease caused or aggravated by workplace exposure. (B)

Summary Statement 243. There are 7 generally acceptable criteria for establishing causation and aggravation of OCD. (C)

Summary Statement 244. Among health care professionals, ACD may occur as part of the spectrum of immunoreactivity to NRL in latex gloves. (A)

Summary Statement 245. Allergic contact dermatitis from exposure to plants is the result of specific cell-mediated hypersensitivity induced by previous contact with that family of plants. (A)

Summary Statement 246. Contact dermatitis is commonly implicated after exposure to topical medications, including lanolin, para-aminobenzoic acid (PABA), caine derivatives, antihistamines, iodochlorhydroxyquin, NSAIDs, and corticosteroids. (A)

Summary Statement 247. Allergic contact dermatitis due to topical corticosteroids may occur in up to 5% of patients with suspected CD. (A)

Summary Statement 248. Simultaneous exposure to allergens and irritants may produce both additive and synergistic ACD responses due to their interaction. (A)

Summary Statement 249. The role of detergents in hand dermatitis is a reflection of their ability to disrupt the skin barrier. (A)

Summary Statement 250. Allergic contact dermatitis is a significant clinical problem in children. (A)

PART 1

Part 1 is an update of in vivo and in vitro techniques that are available as adjunctive diagnostic instruments for confirmation of common allergic problems. These problems include both IgE and delayed hypersensitivity (ie, tuberculin-like and contactant allergy) adaptive immune responses. Emphasis is placed on reliability of reagents and devices. Quality assurance is also discussed in the context of reproducibility and the need to minimize intertechnician and interlaboratory variability.

IN VIVO DIAGNOSTIC TESTS OF IMMEDIATE HYPERSENSITIVITY REACTIONS

Percutaneous and Intracutaneous In Vivo Diagnostic Skin Tests

Summary Statement 1. First described in 1867 by Dr Charles Blackley, skin tests (prick/puncture and intracutaneous) have evolved as reliable, cost-effective techniques for the diagnosis of IgE-mediated diseases. (B)

History and Background

Although Blackley first documented the diagnostic potential of skin testing by placement of an allergen on abraded skin, the introduction of the cutaneous test for tuberculosis by von Pirquet became the chief impetus for subsequent allergy skin testing.[1,2] The first of these was the scratch test made by rubbing the allergen into a small, blood-free scratched area of the forearm and introduced by Schloss for the diagnosis of food allergy in children.[2] A few years later, Schick and Cooke independently introduced the intracutaneous test as a diagnostic method.[2] Although the scratch method was used extensively in the past, it has fallen out of general use because of greater patient discomfort, poor reproducibility, and the possibility of residual linear pigmented or depigmented areas.[3] As a way to avoid these problems, prick/puncture tests were introduced in the early 1950s. Sir Thomas Lewis had first suggested the puncture technique as an alternative skin test.[4] However, Squire first called attention to the quantitative aspects of the prick/puncture technique as a method of detecting sensitization to various proteins.[5] He estimated that a small amount (3×10^{-6} mL) of the test solution was introduced through the puncture site. Prick/puncture tests have been widely adapted throughout the world, although some practitioners prefer exclusive use of intracutaneous tests.

Prick/Puncture Tests

Summary Statement 2. Prick/puncture tests are used to confirm clinical sensitivity induced by aeroallergens, foods, some drugs, and a few chemicals. (B)

Present application

Prick/puncture tests are widely used for confirmation of clinical immediate hypersensitivity induced by a wide variety of naturally occurring allergens such as inhalants and foods. Under carefully defined circumstances, these tests are also useful in the diagnosis of drug and chemical hypersensitivity (platinum salts, acid anhydrides, polyisocyanates, sulfonechloramide, and succinylcholine analogs) reactions. They are frequently used as reference standards for evaluating specificity and sensitivity of specific in vitro tests for IgE, and they

may also be used to determine bioequivalent potencies of allergenic extracts in European countries.

Technique

Summary Statement 3. A number of sharp instruments (hypodermic needle, solid bore needle, lancet with or without bifurcated tip, and multiple-head devices) may be used for prick/puncture tests. (C)

Summary Statement 4. Although a number of individual prick/puncture comparative studies have championed a particular instrument, an objective comparison has not shown a clear-cut advantage for any single or multitest device. Furthermore, interdevice wheal size variability at both positive and negative sites is highly significant. (C)

Summary Statement 5. Optimal results can be expected by choosing a single prick/puncture device and properly training skin technicians in its use. (C)

Summary Statement 6. Although prick/puncture tests are generally age, sex, and race independent, certain age (children younger than 2 years and adults older than 65 years) and racial (African American children) factors may affect their interpretation. (C)

Summary Statement 7. Skin test allergens used for prick/puncture tests should be potent and stable. (B)

Summary Statement 8. To ensure proper interpretation, positive (histamine) and negative (saline or 50% glycerinated HSA–saline) should be performed at the same time as allergen tests. (B)

In performing the 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.[6,7] The skin is then gently lifted, creating a small break in the epidermis through which the suspected allergen solution penetrates. Alternatively, the skin device may be passed through the drop at a 90° angle to the skin. This is called a puncture test. 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. Devices with multiple heads have also been developed to apply several skin tests at the same time.[8] Several of these devices may also be used for modified scratch tests by applying a slight rotating twist after the puncture is made. Lancet instruments, either coated or submerged in a well containing the allergen extract (Phazet, Prilotest), are not used in the United States.[6,7]

In 1995, the Occupational Safety and Health Administration (OSHA) called attention to the possible safety and health risks to bloodborne pathogens that may arise with the practice of using a single device for multiple applications and wiping the device between tests.[9] OSHA opined that the technician could unintentionally be pricked with the device when wiping it between tests. This notice led many allergists to abandon the use of solid-bore needles for percutaneous testing, resulting in greater use of the newer devices, each of which is discarded after use.

Numerous studies have compared the reliability and variability of various devices.[10–29] Analysis of the results of these combined studies plus several recent prick/puncture comparative studies does not reveal a clear-cut advantage for any single or multitest device because interstudy results are variable. This is partially due to the degree of trauma that the device may impart to the skin, thereby accounting for different sizes of positive reactions and even the possibility of producing a false-positive reaction at the site of the negative control. Thus, prick/puncture devices require specific criteria for what constitutes a positive reaction (Table 2).[27,30] What is readily apparent from this table is the fact that wheal size variability between the studies is highly significant at both positive and negative test result sites.[27] In addition, considerable care should be given to proper training of skin test technicians. To achieve quality assurance among technicians, consistency in skin test performance should be demonstrated by skin testing proficiency protocols. In Europe, a coefficient variation of less than 20% after histamine control applications has been suggested, whereas a coefficient variation of less than 30% was used in a recent Childhood Asthma Management Study.[30] Table 3 outlines a suggested proficiency testing protocol. Criteria (diameter or wheal area ± SD) for positive and negative test results should be preestablished with the device selected by each clinical test site. Under clinical conditions, it is impossible to quantify the exact amount of

Table 2. 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[a]

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	DuoTip (Lincoln) twist	3.5
Smallpox needle (HS) prick	0	Bifurcated needle (ALO) prick	4.0
DuoTop (Lincoln) prick	1.5	MultiTest (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

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

Table 3. Suggested Proficiency Testing and Quality Assurance Technique for Prick/Puncture Skin Testing

• 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 = orthogonal or perpendicular diameter at the largest width of D.
• Histamine
Calculate the mean and SDs of each mean wheal diameter
Determine coefficient of variation (CV) = SD/mean
Quality standard should be CV less than 30%
• Saline
All negative controls should be <3-mm wheals and <10-mm flares.

injected material by prick/puncture tests. However, a recent gamma camera–based method to measure microvolumes labeled with radioisotopes attained a high degree of precision and accuracy in measuring microvolumes.[31] This degree of precision of allergen delivery might be useful for bioequivalency standard assays by prick/puncture methods.

In the past decade, a number of prospective epidemiologic studies have relied heavily on the prick/puncture test for evaluating increase or decrease in atopy over time.[32,33] Obviously, as discussed herein, such studies require the use of a single device with predetermination of the variability of wheal-and-flare diameters elicited by allergen, histamine, and saline, a proficient operator, and potent, stable test extracts. Several epidemiologic studies of this type have confirmed highly repeatable results in the short term (ranging from 1 week to 11 months).[34,35] In addition, other studies reported repeatability during a 2- to 3-year period.[36,37]

Concurrent drugs may affect the validity of prick/puncture and intracutaneous tests. Antihistamines vary considerably in their ability to suppress wheal-and-flare responses (Table 4). Furthermore, the studies that evaluated degree and duration of antihistamine suppression were not directly comparable because they used different pharmacodynamic models (eg, histamine vs allergen induced). The general principle to be gleaned from various studies is that the use of first- and second-generation antihistamines should be discontinued 2 to 3 days before skin tests with notable exceptions being cetirizine, hydroxyzine, clemastine, loratadine, and cyproheptadine (Table 4).[38–40] The tricyclic antidepressant doxepin may also suppress the wheal-and-flare response for as long as 6 days.[41] Histamine$_2$ antagonists may cause mild suppression, and their use should be discontinued for 24 hours before testing.[42,43] Oral prostaglandin D2 inhibitors, (eg, indomethacin) given several hours before testing may increase the wheal area by 17%, whereas cysteinyl leukotriene antagonists (eg, zafirlukast, montelukast) had negligible effects.[44,45] Short-term oral corticosteroids (30 mg of prednisone daily for 1 week) do not suppress skin tests.[46] There are dissenting opinions about the effect of long-term and relatively high-dose corticosteroids (>20 mg/d) on suppression of immediate skin test reactions.[47,48] By contrast, repetitive and prolonged application of potent topical corticosteroids for greater than 3 weeks may suppress immediate skin tests over areas where they have been applied.[49–51] Skin tests should be avoided in these sites or corticosteroids should be avoided in such sites for 2 to 3 weeks before testing. This effect is attributed to a combination of a decrease in mast cell recruitment and an increase of mast cell apoptosis.[49,50]

Several physiologic factors may affect interpretation of skin test results. Suppression of endogenous cortisol may affect late-phase reactions (skin and pulmonary) without a change in early-phase responses.[52,53] Although both prick/puncture and intracutaneous histamine tests were not formerly considered to be affected by menstrual phase, a recent study demonstrated optimal reactions to allergens when prick/puncture tests were performed at midcycle.[54] Histamine wheals are significantly larger in darkly pigmented skin compared with light skin, thus emphasizing the importance of a histamine control.[55] A recent investigation of school aged children revealed that histamine skin reactivity differed markedly in 3 different countries (Italy more than Poland more than Libya).[56] Short-term UV-B radiation may reduce wheal-and-flare intensity by as much as 50%.[57]

Prick/puncture tests can be performed on the upper back or volar surface of the forearm.[58] Not only is the back more reactive than the forearm, but specific locations on the back and forearms vary in reactive intensity.[58] Regardless of location, it is recommended that there should be sufficient space (eg, approximately 2 to 2.5 cm) between each applied allergen, and tests not be placed in areas 5 cm from the wrist or 3 cm from the antecubital fossae.[21,35,59–60] Skin tests should not be performed in skin sites with active dermatitis or severe dermatographism. If they are performed in the presence of mild dermatographism, the results should be interpreted with caution.

Prick/puncture tests may be performed in infants as young as 1 month. Although an early study reported that positive reactions tend to be smaller in infants and younger children (<2 years) than in adults, a recent investigation of prick/puncture tests in infants revealed that they exhibit a high degree of reliability.[61,62] The prevalence of positive skin test results increases until the third decade, after which there is a slow decline, particularly after the age of 50 years.[63] Nevertheless, significant positive skin test results may still be demonstrated in patients well older than 65 years. Several investigations reported that African American children with or without asthma were more likely to exhibit positive prick/

Table 4. Suppressant Effects of Drugs on Immediate Skin Tests[a]

Antihistamine generic name	Mean days suppressed	Maximum days suppressed	Dose
First generation			
Chlorpheniramaine	2[1b]; 3[2c]	6[1c]	4 mg 4 times daily
Clemastine	5[3c]	10[3c]	1 mg twice daily
Cyproheptadine	9[4c]	11[4c]	8 mg/d
Dexchlorpheniramine	4[4c]	4[4c]	4 mg/d
Diphenhydramaine	2[2c]	5[2c]	50 mg 4 times daily
Hydroxyzine	5[2c]	8[2c]	25 mg 4 times daily
Promethazine	3[2c]	5[2c]	25 mg 4 times daily
Tripelennamine	3[2c]	7[2c]	50 mg 4 times daily
Second generation			
Azelastine nasal	2[5,6c]		1% twice daily
Cetirizine	3[5c]		10 mg/d
Fexofenadine	2[5c]		60 mg twice daily
Loratadine	7[5c]		10 mg/d
Levocabastine nasal	0[5c]		50 micro/sp twice daily
Levocabastine Opth	0[5c]		0.05% twice daily
Tricyclic antidepressants and tranquilizers			
Desipramine	2[7b]		25 mg single dose
Imipramine	>10[8c]		
Doxepin	6[7b]		25 mg single dose
Doxepin topical	11[9c]		
Histamine$_2$ antihistamines			
Ranitidine	<1[10c]		150 mg single dose
Cysteinyl leukotriene antagonists			
Monteleukast	0[11,12]		10 mg
Zafirlukast	0[13]		20 mg
Local anesthetic			
EMLA cream	0 wheal[14] 150–100%[14]		5 g over volar surface of arm 1 hour before test suppression of erythema

[a] When study reports in fractions of days, the total is rounded up. Maximum days would apply to most patients, but there may be exceptions where this would be longer.

[b] Single-dose study.

[c] Multiple-dose study

1. Long WF, Taylor RJ, Wagner CJ, Leavengood DC, Nelson HS. Skin test suppression by antihistamines and the development of subsensitivity. *J Allergy Clin Immunol.* 1985;76:113–7.(III).

2. Cook TJ, MacQueen DM, Wittig HJ, Thornby JI, Lantos RL, Virtue CM. Degree and duration of skin test suppression and side effects with antihistamines: a double blind controlled study with five antihistamines. *J Allergy Clin Immunol.* 1973;51:71–7.(III).

3. Phillips MJ, Meyrick Thomas RH, Moodley I, Davies RJ. A comparison of the in vivo effects of ketotifen, clemastine, chlorpheniramine and sodium cromoglycate on histamine and allergen induced weals in human skin. *Br J Clin Pharmacol.* 1983;15:277–86.(IIa).

4. Imind M, Dirksen A, Nielsen NH, Svendsen UG. Duration of the inhibitory activity on histamine-induced skin weals of sedative and non-sedative antihistamines. *Allergy.* 1988;43:593–6. (III).

5. Simons FE, Simons KJ. Clinical pharmacology of new histamine H1 receptor antagonists. *Clin Pharmacokinet.* 1999;36:329–52. (LB).

6. Pearlman DS, Grossman J, Meltzer EO. Histamine skin test reactivity following single and multiple doses of azelastine nasal spray in patients with seasonal allergic rhinitis. *Ann Allergy Asthma Immunol.* 2003;91:258–62. (Ib).

7. Rao KS, Menon PK, Hilman BC, Sebastian CS, Bairnsfather L. Duration of the suppressive effect of tricyclic antidepressants on histamine-induced wheal-and-flare reactions in human skin. *J Allergy Clin Immunol.* 1988;82:752–7. (III).

8. Wolfe HI, Fontana VJ. The effect of tranquilizers on the immediate skin wheal reaction: a preliminary report. *J Allergy Clin Immunol.* 1964;35:271–3. (III).

9. Karaz SS, Moeckli JK, Davis W, Craig TJ. Effect of topical doxepin cream on skin testing. *J Allergy Clin Immunol.* 1995;96:997–8. (III).

10. Miller J, Nelson HS. Suppression of immediate skin tests by ranitidine. *J Allergy Clin Immunol.* 1989;84:895–9. (III).

11. Simons FE, Johnston L, Gu X, Simons KJ. Suppression of the early and late cutaneous allergic responses using fexofenadine and montelukast. *Ann Allergy Asthma Immunol.* 2001;86:44–50.(Ib).

12. Hill SL III, Krouse JH. The effects of montelukast on intradermal wheal and flare. *Otolaryngol Head Neck Surg.* 2003;129:199–203. (Ib).

13. Saarinen JV, Harvima RJ, Horsmanheimo M, Harvima IT. Modulation of the immediate allergic wheal reaction in the skin by drugs inhibiting the effects of leukotriene C4 and prostaglandin D2. *Eur J Clin Pharmacol.* 2001;57:1–4. (IIb).

14. Sicherer SH, Eggleston PA. EMLA cream for pain reduction in diagnostic allergy skin testing: effects on wheal and flare responses. *Ann Allergy Asthma Immunol.* 1997;78:64–8. (IIb).

puncture test results to outdoor aeroallergens than their counterpart white cohorts.[64,65]

Allergen extracts used for percutaneous and intracutaneous testing ideally should be of known composition and potency. Although a limited number of standardized extracts are commercially available, most inhalant and food extracts are not standardized. Before the recent availability of standardized extracts, the composition of nonstandardized, commercially available extracts varied greatly between the manufacturers.[66] This situation is slowly improving with the introduction of bioequivalent extracts.[67,68] In Nordic countries, the wheal and flare of a positive control histamine test are used to assign biologic equivalency to allergen materials.[69,70] Bioequivalency by this system is defined as histamine equivalent prick (HEP) units. Although relatively few commercialized extracts are yet designated in bioequivalent allergy units (eg, grass, cat), the trend toward universal bioequivalency is well under way, as evidenced by more recent attempts to standardize commercial food antigen extracts not only by wheal area but also by objective organ challenges.[71,72]

Stability and potency of allergenic test extracts are also important issues. Since it is known that allergen extracts deteriorate with time, accelerated by dilution and higher temperatures, allergen skin test extracts are usually preserved with 50% glycerin.[73,74] If dilutions are required for skin test threshold testing, the diluent should be HSA (0.03%)–saline.[73] All extracts should be stored under cold (4°C) to ensure stability.[74] In vivo biologic activity of genetically engineered recombinant allergens has been evaluated and compared with specific allergens from which they were derived.[75,76] In general, they appear to be highly specific and safe. However, the sensitivity of single recombinant allergens is usually lower than those obtained with natural allergen extracts.[75] The precise role of recombinant allergens as in vivo diagnostic tools remains to be determined.

Positive and negative controls should be performed with all tests. In the United States for many years the only available positive control was histamine phosphate (2.7 mg/mL equivalent to a 1.0-mg/mL histamine base). Wheal diameters with this preparation range from 2 to 7 mm.[59] Currently, a 10-mg/mL histamine dehydrochloride control is available, and this is the preferred positive control for prick/puncture skin tests. The negative control consists of 50% glycerinated HSA–saline if concentrated extracts are used.

Reading the test results

Summary Statement 9. 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)

Summary Statement 10. Qualitative scoring (0 to 4+; positive or negative) is no longer used by many clinicians because of interphysician variability in this method of scoring and interpretation. (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.[77] No essential difference has been found between these techniques. Histamine control tests should be read 15 minutes after application at the peak of reactivity.[69] The peak of allergen prick/puncture tests is usually 15 to 20 minutes after application. Although some investigators have advocated the primary importance of the wheal diameter,[70] both erythema and wheal should be measured and recorded in millimeters for appropriate comparisons with positive (ie, histamine) and negative controls (ie, buffered diluent or 50% glycerinated extracts). Since trauma may affect wheal size (Table 2), an allergen response less than 3 mm generally should not be regarded as positive.[27,30,70] Devices that produce wheals that exceed 3 mm at negative control sites should be avoided.[11] Unfortunately, there is variability among physicians and investigators in recording the dimensions of flare, wheal, or both. 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 orthogonal to D), planimetry (either direct or from a traced copy), minimal diameter of a significant wheal ≥3 mm, comparison to an HEP test caused by 1 or 10 mg/mL of histamine dihydrochloride (defined as 1 HEP with 10 mg/mL being the preferred reference standard), or a score related to a codeine phosphate control defined as a wheal of 75% or greater of a control codeine phosphate solution (25 mg/mL).[17,24,78] Qualitative scoring (0 to 4+; 0 or +) is no longer used by many clinicians because of marked interphysician variability in scoring and interpretation of this method.[79]

To summarize, 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. There is a recent trend to develop more precise methods of measuring wheal area, such as handheld scanners with appropriate computer software, end point titration, and morphometry because wheal size (area or diameter) has assumed greater diagnostic significance.[80–84] Several investigators have determined that specific cutoff values (eg, ≥8 mm for peanut) obviate the need to confirm clinical sensitivity by organ challenge tests.[85,86] If similar pretest probabilities for clinical sensitivity can be developed for more allergens by defining precise cutoff prick/puncture measurement results, the clinical utility of prick/puncture tests will be greatly enhanced.

Clinical relevance

Summary Statement 11. The diagnostic validity of prick/puncture tests has been confirmed not only in patients exposed to allergens under natural conditions but also in patients undergoing controlled organ challenge tests. (B)

Summary Statement 12. Although prick/puncture testing often correlates with exposure history, there are significant exceptions to this observation. (B)

The diagnostic value of prick/puncture tests has been determined chiefly by comparing the test to history of symptoms associated with exposure. They have been used most frequently to evaluate individual cases and populations of allergic patients. The diagnostic validity of prick/puncture tests has been confirmed as a correlate of clinical sensitivity in double-blind, randomized control studies under outdoor (parks) and indoor (controlled environmental exposure units) exposure conditions.[87] The diagnostic accuracy in prick/puncture tests has also been confirmed in groups of clinically allergic patients undergoing specific nasal bronchoprovocation challenge measured by nasal resistance or acoustic rhinometry under controlled laboratory conditions.[88–92] Likewise, in the case of foods, prick/puncture tests have been demonstrated to correlate with clinical symptoms that occur after either open or double-blind food challenges.[93–97] However, 1 of these studies revealed that this correlation did not necessarily apply to all foods.[93] When used as a diagnostic test for potential symptoms based on exposure and/or history alone, the utility of prick/puncture tests is highly allergen dependent, giving concordant results with certain allergens, such as cat dander, but not with others.[97–99] Nevertheless, given their generally favorable diagnostic characteristics, other tests (intracutaneous, atopy patch, various specific IgE tests) are often compared with prick/puncture tests as a reference.[100–108] Interestingly, a recent prospective study reported that 60% of skin sensitive (wheal ≥4 mm) asymptomatic subjects developed clinical allergy. These results suggested that a positive prick/puncture test result in an asymptomatic person may predict subsequent clinical allergy.[108]

Sensitivity, specificity, and positive and negative predictive indices

Summary Statement 13. 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)

Summary Statement 14. 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)

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 or by adding small amounts of Tween 80 (0.0005%).[15] 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. Because of the uncertainty created by this relationship between prick/puncture and intracutaneous tests, 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 it is possible to establish more scientific guidelines for interpreting the tests and what they predict.[90,91,97,109] 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%.[90,91] A recent meta-analysis comparing prick/puncture tests to nasal challenge revealed positive likelihood ratios of 4.93, 16.17, 3.23, and 4.06 for cat, tree pollen, grass pollen, and house dust allergen, respectively, whereas the corresponding negative likelihood ratios were 0.08, 0.03, 0.04, and 0.03.[109–113] In a single mold investigation (*Alternaria* sp) that compared skin testing to challenge tests, positive and negative likelihood ratios were similar for both prick/puncture and intracutaneous tests (prick: positive likelihood ratio of 11.75, negative likelihood ratio of 0.05; intracutaneous: positive likelihood ratio of 8.80, negative likelihood ratio of 0.05).[114] (Refer to Evaluation of Inhalant Allergy, Part 2 for clinical significance.)

A comparative study of allergic asthmatic patients undergoing nonspecific methacholine challenge causing a 20% fall in FEV_1 of 4 mg/mL or less (wt/vol) or 8 mg/mL or less (wt/vol) revealed that the sensitivity, specificity, and negative predictive value of prick/puncture tests were 91%, 52%, and 85%, respectively, with the cutoff value of provocation concentration that caused a decrease in FEV_1 of 20% (PC_{20}) 8 mg/mL or less (wt/vol).[108] The lower cutoff PC_{20} of 4 mg/mL or less (wt/vol) increased the sensitivity and negative predictive value to 98.2% and 97.8%, respectively. This suggests that positive prick/puncture skin test results are more likely to be associated with asthma of greater severity, as indexed by the lower cutoff methacholine PC_{20} value of less than 4 mg/mL (wt/vol). 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.[109] 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.[85,93–97,115–118] 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, ≥8 mm for milk; ≥7 mm for egg; ≥8 mm for peanuts).[94–99,115] These specific food cutoff values also indicate the probability of more severe food allergy because the 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. Once cutoff values are ascertained and validated, both likelihood ratios and the area under the receiver operating characteristic curve can be calculated with the goal of eliminating the need for confirmation by provocative challenges.[85,86,115]

Sensitivity, specificity, and the predictive indices have also been compared with clinical history, both for inhalant and food allergens.[97,98,119–122] Several of these investigations used the area under the receiver operating characteristic curves to determine optimal cutoff values and to evaluate the ability of various allergens to predict symptom histories of hay fever and asthma.[120,121] Analysis of these studies revealed no unifying principle about the accuracy of prick/puncture skin tests as predictors for hay fever and asthma. Thus, 1 study concluded that even the combination of history to common allergens and physical examination is not diagnostic with respect to skin prick/puncture and specific IgE tests.[98] There are exceptions, one of which concluded that a skin prick/puncture wheal size of 3 mm or larger to cat elicited a sensitivity of 0.9, a specificity of 0.9, and a diagnostic accuracy of 0.9.[97] The limited capacity of skin prick/puncture tests for predicting clinical symptoms was also tested by structured interviews with patients undergoing aeroallergen skin tests.[123] Patients were found to have limited ability to correctly predict positive skin test results to aeroallergens based on their own clinical symptom experiences.

Limitations

Summary Statement 15. The reliability of prick/puncture tests depends on the skill of the tester, the test instrument, color of the skin, skin reactivity on the day of the test, potency, and stability of test reagents. (C)

Summary Statement 16. False-positive prick/puncture tests may occur (1) to tree pollens in honey bee–sensitive patients due to cross-reactive carbohydrate determinants present in honey bee venom and (2) in tree-sensitive patients being tested to tree pollens no longer indigenous to the area. (C)

Summary Statement 17. The rare occurrence of specific positive organ challenge test results in patients with both negative prick/puncture and intracutaneous tests suggests that alternative pathways, including locally secreted IgE, IgE-independent, or nonimmune stimuli may activate mediator release in the end organ. (C)

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, potency and stability of test extracts, especially the optimum concentrations used for the test, and experimental differences between duplicate prick tests.[13,19,30] Appropriate proficiency test methods for evaluating accuracy, precision, and reproducibility of skin testing are encouraged in the training of personnel (Table 3).[30,124]

If quality controls are not used, interpretation of the test results varies from one technician to another. The hazards of blood contamination with the use of all instruments must be given appropriate attention, and all technicians must be carefully trained in appropriate barrier techniques, as well as avoidance of accidental needle punctures. Reliability of prick/puncture tests requires that allergen extracts be potent and of known composition. Whenever possible, extracts with known biologic potency should be used.[66,124,125] For example, commercial extracts of fruits and vegetables are likely to lose potency over relatively short periods. Therefore, prick/puncture tests for these potential allergens should be performed either with freshly made food extracts or by the prick-prick method in which the tester first pricks the fresh food and then the skin. This method may be particularly helpful when there are differences in the allergenicity of different cultivar strains (eg, apples).[126,127]

If interpretation of allergen prick/puncture tests are expressed as a ratio of equivalency to a positive control (eg, HEP), selection of the positive control may affect the diagnostic accuracy of the test. It has been shown that using a ratio of allergen to positive histamine control for grading ragweed reactivity elicited better diagnostic accuracy than the ratio of allergen to a codeine phosphate control.[128] By contrast, relating allergen-induced and control histamine wheals reduced intertechnician reproducibility.[129]

Several confounding issues concerning test extracts could limit diagnostic accuracy of prick/puncture tests. A recent report demonstrated that approximately 16% of honeybee venom allergic patients may be misdiagnosed as having multivalent pollen sensitization because they reacted to nonspecific cross carbohydrate determinants in venom extracts.[130] A recent study of patients sensitive to multiple tree pollens revealed a lack of correlation between prick/puncture tests using commercial extracts of 15 previously reported indigenous tree species compared with actual mean tree species pollen counts samples in the local aerobiology system.[99] This study indicated that prick tests to tree pollens should only be performed with those species that have been confirmed as being current airborne aeroallergens by aerobiologic sampling (see nationalallergybureauwww.aaaai.org/nab/).

For unknown reasons, the diagnostic accuracy of intracutaneous testing is superior to that of prick/puncture tests in several well-established IgE-mediated anaphylactic reactions (eg, penicillin, muscle relaxant, and venom hypersensitivity). In recent years, however, even intracutaneous negative *Hymenoptera* allergic patients have been reported to experience anaphylaxis.[131,132]

Although the accuracy of prick/puncture tests in predicting the presence or absence of clinical allergy has been generally confirmed by previously cited studies, provided the proper cutoff levels of interpretation are used, there are specific reports of proven end-organ sensitivity in the absence of positive prick/puncture or intracutaneous test results.[133–143] This occurrence has not been explained, although pathways such as locally secreted IgE, IgE-independent, or non-IgE stimuli have been suggested.[139,144,145] In the case of reactions to foods despite negative test results, the trigger protein in the test reagent may not have

been fully extracted (with respect to the appropriate epitope) or the causal protein was rapidly degraded.[140–143]

Safety

Summary Statement 18. Life-threatening generalized systemic reactions are rarely caused by prick/puncture tests. In a recent retrospective survey, 1 death was reported in a patient who received 90 food prick/puncture tests at one time. (C)

In a retrospective analysis of children being tested for atopy, 6 cases of generalized reactions occurred in infants younger than 6 months who showed positive skin prick test results to fresh food specimens. Other common features in this group of patients were active eczema and a family history of allergic diseases. All infants received prompt treatment and recovered well.[146] The overall rate of generalized reactions was 521 per 100,000 tested children. In a 12-year survey of fatal reactions to allergen injections and skin testing in both adults and children from 1990 to 2001, one fatality was confirmed after skin prick testing with multiple food allergens.[147] This patient also had moderately persistent asthma, and 90 food prick tests were applied at one time. Analysis of near or life-threatening reactions in the same survey revealed no instances of reactions attributed to inhalant prick/puncture tests. In the recently published Practice Parameter, The Diagnosis and Management of Anaphylaxis, the concurrent use of β-blockers and angiotensin-converting enzyme inhibitors is cited as a relative contraindication to skin testing.[148–151]

Intracutaneous Tests

Present applications

Summary Statement 19. Intracutaneous tests will identify a larger number of patients with lower skin test sensitivity and are used when increased sensitivity is the main goal of testing. (B)

Summary Statement 20. Intracutaneous tests are useful for evaluation of anaphylaxis, particularly drug (ie, penicillin) and *Hymenoptera* venom anaphylaxis. (A)

Summary Statement 21. When compared with specific nasal challenge, skin end point titration (SET) is equivalent to prick/puncture skin tests. (B)

Intracutaneous tests are generally used when increased sensitivity is the main goal of testing (ie, when prick/puncture test results are negative despite very convincing history of exposure).[152] They permit identification of a larger number of clinically reactive patients, especially those with lower skin test sensitivity. In addition, skin sensitivity to low potency allergenic extracts may best be evaluated by this method.

As previously discussed, intracutaneous tests are preferable for diagnosis of drug and venom anaphylaxis.[153–161] The utility of intracutaneous tests for diagnosis of drug-induced penicillin anaphylaxis has been extended to a variety of drug classes, including cancer chemotherapeutic agents, muscle relaxants, insulin, and heparin.[162–166] Although experience and standardization of these drug categories are limited compared with penicillin and venoms, their negative and positive predictive values appear to be comparable.[163]

Although intracutaneous tests at strengths customarily performed (1:100 to 1:1,000 [wt/vol] from manufacturer's concentrate) are more sensitive, there are conflicting results about their ability to predict clinical allergy. Several studies in the previously cited meta-analysis investigated how well intracutaneous tests predict symptoms after natural or laboratory allergen challenges.[110] Two high-quality studies conducted in cat- and grass-sensitive patients concluded that positive likelihood ratios were poor (0.89 and 1.05 for cat and grass, respectively) as were negative likelihood ratios (1.24 and 0.98 for cat and grass, respectively).[111,167] By contrast, the accuracy of intracutaneous tests was excellent for *Alternaria* species, as evidenced by positive and negative likelihood ratios of 8.80 and 0.05, respectively.[114] These disparate results probably reflect the intrinsic variability of individual allergens among investigators and their abilities to predict clinical allergy.[113]

One recent investigation demonstrated that SET, which is a modified quantitative testing method, is equivalent to prick/puncture testing for both positive and negative predictability of clinical allergy when both are compared with nasal challenge.[90] The end point response in SET is the lowest concentration of allergen that produces a wheal: (1) that is the first wheal 2 mm larger than the negative control wheal and (2) is followed by a second wheal that is at least 2 mm larger than the preceding one.[90] It should be stressed, however, that SET is roughly equivalent to new skin prick tests only at dilutions ranging from 1:12,500 (wt/vol) to 1:312,000 (wt/vol). By comparison, most physicians who perform intracutaneous testing use dilutions ranging from 1:100 (wt/vol) to 1:1,000 (wt/vol).[90] Indeed, a study designed to test the predictive response of timothy prick/puncture and intracutaneous tests to nasal provocation revealed that the addition of a single intracutaneous test at a dilution of 1:500 (wt/vol) (No. 2 in the Rinkel nomenclature) adds no additional predictability when the prick test result is negative and therefore appears to be unwarranted.[91] Similar disappointing results were obtained when *Alternaria* intracutaneous tests at a dose of 1:500 (wt/vol) were compared with specific nasal challenge[93] and contrasted sharply with a previous *Alternaria* study.[114]

Techniques

Summary Statement 22. Intracutaneous tests should be performed with small volumes (approximately 0.02 to 0.05 mL) of allergens injected intracutaneously with a disposable 0.5- or 1.0-mL syringe. (C)

Summary Statement 23. As a general rule, the starting dose of an intracutaneous allergen test ranges from 100- to 1,000-fold more dilute than the allergen concentration used for prick/puncture tests. (C)

A single-unit, 0.5- or 1.0-mL disposable syringe with an attached hypodermic needle is preferred. The gauge of the attached hypodermic needle may vary from 26 to 30.[168] The use of a Hamilton calibrated syringe ensures a reproducible

injected volume, but this appears to offer little advantage to careful injections that produce wheals of similar size.[168,169] The reproducibility of intracutaneous tests is affected by the same variables as those described for prick/puncture tests.[57] These include the age of the skin, the area of the body where the tests are applied, skin pigmentation, interference by concurrent medications, and potency and biologic stability of the allergen test extracts. Intracutaneous tests are usually placed on the upper arm or volar surface of the forearm rather than the back to allow for application of a tourniquet should systemic symptoms occur. The back also reveals considerable differences in skin reactivity between different areas of the back of individual patients.[168] There may be leakage of the allergen at the injection site because of improper technique, but this can be prevented by the use of unitized syringes and needles. Concurrent tests with diluent control solutions also should be performed. In addition, a positive histamine control (equivalent to 0.10 mg/mL [wt/vol] of histamine base) should be included to evaluate the degree of skin response at the time of the test. The volumes of intracutaneous test solutions may vary from 0.02 to 0.05 mL, depending on the purpose of the test. Delivery of small volumes (<0.03 mL) is difficult to attain with regularity. Because of the greater possibility of systemic reactions after intracutaneous testing, special care should be given to preparing less potent test dilutions. As a general rule, the starting dose of intracutaneous extract solutions in patients with a preceding negative prick test result should range from 100- to 1,000-fold dilutions of the concentrated extracts used for prick/puncture tests.[58] In the case of standardized allergens, such as ragweed, grass, dust mite, and cat, the range of starting intracutaneous test solutions in patients with preceding negative prick/puncture test results is between 10 and 100 BAU.[58,170]

Most of the factors that affect the reliability of prick/puncture tests also apply to intracutaneous tests. Several of these have already been discussed (ie, smaller dose of the positive histamine phosphate control and the unsuitability of the back for intracutaneous tests). Technical training for precision and reproducibility of intracutaneous tests should also be emphasized, especially for those persons performing biologic equivalency tests. A recent investigation of intracutaneous skin tests noted that intracutaneous testing had poor reproducibility, appearing to confirm a much earlier study.[171,172] The effects of drugs on intracutaneous testing are similar to the agents discussed under prick/puncture tests. Although immediate-phase reactions are not affected by cysteinyl leukotriene modifiers, the late-phase cutaneous reaction is reduced.[45]

Reading the test results

Summary Statement 24. Intracutaneous tests are read 10 to 15 minutes after injection, and both wheal and erythema (in millimeters) should be recorded. (B)

For intracutaneous tests, histamine controls and allergen sites are usually read 10 to 15 minutes, respectively, after the injections. Similar to prick/puncture tests, various indices, such as the longest diameter, the sum of the largest diameter and its orthogonal diameter divided by 2, products of the diameters, planimetry, and measurement of paper traced from skin responses, have been used to interpret intracutaneous results. Both erythema and wheal diameters should be measured and recorded. Erythema can be measured as reliably as wheal reactions and is the sole criterion for bioequivalency tests in the United States.[59,124] Any reaction larger than the negative control may indicate the presence of specific IgE antibody. Given the greater sensitivity and equivocal reproducibility of intracutaneous testing, however, small positive reactions may not be clinically significant.[173] There are no evidence-based studies on standardized intracutaneous test grading. Eighty-five percent of board-certified allergists recently surveyed reported that they used the criterion of 3 mm above the negative control as a threshold for a positive intracutaneous test result.[174] The criteria for determining the SET titration threshold stipulate a measurement of 4 mm above the negative control.[90]

Clinical relevance

Summary Statement 25. The diagnostic sensitivity of intracutaneous tests is probably greater than prick/puncture tests when testing for penicillin, insect venom, or certain drug class (eg, insulin, heparin, muscle relaxants) hypersensitivity. (C)

Summary Statement 26. The greater sensitivity of titrated intracutaneous tests, especially in the erythema component, is an advantage for determining biologic potency of allergen extracts and biologic allergy units (BAU) as based on intracutaneous erythema assays in sensitive human volunteers. (B)

In general, intracutaneous tests are useful in detecting patients with lower levels of clinical sensitivity when evaluating allergens (both natural and recombinant) of low skin reactive potency (eg, *Hymenoptera*). They have been evaluated and validated in diagnosis of several important IgE-mediated drug reactions, including anaphylactic reactions induced by penicillin, succinylcholine analogs, and cancer chemotherapeutic agents. In the case of penicillin anaphylactic hypersensitivity, intracutaneous testing (after initial prick testing) is a first-line approach. Under the proper test conditions[1] (use of both major and minor penicillin determinants), these tests were found to have a negative predictive value of almost 99% in a large, multicentered clinical trial.[153] Recent reports suggested that intracutaneous tests might also be useful adjuncts for the diagnosis of nonimmediate allergic reactions to aminopenicillins.[175] The diagnostic accuracy of intracutaneous tests for predicting anaphylaxis associated with cephalosporins and other non–β-lactam antibiotics is limited because standardized reagents are not available for most of these antibiotics.[176] Intracutaneous tests are used mostly as a complement to prick/puncture tests in the evaluation of anaphylaxis to muscle relaxants.[163,166] Test concentrations between 10 and 1,000 μg/mL (wt/vol) have shown 97% concordance with prick/puncture tests.[177] Although systemic IgE-mediated reactions are much less frequent with

commonly used biologics (eg, protamine, human insulin, heparin), a number of case reports suggest that they may be useful for confirming the immunologic nature of these reactions.[166,178–180] Venom intracutaneous skin testing is the most useful in vivo immunologic procedure for confirming immediate hypersensitivity to venoms.[181]

The greater sensitivity of intracutaneous tests offers an advantage for determination of biologic potency of allergenic extracts and their respective recombinant allergens.[75,182] Diagnostic markers for ABPA were identified by intracutaneous testing of a panel of recombinant antigens derived from *Aspergillus* fumigatus.[182] Variability among commercial venom extracts may also be evaluated by intracutaneous testing.[183]

Dose response assays of erythema in response to intracutaneous testing in sensitive human volunteers are the basis of BAU in the United States.[124] In Europe, it has been suggested that bioequivalency could be based on prick/puncture wheals[125]

Sensitivity, specificity, and positive and negative predictive indices

Summary Statement 27. At dilutions between 10^{-2} and 10^{-3} (wt/vol), intracutaneous tests for most allergens exhibit poor efficiency in predicting organ challenge responses and correlating with the presence of detectable serum specific IgE. (C)

Summary Statement 28. There are limited data about equivalency of sensitivity, specificity, and predictive indices between intracutaneous and prick/puncture tests when compared with organ challenge tests. One study demonstrated that more dilute intracutaneous concentrations were comparable to prick/puncture tests in predicting positive nasal challenges. (C)

Summary Statement 29. Similar comparative equivalency studies based on history and symptoms alone revealed that intracutaneous tests were comparable to prick/puncture tests only at intracutaneous titration end points between 10^{-5} and 10^{-6} g/mL (wt/vol). (B)

Summary Statement 30. Because clinical use of intracutaneous tests is usually restricted to a single dose (ie, 1:1,000 wt/vol), which may be irritant, predictive accuracy of these tests at this concentration is often confounded by false-positive results. (C)

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 example, a recent investigation of potential clinical mold allergy could not distinguish between atopic and nonatopic phenotypes in patients being tested to molds at this dilution.[171] However, a study comparing intracutaneous titration end point with prick testing showed a modest correlation for a panel of 8 allergens.[106] Although these data suggest that intracutaneous end point titration might achieve sensitivity and specificity values equivalent to the prick test, there is only one head-to-head comparison of the 2 methods with clinical history and/or provocation testing.[91] By comparing intracutaneous end point titration, skin prick/puncture tests, and nasal provocation determined by acoustic rhinometry, this investigation revealed that skin prick/puncture tests were more sensitive (85.3% vs 79.4%) and more specific (78.6% vs 67.9%) than intracutaneous end point titration as a screening procedure. The positive and negative predictive values of intracutaneous end point titration were 75% and 73%, respectively.[91] Another study comparing intracutaneous tests to skin prick/puncture tests at 30 and 3,000 biologic units/mL, respectively, found positive predictive values of 87.1% and 79.1% for intracutaneous and prick/puncture tests, respectively.[170] The same investigators also established optimum intracutaneous and prick/puncture cutoff values of 0.7 and 0.4 HEP equivalents, respectively.[184] Compared with clinical history, the positive predictive value for detection of allergic sensitization was 77% for intracutaneous tests and 86% for prick/puncture tests.[184] End point intracutaneous titrations to 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.[185] At intracutaneous titration end points between 10^{-6} and 10^{-8} g/mL (wt/vol), 70% of the patients had a positive history and approximately 50% of the patients had a positive RAST result. At intracutaneous titration end points between 10^{-5} and 10^{-3} g/mL (wt/vol), only 60% of patients gave positive histories and 15% exhibited specific IgE.[185] This study indicated that more dilute end point threshold levels of intracutaneous tests could approach the diagnostic accuracy of prick/puncture tests. In a more recent investigation using recombinant birch pollen Bet v 1 as the allergen, the endpoint intracutaneous titration method correlated modestly with basophil histamine release but not with specific serum IgE.[186] Thus, in this study, the biologic sensitivity of the intracutaneous end point titration threshold appeared to outperform both basophil histamine release and serum specific IgE.

Limitations

Summary Statement 31. For most allergens, a fixed dilution (1:1,000 [wt/vol]) of intracutaneous tests has poor efficiency in predicting organ challenge responses. (A)

Summary Statement 32. Intracutaneous tests are occasionally negative in venom-sensitive patients who experience life-threatening reactions. (C)

Summary Statement 33. Repetitive ($\geq$2) intracutaneous penicillin testing may sensitize a small number of individuals to penicillin. (C)

The chief problem with intracutaneous tests performed at a fixed dilution (1:1,000 [wt/vol]) for most allergens is relatively poor efficiency in predicting organ challenge responses, the most reliable predictors of clinical sensitivity. In a recent study that specifically evaluated this relationship, this limitation appeared to apply to most of the common indoor and outdoor allergens.[171] Similar findings were reported in an exposed population being evaluated for mold-

related health effects.[187] In venom allergy, intracutaneous skin test results are occasionally negative, even in patients with near life-threatening reactions to the specific venom.[188] A deliberate sting challenge under controlled conditions should be considered in such an unusual circumstance.

There is some evidence that anaphylactoid reactions to venom occur in a substantial number of patients with mastocytosis or urticaria pigmentosa having relatively high constitutive levels of serum tryptase.[189,190] Also, as discussed under Summary Statement 17, skin test results to inhalants and foods may rarely be negative despite positive end organ challenge test results. Repetitive ($\geq$2) intracutaneous penicillin testing may sensitize a small number of individuals to penicillin.[191] Six of 239 (2.5%) volunteers who were skin tested to penicillin on 2 occasions converted to a positive skin test result. Intracutaneous tests often do not correlate well with serum specific IgE levels. One possible explanation for this disparity was a recent study in which binding of allergen specific IgE antibodies to the α chain of FcεI receptor was suboptimal and did not correlate with either intracutaneous tests or specific basophil sensitivity.[186,192]

Safety

Summary Statement 34. Immediate systemic reactions are more common with intracutaneous tests; 6 fatalities were reported in a recent retrospective survey. (C)

Summary Statement 35. Prescreening with prick/puncture tests is a practical way to avoid life-threatening reactions to intracutaneous tests. (C)

Summary Statement 36. If prick/puncture prescreening is not used, preliminary serial threshold titrations should be considered, starting at high dilutions (10^{-5} to 10^{-8} g/mL [wt/vol]). This is of particular importance if exquisite sensitivity (eg, anaphylaxis to foods and drugs) is suspected. (D)

Although adverse events occurring after intracutaneous tests are rare, they can occur.[193,194] Large local reactions, both immediate and late, may cause discomfort and occasionally mild, nonprogressive systemic reactions may be associated with the latter. Immediate systemic reactions are more common with intracutaneous tests because larger volumes are injected. Six fatalities attributed to intracutaneous skin tests were reported by the Committee on Allergen Standardization of the AAAAI.[195] Five of these patients had asthma and were tested without preceding prick/puncture tests. No fatalities were associated with intracutaneous testing in the most recent 12-year survey of fatal reactions from 1990 to 2001.[147]

To reduce the likelihood of adverse reactions during skin testing, several precautions may be taken. Prescreening with prick/puncture test is a practical way to avert an untoward number of adverse local and/or systemic responses in routine skin testing of patients. If prick/puncture tests are not performed routinely, preliminary threshold intracutaneous testing should be considered, beginning at higher dilutions (ie, 10^{-5} to 10^{-8} g/mL [wt/vol]). Even greater precautions should be observed if patients are suspected of having exquisite sensitivity, such as anaphylaxis, to certain foods and drugs. In such cases, even prick/puncture tests should be initiated with several serial 10-fold dilutions of the usual test concentration. Patients receiving β-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 β-blockers, and epinephrine may adversely affect patients taking monoamine oxidase inhibitors.[59]

Late-Phase Cutaneous Reactions

Definition and description

Summary Statement 37. The late-phase cutaneous response is a continuation of either prick/puncture or intracutaneous testing, generally the latter, and is characterized by erythema, induration or edema, and dysesthesia. (B)

The late-phase cutaneous reaction develops progressively at sites of immediate wheal-and-flare reactions and is characterized by erythema, induration or edema, and dysesthesia.[196–201] Histopathologically, it is characterized by the presence of edema, mixed cellular infiltrates, and sometimes fibrin deposition scattered throughout the dermis without the deposition of complement, IgG, IgA, IgM, or vascular damage. Less frequently, the late-phase cutaneous response may occur in the absence of an immediate skin test response and may be confused with cell-mediated, delayed hypersensitivity.[202,203] Isolated late cutaneous reactions were observed in approximately 36% of children undergoing skin tests for suspected allergies. Most of these isolated late-phase cutaneous responses were due to inhalant allergens, such as cockroach and various mold spores.[204] The clinical significance of this is as yet unknown.

Causes

Summary Statement 38. The late-phase cutaneous response may occur after both immune and nonimmune activation. Many allergens have been implicated. (B)

Late-phase cutaneous reactions occur after both immune and nonimmune (eg, 48/80, kallikrein) mast cell activation. Agents stimulating immunologic activation of the mast cells that have induced the late-phase cutaneous response include anti-IgE antibodies and the following allergens or antigens: aeroallergens (molds, pollens, danders, mites, and enzymes), penicillin, heparin, insulin, and possibly some foods.[205,206] The propensity to develop the late-phase cutaneous response may be dependent on the type of antigen, host sensitivity, and the concentration of injected antigen or allergen.[207]

Reading the test results

Summary Statement 39. The late-phase cutaneous response should be read between the 6th and 12th hours after the skin tests are applied; measurements of mean diameter and/or area of induration or edema should be recorded. (B)

After challenge with diverse stimuli causing immediate wheal-and-flare responses, the intensity of the late-phase cutaneous response increases rapidly (doubling or tripling in size) during the first 2 hours.[198–201,208] The response plateaus between the 6th and 12th hours, is present at 24 hours, and

usually disappears by 48 hours after challenge. Accordingly, these reactions should be quantified between the 6th and 12th hours (most commonly at the 6th or 8th hour) by measurements of mean diameter and/or area of induration or edema.

Although the minimum size of induration or erythema of the late-phase cutaneous response has not yet been standardized, the extent of these measurements should be compared directly with previously applied diluent or histamine sites, which typically demonstrate neither induration nor erythema 6 to 8 hours later.[197,207] One investigator suggests a minimum of 5 mm of induration and/or erythema be considered.[204] The late-phase cutaneous response is in part mediated by antigen-specific major histocompatibility complex restricted T cells, which in the past were thought to be prototypic of tuberculin-induced delayed-type hypersensitivity. However, it has been demonstrated that both characteristic histologic features and the occurrence of isolated late-phase cutaneous response after immunization with T-cell–specific small overlapping allergenic (eg, from Fel d 1) peptides can distinguish between a late-phase cutaneous response and delayed-type hypersensitivity.[202,209] Immunochemical histologic analysis at various stages of the inflammatory milieu of a late-phase cutaneous response reveals a diversity of cells, including macrophages, eosinophils, neutrophils, tryptase positive mast cells, Langerhans cells, and, interestingly, large numbers of basophils.[210,211] T cells are also present and the late-phase cutaneous response is thought to be partially dependent on them, possibly through effects of cytokines, particularly IL-4, IL-5, and IL-10.[210,212–214] Also noteworthy is up-regulation of the CCR3L (eotaxin) and CCR4L chemokines in T cells (skin, lung, and blood) after allergen-induced late-phase cutaneous response.[215,216] Not surprisingly, a variety of other mediators and proinflammatory cytokines have also been described in association with the late-phase cutaneous response.[217–219]

Clinical relevance

Summary Statement 40. Although the clinical relevance of late-phase cutaneous response is not as yet fully established, several randomized, controlled studies suggest that reduction in sizes of late-phase cutaneous response may parallel clinical response to immunotherapy. (B)

Although the clinical relevance of late-phase cutaneous response cannot yet be delineated with certainty, there has been preliminary progress about some potential clinical applications. At least 4 randomized, controlled clinical trials of immunotherapy in patients with allergic rhinitis have shown marked reductions in late-phase cutaneous response in patients who experience successful reduction of clinical symptoms.[212,220–222] Furthermore, reduction in size of the late-phase cutaneous response was also associated with recruitment of $CD4^+$ $CD25^+$ regulatory T cells and $CD4^+$ interferon-γ^+ T_H1 cells to sites of allergen-induced late-phase cutaneous response in cat-allergic subjects.[223] It has been suggested that patients with atopic dermatitis may be classified phenotypically into either positive or negative late-phase cutaneous response reactors.[208] Atopic dermatitis patients with significant late-phase cutaneous response reactions were more likely to demonstrate higher levels of IL-5 and specific IgE to house dust mite antigens.[214,224] Of related interest was a study in which birch pollen–sensitive patients with atopic dermatitis and isolated late eczematous reactions to birch pollen–related foods demonstrated up-regulation of specific T cells in biopsy specimens of delayed skin lesions.[225] Recently, there have been numerous anecdotal case reports that suggest delayed-type intracutaneous tests are useful for the diagnosis of various drug allergies, including nonimmediate allergic reactions to muscle relaxants, penicillins, non–β-lactam antibiotics, antiepileptics, and heparins.[177,226–230] However, many of these delayed intracutaneous tests were not interpreted within the 6- to 12-hour range of the late-phase cutaneous reaction, so it is not clear whether such testing represents cell-mediated hypersensitivity reactions or variants of the late-phase cutaneous response. Further research is needed to clarify this issue.

Sensitivity, specificity, and positive and negative predictive indices

None of these indices are available for late-phase cutaneous response because there are too few clinical trials to provide a practical basis for determining sensitivity, specificity, predictability, or likelihood ratios.

Safety

Summary Statement 41. The same principles that pertain to safety of skin tests apply to late-phase cutaneous responses. (C)

The same principles that pertain to safety of prick and intracutaneous tests used to detect immediate hypersensitivity apply to late-phase cutaneous responses. Possible severe immediate reactions would only occur during the initial immediate phase and not during the late-phase cutaneous response–evolving reaction in the 6- to 12-hour period after application of the test. However, systemic reactions that occur during the reading period of intracutaneous testing could possibly persist or worsen and present a clinical problem if the mediator release was intense enough. This could occur at the same time as the late-phase cutaneous response might be expected to peak. In both safety surveys previously discussed, no evidence of life-threatening events or fatalities to late-phase cutaneous responses has been reported. Antihistamines may offer symptomatic relief for persistent erythema and pruritus, presumably due to histamine newly released from previously unstimulated mast cells recruited to the lesion.

Inhibitors of the late-phase cutaneous response

Summary Statement 42. Preadministration of drugs, such as calcineurin inhibitors, misoprostol, prednisone, and azelastine, before application of skin tests partially or completely inhibit the late-phase cutaneous response. (B)

Preadministration of calcineurin inhibitors and misoprostol results in complete inhibition of late-phase cutaneous response, whereas prednisone and azelastine are partial inhib-

itors.[231–234] However, none of these agents has been proven to be inhibitory once late-phase cutaneous response is fully established.

Number of Skin Tests

Summary Statement 43. 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 (eg, region of the country), 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)

Although recommending a standard panel of skin tests that would encompass all possible clinical situations in North America may prove to be unattainable, expert consensus panels have ventured opinions with the expectation that relative consistency of skin testing, including number of tests, is a desirable goal for both clinical practice and research.[235,236] These opinions are based on current principles regarding constitutive allergenicity, cross-allergenicity, aerobiologic monitoring, and correlation with organ challenge testing or supervised natural exposure (ie, a park study or environmental exposure unit). Wherever possible, evidence-based sources should be used to determine whether specific allergen tests based on pretest probability are likely to confirm a suspected clinical diagnosis.

Special clinical situations and exposures must be considered in selecting skin test reagents. Prick/puncture or intracutaneous skin tests are important for diagnosis of inhalant allergy. Some clinicians prefer to initially screen with prick/puncture followed by intracutaneous tests if the results of the former are negative, whereas others exclusively use intracutaneous tests. Initial prick/puncture screening followed by end point intracutaneous serial titration is an accepted regimen for evaluation of *Hymenoptera* and several clinical drug sensitivities. Only prick/puncture tests should be performed to define food sensitivity. Each of these situations involves special approaches, which will be addressed in the following discussion.

Restricted allergen panels may be adequate for epidemiologic cross-sectional or prospective population studies.[236,237] Similarly, baseline atopic phenotype, as determined by selected skin test allergen panels, is a necessary prerequisite for evaluation of genetic or environmental interactions. For individual patient evaluations, a larger number of skin tests is usually necessary in the rational planning of avoidance measures and immunotherapy if that should be required.

Although recognizing that the history may be a relatively insensitive predictor of clinical sensitivity in some situations, certain historical features serve as important pretest probability guides to the numerical extent of skin tests. Generally, fewer prick/puncture tests need to be performed in infants and very young children (<2 years of age) because these children are not likely to be sensitized to as many allergens as older children and adults. In toddlers, sensitization is more apt to reflect intense and prolonged exposure to allergens encountered earliest in life, such as foods, house dust mites, indoor molds, and animal danders rather than pollen.

If inhalation allergy is narrowly confined to a single season (eg, ragweed in North America or birch in European northern countries), a limited number of relevant skin tests would suffice for confirmation of the clinical diagnosis and testing to irrelevant inhalant and food allergens would be inappropriate. By contrast, perennial symptoms would require a more extended skin test panel of both indigenous outdoor and indoor inhalants but not foods unless a history of food allergy happened to be a concurrent problem of the patient.

Occupationally related clinical allergy (eg, latex, food inhalants, chemicals) is a special circumstance for which limited skin test reagents would be satisfactory. Similarly, skin tests for a few drugs that cause anaphylaxis (eg, penicillin, succinylcholine analogs) reliably predict life-threatening anaphylactic reactions. A history of anaphylactic reactions to insect venom stings requires skin test confirmation. There are 6 commercially available skin test preparations for stinging and biting insects (eg, honey bee, wasp, yellow jacket, yellow faced hornet, white faced hornet, and imported fire ant).

The most controversial aspect of defining a standardized skin test panel relates to inhalant allergens. Of these, 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. Pollens may also be found indoors when windows are kept open.[238] The geographic variability of airborne-pollinating plants throughout the floristic zones of the world, particularly in North America, raises a cogent 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. First and foremost, all 5 of the postulates regarding clinically significant pollen allergens originally proposed by Thommen should be satisfied: (1) constitutive allergenicity of the pollen, as determined by symptoms occurring during its exposure in addition to the presence of a positive skin test result; (2) the pollen is anemophilous; (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.[239] Thus, pollen of water and insect pollinated plants are automatically eliminated from further consideration. This applies to such plants as goldenrod, daisy, sunflower, dahlia, and rhododendron. Although pine pollen satisfies postulates 2 to 5, it is not a clinically important allergen because its constitutive allergenicity is weak. A cardinal principle of Thommen's postulates is that skin test reactivity alone does not define clinical sensitivity. Skin test positivity must be combined with observable symptoms, increased symptom scores or physical signs during a known pollen season, controlled laboratory, or environmental exposure unit challenges.[240,241] Pollen quantitative sampling

by Burkard traps, Hirst traps, Rotorods, or personal sampling must be sufficiently high to fulfill the other Thommen postulates. Annual pollen sampling data in various regions of the country are available at the National Allergy Bureau web site (www.aaaai.org).

Aerobiologic variability also affects pollen distribution. Masting, the simultaneous production of large numbers of pollens by a plant population, is a common feature among trees in temperate forests.[242] Allergenicity is enhanced with higher daily mean temperatures.[243] Successive wetting and drying cycles release not only pollen but also very small cytoplasmic fragments (30 nm to 4 μm) that retain allergenic activity.[244,245] The latter are detected by immunostaining of personal cascade impactors.[244–246] Pollen distribution also varies with altitude.[247,248]

The difference between cosensitization and cross-sensitization is often misunderstood in the selection of relevant skin test and extract reagents. The Allergome database revealed that pollen allergens can be classified into 29 of 7,868 protein families.[249] Panallergens such as profilin, polcalcin, 1,3-β-gluconase, and cross-reactive carbohydrate determinants account for extensive cross-reactivity among pollen-sensitized patients.[249–254] Thus, the relevant allergen profile of ash shares epitopes with pollen allergens not only from other tree pollens but also from grass and weed pollen species.[255] Similarly, pollen allergy to white birch, a member of the order Fagales, can be found in birch-free geographic areas that have other non–birch tree species of the Fagales order.[256] This is particularly germane to the decision about number of tests because it demonstrates clearly that skin test reactivity alone cannot decide the clinical significance of an allergen. A high percentage of reactions to the ornamental black locust in pollinosis patients is ascribed to cross-sensitization to panallergens in other common pollens.[257] This is termed an *allergy mirage*.[257] Cross-sensitivity to pollen profilins has been demonstrated in $CD4^+$ T_H2 clones, which promiscuously recognize homologously conserved regions on birch and grass profilins.[258] This may be in part due to conserved allergen-specific motifs.[259] Cross-sensitization to profilin and/or bromelain-type cross-reactive carbohydrate determinants caused by timothy grass or mugwort pollen has also been reported in venom sensitization.[130,260] These recent demonstrations of extensive cross-sensitivity among all pollens must be critically reviewed either when selecting a skin test panel or when interpreting the results.

The reported prevalence of outdoor airborne fungi depends on sampling technique (viable vs nonviable; bioaerosol vs surface) and the collecting device.[261–266] Viable cultures also vary depending on media and duration of culture. In general, *Cladosporium* and *Alternaria* species are predominant in the summer months. Indoor mold sampling almost always detects species of *Aspergillus* and *Penicillium*.[267,268] Recent molecular cloning of airborne fungal spores can also identify many *Basidiomycetes* and *Ascomycetes*.[269] Although many other species have been identified, their comparative significance is difficult to ascertain. Cross-allergenicity among major classes of airborne fungi has not been well delineated.

These facts about cross-allergenicity are particularly germane to formulation of treatment extracts for a particular floristic region, which should reflect the validity of aerobiologic sampling, the constitutive allergenicity of pollens, as evaluated by direct skin, exposure, and/or challenge tests, and how well positive test results correlate with the patient's clinical symptoms. Other factors that may need to be incorporated into the final formulation decision include unexpected allergen exposure because of frequent travel to other floristic zones and commercial availability of appropriate allergen extracts.

Food prick and puncture skin tests are excellent diagnostic modalities for the diagnosis of IgE-mediated clinical entities, which include anaphylaxis, food-dependent exercise-induced anaphylaxis, acute urticaria, atopic dermatitis, and the oral allergy syndrome. The last is often associated with cross-sensitivity to panallergens in pollens.[270] In many instances, the history suggests appropriate allergen testing; in other situations, a preliminary diet history and diaries provide additional clues. At times, reconstruction of a suspected etiologic meal may direct suspicion to specific food components in that meal. Relatively few foods account for most IgE-mediated allergic reactions in both children and adults. The more common food allergens in infants and young children are cow's milk, hen's egg, peanuts, tree nuts, soybeans, and wheat, whereas the adult counterparts are peanuts, tree nuts, fish, crustaceans, mollusks, fruits, and vegetables. Commercial fruit and vegetable extracts rapidly lose potency so many clinicians either prepare fresh extracts of these classes of foods or test by the prick-prick method. This method is preferred to detect strain differences in fruit allergens (eg, apple).[126] Because of many false-positive test results and potential risks, intracutaneous tests to foods are not recommended. Food tests are inappropriate for investigation of chronic idiopathic urticaria (CIU) or angioedema.

The scope and number of skin tests for allergy diagnosis reflect the clinician's scientific knowledge and clinical experience. The choice and number of test allergens should be continuously refined in accord with scientific advances, botanic and aerobiologic surveys, demographic trends, and availability of relevant, defined reagents. Practice must be directed to the best documented concepts of allergen prevalence, geographic distribution, and immunochemical relationships.

Although no prospective studies provide direct evidence for these issues, the literature concerning clinically relevant allergens suggests a rationale and evidence-based process for determining the number of skin test reagents. This issue had received serious consideration by the Joint Task Force on Practice Parameters in conjunction with expert consultants during the preparation of Allergen Immunotherapy: A Practice Parameter, at which time the Joint Task Force suggested a core panel of indoor and outdoor inhalant allergens. This list includes representative species of the major classes of

trees, grasses, and weeds, commonly sampled species of the *Deuteromycetes* fungal class, and a group of well-recognized indoor allergens.

After full consideration of the previously discussed variables and confounders that may affect clinical sensitization to inhalants, the Joint Task Force on Practice Parameters concludes the number of skin tests (eg, ≤70 prick/puncture and 40 intracutaneous tests) for inhalant allergens, as published in the Practice Parameters on Allergy Diagnostic Testing in 1995, is justified as an initial diagnostic evaluation. However, routine annual tests without a definite clinical indication are clearly not indicated.

Based on recent extensive food allergy research reviewed in Food Allergy: A Practice Parameter, relatively few foods are responsible for most clinical food allergy suggested by the patient's history and pretest probability. However, this generality does not exclude the possibility that larger numbers of tests may be required in certain disease states in which multiple or hidden food allergy is possible (eg, eosinophilic esophagitis; anaphylactic reactions after a restaurant meal; food-dependent, exercise-induced anaphylaxis) or for evaluating the potential that allergy to additional allergenic foods may exist or occur once a diagnosis of food allergy is confirmed to be likely. Tests for venom and drug sensitivities are not included in this calculation because these tests are performed only in patients with a strongly suggestive history of anaphylaxis and not routinely in patients who present with inhalant or food allergy.

Exceptions to these recommendations may occur based on causal factors suggested by the patient's history. Additional test allergens may be required for exposures to occupational allergens, in patients with unusual hobbies or personal contact with less common pets (eg, rodents) or livestock. In some cases, the history may be misleading. For example, some patients with predominantly seasonal symptoms and an indefinite history after exposure to house dust may exhibit positive skin test results to house dust mites, subsequent avoidance of which may decrease both seasonal and nonseasonal symptoms.

From time to time, patients may present with symptoms caused by previously unidentified substances that potentially are new allergens. There is a role for testing such patients with properly prepared extracts of a new allergen. There is insufficient evidence, however, to justify tests for nonproven agents, such as newsprint, sugar, cornstarch, orris root, tobacco smoke, cotton, formaldehyde, and smog.

If a patient presents with idiopathic anaphylaxis, up to 30 screening prick/puncture tests have been reported to identify causal foods in a small percentage of such patients.[271] A subsequent overview of this study questioned whether the diagnostic yield of such a strategy was worthwhile.[272] Nevertheless, in the diagnostic evaluation of suspected anaphylaxis, it would be prudent to distribute the total number of screening tests over several clinic visits to avoid the possibility of severe anaphylaxis if multiple reactions occurred.

Apart from the exceptions noted herein, the consensus of the Joint Task Force is that it is rarely necessary to exceed the number of tests cited in the previously published statement.

ORGAN CHALLENGE TESTS

Introduction

Summary Statement 44. Respiratory challenge tests are used when an objective gold standard for establishing clinical sensitivity is indicated. (B)

Historically, provocation challenge tests with inhalant allergens have been used to clarify the role of allergens in specific organs. They may occasionally facilitate or confirm the diagnosis of clinical sensitivity when the history is suggestive but skin and/or specific IgE test results are negative.[133–138,145,185,188] If negative, a nonallergic trigger is likely. They also are used to evaluate response to therapy, either pharmacologic or immunologic.[273] In general, these tests require cooperative patients with respect to both age and mental status. The site of specific organ challenge is history dependent (ie, conjunctival, nasal, bronchial, or skin) (eg, patch tests for ACD; supervised insect stings).[189,274,275] These tests are often the tools of research protocols that require an objective gold standard for establishing clinical sensitivity. They are often required to substantiate clinical sensitivity of occupationally induced diseases after cutaneous and respiratory exposure to proven and possible new workplace allergens.[276] Since occupational exposures may occur via fluids, aerosols, vapors, or dust, special exposure apparatuses for such tests are necessary and may only be available in tertiary medical centers. New techniques for assessing local and systemic inflammatory biomarkers are emerging as useful clinical diagnostic adjuncts for both immediate and delayed hypersensitivity diseases. In this regard, components of external secretions (ie, tears, nasal lavage, induced sputum, BAL), exhaled nitric oxide, and breath condensates are currently being used independently or in conjunction with challenge regimens.

Conjunctival Challenge

Summary Statement 45. Conjunctival challenge tests are usually conducted for suspected localized eye allergy but in some cases they may also be helpful in investigating nasal allergy. (B)

Summary Statement 46. Conjunctival challenge tests are evaluated by symptoms of itching and objective indices, including tear volume, amount of mucus, and palpebral or bulbar erythema. (B)

Although conjunctival tests are used primarily for suspected localized eye allergy, some clinicians also consider them to be confirmatory for nasal allergy.[274,277,278] The inferior conjunctival fornix is a point where the inferior palpebral and bulbar conjunctivae meet and is the most convenient area to apply either dry or fluid challenge allergens.[279,280] Dry materials may be placed directly with an applicator (eg, toothpick), whereas fluid materials may be applied with an eye dropper, with a pipette, or through a high-water-content con-

tact lens. For solutions, a starting concentration is usually 3- to 4-log fold less than prick/puncture allergen concentrations (ie, 1:10 [wt/vol]). If test results are negative at these dilutions, serial log fold increasing concentrations are tested up to a final concentration of 1:1,000 (wt/vol). Before beginning the challenge, placebo tests (isotonic sodium chloride solution or an inert, nonirritant dust particle) are applied to the opposite eye. Subjective and objective responses can be measured before and 5, 10, and 15 minutes after the challenge. Subjective symptom scores for erythema, edema, and sensation may be obtained for the subject. These 3 features are usually graded on a scale of 0 to 4. Sensation (usually itching) is usually the first to occur followed by erythema and edema within 10 minutes of the challenge. Recording the duration of itching may add more objectivity to this measurement.[281] Quantitative measures include tear volume, amount of mucus, palpebral and/or bulbar conjunctival hyperemia or erythema, edema, or surface sensation of itching. Objective conjunctival changes can be examined with slit-lamp magnification. A more precise technique is spectroradiometry, which uses the chromaticity of light reflected from the conjunctivae to quantify erythema.[281] Edema can be measured with the fractional millimeter reticule of the slit-lamp microscope. Measurements are made of the lower lid and bulbar conjunctivae.[281] Tears and secretions can be further evaluated according to composition and cytology (ie, inflammatory cells, mediators, cytokines, specific IgE antibodies).[282,283]

Nasal Challenge

Summary Statement 47. Nasal challenges provide objective evidence of clinical sensitivity when the diagnosis is in question or in situations when it is desirable to evaluate efficacy of therapeutic management. (B)

Summary Statement 48. Nasal challenge responses are evaluated by subjective symptoms and objective measurements of nasal airway resistance, the number of sneezes, and the measurement of inflammatory mediators in nasal secretions. (B)

Blackley first reported the effects of applying allergen in the nose as a diagnostic and research tool.[1] In clinical practice today, nasal challenge testing is infrequent but may be used as an objective test of clinical sensitivity when the diagnosis is in question or to evaluate efficacy of therapeutic management.[90,185,133–138] Clinical investigators consider it to be an especially valuable technique of evaluating new therapeutic agents.[284,285]

Almost any allergen may be used for a nasal challenge. Over the years, many procedures for delivering allergen have been used. The allergen can be applied as a dry or fluid preparation. Dry grains of pollen and other allergens have been placed or inhaled directly in the nasal mucosa but can be difficult to distribute evenly and prevent inhalation into the lower airways.[286] Allergen extracts can be directly applied to the nasal mucosa with paper disks, pipettes, syringes, or spraying with an atomizer. The particles should be large enough to permit trapping in the nose because fine particles may tend to go beyond the nasal passages into the lower airways and produce undesirable effects.[287,288] Paper disks soaked with fluid appear to provide the most localized delivery and avoid the spread of fluid droplets to other areas, especially the lower airways.[279] Dose responses using nasal solutions are similar to those described for conjunctional tests. Fluid allergen preparations can also be sprayed intranasally by aerosol. Spraying aerosol particles (0.1 to 0.4 mL) with an atomizer reaches a wider area of the nasal passages and has been referred to as a whole-nose challenge.[280] The diluent often used is 0.9% saline. For research purposes, pollen exposure simulating natural exposures has been conducted in large exposure chambers or rooms.[281]

Procedure

Nasal challenges should be conducted in a quiet room with temperature and humidity being recorded. The subject should be allowed to accommodate to the environment for at least 30 minutes before testing is started. Inasmuch as nasal congestion is the primary response, a baseline measurement of nasal airway resistance is first performed (eg, anterior [ie, inspiratory and expiratory nasal PEFR], posterior, or acoustic rhinometry).[286,288–300] This is followed by a control challenge most often with the saline diluent. If the nasal airway resistance increases by more than 30% from baseline, the testing is deferred. Otherwise, testing continues with increasing concentrations of the allergen challenge material and measurement of nasal airway resistance or ancillary tests at regular intervals (eg, every 1 minute for the first 5 minutes, every 2 minutes for the next 10 to 15 minutes, every 5 minutes if testing is continued beyond 15 minutes).[287,288,294–297]

Supplementary measurements

Subjective responses may be obtained by symptom scores or visual analog scales.[298,299] Objective ancillary measures include counting of sneezes and measurement of secretions. The volume of secretions can be measured by collecting all secretions within a specified period by suction, lavage, handkerchiefs, filter paper, or simply gravity drainage from the subject's nose into a container.[290,300] Nasal secretions, as well as specimens obtained by lavage, biopsies, and brushings after a challenge can be studied and analyzed for inflammatory mediators, cytokines, cells, and other components.[301–304] Nasal specific IgE may suggest local production of IgE antibody.[301]

Specific Bronchial Challenge

Summary Statement 49. Specific (allergic) bronchial challenge provides a measure of lower airway clinical sensitivity when there is uncertainty or dispute. (B)

Summary Statement 50. Guidelines for the performance of specific bronchial challenge include factors such as withholding certain medications before the test, determining the initial allergen dose by preliminary skin or methacholine challenge testing, a beginning forced expiratory volume in 1 second (FEV_1) baseline of 70% or better, the amount or duration of exposure to allergen, measurement of FEV_1 at intervals after

the exposure, careful observation for late-phase responses, comparison to a placebo-controlled challenge usually performed the day before the specific challenge, and, optionally, repetition of methacholine challenge 24 to 48 hours after specific challenge for evaluation of induced bronchial hyperresponsiveness. (B)

General considerations

In general, specific bronchial challenge testing is most often performed for research or when there is diagnostic uncertainty or dispute.[305] Additionally, possible new asthma triggers can be investigated and confirmed with specific bronchial challenge. Before implementation of a specific bronchial challenge, many centers elect to determine the degree of nonspecific bronchial hyperresponsiveness as a guide to allergen dosage and duration of allergen challenge. This is usually scheduled 1 day before specific bronchial challenge. After baseline and control FEV_1 tests are measured, dose increments of methacholine or histamine are inhaled every 10 minutes and followed by FEV_1 tests. The end point PC of either methacholine or histamine is extrapolated from the respective dose response curves at the point where the FEV_1 decreases 20% from the control (saline) test, and these are designated as $PC_{20\ METH}$ or $PC_{20\ HIST}$. Bronchial hyperresponsiveness is defined as $PC_{20\ METH}$ of 10 mg/mL or less or $PC_{20\ HIST}$ of 8 mg/mL or less. The precautions and preparations recommended for specific new challenges are identical to those for nonspecific testing (eg, methacholine, histamine).[273,306] Use of short-acting β_2-agonists should be stopped 8 hours before the challenge, whereas long-acting β_2-agonists, leukotriene antagonists, and sustained-release theophylline should be withheld 48 hours before the test. Use of inhaled cromolyn and steroids is preferably discontinued 1 month before the challenge if the purpose is to identify or confirm a specific allergenic trigger. Antihistamines should be withheld for at least 72 hours. Systemic steroids inhibit mainly the late response and should be withheld for at least 24 to 48 hours if the presence of a late response needs to be observed. If medications cannot be withheld without worsening of symptoms and maintaining the FEV_1 at 70% or more of the normal predicted value, the test should be postponed because symptomatic, unstable asthma may lead to false-positive results. Ideally, if testing is being performed for occupational allergens, the subject should have been away from work for at least a week or until the asthma disappears. Specific bronchial challenge should be performed only in a medically supervised setting, usually a hospital or research facility, with resuscitation equipment readily available in the event of life-threatening anaphylactic or asthmatic reactions.[307]

In the case of natural allergens (eg, pollen, molds, house dust mite), there are no clear guidelines for the initial concentration of allergen or exposure time to be used for testing.[307,308] Standardized (AU or BAU) or conventional (wt/vol or protein nitrogen units [PNU]) allergenic extracts can be used. A prior intracutaneous skin test SET may be performed to estimate the initial challenge dose. In general, the initial concentration can be 10 to 100-fold more concentrated than the concentration that produced a 2+ reaction with a wheal greater than 5 mm (eg, an initial concentration of 0.05 μg/mL if the SET was 0.0005 μg/mL).[45] Exposure to ambient and particulate allergens (eg, epidermals, pollens) is a more difficult procedure because the combined logistics of locale, ambient measurements and exposure time require special attention. Preliminary nonspecific bronchial challenge results with methacholine or histamine (ie, PC_{20METH} or PC_{20HIST}) may be useful for planning the duration of exposure.[309]

Procedures

Because suspected allergens or agents in the home or workplace have different physical configurations, protocols for exposing a patient during a challenge are variable, and there are currently no standardized or universally accepted protocols for specific bronchial challenge testing. For soluble allergens, aerosolization is the preferred technique. The diluent used in the allergen extract should be used as the control aerosol at the beginning of the specific bronchial challenge. Various types of nebulizers may be used, including the DeVilbiss jet nebulizer, Wright nebulizer, Rosenthal dosimeter, or an ultrasonic nebulizer.[310] The fall in FEV_1 after control exposure, if any, should be less than 10% from the baseline. A greater fall indicates bronchial lability that can affect test results, and further testing should be postponed until the underlying asthma is stabilized.

Since the early airway response usually occurs within 10 to 12 minutes after challenge, the subject is dosed with increasing concentrations (2- to 5-fold) of allergen every 15 to 20 minutes. Pulmonary function tests are best performed 10 to 15 minutes after aerosol challenge.[311] A sustained fall in FEV_1 of 20% or more from the baseline at any time is considered a positive response, and the testing is stopped if this occurs. The results of the challenge can be expressed as PC_{20}, which is derived from a log dose-response curve. Inhaled short-acting β_2-agonists should be given to restore FEV_1 to within 10% of baseline. Since late-phase asthmatic responses may occur, arrangements should be made for peak flow monitoring or direct observation of such reactions, which usually appear 6 to 12 hours later.[312] Several doses of systemic steroids may be required if the FEV_1 does not reverse after inhaled β_2-agonist treatment of the late-phase response. Some clinicians repeat methacholine challenge 24 to 48 hours after specific challenge for evaluation of induced bronchial hyperresponsiveness.

Allergen exposure units

Allergen exposure units, also known as challenge chambers, enable a controlled environment where the delivery of the allergen into the atmosphere can closely approximate natural exposure and where the concentration can be rigorously controlled. Such units range from a simple enclosed space to a specially constructed chamber for precisely monitoring variables such as humidity and temperature. The Vienna chal-

lenge chamber was the first chamber developed for controlled allergen exposure of several subjects at one time.[303] Most challenge chambers are currently located only in academic medical centers and research facilities.[313]

Occupational challenge testing

Summary Statement 51. Occupational challenge testing requires special precautions with respect to the innate toxicity of the suspected allergen and special apparatuses used to measure and control the quantity of challenge substances, such as potentially irritating volatile agents and dust. (B)

The American Conference for Governmental Industrial Hygienists sets the threshold limit value and short-term exposure limit for many occupational agents.[314] Ideally, these limits should not be exceeded in any specific bronchial challenge testing. If possible, the level of the suspected agent is measured in the workplace, and this level is used to guide the dose for testing so that unrealistically high concentrations are not inappropriately used. The duration and concentration in the challenge are determined by the investigator based on the subject's clinical history, airway hyperresponsiveness on prior nonspecific bronchial challenge testing, and nature of the test agent. If the subject has a history of a severe, immediate reaction, exposure should be shorter and more incremental. The lower the PC_{20}, the greater the baseline airway hyperresponsiveness and the greater likelihood of an immediate significant reaction. A shorter or longer starting duration of exposure is used if the PC_{20} is 0.25 mg/mL or less or more than 0.25 mg/mL, respectively.[309,315] High-molecular-weight allergens (eg, animal or vegetable proteins) usually cause immediate reactions with an isolated early or biphasic reaction (early and late) and can often adequately be tested in 1 active challenge day.[316] On the other hand, low-molecular-weight agents (eg, polyisocyanates, plicatic acid) induce non–IgE-mediated isolated late or biphasic reactions, necessitating progressive incremental testing over several days.[317] Many OA laboratories conduct follow-up nonspecific bronchial challenge tests to determine if the challenge test itself has caused an increase in bronchial hyperresponsiveness.[316,317]

In 1989, the AAAAI Subcommittee on Bronchoprovocation for Occupational Asthma released the Guidelines for Bronchoprovocation on the Investigation of Occupational Asthma, which reviewed general principles for specific bronchial challenge testing.[318] The Canadian Thoracic Society has also released guidelines on the diagnosis and management of OA.[317] The nature of workplace exposure should be simulated as closely as possible. Special protocols and closed circuit apparatuses for specific types of agents, including dust and vapor challenges, have been developed in OA research centers.[316,317,319]

Evaluation at and away from work

Summary Statement 52. A practical clinical method of assessing OA is prospective monitoring of the worker at and away from work by serial peak expiratory flow rates (PEFRs) or FEV_1 values if this can be arranged by mutual agreement of employee and employer. (B)

A practical clinical method of assessing OA is prospective monitoring of the worker at and away from work if this can be arranged with mutual agreement of employee and employer. After symptomatic asthma has disappeared during absence from work, the worker returns to his/her job for a period of 1 to 2 weeks. During this time, a symptom log is kept and supervised PEFR tests 4 times a day are obtained. Similar data are collected for 1 to 2 weeks away from work. The PEFR records are plotted serially to determine changes over time. This is accomplished by visual inspection, but a computer-based pattern recognition system having the advantage of complete repeatability is available.[320]

Animal exposure challenges

Animal exposure challenges are used primarily in research settings to determine the efficacy of medication regimens or environmental interventions. Exposure challenges using live cats in enclosed rooms, commonly known as cat rooms, are being used more frequently to evaluate medication efficacy in cat-allergic patients.[321] Although levels of cat (Fel d 1) antigens vary widely, the cat room is still considered a convenient and valid challenge technique that closely approximates natural cat exposure.

Workplace challenge is a direct approach to determine animal allergy in the workplace (eg, laboratory workers whose primary research requires exposure to mice, rats, guinea pigs, and rabbits).[322,323] It is estimated that a third of laboratory animal workers have allergy to animals and a third of allergic workers have asthma.[324] The diagnosis is often made by a suggestive history, positive skin test responses to the relevant allergens, and PEFR monitoring inside and outside the workplace. Although specific challenge testing is rarely necessary in the laboratory, as with other occupational allergens specific bronchial challenge may be useful under special circumstances.

Inflammatory Biomarkers of Upper and Lower Airway Fluids

Summary Statement 53. Many inflammatory correlates can be evaluated and studied serially in respiratory and other body fluids, such as nasal smears or lavage, induced sputum, and BAL. These may define specific phenotypes or in some cases predict severity. (B)

Respiratory fluids (in some cases blood and urine) may reflect the presence of both specific sensitivity and nonspecific inflammatory events. Measurement of inflammatory markers is emerging as a common clinical paradigm. Noninvasive techniques, such as nasal or sputum eosinophils, have been previously discussed. However, improved nasal lavage and induced sputum techniques have substantially expanded our ability to measure various inflammatory indices.[303,304,325–332] For example, the number of $CD4^+$ and $CD8^+$ cells, macrophages bearing IL-10 or IL-12, ECP, myeloperoxidase, and cytokines or chemokines may

identify specific asthma phenotypes or differentiate asthma severity.[326,332] In some instances, inflammatory mediators may be serially assayed in blood and urine.[333,334] Various cell populations and inflammatory proteins have also been identified in BAL.[335–341] Soluble factors can be identified by proteonomic analysis.[342,343]

Summary Statement 54. Exhaled nitric oxide is a noninvasive measure of airway inflammation and is useful for monitoring objective responses to topically administered corticosteroids. (B)

Many recent clinical studies have demonstrated that exhaled nitric oxide is a suitable, noninvasive measure of airway inflammation, particularly in atopic subjects.[338–348] Several reports indicate that it is as good a predictor of asthma as nonspecific bronchoprovocation tests.[344,345,347,349] It is particularly useful for monitoring objective responses to topically administered corticosteroids.[338] A recent longitudinal monitoring study of lung injury and reactive airways dysfunction syndrome after short-term chlorine exposure revealed marked reduction of exhaled nitric oxide during the acute toxic phase with gradual return to normal during the next 15 months.[350] This was interpreted as a reflection of acute damage to epithelial cells, which are the chief sources of nitric oxide synthesis.

Summary Statement 55. Although breath condensate analysis is an evolving noninvasive method for evaluation of asthma, results are still variable and further refinements are required before it can be accepted as a valid diagnostic method. (C)

Exhaled breath condensate analysis is an evolving noninvasive method for evaluation of asthma.[351] A number of inflammatory and oxidative stress proteins associated with asthma have been demonstrated by this method.[351–354] However, results in several studies were variable, indicating that further sensitivity adjustments in the technique would broaden its applicability.[355]

Summary Statement 56. Bronchoalveolar lavage obtained through flexible bronchoscopy is useful in phenotyping asthma. The finding of lymphocytic alveolitis may suggest a diagnosis of hypersensitivity pneumonitis. (B)

Flexible bronchoscopy may occasionally be necessary for differential diagnosis of nonasthmatic endobronchial obstruction–induced wheezing in adults.[356–359] Several indications in children include suspected tracheomalacia, persistent middle lobe syndrome, and recurrent wheeze with cough.[360–364] Bronchoscopy is used primarily to obtain BAL. All inflammatory markers previously discussed in the induced sputum section may be readily evaluated in BAL.[335–343] Higher levels of immunoglobulins in BAL may indicate increased permeability of respiratory membranes.[365] Bronchoalveolar lavage is routinely evaluated before and after segmental bronchial challenge, a research procedure.[340] As asthma phenotyping becomes more of a clinical reality, the cellular components of BAL assume paramount importance in distinguishing between eosinophilic and neutrophilic asthma. Atopic asthma is also associated with specific cytokines and chemokines.[333,339,366,367] The finding of lymphocyte alveolitis with increased $CD8^+$ lymphocyte counts may contribute to the diagnosis of hypersensitivity pneumonitis.[368–370]

TESTS TO DISTINGUISH CLINICAL OBSTRUCTIVE DISEASES RESEMBLING ASTHMA

Cystic Fibrosis

Summary Statement 57. Cystic fibrosis may not only be confused with asthma, but certain genetic variants may be associated with increased asthma risks. (B)

Patients with well-defined genetic diseases, such as cystic fibrosis and α_1-antitrypsin deficiency, may require confirmatory tests if a differential diagnosis suggests a reasonable suspicion. In addition, specific allelic inheritance patterns in these patients may predict a higher risk for developing asthma in addition to the underlying disease. Whenever doubt exists, a sweat chloride sample should be obtained, especially in children and young adults. Commercial test kits are now widely available for cystic fibrosis mutation testing.[371] A recent large population survey in Denmark revealed that 5T homozygosity or F508 del heterozygosity of the CF transmembrane conductance regulator gene was associated with increased asthma risk.[372] Cytokine levels (ie, IL-8 and TNF receptor) were higher in cystic fibrosis than asthma patients.[373]

α_1-Trypsin Deficiency

Summary Statement 58. Although major phenotypes of α_1-antitrypsin deficiency do not occur in asthma, recent surveys demonstrated a high prevalence of asthma in young ZZ homozygous α_1-antitrypsin deficiency patients. (B)

The frequent occurrence of asthma symptoms among patients with α_1-antitrypsin deficiency led to a brisk and persistent controversy, with conflicting reports about the scientific advisability of checking for α_1-antitrypsin deficiency in children and young adults.[374–382] For many years, the debate focused on the major phenotypes of α_1-antitrypsin deficiency (MM, MX, MS, MZ), but the distribution of these phenotypes in asthmatic patients does not differ from that found in the general population.[375,378] Nevertheless, recent surveys demonstrated a high prevalence of asthma symptoms in young ZZ homozygous α_1-antitrypsin deficiency patients. Furthermore, a gene-environment interaction may predispose farmers with rare phenotypes (SZ, SS, and ZZ) to develop house mite sensitization and asthma in contrast to what is found in other young people living in rural areas.[380,382] Despite these inconsistencies, α_1-antitrypsin deficiency tests and even α_1-antitrypsin phenotyping may be indicated under special circumstances. Mutational screening for α_1-antitrypsin deficiency may be obtained through pro bono commercial programs.

Specific tests are available to distinguish other wheezing disorders, such as carcinoid (urine 5-hydroxyindole acetic acid) and mastocytosis (serum tryptase).[383–385]

IN VIVO DIAGNOSTIC TESTS OF CELL-MEDIATED IMMUNITY

Intracutaneous Tests

Tuberculin and Recall Intracutaneous Tests

Summary Statement 59. Purified protein derivative (PPD) of tuberculin is the prototype antigen recall test and provides direct evidence that hypersensitivity, as opposed to toxicity, is elicited by the antigens in *Mycobacterium hominis* or related mycobacterial species. (B)

Summary Statement 60. The tuberculin skin test is elicited by the intracutaneous injection of 0.1 mL of standardized PPD starting with the intermediate strength of 5 tuberculin units. (C)

Summary Statement 61. Recall antigen skin tests are used to evaluate cellular immunity in patients with infection (eg, life-threatening sepsis), cancer, pretransplantation screening, end-stage debilitating diseases, and the effect of aging. (C)

Summary Statement 62. Reduced or absent recall antigen tests are termed *anergy,* which develops frequently in certain diseases, such as hematogenous tuberculosis, sarcoidosis, and atopic dermatitis. (C)

Present applications

Purified protein derivative of tuberculin is the prototype recall test antigen.[386] Purified protein derivative provokes a delayed cutaneous reaction in most (but not all) immunocompetent subjects who have had past or present infection with *M hominis.* This test provides direct evidence that hypersensitivity, as opposed to toxicity, is elicited by the antigen. Purified protein derivative–tuberculin is an ammonium sulfate precipitate of the heated aqueous ultrafiltrate from a broth culture of *M hominis*. The skin test is elicited by the intracutaneous injection of 0.1 mL of standardized PPD. The reaction begins within hours and reaches maximum size in 48 hours. The involved skin feels firm or indurated to the touch. Erythema and edema are not necessary components of the tuberculin reaction but are usually present.[387] The reaction can persist for a week or longer. Rarely, vesiculation and blistering indicative of exquisite delayed cutaneous hypersensitivity may occur. A positive tuberculin skin test result identifies prior exposure and sensitization to the tubercle bacillus and/or possible active infection. Prior cross-sensitization to nonpathogenic soil or atypical mycobacteria can produce small or modest size positive tuberculin test results.[388] Although earlier studies recommended that a tuberculin skin test of more than 10 mm in diameter identifies 90% of healthy persons who have been sensitized to the tubercle bacillus, a recent study in 2,848 healthy non–BCG-vaccinated persons revealed that this cutoff value was valid only if the infection prevalence in the tested population was at least 10%, but it lost predictive accuracy at low infection prevalences. Thus, in populations with lower prevalences of latent tuberculosis, a cutoff value of more than 15-mm diameter is proposed.[389–391] When the skin test is applied in immunoincompetent sick patients, smaller reactions, which may be indicative of prior sensitization to *M hominis,* are often observed.[392,393] One of the most important uses of the tuberculin skin tests is to evaluate successful skin conversion after BCG vaccination.[394]

Delayed-type recall antigen skin tests are used to evaluate cellular immunity in patients with infection (eg, life-threatening sepsis), cancer, pretransplantation screening, end-stage debilitating diseases, and the effect of aging.[395–397] Recall antigen skin tests can also be used to predict survival of patients, to detect disease-related changes in immunity, and as a guide to therapy outcome.[398–411] A recent study suggested that anergy appears to be a simple and reliable biomarker of inflammatory activity in sarcoidosis patients.[412] Impaired in vivo reactions to recall antigens occur in atopic dermatitis despite normal in vitro lymphocyte transformation responses.[413] It is postulated that this in vivo defect in cell-mediated immunity may also impair host defense for certain infections, such as chronic *Molluscum contagiosum*.[414] A normal delayed hypersensitivity response provides evidence of intact 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 such as occurs in hematogenous tuberculosis. Discrepancies in interphysician evaluation of delayed-type hypersensitivity skin tests occur because of the use of different antigens, variability of reading times, and lack of standardization of test methods.[414] Despite these differences, recall antigen skin tests are in vivo correlates of lymphocyte- and macrophage-dependent delayed-type hypersensitivity responses and may be used to avoid costly and more labor-intensive laboratory tests of cell-mediated immunity.[415]

Technique

Summary Statement 63. Candida albicans, *Trichophyton mentagrophytes,* and *Tetanus* toxoid, the currently available recall antigens, are injected intracutaneously in the same way as the PPD test. (C)

Currently available recall antigens other than PPD include *C albicans* (Candin Allermed Laboratories Inc; CASTA Greer Laboratories), *Tetanus* toxoid vaccine (Aventis Pasteur), and *Trichophytin* (Allermed Laboratories; Greer Laboratories). The MULTI TEST cell-mediated immunity, which had included 7 recall antigens, mumps skin test antigen, and a number of other bacterial and fungal recall antigens, is no longer commercially available.

The standard Mantoux method for performing recall antigen skin tests consists of the intracutaneous injection of 0.1 mL of antigen solution. The skin test is initiated with the bevel of a No. 27 gauge, 0.5-in needle directed upward and the needle held at a 15° to 20° angle to the skin. The needle should be inserted into the skin and channeled several millimeters through the dermis. When correctly done, the skin will

dimple with slight pressure or downward movement at the tip of the needle. Injection of a 0.1-mL volume of antigen solution usually provokes a transient, mild burning discomfort and a 5- to 10-mm wheal in the skin. Prior high level of natural exposure is the criterion used to select potentially useful delayed-type hypersensitivity antigens. Appropriate delayed-type hypersensitivity skin test reagents include tuberculin, trichophytin, oidiomycin (*C albicans*), and *Tetanus* toxoid. In the case of tuberculin tests, several disposable varieties (tine test and Heaf) are available.[416–418]

Reading the test results

Summary Statement 64. The size of the delayed skin test reaction is measured 48 hours after antigen challenge, and the largest diameter of the palpable firm area that outlines the induration response should be measured to the nearest millimeter. (C)

The size of the delayed skin test reaction is measured 48 hours after antigen challenge. The diameter of the palpable firm area of the induration response should be estimated as the average of orthogonal diameters measured to the nearest millimeter. Gentle pressure with a ballpoint pen can be used to dimple the skin and define the homogeneous area of induration.[419] Although measurement of accompanying erythema was not formerly considered to be essential, recent investigations showed that both erythema and induration measurements were equally effective for identifying tuberculosis hypersensitivity in schoolchildren vaccinated with BCG and active tuberculosis patients.[387,394,420] A reaction diameter of 2 mm or greater should be used as the threshold for a minimal but measurable reaction. The size of all measurable reactions, including immediate ones, which can occur in up to 90% of normal subjects, should be recorded.[420] If immediate reactions to delayed-type hypersensitivity skin tests occur, the diameters of erythema and wheal reactions at 15 to 20 minutes, the erythema, edema, and induration at 6 and 24 hours, and induration at 24, 48, and 72 hours should be measured. Notation of changes in the skin test reactions over time should be used to differentiate immediate, late-phase cutaneous response, and delayed-type hypersensitivity reactions and detect adverse (>40 mm) skin test reactions under these circumstances. Although rare, severe local reactions can include blisters, necrosis, scar formation, changes in pigmentation, local lymphadenopathy, and systemic symptoms, such as fever.[421,422]

Summary Statement 65. When a single intracutaneous antigen (other than PPD) is used to evaluate prior sensitization to a potential pathogen, a reaction of 5 mm or greater may suffice as the cutoff point for positive tests, but smaller reactions (2 to 4 mm) may be clinically important. (C)

Tuberculin reagents and reading criteria have been subjected to extensive prospective investigations. The PPD vaccine is available in 3 strengths (first, intermediate, and second). Widespread use of the intermediate strength of PPD (5 tuberculin units) has demonstrated that reactors can be separated from nonreactors by diameters of 10 mm or greater of induration if the prevalence of active infection in the tested population at large is 10% or more. At this level, the presence of turgidity was associated with a higher occurrence of active tuberculosis.[423] The cutoff value is 15 mm or more when there is a lower prevalence of latent tuberculosis in the general population.[424] Tuberculin skin test readings up to 168 hours after application may still be reliable.[425] Nevertheless, the number of mitigating circumstances to be discussed under "Limitations" may affect the interpretation of the tuberculin skin test.

Most recall antigen tests for evaluation of delayed-type hypersensitivity have not been standardized to the same extent as the tuberculin skin test. However, the potency of several *C albicans* commercial antigens has been determined by delayed-type hypersensitivity skin tests in immunocompetent human volunteers[426] (Candin package insert). A positive response at 48 hours is 5-mm induration or more. When multiple antigens are collectively interpreted, the identification of 2 or more reactions of 2-mm diameter or more can be accepted as reliable evidence of intact delayed cutaneous hypersensitivity. When a single intracutaneous antigen (other than PPD) is used to evaluate prior sensitization to a potential pathogen, a reaction of 5 mm or more may suffice as a cutoff for a positive test result, but smaller reactions (2 to 4 mm) should also be clinically correlated.

Clinical relevance

Summary Statement 66. The absence of delayed-type hypersensitivity to all the test antigens would suggest an anergic state. (C)

The absence of delayed-type hypersensitivity to all of the test antigens suggests an anergic state. Above and beyond the null reactive state, differences in relative levels of delayed-type hypersensitivity in diameters and the ratio of the number of positive to the total number of tests must be compared in an appropriately matched control population within that population's reference range of estimated exposures to the particular test antigen(s). This has not been accomplished for most nontuberculin antigens. Thus, if all test results in the anergy panel are negative, the significance of this finding implies that 95% or more of an appropriate reference population has reacted to 2 or more of the antigens on the same recall test panel. Apart from the tuberculin skin test, quality performance of this type has only been established in the case of *C albicans* delayed-type hypersensitivity.[424,426] The recall panel on which this criterion is based contained Varidase, a test antigen that is no longer available. Since the 3 currently available recall antigens have not been compared en bloc in a large panel of immunocompetent volunteers, anergy may only be inferred if all 3 tested antigens are negative. Nevertheless, in a relatively small study of immunocompetent children ages 6 weeks to 12 years, 73% of subjects tested to 2 recall antigens (*C albicans* and *Tetanus* toxoid) had at least 1 positive response.[427]

Summary Statement 67. The most important use of delayed-type hypersensitivity skin testing is epidemiologic

screening of susceptible populations exposed to bacterial and fungal pathogens. (C)

Summary Statement 68. The widest application of recall antigen testing is the detection of anergy and as an in vivo clinical correlate of cell-mediated immunoincompetency. (C)

Summary Statement 69. Although anergy testing was formerly conducted frequently in HIV patients to determine whether a concurrent negative tuberculin skin test result rules out active tuberculosis, recent evidence mitigates against this approach. Recall antigen anergy in HIV patients has also been investigated as an indicator of staging, progression of disease, and response to therapy. (C)

The most important use of delayed-type hypersensitivity skin testing is to confirm exposure to many bacterial and fungal pathogens such as tuberculosis and histoplasmosis in susceptible populations. Detection of positive reactions to these and other organisms that induce delayed-type hypersensitivity may then lead to the proper diagnosis of active infection (if this is present) or a state of latent or herd infection that may or may not require appropriate therapy.[428–433] The use of delayed-type hypersensitivity skin tests for diagnosis of blastomycosis and coccidiomycosis is no longer considered to be reliable.[434] Although histoplasmin has been used for diagnosis of histoplasmosis in the past, a commercial histoplasmin reagent is no longer available. Although tuberculin skin testing has been used extensively to evaluate the efficacy of BCG vaccination, recent doubts have been raised about the reliability of such tests, either for the protective capacity of BCG or the confounding effect of BCG vaccination in detecting active infection in large populations.[435,436] The widest application of recall antigen testing is the detection of anergy, an in vivo clinical correlate of cell-mediated immunity incompetency. In the case of tuberculosis detection, anergy is purported to obfuscate tuberculin skin sensitivity in approximately 8% of patients with active tuberculosis, particularly those patients with meningitis and miliary tuberculosis.[397,398,437] Because active tuberculosis is so common in HIV-infected individuals, anergy skin tests were often performed at the same time as the tuberculin skin test to determine whether a negative tuberculin skin test result could reliably rule out tuberculosis.[438] However, a number of recent studies have concluded that concurrent anergy is not a reliable way of deciding whether HIV-infected individuals have active tuberculosis.[439–441] Apart from the question of HIV patients being coinfected with tuberculosis, recall antigen anergy has been investigated in AIDS as an indicator of staging, progression of disease, and response to therapy.[442–446] As alluded to in the previous section, anergy investigations have been performed in diseases in which delayed-type hypersensitivity immunoregulatory mechanisms are affected.

Intracutaneous tests are currently being evaluated as diagnostic adjuncts for nonimmediate allergic reactions to various drugs.[175–179] There is no precedent for such testing because metabolites or allergenic determinants have not yet been found for many of these drugs. Nevertheless, anecdotal reports are appearing more frequently with respect to drugs such as lidocaine, heparins, semisynthetic heparinoids, and even iodinated contrast media.[447–450] In a larger prospective study of 947 patients with cutaneous adverse drug reactions, intracutaneous tests were not useful.[228]

Sensitivity, specificity, and positive and negative predictive indices

Summary Statement 70. Although the standardized PPD antigen has been used for many years as a predictor of active or latent tuberculosis infection, confounders, such as susceptible populations, BCG vaccination, and cross-sensitization with other atypical mycobacterial species, have all affected the diagnostic accuracy of the tuberculin skin test and, by extrapolation, other delayed-type hypersensitivity tests. (C)

It would appear that these indices should be readily available in the case of PPD, a standardized antigen used for many years as a predictor of active or latent infection. However, establishing the cutoff value for mean wheal diameter of tuberculin reactions has not been universally accepted because of several confounding factors. First, part of the wheal diameter may be due to cross-sensitization with atypical mycobacterial species, and if these are strongly suspected, specific delayed-type hypersensitivity skin tests for these antigens may need to be evaluated.[387] Other situations that influence and give rise to positive tuberculin reactions are prior BCG vaccination and a delayed boosting effect in health care workers.[451] By contrast, tuberculin skin test reactions may be either reduced or abolished if concurrent anergy exists. Thus, sensitivity, specificity, and predictive indices would not be applicable to large population groups unless these confounders could be eliminated. Standardization of other recall antigens present in anergy panels is incomplete, in respect to the antigens themselves, the diameters of wheal and induration reactions, and the extent of exposure of test populations to various antigens in the panel with the possible exception of *C albicans*.

Limitations

Summary Statement 71. The gross appearance of a late-phase cutaneous response and delayed-type hypersensitivity reactions may not be completely distinguishable except that the latter are more characterized by prolonged induration. (B)

Summary Statement 72. Although systemic corticosteroids will render delayed-type hypersensitivity skin tests uninterpretable, 28 days of treatment with high-dose inhaled fluticasone (220 μg, 2 puffs twice a day) did not suppress delayed-type hypersensitivity to PPD in healthy volunteers. (B)

Summary Statement 73. Neither anergy nor tuberculin testing obviates the need for microbiologic evaluation when there is a suspicion of active tuberculosis or fungal infections. (F)

Summary Statement 74. Several new in vitro assays (ie, interferon-γ and polymerase chain reaction) appear to be more reliable in predicting active tuberculosis in BCG-vac-

cinated persons or when cross-sensitivity to atypical mycobacteria may coexist. (C)

The gross appearance of late-phase cutaneous and delayed-type hypersensitivity reactions may not be completely distinguishable except that the latter reactions are more characterized by prolonged induration. If this should occur with antigens that have both IgE-mediated- and delayed-type hypersensitivity characteristics (eg, trichophytin), histologic studies might be required.[452–455] Systemic corticosteroids will render delayed-type hypersensitivity skin test results uninterpretable.[456] Interestingly, 28 days of treatment with high-dose inhaled fluticasone (220 μg, 2 puffs twice a day) did not suppress delayed-type hypersensitivity to PPD in healthy volunteers.[557] The validity of anergy testing as a guide to interpretation of tuberculin skin testing has been questioned by many experts.[458] Neither anergy testing nor tuberculin testing obviates the need for microbiologic evaluation when there is a suspicion of active tuberculosis infection. In recent years, reliability of delayed-type hypersensitivity skin tests have been compared with polymerase chain reaction assays and a whole-blood interferon-γ assay based on stimulation with *M hominis*–specific antigens. Thus far, these studies appear to be more reliable in predicting active tuberculosis in BCG-vaccinated individuals and situations where cross-sensitivity to atypical mycobacteria may coexist.[459–464] The Centers for Disease Control and Prevention has recommended that one of the recently FDA-approved interferon-γ tests, the Quantiferon (QFT) – TB Gold (Cellestis, Victoria, Australia), replace the tuberculin skin test.[465]

Safety

Summary Statement 75. Immediate hypersensitivity reactions, including anaphylaxis, have been reported after tuberculin skin tests. (D)

Immediate hypersensitivity reactions after tuberculin skin testing have occurred. Within an 11-year period from 1989 to 2000, there were 24 reports that were classified as serious.[421,422] Of these, 9 are identified as being due to anaphylaxis. There were no deaths in this group. Other reactions included paresthesias, seizures, chest pain, syncope, Guillain-Barré syndrome, and vasovagal reaction. In 1% to 2% of positive test results, blistering or even local necrosis may occur, but this is usually self-limited. Local reactions such as regional lymphangitis and adenitis may also occur on rare occasions. There is 1 reported case of acute transverse myelitis associated with tuberculin skin testing.[466]

Number of Cell-Mediated Hypersensitivity Tests

Summary Statement 76. The number of skin tests for delayed, cell-mediated hypersensitivity reactions is relatively limited. (C)

As previously discussed, delayed hypersensitivity skin testing is now limited to tuberculin testing and anergy testing is available for 3 recall antigens (*Candida, Trichophyton,* and *Tetanus* toxoid).

Epicutaneous Tests

History and background

Summary Statement 77. First introduced by Jadassohn in 1896, the epicutaneous patch test has evolved as the definitive diagnostic technique for the diagnosis of ACD. (A)

The patch test was introduced by Jadassohn in 1896 as the definitive method for verifying the presence of ACD.[467] A small area of skin was covered with a semiocclusive bandage that contained the reputed causative agent. A positive test result was declared when the clinical disease state was reproduced. Procedures for performing this deceptively simple test have evolved to provide adequate, nonirritating controlled exposure to a defined amount of substance in a nonsensitizing and nonsensitizer-containing patch test system.

Patch Tests

Present applications

Summary Statement 78. When clinical evaluations suggest that exposure to a specific contactant has occurred either in an occupational or nonoccupational clinical setting, patch testing can be used to confirm the diagnosis. (C)

Summary Statement 79. From a public health perspective, patch testing is useful to identify potential health hazards of unknown and newly introduced contact allergens for the medical community and industrial hygienists. (C)

Patch testing is used to determine the causative agent in any chronic eczematous dermatitis if underlying or secondary ACD is suspected. Dermatitis of the hands, feet, lips, anogenital region, and multiple areas of the body is a clinical situation in which patch testing is useful. Additional indications include chronic occupational dermatitis to differentiate ICD and also when a change of job is being considered. Contact dermatitis due to topical medications may be superimposed on all dermatologic conditions, including atopic dermatitis. When clinical evaluation suggests exposure to a specific contactant has occurred in a clinical setting, patch testing can be used to confirm the diagnosis. Patch testing is also used when ACD is suspected but unproved and the allergen is unknown. Patch testing may also be important to inform a patient that sensitivity to a specific substance is not present. For medicolegal adjudication purposes, it is essential to include or exclude the diagnosis of ACD. From a public health perspective, patch testing is useful to identify potential health hazards of known and newly introduced contact allergens for the medical community and industrial hygienist.[59] This is of particular importance considering that there are more than 85,000 chemicals in the world environment today, and of these, more than 3,700 substances have been identified as contact allergens.[468,469]

Technique

Summary Statement 80. The most common patch test techniques are the individual Finn Chamber and the T.R.U.E. TEST, an FDA-approved screening method for screening contactant allergens. The T.R.U.E. TEST is preloaded with 23 common contactants and vehicle control that have been

previously incorporated into a dried-in-gel delivery system, which is coated onto a polyester backing to form a patch template. (B)

Summary Statement 81. If photocontact sensitivity is suspected, the appropriate allergens should be subjected to photopatch tests primarily in the UV-A range of 320 to 400 nm. (C)

Aluminum is the major substrate for current patch tests because of its low allergenicity.[470] The Finn Chamber is the most popular system and uses small 8-mm (inner diameter) aluminum chambers that are occlusive and permit more accurate quantification of the dose of allergen per unit area of skin. Individual Finn Chambers are filled with contactants and applied at the time of testing. The chamber is applied to the skin and held in place by hypoallergenic tape. The T.R.U.E. (thin layer rapid use epicutaneous) TEST is an FDA-approved method for screening contactant allergens. The T.R.U.E. TEST is preloaded with 23 common contactants and vehicle control that have been previously incorporated into a dried-in-gel delivery system, which is coated onto a polyester backing to form a patch template. When the template is applied to the skin, the allergens are released as the gel becomes moisturized by transepidermal water.

Although many contact allergens have been identified and reported, most cases of ACD are due to relatively few substances. Fewer than 40 allergens produce most cases of ACD. Identification of the actual sensitizer in a complex product can at times be daunting. Contaminating chemicals and minor ingredients may be the actual allergen(s), whereas the parent compound or major component(s) was originally considered to be the sensitizer. In some contactant tests, mixtures such as balsam of Peru, a mixture of various fragrances, the precise chemical antigen is yet to be determined. In other cases, the hapten may be an altered product metabolized after contact of the substance to skin has occurred. The allergens formulated in the T.R.U.E. TEST panel can be estimated to identify approximately 25% to 30% of clinically relevant causes of ACD.[469–471] Selected panels of contactant allergen based on the patient's history may be required to supplement the screening panel of allergens to cover as completely as possible the range of exposures of the patient.[472] Kits for specific occupations (eg, hairdressers, machinists) and exposures (eg, shoes, plants, photoallergens) permit identification of other significant contactant allergens. Each new antigen that is evaluated requires identification, validation, and determination of the MEC and the zero-level irritant concentration for appropriate patch testing.

Petrolatum is the most widely used vehicle for dispersion of allergens. Although it has good stability and simplicity for many antigens, some substances do not disperse well in this medium. The quality of dispersion can be evaluated by light microscopy of a test substance in petrolatum. Substances added to enhance antigen dispersion in petrolatum introduce an additional variable into patch testing and require patch test controls of the additive substance in petrolatum without antigen. Some materials penetrate the stratum corneum to a greater degree in aqueous (hydrogel) or propylene glycol–containing vehicles and will give consistently negative results if tested in petrolatum (eg, NSAIDs).[473] Failure to appreciate the importance of vehicle-dependent delivery of a contact allergen may lead to errors in diagnosis. Therefore, an appropriate vehicle control must always be used.[474] Individual Finn Chambers or the T.R.U.E. TEST template en bloc are placed on the upper or middle back areas (2.5 cm lateral to a midspinal reference point), which must be free of dermatitis and hair. If shaving is required, an electric razor is preferable.

Certain contactants (eg, antibiotics, PABA) may induce photocontact ACD or phototoxic CD (eg, carrot, celery, fennel, lemon-lime, grapefruit). When these are suspected, photo patch tests, primarily in the UV-A range of 320 to 400 nm, are recommended.[475–478]

Reading the test results

Summary Statement 82. Traditionally, patch tests remain in place for 48 hours. After the 48-hour patch test reading, additional readings at 3 to 4 days and, in some cases, 7 days after the original application of the patch yield the best overall reading reliability. (C)

Summary Statement 83. A descriptive reading scale developed by 2 major international ACD research groups is the current standard for interpreting patch test results. (C)

Traditionally, patch tests remain in place for 48 hours.[479] A 24-hour reading time has also been used, but the 48-hour patch test reading will detect a greater number of sensitized persons.[480] Additional reading schedules have also been recommended by 2 collaborative group studies (The International Contact Dermatitis Research Group and the North American Contact Dermatitis Group).[481,482] These large-scale investigations documented that approximately 30% of relevant allergens that are negative at a 48 hour reading become positive at 96 hours.[481,482] If positive reactions at 48 hours disappear by 96 hours, they may be due to irritants. Readings at 96 hours are conducted 48 hours after removal of the original 48-hour occlusive patch. For weak sensitizers, a 7-day reading time may be necessary.

Consensus of the 2 major ACD research groups has led to the development and refinement of the currently used nonlinear descriptive scale, which has been accepted almost universally.[483,484] This reading scale is described in more detail in Contact Dermatitis: A Practice Parameter. With some experience in grading, most observers can replicate the scores of more experienced graders. Although novel bioengineered techniques (laser Doppler or reflectance measurements) are objective and offer the advantage of metric results, they have not supplanted the descriptive scale for routine clinical observations.[485]

Clinical relevance

Summary Statement 84. Although patch tests are indicated in any patient with a chronic eczematous dermatitis if ACD is suspected, patch tests are especially important in identifying both ICD and ACD in the occupational setting. (C)

Summary Statement 85. Other important exposures associated with ACD include the use of topical medication, including corticosteroids, plant-induced ACD, and dermatitis occurring after use of cosmetics and personal hygiene products. (C)

Summary Statement 86. Unprotected work and repetitive exposure to surfactants may predispose patients to occupational dermatitis, including ICD and ACD. (C)

Summary Statement 87. Certain contactant allergens in the T.R.U.E. TEST panel, such as nickel and some rubber chemicals, have a high degree of relevant (approximately 75%) correlation with clinical sensitivity but others do not (eg, hydroxycitronellal, thimerosal). (B)

Patch testing is the gold standard for identification of a suspected contact allergen. It is indicated in any patient with a chronic, pruritic, eczematous, or lichenified dermatitis if underlying or secondary ACD is suspected. Patch tests are especially important in identifying occupational dermatitis. The most common occupations associated with occupational dermatitis (both ICD and ACD) are the health professions (especially nurses), food processors, beauticians/hairdressers, machinists, and construction workers. Medicinal-induced CD is a common cause of ACD. It is estimated that up to 5% of patients using topical corticosteroids may develop ACD[486–489] (see Contact Dermatitis: A Practice Parameter). Other implicated agents include lanolin, PABA, caine derivatives, neomycin, bacitracin, and NSAIDs. Allergic contact dermatitis to cosmetics and personal hygiene products are common because these agents are ubiquitous in today's society. At times, inert formulation excipients in commercial formulations are the sensitizers rather than the main ingredient. Plant-induced ACD is the most common form of ACD, but patch tests for the various varieties of culprit plants are usually not appropriate because of their high sensitization potentials. However, open patch tests are valuable to demonstrate ACD to sesquiterpene lactones and tuliposides in florists, bulb growers, and other workers in the bulb industry.[490]

Unprotected wet work and repetitive exposure to surfactants may predispose patients to occupational dermatitis. Lower irritant thresholds, initially determined by dose response reactions to a common detergent (ie, sodium lauryl sulfate), were associated with subsequent development of hand ACD in a prospective study conducted in apprentice hairdressers.[491]

Certain contactant allergens in the T.R.U.E. TEST panel, such as nickel and some rubber chemicals, have high clinical relevance (approximately 75%) to clinical sensitivity, whereas others such as thimerosal and hydroxycitronellal appear to have decreasing clinical relevance in recent years.[492,493] Correlations between initial patch test reactivity and subsequent ROATs revealed a correlation between MEC of various allergens on patch testing and positive reactions after use testing.[494]

Because sensitivity to multiple allergens in test panels such as the T.R.U.E. TEST occurs frequently, it has been proposed that a susceptibility factor may determine the occurrence of multiple ACD sensitivity in patients. One recent study proposed a "multiple sensitivity index" in patients exhibiting multiple reactions.[495] For 17 allergens examined, 131,072 possible combinations were evaluated in a total of 2,881 patients. A total of 12.4% of these patients had multiple positive patch test reactions ranging from 2 to 7 allergens. No cluster patterns were evident in patients exhibiting 3 to 7 positive combinations. However, dual combinations were most frequently observed with nickel sulfate and potassium dichromate; formaldehyde and quaternium-15; and nickel sulfate and formaldehyde. In this study, nickel sulfate once again was the most frequent sensitizer.

Sensitivity, specificity, and positive and negative predictive indices

Summary Statement 88. Patch tests are most effective when the patients are selected on the basis of a clear-cut clinical suspicion of contact allergy, and they are tested with the chemicals relevant to the problem; these conditions satisfy the prerequisites of high pretest probability. (C)

Summary Statement 89. Although the diagnostic accuracy of contactants cannot be compared with other in vivo or in vitro tests, diagnostic concordance between patch test sensitivity and the outcomes of repeated open provocation tests has been demonstrated for some contactants. (B)

If patch tests are to be considered as hallmarks of the evidence-based diagnosis of ACD, the sensitivity, specificity, predictive indices, and likelihood ratios must ultimately be ascertained in cohorts of patients who have ACD and control populations who do not. To some extent, these data are available for ACD of workers in certain industries. For example, a large industrial investigation revealed significantly higher sensitization rates of employees in the food processing industry compared with the total test population for nickel sulfate (22% vs 17.2%, $P < .0005$), thiuram mix (4.9% vs 2.6%, $P < .0005$), and formaldehyde (3.5% vs 2.1%, $P < .005$).[496] These results were predicated on a final physician diagnosis of ACD, which integrated the history of exposure and clinical appearance of the lesions with patch test results.

In lieu of a valid clinical surrogate of ACD, the clinical relevance of patch tests has been investigated by correlating them with repeated open provocation tests. Of necessity, these are dose response studies and must establish the MEC of contactants before the onset of use testing. Of the reported assays of this type, colophony, cinnamic aldehyde, isoeugenol, and methyldibromoglutaronitrile showed concordance between thresholds of patch test sensitivity and outcomes of use tests with these chemicals.[494,497,498] On the other hand, several chemicals such as hydroxycitronellal and formaldehyde did not show good concordance with use tests, thereby posing the question of what constitutes a suitable gold standard for predictors of clinical diagnosis. Given the current uncertainty with regard to predictors, the opinion of 1 investigator that "patch testing is cost effective only if patients are selected on the basis of a clear-cut clinical suspicion of contact allergy and only if patients are tested with chemicals

relevant to the problem (ie, high pretest probability)" appears to be a reasonable summation of the utility of patch tests in ACD.[499]

Limitations

Summary Statement 90. The chief limitation to traditional patch testing for the diagnosis of ACD is the lack of a suitable gold standard by which it can be evaluated in terms of diagnostic accuracy predictors and likelihood ratios. (C)

Summary Statement 91. Other technical limitations of patch tests include the inclusion of relevant contact allergens, use of the proper vehicle, application to the proper skin area, proper reading and interpretation, and the ability to correlate the tests with the patient's specific exposure. (B)

Summary Statement 92. Other limiting factors concern reproducibility, lack of information about irritant thresholds, and minimal elicitation concentrations (MECs) for many common chemicals in the human environment. (C)

Summary Statement 93. The inability to separate irritants from allergic responses is often encountered in the angry back syndrome, which occurs in approximately 6% of cases and is likely to develop in patients with a longer duration of the primary dermatitis. (C)

Summary Statement 94 Negative patch test reactions may occur even when the tests are performed with the correct sensitizing materials because the test fails to duplicate the conditions under which the dermatitis developed (eg, abrasions, frequent use of irritating soaps, washing the hands with solvents). (C)

The chief limitation to traditional patch testing for diagnosis of ACD is the lack of a suitable gold standard by which it can be evaluated in terms of diagnostic accuracy predictors and likelihood ratios. It itself constitutes a direct organ challenge with the suspected agent, and if irritancy effects can be excluded, it could have the same challenge significance as other double-blinded organ challenge tests. The issue is further clouded by the fact that each of the 3,700 contactant substances has its own unique MEC, which may vary depending on whether it is incorporated into petrolatum or aqueous solvents. Attempts to relate positive reactions to clinical history are not feasible because specific agents are often not suspected by the patient. One clinical approach suggested many years ago was to correlate the test results with clinical response after elimination of the patch test–positive contactant. This can be accomplished by having the patient take a vacation, a change in the nature of his/her work, a change in the home environment, or the use of protective gloves. Even so, this is a painstaking process and does not lend itself to prospective scientific investigations with appropriate cohort controls. Alternatively, after symptoms and signs of dermatitis have subsided by an elimination trial, modified use tests conducted by single- or double-blinded protocols could serve as a challenge gold standard regimen for specified contactants. One of these tests is the ROAT, which is described in greater detail later in this section.

Technical problems of selecting relevant contact allergens using the proper vehicle, applying them to the proper skin area, reading and interpreting them properly, and correlating the tests with the patient's specific exposure constitute the other limitations of patch testing. Spurious outcomes may also be due to the difficulty of identifying a uniform test procedure that reliably separates irritants from allergic responses. The latter problem is especially prevalent in persons with the angry back or excited skin syndrome.[500] Patch test reactions are not uniformly reproducible. The greatest sources of irreproducible reactions are apparently weak 1+ or +/− reactions. The accuracy of 1+ reactions has been estimated to be 20% to 50%, depending on the allergen and vehicle, whereas 2+ and 3+ reactions are accurate 80% to 100% of the time.[501] Data are sparse about irritancy and the MECs for many common chemicals in the human environment. Danish workers, however, have established that nickel-containing objects that release no more than 0.5 $\mu g/cm^2$ per week of nickel pose a minimal risk of sensitization and elicitation of CD to nickel, which is one of the most prevalent sensitizers.[502]

The diagnostic value of patch tests hinges on reproducibility. Although an earlier study found 40% of patch tests to be nonreproducible, recent studies have shown excellent reproducibility and reliability for a test panel of 30 allergens from different commercial sources, with 97.2% concordant negative and 95% concordant positive results.[503] This degree of reproducibility also applies to the T.R.U.E. TEST.[504]

The interpretation of a single test result is susceptible to both interobserver and intraobserver variation. Several large studies have compared the results of simultaneous applications of several allergens tested by different patch test techniques and interpreted by the same observers. Both European and Asian study centers revealed a 64% concordance between Finn Chamber and T.R.U.E. TEST patch test methods. Irritant or questionable reactions occurred in less than 1% of all applied patches, but false-negative and false-positive test results can occur with either technique.[505,506] The ability to separate irritant from allergic responses is often encountered in the angry back or excited skin syndromes. A recent prospective study revealed that this occurred with a frequency of 6.2% and was more likely to develop in patients with a longer duration of the primary dermatitis.[507] The position of contactants in the testing template should be considered, especially if cross-reacting or cosensitizing substances are tested adjacent to a relatively potent sensitizing agent.[508] Not infrequently, negative reactions occur even when the tests are performed with the correct sensitizing materials because the test fails to duplicate the conditions under which the dermatitis developed. Abrasions of the skin, frequent use of irritating soaps, washing the hands with grease solvent, and accompanying infection of the skin are all factors that could act as cofactors for either induction or elicitation of patch test sensitivity.

Safety

Summary Statement 95. Systemic ACD after patch testing is rare, as is reactivation of patch test reactions after oral ingestion of related allergens or even by inhalation of budesonide in patients with sensitization to topical corticosteroids. (B)

Summary Statement 96. It is possible to sensitize a patient who had not been previously sensitized to the allergen being tested. This is particularly true of plant contactants, such as poison ivy or oak and aniline dyes. (B)

Systemic ACD occurring after patch testing is rare.[509,510] However, it is not uncommon for patients to experience local flares over patch test sites after peroral challenges with fragrance-containing foods, Chinese herbs, contactant chemicals (nickel, gold), or drugs.[511–516] Reactivation of patch test reactions caused by budesonide have also been reported after inhalation of the same drug weeks after the positive patch test result.[517] Exaggerated local reactivities may also be encountered if the concentration of the patch test substance is too strong, thereby causing both an irritant and increased allergic reaction. There is also the possibility of sensitizing a patient who has not previously been sensitive to the allergen being tested. This is particularly true of plant allergens, such as poison ivy or oak, and aniline dyes. The possibility of active sensitization can be minimized by testing with dilute concentrations of various materials.

Foods that are prone to cause ACD and also have the ability to cause systemic CD include flavoring agents (eg, oil of cinnamon, vanilla, balsam of Peru), various spices, garlic, and raw cashew nuts.[512]

Modified Epicutaneous APT and RUT

Summary Statement 97. Two major variants of traditional patch tests are available: the atopy patch test (ATP) and repeated use test (RUT). (B)

Present applications

Two major variants of traditional patch tests are available: APTs and RUTs. Evaluation of APTs as a diagnostic adjunct for IgE-mediated inhalant and food allergy[105,518–527] in patients with atopic dermatitis has occurred chiefly in non–North American international centers. The diagnostic value of ATPs has also been investigated in eosinophilic esophagitis.[528] Use tests have been developed for weak sensitizers (ROAT), substances with poor percutaneous absorption (the strip patch test), and several premarketing skin dose response provocation assays for determining the minimal sensitizing dose in human volunteers.

Technique and reading the test results

Summary Statement 98. Atopy patch tests have been evaluated in patients with atopic dermatitis and eosinophilic esophagitis as an adjunct for the diagnosis of inhalant and food allergy. (B)

Summary Statement 99. Atopy patch tests for foods are prepared with dried or desiccated foods mixed into an aqueous solution and placed in 12 mm Finn Chambers before positioning on the patient's back. (B)

Summary Statement 100. Atopy patch tests for the diagnosis of drug allergy are performed by incorporating liquid or powdered drugs into petrolatum or aqueous solvents, which are added to 12-mm Finn Chambers and placed on the back. (B)

Summary Statement 101. Use tests have been developed for weak sensitizers (repeated open application test [ROAT]), substances with poor percutaneous absorption (strip patch test), and several premarketing dose response provocation tests for determining the minimal sensitizing dose of potential contactants in human volunteers. (B)

Summary Statement 102. In the strip patch test penetration of substances is enhanced by repeated adhesive tape stripping before application of the contactant patch to the stripped area. (B)

Summary Statement 103. The ROAT is an exaggerated use test designed to determine a patient's biologic threshold or response to a suspected contactant, especially if this has not been achieved with prior open or closed patch testing. (B)

Evaluation of APTs has occurred, particularly in Europe.[517–528] They have been used as adjuncts for the diagnosis of inhalant and food allergy in atopic dermatitis and eosinophilic esophagitis (only in the United States) and identification of drugs that induce mixed cutaneous reactions.[528–531] For identification of food allergy, on the test day 2 g of dried or desiccated foods is mixed with 2 mL of an isotonic saline solution. The mixtures are placed in 12-mm (internal diameter) Finn Chambers on Scanpore (Allerderm Laboratories Inc, Petaluma, California) and placed on the patient's back. Undiluted samples of commercially prepared single-ingredient foods (foods, vegetables, and meats) are placed directly in the Finn Chambers. The patches are removed at 48 hours and the results read at 72 hours. Patch readings are the same as classic patch test interpretation previously discussed. Although 6-mm chambers might be preferable on small backs of young children, the 12-mm chamber size for APTs yields much better results than the 6-mm chamber size.[532] A ready-to-use food APT (Diallertest) was recently compared with a Finn Chamber APT and was found to have good sensitivity and specificity.[533] However, intercenter APTs are often difficult to compare directly because of the variability of test preparations.[526,528]

For determination of possible drug allergy, drug patch tests are performed with high concentrations of the commercial form of the drug.[531] It has been determined that 30% is the highest concentration possible for preparation of a homogenous dispersion in petrolatum, water, or alcohol. To avoid serious reactions, dilutions ranging from 1% to 10% may be preferable for specific drugs. If a commercial tablet is used, the coating must first be removed before the substance within the tablet is pulverized to a very fine powder. The powder is then incorporated into white petrolatum at a concentration of 30% and also diluted at the same concentration in aqueous solvents. Powder contained in capsules is also tested at 30% in petrolatum or solvent. The jacket portion of the capsule is moistened and tested as is. Liquid formulations are tested

both as is and diluted 30% in solvent. Various formulations are loaded onto Finn Chambers and placed on the upper back. Because some drugs can cause immediate positive reactions, drug patch test results should be read in 20 minutes. For delayed hypersensitivity readings, it is necessary to read the patches at 48 and 96 hours and, if the results are negative, on day 7.[531]

An open topical provocation is also used for the diagnosis of mixed cutaneous drug eruptions. One modification of the open technique is to incorporate drug preparations into dimethyl sulfoxide, which enhances absorption.[531] This method has been successful for the diagnosis of metamizol- and naproxen-induced fixed drug eruptions.[534]

The traditional patch test has been modified in other ways, depending on the purposes for which they are intended. Test chambers of various sizes are commercially available. These include 8-, 12-, and 18-mm Finn chambers and 19- and 25-mm Hilltop chambers.[535] For relatively weak-sensitizing substances, the larger test chambers (12-mm Finn Chamber) may be more useful for detection of ACD.[536] This is also the case in which irritant testing with such substances as sodium lauryl sulfate is required.[535–537]

The strip patch test is a variant of patch testing used for substances with poor percutaneous penetration. Penetration of substances is enhanced by repeated application of adhesive tape before applications of the contactant patch to the skin. Thus, for sequential strips, a 25-mm-diameter Blenderm surgical tape is vertically applied and gently pressed downward with the fingertips for approximately 2 seconds. The tape is then removed in one quick movement at an angle of 45° in the direction of adherence. Each strip is performed with a new piece of tape on exactly the same skin area until the surface starts to glisten.[538] The older Al-Test consists of larger aluminum strips with Webril pads affixed by heat. These have been found to be more useful for retaining substances of high volatility and leachability (eg, ethylene oxide).[539] For premarketing research purposes, several tests, including the human repeat insult patch test (RUT), the 4-day semiocclusive patch test, and an occlusive patch test to the popliteal fossae for 6 hours daily for 4 consecutive days, are available.[540] The ROAT is an exaggerated use test designed to determine a patient's biologic threshold of response to a suspected contactant, especially if this had not been achieved with prior open or closed patch testing. It is often used as a special test for leave-on products (eg, mascara, lotions, henna tattoos) intended for use on the skin.[541] The ROAT is performed by applying a suspected contactant to the antecubital fossae twice daily up to 1 week and observing for dermatitis.[540] At times, ROAT can be performed by applying the patch test to the popliteal areas or on the back of the ear.

Clinical relevance

Summary Statement 104. Although clinical relevance is still evolving with regard to the APT, several investigative groups have reported that this test may be an adjunct in detection of specific allergens in atopic dermatitis and eosinophilic esophagitis. (B)

Because APTs are as yet not standardized, there are ongoing attempts to establish reliable systems for evaluation of clinical relevance. In patients being tested for aeroallergen reactivity, allergen-specific concordance of APTs was compared with prick/puncture tests and Pharmacia CAP tests using 2 different concentrations and 2 different vehicles. In a limited US investigation, optimal concordance was obtained when petrolatum was the vehicle and allergen concentration was [more than 1,000 PNU/g.[542] Reproducibility was also tested with allergens from different commercial sources. Reproducibility was 56% using the same manufacturer's extracts but much less when 2 different commercial extracts were compared.[543] An interesting insight into APTs was provided by a recent report that compared routine histologic analysis and in situ hybridization between involved and noninvolved skin of atopic dermatitis patients who exhibited positive ATP results. Interestingly, a positive APT reaction required the presence of epidermal IgE on the surface of $CD1a^+$ cells in both clinically involved and noninvolved skin.[544]

Although single-center studies have disagreed about the overall reliability of APT for the diagnosis of inhalant allergy in atopic dermatitis patients,[518–520] a large, multicenter European study concluded that APTs had a higher specificity (64% to 91%), depending on the allergen, than skin prick/puncture (50% to 85%) or specific IgE tests (52% to 85%). Positive APT reactions were not seen in 10 nonatopic controls. The conclusion of this study was that the potential for aeroallergens and food as causes of atopic dermatitis flares may be evaluated by APTs in addition to prick/puncture and specific IgE tests.[530]

With respect to the diagnosis of food allergy by APT in atopic dermatitis patients, several European investigative groups show data to support that APTs may be adjunctive diagnostic methods of evaluating food allergy in atopic dermatitis patients, especially those patients having nonimmediate or delayed reactions and in patients younger than 6 years.[522,523] In studies of eosinophilic esophagitis limited to a center in the United States, the combination of prick/puncture tests and APTs led to the discovery of causative foods in 18 of 26 cases.[528] However, it is unlikely that APTs will have wide applicability in North America until issues of standardization and reproducibility of these tests are more fully resolved.

Sensitivity, specificity, and positive and negative predictive indices

Summary Statement 105. The role of the atopy patch in predicting clinical allergy to food is indeterminate. (B)

In contrast to traditional ACD patch tests, both negative and positive predictive indices of APTs can be determined by correlation with gold standard inhalation or oral food challenge tests. When APTs were evaluated in 173 patients receiving double-blinded, placebo-controlled food challenges,

APT was the best single predictive test (positive predictive value of 95%); the combination of positive APT and positive prick/puncture test results optimized the positive predictive value to 100%. For hen's egg allergy, the APT was also the best single predictive test (positive predictive value of 94%).[525] The conclusion of this study was that the combination of positive APT results with high levels of specific IgE for cow's milk or hen's egg, respectively, makes double-blinded, placebo-controlled food challenges unnecessary for these respective food allergies in patients with atopic dermatitis.[525] A recent larger study of suspected food allergy in 437 children (90% with atopic dermatitis) revealed that APT was more specific than a prick/puncture or specific IgE tests but less sensitive, so that oral food challenge was only unnecessary for 0.5% to 14% of the subjects.[526]

Limitations

Summary Statement 106. The lack of standardization of APTs for diagnosis of both food and drug allergy is the chief limitation. (C)

Although progress is being made, the lack of standardization is still the major limitation of APTs. As previously discussed, there is also a lack of uniformity in preparing reagents, vehicles, and how the test should be read in a uniform way. The diagnostic value and reliability of tests are at present restricted to several clinical entities, so it is not possible to extrapolate to allergic conditions other than atopic dermatitis or eosinophilic esophagitis. There have been no collaborative attempts to standardize ATPs for the diagnosis of drug allergy. Results are highly variable at present, and it is impossible to predict whether such testing will ultimately be generally useful in the diagnosis of cutaneous drug reactions.

Safety

Summary Statement 107. Although the purpose of APTs is to test for food and drug nonimmediate reactions, the possibility of anaphylaxis must be considered because there could be significant percutaneous absorption of proteins and/or simple chemicals with high anaphylactogenic potential. (B)

Although the chief purpose of APTs is to test for food and drug nonimmediate reactions, occurrence of anaphylaxis must be considered. The possibility that there could be significant percutaneous absorption of proteins and/or simple chemicals cannot be ignored, particularly in patients with a history of exquisite anaphylactic sensitivity in addition to their nonimmediate reactions.

Number of Epicutaneous Skin Tests

Summary Statement 108. The appropriate number of APTs is indeterminate because they are not routinely performed. (B)

Atopy patch tests are being evaluated as diagnostic adjuncts chiefly to evaluate the role of inhalant and food allergens in atopic dermatitis and less often for the diagnosis of drug hypersensitivity. The use of APTs in the United States is controversial because there is no consensus about their relevance or number. The decision to use them is made on a case-by-case basis, but previously discussed criteria for performing such tests should be reevaluated periodically as their future use increases in the United States.

Summary Statement 109. Because ACD is frequently caused by unsuspected substances, up to 65 patch tests may be required for diagnosis. (D)

The number of patch tests is highly variable and case dependent. The only FDA-approved test device is the T.R.U.E. TEST, which consists of 23 common contactants, but it is only diagnostic in approximately 25% to 30% of cases. Supplementary patch tests are often required as suggested by the patient's exposure history, and up to 65 contactant tests are recommended by the North American Contact Dermatitis Research Group.

IN VITRO DIAGNOSTIC TESTS OF IMMEDIATE HYPERSENSITIVITY

Measurements of IgE Antibodies

Historical Perspective

One of the most important advances in allergy research was the 1966 discovery that reaginic activity resided in a previously unidentified immunoglobulin class.[545–548] After consolidation of the available data, the WHO named this class of immunoglobulin IgE. The availability of an IgE myeloma provided relatively large quantities of IgE and allowed the production of human anti-IgE antibodies, which led to immunoassays capable of measuring both total and allergen specific IgE concentrations in serum and other body fluids. The first assay for allergen specific IgE was reported in 1967 and was termed the RAST.[549] Since its initial description and commercialization, a number of technical improvements have been made in assay technology, including better characterized allergen solid phases, monoclonal antibodies, decreased assay time, less expensive instrumentation, automation, and the substitution of enzyme labels for radioactive labels. More recent, modified allergen specific IgE antibody assays are calibrated using heterologous interpolation against the WHO 75/502 international human serum IgE reference preparation. This common calibration strategy among assay methods permits a uniform system of reporting IgE antibody results in quantitative kIU_A/L units traceable to a common IgE standard.

One of the major controversies in allergy has been the comparison of immunoassays for allergen specific IgE with biologic tests of allergic sensitivity.[186] Much of the controversy results from failing to make a clear distinction about precise questions to be answered by these studies. As is the case with skin tests, a direct correlation cannot be assumed between the presence of specific IgE antibodies and clinical disease. Accepting the skin test as the equivalent of clinical hypersensitivity creates problems because of factors discussed in the previous section: (1) the lack of uniform procedures for performing skin tests, (2) the lack of uniform criteria for grading skin test results as positive or negative, (3) the difference among natural, purified, and recombinant test

allergens, and (4) the differential sensitivity of individuals sensitized to the same allergen. In addition, there has been limited effort to ensure the quality of skin tests, since skin tests are typically performed as a single determination, and the skin test result may be falsely negative or positive. Attempts have been made to resolve the question of false-positive skin test results by performing allergen challenges, but this may not always be relevant since there is general agreement that some patients with allergen specific IgE do not respond to an allergen challenge, an inherent limitation of these procedures.[550] This suggests that a positive skin test result does not necessarily mean clinical allergy.[550] Conversely, some patients who respond to end organ allergen challenge do not have positive specific IgE in vivo or in vitro test results.[90,133,136,185,527,551] Notwithstanding these controversies, there is general agreement that, for most allergens, allergen-specific immunoassays detect IgE antibody in the serum of most but not all patients who are clinically allergic. The precise sensitivity of these immunoassays compared with prick/puncture skin tests has been reported to range from less than 50% to greater than 90%, with the average being approximately 70% to 75% for most studies.[112,13,133,185,186,552–559] In most situations, skin tests are therefore the most clinically useful tests for the diagnosis of IgE-mediated sensitivity.

Total Serum IgE Assays

Summary Statement 110. Total serum IgE concentrations are reported in international units or nanograms per milliliter (1 IU/mL = 2.44 ng/mL). (A)

Summary Statement 111. Total IgE is cross-standardized with the WHO 75/502 human reference IgE serum verified by periodic proficiency surveys. (B)

Summary Statement 112. The clinical applications of total serum IgE are of modest value. High serum IgE concentrations occur in allergic bronchopulmonary Aspergillosis (ABPA), the therapeutic response of which is evaluated by serial IgE values. (B)

Summary Statement 113. Total serum IgE is required for assessing the suitability of a patient for omalizumab therapy and determining the initial dose. (B)

The most frequently used method for measuring total IgE concentrations is a sandwich-type assay. In this assay an anti-IgE antibody bound to a solid support is used to bind all IgE from the test sample. Serum proteins other than IgE are washed away from the support, and the IgE remaining bound to the support is quantified by means of a second, labeled, anti-IgE antibody.[59,549,560]

Total serum IgE concentrations are most frequently reported as international units or nanograms per milliliter (1 IU/mL = 2.44 ng/mL of IgE). Although the Systeme International (SI) specifies that IgE be reported as micrograms per liter with 2 significant digits (XX × 10n), it is still not widely used.[561] There are now a number of national and international reference preparations for total serum IgE.[562–564] However, the WHO 75/502 is the principal human IgE reference serum preparation to which all clinically used total serum IgE assays are currently cross-standardized. The availability of these IgE reference preparations has led to improved interlaboratory concordance of clinical total IgE assay results. For most commercial methods, total serum IgE determinations should be accurate to 2 significant digits, and the coefficient of variation for repeated assays should be less than 10%.[59,564] This level of proficiency has been recently confirmed with data from the College of American Pathologists Diagnostic Allergy External Proficiency Survey.[565] The routine quality control for total serum IgE assays is primarily directed toward assessing accuracy and precision. Previous problems with interference by other serum proteins have been largely eliminated by the availability of commercial antibodies specific for human IgE with high specificity and avidity. Accuracy and precision are evaluated by the inclusion of both internal and external standards in these assays.

One technical problem reported with some sandwich-type immunoassays for total serum IgE has been termed the *hook effect*. This term describes the problem of samples with very high total IgE concentrations that produce results identical to those of samples with much lower IgE concentrations. If increasing quantities of IgE are added in an assay, there should be a linear rise to a plateau. In some assays, however, the plateau may begin to fall to lower levels as increasing amounts of IgE are added. To avoid this problem, some laboratories assay samples at 2 dilutions with the expectation that the more dilute sample will produce a quantitatively lower result. If the more dilute sample does not produce a lower result, the sample needs to be rediluted and reassayed until it is clear that the sample is appropriately diluted. At this point the concentration of IgE in the unknown can be extrapolated from the linear rising portion of the standard calibration curve. Interdilutional coefficients of variation should remain less than 20% for an assay that maintains parallelism between the reference curve and dilutions of test specimens.

Allergen Specific IgE Assays

Summary Statement 114. As with total IgE, commercial specific IgE antibody assays are calibrated using heterologous interpolation against the WHO 75/502 human IgE reference serum, thereby enabling a uniform system of reporting. (E)

Summary Statement 115. In addition to WHO 75/502 calibration, an earlier specific IgE classification system was based on internal positive calibration curves from a positive control heterologous serum containing specific IgE antibodies, which in the original RAST was white birch specific. However, FDA clearance for modified specific IgE tests requires use of homologous internal control allergic sera whenever this is possible to obtain. (E)

Summary Statement 116. The precise sensitivity of these immunoassays compared with prick/puncture skin tests has been reported to range from less than 50% to more than 90%, with the average being approximately 70% to 75% for most studies; similar sensitivity ranges pertain when immunoassays are compared with symptoms induced after natural or controlled organ challenge tests. (C)

Summary Statement 117. As with skin tests, the interpretation of specific IgE results requires correlation with the history, physical examination, and, in some cases, symptoms directly observed after natural or laboratory exposure to allergens. This cannot be accomplished by commercial remote practice laboratories, which base recommendations for immunotherapy on a history form submitted by the patient and specific IgE results. (B)

Summary Statement 118. Because the constitutive allergenicity, potency, and stability are variable among commercial allergen extract reagents, sensitivity and the positive predictive value of both prick/puncture and specific IgE tests generally tend to be higher among pollens, stable anaphylactogenic foods, house dust mite, certain epidermals, and fungi compared with venoms, drugs, and chemicals. (C)

Summary Statement 119. Proper interpretation of specific IgE tests needs to take into consideration variables such as the binding affinity or avidity of allergens, solid-phase systems, cross-reactive proteins and glycoepitopes, specific IgG antibodies in the test system, and high total serum IgE (>20,000 IU). (E)

Summary Statement 120. A multiallergen (up to 15 allergens bound to a linear solid-phase system) test can screen for atopic status, following which allergen specific tests are required for more definitive evaluation.

Summary Statement 121. Specific IgE immunoassays are not recommended as a definitive confirmatory test for several specific clinical conditions. They provide neither diagnostic nor prognostic information when measured in the cord blood of newborn infants. They do not have sufficient sensitivity for foolproof prediction of anaphylactic sensitivity to venoms or penicillins. (B)

Summary Statement 122. Specific IgE immunoassays may be preferable to skin testing under special clinical conditions such as widespread skin disease, patients receiving skin test suppressive therapy, uncooperative patients, or when the history suggests an unusually greater risk of anaphylaxis from skin testing. (B)

Summary Statement 123. Determination of allergen specificity by inhibition of specific IgE binding is a unique attribute of specific IgE testing. (E)

Summary Statement 124. Automated systems using multiplexed allergen assays are being rapidly developed. One of these is cleared by the FDA for the simultaneous measurement of 10 allergens. (E)

Commercially available assays for allergen specific IgE are based on the principle of immunoadsorption.[557,560,562,566] The allergen specific IgE of interest binds to the allergen, which has either been previously bound to a solid phase or becomes bound to a solid phase after the IgE has been bound. IgE that does not bind to the allergen, together with other irrelevant proteins, are then washed away from the solid phase. The amount of the IgE bound to the allergen is quantitated using a labeled anti-human IgE (monoclonal or mixture of monoclonal) antibodies. The label can be a radioactive isotope an enzyme, or a ligand to which an enzyme or antiligand conjugate is bound.

A number of methods have been used throughout the years to report allergen specific IgE results.[562,566–569] First, a qualitative reporting scheme was used in which assay response produced by the test serum was compared with the results obtained with sera from nonatopic individuals who are known to be free of allergen specific IgE antibody. The mean and SD are computed for the IgE antibody–negative sera. Only test sera that produces results greater than the mean ± 2 or 3 SDs are called positive. The results of a test serum also can be expressed as a ratio or a percentage of the mean of the negative sera. In original RAST-type assays, a ratio of more than 3 was considered positive. This qualitative ratio method is presently used only in research IgE antibody assays and is no longer used by clinical laboratories certified by the Clinical Laboratory Improvement Amendments of 1988 reporting patient data.

A second method for classifying IgE antibody results has been to compare the results of a test serum to a calibration curve derived from a serum with a known amount of the same specific IgE. This method is called "homologous interpolation" because the IgE antibody specificity being measured in the test and reference serum are the same. Although a homologous interpolation scheme is considered by some investigators as the most attractive calibration approach, it is not used in many FDA-cleared IgE antibody clinical assays because it was not always possible to find sufficient quantities of serum containing IgE antibody (ie, 35 to 100 serum samples) from patients with relatively rare clinical allergies. However, the FDA Final Guidance for Industry document stipulates specific provisions for using "allergen specific control sera."[570]

The first clinically used RAST incorporated a heterologous interpolation scheme that related all allergen-specific IgE values to a standard curve derived from sera containing IgE anti–birch pollen.[549] To provide a grading scheme, the calibration curve was divided into arbitrary classes from 0 to 4. In an attempt to improve the sensitivity of the RAST, the modified RAST scheme was developed. The modified scoring system relates the number of radioactive counts in each unknown to class scores using a single control point (ie, 750 normalized counts).[571] Although the modified scoring system artificially increases diagnostic sensitivity by lowering the assay threshold, it also reduces diagnostic specificity of the assay (ie, increase of false-positive results). This limitation constitutes a major problem for those who continue to use this system.[59]

The major FDA-cleared semiautomated and automated assays (Table 5) for allergen-specific IgE antibody use a second, heterologous interpolation scheme in which a total serum IgE calibration curve is used to report results as international units of IgE per milliliter. This value can be converted into mass units of IgE per volume (eg, nanograms of IgE per milliliter) because the IgE calibration curve is standardized against the WHO Human IgE International Refer-

Table 5. Representative List of Current Commercial Specific IgE Technology

Method	Detected by	Technique
Phadebas RAST	Radioimmunoassay	Manual
Hitachi CLA (MAST)	Chemiluminescence	Semiautomatic
CAP System	Enzyme/substrate	Semiautomatic
Hycor Turbo-MP	Radioimmunoassay	Semiautomatic
Ala Stat	Enzyme/substrate	Semiautomatic
Hy-Tec E/A	Enzyme/substrate	Automatic
ImmunoCAP Systems	Enzyme/substrate	Automatic
Immulite 2000	Chemiluminescence	Automatic

Abbreviations: MAST, multiple allergen solvent test; RAST, radioallergosorbent assay.

ence Preparation 75/502.[570] There are some data to indicate that 1 IU/mL of allergen specific IgE antibody is equivalent to 1 IU/mL of total serum IgE.[572] However, this needs further confirmation.[559,573] Table 5 is a partial compilation of current commercial assays compared with the original RAST immunoassay. Current methods now use improved matrix binding combined with fluoroenzymatic or chemiluminescence detection systems. In addition, most of them are either semiautomatic or completely automatic.

Apart from the obvious advantage of expressing specific IgE results in mass units, as compared with the WHO human IgE reference standard, most specific IgE classification systems (both radioactive and enzymatic) are currently based on internal positive control curves calibrated with allergen-specific antisera. According to current FDA guidance regulations, regarding RAST-based methods, the source and stability of allergen-specific control sera should be specified.[570] In addition, confirmation of allergen IgE specificity should be identified for each allergen contained in the internal control sera. This implies that there be a homologous internal control positive reference specific IgE serum (eg, test serum specific for ragweed vs internal control ragweed specific IgE serum). A heterologous specific IgE serum control is not ideal and could confound or mislead results inasmuch as allergen preparations are mostly mixtures of proteins that can vary widely in composition, immunogenicity, allergenic potency, and binding to various matrices.[574]

One semiautomatic assay manufacturer stipulates that each laboratory should establish its own expected reference ranges presumably with homologous antisera for various sensitive populations (pollen, mold, or other allergen-sensitive patients) of interest.[575] It is not known whether all technology manufacturers address this issue. Although it is recognized that homologous control sera might be difficult to obtain, store, and maintain stability for many allergens, homologous specific reference sera to 8 major inhalant, 6 major food, and 4 major venom allergens could readily be incorporated into currently available multiarray semiautomatic or automated systems (Table 5). This could be readily accomplished because FDA clearance for commonly available allergosorbents only requires a maximum of 100 specific IgE positive serum samples.

Recent advances in lasers, computational power, DNA technology, component miniaturization, and other technological advances have allowed for the development of allergen specific IgE multiplexing.[576] Multiplexing, or the quantitative measurement of specific IgEs to numerous allergens simultaneously using array technology, is a major potential improvement over present day monoplex methods.[577] Multiplexed arrays for the measurement of specific IgEs use smaller sample sizes and are potentially cheaper, faster, more sensitive, and more accurate than any present day technology. Approaches to multiplexed array allergy testing have been described using glass slides with microdot placement of allergens or allergens covalently attached to microspheres that have been internally dyed and are spectrally distinguishable (liquid suspension arrays).[578,579] One such liquid suspension array assay has been FDA (510K No. K020387) for the simultaneous measurement of specific IgEs to house dust mite, cat, timothy grass, Bermuda grass, mountain cedar, short ragweed, *Alternaria* (mold), milk, egg white, and wheat. Secondary antibody detector systems include chemiluminescence and fluorescence. Amplification methods, such as DNA rolling circle amplification, have also been described.[580]

In terms of quality control, all assays for allergen specific IgE antibody should have known IgE antibody–positive and IgE antibody–negative sera run with each lot of reagents. Known positive and negative sera should be included in each assay for each specific allergen being tested. These quality control serum data confirm the quality and validity of the assay and the accuracy of the calibration curve. Results generated by the assay should not be reported if the results of the positive and negative quality control sera are not within 95% confidence limits for the assay.[562]

An investigation comparing analytic precision and accuracy of specific IgE assays performed by 6 different commercial laboratories using various methods (or modifications thereof) listed in Table 5 was reported in 2000.[563] Coded and blinded serum samples containing different levels of specific IgE antibodies to 17 allergens were picked up from physicians' offices by each laboratory over a span of 6 weeks. Collectively, the statistical analyses of these data revealed that assays performed by 4 laboratories gave different results for different allergens, and there were multiple instances of poor precision, quantitation, and accuracy. Results from 2 laboratories that use the ImmunoCap system could be grouped with results expected from an ideal immunoassay.[563] These disparate results should encourage commercial laboratories to participate in proficiency surveys and to make the results of such surveys readily accessible to the ordering clinician.[565]

Some laboratories report negative IgE antibody results for rare allergens when the laboratory has never obtained specific positive sera, which could demonstrate that the assay is really capable of detecting IgE with the expected specificity. In the United States, commercially available allergen-containing reagents (eg, allergosorbents) are submitted to the FDA and

given FDA clearance when data are supplied by the manufacturer that has analyzed at least specific 35 to 100 IgE antibody–positive serum samples from clinically allergic individuals. When serum samples from 35 different sensitized individuals cannot be identified in the world for a particular allergen specificity, the FDA gives the allergen-containing reagent the designation of an analyte-specific reagent (ASR), which indicates that less than 35 serum samples have been used to quality control the allergen-containing reagent. The ASR reagents are not fully FDA cleared, but they have been quality controlled sufficiently by the manufacturer to validate allergen specificity and permit their use in clinical laboratory testing with the caveat that they are "for research purposes only."

Ideally, a total serum IgE should be performed on all serum samples that are assayed for allergen specific IgE antibody. If the total serum IgE level is high (eg, 20,000 IU/mL for some assays), steps such as automatic dilution should be taken by the laboratory to ensure that the assay results for specific IgE are true positives and not the result of nonspecific binding in the assay. The total serum IgE level that produces a false-positive result due to nonspecific binding is presumably identified by the manufacturer of all commercially available allergen specific IgE assays[563,566,567] and should be made available to the ordering clinician. A clue to possible nonspecific binding is a report of weakly positive IgE antibody results with multiple allergens.[581] Nonspecific binding by glycoepitopes (ie, cross-reactive carbohydrate determinants) is a potential source of a positive test result without clinical significance.[582–584] To check this, IgE reactivity of a glycoprotein to which the patient had not been sensitized (eg, bromelain) should be tested against the patient's serum.[582]

An adequate presentation of the allergen in the assay is essential for optimal sensitivity of an assay.[585] Assay inaccuracies can result from a number of conditions: (1) the protein recognized by IgE may be a minor constituent of the total protein in the allergen preparation and hence it becomes a minor fraction of the protein bound to the solid phase, which leads to insufficient protein for adequate IgE binding; (2) the protein recognized by IgE may be labile because of either its molecular structure or the presence of proteolytic enzymes in the allergen preparation; and (3) the chemical linkage used to bind the protein to the solid phase may destroy the epitope recognized by IgE or the linkage may occur at a site so close to the epitope that steric hindrance occurs.

IgG antibody specific for allergens may occur as a result of natural allergen exposure or active allergen immunotherapy. Since IgG antibody is often present in quantities greatly exceeding the quantity of IgE antibody, specific IgG antibody may bind to available sites of the allergosorbent, thereby preventing subsequent IgE binding and leading potentially to falsely low or negative test results.[586]

Different allergen extracts may have identical proteins or peptide epitopes recognized by IgE antibodies. In this case, a patient who is sensitized to an allergen may have a positive test result to both the original allergen and other allergens that cross-react with the original allergen.[587] The relationship of cross-reactive IgE antibodies evaluated by either skin or specific IgE tests to clinical disease is known for some but not all allergens. Exposure to cross-reactive allergens may or may not provoke symptoms (eg, most grass-sensitive patients tolerate wheat, a potent cross-reactant in grass pollen extracts). This problem of allergen cross-reactivity may also complicate interpretation of skin tests.

The detection of allergen specific IgE antibody in serum with an FDA-cleared assay may be viewed as a risk factor that supports a positive clinical history in making the diagnosis of allergic disease. As with skin testing, IgE antibody specificities involving extracts that contain potent allergenic components such as ragweed, house dust mite, and cat epidermals tend to correlate much better with clinical sensitivity and provocation tests. The use of purified fractions (ie, Amb a 1, Der p I, Der f 1, Fel d 1, Alt a 29, Hev b 5) often fortifies sensitivity and the test's correlation with clinical disease compared with unfortified immunosorbent.[588] On the other hand, extracts with weaker allergenic epitopes may demonstrate substantially less correlation with various indices of clinical sensitization. This situation may be compounded further in the case of foods in which multiple allergenic epitopes are often contained in the crude extract mixture and minor components may actually dilute the major allergen responsible for clinical sensitization. Furthermore, as discussed herein, certain allergenic epitopes in foods (ie, wheat) may strongly cross-react with allergens in 1 of the potent classes of inhalant aeroallergens (ie, grass), leading to spuriously false-positive results. However, the predictive value of anaphylactogenic food specific IgE for outcome of oral food challenge has received considerable attention and is discussed below and further in part 2.[140,589–591] Because the constitutive allergenicity, potency, and stability are variable among commercial allergen extract reagents, sensitivity and the positive predictive value of both prick/puncture and specific IgE tests generally tend to be higher among pollens, anaphylactogenic, stable food proteins, house dust mite, certain epidermals, and fungi compared with venoms, drugs, and chemicals.

Inhibition of Specific IgE Antibody Binding

The most expedient method for determining the specificity of IgE binding is to determine whether the addition of a small quantity of a homologous allergen in the fluid phase will inhibit most IgE binding. Inhibition usually indicates that IgE binding in the assay is a result of the IgE antibody specifically recognizing the allergenic protein.[564] Theoretically, some allergen preparations may contain substances such as lectins, which could nonspecifically bind IgE. If IgE is being nonspecifically bound, either most serum samples are positive in the assay or there is a relationship between the total serum IgE concentration and an increase of assay positivity to multiple lectin-containing allergens. In demonstrating specific inhibition, it should be possible to inhibit at least 80% of the specific IgE binding in a dose response manner.[564]

The degree of binding inhibition produced when a fluid phase allergen is added to a serum containing allergen specific IgE depends on the ratio between the quantity of specific IgE and the quantity of allergen added. When the quantity of specific IgE is kept constant, the percentage of inhibition produced can be used to estimate the quantity of allergen in the fluid phase. Under appropriate experimental conditions, including an adequate supply of potent allergen specific IgE, inhibition can be used to standardize allergen extracts, estimate the quantities of allergens, and evaluate cross-reactivity between allergens.[581]

A specific allergen may be detected in a crude extract of multiple allergens using the inhibition technique.[581] For example, if a patient is known to be allergic to peanuts, and the patient has symptoms of an allergic reaction after eating a piece of candy, the question could be whether the candy contained peanut allergen. If an extract of the candy inhibits the binding of the patient's IgE to a solid-phase peanut allergen preparation, there would be good reason to suspect that the candy contained either peanut or an allergen that cross-reacted with peanut.

Allergen Specific IgG and IgG Subclass Assays

Summary Statement 125. Allergen specific IgG may be measured by immunodiffusion or immunoabsorption. (E)

Summary Statement 126. Immunodiffusion antibodies to cow's milk are associated with Heiner's disease, a non-IgE disorder that presents in infants with pulmonary infiltrates. (B)

Summary Statement 127. IgG and IgG subclass antibody tests for food allergy do not have clinical relevance, are not validated, lack sufficient quality control, and should not be performed. (B)

Summary Statement 128. Although a number of investigators have reported modest increases of IgG4 during venom immunotherapy, confirmation and validation of the predictive value of IgG4 for therapeutic efficacy of venom immunotherapy are not yet proven. (C)

Allergen specific IgG can be measured using immunoassays similar to those used to measure allergen specific IgE.[586] Allergen specific IgG is often easier to detect than specific IgE because it is usually present in a higher concentration. The antibody used to measure the IgG bound in an assay can be either an anti-human IgG or specific for 1 of the subclasses of IgG (IgG1, IgG2, IgG3, or IgG4). When subclass specific IgG antibodies are used, the quantity of the particular IgG antibody subclass can be determined. IgG and IgG subclass antibodies specific for allergens usually are measured in arbitrary units, although mass values may be extrapolated from a total or subclass specific standard curve. An allergen specific IgG assay is subject to the same technical problems as specific IgE assays, and specific IgG assays should be evaluated using the same criteria and techniques as those used for IgE assays. The level of expected precision should be 2 significant figures with variation less than 15%, or lower, since the quantity of IgG to be measured is often relatively large, especially after immunotherapy.[586] Currently, no blinded proficiency surveys are available for evaluating interlaboratory performance of allergen specific IgG or IgG subclass assays.

Clinical Application and Interpretation

Total serum IgE concentration

Several studies have proposed using the total IgE concentration in cord blood as a method for predicting an infant's risk of developing allergic disease.[592] Although the results of the early reports were promising, subsequent studies have not found the cord blood IgE concentration to be a reliable predictor of risk.[592] Even if it were possible to predict the risk of allergic disease, such knowledge would have little clinical value because there are not as yet proven methods for preventing allergic disease in high-risk children.

Measurements of total serum IgE concentration are of modest clinical value when used as a screen for allergic disease or for predicting the risk of allergic disease.[592] Although epidemiologic studies have shown that the risk of asthma is highly correlated with the total serum IgE concentration, the variation from individual to individual is too great to provide much diagnostic value.[592–595] Similarly, the broad range of values and the variation among individuals means that total serum IgE concentrations provide only modest information about the risk of allergic disease on an individual basis.[590] Furthermore, a normal total IgE does not exclude clinical allergy. Evaluation of patients with suspected ABPA is one of the few clear indications for measuring serum IgE concentrations.[596] An extremely elevated total serum IgE concentration is found in nearly all patients with ABPA and is one of the major diagnostic criteria. There is also a suggestion that the serum IgE concentration is an indicator of disease activity and that serial determinations should be used to evaluate the adequacy of treatment.[596]

With the licensing of omalizumab (Xolair) for the treatment of allergic asthma, another application of total serum IgE is verification that the patient is a suitable candidate for anti-IgE therapy with total serum IgE levels between 70 and 800 IU/mL. The total serum IgE level before taking omalizumab combined with the patient's weight will determine the correct dosing to ensure efficacious reduction of free IgE circulating in blood. After 1 month of taking omalizumab, a new assay that measures the level of circulating IgE that is free or unbound with omalizumab can confirm the effectiveness of the dosing regimen.[597] This test is not yet commercially available. Although as much as 62% loss in accuracy was observed in FDA-cleared human IgE assays, the ImmunoCAP system was sufficiently robust to provide accurate and reproducible total and allergen-specific antibody results in the presence of therapeutic levels of serum omalizumab.[598]

Serum IgE concentrations are often abnormal in patients with congenital immunodeficiencies, but these abnormalities are rarely diagnostic.[599] The primary exception to this statement is the syndrome of hyper-IgE, eczematous dermatitis, and recurrent pyogenic infections. In this syndrome, the total

serum IgE level is extremely elevated and is one of the major diagnostic criteria for the disease.[599] Nonspecific elevation of IgE level is also observed in the Wiskott-Aldrich, ataxia telangiectasia, DiGeorge, and Ommen syndromes.[600]

Patients with acquired forms of immunodeficiency may have altered levels of serum IgE, but these alterations are not diagnostic.[601] A few published reports have indicated that serum IgE is elevated in patients with HIV infection and that there is a modest correlation between IgE elevation and clinical course or state of the disease.[602]

IgE myeloma is a rare form of multiple myeloma, with fewer than 40 cases reported worldwide.[603] Some cases of IgE myeloma may have been misdiagnosed as light chain disease because of failure to measure serum IgE concentrations. Since the course of IgE myeloma is distinct from that of light chain disease and other myelomas, IgE should be measured in patients with clinical symptoms suggestive of myeloma and in whom myelomas of other isotypes have been ruled out.[603]

Total serum IgE concentrations have been reported to be abnormally high in a variety of diseases. In drug-induced interstitial nephritis or graft vs host disease, there may be a relationship among the course of the disease, response to therapy, and the IgE level, but none of these relationships are firm enough to recommend total IgE as part of the clinical evaluation of these diseases.[592]

Allergen specific IgE concentration

Summary Statement 129. The probability distribution of specific IgE for several anaphylactogenic foods (peanuts, egg white, cow's milk, and codfish) can define clinical sensitivity as verified by double-blind oral challenge tests; similar relationships have been defined for several respiratory allergens. (A)

Multiple studies have shown that allergen specific IgE is rarely detectable in cord blood.[592] In the few cases in which specific IgE for common allergens was detectable, neither diagnostic nor prognostic significance was demonstrated. Based on current information, there is no clinical indication for attempting to measure allergen specific IgE in cord blood. However, several investigations have shown that elevated food specific IgE in early infancy may predict respiratory sensitization at a later age.[604–607]

A recent study claimed virtually equivalent specific IgE sensitivity results between a blood spot test and serum.[608] The blood spot test was performed using paper-absorbed or -eluted blood obtained by finger prick.[608] Preliminary results suggested this was a successful method for determining IgE sensitization in preschool children. Prototypic, miniaturized, multiarray assays may offer a similar advantage in the future.[577,609]

Efforts to develop a screening procedure have led to tests in which multiple allergens are coupled to a single solid-phase substrate[560,610,611] (Table 5). If the multiple allergen test result is positive, there is a high probability that the patient is allergic to at least 1 of the allergens included in the test. Additional tests that use individual allergens then can be used to determine other allergens to which the patient may be sensitive. In general, these multiallergen screening tests have shown acceptable diagnostic sensitivity and specificity when compared with skin tests.[560,610,611]

The clinical value of multiple allergen screening tests depends on the selection of patients. In a symptomatic self-selected population, a positive test result would significantly increase the probability that the patient was allergic. If multiple allergen tests were used to screen an unselected population, there would be an unacceptable number of false-positive and false-negative results. By itself, a positive multiple allergen test result does not provide sufficient information to make a specific clinical diagnosis or to initiate therapy.[59] In addition, a negative multiple allergen test result does not exclude clinical sensitivity because the commercially-available multiallergen screening tests only screen for approximately 15 aeroallergens.

Recommendations concerning the number of specific IgE tests for confirmation of suspected clinical sensitivity correspond to those discussed for prick/puncture tests in Summary Statement 43.

There are no clinical scenarios in which immunoassays for allergen specific IgE can be considered either absolutely indicated or contraindicated. There are some situations in which immunoassays may be preferable to skin testing for the diagnostic evaluation of patients. If the patient has had a nearly fatal reaction to an allergen, the immunoassay offers the advantage of testing the patient for allergen specific IgE without the risk of inducing a severe reaction from a skin test.[612,613] In this situation, an IgE antibody measurement using immunoassay is less likely to provoke severe patient anxiety about the possible adverse consequences of a skin test. A positive IgE antibody test result strongly supports the clinical impression. A negative test result reduces the probability that the suspected allergen is causally associated, but it is essential that the negative result be confirmed by skin test before the allergen can be excluded as a possible anaphylactogen.[612,613] If both test results are negative, a supervised challenge may still be necessary. If a patient does not have a sufficient large area of normal skin to allow skin testing, immunoassays for specific IgE are useful for confirming clinical impressions.[550,564] Examples would include individuals with severe dermatographism, ichthyosis, or generalized atopic dermatitis. Theoretically, a third situation in which immunoassay may be preferable is during the refractory period immediately after a severe allergic reaction. If it were clinically necessary to determine the patient's sensitivity within a few days after such a reaction, an immunoassay might provide a better way to ascertain the necessary information.[550] Antihistamines and drugs such as tricyclic antidepressants reduce or block skin test reactivity[550] (Table 4). If it is necessary to document allergic sensitization either before the drug has been cleared from the patient's body or if it is inadvisable to stop taking the medication, an immunoassay may provide needed information. When a patient is unable to cooperate with skin testing because of mental or physical

impairment, measurement of specific IgE by immunoassay would be preferable because of reduced risk to an agitated patient or personnel who would normally perform the skin testing.

Quantitative results from clinical IgE antibody assays have allowed investigators to study whether the quantity of serum IgE antibody has any predictive utility in defining clinical sensitivity. In the area of food allergy, several groups have shown that the quantity of specific IgE antibody in serum to peanut, egg white, cow's milk, and fish may define current clinical sensitivity in many patients.[140,526,589–591,614] Probability-based risk of clinical food allergy increases as the quantity of serum food specific IgE increases. Probability curves can define, for some foods, levels at which reactions are highly likely (eg, 95%) and may dissuade the need for an oral food challenge. Thus, the higher the value, the more specific the test becomes in terms of clinical food allergy. Over interpreting values in the class 1 and 2 categories may lead to false assumptions. When levels are undetectable, 5% to 20% may still have reactions, and so the clinical history is important in interpretation of results.[589]

Probability-based risk evaluation has also been extended to respiratory allergy using quantitative allergen specific IgE antibody data previously reported from four European laboratories.[573] A logistic regression model was used to compare the relationship between the physician's final diagnosis of allergic respiratory disease (positive or negative) based on the clinical history, physical examination, and skin testing and serologic testing data and the quantitative level of serum IgE antibody alone. Probability curves were calculated in this study to show the relationship between IgE antibody in blood and the dichotomous clinical diagnosis of the absence or presence of allergic respiratory disease. The probability of obtaining a positive allergy diagnosis at a given serum IgE antibody level by the Pharmacia UniCAP System has been evaluated for different allergens at 4 clinics. Differences in the shape of the IgE antibody level vs probability of clinical disease curves was seen both between allergens within a clinic and between clinics for the same allergen specificity.[573] This indicates that use of specific IgE antibody levels to support the clinical diagnosis of respiratory allergic disease is different for the same allergist depending on the particular inhalant allergen and between allergists for the same allergen specificity. Importantly, however, the authors make the case that quantitation of serum IgE antibody improves the confidence of the clinical diagnosis of inhalant allergies better than simply knowing if IgE antibody is present or absent.

Another group also studied the clinical utility of quantitative serum IgE antibody measurements in the diagnosis of respiratory allergy.[574] They used purified recombinant timothy grass and birch pollen allergens to compare the relative ability of puncture skin testing, nasal provocation, and IgE antibody serology by the CAP System to reflect immediate-type respiratory sensitivity. Although the skin test and nasal provocation results were significantly correlated, the intensity of these biological reactions did not correlate with the level of allergen specific IgE antibody in serum. The authors concluded that factors in addition to IgE influence the extent of allergic tissue reactions.

A recent probability risk evaluation comparing skin tests and serum specific IgE to a panel of saprophytic mold aeroallergens revealed relatively poor correlations.[615] The results of this investigation confirmed the relatively low sensitivity rank order for fungi when evaluated by in vitro serologic tests.

Predictability of both skin and in vitro tests for IgE-mediated anaphylaxis to *Hymenoptera* venoms may also require reconsideration, especially if patients are tested at extended times after the anaphylactic episode. A recent investigation demonstrated relatively poor reproducibility of both venom skin tests and serum specific IgE when 35 patients, who had experienced systemic reactions, were tested on 2 occasions 2 and 6 weeks apart.[616]

Immunoassays for allergen specific IgE offer a unique advantage when compared with skin testing in their ability to use soluble allergen inhibition to examine specificity and cross-reactivity among allergens. Although these assays are used chiefly for research purposes, they may be clinically important in some situations. For example, if a patient has a history of anaphylaxis after an insect sting and the patient is found to be skin test positive to yellow jacket venom at a low concentration and positive to *Polistes* wasp venom at a higher concentration of venom, the question arises whether the patient is sensitive to both insects or whether skin test reactivity to wasp venom is the result of cross-reactivity. An inhibition assay showing that all the reactivity to *Polistes* wasp venom could be inhibited by yellow jacket venom strongly suggests that the positive skin test result to *Polistes* wasp was the result of cross-reactivity. Furthermore, the patient could be successfully treated with yellow jacket venom alone, saving the added expense of treating with *Polistes* wasp venom. Allergen cross-reactivity may also be clinically relevant when deciding how many species of weeds, grasses, trees, and mites need to be included in an immunotherapy regimen.

Allergen specific IgE measurements may be useful in evaluating fatalities that may have resulted from allergic reactions by determining the allergen responsibility for the fatal reaction.[612,613]

Allergic bronchopulmonary aspergillosis is an inflammatory disease of the lungs characterized by severe asthma, sputum production, peripheral blood eosinophilia, and an increased total serum IgE concentration. If untreated, it may progress to central bronchiectasis and, ultimately, pulmonary fibrosis and death. After proper treatment with corticosteroids, total serum IgE levels usually decrease. Total serum IgE should be followed during the disease since an increase in IgE may herald a relapse of disease. *Aspergillus* specific IgE and IgG are usually present in the sera of patients with ABPA. Although the levels of these antibodies do not always correlate with disease activity, they tend to decrease as active disease subsides.[596,617,618]

The initial immune response to many endoparasitic and ectoparasitic organisms is predominantly an IgE response. In many parasitic infections, an increase in both parasite specific IgE and total serum IgE concentrations occurs.[592] Detection and measurement of the parasite specific IgE (eg, *Toxoplasma gondii*) may be useful for diagnosis and potentially for following the course of the infection.[619] The major limitation of using assays for parasite specific IgE has been the availability of adequate allergosorbent preparations from the relevant parasite. Cell-mediated immunity may be an equally important pathogenetic factor in some parasite infections (ie, leishmaniasis). A recent report suggests that antimalarial specific IgE in asymptomatic individuals may be associated with reduced risk for subsequent clinical malaria.[620] It has been postulated that serum IgE may be a prognostic marker for AIDS in HIV-infected adults and that a switch to the T_H2 profile might represent a turning point in HIV.[621,622] Anti-HIV IgE found in the serum of certain long-term pediatric survivors is associated with inhibition of HIV-1 production, possibly through cytotoxicity rather than virus neutralization.[623]

Remote Formulation of Allergen Extracts

Several clinical laboratories offer nonallergists a service of preparing extract mixtures for allergen immunotherapy based on results of specific IgE tests. In some cases, the extract prescription is also based partially on a patient self-administered questionnaire. One study prospectively compared the results of allergy evaluations of 118 patients performed by a group of practicing board-certified allergists vs a laboratory that offered allergy diagnosis and recommendations for immunotherapy.[624] Although this study demonstrated that allergists identified allergy more frequently (53% vs 47%), they actually recommended immunotherapy less frequently than did the laboratory (35% vs 59%). The recommendations of the laboratory were deficient in that they were solely based on the history form and results. The laboratory was unable to clarify answers or to further explore areas that were suggested by patient responses or allergy testing results. On critical analysis of the laboratory-based extract recipes, it was found that the laboratory ignored the history forms and developed an extract formulation based solely on the results of antibody analysis in several cases. Overall, approximately 50% of the recommendations by the laboratory were inappropriate or incomplete. Even more serious errors could occur if the laboratory offering such a service had a record of poor or unsubstantiated quality control for performance of specific IgE tests.[563]

This form of allergen treatment is therefore not in the patient's best interest and should be discontinued.[624]

Allergen Specific IgG

Allergen specific IgG can be produced by persons either as a result of natural allergen exposure or as a result of immunotherapy.[625–629] Allergen specific IgG may have specificity for different allergen proteins or different protein epitopes than those eliciting an IgE response in the same person. There has been no convincing evidence that the quantity of allergen specific IgG produced as a result of natural exposure is related to or predictive of disease.[590,629]

Food specific IgG has been found in many healthy nonallergic individuals, and the quantity detected seems to depend on the quantity of the food ingested.[625,629] Precipitating antibodies to cow's milk are associated with Heiner's disease, hallmarks of which are pulmonary infiltrates that disappear after elimination of cow's milk.[630] No studies have convincingly demonstrated a relationship between the presence of food specific IgG antibodies and allergic disease (see "Unproven Tests"). By substituting an antibody specific for an IgG subclass for the antihuman IgG in the allergen specific IgG assay, it is possible to measure allergen-specific subclass distribution of IgG antibody responses.[586] A single study reported that persons having irritable bowel symptoms after ingestion of foods have increased levels of IgG4 antibody subclass to the offending food.[631] Unfortunately, high levels of the IgG4 subclass of food specific IgG antibody have not been consistent among studies so that the clinical value of measuring subclass specific IgG antibody remains to be determined.[625,632] Thus, IgG and IgG subclass antibody tests for food allergy have not been demonstrated to have clinical relevance, are not validated, lack sufficient quality control, and should not be performed.

Some investigators have shown that 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.[586,633,634] The value of measuring IgG antibody during or after immunotherapy with other allergens has not been demonstrated. Although allergen specificity may occur in each of the 4 IgG subclasses during allergen immunotherapy, there is conflicting evidence concerning the value of equating such antibodies with efficacy.[586,633,634] In the case of immunotherapy with insect venoms, there appears to be a modest relationship between the presence of elevated specific IgG4 for the venom and protection from anaphylaxis after an insect sting.[586,633–637] Other studies have not found any relationship between the quantity or specificity of allergen specific subclass IgG and the outcome of pollen immunotherapy.[564] The general predictive value of subclass IgG4 for successful immunotherapy is not proven at present.

Allergen specific IgG has been reported to be a potentially important biomarker of exposure to specific chemical allergens in the workplace.[638] Thus far, the predictive value for such antibodies and emergence of clinical disease in exposed workers has not been demonstrated.[638]

Immunoprecipitin tests to various causal proteins of hypersensitivity pneumonitis (eg, *Micromonospora faeni*, pigeon serum), allergic bronchopulmonary or sinus mycosis may be useful diagnostic adjuncts.[638–641] Panels of the most common etiologic agents of these diseases are validated and commercially available.[642]

In Vitro Methods of Allergen Standardization

Summary Statement 130. Although allergens can be standardized either by radioimmunodiffusion or immunoassay inhibition based on major allergenic epitopes, the FDA selected BAU instead because in vitro analytic techniques would have been variable from allergen to allergen and would have caused great confusion. (C)

Background

All medicines should have a label that describes quantity and potency. Traditionally, allergenic extracts have been labeled in weight to volume units or PNU, the quantity of phosphotungstic acid precipitable nitrogen. Since allergenic components are known to be a small percentage of the total protein, source material can be manipulated to maximize the content of proteins that contribute to the PNU value without regard to the allergenically active proteins. Consequently, these procedures yield extracts whose labeling cannot be relied on to express the allergenic activity of the contents. In 1970 a program was initiated in the FDA Laboratory of Allergenic Products to develop procedures that would permit a description of the allergenic activity of extracts as determined by comparison of laboratory and skin test reactivities.[59]

Current methods

The first extracts in which an attempt was made to provide lot-to-lot consistency were the insect venoms. These products are labeled in arbitrary units of hyaluronidase enzyme per 100 μg of protein. The next extract was that of short ragweed pollen. This was labeled in units of antigen E (Amb a 1) per milliliter (a unit of antigen E is approximately 1 μg). Amb a 1 was initially measured by a radial immunodiffusion test and currently is measured by an enzyme immunoassay inhibition test.

If this program had continued, there would have been as many types of analytical methods and labeled designations as there are extracts, a situation that would have resulted in considerable confusion in the use of these products. Therefore, a potency unit was developed, BAU, which is based on skin bioreactivity. After this is determined, each lot is evaluated by 1 or more laboratory tests that can be compared and expressed in BAU potency equivalents. In the future, all standardized extracts used in the United States will be verified by carrying out the bioequivalent FDA tests.

Procedures

Standardization methods of allergenic extracts for use as skin and specific IgE reagents involve a series of tests. All tests are described in detail along with statistical methods and variability in the *FDA's Manual of Methods of the Laboratory of Allergenic Products*.[124]

These methods include (1) identification of the source material used for extract production, (2) determination of a satisfactory procedure for preparing an extract, and (3) tests of the extract that include total protein, radial immunodiffusion test for a single allergen (eg, short ragweed antigen E [Amb a 1]) and cat allergen (Fel d 1), enzyme assay (eg, hyaluronidase in venoms), or immunoinhibition (pollen, mite and mold extracts). Where applicable, immunoblotting from polyacrylamide gel electrophoresis or isoelectric focusing techniques are performed to evaluate the allergenic identity of extracts and the number of IgE-binding proteins. Using an extract standardized by these methods, serum pools collected from sensitive patients can be evaluated to determine the validity and reproducibility of specific IgE tests.

Test variability

For the radial immunodiffusion method, the correlation coefficient of the reference dose response line should be at least 0.9. A single concentration for a test extract is reproducible to $\pm$25% when estimated from the calculated regression line.[642]

Immunoinhibition (RAST or ELISA) provides relative potency and consequently requires a reference preparation. The data are analyzed by means of parallel line statistics. Therefore, the frequently used methods of comparing extracts at extrapolated 50% inhibition values is without special meaning when this procedure is used for standardization of extracts. All validity assays must be included in the data statistical analysis protocol as described in detail in the *FDA's Manual of Methods*.[124] Prospective evaluation of the allergen standardization procedure was performed in the FDA laboratory and other laboratories by assessing more than 45 sets of data from 3 individual investigators. The variability is proportional to the number of test methods, all of which should be performed at least in duplicate. For 3 tests, the calculated variability was 47% to 213%, and for 5 tests, it was 56% to 180%.

Histamine and Leukotriene Tests

Summary Statement 131. Histamine and leukotriene release measurements from human basophils after incubation with allergen are valuable research tools for in vitro investigations of allergy. (B)

Summary Statement 132. The recent availability of several sensitive immunoassays for histamine and leukotriene C4 is a significant technological advance for measuring these mediators in various biologic fluids or release from whole blood, isolated basophils, mast cells, or other cultured cells. (B)

Summary Statement 133. Histamine and its N-methyl histamine metabolite may be measured in 24-hour urine samples after suspected anaphylactic episodes. (B)

Background

More than 75 years ago, Dale et al demonstrated the presence and physiologic action of histamine in different tissues.[643] Later work established that histamine in tissue is present in granules of cells and that in human tissue these granules are present only in basophils and mast cells. Over the years it was demonstrated that histamine or histamine-like material was released into the blood of experimental animals during anaphylactic reactions. This approach led to the demonstration that the addition of specific antigen or allergen to the blood or washed leukocytes of either experimentally sensitized ani-

mals or allergic persons can result in the release of histamine from basophils.

Basophils are the only cells in human peripheral blood that contain histamine. The interaction of specific allergen with the IgE antibody fixed to high-affinity Fcε receptors on the basophil membrane initiates release of preformed histamine and other inflammatory mediators associated with immediate hypersensitivity. The release of histamine is modulated by the addition of a number of pharmacologic agents. The addition of plasma or serum factors is not essential for the release reaction, although normal serum will enhance the release reaction when conditions are suboptimal. The serum of allergic persons also contains blocking IgG antibodies that also react with the allergen. When histamine release measurements are done with washed leukocytes, however, these antibodies probably do not influence the results.

Applications

Histamine release from human basophils is primarily a valuable research tool for in vitro investigations of allergy. In most studies of histamine release, allergen or antigen is added to washed leukocytes from venous blood. This can be simplified by eliminating the leukocyte preparation step and adding the allergen to whole heparinized blood.[644] The histamine released into the supernatant then can be determined directly. More recently, leukotriene C4 release has been monitored from basophils exposed to allergen as an indication of the presence of specific IgE antibody.[645,646]

In ragweed-allergic persons there is a good correlation between the severity of the clinical symptoms and the extent of in vitro histamine release.[647] The histamine release also correlates with the magnitude of the skin test and the level of serum IgE specific for ragweed Amb a 1. Both the antigen concentration at which 30% to 50% histamine release (cell sensitivity) occurs and the maximum percentage of histamine release (cell reactivity) correlate with the clinical severity of allergic rhinitis and the skin test. Patients with high levels of serum ragweed specific IgE release histamine with low concentrations of antigen. Similarly, in *Hymenoptera* venom–sensitive patients, there is good correlation between positive histamine release in vitro and the magnitude of the skin test with venom antigen.[648] This procedure is also being used to evaluate the functional characteristics of autoantibodies to IgE or the FcεR1 receptor in patients with chronic idiopathic urticaria (CIU).

Current Methods for Measuring Histamine

The discovery of histamine and the demonstration of its biologic importance were accomplished through the use of biologic assay systems dependent on the contractility of smooth muscle after the addition of this biologically active amine. This early technique has been superseded by chemical (fluorometric) and most recently, immunologic methods.

Chemical

A method for the chemical determination of histamine was first described by Shore et al in 1959.[649] Since then, this method has been modified to increase both its specificity and sensitivity. It is based on the coupling of ophthalaldehyde to histamine at alkaline pH to form a fluorescent product. The fluorescence of the histamine-o-phthalaldehyde complex is more intense and more stable at an acid pH, unlike the complex formed by some other amines. To remove interfering compounds, the histamine is extracted before the condensation step. Protein is removed from the sample to be analyzed by perchloric acid precipitation; the histamine is extracted into n-butanol from the alkalinized salt-saturated solution. The histamine is recovered in an aqueous solution in dilute hydrochloric acid by adding heptane. This dilute hydrochloric acid solution is then used for the condensation of histamine with o-phthalaldehyde. The extraction procedure with organic solvents is essential to remove histidine and other interfering compounds before the condensation step. A completely automated fluorometric technique is capable of analyzing 30 samples per hour with a precision between 1% and 2%.[650] The sensitivity of the method is such that 0.1 to 10 ng/mL of histamine can be accurately determined. This method is convenient in handling large numbers of samples with excellent precision. The methods for both the manual and automated histamine analysis method have been described in detail.[549,650]

Immunoassay

The fluorometric assay has technical requirements that minimize its use to research laboratories. Simpler assay methods have recently been developed that use antibodies to histamine or histamine analogs, and the reagents are available in commercially available kits.[651] A variety of immunoassay kits are available. Many of these are competitive inhibition assays, and most use monoclonal antibodies.[652] As with other immunoassays, the methods used are sensitive, reproducible, and easy to perform.[653]

Interpretation

Histamine release results are expressed as a percentage of total cellular histamine determined after incubation with a calcium ionophore or boiling an aliquot of cells. Control measurements include the histamine released in the absence of added antigen, and this value is subtracted to calculate the specific release. In most experiments, the nonspecific "blank" release should be less than 10% of the total cellular histamine. High spontaneous release of histamine from washed leukocytes has been reported in a small percentage of patients who are highly atopic or sensitive to food.[654] The significance of that finding is not clear.[654] Appropriate controls should also include the testing of the allergen with the cells of nonallergic donors to demonstrate that the allergen does not contain any cytotoxic materials or histamine itself. Similarly, allergens or pharmacologic agents should be tested to see whether they influence the histamine assay procedure nonspecifically and contribute to erroneous results.

Histamine release results can be conveniently expressed by 2 parameters: (1) cell sensitivity: this is the concentration of

antigen or allergen expressed in units (preferably micrograms per milliliter) required to release either 30% or 50% of total cellular histamine; and (2) cell reactivity: this is the maximal amount (percentage) of histamine release obtained with any amount of the antigen.

A positive control in histamine experiments should be the addition of different dilutions of an anti-IgE antiserum to the cells. In general, the cells of most persons release more than 10% histamine after challenge with anti-IgE.

The number of false-positive reactions to allergens determined by histamine release is low. These are defined as subjects having a negative skin test result and a positive histamine release result with an allergen.

The incidence of false-negative reactions is a more critical factor in the interpretation of histamine release tests. Some patients have little histamine release at any concentration of allergen, but nevertheless are sensitive by skin tests. Even under the best of circumstances, for example, skin test studies with pure venom antigens, there is a significant number of people who have positive skin test results and appear to be allergic by a convincing history but fail to release histamine after challenge with appropriate allergens. The percentage of these persons may be as high as 10% to 15%. This raises the issue of how to interpret a negative test result. Cells from patients with CIU frequently do not release histamine. Desensitization of patients may also result in changes in the degree of histamine release from leukocytes. Changes attributable to immunotherapy are variable and inconsistent.

Significance

Histamine release from leukocytes of allergic persons is an excellent in vitro correlate of allergy. At present, it is primarily considered a research test and is not widely available from clinical immunology laboratories. However, in rare instances it may have confirmative value in assessing the presence or absence of allergy. In vitro histamine release can be a useful adjunct by supplying quantitative data on the degree of allergen specificity. Therefore, it can be compared with in vitro serologic methods using direct and inhibition techniques. Both of these assays suffer from the occurrence of false-negative results, that is, patients who are clinically sensitive but exhibit negative findings to these tests.[655] The serologic measurement of IgE antibody has the following advantages: (1) it requires a small amount of serum, (2) samples can be stored and processed at a central laboratory, and (3) the techniques involving immunoassay are well established and FDA cleared. In contrast, histamine release requires a larger blood sample, it must be performed within a relatively short time after the sample of blood is obtained, and the techniques are more complicated and not FDA cleared. The advantages of histamine assays are that they require smaller amounts of allergen, unlike skin testing they do not involve injection of allergen into the subject, and they are not dependent on coupling antigens to immobilized support systems with the inherent problems of antigen modification or unavailability of binding sites. By contrast, using washed leukocyte experiments, there is no competition between IgG and IgE for antigenic binding sites, and, therefore, IgG cannot interfere in the release assays as sometimes is the case with IgE antibody serology.

Histamine and its metabolite, N-methyl histamine, may be measured in urine samples (usually 24-hour collection) after a suspected anaphylactic episode or evaluation of suspected mastocytosis.[636,637] Plasma histamine is more likely to be elevated in patients who present to the emergency department with acute allergic syndromes than tryptase.[656–658]

Plasma Tryptase

Summary Statement 134. Plasma tryptase, particularly the β form, should be obtained within 4 hours after an anaphylactic episode. (B)

Summary Statement 135. Combined α and β species of plasma tryptase are elevated in patients with systemic mastocytosis. (A)

Mast cells that have been activated during an IgE-mediated hypersensitivity reaction release proteases and prestored histamine and newly generated vasoactive mediators into surrounding soft tissue. Tryptase (molecular weight, 134,000 kDa) is a neutral serine esterase with trypsin-like substrate specificity that is found in relatively large quantities in mast cells (approximately 10 pg per lung mast cell and up to 135 pg per skin mast cell). It is stored in the secretory granules as an active enzyme complexed to and stabilized by heparin. Although several forms have been described (I, IIβ, IIIα, T) and all are found in mast cells, only the β and, less frequently, α species are clinically relevant. Since modest amounts are found in basophils (less than 1% than found in tissue mast cells), tryptase is considered to be a good clinical marker of mast cell activation.[657,659] When dissociated from heparin, tryptase rapidly degrades into its monomers and loses enzymatic activity.

Immunoreactive tryptase levels in serum of healthy adults are less than 5 μg/L. Elevated levels of tryptase ($>$10 μg/L as measured by immunoassay) can be detected in serum from 1 to 4 hours after the onset of systemic anaphylaxis with hypotension.[660] In some cases, tryptase may reach levels as high as 1 mg/L. Recommended serum collection times for a serum tryptase are 30 minutes to 4 hours after the onset of an acute event. Although postmortem specimens are difficult to analyze for tryptase due to gross lysis of cells, levels of approximately 10 μg/L in these specimens have been considered abnormal. Elevated tryptase can be detected usually within 15 to 30 minutes after an allergen challenge, and it declines with an approximate half-life of 2 hours. This is in contrast to histamine, which peaks more quickly within 5 to 10 minutes after an event and may return to baseline levels in less than 1 hour. Tryptase has also been detected in BAL fluid, nasal lavage fluid, tears, and skin chamber fluid, but there are currently no clinical indications for such measurements. Like histamine, β-tryptase is released from mast cells within 15 minutes after in vitro degranulation and is the predominant form detected 1 to 2 hours in the serum after

Table 6. Human Cytokine Families and Subfamilies of Special Interest to Allergy/Clinical Immunology

Family	Subfamily	Source	Major function
I. IFN			
Type 1			
IFN-α		N	Activates NK, B; antiviral activity
IFN-β		F, many other cells (virus induced)	Activates NK; antiviral
Type 2			
IFN-γ		T, MC	Activates M/MA, DC, F, T, B; induces MHC class II molecules; up-regulates IgG and CMI; downregulates IgE
	IFN-γ 2 IL-28A	Plasmacytoid DC	Antiviral; up-regulates MHC class I
	IL-28B	Plasmacytoid DC	〃
	IFN-γ 3 IL-29	Plasmacytoid DC	〃
II. TNF			
TNF-α		M/MA, K, EC, MC	Potent T activator similar to IL-1α /β (with few exceptions); induces IL-1 and IL-6
TNF-β		T	Fever, anorexia, wasting, acidosis, hypotension/shock, leukocytosis
	LT-α	B, T, NK	Lymphoid development
	LT-β	B, T, NK	Lymphoid development
	Fas L	T, testis, eye	Apoptosis
III. CSF			
GM-CSF		T, E, MC, many others	Activates hematopoietic cells, M, MA, cytotoxic N, inhibits chemokinesis; perpetuation of eosinophilic inflammation
G-CSF		T, many others	Activates immature N
M-CSF		T, many others	Activates immature M/MA
IL-11 (megakaryocyte-CSF)		BM, stromal cells, mesenchymal cells	Megakaryocytopoiesis
IV. IL			
IL-1α		M/MA, NK, B	Activates T, B, NK, N, EC, F, and other cells; cytotoxic
IL-1β		Langerhans cells, K, EC, ME, SMC, and others	Melanocytes, pancreatic B cells; fever, anorexia, leucocytosis, slow-wave sleep; acute phase protein induction; a variety of metabolic interactions
	IL-18	Variety of cells	Induces IFN-γ production by T, NK; proinflammatory; may be a cofactor in T_H2 inflammation
IL-2			Promotes T, B, NK growth, tumor surveillance
	IL-7	MB, K, thymus stromal cells	Promotes pre-B development; MK maturation; immature and mature T growth
	TSLP	K, EP, SM, F, MC	Master switch of allergic inflammation at the EC-DC interface
	IL-15	Fetal astrocytes in response to IL-1β, IFN-γ , or TNF-α	Similar to IL-2; T-CMI immune responses in CNS; induces cytolytic and LAK cells in vitro
	IL-21	Activated CD4* T cells	Costimulates B-cell proliferation with CD40; T, NK stimulation; proliferation of bone marrow progenitor cells
IL-3		T, MC, K, NK, EC	Proliferation and differentiation of N, MA, MK, MC; histamine releasing factor
IL-4		T_H2 CD4*T, MC, B	Promotes T (T_H2) and B-cell growth; isotypic switch for production of IgE

Table 6. Continued

Family	Subfamily	Source	Major function
IL-5		T, MC, E	Growth and differentiation of eosinophils; promotes B activation and production of IgM and IgA
IL-6		T, B, M/MA, F, EC, MC BM stromal cells, thymocytes, pancreatic islet cells, neoplastic cells	Similar to IL-1β; inflammation; mediator of acute phase reaction; T-cell growth, maturation of B to plasma cells; development of trophoblasts
IL-8 (CXCL8); this is a chemokine (see Table 7)			
IL-9		Activated T_H2 cells; Hodgkin lymphoma	Promotes MC and B growth
IL-10		T_H2 subpopulation; activated CD8 cells	Immune inhibitor (down-regulates IFN-γ); cofactor for proliferation of thymocytes and B
IL-19		Activated M, B	Induces IL-6 and TNF-α by M; induces apoptosis and reactive oxygen species by M; induces IL-4; IL-5, IL-10, IL1β by activated T; pathogenesis of asthma
	IL-20	M, K	Autoregulation of K function, differentiation and proliferation
	IL-22	NK, $CD4^+$ T_H1; induced by IL-9 in thymic lymphomas, T, MC	Proinflammatory; induces synthesis of acute phase proteins
	IL-24	M, SM, NK, B, naive T, melanocytes, breast epithelium	Induces IL-6 and TNF-α by monocytes; megakaryocyte differentiation; apoptosis of breast cancer cells
	IL-26	$CD4^+$ $CD45^+$ ROT; NK, T_H1	Induces secretion of IL-8, IL-10, and expression of ICAM-1
IL-11 (see under III CSF)			
IL-12		M/MA, DC	Regulates CMI immune response; stimulates NK; induces IFN-γ production, cell proliferation and cytotoxicity mediated by NK, T; induces T_H1 responses
	IL-23	Activated DC	Proinflammatory; induces proliferation of memory T; moderates levels of IFN-γ production by memory and naive T; together with IL-17, induction of autoimmune disease
IL-13		Activated $CD8^+$ and $CD4^+$ T_H2, MC, NK	Enhances mucus production; induces SM hyperresponsiveness; activates airway stromal cells to produce eotaxin; with IL-4 enhances IgE production
IL-14		T and some B lines	Mitogen for activated B; selectively expands certain B-cell subpopulations; inhibits immunoglobulin secretion
IL-16 (lymphocyte chemoattractant factor)		Activated $CD8^+$ T, F, E, MC, EP	Chemoattractant for $CD4^+$ T, MA, E; suppresses HIV replication
IL-17		Activated $CD8^+$ T, T_HI7 T; CD4 memory T	Proinflammatory; enhances T priming; stimulates F, EC, MA, and EP to produce proinflammatory mediators; with IL-23, induces autoimmune disease; increased in autoimmune diseases and asthma

Table 6. Continued

Family	Subfamily	Source	Major function
	IL-25	Polarized T_H2 cells, BM stromal cells	Supports L proliferation; supports T_H2 effects through induction of IL-4, IL-5, IL-13; induces serum IgE; increases E production and inflammation
IL-27		Mature DC	Induces proliferation of nave (not memory) T; initial activator of T_H1 responses
IL-30		Activated APC	
IL-31		Activated T	Allergic reactions; dermatitis
IL-32		Activated T and NK	Inflammatory; induces production of TNF-α , IL-8, and MIP-2
IL-33	IL-1	Endothelial cells	Induces TH2 cytokines; induces proinflammatory medicators in most cells
TGF-β		M/MA, L, P, virus-infected cells	Activates M, P, F, EC; chemotactic for M, F; inhibits T, B; crucial in airway remodeling; proliferation of F
	Activan A	$CD4^+$ T, infiltrating L and structural cells of the lung	Associated with early allergen-dependent activation of the immune system; associated with more severe asthma; is linked to and induces TGF-β
SCF		Embryonal cells	Growth of MC from hematopoietic precursors; primordial cell development
MIF		Antigen and mitogen-activated L	Proinflammatory; correlate of CMI diseases; associated with autoinflammatory and autoimmune diseases; elevated in asthma

Abbreviations: CMI, cell-mediated immunity; CNS, central nervous system; DC, dendritic cell; EC, endothelial cell; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; MC, mast cell; MHC, major histocompatibility complex; MIF, macrophage inhibitory factor; MIP, macrophage inflammatory protein; M/MA, monocyte/macrophage; SCF, stem cell factor; SM, smooth muscle; TGF, transforming growth factor; TNF, tumor necrosis factor; NK, natural killer cell; T, T cell; B, B cell; K, killer cell; E, eosinophil; BM, bone marrow; ME, melanocyte; MK, megakaryocyte; EP, epithelial cell; MB, IgM B cell; P, platelet; APC, antigen presenting cell; L, lymphocyte; TSLP, thymic stromal lymphopoietin; T_H, T helper; LT, lymphotoxin; FasL, Fas ligand; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte/macrophage colony stimulating factor; LAK, lymphokine activated killer cell; N, neutrophil; F, fibroblast.

anaphylaxis. However, since α-tryptase is spontaneously secreted from mast cells and often elevated in mastocytosis patients during mast cell burden or activation, quantifying serum ratios of α and β provide the best indication of mast cell activation by a specific allergen.[660–662] Most commercial laboratories report results as a combination of α and β forms. (β tryptase levels can be obtained from the Division of Immunology, University of Virginia Medical College, Charlottesville.)

Eosinophils, Eosinophil-Derived Substances, and Chemoattractants.

Summary Statement 136. Eosinophils in body fluids correlate highly with the diagnosis of allergic rhinitis, allergic asthma, and eosinophilic bronchitis. (B)

A recent investigation demonstrated that eosinophils in the nasal smear correlate best with active allergen exposure symptoms, positive prick/puncture skin tests, specific serum IgE, release of inflammatory cytokines, spirometry, and methacholine responses.[302] There is also increased appreciation in the clinical utility of sputum eosinophils for diagnosis of asthma and eosinophilic bronchitis.[663]

Summary Statement 137. Elevated eosinophil derived substances (ie, ECP) and chemoattractants (ie, eotaxin) in body fluids are indicators of allergic inflammatory disease. (B)

Eosinophils are key cells in allergic inflammation. Eosinophilic cationic protein is a basic protein that can be detected in the granules of the eosinophil in different forms, with molecular weights ranging from 18.5 to 22 kDa.[664] Elevated levels of ECP have been detected in the serum, sputum, and nasal secretions of individuals undergoing a late-phase allergic reaction (usually 6–24 hours after exposure), when an eosinophil influx is predominant at the reactive site.[665,666] Levels of immunoreactive ECP detected in the serum of 100 healthy subjects ranged from 2.3 to 16 μg/L (95% range,

Table 7. Human Chemokine Receptor and Ligand Families of Special Interest to Allergy/Clinical Immunology

Family receptor	Receptor	Ligand/agonist	Source	Major function
I. CC				
	CCR1	CCL3/MIP-1α CCL5/RANTES CCL7/MCP-3	M, L, N, E, MC, ME T, M, F, ME, P, M, MC, F, EC, EP	T, M migration; innate and adaptive immunity, inflammation
		CCL8/MCP-2	M, F	
		CCL13/MCP-4,	DC, EP, lung, thymus	
		CCL14–16/HCC1–3	Intestine	
		CCL23/MPIF	DC, M, lung, liver	
	CCR2	CCL2/MCP-1 CCL7/MCP-3, MARC	M, L, EC, EP, N, MC, G, ME, DC, F	T, M migration; innate and adaptive immunity; T_H1 inflammation
		CCL8/MCP-2	M, F	
		CCL13/MCP-4	DC, EP, lung, thymus, intestine	
	CCR3	CCL5/RANTES	T, M, F, ME	E, B, T migration, allergic inflammation
		CCL7/MCP-3, MARC	P, M, MC, F, EC, EP	
		CCL8/MCP-2	M, F	
		CCL11/Eotaxin-1	EC, EP, E, lung	
		CCL13/MCP-4	DC, EP, lung, thymus, intestine	
		CCL15/HCC-2, MIP-5	DC, M, T, B, NK	
		CCL24/Eotaxin 2	M, T, lung, spleen	
		CCL26/Eotaxin 3	EC, heart, ovary	
	CCR4	CCL17/TARC	DC, M, EP, F, SM	T, M migration; allergic inflammation
		CCL22/MDC	DC, M, B, T, NK, EP	
	CCR5	CCL3/MIP-1α CCL4/MIP-1β CCL5/RANTES	M, L, N, E, MC, ME M, L, N, E, F, MC, BA, NK T, M, F, ME	T, M migration; innate and adaptive immunity; HIV infection
		CCL8/MCP-2	M, F	
		CCL14/HCC-1	Bone marrow, gut, spleen, liver, SM	
	CCR6	CCL20/MIP-3α, LARC	M, T, N, EC, liver, lung, thymus, placenta appendix	Dendritic cell migration
	CCR7	CCL19/MIP-3β	N, lymph node, spleen, thymus, intestine	T, DC migration; lymphoid development; primary immune response
		CCL21/SLC	EC, lymph node	
	CCR8	CCL1/Groα	M, T, MC	T trafficking
		CCL4/MIP-1β	M, L, N, E, F, MC, BA, NK	
		CCL17/TARC	DC, M, EP, F, SM	
	CCR9	CCL25/TECK	DC, EP, EC, gut	T homing to gut
	CCR10	CCL26/eotaxin-3	EC, heart, ovary	T homing to skin
		CCL27/CTACK, ILC	K, placenta, skin	
		CCL28, MEC	EP, EC	
III. CXC				
	CXCR	CXCL8/IL-8, NAP-1, MDNCF, MIP-2	EC, N, P, G, ME, BA, NK	N migration; innate immunity; acute inflammation
	CXCR2	CXCL1–3/Groα, Groβ	M, N, EC, F, M, N, EC, F	N migration; innate immunity; acute inflammation;
		Groγ	M, N, EC, F	Angiogenesis
		CXCL5–8/ENA, GCP, NAP-2	EC, P, E, F, M (thymus)	
	CXCR3	CXCL9–11/MIG, 1P-10, I-TAC	M, N, K, N, F, EC, G	T migration; adaptive immunity; T_H1 inflammation

Table 7. Continued

Family receptor	Receptor	Ligand/agonist	Source	Major function
	CXCR4	CXCL12/SDF-1, PBSF	EC, EP, lung	B-cell development; myeloid cell development
	CXCR5	CXCL13/BLC, BCA-1	EC, M, DC, lymph node, spleen	B trafficking; lymphoid development
	CXCR6	CXCL16	B, M, DC	T migration
	?	CXCL4/PF4	P, MK	Inflammation
IIII. CX3C				
	CX_3CR1	CX3CL1/FRACTALKINE	APC, DC, EC, T, SM	T, NK trafficking and adhesion; innate and adaptive immunity, T_H1 inflammation, MC chemotaxis
IV. XC				
XCRI				
		XCL1/ lymphotactin a, SCMIα	T, MC, NK	T trafficking
		XCL2/ Lymphotactin b, SCMIβ	T, NK, spleen	

Abbreviations: Same as Table 6; BA, pro-B cell line.

geometric mean of 6 μg/L). The ECP measurements have limited clinical utility as an analyte for monitoring patients with extrinsic asthma and other allergic diseases in which eosinophils may play a role in tissue damage.[666–672]

Several eosinophil chemoattractants (eg, IL-5, eotaxin) are elevated in nasal and BAL samples in patients with active allergic inflammatory disease associated with recent or concurrent exposure to aerogenic allergens.[303,304,672]

Basophil Activation Test

Summary Statement 138. A basophil activation test measured by expression of CD63 and CD203c and detected by flow cytometry is being evaluated for many IgE-mediated disorders. (C)

The degree of basophil activation based on the expression of CD63 and recently CD203c is determined by flow cytometry.[672] It has been evaluated in IgE-mediated pollen, food, drug, *Hymenoptera* venom, and latex reactions.[673–681] Sera of patients with CIU also demonstrate basophil-activating autoantibodies.[682] High sensitivity and specificity have been observed in most of these studies. Recently, a CD63 sensitivity assay was shown to be useful in monitoring clinical effects in patients who receive omalizumab (Xolair) treatment.[683]

IN VITRO DIAGNOSTIC TESTS OF CELL-MEDIATED IMMUNITY

Background and Present Application

Summary Statement 139. Tests that quantify lymphocyte function measure the ability of lymphocytes to (1) proliferate, (2) produce inflammatory mediators and cytokines or chemokines, (3) mount cytotoxic responses, and (4) regulate immune responses. (B)

Summary Statement 140. Lymphocyte proliferative responses may be evaluated by either nonspecific mitogens (eg, phytohemagglutinin, concanavalin A, or pokeweed) or specific soluble and cell-bound antigens. (B)

Summary Statement 141. 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. (B)

Summary Statement 142. Cytokines (IL-1 through IL-33) and growth factors are glycoproteins produced by a variety of cells that are capable of altering activities of other cells through interaction with specific surface receptors. (E)

Summary Statement 143. Chemokines are small (8 to 10 kDa) proteins secreted by many immune and nonimmune cells with essential roles in inflammatory and immune reactions, including the late-phase cutaneous response. (E)

Summary Statement 144. Cytokine and chemokine profiles play essential roles in allergic inflammation and are being increasingly evaluated as phenotypic markers and in the differential diagnosis of human hypersensitivity disorders. (B)

The cell types that contribute to the cellular hypersensitivity reaction include lymphocytes, macrophages, dendritic cells, Langerhans cells, and granulocytes. In vitro tests of cell-mediated immunity may be used to evaluate (1) cellular function in patients who may have recurrent or multiple serious infections (eg, fungal, mycobacterial and protozoan); (2) depressed cellular immunity (eg, acquired immune deficiency syndrome, sarcoidosis, and cancer); (3) certain cases of drug hypersensitivity; (4) chemical hypersensitivity (eg, toluene diisocyanate, beryllium); (5) autoimmune diseases (eg, rheumatoid arthritis, Guillain-Barré syndrome, chronic hepatitis, and thyroiditis); and (6) many other inflammatory entities.

Table 8. Common Autoantibodies and Corresponding Autoimmune Diseases[a]

Autoantibody	Disease	Prevalence, %[a]
ANA	SLE	95–98
Anti–double-stranded DNA	SLE	50–80
anti-Sm	SLE	15–20
Anti-C1q[b]	SLE nephritis	97
Anti-RNP	MCTD	30–40
Anti-histone	Drug-SLE	70
Anti-Ro/SS-A	Sjögren's syndrome	30–90
Anti-La/SS-B	Sjögren's syndrome	15–20
Rheumatoid factor	RA	80
Anti-CCP	RA	99
Anti-Centromere	CREST	80
Anti-Scl 70	Systemic sclerosis 70	

[a]Adapted from D'Cruz D. Testing for autoimmunity in humans. *Toxicol Lett.* 2002;127:93–100.

[b]Trendelenburg M, Lopez-Trascasa M, Potlukova E, et al. High prevalence of anti-C1q antibodies in biopsy-proven active lupus nephritis. *Nephrol Dial Transplant.* July 28, 2006 (Epub ahead of print).

Tests that quantify lymphocyte function detect 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 can be evaluated by the use of nonspecific mitogenic stimulants such as phytohemagglutinin, concanavalin A, or pokeweed mitogen and by specific stimuli such as soluble and cell bound antigens. The nonspecific activation of lymphocytes measures both T (ie, phytohemagglutinin, concanavalin A) and B (ie, pokeweed mitogen) cell function, although the kinetics of these responses differ. In contrast, specific antigenic challenge appears to measure only T-cell function. In addition, by using autologous and homologous serum in the cultures, one can also determine whether the patient's serum contains factors that may interfere with or enhance the proliferative response.

Cytokines and growth factors are glycoproteins produced by a variety of cells that are capable of altering activities of other cells through interaction with specific surface receptors.[684–694] They are secreted by lymphocytes, macrophages, epithelial cells, and a variety of effector cells (eg, eosinophils, mast cells) among others. They have significant growth differentiation and activation functions on contiguous or distant cells and tissues. There are 4 major families of cytokines that have been identified: (1) interferons, (2) colony-stimulating factors, (3) TNFs, and (4) ILs (Table 6). The last is divided into subfamilies, which consist of an expanding list of new cytokines, now numbering from IL-1 to IL-33. Historically, cytokines have been called lymphokines if they were produced by lymphocytes or monokines if they were produced by monocytes or macrophages. Many cytokines produced by lymphocytes have also been termed *interleukins* even though most of their functions are not restricted to between cells. Both immune and nonimmune cells produce chemokines and smaller proinflammatory proteins (Table 7).

The elaboration of cytokines or chemokines by lymphocytes and monocytes indicates that these cells are capable of producing factors that are involved in both afferent and efferent limbs of the cellular hypersensitivity response.[695,696] Proinflammatory cytokines and chemokines derived from activated macrophages (eg, TNF-α, IL-6, IL-8, IL-12, transforming growth factor β1, MIF, RANTES, eotaxin, MCP-1, MCP-2, MCP-3, MIP-1, MIP-2, and MIP-3) have diverse activating and chemotactic properties.[697] One of the lymphocyte- or macrophage-derived inflammatory mediators, macrophage MIF, has an essential role in the expression of cell-mediated immunity and a number of inflammatory diseases.[698–707] In the past, MIF and its correlate, leukocyte inhibitory factor, were shown to correlate with in vivo delayed-type hypersensitivity skin reactivity, although they do not necessarily measure the function of a particular cell type (T or B cell).[708,709]

The ability of lymphocytes to act as cytotoxic killer cells in response to either allogeneic, target, or malignant cells has clinical relevance in patients undergoing a transplant procedure and patients with cancer. T-cell regulation of immunoglobulin synthesis or antibody production, as well as lymphocyte proliferation, also has clinical application. Excessive or diminished regulation of these immune responses can result in disorders associated with humoral immunity, cell-mediated immunity, or both. In 1960, Nowell described that phytohemagglutinin, a lectin extracted from kidney beans, nonspecifically transformed small lymphocytes into proliferating lymphoblasts in vitro.[710] Subsequently, in addition to plant lectins that activate all normal T cells, it was shown that a variety of antigens could also induce proliferation.[711] This occurred, however, only in those persons who had positive delayed skin test reactions to these antigens. In vitro proliferation to some soluble antigens, but not to mitogens, has been shown to be a good correlate of specific in vivo delayed-type hypersensitivity. However, the role of lymphocyte proliferation in the pathogenesis of delayed-type hypersensitivity skin reactions is unclear.

On the basis of what is known about the biologic activities of cytokines and chemokines, these factors appear to be better candidates for investigating pathogenesis. In fact, when these in vitro tests are correlated with skin testing in normal subjects and in patients with diseases associated with defects in delayed-type hypersensitivity, lymphokine levels more often closely parallel the results of delayed skin tests than does lymphocyte proliferation.[712] Effector lymphokines, particularly MIF, therefore are assuming greater significance as in vitro correlates of delayed-type hypersensitivity.

Antigen-induced inhibition of cell migration has been used as a bioactivity index of delayed-type hypersensitivity since its original description in 1932. Development of the capillary tube method for measuring macrophage migration has facilitated the significance of migratory inhibitors, particularly MIF.[713] The latter was first described by David, Bloom, and Bennett[714,715]; it is a protein produced by sensitized lymphocytes after activation by specific antigens or mitogens. In

Table 9. Autoantibodies Associated with Systemic Vasculitis[a]

Autoantibody	Vasculitis	Specificity	Prevalence, %
c-ANCA	Wegener's syndrome	Proteinase-3	95–98
p-ANCA	Churg-Strauss, microscopic polyangiiitis, overlap syndromes	Myeloperoxidase, elastase, lactoferrin cathepsin G	50–90
Anti-GBM	Goodpasture syndrome	Type IV collagen	60–75
Anti-C1q[b]	Hypocomple mentemic urticarial vasculitis	Collagen-like region C1q	50–90

Abbreviations: c-ANCA, antineutrophil cytoplasmic antibody; GBM, glomerular basement membrane; p-ANCA, peripheral nuclear antineutrophil cytoplasmic antibody.

[a] From D'Cruz D. Testing for autoimmunity in humans. *Toxicol Lett.* 2002;127:93–100.

[b] From Wisnieski JJ, Jones SM. Comparison of autoantibodies to the collagen-like region of C1q in hypocomplementemic urticarial vasculitis syndrome and systemic lupus erythematosus. *J Immunol.* 1992;148(5):1396–403.

initial studies, human MIF exhibited heterogeneity in its molecular weight, isoelectric point, and glycosylation.[716] Some of the heterogeneity was found to be due to contamination with other cytokines (ie, interferon-γ, IL-4, and TNF-α also having macrophage migration inhibitory activities) in conditioned media samples from stimulated lymphocytes.[717] A small gene for purified, monomeric MIF, which has a molecular weight of 12,000 kD and does not share homology with IL-4 or interferon-γ, has been cloned.[717,718] Further, MIF protein has been crystallized and analyzed by x-ray diffraction, thereby confirming that it is clearly distinguishable from other cytokines with migration inhibitory activities.[719] Native and recombinant human MIF are biochemically and bioactively identical.[720] Interestingly, MIF is also secreted from anterior pituitary cell lines.[721]

Leukocyte inhibitory factor, a related but higher-molecular-weight cytokine originally isolated by molecular sieve chromatography, was found to inhibit neutrophil but not macrophage migration.[722] Similar to MIF, it was antigen specific and associated with delayed-type hypersensitivity. However, its significance was overshadowed by MIF, and it is no longer used as a bioactivity index of delayed-type hypersensitivity.

Extensive research of purified MIF has fully established its importance as a critical T-cell proinflammatory cytokine in a diversity of human diseases, including rheumatoid arthritis, sepsis, the systemic inflammatory response syndrome, renal infections, blunt trauma, renal allograft reaction, diabetes, and atopic dermatitis.[698–723] Recently, a significant association between mild asthma and the low expression, 5-CATT MIF allele, suggests that MIF may also play a role in asthma via promotion of T_H2 responses.[724] As a result of these associations, the bioactive profile of in vitro cell-mediated immunity may now be estimated by both functional and biochemical assays of MIF.

Current Methods

Summary Statement 145. Other bioactive indices of cell-mediated immunity include cytotoxic assays, cultures of mixed lymphocytes, and macrophage inhibition. (E)

Summary Statement 146. Most cytokines and chemokines can be measured by commercial ELISA and ELISpot immunoassays. (E)

Summary Statement 147. Proinflammatory cytokines or chemokines, which are particularly associated with cell-mediated immunity, include interferon-γ, IL-12, tumor necrosis factor α (TNF-α), IL-16, MIF, macrophage inflammatory protein 1 (MIP-1), and MCP 1, 2, and 3. (B)

Functional Assays

Lymphocyte activation and proliferation

Nonspecific assays are performed to evaluate the general responsiveness of peripheral blood mononuclear cells (PBMCs) or isolated T cells (10^6 per well).[725] Appropriate polyclonal reagents consist of phytohemagglutinin (2 μg per well), concanavalin A (2 dilutions [100 and 50 μg per well]), and pokeweed mitogen (20 μL [1:200] per well). Phytohemagglutinin and concanavalin A primarily test T-helper cell function, whereas pokeweed mitogen stimulates B cells.

Varying dilutions of recall antigens (*Candida, Tetanus* toxoid, and trichophyton) or other specific antigens are added to test wells containing isolated lymphocytes in accordance with an optimal dose response protocol. In both specific and nonspecific assays, unstimulated lymphocytes serve as controls.

Each well is pulsed with 0.4 μCi of ^{3}H thymidine.[726] Plates are incubated at 37°C in a humidified carbon dioxide incubator and followed 0 hours for spontaneous blastogenesis, 3 days for phytohemagglutinin and concanavalin A, 5 days for mixed lymphocyte culture assay, and 6 days for pokeweed mitogen and specific soluble antigens. After an additional ^{3}H thymidine pulse for 4 hours, cells are placed on a glass fiber mat using a cell harvester, scintillation fluid is added, and cells are counted for 1 minute. Proliferative responses are reported as mean net counts per minute (cpm) (experimental cpm − control cpm) or preferably as a stimulatory index (experimental cpm ÷ control cpm).[726]

To circumvent the use of using radioactive reagents, nonradioactive alternative methods have been developed. One of these is based on the incorporation of a pyrimidine analog, 5-bromo-2[1]-deoxyuridine (BrdU) instead of thymidine into the DNA of proliferating cells. After suitable incubations, as described herein, BrdU is detected by standard or chemilu-

Table 10. The Major Clinically Relevant Aeroallergens of North America[a]

Tree pollen
Chinese elm *(Ulmus parvifolia)*[b,c]; Siberian elm (*Ulmus pumila)*[b,c]; elm (*Ulmus americana)*[b,c]
Red oak (*Quercus rubra)*[b]; white oak (*Quercus alba)*[b]
Paper birch (*Betula papyrifera)*
Alder (*Alnus rubra)*
Box elder *(Acer negundo)*[b]; red maple (*Acer rubra)*[b]
Eastern cottonwood (*Populus deltoides)*
Sycamore (*Platanus occidentalis)*
White ash (*Fraxinus Americana)*[b]; olive *(Olea europaea)*[b,c]
Black walnut (*Juglans nigra)*
Mulberry (*Moras rubra)*
Mountain cedar (*Juniperus ashei)*
Pecan (*Carya illinoensis)*
Grass pollen
Rye (*Lolium perenne*)[d,e]
Timothy (*Phleum pretense)*[d,e]
Meadow fescue (*Festuca elatior)*[d,e]
Bermuda (*Cynodon dactylon)*[e]
Johnson (*Holcus halepensis)*
Bahia (*Paspalum notatum)*
Weed pollen
Short ragweed (*Ambrosia artemisiifolia)*[e,f]
English (narrow leaf) plantain (*Plantago lanceolata)*
Mugwort (*Artemisia vulgaris)*
Russian thistle (*Salsola kali)*
Burning bush *(Kochia scoparia)*
Sheep (common, red) sorrel (*Umex asetosella)*
Red root pigweed (*Amaranthus retroflexus)*
Indoor aeroallergens
Cat epithelium (*Felis domesticus)*[e]
Dog epithelium (*Canis familiaris)*
Arthropods (domestic mites: *Dermatophagoides farinae*)[e]
Dermatophagoides pteronyssinus)[e]
Insects (German cockroach: *Blattella germanica)*
Fungi
Alternaria alternata[g]
Cladosporium (Cladosporium cladosporioides, Cladosporium herbarum)[g]
Penicillium (Penicillium chrysogenum, Penicillium expansum)[g]
Aspergillus fumigatus[g]
Epicoccum nigrum
Drechslera or *Bipolaris* type (eg, *Heiminthosporium solani)*[g]

[a] Compiled and selected in collaboration with the American Academy of Allergy, Asthma, and Immunology Immunotherapy committee and Allergen subcommittee for the identification of 36key allergens in North America.

[b] Extensive cross-reaction of species within the genus.

[c] Apart from regional prevalences, are limited to local sites with substantial stands of these trees.

[d] Extensively cross-react with one another and bluegrass, orchard, red top, and sweet vernal.

[e] Allergens for which standardized extracts are commercially available.

[f] Like all ragweeds, extensively cross-react with other species within their genus.

[g] Fungal species that are widely distributed and clinically important.

minescent ELISA detection systems.[727,728] T-cell activation can also be determined by an increase in intracellular adenosine triphosphate, which occurs when cells proliferate. After cellular proliferation, adenosine triphosphate levels increase and a linear relationship between cell concentration and adenosine triphosphate level is proportional to light intensity, which can be measured by a luminescence assay. This technique has the added advantage of requiring only small volumes of whole blood and results can be reported in 24 hours.[729] Currently, this technology has been cleared by the FDA for an in vitro phytohemagglutinin test (www.ibtreflab.com).

Cellular cytotoxicity

Cytotoxic function can be readily demonstrated in mixed lymphocyte culture techniques wherein irradiated effector cells are incubated with varying proportions of ^{51}Cr-labeled target cells. Cytotoxicity of target cells is detected by radioactive chromium release after suitable incubation. An alternative, nonradioactive toxicity assay labels target cells with a europium (EU) diethylenetriamine penta-acetic acid (DTPA) chelate. Lysis of labeled targets by effector T cells releases the EU-DPTA complex, which is measured in a time-resolved fluorometer.[730,731]

Cytokine or chemokine release and histopathologic analysis

Depending on their stability in various media, most but not all cytokines can be measured by commercial ELISA and ELISpot immunoassays.[732] Many of these immunoassays have sufficient sensitivity to be detected in small fluid samples (eg, IL-5, IL-6, IL-8, RANTES, eotaxin, MCP-1, IL-10, IL-12, IL-13, interferon-γ, TNF-α, and MIF). Thus, these assay procedures can also be used to detect in vitro cytokine and chemokine synthesis and release from PBMCs cultured for varying periods. ELISpot assays, which measured interferon-γ release from PBMCs stimulated with nickel sulfate, positively correlated with both positive patch test results and lymphocyte proliferation.[732] Simultaneous measurement of multiple cytokines or chemokines in small fluid samples of antibody-array techniques is emerging as a potentially useful method.[733,734] This assay combines the specificity of ELISAs, sensitivity of enhanced chemiluminescence, and high-throughput of microspot assays.[733]

Macrophage inhibition, a measure of MIF bioactivity

Two assays are available to measure macrophage migration: (1) indirect (2 step) and (2) direct (1 step).[59,735] In the indirect method, PBMCs are cultured with antigen to produce MIF, which is then assayed on indicator cells (human monocytes) at a different time. The direct method entails mixing PBMCs, antigen, and indicator cells (monocytes). MIF is produced locally, and its effects are measured at the same time. Inhibition of migration is read at 18 to 24 hours. Functional assays of MIF may be subject to false-positive results due to nonlymphokine factors, such as antigen antibody complexes and the antigen itself, both of which might inhibit migration.

False-negative results may also occur because of individual variation of indicator cells. Because of their complexity and technical skill requirements, functional MIF in vitro assays are not commercially available. They are used chiefly for research of patient populations rather than individual patients. However, they may be of adjunctive clinical value for purposes of identifying certain pathogenetic factors, monitoring the results of therapy and the clinical course of patients with depressed cell-mediated immunity.

Immunoassays

As discussed herein, direct measurement of several cytokines in blood, BAL, sputum, and other body fluids by commercial ELISAs may act as biologic markers of acute or chronic inflammation and/or delayed-type hypersensitivity. Proinflammatory cytokines or chemokines and their subfamilies include interferon-γ, TNF-α, IL-4, IL-5, IL-6, IL-8, RANTES, eotaxin, IL-12, IL-13, IL-15, IL-18, IL-19, IL-22, IL-23, IL-25, IL-31, IL-32, IL-33, and TNF-α. Proinflammatory cytokines or chemokines, which are particularly associated with cell-mediated immunity, include interferon-γ, IL-12, TNF-α, IL-16, MIP-1, MCP-1, MCP-2, MCP-3, and MIF. A test of interferon-γ release by peripheral lymphocytes has been recommended by the Centers for Disease Control and Prevention for diagnosis of latent tuberculosis.[465] Increased serum levels of MIF have been found in a wide range of exogenous and autoinflammatory T-cell–mediated diseases.[698–707] Normal serum concentrations of MIF range from 0.5 to 8 μg/mL. In situ hybridization of MIF messenger RNA in macrophages can also be documented in delayed-type hypersensitivity skin lesions.[698]

Nonspecific Screening Tests for Cellular Immune Competency

Summary Statement 148. Simple, cost-effective tests include (1) an absolute lymphocyte count, (2) the absolute number of $CD4^+$ T cells, and (3) the $CD4^+/CD8^+$ ratio. (B)

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 less than 1,200/mm^3 suggests an abnormal immune response); (2) number of total T cells, as measured by anti-CD3 surface markers; (3) estimation of $CD4^+$ helper cell and $CD8^+$ cytotoxic populations by immunofluorescence 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; (5) determination of the relevant percentage of CD45 RO^+ $CD29^+$ T-cell lymphocytes as an indication of memory cells; and (6) flow cytometric assay of the percentage of $CD4^+$, $CD25^+$ Fox $P3^+$ immunoregulatory T cells. The latter cells may be increased after successful allergen immunotherapy.

Current Status of Cytokines and Chemokines

These cellular products were first identified more than 25 years ago and new ones are being discovered almost every year. As of 2007, there are 33 recognized IL cytokines and many more that do not have the IL designation.[696,741] Tables 6 and 7 are summaries of selected cytokines and chemokines, respectively. They are derived from multiple cell sources and often have redundant and overlapping biologic functions, which have been investigated extensively in knockout and transfected animal models. Their chief utility in diagnosis of human hypersensitivity is to determine the relative significance of cellular interactions involving dendritic cells, T-cell subsets, NK-T cells, macrophages, NK cells, mast cells and epithelial cells in various immune inflammatory processes. For example, body tissue secretions that contain IL-4, IL-5, IL-10, and IL-13 are associated with IgE-mediated allergic inflammation, whereas the predominant cytokines found in cell-mediated immunity inflammation are IL-12 and interferon-γ. Likewise, in situ hybridization techniques enable identification of specific cytokines in tissue biopsy samples. Certain cytokines such as TNF-α and IL-5 are nonselective and are associated with various types of inflammation. Chemokines (eg, eotaxin, RANTES, MCP-1, MCP-2, MCP-3) can also be identified by similar techniques.[688,693]

For the most part, cytokine and growth factor identification and profiling in human disease are still research oriented.[736–741] However, a number of reports suggest that cytokine profiles may be useful in differential diagnosis of a variety of human diseases.[742–749] For example, serologic profiles may differentiate infectious from noninfectious uveitis and may be of prognostic value in acute pancreatitis.[742,743] Similar reports concerning the utility of cytokine profiling for the diagnosis and follow-up of allergic or allergy-related diseases have also recently appeared.[734,750–760]

Chemokines are small (8 to 10 kDa) proteins secreted by many immune and nonimmune cells with essential roles in inflammatory and immune reactions, including the late-phase cutaneous response.[697,761] Although principally chemoattractants, they have diverse functions during an inflammatory or immune response, which involve cellular recruitment, activation, and differentiation.[762] The processes of intravascular rolling, tethering, and diapedesis of inflammatory cells are complex and also involve integrins, adhesion molecules, and selectins.[763–766] The approximately 50 human chemokine receptors and their corresponding ligands are classified on the basis of the number and structural characteristics of canonical cysteine residues (Table 7).[697,761,762] Understanding chemokine biology is at times confusing because a single chemokine can bind to multiple receptors and vice versa.

High serum levels of thymus and activation regulatory chemokine (TARC) (CCL17) and macrophage-derived chemokine (MDC) (CCL22) preferentially attract T_H2 subsets of T-helper cells in patients with allergic disease.[758] Once activated and differentiated, T_H2 cells express CCR3 and CCR4, receptors for eotaxin, RANTES, and TARC. Human CD25 regulatory T cells express higher levels of CCR4, CCR5, and CCR8 compared with CD25 cells. Eotaxin (CCL11), its receptor CCR3, and other ligands of CCR3 (CCL5, CCL7, CCL8, and CCL13) are coattractants for eosinophils in asthma.[767] Lymphocytes of nonallergic asthma patients strongly

express CXCR4. Certain chemokines (CCL1, CCL2, CCL11, CCL17, CCL22) are preferentially produced in the presence of T_H2 cytokines. CCL2 may enhance IL-4 production in activated T lymphocytes.

Specific chemokines have been reported to be associated with T_H1 or T_H2 cytokine production.[768] Both CCR4 and CCR8 are associated with allergen-induced late asthmatic responses.[769] Both IL-8 and eotaxin levels were increased in patients with severe asthma compared with patients with mild asthma.[770,771] Fracktalkine (CX3CL1) contributes to mast cell recruitment in asthma.[772] Eotaxin levels are elevated in both aspirin tolerant and intolerant patients.[773]

Although there is increased expression of RANTES, CCR3, and CCR5 in lesional skin, CC4R expression reflects greater severity in atopic dermatitis patients.[773,774] Two chemokine receptors (TARC, CCR3) appear to be targets for treatment in atopic diseases.[775,776]

OTHER DIAGNOSTIC IMMUNOLOGIC TESTS

Summary Statement 149. Investigation of non-IgE and non–cell-mediated clinical immunologic disorders may require tests that indicate abnormal adaptive and innate immune reactions. (B)

Evaluation of non-IgE and non–cell-mediated clinical immunologic diseases may include laboratory screening for (1) primary and acquired immunodeficiencies, (2) immune-mediated gammopathies, (3) complement activation disorders, and (4) a diverse spectrum of autoimmune and vasculitic disorders.

Immunodeficiency

The scope of diagnostic procedures for primary immunodeficiency has been reviewed in the recently published Practice Parameter for Primary Immunodeficiencies. Current status of immune-based diagnostic and monitoring of HIV-acquired immunodeficiency and fully developed AIDS have been reviewed elsewhere.[777,778] It is generally agreed that HIV-1 RNA levels and CD4 cell counts are important predictors of subsequent virologic and clinical outcomes.[777]

Immune-Mediated Gammopathies

Summary Statement 150. Abnormal serum and urine proteins, including cryoglobulins, may be associated with several abnormal immune syndromes. (B)

Drug-induced dysgammaglobulinemia, hypogammaglobulinemia, and leukocytoclastic vasculitis may occasionally be confused with premalignant or malignant gammopathy.[779,780] Abnormal serum and urine protein levels are detected by electrophoresis and immunofixation. Free light chains may also be demonstrated in serum.[781] Cryoglobulins may also develop, and these are usually classified as type I, type II, or type III.[782,783] Type I contains a single monoclonal IgG, type II is a mixture of monoclonal IgG with polyclonal IgGs, and type III is a mixture of polyclonal IgGs of different isotypes, most frequently IgG and IgM. Types II and III are also called mixed cryoglobulins.[784,785] Cryofibrinogenemia may also have to be considered in the differential diagnosis of cold precipitable proteins.[786]

Nonspecific Tests of Immunologic Inflammation

Summary Statement 151. The inflammatory consequences induced by immune functions may be detected by nonspecific tests, such as complete blood cell count with differential, sedimentation rate, C-reactive protein, and other acute-phase reactants. In some instances, functional assays of neutrophils and macrophages may be necessary to pinpoint inflammatory responses. (B)

Routine laboratory tests, such as a complete blood cell count with differential, sedimentation rate, C-reactive protein, and other acute-phase reactants (eg, fibrinogen, ferritin), are useful in determining the inflammatory consequences of innate immunity.[787–789] Functional assays of macrophages and neutrophils, the primary host defense cells, may indicate either impaired defense (eg, decreased or absent chemotaxis, phagocytosis, bacterial killing, and cytokine or chemokine synthesis) or uncontrolled inflammatory responses (ie, the macrophage activation syndrome).[790–793] Neutrophil and macrophage function tests may only be available in specialized medical centers. High ferritin levels associated with the macrophage activation syndrome can be used to monitor treatment of this disorder[792]

Complement Activation

Summary Statement 152. Evaluation of complement activation with a decrease of C3 and C4 may indicate complement deficiency, drug reactions, or the presence of immune complexes, which often are associated with increases in serum cryoglobulins and C1q binding. (B)

Although complement is a major component of innate immunity to pathogenic microorganisms, it may also be abnormally activated by adaptive immune pathways such as immune complexes or cytotoxic antibodies. In most cases, these pathways of complement function can be estimated by immune hemolysis (CH_{50} and AH_{50}) and a functional ELISA of the mannan lectin binding pathway.[794,795] Decrease of C4 and C3 and increase in factor B are general screening tests for complement activation.[796–798] Specialized laboratory centers can also determine individual complement components. Evaluation of both inherited and acquired forms of complement deficiencies, including C1 esterase inhibitor, have been discussed in Practice Parameters of Immunodeficiency. Immune complex activation of complement may be associated with an increase in serum cryoglobulins and/or an increase of C1q binding.[784,785,799]

Autoimmunity

Summary Statement 153. Autoantibody profiles offer important diagnostic adjuncts in the diagnosis of collagen vascular diseases, vasculitides, and cytotoxicity disorders. (B)

Many tissue antigens are capable of provoking autoimmune responses when the milieu of genetic susceptibility (ie, major histocompatibility subtypes) and environmental interaction is apropos. The discoveries of rheumatoid factor and

antinuclear antibody in association with rheumatoid arthritis and systemic lupus erythematosus, respectively, provided impetus for the clinical significance of autoimmunity.[800] Table 8 is a partial list of common autoantibodies and their prevalence in corresponding autoimmune diseases.[800,801] Thyroid autoantibodies may occur in up to 30% of patients with CIU.[802,803] IgG autoantibodies to IgE or the α subunit of FcεR1 may also be demonstrated in approximately 40% to 50% of patients with CIU.[804,805] The diagnostic or pathophysiologic significance of these autoantibodies in CIU is as yet indeterminate.

Selection of any one or a combination of these tests should be predicated on a reasonable clinical pretest probability.

Vasculitides

Small, medium, and large vessel vasculitides are most commonly diagnosed by characteristic clinical features and biopsy with demonstration of appropriate immune complexes within vessel walls.[800] Some cases of palpable purpura are associated with type III cryoglobulins.[780] A low C1q and anti-C1q antibodies[806] may be associated with hypocomplementemic urticarial vasculitis. Several types of small and medium vessel vasculitides are associated with antineutrophilic cytoplasmic and glomerular basement membrane antibodies (Table 9).

Human Cytotoxic Antibodies

Antibodies of this type may induce hemolytic anemia, neutropenia, or thrombocytopenia. These conditions may occur spontaneously or in association with drug therapy. Immune-mediated hemolytic anemia includes paroxysmal nocturnal hemoglobinuria, paroxysmal cold hemoglobinuria, and cold agglutinin disease.[807–810] Direct and indirect Coombs tests are useful screening tests for red cell autoantibodies. A gel microcolumn assay is purported to increase sensitivity.[811] A quantitative antiglobulin consumption technique can detect IgG on granulocyte cell membranes, which occurs in the Felty syndrome.[812] Because definitive tests are not generally available in most clinical laboratories, immune-induced neutropenia and thrombocytopenia are less well studied.[813–817] Drug-induced autoantibodies to red cells, neutrophils, and thrombocytes have been induced by a number of drugs but most often they are associated with penicillin, propylthiouracil, and quinine/quinidine, respectively.[818–827]

Analytic Techniques

The clinician should be aware that there is considerable interlaboratory variation of the methods described in this section. Techniques that originally used agglutination, turbidimetry, nephelometry, double immunodiffusion, counterimmunoelectrophoresis, and indirect immunofluorescence have evolved to ELISA, Western blotting, and in some situations immunoblot assays.[828] However, ELISA antinuclear antibody screening assays may lack sensitivity for certain collagen vascular diseases and therefore require confirmatory indirect immunofluorescence tests.[829] In recognition of these possible confounders, future diagnostic accuracy of these tests will be based on likelihood ratios.[830] Because multiple autoantibodies tend to occur in autoimmune diseases by a process known as epitope spreading and specific autoantibody profiles may have greater diagnostic predictability or prognostication, multiplexed proteomic platforms are in current development for SLE and rheumatoid arthritis.[831,832] As yet, optimal conditions for autoantigen arrays have not been established so these high throughput measures will require thorough validation in the future.[832]

UNPROVEN TESTS

Summary Statement 154. Procedures for which there is no evidence of diagnostic validity include cytotoxic tests, provocation-neutralization, electrodermal testing, applied kinesiology, iridology, hair analysis, or food specific IgG, IgG4, and IgG/IgG4 antibody tests. (B)

Cytotoxic Tests

The cytotoxic test is performed by placing a drop of whole blood or buffy coat as an unstained wet mount on a microscope slide precoated with a dried food extract. The technician observes the unstained cells for changes in shape and appearance of the leukocytes. Swelling, vacuolation, crenation, or other cytotoxic changes in leukocyte morphology are taken as evidence of allergy to the food.[833,834] The test is time consuming and entirely subjective, and there are no standards for time of incubation, pH osmolarity, temperature, or other conditions of the test.[835] Controlled studies have shown that results are not reproducible and do not correlate with clinical evidence of food allergy.[836–840] It offers no reliable help in establishing a diagnosis of food allergy.[836,837]

Provocation-Neutralization

This procedure is purported to diagnose allergy to foods, chemicals, inhalant allergens, and endogenous hormones. Varying concentrations of test extracts of these substances are given to the patient by intracutaneous or subcutaneous injection or sublingually. The patient records all subjective sensations for 10 minutes afterward, and any reported sensation is taken as a positive test result for allergy. In the event of a positive test result, other doses of the same substance are given until the sensation has disappeared, at which point the action is said to be "neutralized." Some proponents recommend measuring increase in the size of the injected wheal in the intracutaneous provocation procedure, but the primary indication of a positive result is the provocation and neutralization of symptoms. This procedure has been evaluated by double-blind, placebo-controlled trials, which showed that responses to test substances are no different from responses to placebo.[841] Furthermore, there is no rational immunologic explanation for provocation and prompt neutralization of subjective symptoms under these conditions.[841] Application of neutralizing injections of milk and wheat in a patient with unsuspected urticaria pigmentosa resulted in a potentially life-threatening reaction.[842]

Electrodermal Diagnosis

This procedure measures changes in skin resistance while the patient is exposed to an allergen, either food or inhalant. Allergen exposure is done in various ways, the most common of which is placing a sealed glass vial containing allergen extract onto an aluminum plate inserted in the electrical circuit between the skin and the galvanometer. A drop in the electrical resistance of the skin is said to indicate allergy. Although promoted by a single study, electrodermal testing or "Vega" cannot be recommended because its rationale is unsound and not evidence based.[843–845] Two double-blinded, placebo-controlled, prospective studies revealed no significant differences between allergic patients and control, nonatopic volunteers.[846,847]

Applied Kinesiology

This technique is one in which change in muscle strength in extremities is measured before and after the patient is exposed to a test allergen. The usual exposure is performed by placing a sealed glass vial of allergen extract on the patient's skin. Measurement of muscle strength is measured in the contralateral arm.[848] Two controlled and blinded studies demonstrated that the technique was no more useful than placebo.[848,849]

Iridology

Iridology attempts to relate the anatomical features in the iris to various systemic diseases.[850] Several systematic reviews concluded that iridology as a diagnostic tool does not have scientific validity.[851,852]

Chemical Analysis of Body Fluids, Hair, or Other Tissues

Based on unsupported theories that environmental chemicals induce allergies or a toxic effect on the immune system, certain practitioners have recommended measurement of various exogenous environmental chemicals, particularly organic solvents and pesticides in such endogenous substances as amino acids, minerals, and various cytokines. These measurements have been made in samples of blood, urine, fat, and air. Exquisitely sensitive analytic chemistry techniques permit detection of quantitation of almost any chemical at extremely low levels, but to date there has been no evidence that allergic patients differ from nonallergic controls in their body burden of any of these compounds.

Hair analysis has important uses in screening for metal intoxication, but this does not necessarily carry over to its utility for nutritional deficiencies or chronic diseases. In one study, duplicate hair samples of 2 healthy volunteers were sent to 13 different laboratories that performed multimineral hair analysis. Reported levels of most minerals varied considerably among identical samples. Six laboratories recommended food supplements, but the types and amounts varied widely.[853] In another study, hair analysis samples in patients proven to be fish allergic by oral provocation were sent to several laboratories, which did not recognize fish-allergic patients and, in fact, reported that other allergies were found in these individuals.[854]

Specific IgG Antibodies

IgG antibodies to allergens such as foods can be detected and quantified by Unicap or ELISA techniques. The presence of IgG antibodies, however, does not indicate allergy to these environmental substances. Detection of IgG antibodies, IgG subclasses, or IgG/IgG4 antibody ratios were discredited as reliable diagnostic tools.[855,856] IgG antibodies to common foods can be detected in health and disease. This reflects the likelihood that circulating immune complexes to foods occur in most normal individuals, particularly after a meal that would be considered a normal physiologic finding. It was therefore concluded that food specific IgG or IgG subclasses should not be used in the diagnostic evaluation of food allergy.[857,858]

PART 2

The purpose of this section is to provide evidence-based guidance about the application of in vivo and in vitro diagnostic tests to the evaluation of 5 common clinical entities of unique interest to the allergist/immunologist. An essential prerequisite to understanding the variables posed during individual clinical assessments is the potency and availability of allergens that are used in both in vitro and in vivo test procedures. Only a few of the protein allergen extracts have been standardized in biologic or mass units (ie, house dust mite, cat, ragweed/grass pollens, and insect venoms). Customized extraction of unusual pollens or testing with fresh foods is sometimes necessary. Patch tests are often applied using nonirritant concentrations of commercial products to which a patient is exposed. If positive, special patch tests to relevant components of the product are subsequently tested. Although some laboratories can prepare a customized solid-phase immunosorbent for unusual allergens (eg, occupational chemicals, latex, drugs), the validity of such tests is unknown. Since keeping every available extract or patch test reactant in the office stock is impractical and testing every patient for every known allergen is unnecessary, the practicing allergist must choose from current catalogs of commercially available test reagents. This poses a considerable dilemma for evaluation of CD since the commercially available FDA-approved patch test reagents (T.R.U.E. TEST) may only account for 25% to 30% of clinical contact sensitivity problems so further testing may be needed. Each of the foregoing sections will address specific issues that are germane to that particular clinical topic.

The situation with respect to diagnostic reagents required for specific IgE testing is somewhat more complicated because of the rapidly changing technology. For example, in the case of new, multiplexed arrays for measurement of specific IgE and the ability to test for many allergenic determinants simultaneously, the anticipated use of component-resolved diagnosis may pave the way for using recombinant markers or partially purified allergens. As each of these diagnostic reactants is introduced into clinical practice, prospective correlative studies will be required to validate their respective clinical utility.

ALLERGENS

Introduction and General Considerations

Summary Statement 155. Although North American inhalant allergens are botanically and ecologically diverse, several expert committees consisting of members with botanic and mycologic expertise have compiled and selected 36 key allergens in North America, based on Thommen's postulates. (D)

Summary Statement 156. For individual patients, the choice of test allergens is guided by the history and physical examination and the physician's knowledge, training, and experience. (B)

The catalogs of manufacturers that produce allergen extracts or market in vitro tests list a wide range of pollens, molds, epidermals, insects, foods, and other substances available for diagnostic allergy testing. Allergen extracts are commercially available for most recognized allergenic materials, although field collection and customized extraction of unusual pollens or other substances or testing with fresh foods is sometimes necessary. Allergen-bound solid matrix materials for in vitro immunoassay are available for approximately the same number of substances as skin test diagnostic allergen extracts, but some laboratories can prepare custom solid-phase immunosorbents for unusual allergens. Since keeping every available extract in the office stock is impractical, and testing every patient for every known allergen is unnecessary, the practicing allergist must choose from the variety of available extracts.

Because North America is diverse botanically and demographically, it is not possible to devise a universal list of appropriate inhalant, food, and other allergens for testing in all patients with a given symptom complex. For pollens, regional lists are available but have generally been unsatisfactory because such lists are either too broad for a given area in the region or are incomplete and inaccurate. Thus, the selection of extracts for testing can be guided, but not directed, by the sciences of botany and mycology. As previously discussed, the range of extracts stocked in the allergy office should reflect the following: (1) recent local aerobiologic data obtained by a qualified counting station; (2) correlation between patients' symptoms and aerobiologic data; (3) results of local and regional botanical and mold surveys conducted by a qualified botanist and mycologist, respectively; (4) knowledge of locally and regionally indigenous allergenic plants and other flora; (5) knowledge of foods in patients' diets; (6) knowledge of fungi prevalent in outdoor and indoor air; (7) knowledge about the clinical significance (sensitization) of allergens in the region; and (8) knowledge of cross-reactivity patterns between allergens.

Unfortunately, this information is not available for all potential allergens, particularly the fungi, in all areas. Often one must rely on the opinions and experience of local colleagues in stocking the skin testing laboratory. In some areas, consultation with a local or regional allergy-immunology training program might prove useful. The Immunotherapy Committee and the Allergen Subcommittee of the AAAAI compiled and selected 36 key allergens in North America (Table 10).

For an individual patient, the choice of allergens for testing should be guided primarily by the patient's history and physical examination and will reflect the physician's knowledge, training, and experience. Indiscriminate testing is inconvenient to patients and office staff and is an unwise use of health care resources. Parameters for appropriate numbers of skin tests have been suggested by the Joint Task Force on Practice Parameters for Allergy and Immunology, distributed by the Joint Council of Allergy, Asthma and Immunology and reconsidered in the previous section on "Numbers of Skin Tests."

The Skin Testing Form

Summary Statement 157. A well-designed skin test or laboratory ordering form should provide useful information to the ordering physician, his/her staff, health care providers, and other physicians who may be consulted in the future. (B)

A well-designed skin testing form or laboratory test order form should reflect the physician's knowledge of local aerobiology, foods, and possibly other substances that may have clinical relevance. Knowledge about cross-reactivity is also an essential prerequisite in the design of this form. As an important part of the medical record, the form should provide useful information to the ordering physician, as well as to other physicians and health care providers.

At a minimum, a skin/laboratory testing form should include the following: (1) name, address, and telephone number of the physician; (2) the patient's name and the date of testing; (3) name or initials of the person performing testing; (4) method(s) used for testing, (ie, prick, puncture, intracutaneous; laboratory method); (5) measurement of reaction sizes of both wheal and erythema in mm should be recorded, and numerical grades (0-+4) are not recommended; (6) concentration at which allergens are tested for both percutaneous (eg, 1:10 wt/vol or 100,000 AU/mL) and intracutaneous (eg, 1:1,000 wt/vol or 1000 AU/mL) methods; (7) concentration of histamine phosphate, histamine dihydrochloride, or other substances (ie, codeine phosphate) used for positive control (composition of material used for negative control); (8) results of positive and negative control tests (in millimeters); (9) an unambiguous common name for all allergens tested; (10) when allergen mixes are used, a listing of the individual components and precise ratio of the extract mix; (11) since it is not practical to record extract source, manufacturer's lot number, and expiration date on the skin testing form for many clinicians, these records may be kept separately; and (12) for specific IgE testing, the quantitative result (in kIU/L) is preferred to "class" results.

The following are optional: (1) abbreviated binomial Latin nomenclature (ie, *Poa pratensis*) for pollens and fungi in addition to common names (Kentucky bluegrass) or generic designations (*Baccharis* spp). The manufacturers of allergen

extracts and in vitro materials are encouraged to provide this information both in their catalogs and on product labels; (2) arrangement of test allergens by botanical classification; (3) especially in academic centers, it is useful to include timing of pollination periods. A typical example of a skin test form is included in the subsequent section on "Evaluation of Inhalant Allergy."

Specific Allergen Types

Pollens

Summary Statement 158. The best indicators in the selection of appropriate pollens for clinical use are extensive prevalence in the air and concurrent allergy symptoms during annually recurrent seasons when such pollens are expected to be present in the ambient air. (B)

Regional native plant geography is relatively well defined for most areas, and lists of prevalent plants in various floristic zones are available from various sources.[235,239,859,860] Such lists, however, do not generally consider various plants such as trees being introduced as ornamental species. Therefore, lists are only a starting point and should not be taken literally or overinterpreted.

In selecting appropriate pollens for clinical use, the combination of extensive prevalence in the air and accompanying allergic symptoms during annually recurring periods remains the best indicator of potential importance. Well-standardized aeroallergen collection procedures can generate comparative data from year to year and from collection station to collection station in various localities and floristic regions. These data can be used to establish the dates of onset and ending of a flowering season, identify peak days of pollination, and quantitate the types and numbers of pollen and spores during the seasons of the year. Clinical application of these data should consider the following.

First, collection sites relatively close to each other can have both quantitative and qualitative differences in data.[239,247,262,263,861] Second, single-site collection in a community can only roughly estimate an individual patient's actual exposure. Use of a personal sampler worn throughout the day at home or at work may provide more meaningful data in selected cases. Spore and pollen counts may vary by as much as 1,000 times at certain sites of activity.[248,862] Third, in many regions, several plant species concurrently shed windborne pollens that are both antigenically similar and morphologically indistinguishable, making it impossible to discriminate major from minor source species even after extensive source surveys. Botanical groups that especially reflect this difficulty in North America include the grasses, the chenopod-amaranth complex, and the ragweeds.[863] Many families of anemophilous plants, however, include 2 or more (and often *many* more) species with pollens that are similar in form, allergenicity, and distribution.[863] In some of these groups, 1 or more shared allergens determine partial or complete cross-reactivity, but pollens of related species may contain distinctive or unique allergenic epitopes, as well as shared determinants.[251,252–256,260,863] When available, cross-reactivity data can simplify testing when sensitization has been demonstrated not to be species specific. These principles of cross-reactivity are best illustrated among grass and tree pollens. Rye, timothy, blue, and orchard grasses share common allergens and are frequently considered to be cross-reactive, although timothy also has unique allergenic epitopes. Bermuda, Bahia, and Johnson grasses have separate and distinct allergenic proteins and therefore should be tested separately when there is significant clinical exposure. Various trees also share major allergens, including elder, birch, oak, hazel, and beech. Juniper and cedar, trees of the cypress family, share common allergens with the elder-birch family despite the fact that they are separate and distinct species.

Unfortunately, characterization of cross-reactivity patterns between species or within families or other botanic groups has proceeded slowly. Since clinically relevant allergenic difference among related pollens occur commonly, valid single "representatives" of such groupings seldom can be identified.[256,260] When common airborne pollens cannot be judged to be interchangeable in allergen content, testing and treatment with multiple locally prevalent pollens may be required to avoid clinically significant omissions.

Fourth, the dose response relationship in pollinosis or asthma is often difficult to define. The degree of clinical sensitivity varies from the very sensitive patient with overt symptoms at low pollen concentrations to those only developing symptoms at high concentrations. Data on threshold levels are available only for grass and ragweed pollens and animal epidermals.[286,322,864–866] Although grass pollen concentrations of 20 grains/m^3 elicited rhinitis in some patients, a level of 50 grains/ m^3 was required to affect all sensitized grass patients.[860] In some patients who are ragweed sensitive and already "primed," 7 to 15 pollen grains/m^3 provoke symptoms.[865,866]

Fifth, pollens exposed to atmospheric pollutants may have increased allergenicity.[867,868] The increased number of allergic individuals in regions with high levels of air pollution may be a function of this factor rather than simply an alteration of airway permeability to allergens.[869]

Sixth, diverse factors, including air temperature, relative humidity, body position, ocular stimulation, respiratory infection, simultaneous exposure to airborne irritants, and other pollens, may modify response to a specific pollen challenge.[235,243,262,264]

Seventh, sensitization and allergy in children may depend on pollen load, with the influence of pollen load strongest on the development of specific IgE and less on skin test reactivity and manifest allergies.[870–872]

Eighth, magnitude of pollination or spore production does not alone denote clinical relevance of airborne pollens. Pine pollens and *Cladosporium* spores appear in great numbers but seem less sensitizing than grass and tree (eg, oak and birch) pollens or *Alternaria* spores, which seem to be sensitizing at low levels in ambient air.[836] Pine pollen, in fact, is nonsensitizing.

Ninth, skin reactivity or specific IgE tests may be shown to pollens that occur at low levels or not at all in aerobiologic surveys. In such cases, it is arguable that sensitization has occurred at levels not generally considered high enough to provoke symptoms, that apparent sensitization reflects sharing of 1 or more allergenic epitopes with other important pollen types (ie, cross-reactivity), that sensitization reflects exposure to sufficiently high concentrations of a clinically relevant anemiophilous pollen, that the results of the aeroallergen survey are not applicable to the patient's actual environment, or that the demonstrated sensitization is not clinically relevant. In most of such situations, the truth remains obscure and may vary from site to site. When a patient is having clear-cut seasonal symptoms unexplained by results of routine testing, the clinician might consider testing with more esoteric allergens, perhaps based on a home visit.

Fungi

Summary Statement 159. The clinical significance of a single fungus test reagent may be difficult to ascertain because of important confounders, such as sampling method, culture conditions, nonculturable species, allergenic differences between spores and hyphae, and preferential ecologic niches. (A)

Summary Statement 160. For clinical purposes, molds are often characterized as outdoors (*Alternaria* and *Cladosporium* species), indoors (*Aspergillus* and *Penicillium* species), or both (*Alternaria, Aspergillus,* and *Penicillium* species). (B)

Although there is diversity of opinion regarding the prevalence and clinical importance of fungal allergy, there is a strong sense among clinicians that fungi contribute to symptoms of respiratory allergy, with the dark-spored (dematiaceous) asexual forms especially implicated. Apart from the association of spore blooms with thunderstorms, there is little evidence, however, linking exposure to well-defined single agents with isolated or recurring periods of morbidity.[873,874]

Establishing the practical importance of the various allergenic fungi involves many of the same basic problems faced in pollen allergy.[261,262,264,265,267–269,872] These include a large number of potentially sensitizing species, uncertain allergenic relationships, and limited aerobiologic data. In addition, fungi present distinctive concerns such as the limited value of field studies when sources are microscopic, the apparent extreme biologic diversity of the organisms within the deuteromycete form genera, the need to identify many fungus colonies in culture, the relative importance of apparently potent sensitizers in both indoor and outdoor environments, and difficulty in identifying many spores of the fleshy fungi.

For clinical purposes, molds are often characterized as outdoor (*Alternaria* and *Cladosporium* species) and/or indoor (*Aspergillus* and *Penicillium* species). Traditional gravity or Rotorod air sampling equipment does not collect fungal spores as efficiently as pollens, and many counting stations have made no attempt at differentiating among the various fungal forms.[263,266]

Several taxa of deuteromycetes (eg, major *Cladosporium* species) occur universally in all but the coldest regions. The relative prevalence of even the common types, however, remains to be defined by aeroallergen sampling methods having high sampling efficiency for small particles (ie, spore traps). Since isolated spores within and among many groups of fungi are similar, species determinations require careful studies of isolates in culture. Present efforts to achieve these basic and essential goals are still at an early stage of development for the deuteromycetes, although molecular characterization of airborne fungal spores appears to be a promising advance.[269] The identification of the *Ascomycetes* and *Basidiomycetes* is complicated by failure of many of these organisms to produce distinctive growth on laboratory media. For most *Basidiomycetes*, this is a modest limitation since field collections readily provide spores for extraction and insight into relative species prevalence. Ascospores, however, typically originate from minute fruiting bodies that are not easily found or identified. Even when laboratory propagation is feasible, the spore harvest tends to be sparse. As a result, few studies of human reactivity to ascospores or basidiospores have been recorded, although IgE-mediated sensitization to representatives of both groups is demonstrable.[875] These current investigations may lead to the inclusion of some of these organisms in future routine clinical testing.

When the fungal extracts are grown in the laboratory, the source of specific organism for propagation and the culture media selected for use should be explicitly noted. Increasing evidence suggests that, at least among the *Deuteromycetes*, individual species should be assumed to differ allergenically until proven otherwise. Fungal extracts should derive from authenticated species and be labeled accordingly; extracts bearing only generic designations should be rejected. Labels, skin testing sheets, and specific IgE test reports should reflect contemporary nomenclature, avoiding discarded designations even if these are familiar. When recent taxonomic change threatens confusion, both the new and old names should be supplied. At no time should extracts of a single organism be supplied under 2 or more deuteromycetal synonyms. The historic tendency of taxonomists to give distinctive names to different life cycle stages of single fungus organisms is a fact that all must recognize. Although it is often proper to relate selected *Deuteromycetes* to their sexual stages, which are more reliably classified, product labeling should show from which growth form(s) the extract was derived, that is, the sexual or asexual stages.

At present, the optimal preparative approach for fungus extracts has not been defined, although a systematic evaluation of available options is clearly overdue. Such comparisons must confront the tendency, especially among the *Deuteromycetes*, to undergo somatic mutation and antigenic shifts under extended culture conditions. In addition, possible immunospecific differences among strains of single taxa, on primary isolation, are suggested by limited experience. These sources of diversity are of special concern because methods for assaying extracts for major fungal allergens are still

rudimentary. Similarly, the potential of fungus enzymes to degrade allergens of diverse origins (including other fungi) should be continually evaluated in practice. The apparent primary importance of airborne spores as dispersive vectors for many common fungi is evident, but this does not preclude a clinical role for hyphal and secreted products. Possible allergenic difference among spores, hyphae, and metabolic products have been suggested without the emergence of definitive proof. Pending resolution of this issue and recognizing that advantages of using separated spores may still evolve, it seems appropriate, at least for *Deuteromycetes*, to base fungal extracts on actively sporulating whole colonies.[876] On the basis of this approach, the major allergen of *Alternaria alternata (Alt a* 29 subsequently Alt a 1) has been identified.[877]

Insect and acarid allergens

Summary Statement 161. Five Hymenoptera venom extracts are available for evaluation of anaphylactic reactions to honeybee, yellow jacket, yellow hornet, white faced hornet, and *Polistes* wasp. A whole-body extract is the only currently available diagnostic reagent for fire ant sting allergy. (A)

Summary Statement 162. Major inhalant acarid and insect allergens include several species of house dust mite and cockroach. (A)

Venom extracts are widely accepted as the standard reagents for diagnostic testing and immunotherapy for *Hymenoptera* anaphylactic allergy. The major allergenic venom proteins have been identified and most have enzymatic activities. Honeybee venom, the most thoroughly studied, contains the major allergen, Api m I, phospholipase A, hyaluronidase, mellitin, apamin (an acid phosphatase), and several higher-molecular-weight molecules.[158,878] Trace amounts of kinins and histamine have been found in wasp venom.[879,880]

Five *Hymenoptera* venom extracts are available for clinical use: honeybee, yellow jacket, yellow hornet, white-faced hornet, and *Polistes* wasp. Honeybee venom is collected by inducing bees to sting against an electric grid. Commercial vespid venoms are primarily collected by microscopic dissection of individual venom sacs. Venoms are supplied as lyophilized powder to be reconstituted with special diluent containing pasteurized HSA as a stabilizing agent.

Venom skin testing is the most useful and sensitive immunologic procedure for confirming immediate hypersensitivity to venoms.[183,881] Circulating levels of venom specific IgE may be measured in the laboratory.[132] The original RAST method is less sensitive than skin tests, but current methods (eg, ImmunoCap) demonstrate substantial improvement of sensitivity.[882,883] The significance of skin or in vitro sensitization is unclear when the sting history is negative, but the converse situation (negative test, results positive history) is also problematic.[131,159]

The venoms used for treatment are the same as those used for skin testing. In cases of sensitivity to multiple venoms, a mixture of yellow jacket, yellow hornet, and white-faced hornet venoms is available for treatment.[158,884]

Imported fire ant whole body extract is the only reagent presently available for diagnostic testing and immunotherapy for fire ant sting allergy.[885] Although most imported fire ant whole body extracts appear to be useful for diagnosis and treatment, some preparations contain variable concentrations of relevant venom allergens. One possible explanation for variability among imported fire ant whole body extract preparations may be seasonal variation in antigenic activity. One study revealed a more than 100-fold difference in phospholipase activity in extracts prepared from ants collected in early summer compared with winter.

Solenopsis invicta imported fire ant venom is currently available only for research purposes. Unique among *Hymenoptera* venoms, fire ant venom contains 95% piperidine alkaloids.[886] The small protein fraction contains phospholipase (0.1%) and hyaluronidase (0.1%). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the commercial imported fire ant venom product revealed bands identical to those found in pure fire ant venom at approximately 15, 26, 28, and 37 kD. These bands represent the molecular weights of the 4 allergens identified in imported fire ant venom (Sol i, I, II, III, and IV).[887] Monoclonal antibody assays have been developed for all of the antigens. Sol i 1, the venom phospholipase of imported fire ant venom, cross-reacts with vespid venom.[888] All 4 Soli proteins are important allergens. Most patients react to all 4 allergens, whereas some react almost exclusively to a single allergen.

Mosquitoes (Diptera) and fleas (Siphonaptera) pierce the skin with their needle-like mouth parts and feed directly in a capillary or a pool of extravasated blood.[889] Antigens responsible for mosquito bite reactivity include 4 different nondialyzable components extracted from the head-thorax portion of the mosquito and, more specifically, a high-molecular-weight protein fraction isolated from the oral secretions. A dialyzable low-molecular-weight material has been extracted from the oral secretion of the flea. Antigens involved in these reactions have been obtained from extracts of whole body, body segments, and oral secretions of both mosquitoes and fleas. Studies of the efficacy of immunotherapy with whole-body extract of these insects have yielded variable results. The use of the low-molecular-weight hapten isolated from flea saliva has been anecdotally effective.[889]

The most commonly reported and well-characterized anaphylactic type of reaction to biting insects (*Hemiptera*) is caused by the saliva of the genus *Triatoma* of the reduviid group.[890] These are called kissing bugs, cone nose bugs, or assassin bugs and are commonly found in the southwestern United States from Texas to California. Rohr et al have reported the successful treatment of 5 patients with anaphylactic reactions to *Triatoma*, using immunotherapy with salivary gland extract.[891]

Several other biting insect allergens have been identified and partially purified. Baur and coworkers have isolated, characterized, and analyzed the amino acid sequences of the specific antigenic proteins from the midge, *Chironomus thummi*, already shown by other investigators to cause asth-

ma.[892] Baur et al demonstrated that chironomid hemoglobins were the major inhalant allergens and that several different species of midge hemoglobins were cross-reactive in RAST inhibition studies. Exposure to fish food containing midge larvae is a possible inhalant source of sensitization.[893]

In the past decade, there has been an upsurge of home infestations by the multicolored Asian lady beetle, *Harmonia axgridis*. Hypersensitivity symptoms, including rhinitis, wheezing, and urticaria, have been reported.[894]

Inhalant insect allergens have long been recognized as possible causes of clinical sensitivity. The earliest recognition of this problem occurred in patient populations exposed to swarming mayflies or caddis flies at certain seasons of the year.[895] Localized mini-epidemics of asthma have also occurred after exposure to dust from insect larvae.[896] More recently, the importance of cockroaches as an indoor allergen has been emphasized.[897] The major cockroach allergens (Bla g I and Bla g II) have been isolated. Other inhalant allergen sources have also been identified, although not all have been purified. The proteins share similar immunochemical properties with other known allergens in that they are abundantly available, retain their tertiary structure, and have the appropriate size for airborne dispersal and inhalation.

House dust mites are a major source of the house dust allergen worldwide.[898,899] House dust mites also are major causes of asthma.[900] The pyroglyphid mites dominate and these include *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Euroglyphus maynei*.[901] *Blomia tropicalis* is endemic to Florida. The major allergens of *D pteronyssinus* (Der p I, Der p II) and *D farinae* (Der f I, Der f II) have been characterized. Tovey et al showed that dust mite feces contain the clinically significant aeroallergen of house dust.[902] Krills et al demonstrated many distinct *D pteronyssinus* allergens.[903]

Epidermals

Summary Statement 163. Animal clinical sensitivity is most often associated with domestic pets (cats, dogs, birds) and laboratory animals (rodents, rabbits). Specific testing is guided by history of appropriate animal exposure. (A)

Animal allergen sensitization may result in either trivial or severe symptoms and may be both socially and occupationally important. Selection of animal allergens for testing is generally guided by history of animal exposure. Exhaustive testing is rarely indicated. Cat allergen extracts have been well characterized (ie, Fel d l) and are derived from sebaceous glandular protein in skin. To a lesser extent, allergens can also be identified in cat saliva. Standardized cat extracts are commercially available. Similar antigen characterization studies are now available in several rodent laboratory animals. Urinary allergens have also been demonstrated in these species. Other mammalian extracts have been less carefully studied, but clinically relevant sensitizations are easily demonstrable to dog (the major allergen of which is Can f I), birds, and farm animals. On occasion, customized extract preparation is indicated for evaluation of sensitization to more exotic animals, including those found in zoos.

Foods

Summary Statement 164. Selection of food tests for IgE-mediated clinical sensitivity is usually tailored to the patient's temporal history, which may be supplemented by a food diary. (A)

Immediate or IgE-mediated hypersensitivity to foods may be particularly important in infants and young children. Many of these clinical responses are severe and result in anaphylaxis.[613] In children younger than 3 years, the most important food allergens include milk, egg white, peanuts, soybean, and wheat. The prevalence of clinical sensitivity to food appears to decline with age based on evidence that positive skin reactivity confirmed by double-blind oral food challenge is less than 2% of the general adult population. Despite these observations on relative prevalence, any individual at any age can develop IgE-mediated sensitization to foods that can trigger symptoms in gastrointestinal, skin, or respiratory organs. All clinicians caring for allergic patients must therefore be aware of the indications for and limitations of food testing. Nearly any food can be allergenic, and cross-reactivity cannot generally be assumed. The absence of reactivity to one member of a group of botanically 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 available.

Patient history is important in the selection of foods for testing, and a patient's spontaneous history may 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 and food-dependent exercise-induced anaphylaxis. Testing for the most common allergenic foods usually detects most suspected food hypersensitivity, although more tests may be required depending on the clinical situation. Many clinicians test with a limited panel of commonly allergenic foods when food allergy is suspected and there is no clear-cut history of symptoms occurring after exposure to specific foods. Exhaustive testing to the 200 or so foods available for skin or specific IgE testing in patients presenting with anaphylactic reactions is rarely indicated. The diagnostic yield and cost effectiveness of such a strategy have been seriously questioned.[271,272] The routine use of food prick tests when the history is negative for food allergy is not justified. Testing to a food product not commercially available may be indicated in certain clinical situations. Furthermore, testing with commercially available materials may produce false-negative results because of alteration of relevant allergens by storage, cooking or the digestive process. Additionally, the allergens of many plant-derived foods are labile, and testing with the fresh product or prick-prick testing may be necessary. This is especially germane in the case of fruits and berries. Aerosolized food proteins in certain occupational settings such as bakeries,

crab processing, or spice factories may induce severe forms of respiratory sensitization.

Antibiotics, other drugs, and chemicals

Summary Statement 165. Although commercial skin tests for drugs, biologics, and chemicals are not available, specialized medical centers prepare and use such tests under appropriate clinical situations. The validity of such tests is adjudged on a case by case basis. (C)

IgE-mediated mechanisms are implicated in adverse reactions to many antibiotics, pharmaceuticals, and biologic proteins such as insulin, protamine, heparin, streptokinase, and chymopapain. Penicilloyl polylysine, the skin test reagent that tested for the major allergenic determinant in penicillin (ie, a penicilloyl major catabolic product) and detected 80% of penicillin-sensitive patients, is no longer commercially available. Many severe reactions, however, can only be confirmed by minor penicillin metabolic determinants, which occur in lower (minor) concentrations.[153,154] Routine clinical testing for standardized minor determinant mixtures is not feasible because they are not commercially available. Some physicians test with aged penicillin as a surrogate for minor determinants in the hope that minor determinants will form on aging; however, studies of these aged test preparations have not confirmed that this occurs. Some medical centers prepare major and minor determinants in their own laboratories. Cross-sensitivity to penicillin analogues, including amoxicillin and imipenem, presumably occurs because of reactive metabolites derived from the β-lactam ring.[156] Cross-sensitivity to 1 of the monocyclic β-lactam antibiotics, aztreonam, has not been demonstrated thus far, and this drug can be used with caution in patients with known prior penicillin sensitivity. It is also estimated that 6% to 15% of penicillin-sensitive patients will exhibit cross-sensitivity to the first generation of the cephalosporin family of drugs, but this may be as low as 1% to 2% in the case of second- and third-generation analogs. Standardized skin test reagents for prediction of cephalosporin sensitivity are not available. Recently, it has also been demonstrated that a significant proportion of patients with clinical histories of β-lactam antibiotic sensitivity and immediate skin test reactivity respond only to side-chain specific determinants.[904] The diagnostic validity of commercially available specific IgE tests for all drugs, including penicillin, has not been confirmed. It is emphasized that negative specific IgE test results do not rule out the possibility of penicillin allergy, and therefore such tests should not be used to detect penicillin allergy.

In centers that have proper reagents, penicillin skin tests should be used to evaluate the likelihood of an immediate hypersensitivity reaction in a patient with a history of a penicillin reaction when the clinical requirement for penicillin is strongly indicated and an effective alternate antibiotic cannot be substituted.[905] Under these conditions, 2 scenarios are possible. A skin test–negative patient may receive penicillin without anticipated problems. A skin test–positive patient will require rapid oral or parenteral desensitization to penicillin with close monitoring in the hospital. Skin tests may also be used to determine whether IgE-medicated mechanisms were involved in a reaction that occurred in the recent past. It has been argued that penicillin skin testing could be performed in individuals who have had a history of non–life-threatening reaction to penicillin to prevent antibiotic resistance (eg, vancomycin-resistant enterococcus) and allow more efficacious and cost-effective selection of antibiotics since, in most instances, such patients have been found not to be penicillin-sensitive when skin tested and subsequently challenged.[905] This has been considered to be especially useful in the pediatric age group in which antibiotic use for pharyngitis, otitis media, and various other infections is frequent and recurrent. Routine penicillin testing before administration of penicillin or related analogs in a history-negative patient is not recommended.

Sulfonamide adverse reactions have increased significantly since the advent of AIDS. Approximately 30% of these reactions are attributed to IgE mechanisms, as detected by positive skin test results with an immunogenic metabolite, sulfamethoxazoyl-poly-L-tyrosine.[906] Most adverse reactions are due to toxic hydroxylamine metabolites, which induce in vitro cytotoxic reactions in peripheral blood monocytes of patients with AIDS.[907–909] Clinical confirmation of these reactions may be accomplished by a cautious graded oral challenge protocol. If a positive response is obtained, an extended oral desensitization or graded tolerance regimen is begun.[910] Similar oral challenge testing and graded challenges have been accomplished with aspirin, isoniazid, rifampicin, sulfasalazine, and allopurinol.[911]

Skin and in vitro tests may be used to detect sensitivity to vaccines that contain egg protein, other biologic large-molecular-weight materials (enzymes, protamine, insulin, heterologous monoclonal antibodies, heparin, intravenous immunoglobulin preparations, and other blood products), and certain other drugs such as suxamethonium muscle relaxants. These materials are not available commercially. and test materials are usually prepared locally with fresh materials. Appropriate concentrations for testing with these materials have not been well studied in large groups of patients and controls. The predictive value of negative skin or in vitro test results to these substances is therefore not known, and tests can only be interpreted in the context of an individual patient's clinical situation. For example, in the case of reactions to vaccines that contain traces of egg protein, other proteins unrelated to egg may account for some reactions and that most egg-allergic children may tolerate such vaccines (ie, MMR vaccine).[912]

Large- and small-molecular-mass additives in foods and drugs

Although rarely considered, many chemicals (sulfonechloramide, azodyes, fragrances, parabens, vegetable gums) contained in food and drugs or used for processing biologic materials may induce classic IgE-mediated reactions. Large-molecular-weight substances may also induce similar reac-

tions. Excipient chemicals may also induce contact urticaria (ie, butylated, hydroxyanisole). In addition to IgE-mediated reactions, many excipients in food and drugs contain a sizable number of agents that may cause ACD. Allergenic food and drug additives and excipients have been reviewed extensively elsewhere.[913]

Occupational Allergens

Summary Statement 166. More than 300 low- and high-molecular-weight occupational allergens have been identified. Test reagents for these agents are generally available in specialized occupational allergy centers. (A)

More than 300 occupational allergens have been reported.[266] Many of these are large molecular weight substances that only occur as significant inhalant agents in certain industrial locales. In addition, a large number of low-molecular-weight substances, including polyisocyanates, acid anhydrides, metallic salts, aromatic amines, and azo dyes, have also been shown to cause allergic symptoms by classic immunologic mechanisms. A complete listing of these agents is available.[276] If exposure to these compounds is by respiratory route, asthma and/or hypersensitivity pneumonitis may ensue. The epidermal route of exposure may induce ACD, the most common immunologic occupational disease. Less commonly, skin sensitization may also evoke clinical respiratory allergy.[276]

Miscellaneous Plant Products

Summary Statement 167. A variety of plant or plant-derived proteins or glycoproteins may be associated with systemic allergic symptoms. (A)

A variety of other plant products has been associated with allergic symptoms. These include kapok, papain, chymopapain, pyrethrum, cottonseed, flaxseed, condiments, psyllium, and bean products. Latex allergens contained in hospital gloves, airborne sources, and medical appliances have increased in clinical importance since the introduction of universal barrier precautions. Although NRL standardized skin test extracts are not commercially available, they exhibit superior sensitivity for detecting NRL clinical sensitivity in comparison to FDA-approved specific IgE assays.[914–916] Testing with nonstandardized reagents from commercial sources or prepared locally is generally guided by clinical history of symptoms after exposure. Various vegetable gums are "hidden" ingredients of commercial food and drug products, and a clear patient history of exposure is rare. In fact, they are so prevalent in the diet that exposure may be assumed. Testing with vegetable gum extract may be indicated in selected patients with clear-cut symptoms not otherwise explained.

Contactant Allergens

Summary Statement 168. Chemicals, plant resins, and lipid constituents are the chief causes of ACD, which requires patch testing for confirmation. (A)

Chemicals, plant resins and lipid materials are the chief causes of classic ACD. Complex topical medications may contain potential antigens and additives. The major component of a complex mixture may not be the sensitizer. The 23 antigens in the FDA-certified T.R.U.E. TEST detect approximately 25% to 30% of all cases of ACD.[469,504] For this reason, an updated panel of 65 allergens has been designated by the North American Contact Dermatitis Group. After this screening test panel, selected allergens based on the patient's history must then be used to supplement the screening panel (see section, "Evaluation of Contact Dermatitis").

General Principles of Cross-reactivity of Plant-Derived Allergens

Summary Statement 169. As previously emphasized, knowledge of specific patterns of cross-reactivity among tree, grass, and weed pollens is essential in preparing an efficient panel of test reagents. (A)

Summary Statement 170. Although cross-reactivity among related pollen families can usually be ascribed to specific epitopic determinants, more diffuse cross-reactivity due to plant profilins and cross-reactive carbohydrate determinants may also be present. (A)

Summary Statement 171. Cross-reactivity data on fungi are extremely sparse. (C)

Botanical taxonomy may be used to infer cross-reactivity, but this assumption depends on 2 premises. The first is that the more closely related plants are, the greater will be their similarities and shared antigens. The second premise is that the present botanical classification truly reflects phylogeny. Two plants in the same genus might therefore be expected to share at least some allergens, 2 in the same family should share a lesser number. Distantly related plants would be expected to show little if any cross-reactivity. However, even in closely related species, unique allergenic epitopes may exist and have clinical relevance.[239]

Cross-reactivity data on pollens are limited and extremely sparse on fungi. Pollen data suffer in some cases from being derived from older techniques, being incomplete, or being occasionally contradictory. Several more recent studies investigating characterized allergens have addressed cross-reactivity in a limited fashion. There have been few attempts to collate this information[859,917] (see Allergome.com). Data on conserved epitopes between genera and families have been discussed previously under "Number of Skin Tests."

Trees

Available information reveals marked diversity, with little cross-reactivity except some notable exceptions.[253,255–257] Conifers of the Cypress family (including cypresses, cedars, and junipers) strongly cross-react. Thus, testing with a single member is probably adequate in most clinical situations. The other conifers do not cross-react.[917] Members of the 2 closely related birch and beech families (beech, oak, birch, alder, hazel) cross-react with each other within the family but not completely across family boundaries. The birch family members appear to be the most closely related[918,919]; testing with 1 or 2 should be adequate in most clinical situations. The olive family shows moderate cross-reactivity among olive, privet,

and ash.[255,920] Information on other tree families is too fragmentary to make useful recommendations. Indeed, a recent study failed to show correlation between regional pollen counts and percutaneous reactivity to tree pollens in patients with seasonal allergic rhinitis.[99]

Grasses

Most allergenically incriminated grasses belongs to the large Fescue subfamily or the northern pasture grasses. Extensive research with the rye group antigens (eg, Lol p I) suggests shared antigens and strong cross-reactivity across most of the members of this subfamily that have been studied[921–925]; however, complete cross-reactivity rarely occurs. Timothy and Johnson grasses may possess additional unique antigens.[926] In most clinical circumstances it is reasonable to test with Timothy and/or Johnson grasses and 1 or 2 locally prevalent northern pasture grasses. Southern grasses, such as Bermuda grass, show greater diversity and should be tested separately in areas where these are common or when dealing with a mobile population. Bermuda, although not sharing major allergens with the northern pasture grasses, has been shown to cross-react with some western prairie grasses of minor significance.[927,928] Bahia grass likewise does not appear to cross-react with northern grasses.[929]

Weeds

The composite family contains a number of potent sensitizers, the most important of which are the ragweeds of the genus *Ambrosia*. Short ragweed has been the most extensively studied, and several major and minor allergens are described. Information on which ragweeds contain the major allergens Amb a I (antigen E) is conflicting, but by RAST inhibition the 4 major species (short, giant, western, and false ragweed) all strongly cross-react.[587] Other members of the same tribe and other composites do not cross-react.[930,931] Recent data on the sages and mugworts(genus *Artemisia*) suggest strong cross-reactivity.[252] Thus, in many critical circumstances it may be reasonable to test for 1 or 2 *Ambrosia* species and a single *Artemisia*. Other compositae should betested separately. The Chenopod and Amaranth families are closely linked and contain plants of major importance, especially in the western United States. Members show varying degrees of cross-reactivity, even across family lines.[859,932] The *Atriplex* weeds (salt bushes, wing scale, shad scale) are nearly identical antigenically, and testing for a single locally prevalent species should be adequate in most cases. The *Amaranthus* weeds (eg, redroot pigweed and Palmer's amaranth) are likewise almost identical, whereas another member of the family, western water hemp, shows some differences. The 2 major tumbleweeds, Russian thistle and burning bush, show only partial cross-reactivity. Lamb's quarters shows the largest degree of cross-reactivity with other family members. In endemic areas, testing for Russian thistle, burning bush, an *Amaranthus,* and an *Atriplex* should be sufficient in most clinical situations.

Allergen Compendium

The choice of extracts for testing and treatment should be continuously refined in accord with scientific advances, botanic and aerobiologic surveys, demographic trends, and availability of relevant, defined reagents. Practice must be directed by the best documented concepts of allergen prevalence, geographic distribution, and immunochemical relationships.

From time to time patients may present with symptoms caused by previously unidentified substances that could be potential new allergens. There is a role for testing these patients with properly prepared extracts of a new allergen. There is insufficient evidence, however, to justify tests for such agents as newsprint, sugar, cornstarch, tobacco smoke, orris root, cotton, smog, formaldehyde, and human dander.

An entirely satisfactory basis for establishing guidelines for choice of allergen extracts is not currently available. A broad listing of allergens, based on botanic and aerobiologic surveys of North America, the catalogs of various extract, and specific IgE test manufacturers and miscellaneous other sources is presented in Table 11. For the pollens, fungi (currently alphabetical by genus), and foods, the list is organized taxonomically. For other allergens, the list is alphabetical within categories. The most current Latin binomial nomenclature is used, and older names are listed in parentheses, for example, *Aureobasidium* (*Pullularia*). Likewise, the most commonly encountered vernacular names are listed and synonyms (some of which are more colloquial than factual) are provided in brackets. The use of the common English names for definitive identification of regional plants is not advised. For instances, the tree commonly called Chinese elm (implying *Ulmus parvifolia*) is properly termed Siberian elm (*Ulmus pumillia*). Similarly, the term cottonwood may apply to 5 or more species in the genus *Populus*. Although reasonably comprehensive, the list is not exhaustive. Pollens and other allergens not in these lists were omitted because they were judged to lack current evidence of allergenic impact. Numerous substances on the list are included even though they probably are of minor importance.

It is difficult to make clinically relevant recommendation for testing with fungal extracts. In the listing of fungi, various organisms have been classified on the basis of what is currently known about prevalence and clinical activity. Primarily because of problems of procurement and manufacture, however, the capacity of many commonly prevalent spores to elicit IgE-mediated sensitization has not been evaluated. It also seems likely that sampling in previously unstudied situations will reveal new important forms; therefore, any list must admit later additions and corrections, as analyses of collections become more factual.

ASSESSMENT OF INHALANT ALLERGY

Summary Statement 172. The skin prick/puncture test is su perior to intracutaneous testing for predicting nasal allergic symptoms triggered by exposure to pollen. (B)

Table 11. Selected Compendium of Botanical, Animal, Food, and Chemical Allergens

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Grasses									
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Agropyron*	*desertoram*	Wheatgrass	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Agropyron*	*repens*	Quackgrass, (couch)	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Agropyron*	*smithii*	Wheatgrass, western	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Agropyron*	*spicatum*	Bunchgrass, blue	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Agropyron*	*stolonifera*	Redtop (bentgrass)	IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Agrostideae	*Agrostis*	*alba (gigantea)*	Redtop	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Agrostideae	*Agrostis*	*tenius*	Bent grass, colonial	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Agrostideae	*Alopecurus*	*pratensis*	Foxtail, meadow	IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Panicoideae	Andropogoneae	*Andropogon*	*virginicus*	Beardgrass, Virginia	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Phalarideae	*Anthoxanthum*	*odoratum*	Sweet vernalgrass	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Aveneae	*Arrhenatherum*	*elatlus*	Oatgrass, tall	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Aveneae	*Avena*	*fatua*	Oats, common wild	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Aveneae	*Avena*	*sativa*	Oats, cultivated (wild)	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Eragrostoideae	Chlorideae	*Bouteloua*	*gracila*	Grama grass, blue	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Bromus*	*inermis*	Brome, smooth	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Bromus*	*secalinus*	Brome,cheat	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Eragrostoideae	Chlorideae	*Buchloe*	*dactyloides*	Buffalo grass	
Monocotyledonae	Commelnidae	Cyperales	Cyperaceae			*Carex*	*aquatile*	Sedge	
Monocotyledonae	Commelnidae	Cyperales	Cyperaceae			*Carex*	*barbarae*	Sedge	ST
Monocotyledonae	Arecidae	Arecales	Areceae (Palmaceae)			*Cocos*	*nucifera*	Coconut palm	
Monocotyledonae	Commelnidae	Poales	Graminae	Arundinoideae	Arundineae	*Cortaderia*	*selloana*	Pampas grass	
Monocotyledonae	Commelnidae	Poales	Graminae	Eragrostoideae	Chlorideae	*Cynodon*	*dactylon*	Bermuda grass	ST, IVT
Monocotyledonae	Commelnidae	Cyperales	Cyperaceae			*Cypreus*	*esculentus*	Yellow nut sedge	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Dactylis*	*glomerata*	Orchard grass (cocksfoot)	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Eragrostoideae	Aeluropideae	*Distichlis*	*spicata*	Saltgrass	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Eragrostoideae	Aeluropideae	*Distichlis*	*stricta*	Saltgrass	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Elymus*	*cinereus*	Ryegrass, giant wild	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Elymus*	*condensatus*	Rye, giant wild	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Elymus*	*triticoides*	Rye, wild (alkali)	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Festuca*	*elatlor (pratensis)*	Fescue, meadow	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Festuca*	*rubra*	Fescue, red	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Aveneae	*Holcus*	*lanatus*	Velvet grass (Yorkshire fog)	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Aveneae	*Holcus*	*sudanensis*	Sudan grass	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Hordeum*	*vulgare*	Barley	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Triticeae	*Koeleria*	*cristata*	Hairgrass, crested (Koeler's grass)	
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Lolium*	*multiforum*	Ryegrass, Italian	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Festuceae	*Lolium*	*perenne*	Ryegrass, perennial (common)	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Oryzoideae	Oryzeae	*Oryza*	*sativa*	Rice	
Monocotyledonae	Commelnidae	Poales	Graminae	Panicoideae	Paniceae	*Panicum*	*purpurascens*	Para grass	ST
Monocotyledonae	Commelnidae	Poales	Graminae	Panicoideae	Paniceae	*Paspalum*	*notatum*	Bahia grass	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Phalarideae	*Phalaris*	*arundinacea*	Canry (reed) grass	ST, IVT
Monocotyledonae	Commelnidae	Poales	Graminae	Festucoideae	Phalarideae	*Phalaris*	*canariensis*	Canary grass	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Monocotyledonae	Commellnidae	Poales	Graminae	Festucoideae	Phalarideae	*Phalaris*	*minor*	Canary grass	
Monocotyledonae	Commellnidae	Polaes	Graminae	Festucoideae	Agrostideae	*Phleum*	*pratense*	Timothy	ST,IVT
Monocotyledonae	Arecidae	Arecales	Arecae (Palmaceae)			*Phoenix*	*dactylifera*	Date palm	
Monocotyledonae	Commellnidae	Poales	Graminae	Arundinoideae	Arundineae	*Phragmites*	*communis*	Common reed	IVT
Monocoytledonae	Commellnidae	Poales	Graminae	Festucoideae	Festuceae	*Poa*	*compressa*	Bluegrass, Canada	ST
Monocoytledonae	Commellnidae	Poales	Graminae	Festucoideae	Festuceae	*Poa*	*pratensis (palustris)*	Bluegrass, Kentucky (June)	ST, IVT
Monocoytledonae	Commellnidae	Poales	Graminae	Festucoideae	Festuceae	*Poa*	*sandbergii*	Bluegraass, Sandberg's	ST
Monocoytledonae	Arecidae	Arecales	Areceae (Palmaceae)			*Roystonea*	*regia*	Royal Palm	
Monocoytledonae	Commellnidae	Poales	Graminae	Panicoideae	Andropogoneae	*Saccharum*	*officinarum*	Sugar cane	
Monocoytledonae	Commellnidae	Cyperales	Cyperaceae			*Scirpus*	*validus*	Bulrush	
Monocoytledonae	Commellnidae	Poales	Graminae	Festuciodeae	Triticeae	*Secale*	*cereale*	Ryegrass, cultivated	ST, IVT
Monocoytledonae	Commellnidae	Poales	Graminae	Panicoideae	Andropogonoea	*Sorghastrum*	*nutans*	Indian grass	
Monocoytledonae	Commellnidae	Polaes	Graminae	Panicoideae	Andropogonoea	*Sorghum*	*halepense*	Johnson grass	ST, IVT
Monocoytledonae	Commellnidae	Poales	Graminae	Panicoideae	Andropogoneae	*Sorghum*	*sudanense (vulgare-sudanense)*	Sudan grass	
Monocoytledonae	Commellnidae	Poales	Graminae	Panicoideae	Andropogoneae	*Sorghum*	*vuigare(bicolor)*	Sorghum, common cultivated	ST
Monocoytledonae	Commellnidae	Poales	Graminae	Eragostoideae	Sporoboleae	*Sporobolus*	*cryptandrus*	Sand dropseed	
Monocoytledonae	Commellnidae	Poales	Graminae	Festucoideae	Triticeae	*Triticum*	*aestivum*	Wheat, cultivated	ST
Monocoytledonae	Commellnidae	Poales	Graminae	Festucoideae	Triticeae	*Triticum*	*sativum*	Wheat, cultivated	IVT
Monocoytledonae	Arecidae	Typhales	Typhaceae			*Typha*	*angustifolla*	Narrow Leaf cattail	
Monocoytledonae	Arecidae	Typhales	Typhaceae			*Typha*	*latifolla*	Common cattail	
Monocoytledonae	Commellnidae	Poales	Graminae	Panicoideae	Andropogoneae	*Zea*	*mays*	Corn	ST, IVT
Trees, shrubs, related plants									
Gymnospermae		Coniferales	Pinaceae			*Abies*	*concolor*	Fir, white	ST
Gymnospermae		Coniferales	Pinaceae			*Abies*	*procera*	Fir, red	ST
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Acacia*	*baileyana*	Acacia, golden (Bailey's)	ST
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Acacia*	*dealbata*	Mimosa	STsp
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Acacia*	*farnesiana*	Acacia, sweet	
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Acacia*	*longfolia*	Acacia (wattle), golden	ST, IVT
Dicotyledonae	Rosidae	Sapindales	Aceraceae			*Acer*	*macrophyllum*	Maple, coast (bigleaf)	ST
Dicotyledonae	Rosidae	Sapindales	Aceraceae			*Acer*	*negundo*	Box elder	ST, IVT
Dicotyledonae	Rosidae	Sapindales	Aceraceae			*Acer*	*platanoides*	Maple, Norway	
Dicotyledonae	Rosidae	Sapindales	Aceraceae			*Acer*	*rubrum*	Maple, red	ST
Dicotyledonae	Rosidae	Sapindales	Aceraceae			*Acer*	*saccharinum*	Maple, silver (soft)	ST
Dicotyledonae	Rosidae	Sapindales	Aceraceae			*Acer*	*saccharum*	Maple, sugar (hard)	ST
Dicotyledonae	Rosidae	Sapindales	Hippocastanaceae			*Aesculus*	*flava*	Buckeye, yellow	
Dicotyledonae	Rosidae	Sapindales	Hippocastanaceae			*Aesculus*	*hippocastanum*	Chestnut, horse	ST
Dicotyledonae	Rosidae	Rutales	Simaroubaceae			*Allanthims*	*altissima*	Tree of heaven	ST
Dicotyledonae	Roasidae	Fabales	Mimosaceae			*Albizzia*	*julibrissin*	Acacia, Persian	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*crispa*	Alder, green	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*glutinosa*	Alder, European	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*incana (tenuifolia)*	Alder, white (speckled) (grey)	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*rhorrbifolia*	Alder, white	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*rubra*	Alder, red	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*rugosa*	Alder, speckled (lag) (smooth)	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*serrulata*	Alder, hazel	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Alnus*	*viridis*	Alder, Sitka	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*alleghaniensis (lutea)*	Birch, yellow	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*fontinalis*	Birch, spring	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*lenia*	Birch, sweet (black)	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*lenta*	Birch, cherry	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*lutea (alleghaniensis)*	Birch, yellow	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*nigra*	Birch, river (gray) (red)	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*occidentalis*	Birch, water (spring)	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*papyrifera*	Birch, paper	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*pendula*	Birch, European white	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*populifolia*	Birch, white	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Betula*	*verrucosa*	Birch, silver	IVT
Dicotyledonae	Hamamelldidae	Urticales	Urticaceae			*Boehmeria*	*cylindrica*	Boghemp	
Dicotyledonae	Hamamelldidae	Urticales	Moraceae			*Broussoneria*	*papyrifera*	Mulberry, paper	ST
Dicotyledonae	Hamamelldidae	Myricales	Myricaceae			*Callistemon*	*citrinus*	Bottlebush tree	ST
Gymnospermae		Coniferales	Cupressaceae			*Libocedrus (Calocedrus)*	*decurrens*	Cedar, incense	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Carpinus*	*betulus*	Hornbeam	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Carpinus*	*caroliniana*	Bluebeech (American hornbeam)	
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Carya*	*alba*	Hickory, white	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Carya*	*aquatica*	Hickory, water	
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Cayra*	*cordiformis*	Hickory, bitternut	
Dicotyledonae	Hamamellidade	Juglandales	Juglandaceae			*Cayra*	*tomeniosa*	Hickory, mockernut (white)	ST
Dicotyledonae	Hamamellidade	Juglandales	Juglandaceae			*Cayra*	*glabra*	Hickory, pignut	
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Cayra*	*illinoensis (pecan)*	Pecan	ST, IVT
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Cayra*	*lacinosa*	Hickory, shellbark	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Cayra*	*ovata*	Hickory, shagbark	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Cayra*	*texana*	Hickory, black	
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Castanea*	*sativa*	Chestnut, sweet	
Dicotyledonae	Hamamelldidae	Casuarinales	Casuarinaceae			*Casuarina*	*equisetifolia*	Pine, Australian (beetwood)	ST, IVT
Gymnospermae		Coniferales	Pinaceae			*Cedrus*	*deodara*	Cedar, deodar	ST
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Celtis*	*australis*	Nettletree, southern	
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Celtis*	*laevigata*	Sugarberry	
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Celtis*	*occidentails*	Hackberry	ST
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Ceratonia*	*sillqua*	Carob (algarroba)	ST
Dicotyledonae	Rosidea	Fabales	Caesalpinioideae			*Cercidium*	*floridium*	Palo verde	ST
Gymnospermae		Coniferales	Cupressaceae			*Chamaecyparis*	*lawsoniana*	Cypress (cedar), Port Orford	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Gymnospermae		Coniferales	Cupressaceae			*Chamaecyparis*	*nootkatensis*	Cedar, Alaska	
Gymnospermae		Coniferales	Cupressaceae			*Chamaecyparis*	*obtusa*	Cedar, hinokl white	
			Arecaceae			*Chamaerops*	*humulis*	Palm, dwarf	ST
			Rutaceae			*Citrus*	*sinensis*	Orange	ST
			Arecaceae			*Cocos (Arecastrum)*	*plumosa (romanzoffianum)*	Palm, queen	ST, IVT
Dicotyledonae	Hamamelldidae	Myricales	Myricaceae			*Comptonia*	*peregrina*	Fern, sweet	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Corylus*	*americana*	Hazelnut (filbert), American	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Corylus*	*avellana*	Hazelnut, (filbert) European	ST, IVT
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Corylus*	*comuta*	Hazel, beaked	
Dicotyledonae	Rosidae	Rosales	Rosaceae			*Crategus*	*crus-galli*	Hawthorn, cockspur	
Gymnospermae		Coniferales	Taxodiaceae			*Cryptomeria*	*japonica*	Cedar, Japanese red(sugi)	ST, IVT
Gymnospermae		Coniferales	Cupressaceae			*Cupressus*	*arizonica*	Cypress, Arizona	ST
Gymnospermae		Coniferales	Cupressaceae			*Cupressus*	*macrocarpa*	Cypress, Monterey	ST
Gymnospermae		Coniferales	Cupressaceae			*Cupressus*	*sempervirens*	Cypress, funeral (Italian)	ST, IVT
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Cytisus*	*scoparius*	Scotch broom	
Dicotyledonae	Rosidae	Elaeagnaies	Elaeagnaceae			*Elaegnus*	*angustifolia*	Russian olive (oleaster)	ST
Dicotyledonae	Rosidae	Myrtales	Myrtaceae			*Eucalyptus*	*globulus*	Gum, blue	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Fagus*	*americana*	Beech, American	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Fagus*	*grandifolia*	Beech, American	ST, IVT
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Fagus*	*sylvatica*	Beech, European	
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*americana*	Ash, white	ST, IVT
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*arizonica*	Ash	
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*excelsior*	Ash, European	
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*latifolia*	Ash, Oregon	ST
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*nigra*	Ash, black	ST
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*oregona*	Ash, Oregon	ST
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*pennsylvanica*	Ash, green (red)	ST
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Franxinus*	*velutina*	Ash, velvet (Arizona)	ST
Gymnospermae		Ginkgoales	Ginkgoaceae			*Ginkgo*	*biloba*	Maidenhair tree	
Dicotyledonae	Rosidae	Elaeagnales	Elaeagnaceae			*Hippophae*	*rhamnoides*	Buckthorn, sea	
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Juglans*	*californica*	Walnut, California black	ST, IVT
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Juglans*	*cinerea*	Butternut	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juslandaceae			*Juglans*	*hindsii*	Walnut, Hind's California black	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Juglans*	*nigra*	Walnut, black	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Juglans*	*regia*	Walnut, English	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Juglans*	*rupestris (microcarpa)*	Walnut, Arizona	St
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*ashel*	Juniper, ash (mountain)	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*californica*	Juniper California	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*chinensis*	Pfitzer (Juniper, Chinese)	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*communis*	Juniper, common	
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*monosperma*	Juniper, oneseed	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*occidentails*	Juniper, western	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*osteosperma*	Juniper, Utah	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*pinchotii*	Juniper, pinchot	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*sabinoides (ashel)*	Cedar, mountain	IVT
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*scopulorum*	Cedar, Rocky Mountain	ST
Gymnospermae		Coniferales	Cupressaceae			*Juniperus*	*virginiana*	Cedar, red	ST
Dicotyledonae	Hamamelldidae	Urticales	Urticaceae			*Laportea*	*canadensis*	Nettle, wood	
Gymnospermae		Coniferales	Pinaceae			*Larix*	*occidentalis*	Tamarack (larch)	ST
Gymnospermae		Coniferales	Cuppressaceae			*Libocedrus Colocedrus*	*decurrecms*	Cedar, incense	
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Ligustrum*	*vulgare*	Privet, common	ST
Dicotyledonae	Hamamelldidae	Hamamelldales	Hamamellidaceae			*Liquidambar*	*styraciflua*	Sweet gum	ST
			Caprifoliaceae			*Lonicera*	*caprifolium caprifolium*	Honeysuckle	STsp
Dicotyledonae	Hamamelldidae	Urticales	Moraceae			*Maclura*	*pomisera*	Orange, osage	ST
Dicotyledonae	Rosidae	Rosales	Rosaceae			*Malus*	*pumila (sylvestris)*	Apple	ST
			Anacardiaceae			*Mangifera*		Mango blossom	STsp
Dicotyledonae	Rosidae	Myrtales	Myrtaceae			*Melaleuca*	*leucadendron*	Cajeput tree	ST, IV
Dicotyledonae	Dillenlidae	Euphorbiales	Euphorbicaceae			*Mercurialis*	*annua*	Mercury, annual	
Dicotyledonae	Hamamelldidae	Urticales	Moraceae			*Morus*	*alba*	Mulberry, white	ST, IVT
Dicotyledonae	Hamamelldidae	Urticales	Moraceae			*Morus*	*microphylia*	Mulberry, Texas	
Dicotyledonae	Hamamelldidae	Urticales	Moraceae			*Morus*	*rubra*	Mulberry, red	ST
Dicotyledonae	Hamamelldidae	Myricales	Myricaceae			*Myrica*	*cerifera*	Bayberry	ST
Dicotyledonae	Hamamelldidae	Myricales	Myricaceae			*Myrica*	*gale*	Gale, sweet (bayberry)	ST
Dicotyledonae	Hamamelldidae	Myricales	Myricaceae			*Myrica*	*pennsylvanica*	Bayberry	
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Olea*	*europaea*	Olive (Mediterranean)	ST, IVT
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Ostrya*	*caroliniana*	Hornbeam, American	ST
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Ostrya*	*carpinifolia*	Hophornbeam, European	
Dicotyledonae	Hamamelldidae	Fagales	Betulaceae			*Ostrya*	*virginiana*	Ironwood (eastern hophornbeam)	ST
			Saxifragaceae			*Philadelphus*	*lewisii*	Orange, mock, wild (syringa)	ST
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Phillyrea*	*angustifolia*	Phillyrea	
			Arecaceae			*Phoenix*	*canariensis*	Palm, Canary Island date	ST
			Arecaceae			*Phoenix*	*dactylifera*	Palm, date	ST
Gymnospermae		Coniferales	Pinaceae			*Picea*	*abies*	Spruce, Norway	
Gymnospermae		Coniferales	Pinaceae			*Picea*	*excelsa*	Spruce	IVT
Gymnospermae		Coniferales	Pinaceae			*Picea*	*mariana*	Spruce, black	
Gymnospermae		Coniferales	Pinaceae			*Picea*	*rubens*	Spruce, red	ST
Gymnospermae		Coniferales	Pinaceae			*Picea*	*sitchensis*	Spruce, Sitka	ST
Dicotyledonae	Hamamelldidae	Urticales	Urticaceae			*Pilea*	*pumila*	Richweed	
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*banksiana*	Pine, scrub	
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*contorta*	Pine, lodgepole	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*echinata*	Pine, short leaf (yellow)	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*edulis*	Pine, pinyon	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*elliotti*	Pine, slash	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*lambertiana*	Pine, sugar	
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*monticola*	Pine, western white	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*nigra*	Pine, Austrian	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*palustris*	Pine, longleaf	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*ponderosa*	Pine, ponderosa (western yellow)	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*radiata*	Pine, Monterey	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*resinosa*	Pine, red	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*sabiniana*	Pine, digger	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*strobis*	Pine, (eastern) white	ST, IVT
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*sylvestris*	Pine, Scotch	
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*taeda*	Pine, loblolly	ST
Gymnospermae		Coniferales	Pinaceae			*Pinus*	*virginiana*	Pine, Virginia (scrub)	ST
Dicotyledonae	Hamamelldidae	Hamamelldales	Platanaceae			*Platanus*	*acerifolia*	Planetree, London (sycamore, mapleleaf)	ST, IVT
Dicotyledonae	Hamamelldidae	Hamamelldales	Platanaceae			*Platanus*	*occidentalis*	Sycamore, American (eastern)	ST
Dicotyledonae	Hamamelldidae	Hamamelldales	Platanaceae			*Platanus*	*orientalis*	Planetree, Oriental	
Dicotyledonae	Hamamelldidae	Hamamelldales	Platanaceae			*Platanus*	*racemosa*	Sycamore, California (western)	ST
Dicotyledonae	Hamamelldidae	Hamamelldales	Platanaceae			*Platanus*	*wrightii*	Sycamore, Arizona	
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*alba*	Poplar, white	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*balsamifera*	Poplar, balsam	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*deltoides*	Cottonwood (poplar), eastern	ST, IVT
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*fremontii*	Cottonwood, Fremont	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*lombardy*	Poplar, Lombardy	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*nigra-italica*	Poplar, Lombardy	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*sargentii*	Cottonwood, western	
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*tremuloides*	Aspen, quaking	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*trichocarpa*	Cottonwood, black (western balsam)	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Populus*	*wislizenii*	Cottonwood, Rio Grande	ST
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Prosopis*	*glandulosa*	Mesquite	
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Prosopis*	*juliflora*	Mesquite	ST
Dicotyledonae	Rosidea	Rosales	Rosaceae	Prunoideae		*Prunus*	*armeniaca*	Apricot	ST
Dicotyledonae	Rosidea	Rosales	Rosaceae	Prunoideae		*Prunus*	*cerasus*	Cherry	ST
Dicotyledonae	Rosidea	Rosales	Rosaceae	Prunoideae		*Prunus*	*domestica*	Plum (prune)	ST
Dicotyledonae	Rosidea	Rosales	Rosaceae	Prunoideae		*Prunus*	*dulcis*	Almond	ST
Dicotyledonae	Rosidea	Rosales	Rosaceae	Prunoideae		*Prunus*	*persica*	Peach	ST
Gymnospermae		Coniferales	Pinaceae			*Pseudotsuga*	*menziesii (taxifolia)*	Fir, Douglas	ST
Dicotyledonae	Hamamelldidae	Juglandales	Juglandaceae			*Pterocarya*	*fraxinifolia*	Wingnut	
Dicotyledonae	Rosidea	Rosales	Rosaceae	Maloideae		*Pyrus*	*communis*	Pear	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*agrifolia*	Oak, California (coast) live	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*alba*	Oak, white	ST, IVT
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*bicolor*	Oak, blue	

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*chrysolepsis*	Oak, canyon live (Arizona scrub)	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*dumosa*	Oak, California scrub	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*engelmanii*	Oak, Engelmann	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*falcata*	Oak, southern red	
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*gambelii*	Oak, gambel (Arizona)	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*garryana*	Oak, garry (Oregon, western, white)	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*ilex*	Oak, holly	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*kelloggi*	Oak, California black	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*laurifolia*	Oak, laurel	
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*lobata*	Oak, valley (California) white	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*macrocarpa*	Oak, bur (mossy)	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*marilandica*	Oak, black jack	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*nigra*	Oak, water	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*palustris*	Oak, swamp (pin)	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*phellos*	Oak, willow	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*rubra*	Oak, northern red	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*stellata*	Oak, post	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*velutina*	Oak, black	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*virginiana*	Oak, Virginia live	ST
Dicotyledonae	Hamamelldidae	Fagales	Fagaceae			*Quercus*	*wislizenii*	Oak, interior live	ST
Dicotyledonae	Rosidae	Fabales	Mimosaceae			*Robinia*	*pseudoacacia*	Locust, black locustblossom	ST
Dicotyledonae	Rosidae	Rosales	Rosaceae	Rosoideae		*Rosa*	*multiflora*	Rose	ST
Dicotyledonae	Dillenlidae	Salicales	Salicaceae			*Salix*	*caprea*	Sallow	IVT
Dicotyledonae	Dillenlidae	Salicales	Salicacea			*Salix*	*discolor*	Willow, pussy	ST
Dicotyledonae	Dillenlidae	Salicales	Salicacea			*Salix*	*laevigata*	Willow, red	ST
Dicotyledonae	Dillenlidae	Salicales	Salicacea			*Salix*	*lasiandra*	Willow, yellow	ST
Dicotyledonae	Dillenlidae	Salicales	Salicacea			*Salix*	*lasiolepis*	Willow, arroyo	ST
Dicotyledonae	Dillenlidae	Salicales	Salicacea			*Salix*	*nigra*	Willow, black	ST
			Caprifollaceae			*Sambucus*	*glauca (cerulea)*	Elderberry	ST
			Anacardiaceae			*Schinus*	*molle*	Pepper tree, California	ST
			Anacardiaceae			*Schinus*	*terebinthifolius*	Pepper tree, Brazillian	ST
Gymnospermae		Coniferales	Taxodiaceae			*Sequoia*	*sempervirens*	Redwood, coastal	ST
Dicotyledonae	Rosidae	Elaeagnales	Elaeagnaceae			*Shepherdia*	*argentea*	Buffaloberry	
Dicotyledonae	Rosidae	Oleales	Oleaceae			*Syringa*	*vulgaris*	Lilac	ST
			Tamaricaceae			*Tamarix*	*aphylla*	Tamarisk	
			Tamaricaceae			*Tamarix*	*galilca*	Cedar, salt (tamarisk)	ST
Gymnospermae		Coniferales	Taxodiaceae			*Taxodium*	*distichum*	Cypress, bald	ST
Gymnospermae		Coniferales	Cupressaceae			*Thuja*	*occidentails*	Cedar, white (arbor vitae)	ST
Gymnospermae		Coniferales	Cupressaceae			*Thuja*	*orientails*	Arbor vitae, oriental	ST
Gymnospermae		Coniferales	Cupressaceae			*Thuja*	*plicata*	Cedar, western red (giant)	ST
Dicotyledonae	Dillenlidae	Malvales	Malvaceae			*Tilla*	*americana*	Basswood (linden), American	ST

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Dicotyledonae	Dillenlidae	Malvales	Malvaceae			*Tilla*	*cardata*	Linden (lime)	
Gymnospermae		Coniferales	Pinaceae			*Tsuga*	*canadensis*	Hemlock, eastern	ST
Gymnospermae		Coniferales	Pinaceae			*Tsuga*	*heterophylla*	Hemlock, western	ST
Gymnospermae		Coniferales	Pinaceae			*Tsuga*	*mertensiana*	Hemlock, mountain	
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Ulmus*	*americana*	Elm, white (American)	ST, IVT
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Ulmus*	*crassifolia*	Elm, cedar (fallblooming)	ST
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Ulmus*	*parvifolia*	Elm,Chinese	ST
Dicotyledonae	Hamamelldidae	Urticales	Ulmaceae			*Ulmus*	*pumila*	Elm, Siberian (Chinese)	ST
Dicotyledane	Hamamelldiae	Urticales	Ulmaceae			*Ulmus*	*rubra*	Elm, slippery	ST
Weeds, flowers, miscellaneous plants									ST, IVT
Dicotyledonae	Caryophyllidae	Chenopodiales	Amaranthaceae			*Acnida*	*tamariscina*	Waterhemp, western	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Allenrolfia*	*occidentalis*	Iodine bush (burroweed)	ST
Dicotyledonae	Caryophyllidae	Chernopodiales	Amaranthaceae			*Amaranthus*	*albus*	Pigweed, tumble	
Dicotyledonae	Caryophyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*blitoides*	Pigweed, prostrate	
Dicotyledonae	Caryophyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*hybridus*	Careless weed	ST
Dicotyledonae	Caryophyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*palmeri*	Amaranth, Palmer's (careless weed)	ST
Dicotyledonae	Caryophyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*retroflexus*	Pigweed, redroot (rough)	ST, IVT
Dicotyledonae	Caryophyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*spinosus*	Pigweed, spiny	ST
Dicotyledonae	Caryophlyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*tamariscinus*	Waterhemp, western	ST
Dicotyledonae	Carophyllidae	Chemopodiales	Amaranthaceae			*Amaranthus*	*tuberculata*	Amaranth	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia*	*artemisiifolia (elatior)*	Ragweed, short	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia*	*bidentata*	Ragweed, southern	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia*	*coronopifolia*	Ragweed, western	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia*	*psilostachya*	Ragweed, western (perennial)	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia*	*trilida*	Ragweed, giant	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*acanthicarpa*	Ragweed, bur (false)	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*ambrosioides*	Ragweed, canyon	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*bipinnatifida*	Sandbur, beach	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*chamissonia*	Bur, beach	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosida (Franseria)*	*confertiflora*	Ragweed, slender	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*deltoidea*	Rabbit bush (bur ragweed)	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*discolor*	Bursage	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*dumosa*	Ragweed, desert (burroweed)	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Ambrosia (Franseria)*	*tenuifolia*	Ragweed, slender	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Anthemis*	*cotula*	Chamomille (dog fennel)	ST
						Antirhinum	*majus*	Snapdragon	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*absinthium*	Wormwood (absinthe)	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*annua*	Wormwood (sagebrush), annual	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artenmsia*	*californica*	Sage, (sagebrush), coastal	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artenmsia*	*camperstre*	Southernwood, field	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*dracunculus*	Sage (sagebrush), green	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*filifolia*	Sagebrush, sand	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*frigida*	Sage, pasture	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*graphlodes*	Sage, prairie	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*heterophylla*	Mugwort, California	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*ludoviciana*	Sage (sagebrush), prairie (darkleaved)	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*pycnocephala*	Sagebrush, sanddune	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*trindentata*	Sagebrush, giant (common)	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Artemisia*	*vulgaris (douglasiana)*	Mugwort, common	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Aster*	*chinensis*	Starwort (aster)	STsp
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*argentea*	Scale, silver	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*canescens*	Wingscale (shadscale)	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*confertifolia*	Shadscale (sheepfat)	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*lentiformis*	Lenscale (quailbush)	ST, IVT
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*patula*	Orache, common (spear scale)	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*polycarpa*	Allscale	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*rosea*	Scale, red	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*serenana*	Scale, bract	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*watsoni*	Saltbush, coast	
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Atriplex*	*wrightii*	Saltbush, annual	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Axyris*	*amaranthoides*	Pigweed, Russian	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Baccharis*	*hallmifolia*	Groundsel	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Baccharis*	*viminae*	Mulefat (Baccharis)	ST
						Balsamorhiza	*sagittata*	Balsam root	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Bassia*	*hyssopifolia*	Smotherweed (Bassia)	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Beta*	*vulgaris*	Sugar beet	ST
Dicotyledonae	Dillenlidae	Capparales	Brassicaceae (Cruciferae)			*Brassica*	*campestris (rapa)*	Mustard, common yellow	ST
Dicotyledonae	Dillenlidae	Capparales	Brassicaceae (Cruciferae)			*Brassica*	*nigra*	Mustard, black	ST
Dicotyledonae	Dillenlidae	Capparales	Brassicaceae (Cruciferae)			*Brassica*	*napus*	Rape	IVT
Dicotyledonae	Hamamelldidae	Urticales	Cannabaceae			*Cannabis*	*sativa*	Hemp	ST
Dicotyledonae	Asteridae	Asterales	Compositae		Cynareae	*Centaurea*	*maculosa*	Knapweed	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Chenopodium*	*album*	Lamb's quarter	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Chenopodium*	*ambrosiodes*	Mexican tea	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Chenopodium*	*berlandierl*	Goosefoot	
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Chenopodium*	*botrys*	Jerusalem oak (wormseed)	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroidene	Anthemideae	*Chrysanthemum*	*leucanthemum*	Daisy, ox-eye (Marquerite)	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroidene	Anthemideae	*Chrysanthemum*	*morifolium*	Chrysanthemum	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroidene	Astereae	*Chrysanthemnus*	*nauseosus*	Rabbit brush	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Chrysanthemnus*	*viscidiflorus*	Rabbitbush, Douglas	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Hellantheae	*Cosmos*	*bipinnatus*	Cosmos	ST
						Cytisus	*scoparius*	Scotch broom	ST
						Dahlia	*pinnata coccinea*	Dahlia	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Dicoria*	*canescens*	Ragweed, silver	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Dondia*	*suffrutescens*	Alkaliblite	ST
Dicotyledonae	Asteridae	Polemonales	Boraginaceae			*Echium*	*plantagineum*	Paterson's curse	
			Onagraceae			*Epilobium*	*angustifolium*	Fireweed	ST
			Papaveraceae			*Eschscholzia*	*californica*	Poppy, California	ST
Dicotyledonae	Asteridae	Asterales	Compositae		Eupatorieae	*Eupatorium*	*capillifolium*	Fennel, dog, eastern	ST
Dicotyledonae	Caryophyllidae	Chenopodiales	Chenopodiaceae			*Eurotia (Ceratoides)*	*lanata*	Winterfat	ST
						Gladiolus	*xhortulanus*	Gladiolus	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Heliantheae	*Helianthus*	*annuus*	Sunflower	ST, IVT
Dicotyledonae	Hamamelldidae	Urticales	Cannabaceae			*Humulus*	*lupulus*	Hops	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Hymenocleae*	*salsola*	Burroweed (greasebush) (burrobush	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Iva*	*angustifolia*	Marshelder, narrowleaf	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambroiseae	*Iva*	*axillaris*	Povertyweed, small	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambroiseae	*Iva*	*cillata (annus)*	Marshelder, rough (true)	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambroiseae	*Iva*	*frutescens*	Marshelder, coast	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambroiseae	*Iva*	*xanthifolia*	Ragweed, prairie (burweed)	ST
Dicotyledonae	Caryophyllidae	Chenopodiaies	Chenopodiaceae			*Kochia*	*scoparia*	Burning bush (firebush)	ST, IVT
Dicotyledonae	Asteridae	Asterales	Compositae	Cichorioideae	Cichorieae	*Lactuca*	*serriola*	Lettuce, prickly	
						Lilum	*longiflorum*	Lily, Easter	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Anthemideae	*Matricaria*	*chamomilla*	Chamomille, wild	IVT
Dicotyledonae	Rosidae	Fabales	Fabaceae			*Medicago*	*sativa*	Alfalfa (lucerne)	ST
Dicotyledonae	Rosidae	Fablales	Fabaceae			*Melilotus*	*officinalis*	Clover, sweet yellow	ST
						Narcissus	*pseudonarcissus*	Daffodil	ST
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Hellanthea	*Parthenium*	*argentatum*	Guayule	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Hellanthea	*Parthenium*	*hystererophorus*	Feverfew	
Dicotyledonae	Hamamelldidae	Urticales	Urticaceae			*Parietaria*	*judaica*	Wall pellitory	IVT
Dicotyledonae	Hamamelldidae	Urticales	Urticaceae			*Parietaria*	*officinalis*	Wall pellitory	IVT
Dicotyledonae	Asteridae	Scrophulariales	Plantaginaceae			*Plantago*	*lanceolata*	Plantain, English (narrow leaved)	ST, IVT
Dicotyledonae	Asteridae	Scrophulariales	Plantaginaceae			*Plantago*	*majora*	Plantain, common	
Dicotyledonae	Asteridae	Scrophulariales	Plantaginaceae			*Plantago*	*rugelii*	Plantain, blackseed	
Dicotyledonae	Carophyllidea	Polygonales	Polygonaceae			*Rhuem*	*officinale*	Rhubarb	
			Euphorbiaceae			*Ricinus*	*communis*	Castor bean	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT	
Dicotyledonae	Carophyllidea	Polygonales	Polygonaceae			*Rumex*	*acetosa*	Sorrel, garden		
Dicotyledonae	Carophyllidea	Polygonales	Polygonaceae			*Rumex*	*acetosella*	Sorrel, common (sheep) (red)	ST, IVT	
Dicotyledonae	Carophyllidea	Polygonales	Polygonaceae			*Rumex*	*crispus*	Dock, yellow (curly)	ST	
Dicotyledonae	Carophyllidea	Polygonales	Polygonaceae			*Rumex*	*obtusifolius*	Dock, broadleaf (bitter)	ST	
Dicotyledonae	Carophyllidea	Polygonales	Polygonaceae			*Rumex*	*orbiculatus*	Dock, water		
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae	Chenopodlodeae		*Salicornia*	*ambigua (virginica)*	Pickleweed	ST	
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Salsola*	*pestifer (kali)*	Russian thistle	ST, IVT	
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Salsola*	*soda*	Saltwort		
Dicotyledonae	Asteridae	Dipsacales	Caprifoliaceae			*Sambucus*	*nigra*	Elder		
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Sarcobatus*	*vermiculatus*	Greasewood	ST	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Solidago*	*canadensis*	Goldenrod	ST	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Solidago*	*gigantean*	Goldenrod		
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Astereae	*Solidago*	*virgaurea*	Goldenrod	IVT	
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Spinacia*	*oleracea*	Spinach		
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Suaeda*	*californica*	Seablite, California	ST	
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Suaeda*	*fruticosa*	Seablite, alkall	ST	
Dicotyledonae	Carophyllidea	Chenopodiales	Chenopodiaceae			*Suaeda*	*maritima*	Seablite		
Dicotyledonae	Asteridea	Asterales	Compositae	Asteroideae	Hellantheae	*Tagetes*	*patula*	Marigold	ST	
Dicotyledonae	Asteridea	Asterales	Compositae	Asteroideae	Anthemideae	*Tanacetum*	*vulgare*	Tansy		
Dicotyledonae	Asteridea	Asterales	Compositae	Cichorioideae	Cichorieae	*Taraxacum*	*officinale*	Dandelion	ST	
Dicotyledonae	Asteridea	Asterales	Compositae	Cichorioideae	Cichorieae	*Taraxacum*	*vulgare*	Dandelion	IVT	
Dicotyledonae	Rosidea	Fabales	Fabaceae			*Trifolium*	*pratense*	Clover, red	ST	
Dicotyledonae	Rosidea	Fabales	Fabaceae			*Trifolium*	*repens*	Clover, white	ST	
			Typhaceae			*Typha*	*latifolia*	Cattail, common	ST	
Dicotyledonae	Hamamelldidae	Urticales	Urticaceae			*Urtica*	*dioica*	Nettle, stinging	STsp, IVT	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Xanthium*	*commune*	Cocklebur	ST, IVT	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Xanthium*	*spinosum*	Cocklebur, spiny	ST	
Dicotyledonae	Asteridae	Asterales	Compositae	Asteroideae	Ambrosieae	*Xanthium*	*strumarium*	Cocklebur, common	ST	
Molds, fungi, yeasts, etc. – alphabetized by genus										**Importance[a]**
								Smut, barley	ST	1
								Smut, Bermuda grass	ST	1
								Smut, corn	ST	1
								Smut, Johnson grass	ST	1
								Smut, oat	ST	1
								Smut, sorghum	ST	1
								Smut, wheat	ST	1
								Rust, wheat	ST	1
								Rust, oat	ST	1
						Agaricus				1
Deuteromycetes		Monilliales	Dematiaceae			*Altemaria*	*alternata (tenuis)*		ST, IVT	3
Deuteromycetes		Monilliales	Dematiaceae			*Altemaria*	*brassicicola*		ST	1
Deuteromycetes		Monilliales	Dematiaceae			*Altemaria*	*tenuissima*			3
						Armillarea				1

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT	
						Arthrinium	*sphaerospermum*			1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*repens/chevaller/ amstelodaml*			3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*candidus*			1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*clavatus*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*flavus (var. columnaris)*		ST	3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*fumigatus*		ST, IVT	3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*glaucus (repens)*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*nidulans*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*niger*		ST	3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*ochraceus*			1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*omatus*			3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*oryzae*			1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*penicilloides*			1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*restrictus*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*sydowi*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*terreus*		ST	3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*versicolor*			3
Deuteromycetes		Monilliales	Moniliaceae			*Aspergillus*	*wentii*			3
Deuteromycetes		Monilliales	Dematiaceae			*Aureobasidium (Pullularia)*	*pullulans*		ST, IVT	3
						Botrytis	*aclada (allii)*			1
						Botrytis	*cinerea*		ST, IVT	3
						Brachysporium				1
						Bullera				1
						Candida	*albicans*		ST, IVT	1
						Candida	*tropicalis*			1
Deuteromycetes		Monilliales	Moniliaceae			*Cephalosporium*	*acremonium*		ST, IVT	1
Deuteromycetes		Monilliales	Moniliaceae			*Cephalosporium*	*recifel*		ST	1
						Cercaspora				1
Ascomycetes		Sphaeriales	Chaetomiaceae			*Chaetomium*	*globosum*		ST	3
Deuteromycetes		Monilliales	Dematiaceae			*Cladosporium (Hormodendrum)*	*cladosporoides*		ST	3
Deuteromycetes		Monilliales	Dematiaceae			*Cladosporium (Hormodendrum)*	*herbarum*		ST, IVT	3
Deuteromycetes		Monilliales	Dematiaceae			*Cladosporium (Hormodendrum)*	*macrocarpum*			1
Deuteromycetes		Monilliales	Dematiaceae			*Cladosporium (Hormodendrum)*	*sphaerospermum (hordei)*		ST	2
Deuteromycetes		Monilliales	Dematiaceae			*Cochliobolus (Helminthosporium)*	*sativus*			3
Deuteromycetes		Monilliales	Dematiaceae			*Cochliobolus (Helminthosporium)*	*heterostrophus (maydis)*		ST	3
Basidiomycetes						*Coprinus*	*micaceous*			3
						Cryptococcus	*terreus*		ST	1
						Cryptostroma	*corticale*			1
						Cunninghamella	*elegans*		ST	1

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT	
Deuteromycetes		Monilliales	Dematiaceae			*Curvularia*	*lunata*		ST, IVT	3
Deuteromycetes		Monilliales	Dematiaceae			*Curvularia*	*spicifera*		ST	1
Deuteromycetes		Monilliales	Dematiaceae			*Cylindrocarpon*				1
Deuteromycetes		Monilliales	Dematiaceae			*Daldinia*				1
Deuteromycetes		Monilliales	Dematiaceae			*Dematium*	*nigrum*		ST	1
Deuteromycetes		Monilliales	Dematiaceae			*Dendryphion*				1
Ascomycetes						*Didymella*	*exitialis*			3
Deuteromycetes		Monilliales	Dematiaceae			*Drechslera*	*sorokiniana*		ST	1
Deuteromycetes		Monilliales	Tuberculariaceae			*Epicoccum*	*nigrum*		ST, IVT	3
							(purpurascens)			
						Epidermophyton	*floccosum*		ST	1
						Fomes	*rimosus*		ST	1
						Fulvia	*fulva*			1
Deuteromycetes		Monilliales	Tuberculariaceae			*Fusarium*	*moniliforme*		ST, IVT	3
Deuteromycetes		Monilliales	Tuberculariaceae			*Fusarium*	*nivale*			1
Deuteromycetes		Monilliales	Tuberculariaceae			*Fusarium*	*roseum*			3
Deuteromycetes		Monilliales	Tuberculariaceae			*Fusarium*	*solani*		ST	1
Deuteromycetes		Monilliales	Tuberculariaceae			*Fusarium*	*vasinfectum*		ST	1
Basidiomycetes						*Gandoderma*	*applanatum*			3
Deuteromycetes		Monilliales	Tuberculariaceae			*Geotrichum*	*candidum*		ST	1
Deuteromycetes		Monilliales	Moniliaaceae			*Gliocladium*	*deliquescens*		ST	1
Deuteromycetes		Monilliales	Moniliaaceae			*Gliocladium*	*roseum*			1
						Helicomyces				1
Deuteromycetes		Monilliales	Dematiaceae			*Helminthosporium*	*halodes*		IVT	1
Deuteromycetes		Monilliales	Dematiaceae			*Helminthosporium*	*interseminatum*		ST	1
Deuteromycetes		Monilliales	Dematiaceae			*Helminthosporium*	*velutinum*			1
						Hypholoma				1
						Leptosphaeria	*doliolum*			2
						Merulius	*lacrymans*			1
						Microsphaera	*aini*			1
						Microsphaeropsis	*olivaceae*			2
						Microsporum	*canis*		ST	1
						(Lanosum)				
Deuteromycetes		Monilliales	Moniliaceae			*Monilia*	*sitophila*		ST	1
Zygomycetes		Mucorales	Mucoraceae			*Mucor*	*mucedo*		ST	1
Zygomycetes		Mucorales	Mucoraceae			*Mucor*	*plumbeus*		ST	1
Zygomycetes		Mucorales	Mucoraceae			*Mucor*	*racemosus*		STsp	1
						Mycogone			STsp	1
						Nectria				1
						Neurospora	*crassa*		ST	1
						Nigrospora	*sphaerica*		ST	3
						Oidiodendrum			STsp	1
						Ophiobolus				1
Deuteromycetes		Monilliales	Moniliaceae			*Paecilomyces*	*lilacinum*			3
Deuteromycetes		Monilliales	Moniliaceae			*Paecilomyces*	*varioti*		ST	3
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*atramentosum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*biforme*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*brevicompactum*			3
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*carminoviolaceum*		ST	1

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT	
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*chrysogenum (notatum)*		ST, IVT	3
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*cyclopium*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*decumbens*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*digitatum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*expansum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*frequentans*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*funiculosum*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*glabrum*			3
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*glaucum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*herquei*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*implicatum*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*intricatum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*italicum*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*luteum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*oxallcum (citrinum)(steckii)*			3
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*purpurogenum*			3
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*roqueforti (casel)*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*roseum*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*simplicissimum*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*terrestre*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*verrucosum var. cyclopium (aurantiogriseum)*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*verrucosum var. verrucosum (viridicatum)*			1
Deuteromycetes		Monilliales	Moniliaceae			*Penicillium*	*waksmanii*			1
						Periconia	*byssoides*			2
Deuteromycetes		Spheropidales	Sphaeriodaceae			*Phoma*	*betae*		ST, IVT	1
Deuteromycetes		Spheropidales	Sphaeriodaceae			*Phoma*	*glomerata (exigua)*			3
Deuteromycetes		Spheropidales	Sphaeriodaceae			*Phoma*	*herbarum*		ST	1
						Phytophthora	*infestans*			1
						Pithomyces	*chartarum*			2
						Pityrosporum	*orbiculare*		IVT	1
						Pleospora			STsp	1
						Polyporus				1
						Polythrincium	*trifolii*			1
						Poria	*monticola*		ST	1
Basidiomycetes						*Puccinia*	*graminis*			3
						Pyrenophora (Helminthosporium)	*tritici-repentis*			3
Zygomycetes		Mucorales	Mucoraceae			*Rhizopus*	*arrhizus (oryzae)*		ST	1
Zygomycetes		Mucorales	Mucoraceae			*Rhizopus*	*nigricans (stolonifer)*		ST, IVT	1
Deuteromycetes						*Rhodotorula*	*brevicaulis*			1
Deuteromycetes						*Rhodotorula*	*glutinis*			1

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT	
Deuteromycetes						*Rhodotorula*	*roseus*			1
Deuteromycetes						*Rhodotorula*	*rubrum*		ST	3
						Saccharomyces	*cerevislae*		ST, IVT	1
						Scopulariopsis	*brevicaulis*		ST	3
Deuteromycetes		Monilliales	Dematiaceae			*Spondlyocladium*	*atrovirens*			1
Deuteromycetes		Monilliales	Dematiaceae			*Spondlyocladium*	*australe*		ST	1
						Sporidesmium				1
						Sporobolomyces	*salmonicolor*		ST	1
						Sporobolomyces	*roseus*			3
						Sporothrix				1
Deuteromycetes		Monilliales	Dematiaceae			*Stemphylium*	*botryosum*		ST, IVT	3
Deuteromycetes		Monilliales	Dematiaceae			*Stemphylium*	*solani*		ST	1
						Streptomyces	*griseus*		ST	1
						Suillus	*granulatus*			2
Zygomycetes						*Syncephalastrum*				1
						Tilletia				1
						Torula				1
						Torulopsis				1
						Trichoderma	*reesel*		ST	1
Deuteromycetes		Monilliales	Moniliaceae			*Trichoderma*	*viride*		ST, IVT	1
						Trichophyton	*mentagrophytes*		ST	1
						Trichophyton	*rubrum*		ST, IVT	1
						Trichosporon	*pullulans*		IVT	1
						Trichothecium (Cephalothecium)	*roseum*		ST	1
						Ulocladium	*chartarum*		IVT	1
						Urocystis				1
Basidiomycetes						*Ustilago*	*nuda (tritici)*		IVT	3
Basidiomycetes						*Ustilago*	*zeae*			3
						Verticillium	*albo-atrum*		ST	1
						Verticillium	*lecanii*			1
						Wallemia	*sebi*			2
						Xylaria				1
Insects, arthropods, insect products, venom (sorted by vernacular name)										
						Camponotus	*pennsylvanica*	Ant, black	ST	
						Camponotus	*floridanus*	Ant, carpenter	ST	
						Solenopsis	*richteri*	Ant, fire	ST	
						Solenopsis	*invicta*	Ant, fire	ST, IVT	
						Myrmecia	*pilosula*	Ant, jumper (Jack juniper)	IVT	
						Aphidius	*ervi*	Aphid	ST	
						Trogoderma	*angustum solier*	Berlin beetle	IVT	
						Simulium	*ubiquitum*	Black fly	ST	
						Chironomus	*thummi thummi*	Blood worm	IVT	
						Psilotreta	*labida*	Caddisfly	STsp	
						Periplaneta	*americana*	Cockroach, American	ST	

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
						Blatella	*germanica*	Cockroach, German	ST, IVT
						Gryllus	*bimaculatus*	Cricket	ST
						Chrysops	*relictus*	Deerfly	STsp
						Ctenophthalmus	*pseudagyrtes*	Flea	STsp
						Drosophila	*melanogaster*	Fruitfly	STsp
						Cladotanytarsus	*lewisi*	Green nimitti midge	IVT
						Apis	*millifera*	Honey bee	ST
						Hybomitra	*bimaculata*	Horsefly	STsp
						Musca	*domesitca*	Housefly	ST
						Ephemera	*danica*	Mayfly	ST
						Dermatophagoides	*farinae*	Mite, dust	ST, IVT
						Dermatophagoides	*microceras*	Mite, dust	IVT
						Dermatophagoides	*pteronyssinus*	Mite, dust	ST, IVT
						Acarus	*Siro*	Mite, storage	IVT
						Tyrophagus	*putrescentiae*	Mite, storage	IVT
						Eurogylyphus	*Maynei*	Mite, storage	IVT
						Lepidoglyphus	*destructor*	Mite, storage	IVT
						Aedes	*aegyptii*	Mosquito	ST
						Aedes	*communis*	Mosquito	IVT
						Aedes	*culex-pipiens*	Mosquito	ST
						Aedes	*taenior*	Mosquito	ST
						Lymantria	*obfuscata*	Moth, gypsy	STsp
						Bombyx	*mori*	Silk moth	ST, IVT
						Lasioglossum	*malachurum*	Sweat bee	ST
						Vespula	*vulgaris*	Venom, common wasp (yellow jacket)	ST, IVT
						Apis	*mellifera*	Venom, honey bee	ST, IVT
						Vespa	*crabro*	Venom, hornet, European	IVT
						Polistes	*dominulus*	Venom, paper wasp	ST, IVT
						Dolichovespula	*maculata*	Venom, white-faced hornet	ST, IVT
						Dolichovespula	*arenaria*	Venom, yellow hornet	ST, IVT
Epidermals, miscellaneous animal products, etc									
								Ascaris	IVT
								Camel, hair, dander	ST
								Canary feathers	ST, IVT
								Cat dander	IVT
								Cat epithelium	ST
								Chicken feathers	ST, IVT
								Chinchilla	IVT
								Cow (cattle) dander	IVT
								Cow (cattle) epithelium	ST
								Deer, hair, dander	ST
								Dog dander	IVT
								Dog epithelium	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
								Duck feathers	ST, IVT
								Echinococcus	IVT
								Fink feathers	IVT
								Fish feed (*Art salina*)	IVT
								Fish feed (*Cho arcuatus*)	IVT
								Fish feed (*Daphnia*)	IVT
								Fish feed (*Tetramin*)	IVT
								Fox	IVT
								Gerbil epithelium	ST, IVT
								Glue, fish	ST
								Goat epithelium	ST, IVT
								Goat, Angora (mohair)	ST
								Goat, hair dander	ST
								Goose feathers	ST, IVT
								Guinea pig epithelium	ST, IVT
								Guinea pig, hair, dander	ST
								Hamster epithelium	ST, IVT
								Horse dander	ST, IVT
								Horse epithelium	ST
								Horse serum	IVT
								Mink epithelium	IVT
								Monkey, hair, epithelium	ST
								Mouse	IVT
								Mouse epithelium	ST, IVT
								Mouse serum proteins	IVT
								Mouse urine proteins	IVT
								Parakeet (budgerigar) droppings	IVT
								Parakeet (budgerigar) feathers	ST, IVT
								Parakeet (budgerigar) serum proteins	IVT
								Pigeon droppings	IVT
								Pigeon feathers	ST
								Poodle, hair, dander	ST
								Rabbit epithelium	ST, IVT
								Rabbit serum	IVT
								Rabbit urine	IVT
								Rat	IVT
								Rat epithelium	ST, IVT
								Rat serum proteins	IVT
								Rat urine proteins	IVT
								Reindeer epithelium	IVT
								Schistosoma	IVT
								Seminal fluid (human)	IVT
								Sericin silk glue	IVT
								Sheep epithelium	ST, IVT
								Swine urine	IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
								Swine (hog) (pig) epithelium	ST, IVT
								Swine (hog) (pig) hair, dander	ST
								Turkey feathers	ST, IVT
								Wool sheep	ST
Dusts									
								Dust, barley	ST
								Dust, corn	ST
								Dust, grain mill	ST
								Dust, mattress	ST
								Dust, oat	ST
								Dust, rye	ST
								Dust, soybean	ST
								Dust, upholstry	ST
								Dust, wheat	ST
								Dust, wood, cedar/juniper	ST
								Dust, wood, fir/hemlock	ST
								Dust, wood, gum	ST
								Dust, wood, mahogany	ST
								Dust, wood, maple	ST
								Dust, wood, oak	ST
								Dust, wood, pine	ST
								Dust, wood, redwood	ST
								Dust, wood, spruce	ST
								Dust, wood, walnut	ST
								House dust (Allergopharma)	IVT
								House dust (Bencard)	IVT
								House dust (Greer)	ST, IVT
								House dust (Hollister-Stier)	ST, IVT
								House dust (Pasteur A600)	IVT
								Mattress dust	ST
Foods									
			Actidiniaceae					Kiwi fruit	IVT
			Agaricaceae					Mushroom	ST, IVT
			Amygdalaceae					Almond	ST, IVT
			Amygdalaceae					Cherry fruit	ST, IVT
			Amygdalaceae					Peach	ST, IVT
			Amygdalaceae					Nectarine	ST
			Amygdalaceae					Apricot	ST, IVT
			Amygdalaceae					Plum (prune)	ST, IVT
			Anacardiaceae					Mango fruit	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
			Anacardiaceae					Cashew nut	ST, IVT
			Anacardiaceae					Pistachio	ST, IVT
			Ananaceae					Pineapple	ST, IVT
			Apiaceae					Lovage	IVT
			Apiaceae					Celery	ST, IVT
			Apiaceae					Coriander	
			Apiaceae					Caraway	ST, IVT
			Apiaceae					Cumin	
			Apiaceae					Fennel seed	IVT
			Apiaceae					Fennel, fresh	IVT
			Apiaceae					Dill	ST, IVT
			Apiaceae					Carrot	ST, IVT
			Apiaceae					Parsnip	ST
			Apiaceae					Anise	ST, IVT
			Apiaceae					Parsley	ST, IVT
			Aves					Chicken meat	ST, IVT
			Aves					Egg, yolk	ST, IVT
			Aves					Egg, whole	ST, IVT
			Aves					Egg, white	ST, IVT
			Aves					Turkey meat	ST, IVT
			Aves					Duck	ST
			Aves					Ovalbumin	IVT
			Aves					Ovomucoid	IVT
			Betulaceae					Hazelnut (filbert)	ST,IVT
			Capparidaceae					Capers	
			Caricaceae					Papaya	ST, IVT
			Chenopodaceae					Sugar, beet	ST, IVT
			Chenopodaceae					Beet	ST
			Chenopodaceae					Spinach	ST, IVT
			Compositae					Chicory	ST
			Compositae					Endive	ST
			Compositae					Safflower seed	ST
			Compositae					Tarragon	IVT
			Compositae					Artichoke	ST
			Compositae					Lettuce	ST, IVT
			Convolvulaceae					Potato, sweet	ST
			Cruciferae					Mustard	ST, IVT
			Cruciferae					Mustard greens	ST
			Cruciferae					Broccoli	ST, IVT
			Cruciferae					Brussels sprout	ST, IVT
			Cruciferae					Cauliflower	ST, IVT
			Cruciferae					Cabbage	ST, IVT
			Cruciferae					Radish	ST
			Cruciferae					Turnip	ST
			Cruciferae					Horseradish	ST
			Cruciferae					Kale (collards)	ST
			Crustaceae					Lobster	ST, IVT
			Crustaceae					Crab	ST, IVT
			Crustaceae					Shrimp	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
			Cucurbitaceae					Cucumber	ST, IVT
			Cucurbitaceae					Squash, zucchini	ST
			Cucurbitaceae					Pumpkin seed	IVT
			Cucurbitaceae					Honeydew	ST
			Cucurbitaceae					Cantaloupe	ST
			Cucurbitaceae					Squash	ST
			Cucurbitaceae					Pumpkin	ST, IVT
			Cucurbitaceae					Watermelon	ST
			Cucurbitaceae					Melons, unspecified	IVT
			Ericaceae					Cranberry	ST
			Ericaceae					Blueberry	ST
			Euphorbiaceae					Tapioca	ST
			Fagaceae					Chestnut	ST
			Graminae					Rice	ST, IVT
			Graminae					Rye	ST, IVT
			Graminae					Rice, Wild	ST
			Graminae					Millet seed	IVT
			Graminae					Wheat	ST, IVT
			Graminae					Barley	ST, IVT
			Graminae					Oat	ST, IVT
			Graminae					Malt	ST, IVT
			Graminae					Gluten	ST, IVT
			Graminae					Corn (maize)	ST, IVT
			Juglandaceae					Walnut, unspecified	IVT
			Juglandaceae					Walnut, black	ST
			Juglandaceae					Walnut, English	ST
			Juglandaceae					Pecan, nut	ST
			Labiatae					Peppermint	ST
			Labiatae					Majoram	IVT
			Labiatae					Oregano	ST, IVT
			Labiatae					Thyme	ST, IVT
			Labiatae					Basil	ST, IVT
			Labiatae					Sage	ST
			Lauraceae					Bay leaf	ST
			Lauraceae					Cinnamon	ST, IVT
			Lauraceae					Avocado	ST, IVT
			Lecythidaceae					Brazil nut	ST, IVT
			Leguminosae					Pea, green	ST
			Leguminosae					Lentils	ST, IVT
			Leguminosae					Bean, lima	ST
			Leguminosae					Bean, pinto	ST
			Leguminosae					Bean, red kidney	IVT
			Leguminosae					Garbanzo (chick pea)	
			Leguminosae					Pea, unspecified	IVT
			Leguminosae					Peanut	ST, IVT
			Leguminosae					Bean, navy	ST
			Leguminosae					Bean, white	IVT
			Leguminosae					Soybean	ST, IVT
			Leguminosae					Carob	IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
			Leguminosae					Guar, guar gum	IVT
			Leguminosae					Bean, string (green)	ST
			Leguminosae					Tragacanth	ST, IVT
			Leguminosae					Pea, black-eyed	ST
			Leguminosae					Bean, kidney	ST
			Liliaceae					Garlic	ST, IVT
			Liliaceae					Chives	ST
			Liliaceae					Asparagus	ST, IVT
			Liliaceae					Onion	ST, IVT
			Malvaceae					Okra	ST
			Malvaceae					Cottonseed	ST, IVT
			Mammalia					Alpha-lactalbumin	IVT
			Mammalia					Whey	IVT
			Mammalia					Mutton (lamb)	ST, IVT
			Mammalia					Liver, beef	ST
			Mammalia					Beta-lactoglobulin	IVT
			Mammalia					Beef	ST, IVT
			Mammalia					Casein	ST, IVT
			Mammalia					Pork	ST, IVT
			Mammalia					Gelatin	ST
			Mammalia					Elk meat	IVT
			Mammalia					Rabbit	ST, IVT
			Mammalia					Milk, powdered (Alfare, Nestle)	IVT
			Mammalia					Milk, mare	IVT
			Mammalia					Veal	ST
			Mammalia					Milk, boiled	IVT
			Mammalia					Milk, cow	ST, IVT
			Mammalia					Milk, goat	ST
			Marantaceae					Arrowroot	ST
			Moraceae					Hops	ST
			Moraceae					Fig	ST
			Musaceae					Banana	ST, IVT
			Myrtaceae					Allspice	ST
			Myrtaceae					Cloves	ST, IVT
			Myrtaceae					Guava	IVT
			Oleaceae					Olive, green	ST
			Oleaceae					Olive, black	ST
			Orchidaceae					Vanilla	ST, IVT
			Palmae					Dates	ST
			Palmae					Coconut	ST, IVT
			Solanaceae					Pepper, bell, green	ST
			Papaveraceae					Poppy seed	ST, IVT
			Passifloraceae					Passion fruit	IVT
			Pedaliaceae					Sesame seed	ST, IVT
			Pelecypoda					Scallop	ST
			Pelecypoda					Mussel, blue	IVT
			Pelecypoda					Oyster	ST, IVT
			Pelecypoda					Clam	ST

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
			Pinaceae					Pine nut (pinon nut) (pignola)	IVT
			Piperaceae					Pepper, green	ST, IVT
			Piperaceae					Pepper, black	ST, IVT
			Polygonaceae					Rhubarb	ST
			Polygonaceae					Buckwheat	ST, IVT
			Pomaceae					Pear	ST, IVT
			Pomaceae					Apple	ST, IVT
			Rosaceae					Blackberry	ST, IVT
			Rosaceae					Strawberry	ST, IVT
			Rosaceae					Raspberry	ST
			Rubiaceae					Coffee	ST, IVT
			Rutaceae					Orange	ST, IVT
			Rutaceae					Tangerine	ST
			Rutaceae					Lemon	ST, IVT
			Rutaceae					Lime	ST
			Rutaceae					Grapefruit	ST, IVT
			Saccharomycetaceae					Yeast, bakers'	ST
			Saccharomycetaceae					Yeast (S. cerevisiae)	IVT
			Saccharomycetaceae					Yeast, brewers'	ST
			Sapindaceae					Litchi	
			Solanaceae					Pepper, red	ST
			Solanaceae					Eggplant (aubergine)	ST, IVT
			Solanaceae					Potato, white	ST
			Solanaceae					Tomato	ST, IVT
			Solanaceae					Chili pepper	ST, IVT
			Solanaceae					Paprika/Sweet pepper	ST, IVT
			Sterculiaceae					Karaya gum	
			Sterculiaceae					Cacao (chocolate)	ST, IVT
			Teleostomi					Trout	ST, IVT
			Teleostomi					Tuna	ST, IVT
			Teleostomi					Whitefish	ST
			Teleostomi					Anchovy	ST
			Teleostomi					Catfish	ST
			Teleostomi					Sardine	ST
			Teleostomi					Pickerel	ST
			Teleostomi					Perch	ST
			Teleostomi					Bass	ST
			Teleostomi					Mackerel	ST, IVT
			Teleostomi					Haddock	ST
			Teleostomi					Salmon	ST, IVT
			Teleostomi					Herring	ST, IVT
			Teleostomi					Flounder	ST
			Teleostomi					Snapper	ST
			Teleostomi					Sole	ST
			Teleostomi					Eel	IVT
			Teleostomi					Halibut	ST
			Teleostomi					Codfish	ST, IVT
			Theaceae					Tea	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
			Urristicaceae					Mace	IVT
			Urristicaceae					Nutmeg	ST, IVT
			Vitaceae					Grape	ST, IVT
			Zingiberaceae					Ginger	ST, IVT
			Zingiberaceae					Turmeric	
			Zingiberaceae					Cardamon	IVT
Miscellaneous plant products									
								Acacia gum	ST
								Carragheen gum	ST
								Castor bean	IVT
								Flaxseed	ST
								Green coffee bean	IVT
								Gum karaya	ST
								Gum, India	ST
								Hemp	ST
						Plantago	ovata	Ispaghula (psyllium)	IVT
								Jute	ST
								Kapok	ST
								Kapok seed	ST
						Hevea	brasliensis	Latex	IVT
								Lycopodium	ST, IVT
								Sunflower seed	ST, IVT
								Tobacco leaf	ST, IVT
Antibiotics, other drugs/biologicals, chemicals, haptens, etc									
								Amoxicillin	IVT
								Ampicillin	IVT
								Penicoilloyl polylysine	ST
								ACTH	ST, IVT
								Alpha amylase	IVT
								Subtilopeptidase	ST, IVT
								Ammonium tetrachloroplatinate	ST, IVT
								BSA (bovine serum albumin)	IVT
								Chloramine T	ST, IVT
								Chymopapain (Chymodiactin)	ST, IVT
								Diphenylmethane diisocyanate (MDI)	ST, IVT
								Ethylene oxide	IVT
								Formaldehyde	IVT
								Hexahydrophthalic anhydride	IVT
								Hexamethylene diisocyanate (HDI)	ST, IVT

Class	Subclass	Order	Family	Subfamily	Tribe	Genus	Species	Common name	ST, IVT
								Himic anhydride	ST, IVT
								Insulin, bovine	ST, IVT
								Insulin, human	ST, IVT
								Insulin, porcine	ST, IVT
								Phthalic anhydride	ST, IVT
								Plicatic acid (western red cedar)	IVT
								Succinylcholine or analogs	ST, IVT
								Tetrachlorophthalic anhydride	ST, IVT
								Toluene diisocyanate (TDI)	IVT
								Trimellitic anhydride (TMA)	IVT

Abbreviations: IVT, in vitro tests available; ST, skin tests available.

[a] Key to "importance": 3, agents demonstrating broad prevalence and clinical activity; 2, agents prevalent in air samples but not studied clinically; and 1, all others.

Summary Statement 173. A skin prick/puncture test is superior to intracutaneous testing for predicting allergic rhinitis and allergic asthma triggered by cat allergen exposure. (B)

Summary Statement 174. The skin prick/puncture test can be used to rule out allergic rhinitis and allergic asthma triggered by cat allergen exposure. (B)

Summary Statement 175. Knowledge of allergen cross-reactivity and local aerobiology is important in selecting appropriate allergens and in minimizing the number of allergens required for skin and specific IgE tests. (D)

Summary Statement 176. In general, skin prick/puncture testing is more sensitive for identifying sensitization to inhalant allergens and confirming clinical allergy. However, dated specific IgE assays with defined quantifiable threshold levels can also predict positive respiratory responses after allergen exposure. (B)

Summary Statement 177. Demonstration of sensitization to an occupational agent by specific IgE and/or skin testing alone is insufficient to establish a diagnosis of OA. (B)

Summary Statement 178. Skin prick testing with certain well-characterized occupational protein allergens possesses adequate sensitivity such that a negative skin test result (<3-mm-wheal diameter) can be used to rule out clinical allergy. (B)

Summary Statement 179. Test performance characteristics of specific IgE assays and skin testing for detection of chemical IgE-mediated sensitization must undergo validation and reproducibility in controlled studies using standardized antigens and assay protocols before these can be considered reliable for routine evaluation of workers suspected of OA. (B)

Summary Statement 180. In patients undergoing evaluation for suspected work-related natural rubber latex (NRL) allergy, a positive skin prick test result with a NRL extract (if available) is preferred to demonstration of elevated specific IgE with an FDA-cleared assay due to higher sensitivity of the former. Current IgE-mediated allergy and asthma caused by NRL allergens is highly unlikely in the presence of a negative skin prick test result with a reliable crude NRL allergen extract. Elevated in vitro specific IgE levels can be used to confirm NRL allergy, but a negative result does not exclude NRL allergen sensitization. (B)

Clinical Indications and Utility

Skin testing and specific IgE evaluation of specific IgE are methods used to demonstrate IgE-mediated sensitization to inhalant allergens. In clinical practice, skin and/or specific IgE testing that demonstrate specific IgE for inhalant aeroallergens are utilized to (1) confirm or exclude a suspected diagnosis of allergic rhinitis, allergic conjunctivitis, or asthma triggered by aeroallergens; (2) determine the need for environmental control recommendations to reduce exposure to outdoor or indoor aeroallergens; (3) demonstrate sensitization to inhalant occupational allergens, which cause OA or rhinitis; and (4) guide selection of inhalant allergens for inclusion in allergen immunotherapy extracts.

A clinician must be familiar with performance characteristics of skin testing and specific IgE measurement so that test results are applied accurately to diagnose and treat allergic respiratory disorders. To optimally define test performance, a method should be reproducible and validated against a diagnostic benchmark or gold standard. A medical history is subjective and not adequate alone for defining clinical sensitivity or specificity of in vivo or specific IgE tests.[101,120–123,933] Evaluation of symptoms or physiologic responses during direct allergen challenge tests under supervision of a physician or in association with natural exposure to inhalant allergens (ie, pollen, cat, house dust mite) are appropriate ways to validate skin prick/puncture tests, intracutaneous tests, and specific IgE assays.[111,167,934] Although such validation studies are limited both in number and scope of allergens evaluated, they provide objective evidence for defining test characteristics, including sensitivity, specificity, predictive values, and likelihood ratios.

Performance of Skin Tests in Evaluation of Inhalant Allergy

In clinical practice, skin prick/puncture testing is used as an initial screening test and is often followed by intracutaneous testing for inhalant allergens eliciting negative prick test results. This long-standing practice is based on the assumption that intracutaneous testing has greater sensitivity than skin prick/puncture testing. However, there is evidence that a positive intracutaneous test result at a fixed dose of 1:500 or 1:1,000 (wt/vol) in the absence of skin prick reactivity correlates poorly with clinical sensitivity.[167,171] When seasonal allergic rhinitis was confirmed by nasal challenge with grass pollen allergen, only 11% of patients with positive intracutaneous test results (and negative prick/puncture test results) exhibited a positive challenge test result, and this outcome was identical to symptomatic patients with negative intracutaneous test results. In contrast, 68% of symptomatic patients with skin prick/puncture test reactivity to grass pollen exhibited positive nasal challenge test results.[167] Based on history alone, a positive skin prick/puncture test result to cat defined by a wheal 3 mm or greater than the negative control possessed 90% sensitivity and 90% specificity.[97] Wood et al reported that skin prick/puncture testing using a 27-gauge hypodermic needle exhibited 94% sensitivity, 80% specificity, 90% positive predictive value, and 87% negative predictive value for identifying subjects with increased upper respiratory tract symptoms elicited by live cat exposure. For identifying subjects with lower respiratory tract symptoms after cat exposure, skin prick testing had 84% sensitivity (vs 35% for intracutaneous tests), 87% specificity, 88% positive predictive value, 82% negative predictive value, and 97% sensitivity for predicting a reduction in FEV_1 during live cat challenge.[111]

Table 12. Performance Characteristics of Skin Prick Test to Cat Dander Based on Optimal Cutoff Values Determined from Receiver Operating Characteristic Analysis[a]

Standard	Cutoff, mm	Sensitivity, %	Specificity, %	Efficiency, %	PPV, %	NPV, %
History	5.5	81.0	91.7	96.0.7	95.6	68.4
Symptom score	5.5	88.0	88.9	88.9	94.7	78.3
Specific IgE	6.0	93.3	86.7	91.1	94.0	85.3
Tryptase	6.0	91.7	75.8	80.0	89.4	80.4
PGD_2	6.0	100	89.7	91.1	95.6	100

Abbreviations: NPV, negative predictive value; PGD_2, prostaglandin D_2; PPV, positive predictive value.
[a] Optimal decision points for a positive skin test result using receiver operating characteristic analysis. The optimal cutoff wheal diameter for a positive skin test result to cat dander was 6.0 mm (using specific IgE, postchallenge tryptase, or PGD_2 levels) or 5.5 mm (using symptom or clinical history) as reference standards for cat allergy. Reprinted with permission from the *Annals of Allergy, Asthma and Immunology*.

In allergic rhinitis patients, skin prick/puncture testing with a standardized cat extract was validated by symptom scores and nasal mediators collected after direct intranasal allergen challenge (Table 12).[935] In this study, a 3.0-mm-diameter wheal cutoff for a positive test result provided 100% sensitivity and 100% negative predictive value. As indicated in Table 12, receiver operating characteristic (ROC) analysis was used to identify optimal wheal size cutoff points for different validation methods. For example, when increased symptom scores after nasal allergen challenge were used as the gold standard, a prick/puncture wheal diameter cutoff of 5.5 mm increased test specificity to 89%, albeit at the expense of sensitivity (88%). This study illustrates the potential value of measuring prick/puncture wheal dimensions for predicting clinical respiratory sensitivity.[936]

As discussed in part 1, SET of intracutaneous tests achieves similar sensitivity and specificity compared with nasal challenge, but the intracutaneous threshold dose is 1 to 2 logs more dilute than 1:1,000 (wt/vol).[90]

In Vitro IgE Immunoassays for Evaluation of Allergic Respiratory Disorders Due to Inhalant Allergens

Since introduction of the Phadebas RAST assay in 1967, specific IgE assay technologies have evolved considerably.[937] Performance characteristics of in vitro specific IgE tests have been determined largely by studies that rely on medical history and questionnaire-derived diagnoses of allergic rhinitis and prick/puncture tests as the standards for test validation.[104,122]

The ROC analysis has been used to determine optimal thresholds for defining a positive PHADEZYME assay. Class 2 binding or 0.7 to 3.5 Phadebas units (PRU)/mL exhibited optimal sensitivity and specificity in identifying skin prick test–positive patients with clinical history of allergy to grass pollen, cat epithelium, and birch pollen.[934] The ROC analysis has been applied to determine optimal cutoff values for the Pharmacia CAP specific IgE assay. In a case-control study, optimal cutoff values differentiated between symptomatic and asymptomatic patients with positive skin prick test results to allergens of interest.[120] These thresholds were 10.7 kU/L for seasonal allergens and 8.4 kU/L for perennial allergens. In 1 report, estimated sensitivity and specificity of the RAST-CAP-FEIA for identifying pollen allergy were 79% and 72%, respectively, and the positive predictive value was only 9.3%.[122] In a validation study of subjects with clinical allergy to house dust mites, an optimal cutoff threshold value for Phadezyme RAST for mite was determined to be more than 3.5 PRU/mL by ROC analysis or at least class III. Using 3.5 PRU/mL as a positive threshold, RAST sensitivity and specificity were 84% and 77%, respectively, vs 100% and 32% for dust mite skin prick testing.[934]

The Phadezyme RAST and CAP-RAST system have been well validated for predicting cat allergy confirmed by live cat room challenges.[111] In this model, increases in upper respiratory tract symptoms or lower respiratory tract symptoms and reduction in FEV_1 of 15% are defined as positive challenge responses. When a positive test result was defined as values greater than 0.35 kIU/L, RAST had 87% and 91% sensitivity and specificity, respectively, in identifying those subjects with confirmed upper respiratory tract symptoms to cat challenge. Sensitivity of RAST was 76% in subjects with lower respiratory tract symptom responses after cat challenge and test specificity was 95%. In this study, skin prick testing had greater sensitivity (79%) than RAST (69%) for identifying positive challenge responders, and both tests were highly specific (>90%).[111]

In summary, the precise role of in vitro testing in clinical diagnosis of allergy to common inhalant aeroallergens is uncertain. Based on limited data, validated specific IgE assays tests can be useful in confirming clinical sensitization to certain allergens (eg, cat). Because skin testing has greater sensitivity than in vitro IgE tests, a negative serologic test result cannot be relied on for excluding clinical sensitivity to inhalant allergens.

In Vivo and In Vitro Testing in Diagnosis of Occupational Allergic Disorders

Agents that cause OA and related disorders are broadly classified into categories of low-molecular-weight (principally chemicals) and high-molecular-weight substances (animal and plant proteins) (Table 13). Both OA and rhinitis due to high-molecular-weight proteins are usually IgE mediated, whereas OA caused by chemical agents is

Table 13. Sensitivity and Specificity of Selected High- and Low -Molecular-Weight Occupational Allergens

Reference	High-molecular-weight allergens	Diagnostic test	Gold standard	Sensitivity, %	Specificity,
954,956	Natural rubber latex	Skin prick test	SIC	100	21
957	Commercial bovine extract 1:100 wt/vol	Skin prick test	SIC	100	50
957	Bovine specific IgE-	Unicap	SIC	82	100
958	Industrial enzyme 10 mg/mL	Skin prick test	SIC	100	93
958	Industrial enzyme	IgE-RAST	SIC	62	96
	Low-molecular-weight antigens				
947	Acid anyhydride-HSA	Skin prick test	SIC	71	80
943	Vinyl sulfone dyes	Skin prick test	SIC	76	91
		ELISA-IgE			
930	Green tea (epigallocatechin gallate)	IC ≤1 mg/mL	SIC	100	80
949	Diisocyanates	ELISA-IgE	SIC	31	97
949		ELISA-IgG		72	76
950	Diisocyanate-HSA	Phadebas RAST-IgE class ≥2		41	100
945	Complex platinum salts	Skin prick test	SIC	82	
	Antigen-specific cellular immune responses				
802	Plicatic acid-HSA	In vitro proliferation	Red cedar asthma	24	100
951	Diisocyanate-HSA	↑ MCP-1 by mononuclear cells	SIC	79	91

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HSA, human serum albumin; IC, intracutaneous; MCP-1, monocyte chemoattractant protein 1; RAST, radioallergosorbent assay; SIC, specific inhalation challenge test.

less often IgE mediated. In the latter case, immunologic testing has not been shown to be diagnostically useful. Although proving sensitization to occupational agents is informative, demonstration of decrements in lung function with exposure to the causative agent is necessary to confirm a diagnosis of OA. There are few commercially available occupational protein antigens. Test antigens for assessing sensitization to chemicals have been prepared and evaluated in individual research laboratories and are not generally available.

Chemicals

Chemical antigens that have been used to evaluate specific antibody responses use antigens that are prepared by conjugating chemicals with a protein (eg, HSA). However, because protocols for assays, reference positive and negative controls, and conjugate preparation methods have not been standardized, results obtained from different laboratories are not comparable. For most chemicals that cause OA, skin prick testing is not indicated with the exception of a few agents that are known to induce IgE-mediated sensitization; these agents include acid anhydride compounds (eg, phthalic anhydride, trimellitic anhydride), sulfonechloramide, vinyl sulfone reactive dyes, persulfate salts, and platinum salts.[938–945]

Acid anhydrides are prototypic chemical haptens that form protein conjugates in vivo by combining with autologous respiratory proteins.[946] Phthalic anhydride–HSA conjugates, and not phthalic anhydride alone, which is inactivated by hydrolysis, are suitable reagents for detection of percutaneous and in vivo sensitization to phthalic anhydride.[947] In a small study in which occupational rhinitis and OA were confirmed by challenge testing, acid anhydride–HSA skin prick testing exhibited 71% sensitivity and 80% specificity.[947] Persulfate salts are common ingredients in hair bleaching products. Positive skin prick test results with ammonium persulfate salt solutions indicate that IgE-dependent mechanisms appear to play a role in persulfate induced OA, but there is inadequate experience to define test performance characteristics.[942] Although positive skin test results have been detected in anecdotal cases of diisocyanate asthma, skin prick testing with diisocyanate-HSA antigens has low diagnostic sensitivity.[947] Skin prick testing with hexachloroplatinate salts, (PtCl6)2-, has been used for many years to confirm sensitization to platinum salts in refinery workers.[944] In a validation study, 82% of workers responding to the specific inhalation challenge had positive skin prick test results with (PtCl6)2-, whereas 18% were skin test negative.[945] Thus, specific challenge testing was necessary to confirm OA.

In several studies of workers suspected of diisocyanate asthma, performance characteristics of in vitro IgG and IgE immunoassays were evaluated against results of specific inhalation challenge tests, which is considered to be the diagnostic gold standard for diisocyanate asthma.[948–950] Elevated serum specific IgE levels by the ELISA method exhibited 31% sensitivity and 97% specificity for identifying workers with confirmed diisocyanate asthma, whereas sensitivity and specificity for IgG was 72% and 76%, respectively.[900] Tee et al reported diagnostic sensitivity of 32% and 100% specificity for elevated diisocyanate HSA specific IgE measured by Phadebas RAST (positive test result defined as a RAST ratio of ≥3) and that assay sensitivity was optimized when sera were collected during active workplace exposure to diisocyanates.[950] There are limited data pertaining to in vitro cellular immune assays for diagnosing of OA due to chemicals. Elevated in vitro production of MCP-1 production by mono-

nuclear cells after stimulation with diisocyanate-HSA antigens exhibited 79% sensitivity and 91% specificity in identification of exposed workers with confirmed OA. Replication of these results, however, in larger diisocyanate-exposed populations is needed before this assay is adopted for clinical use.[951]

Protein Allergens in the Workplace

Inhalant proteins encountered at work readily induce sensitization and elicit allergic contact urticaria, rhinoconjunctivitis, and/or asthma at work. In general, skin prick testing with protein allergens (or high-molecular-weight sensitizers) are highly sensitive tools for evaluating workers with suspected OA. In the case of NRL, skin prick testing has greater sensitivity compared with FDA-approved in vitro specific IgE assays.[916]

A nonammoniated latex extract was reported to possess both high sensitivity (99%) and specificity (100%) at a test concentration of 100 μg/mL in identifying health care workers with latex allergy based on medical history.[952,953] In a study that confirmed NRL induced OA based on responses to controlled challenge with NRL powdered gloves, skin prick testing with a well-characterized nonammoniated NRL extract exhibited 100% sensitivity, 100% negative predictive value, and 21% specificity. Specific IgE reactive with NRL was not evaluated in this study.[954] However, a negative skin test result alone virtually excluded NRL-induced OA defined by a positive specific inhalation challenge.[954] Among health care workers reporting a history of NRL allergy, skin prick testing with standardized commercial NRL extracts has superior sensitivity for detecting NRL sensitization compared with FDA-approved in vitro specific IgE assays.[914–916] An important practical limitation in the United States is the lack of a standardized commercial NRL skin test reagent. Because uncharacterized powdered glove extracts prepared by physicians have variable diagnostic sensitivity (64% to 96%), a negative test result must be interpreted cautiously.[955] In a recent study, CAP and UniCAP immunoassays identified 53% to 77% of NRL skin test prick–positive health care workers with latex allergy.[956]

A similar study design was used to investigate test characteristics of skin prick testing with a commercial bovine allergen extract (1:100 wt/vol), which provided 100% sensitivity, 50% specificity, and 100% negative predictive value in identifying OA based on specific challenge testing with bovine dander extract. On the other hand, bovine dander specific IgE measured by quantitative fluoroenzymatic immunoassay (UniCAP) exhibited 82% sensitivity and 100% specificity.[957]

Finally, in a study of workers exposed to industrial enzymes that used specific inhalation testing as the diagnostic gold standard, investigators reported 100% sensitivity and 93% specificity for skin prick testing with enzyme solutions (10 mg/mL) compared with 62% sensitivity and 96% specificity for an enzyme allergosorbent IgE assay.[958]

ASSESSMENT OF FOOD ALLERGY

An adverse reaction to food can result from nonimmune (eg, intolerance, pharmacologic effects) or immune (allergy) origins. Immune-mediated adverse reactions to foods (food allergy) may be attributable to IgE antibody–mediated mechanisms (eg, food-induced anaphylaxis), cellular mechanisms with no detectable food specific IgE antibodies (primarily gastrointestinal disorders), and disorders in which both IgE antibody–mediated and cellular mechanisms have been identified (eosinophilic gastrointestinal disorders, atopic dermatitis).[959]

Summary Statement 181. The primary tools available to evaluate patients' adverse reactions to foods include history (including diet records), physical examination, prick/puncture skin tests, serum tests for food specific IgE antibodies, trial elimination diets, and oral food challenges. (B)

The general aims of diagnosis are to determine if food is causing the disorder under evaluation and, if so, to identify specific causal food(s). A proper diagnosis will allow the patient to receive instructions regarding avoidance of problematic foods. Just as important, specific diagnosis will prevent unnecessary and potentially deleterious dietary restrictions when a suspected food allergy is not present. The diagnostic tools available to the clinician include simple and relatively inexpensive tests, such as the clinical history, physical examination (that may reveal associated atopic disorders and raise the likelihood of a food allergy[960]), prick/puncture skin tests, and serum tests for food specific IgE. Additional tests (oral food challenges) are more involved timewise, may be more expensive, and may carry additional risks. The rational selection and interpretation of diagnostic tests require an appreciation for the utility of the tests themselves and an evaluation of the level of certainty required for the diagnosis.[959]

Summary Statement 182. A detailed dietary history, at times augmented with written diet records, is necessary to determine the likelihood that food is causing the disorder, identify the specific food, and determine the potential immunopathophysiology. (D)

The history is the starting point at which the clinician must decide on the possibility that food is a potential cause of a disorder or reaction. The features of the reaction may also indicate whether the pathophysiology of the disorder may be non–immune-mediated (intolerance, pharmacologic reaction) or allergic, and if the latter, whether it is IgE mediated or associated or not (thereby guiding further diagnostic evaluation). Historical points of interest include age of the patient; a list of suspect foods, ingredients, or labels for manufactured products; the amount of food necessary to elicit a reaction; the route of exposure eliciting a reaction; the typical time interval between exposure and onset of symptoms; clinical manifestations of reaction(s) after exposure to each food; duration of symptoms; ancillary events (exercise, use of NSAIDS, alcohol); treatment of reactions and patient re-

sponse; and the consistency with which a reaction occurs on exposure.

Key points in the history, such as symptoms and timing of onset after ingestion or chronicity, may identify reactions likely to be dependent on IgE antibody (eg, sudden reactions such as anaphylaxis), those that are associated with IgE to particular foods in many but not all cases (eg, chronic disorders such as atopic dermatitis), or disorders not associated with IgE antibodies or ones unlikely to be allergic in nature (eg, lactose intolerance, isolated gastrointestinal disorders of infancy).[959]

In addition to identifying a pathophysiologic basis, the history may indicate specific food triggers and a starting point to estimate the probability that a particular food is causal (eg, prior probability of an association). Diet records, including review of labels from packaged foods, may facilitate identification of specific triggers.[961,962] Common reasoning would indicate that a food previously tolerated on a routine basis is less likely to be a trigger than one eaten rarely. Similarly, for a person with a previously confirmed food allergy to a ubiquitous food (eg, milk, peanut) who reacts to a specific meal, consideration that the previously identified allergen may be present as a hidden ingredient or contaminant should be entertained. Age is important since the epidemiology of food allergy indicates a higher probability of reactions to cow's milk, egg, wheat, and soy in infants; peanuts, tree nuts, seafood, and raw fruits in older children and adults; and a predilection of certain food-related disorders in infants and children (atopic dermatitis, enterocolitis).[959] Consistent reactions, particularly acute ones, to a specific food raise the probability that the food is causal; in 1 study of infants, specificity of urticaria after ingestion of cow's milk was 0.77, but specificity of atopic dermatitis was only 0.22.[95] Indeed, the history is notoriously poor in identifying causal foods for chronic disorders such as atopic dermatitis when compared with outcomes of definitive oral food challenges.[590,960,963] Food allergy is commonly suspected but rarely incriminated in chronic urticaria and/or angioedema.

Summary Statement 183. With regard to evaluations for IgE antibody–associated food allergies, tests for food specific IgE antibody include percutaneous skin tests (prick/puncture tests) and serum assays. In general, these tests are highly sensitive (generally >85%) but only modestly specific (approximately 40% to 80%) and therefore are well suited for use when suspicion of a particular food or foods is high. They are not effective for indiscriminate screening (eg, using panels of tests without consideration of likely causes) and therefore generally should not be used for that purpose. (B)

Modalities to determine the presence of IgE antibody to specific foods include prick/puncture tests and serum assays. Both techniques merely detect the presence of antibody (sensitization) and do not necessarily indicate, by themselves, that ingestion would result in clinical reactions. Even infants can be tested.[95,964] Commercial reagents for food allergy skin testing have not yet been standardized and may have varying concentrations of relevant proteins.[965,966] Clinically important but labile proteins, particularly ones in fruits and vegetables, may degrade, making extracts inadequate for an evaluation; fresh extracts may therefore be needed to evaluate sensitization to these allergens.[965] A retrospective review of medical records (1,152 children) concerning prick/puncture tests with foods indicate a very low rate of generalized reactions (521 per 100,000 tested; 95% confidence interval, 105–937), but all reactions in infants (n = 6) occurred in infants younger than 6 months of age tested with fresh food specimens.[967]

Another means to detect food specific IgE is serologic to determine the presence of food specific IgE antibodies in the serum. There are a variety of manufacturers, substrates, and manners of reporting results as discussed in part 1.

The clinical utility of prick/punctures testing and serum food specific IgE has been evaluated in various referral populations of infants and children evaluated by oral food challenges for suspected food allergy.[140,589–591,960,963,968–970] Sensitivity of a positive test result is generally more than 0.7 and in most studies exceeds 0.85; specificity is lower, generally in the range of 0.4 to 0.8. Test utility varies by intrinsic features of the test (technique, definition of positive, type of food) and features of the population tested (age, disease). These test characteristics generally indicate that a negative test result has a high utility to rule out IgE-mediated reactions to the food tested but that a positive test result may not be associated with true clinical reactions. Consequently, panels of food allergy tests should not be performed without consideration of the history because one may be faced with numerous irrelevant positive results (particularly in disorders with high total IgE antibody).[960] The serum assay for specific IgE may be less sensitive than skin prick tests,[970,971] so if there is a suspicion of a reaction when in vitro test results are negative, a skin test may detect sensitization.[589] However, in many cases the sensitivity is similar.[589,590,960]

Summary Statement 184. Intracutaneous skin tests for foods are potentially dangerous, are overly sensitive, increase the chance of a false-positive test result, and are not recommended. (D)

Intracutaneous allergy skin tests with food extracts give an unacceptably high false-positive rate, can elicit systemic reactions (rarely an issue for prick tests), and should generally not be used.[972]

Summary Statement 185. Based on studies in infants and children, increasingly higher concentrations of food specific IgE antibodies (reflected by increasingly larger percutaneous skin test size and/or higher concentrations of food-specific serum IgE antibody) correlate with an increasing risk for a clinical reaction. (B)

Studies in children support the notion that increasingly higher concentrations of food specific IgE antibody, reflected by increasingly larger prick/puncture test results or high serum IgE antibody concentrations, are correlated with increased risks for clinical reactions.[85,589,590,968–973] Thus, instead of considering a test result for IgE as positive or negative with one decision point (positive-negative at "detectable" serum food specific IgE or a particular skin test size such as 3 mm),

additional clinical utility may be achieved through consideration of prick/puncture test result size and serum food-specific antibody concentration. Various studies have correlated reaction likelihood with test results in this regard,[85,140,589,590,964,968–973] but it is clear that results may vary by technique, food involved, age group studied, the specific patient history, and the disorder under consideration. Although the size of the prick/puncture skin test result or concentration of food-specific IgE antibody by in vitro assay may be positively correlated with an increasing likelihood of a clinical reaction, the level of IgE is poorly correlated with clinical manifestations of the allergy (eg, severity or dose causing a reaction).[974–976]

Summary Statement 186. A trial elimination diet may be helpful to determine if a disorder with frequent or chronic symptoms is responsive to dietary manipulation. (D)

In the evaluation of disorders with chronic symptoms for which foods may be causal (eg, atopic dermatitis, gastrointestinal symptoms), elimination of suspected causal foods may be undertaken to determine whether symptoms are diet responsive. There are no studies to define the utility of this approach. Factors that may complicate interpretation of such a trial (eg, a trial failure when the disorder is truly food responsive) include incomplete removal of causal foods, selection of the wrong foods to eliminate, inadequate time allowed for resolution of chronic inflammation (eg, atopic dermatitis), and additional triggers may be causing symptoms (eg, skin infection in atopic dermatitis). The underlying pathophysiology is not a significant consideration in using elimination trials. Selection of foods to eliminate may be based on a variety of factors, including historical features, results of tests, and epidemiologic considerations. Information concerning strict adherence to the diet must be carefully reviewed, similar to what is needed for treatment of food allergy after a definitive diagnosis. Diets may vary from directed ones (removal of one or a few targeted foods), even more restricted ones with elimination of most allergenic foods (eg, a prescribed diet without major allergens and limited numbers of allowed foods), or even to extreme ones with essentially no source of potential allergen (eg, use of amino acid–based formula alone or with a few other proven safe foods). A positive response to an elimination diet should not be construed as a definitive diagnosis unless there is compelling supportive evidence regarding specific foods. Another use for an elimination diet is to establish baseline status before undertaking oral food challenges; the response to oral food challenge is potentially definitive but must be performed for each food under consideration. Severe reactions have occurred when previously ingested, IgE antibody–positive foods were added back to the diet after they had been removed from the diet for a period.[977]

Summary Statement 187. Graded oral food challenge is a useful means to diagnose an adverse reaction to food. (B)

The oral food challenge is performed by having the patient ingest increasing amounts of the suspected food under physician observation over hours or days.[961,962] This represents a definitive test for tolerance since ingestion of a relevant amount of the food with no reaction excludes the diagnosis of an adverse reaction to the tested food. The test result is open to misinterpretation when not done in a masked manner. Therefore, procedures to reduce this possibility need to be implemented, such as masking the challenge substance (blinding) and using placebos. The format of a food challenge can be applied to evaluate any type of adverse event attributed to foods due to both allergic and nonallergic hypersensitivity mechanisms.

The challenge procedure, its risks, and its benefits must be discussed with the patient and/or the caregiver. Several factors are considered, including the evaluation of the likelihood that the food will be tolerated, the nutritional and social need for the food, and ability of the patient to cooperate with the challenge. In limited circumstances, the food could be administered with potential adverse reactions monitored at home by the patient and parents. This may be considered if the expected adverse reactions are delayed in onset, non–IgE mediated, atypical (eg, headache, behavioral issues), mild gastrointestinal, and not potentially anaphylactic. On the other hand, if there is a reasonable potential for an acute and/or severe reaction, or if there is strong patient anxiety, physician supervision is recommended.

Except in the uncommon circumstances described previously, oral food challenges are undertaken under direct medical supervision. A risk evaluation must be made regarding location of challenge (office, hospital, intensive care unit) and preparation (eg, with or without an intravenous line in place). These decisions are based on the same types of data evaluated for the consideration of food allergy in the early diagnostic process: the history of the possible food allergic reaction, patient's medical history, and prick/puncture skin test results. Although generally considered a safe procedure when undertaken by qualified personnel, it must be appreciated that oral challenges can elicit severe, anaphylactic reactions, so the physician must be immediately available and comfortable with this potential and be prepared with emergency medications and equipment to promptly treat such a potentially life-threatening reaction.[978] In high-risk challenges, it may also be prudent to have intravenous access before commencing challenges.[962,979] Even non–IgE antibody–mediated food allergic reactions can be severe, such as food protein–induced enterocolitis syndrome that may include lethargy, dehydration, and hypotension, and may be complicated by acidosis and methemoglobinemia.[980] The details regarding undertaking an oral food challenge are described in the published Food Allergy Practice Parameter and other resources.[961,962,981] Challenges can be performed openly, with the patient ingesting the food in its natural form; single-blind, with the food masked and the patient unaware if the test substance contains the target food; or double-blind and placebo-controlled, with neither patient nor physician or medical professional knowing which challenges contain the food being tested. Although the open challenge is most prone to bias, it is easy to perform since no special preparation is needed to mask the food.

Indeed, if the patient tolerates the ingestion of the food, there is little concern about bias. Bias becomes an issue when the challenge food causes symptoms, particularly subjective ones. Therefore, open challenges are a good option for screening when several foods are under consideration, and if a food is tolerated, nothing further is needed. If there is a reaction to an open challenge used in the clinical setting, and there is concern that the reaction may not have been physiologic, the format could be altered to include blinding and controls. False-negative rates for double-blind, placebo-controlled food changes are low (usually ≤3%).[982] After a negative challenge, consideration should be given to having the patient eat the food prepared in the same manner and amount that caused the original reaction.

Summary Statement 188. A number of additional diagnostic tests are under investigation, including APTs (APTs) and tests for IgE binding to specific epitopes. (B)

Various additional diagnostic tests, particularly APTs, are under evaluation and are at various stages of acceptance or still under research scrutiny investigation of food allergy.[95,523–530,983–985] The test response is noted in the days after application and may potentially identify food triggers that are not associated with IgE antibodies, which is a particular issue for gastrointestinal food allergies.[945,947] Although increasing studies, primarily from Europe, are assessing the utility of these tests, more work is needed on standardization and clinical correlation before widespread routine clinical use can be advocated.[946] Several recent European experiences suggest that the ultimate utility of APTs in reducing the need for oral food challenges may be limited.[526,530] Additional diagnostic tests under development may use proteins of particular relevance or map informative protein epitopes for improved diagnosis of IgE antibody–mediated reactions.

Summary Statement 189. The rational selection, application, and interpretation of tests for food specific IgE antibodies require consideration of the epidemiology and underlying immunopathophysiology of the disorder under investigation, estimation of prior probability that a disorder or reaction is attributable to particular foods, and an understanding of the test utility and limitations. (D)

Tests for food allergy, like other medical tests, are neither 100% sensitive nor 100% specific. The diagnostic utility of a test in regard to making a diagnosis in an individual patient is influenced by (1) the possibility of the disease existing in the individual being tested (prior probability) and (2) the characteristics of the test itself (sensitivity, specificity). To determine prior probability, it is necessary to undertake a careful history and to understand the epidemiologic features of food allergic disorders. Description of the latter is beyond the scope of this Practice Parameter but may be found in reviews[959,986,987] and the Food Allergy Practice Parameter.[981]

To evaluate the clinical utility of a test, studies are performed comparing outcomes of oral food challenges (preferably double-blind, placebo-controlled food challenges). Many studies use open food challenges with objective symptoms as an end point, or sometimes they rely on convincing clinical histories.

Such studies have reported skin test sizes above which clinical reactions are virtually 100% likely (eg, wheal diameter ≥8 mm for cow's milk or peanut and ≥7 mm for egg in infants) or levels of IgE antibody at or above which reactions are more than 95% likely (eg, 7 kIU/L for egg, 15 kIU/L for milk, 14 kIU/L for peanut and 20 kIU/L for cod fish, measured by Pharmacia CAP-System FEIA) in atopic children at a median age of 3.8 years.[85,589] This approach is helpful in identifying persons who are likely to be clinically allergic, for whom an oral food challenge is not indicated. Conversely, progressively lower levels of food specific IgE (reflected by smaller skin test results or lower serum test results) are associated with a better chance to tolerate the food.[589,974,988]

As described herein, the clinician must appreciate that the predictive data are reflective of the characteristics of the tested population. For example, studies of young children (younger than 2 years) show that 95% reacted to egg if their IgE level was more than 2 kIU/L or milk if their IgE level was more than 5 kIU/L, values lower than those calculated for older children.[969,970] Additional studies have confirmed age-related differences among children in regard to the food specific IgE concentrations indicative of a high risk of reaction.[591,989] Another study also confirmed the utility of threshold values,[589,990] although there are some discrepancies in the actual values associated with specific outcomes (eg, predictive values were higher in a German study of children).[591] As a note of caution, reactions may occur when at risk children have undetectable food specific IgE (eg, approximately 20% with egg or peanut specific IgE <0.35 kIU/L have clinical reactions to these foods); most[970,971] but not all[971,976] such patients have a positive prick/puncture test results, indicating higher sensitivity of the test. As more studies emerge comparing serum and prick/puncture skin test results with clinical outcomes in wider age groups and populations with various disorders, further conclusions of test utility will be possible. In regard to skin prick testing, various reagents (fresh food, commercial extracts) and techniques of testing (probe type, location on the body, method of measurement, timing of measurement) are variables that affect final results and are additional obstacles in regard to applying study results to a particular patient.[141]

An additional and complementary way to interpret tests takes into consideration the individual's history to establish a prior probability on which to interpret a test result. For example, at a serum concentration of peanut specific IgE of 2 kIU/L, children with a history of a peanut allergic reaction had approximately a 50% chance of tolerating peanut.[974] However, in a group of children with a positive test result but no history of a reaction, 50% tolerated peanut at a peanut specific IgE concentration of 5 kIU/L. Similarly, a wheal size of 3 mm to peanut in children with atopic dermatitis was associated with a positive predictive value of 61%, whereas the same wheal size had a positive predictive value of 28% in children at low risk according to their clinical histories.[93,973]

These examples illustrate the importance of clinical history (including age and frequency of reactions) and calculation of a prior probability for allergy in regard to test interpretation.

A means to apply prior probability and test results in a particular patient to improve diagnostic accuracy is through the use of a calculated likelihood ratio. The likelihood ratio is simply the ratio of the odds that the patient whose test results fall within a particular range has the disease divided by the odds that they do not. The formula can most conveniently be expressed as: (likelihood ratio = sensitivity)/(1 − specificity) as applies to a positive test result. To be useful, a likelihood ratio needs to be determined for each diagnostic test used in evaluating the probability of food allergy. Unfortunately, this is not available for most food allergy tests. When the likelihood ratio is known, a pretest probability (based for example on the medical history) is estimated and a nomogram can be used to determine the posttest probability that a person has the disorder.

Although likelihood ratios are not calculated for most tests of food allergy, the concept of likelihood ratio and pretest probability has practical implications for routine practice. Consider, for example, 3 individuals: (1) a child with 3 severe allergic reactions to peanut requiring epinephrine, (2) a child with chronic atopic dermatitis who eats peanuts but has no history of a reaction to peanut, and (3) a nonatopic child who sometimes has headaches on days he eats peanut. Each patient is tested by prick/puncture testing to peanut and has a 4-mm wheal, a positive test result with modest sensitivity (approximately 50%), and good specificity (approximately 90%). The meaning of a 4-mm wheal to peanut when there has been recurrent anaphylaxis in patient 1 (high prior probability of peanut allergy, virtually 100%) is that it confirms reactivity and no food challenge should be undertaken. In a chronic condition like atopic dermatitis in patient 2, a modest size skin test may reflect clinical reactivity in only approximately half of patients (depending also on age) and may be a relevant positive in this scenario, needing confirmation by other means (oral food challenge) or additional testing to improve diagnostic accuracy (serum test). The test result in patient 3 with headaches is most likely of no clinical concern because the pretest probability is essentially zero. Considering again the patient with multiple episodes of peanut-related anaphylaxis, if there were no wheal to peanut, the clinician would not be likely to trust the result because the pretest probability is so high that the correct course of action would be to repeat the skin test or perform an in vitro test and consider a supervised oral food challenge if the test result were negative. Similarly, one could argue that a test for peanut causing migraines is not necessary since the prior probability is so low. Thus, 1 test (eg, prick/puncture) can provide pretest probability for another test (eg, oral food challenge).

It therefore is important to remember that every patient must be evaluated individually and the history taken as carefully as possible. Otherwise one risks obtaining a falsely positive or negative history that could skew interpretation of subsequent tests since they depend on the pretest probability generated by the history. This emphasizes the fact that face-to-face evaluation of the patient is essential and that remote practice of allergy is not valid.

At this time, there are a number of publications about the diagnostic utility of IgE antibody tests for egg, milk, and peanut for children at a range of ages and clinical circumstances that show excellent predictive ability. The studies on these same age groups have not determined strong diagnostic utility for soy or wheat,[589,591,974–990] and more studies are needed for determination of results for additional foods, clinical problems and ages, and the specific impact of cross-reactive homologous proteins in reagents currently used for testing.

ASSESSMENT OF STINGING INSECT ALLERGY

Clinical Indications and Utility

Summary Statement 190. Diagnostic skin and/or specific IgE tests are used to confirm clinical sensitivity to venoms in a patient with a history of a prior systemic reaction. (B)

Summary Statement 191. Although diagnostic tests identify species specificity of venom sensitization, they do not reliably predict severity of the sting reaction. (B)

The diagnosis of insect sting allergy requires confirmation of the clinical history with an accurate diagnostic test. This is most important in patients who require venom immunotherapy such as those with a history of systemic reactions to stings. Testing is not usually performed in those who have had only large local reactions to stings because they have only a 5% risk of systemic reaction to subsequent stings.[991–993] Testing is also not recommended to individuals without a history of a systemic reaction to stings because 25% of such persons will have positive diagnostic venom test results.[994] The overall clinical significance of this finding (asymptomatic skin sensitization) is uncertain, but there is an estimated 15% chance of systemic reaction in such individuals.[995] Diagnostic tests are also used during venom immunotherapy to determine whether the sensitivity has diminished or disappeared.[996]

The utility of diagnostic tests for insect allergy is limited to identifying the presence and species specificity of venom sensitization. Although there is a statistical correlation, the strength of the venom sensitivity shown by either skin or specific IgE diagnostic tests does not reliably predict the clinical severity of the sting reaction. Some patients have very strong test results but only local swelling reaction to a sting, whereas others have barely detectable sensitivity and yet have life-threatening anaphylaxis when stung.

Diagnostic Reagents for Hymenoptera and Fire Ants

Summary Statement 192. Standardized honeybee, *Polistes,* and *Vespula antigens* are commercially available as skin test reagents. (A)

Summary Statement 193. The skin test reagent available for evaluation of imported fire sting allergy is a nonstandardized whole-body extract. (C)

Summary Statement 194. In the case of a history of anaphylaxis to *Hymenoptera* venoms, intracutaneous skin tests are generally performed to 5 of the available venoms in a dose response protocol (up to 1 μg/mL [wt/vol]) when preliminary prick/puncture test results are negative. (B)

Summary Statement 195. The FDA-cleared specific IgE assays have comparable specificity but decreased sensitivity compared with venom skin tests. (B)

Hymenoptera venom extracts are widely accepted as the standard reagents for diagnostic testing and immunotherapy for insect sting allergy. The commercially available products are lyophilized protein extracts for honeybee, *Vespula* (yellow jacket), and *Polistes* wasp venoms. The last 2 are mixtures of clinically relevant species. Also available are 2 *Dolichovespula* venoms (yellow hornet and white-faced hornet). A mixed vespid venom product that contains equal parts of the 3 *Vespula* venoms (yellow jacket, yellow hornet, and white-faced hornet) is available for treatment but is not recommended for diagnostic use. Honeybee venom is standardized for the content of phospholipase A (Api m 1), the major allergen in honeybee venom. *Vespula* venoms are standardized for their content of hyaluronidase. However, the primary vespid venom allergen is a nonenzymatic protein designated as antigen 5 (eg, Ves v 5). This could be a basis for a possible discrepancy between skin test results and sting response. Lyophilized venom products are reconstituted and diluted with buffered saline diluent that contains 0.03% HSA, which functions to stabilize the small amounts of protein allergens in the solutions and prevent adsorption to the walls of the vials. Although the activity of the allergens is stable for 12 months at the full concentration (100 μg/mL [wt/vol]), it may decay more rapidly at lower concentrations used for skin testing or early immunotherapy. Venoms for laboratory use are typically dialyzed to remove small-molecular-weight components, which can interfere in some assays. Dialyzed venoms may be more accurate for skin testing and are available in Europe but not in the United States.

Imported fire ant whole-body extract is the only reagent presently available for diagnostic skin testing and immunotherapy for fire ant sting allergy. Most imported fire ant whole-body extracts have been shown to contain sufficient venom allergens to be useful for diagnosis and treatment, but some preparations contain variable quantities of the relevant allergens.[997–999] Fire ant prick/puncture tests are performed first at the dose recommended by the manufacturer. If the results are negative, intracutaneous skin tests may be started with concentrations as low as 1:1,000,000 (wt/vol) in highly sensitive patients but are considered to be indicative of the presence of specific IgE antibodies if a positive response occurs at a concentration of 1:500 (wt/vol) or less[1000]

Venom skin tests are generally performed using the intracutaneous technique of injecting a small volume (0.02 to 0.03 mL) superficially in the skin to raise a bleb of 3 to 4 mm. The prick/puncture method (with a venom concentration of 1.0 μg/mL [wt/vol]) is used for preliminary skin testing, especially in patients with a history of very severe anaphylaxis, but when these results are negative, intracutaneous tests are required for diagnosis. Venom skin tests are generally performed with all 5 of the available venoms (and/or fire ant whole-body extract when indicated). The prick/puncture method is unable to detect the allergy in most patients (using concentrations $\leq$1.0 μg/mL [wt/vol]), but like all venom skin tests, can cause false-positive irritative reactions when very high concentrations are used ($>$1 μg/mL [wt/vol]). Intracutaneous skin tests are generally performed beginning with concentrations of 0.01 μg/mL (wt/vol) or less, but when they give negative results at the lowest concentration, the skin tests are repeated serially at 10-fold higher concentrations until a positive response occurs or until the result is negative at a concentration of 1.0 μg/mL. Higher concentrations can cause false-positive reactions in some cases.

Serologic diagnostic tests provide measurements of venom-specific IgE antibodies in serum using the FDA-cleared assays, including RASTs and RAST modifications. Modified assays (see part 1) have shown improved accuracy but are still subject to the validity and reproducibility of the clinical laboratory performing the assays.[132] This means that homologous internal controls (eg, specific venoms) are essential for in vitro venom tests.

Performance Characteristics of Insect Venom Tests (Prick, Intracutaneous, Specific IgE)

Summary Statement 196. Paradoxically, as many as 16% of insect allergic patients with negative venom skin test results have positive results in currently available specific IgE in vitro tests. (B)

Summary Statement 197. A small percentage of patients (1%) with negative results to both skin and in vitro tests may experience anaphylaxis after a field sting. (B)

Summary Statement 198. A skin test refractory period lasting up to 6 weeks after a venom sting has been demonstrated by recent data. (B)

Although venom skin tests have been said to be highly accurate, recent studies have focused on deficiencies in both sensitivity and specificity, which are related to the testing reagents and to the natural history of this condition. Venom skin tests are more sensitive than specific IgE tests, since insect allergic patients with positive skin test results have negative specific IgE results in 15% to 20% of cases. However, it has been reported that skin test results are negative in 10% to 30% of patients with a convincing history of systemic reaction to a sting.[188,1000,1001] This may occur in patients with near life-threatening reactions to a specific venom, as previously noted under Summary Statement 32.[188] Some of these patients will have a positive specific IgE test result so that comprehensive testing (both skin and specific IgE tests) rarely misses the diagnosis. Some patients with negative skin and specific IgE test results but a positive history may not experience a reaction to a field sting. This suggests that either the history itself is a poor predictor or the specific IgE that was initially present has disappeared over time. Conversely, 1% of patients with a positive history of an allergic sting

reaction have negative results to both skin and specific IgE diagnostic tests and yet may have a systemic reaction to a subsequent sting.[1001] Absolute reconciliation of these results is difficult. Stated otherwise, although available tests are almost always sufficient for diagnosis, they are not 100% foolproof.

One possible reason for negative venom skin test results in insect allergic patients is the refractory period that occurs after an allergic reaction to a sting. One published report describes this phenomenon in 50% of patients tested within 1 week after the sting reaction, but more than half of the skin test–negative patients had positive specific IgE results at the same time, such that the diagnosis was made in 79% using both test methods.[1002] In the other 21% of patients the test results were positive only 6 weeks after the sting reaction. When venom skin test and specific IgE test results are negative more than 6 weeks after the sting reaction, it has been recommended that the tests should be repeated at a later date. This is based on the observation that venom skin test responses may vary over time such that relatively mild sensitivity may fluctuate around the lower level of detection and give negative results on one occasion and positive on another. This observation has been reported in one clinical investigation.[616]

The immunologic specificity of venom skin tests is excellent but clinically limited. Positive skin test results invariably demonstrate the presence of venom specific IgE antibodies but are not absolute indicators of clinical allergic reactions to stings. Studies of the natural history of insect allergy have revealed that clinical reactivity is variable and can disappear despite the persistence of sensitization demonstrated with diagnostic tests.[995,1003–1006] The risk of systemic reaction to a sting in patients with positive venom skin test results and a history of previous systemic reactions has been reported to be as high as 61% and as low as 30%.[1005–1007] This broad range has been explained by multiple factors, including the age of the patient, the severity of prior sting reactions, the degree of skin test (or specific IgE) sensitivity to venoms, and variables relating to the insects themselves.

Specific IgE in vitro tests have shown improved sensitivity using modified assay methods.[134] The sensitivity of these tests is still not as good as skin tests, but the specificity is comparable. Many such assays show reduced accuracy when the level of venom IgE is in the low range. Compared with venom skin tests, current serologic tests still give false-negative results in some cases, but the converse has become equally important. As many as 10% of insect allergic patients with negative venom skin test results have positive results in the most highly sensitive specific IgE in vitro assays.[1001] A single report of a Western blot technique claims equivalent sensitivity and specificity to skin tests, provided that specific bands for antigen 5 or hyaluronidase are measured.[1008] The clinical significance of positive specific IgE tests in a patient with negative skin test results is uncertain, but this situation clearly indicates the potential for insect sting reactions as shown in case reports of such patients who had systemic reactions to challenge stings.[188]

Complementary Skin and Specific IgE Testing

Summary Statement 199. Because of predictive inconsistencies of both skin and serum specific IgE tests, patients with a convincing history of venom-induced systemic reactions should be evaluated by both methods. (D)

The performance characteristics of the diagnostic tests described herein provide a clear rationale for the combined use of the skin tests and serologic tests. Neither test alone is fully accurate, and some insect allergic patients (by history) show positive results to only one but not the other test. It is therefore important to perform the other test when 1 test result is negative in a patient with a clear history of severe reaction to stings. This is also true for individual venoms. The need to perform specific IgE tests or repeat skin testing when initial skin test results are negative is most clear in patients who have had severe anaphylactic reactions, but there is no consensus about whether this should be done in all patients with negative skin test results who are candidates for venom immunotherapy based on their history of systemic allergic reactions to stings.[132,1019]

Cross-allergenicity

Summary Statement 200. Cross-allergenicity among insect venoms is (1) extensive among vespid venoms, (2) considerable between vespids and *Polistes*, (3) infrequent between bees and vespids, and (4) very limited between yellow jackets and imported fire ants. (B)

Venoms of different species and genera may demonstrate cross-allergenicity consistent with phylogeny or across phylogenetic lines. There is infrequent specific IgE cross-allergenicity between the venoms of honeybees and vespids.[1010] Both hyaluronidase and cross-reacting carbohydrate determinants have been attributed as the basis for this cross-allergenicity.[1010,1011] The clinical significance of IgE antibodies with such cross-allergenicity is unclear but is thought to be minimal. There is also very limited cross-allergenicity between yellow jacket and fire ant venoms, which is of unknown clinical significance.[1010] Bumblebee venom allergy can be diagnosed by specific IgE testing, but there is no approved diagnostic material for skin testing in the United States. Although some cross-allergenicity exists with honeybee venom, most bumblebee allergic patients have negative test results for honeybee venom skin test reagents.[1012,1013]

There is more extensive cross-allergenicity among the vespid venoms. *Vespula* venoms (yellow jacket species, hornets) show almost complete cross-allergenicity, which is manifested by positive diagnostic test results to all 3 vespid venom reagents in most yellow jacket allergic patients. There are some individuals who show positive test results to only 1 of these venoms, and it is clear that some species have unique allergenic determinants.[1014] *Polistes* wasp venom is not as closely related to the other vespids. More than half of yellow jacket allergic patients have positive venom test results to

Polistes venom as well. In almost half of these cases, the IgE antibodies can be shown to be fully cross-allergenic by demonstrating complete inhibition of the *Polistes* specific IgE test by the addition of yellow jacket venom in the assay.[1015] The other half of the patients have separate and distinct sensitivities to yellow jacket and *Polistes* venom allergens. A specific IgE inhibition test can be used to exclude the need for wasp venom immunotherapy in many patients whose tests show multiple vespid venom sensitivities. Unfortunately, this test is not commercially available.

Number and Frequency of Tests

Summary Statement 201. If Hymenoptera venom sensitivity is suspected, initial prick/puncture tests followed by serial end point titration with intracutaneous tests may be required. (B)

Summary Statement 202. Venom skin test may be repeated once or twice at 3- to 6-month intervals to confirm the diagnosis in a patient who initially had negative test results. (D)

When testing is started with prick/puncture tests, the complete set of 5 Hymenoptera venoms should be used, as well as positive and negative controls. If fire ant sting has been confirmed, prick/puncture and intracutaneous testing is limited to this single insect. When prick/puncture test results for Hymenoptera venoms are negative, as they are in most cases, serial intracutaneous tests that use the same materials (including intracutaneous controls) may begin at concentrations of 0.001 and end at 1.0 μg/mL.[1016]

Venom skin tests may be repeated once or twice at 3- to 6-month intervals when necessary to make the diagnosis in a patient who has negative initial diagnostic test results. This may also be useful when initial skin tests show inconsistent results for the vespid venoms, so as to clarify whether additional venoms should be included in immunotherapy. In patients who are treated with venom immunotherapy, some clinicians may repeat skin tests every 2 to 5 years to determine whether the patient has lost sensitivity. In patients who are not treated, there is generally no need to repeat skin tests, but examination for loss of sensitivity may be of interest after 2 to 5 years.

Challenge Testing

Summary Statement 203. When the diagnosis is highly suspected but not proved by skin and specific IgE tests, supervised live insect challenge sting may confirm clinical sensitivity. Nevertheless, most of patients with suspected venom allergy do not require live stings. (D)

The importance of supervised challenge testing in clinical practice has been established for food and drug allergy, and a similar rationale may be used in some cases of insect sting allergy. In research studies for the efficacy of venom immunotherapy and to determine the relapse rate after discontinuing venom immunotherapy, live sting challenge has been used as the gold standard. However, even among untreated patients with a compelling history of allergic sting reactions and positive venom skin test results, approximately half will not react to a challenge sting.[1003,1005,1006] Some investigators have therefore suggested that many patients do not need venom immunotherapy and that these individuals should be identified by deliberate sting challenge. Although it is true that no available test can reliably distinguish those who will react to a sting from those who will not in every case, the outcome of sting challenge is also not fully reproducible. Up to 20% will react to a subsequent sting after experiencing an initial negative challenge sting.[1003] Therefore, sting challenge is less sensitive than venom skin tests and only somewhat more specific. Although deliberate sting challenge in the United States is limited because of both practical and ethical concerns, it is clear that specific patients would benefit from supervised challenge with live insects if this procedure was more widely available in regional allergy and anaphylaxis centers. Nevertheless, most patients with suspected venom allergy do not require live sting challenges.

ASSESSMENT OF DRUG ALLERGY

Summary Statement 204. Evaluation of drug specific IgE antibodies induced by many high-molecular-weight and several low-molecular-weight agents is often highly useful for confirming the diagnosis and prediction of future IgE-mediated reactions, such as anaphylaxis and urticaria. (B)

Summary Statement 205. Neither immediate skin nor tests for specific IgE antibodies are diagnostic of cytotoxic, immune complex, or cell-mediated drug-induced allergic reactions. (B)

IgE-mediated mechanisms are important in adverse reactions to many antibiotics, pharmaceuticals, and biologic proteins, such as insulin, protamine, and heparin. In the case of immediate hypersensitivity reactions mediated by IgE antibodies, demonstration of the presence of drug specific IgE is usually taken as sufficient evidence that the individual is at significant risk of having an anaphylactic reaction if the drug is administered. This is helpful in the case of high-molecular-weight agents. However, insufficient knowledge about drug degradation products and/or metabolites and how they are conjugated with body proteins has been an impediment to developing either skin or specific IgE assays for most small-molecular-weight drug chemicals.

Evaluation of drug specific IgE antibodies induced by many high-molecular-weight and several low-molecular-weight agents is often highly useful for confirming the diagnosis and prediction of future IgE-mediated reactions, such as anaphylaxis and urticaria.[1017–1019] Immediate-type skin tests are usually the most sensitive diagnostic tests but in certain cases where skin testing is not possible (ie, a negative histamine control test result, dermatographism, or generalized eczema), specific IgE assays (eg, Immulite, ImmunoCap) are available though not adequately standardized for either negative or positive predictability. In the case of small-molecular-weight drugs, validated and reliable skin test reagents are only available for penicillin.[1020] They have excellent negative predictive values in predicting that severe reactions to peni-

cillin will not occur. Immunoassays for penicillin specific IgE antibodies are less sensitive than skin tests, and therefore skin testing is preferred. Neither immediate skin nor specific IgE tests for IgE antibodies are diagnostic of cytotoxic, immune complex, or cell-mediated drug-induced allergic reactions.

Summary Statement 206. The availability of specific laboratory tests for non–IgE-mediated drug allergies is limited. (C)

Summary Statement 207. Atopy patch tests, lymphocyte proliferation tests, and basophil activation tests are additional diagnostic tests for drug allergy. Further studies are required to confirm their clinical utility in the evaluation of drug allergic patients. (B)

Both direct and indirect Coombs test results are often positive in drug-induced hemolytic anemia. This may reflect the presence of complement and/or drug on the red cell membrane or an Rh determinant autoantibody (eg, as occurs with α-methyldopa). Although these may be useful as diagnostic adjuncts, elevated levels can occur in individuals who receive the drug and do not experience a clinical reaction.[1020] Specific antibody tests for drug-induced neutropenia or thrombocytopenia have been reported from specific research laboratories but are not clinically available. Furthermore, using small-molecular-weight native drugs for these in vitro cytotoxicity tests may be insufficient because they may not be immunogenoric unless coupled to protein or patients may only react to specific drug metabolites.[1021] Furthermore, testing with the native drug may be insufficient or patients may react to a variety of drug metabolites.[981] Drug-specific tests are generally available in specific research laboratories and therefore are not clinically applicable for most drugs.

Summary Statement 208. A graded challenge (test dose) is a procedure to determine if a drug is safe to administer and is intended for patients who are unlikely to be allergic to the given drug. In contrast to desensitization, a graded challenge does not modify the immune response to a drug. (B)

Graded challenge (ie, test dosing), is intended for patients unlikely to have an IgE-mediated reaction to a drug and does not modify an individual's immune response to a given drug.[1022–1025] The objective of graded challenge is to introduce a medication cautiously so as not to induce a severe reaction. Although it is not possible to be absolutely certain that a patient is not allergic to a drug because valid diagnostic tests are not available for most drugs, the procedure is intended for patients who, after a full evaluation, have low pretest probability of being allergic to the given drug. The starting dose for graded challenge is generally higher than for drug desensitization, and the number of steps in the procedure may be 2 or several. It is postulated that a graded challenge consisting of more than 4 or 5 steps may induce modifications of immune effector cells and therefore induce tolerance in the patient. Since tolerance status is impossible to predict, future administrations of the drug should be given cautiously. The time intervals between doses are dependent on the type of previous reaction, and the entire procedure may take hours or days to complete. Readministration of a drug via graded challenge is absolutely contraindicated if it caused a severe non–IgE-mediated reaction such as Stevens-Johnson syndrome, toxic epidermal necrolysis, or exfoliative dermatitis.

Summary Statement 209. Atopy patch tests, lymphocyte proliferation tests, and basophil activation tests are additional diagnostic tests for drug allergy. Further studies are required to confirm their clinical utility in the evaluation of drug allergic patients. (B)

In recent years there have been reports concerning the diagnostic utility of APTs with drugs in non–IgE-mediated cutaneous drug reactions.[1026,1027] A positive reaction may be useful by identifying a specific drug in a patient receiving multiple drugs, provided that it is properly compared with a group of negative controls. The lack of standardization of reagent concentrations may limit the clinical usefulness of this procedure. The lymphocyte proliferation test has been studied as an in vitro correlate of drug-induced cellular reactions.[1028] This is used primarily as a retrospective test and is not clinically available in most medical centers. There is considerable disagreement among investigators about the value of this assay in evaluating drug allergies because neither its positive nor negative predictive values have been systematically investigated. One potential advantage of the test for some patients is that it is possible to obtain in vitro evidence of lymphocyte transformation by the parent drug itself and liver microsomal products of the drug, thereby bypassing the need for precise knowledge of metabolic determinants.[1028,1029] Although the general clinical applicability of these tests has not been validated in any large-scale study, a number of investigators have shown that drugs may induce both $CD4^+$ and $CD8^+$ T-cell responses and drug-specific T_H1 and/or T_H2 responses.[1030–1034]

Basophil activation tests have recently been used in the diagnosis of drug allergy. Basophil activation tests are in vitro tests that measure expression of activation markers, principally CD63 203C on the surface of basophils. These tests are typically performed by incubating peripheral blood samples with allergen and IL-3 to enhance the expression of CD63, which is detected by flow cytometry. This method has been reported by European investigators in a variety of cases of drug hypersensitivity reactions with drugs such as neuromuscular blocking agents, β-lactams, and NSAIDs and by Australian investigators for succinylated gelatin.[677,1035–1037] In a study of patients with perioperative allergic reactions to neuromuscular blocking agents, intracutaneous skin testing with a 1:100 dilution was more sensitive (100%) compared with basophil activation tests using CD63 (64%).[1040] The specificity of the basophil activation test was relatively high (93%), whereas the specificity of intracutaneous tests varied between 63% (1:100 dilution) and 100% (1:1,000 dilution) for muscle relaxant allergy. Another study evaluated basophil activation tests in 60 subjects with a history of aspirin or NSAID-induced respiratory and/or cutaneous reactions.[1037] In this study, the basophil activation test with aspirin had a specificity of 100% and a sensitivity of 43%. Further confir-

matory studies, especially with commercially available tests, are needed before its general acceptance as a diagnostic tool.

Penicillin

Summary Statement 210. Penicillin skin testing is the most reliable method for evaluating IgE-mediated penicillin allergy provided that the necessary reagents are available. When performed with both major and minor determinants, the negative predictive value of penicillin skin testing for immediate reactions approaches 100%, whereas the positive predictive value is between 40% and 100%. (B)

Penicillin is the only low-molecular-weight agent for which validated testing has been documented.[1019,1020] The major determinant of penicillin, penicilloyl polylysine, is the only skin test reagent licensed for antibiotic skin testing. Currently, neither minor nor major determinant reagents are available commercially in the United States. Some medical centers prepare these reagents for their own local institutional use.

Immediate-type penicillin allergy cannot be accurately diagnosed by history alone. This observation is partially explained by the fact that patients with documented penicillin specific IgE may lose their sensitivity over time.[1038] Additionally, patients with vague reaction histories may be allergic and demonstrate positive skin test results. Overall, approximately one third of patients with positive penicillin skin test results report vague reaction histories.[1039] Penicillin skin testing is the most reliable method for evaluating IgE-mediated penicillin allergy. Specific IgE tests (RAST, ImmunoCAP, or ELISA) are less sensitive and specific compared with skin testing.[1040–1042] Penicillin skin testing detects the presence or absence of penicillin specific IgE antibodies, and it is neither useful nor indicated for clearly non–IgE-mediated reactions (ie, penicillin-induced hemolytic anemia, serum sickness-like reaction, or ACD).

Ideally, both major and minor determinant reagents are used for skin testing. Currently, the major determinant is not commercially available as penicilloyl-polylysine (PrePen) in a premixed 6×10^{-5}M solution but, as cited herein, it has been prepared for local use in various medical centers. Although not actually a minor determinant, penicillin G is commercially available and traditionally has been used for skin testing at a concentration of 10,000 U/mL. The other minor determinants (penicilloate and penilloate) are used for skin testing at 0.01M but have never been commercially available in the United States. Penicillin G left in solution ("aged" penicillin) does not spontaneously degrade to form separable minor determinants and therefore cannot be used as a substitute for the other minor determinants.[1043] The negative predictive value of penicillin skin testing (using penicilloyl-polylysine, penicillin G, and penicilloate and/or penilloate) for serious immediate-type reactions approaches 100%,[1019,1044,1045] and the positive predictive value (based on limited challenges of skin test–positive patients) is between 40% and 100%.[1019,1045,1046]

Likelihood ratios for positive skin test results based on a history of penicillin allergy have been calculated based on the results of 4 studies involving a total of 9,526 patients who had penicillin skin testing performed.[1047] The overall likelihood ratio for a patient with a history of penicillin allergy to have a positive penicillin skin test result was 1.9 (95% confidence interval, 1.5–2.5). Conversely, the negative likelihood ratio was 0.5 (95% confidence interval, 0.4–0.6), indicating the likelihood that a patient without a history of penicillin allergy would have a positive penicillin skin test result.

Summary Statement 211. Skin testing with penicilloyl-polylysine and penicillin G appears to have adequate negative predictive value in the evaluation of penicillin allergy. (C)

When penicilloyl-polylysine was available, most allergists performed penicillin skin tests with only penicilloyl-polylysine and penicillin G. However, some studies report that approximately 10% to 20% of penicillin allergic patients show skin test reactivity only to penicilloate or penilloate.[1019,1044,1048–1053] The clinical significance of these findings is uncertain. Penicillin challenges of individuals skin test negative to penicilloyl-polylysine and penicillin G[1046,1049] have similar reaction rates compared with individuals skin test negative to the full set of major and minor penicillin determinants.[1019,1044,1045] Therefore, based on the available literature, skin testing with penicilloyl-polylysine and penicillin G appears to have adequate negative predictive value in the evaluation of penicillin allergy. To date, the positive predictive value of penicillin skin tests has not been carefully studied.

Penicillin skin testing should only be performed by personnel skilled in the application and interpretation of this type of skin testing, with preparedness to treat potential anaphylaxis. Appropriate positive (histamine) and negative (saline) controls should be placed. First, full-strength reagents are applied by the prick/puncture technique, and if the results are negative, intracutaneous testing should be performed. There is no uniform agreement on what constitutes a positive skin test response, but most experts agree that it is defined by the size of the wheal that should be 3 mm or greater than that of the negative control for either prick/puncture or intracutaneous tests. Penicillin skin testing, using the reagents described herein and proper technique, are safe, with only a rare risk (0.1%–2%) of a systemic reaction occurring.[1044,1050] Patients with a history of anaphylaxis to β-lactams and a history of drug reactions occurring within an hour may be at greater risk for systemic reactions to skin testing with β-lactams.[1051]

Summary Statement 212. Penicillin skin test–negative patients (as determined by testing with major and minor determinants) may receive penicillin, and depending on which skin test reagents are used and the reaction history, the first dose may need to be given via a test challenge with a lower dose under observation. (D)

Penicillin skin testing is indicated in patients who have a reaction history consistent with a possible IgE-mediated mechanism. Penicillin skin testing may be performed electively (when patients are well and not in immediate need of

antibiotic therapy) or only when treatment with a penicillin compound is contemplated.

There is lack of agreement regarding the need to perform an elective challenge with penicillin immediately after a negative penicillin skin test result. Surveys of patient who exhibited negative penicillin skin test results (without subsequently being challenged with penicillin) found that a large proportion were not given β-lactam antibiotics because of fear expressed by either the patient or the treating physician.[1052] In an enclosed health maintenance organization setting, review of medical records found that subsequent prescriptions for penicillins in penicillin skin test–negative patients were comparable in those individuals who were or were not challenged with oral penicillin after their skin test (47% vs 48% during the year after the skin test).[1053] If penicillin skin testing is performed with only penicilloyl-polylysine and penicillin G, initial administration of penicillin, depending on the pretest probability of the patient being allergic, may need to be done via graded challenge (ie, 1/100 of the dose, followed by the full dose, assuming no reaction occurs during a brief observation period).

Summary Statement 213. In the absence of validated skin test reagents, the approach to patients with a history of penicillin allergy is similar to that of other antibiotics for which no validated in vivo or in vitro diagnostic tests are available. Therapeutic options include (1) prescribing an alternative antibiotic, (2) performing a graded challenge, and (3) performing penicillin desensitization. (D)

Currently, penicilloyl-polylysine, the major determinant of penicillin, is not commercially available. Penicillin testing without the major determinant fails to identify most penicillin allergic patients. Therefore, some medical centers prepare these reagents for local, institutional use only. In the absence of validated commercial or locally prepared skin test reagents, therapeutic options include (1) prescribing an alternative antibiotic, (2) performing a graded challenge, and (3) performing penicillin desensitization. If a therapeutically equivalent antibiotic is available, this would typically be the safest choice. However, in some cases penicillin would be the drug of choice. In this scenario, the decision of performing a graded challenge or desensitization would be based on factors such as the documentation and description of the reaction to penicillin, the time elapsed since the allergic reaction, and presence of comorbid conditions (eg, coronary artery disease). For example, in a healthy patient with a childhood history of a morbilliform eruption to penicillin 30 years prior, a graded challenge could be considered. In contrast, a patient with congestive heart failure and a history of anaphylaxis to penicillin 2 years ago should likely undergo an empiric penicillin desensitization.

Summary Statement 214. In patients who have reacted to semisynthetic penicillins, consideration should be given to skin test the implicated antibiotic and penicillin determinants. (B)

Some patients with immediate-type reactions to amoxicillin or ampicillin are able to tolerate other penicillin-class compounds.[1054,1055] These individuals appear to have reactions directed at the R-group side chain, which distinguishes the chemical structure of different penicillin-class compounds. These patients may have skin test results that are positive to a nonirritating concentration of either amoxicillin or ampicillin but test negative to penicillin major and minor determinants. Therefore, when skin testing patients who have reacted to semisynthetic penicillins, consideration should be given to include the implicated antibiotic and penicillin determinants. The negative predictive value of skin testing with native semisynthetic penicillins is unknown, and there is no consensus regarding the appropriate concentration that should be used.

Other Antibiotics

Summary Statement 215. There are no validated diagnostic tests of sufficient sensitivity for evaluation of IgE-mediated allergy to antibiotics other than penicillin. (C)

Summary Statement 216. Skin testing with nonirritating concentrations of other antibiotics is not standardized. A negative skin test result does not rule out the possibility of an immediate-type allergy. A positive skin test result suggests the presence of drug specific IgE antibodies, but the predictive value is unknown. (C)

Most patients with immediate allergic reactions to cephalosporins react to the R1 side chain rather than the β-lactam ring, and skin test results are often positive in such patients.[1056] Specific IgE test results have been positive in some patients with histories of cephalosporin allergy, some of whom had negative skin test results.[1057] A positive cephalosporin skin test result (using a nonirritating concentration) implies the presence of drug specific IgE antibodies, and the patient should receive an alternate drug or undergo desensitization. A negative cephalosporin skin test result (using a nonirritating concentration) does not rule out the presence of drug specific IgE antibodies. IgE antibodies to cephalosporin metabolic products not used in the testing may be present but not detectable. Therefore, since the negative predictive value of cephalosporin skin testing is unknown, a cautious graded challenge should be performed (eg, 1/100 of the therapeutic dose, increasing tenfold every 30 to 60 minutes up to the full therapeutic dose) in cases of negative skin test results. The number of steps in the graded challenge and the pace of the challenge are determined by the reaction history. If the previous history is consistent with a severe IgE-mediated reaction, rapid desensitization may be undertaken instead. Evaluation of IgE-mediated allergy to other β-lactams (eg, aztreonam, carbapenems) is analogous to cephalosporins in that relevant degradation products are unknown, and thus there are no standardized skin test reagents available. Skin testing with a nonirritating concentration of non-β-lactams has the same limitation and questionable predictive value as with cephalosporins.

For most non–β-lactam antibiotics, there are case reports of positive skin test results with the native drug; however, large-scale validation of such skin testing has not been ac-

Table 14. Nonirritating Concentrations for Antimicrobial Intracutaneous Testing

Antimicrobial drug	Full-strength concentration, mg/mL	Dilution from full-strength concentration
Cefotaxime	100	10^{-1}
Cefuroxime	100	10^{-1}
Cefazolin	330	10^{-1}
Ceftazidime	100	10^{-1}
Ceftriaxone	100	10^{-1}
Tobramycin	40	10^{-1}
Ticarcillin	200	10^{-1}
Clindamycin	150	10^{-1}
Gentamycin	40	10^{-1}
Cotrimoxazole	80	10^{-2}
Levofloxacin	25	10^{-3}
Erythromycin	50	10^{-3}
Azithromycin	100	10^{-4}
Nafcillin	250	10^{-4}
Vancomycin	50	10^{-4}

complished. It is well recognized that most antibiotics have multiple end products, and therefore it is possible that the relevant allergens may be metabolites and not the parent drug. Although no validated in vivo or in vitro diagnostic tests are available for non–β-lactam antibiotics, skin testing with nonirritating concentrations of the drug (ie, negative skin test reactivity in a panel of normal, nonexposed volunteers) may provide useful information, and nonirritating concentrations for 15 commonly used antibiotics have been published (Table 14).[1058] If the skin test result is positive under these circumstances, it is likely that drug specific IgE antibodies are present. Some clinicians also verify this interpretation by demonstrating a negative skin test result in a nonallergic control subject tested at the same time as the patient. Therefore, the patient should receive an alternative non–cross-reacting antibiotic or undergo rapid desensitization. On the other hand, a negative skin test result does not denote that drug specific IgE antibodies are absent, since it is possible that a drug metabolite not present in the test reagent may be the relevant allergen. However, if this particular antibiotic is required for treatment, the amount of drug injected intracutaneously can be used as the initial starting dose for rapid desensitization. In skin test–negative patients who have mild reaction histories, a graded challenge procedure may be considered. Readministration of drugs that caused severe non–IgE-mediated reactions (such as Stevens-Johnson syndrome, toxic epidermal necrolysis, and others), either by desensitization or graded challenge, is absolutely contraindicated.

Aspirin and NSAIDs

Summary Statement 217. A presumptive diagnosis of aspirin-exacerbated respiratory disease (AERD) can often be made by history; however, in some cases, aspirin provocation tests might be considered for a definitive diagnosis. (B)

One type of adverse reaction to aspirin or NSAIDs is AERD, a clinical entity characterized by aspirin- or NSAID-induced respiratory reactions in patients with underlying asthma. There is no diagnostic skin prick/puncture or intracutaneous test for AERD. The diagnosis is usually established by history, but if the history is unclear or, when definite diagnosis is required, a provocation test with aspirin or acetylsalicylic acid may be performed. Aspirin or acetylsalicylic acid provocation tests have been performed using various routes of administration, including oral, bronchial, nasal, and rarely intravenous.[1059] In the United States, only oral challenges are available. Twenty-four hours before the challenge, use of anticholinergics, antihistamines, cromolyn, and short-acting β-agonists should be discontinued.[1060] Because of the potential for exacerbating a patient's asthma, use of oral and inhaled corticosteroids, intranasal corticosteroids, theophylline, and long-acting bronchodilators should be continued at the time of the challenge. Leukotriene modifiers may block bronchospastic responses but often do not inhibit aspirin or acetylsalicylic acid–induced lower respiratory tract reactions.[1061,1062]

Oral aspirin or acetylsalicylic acid challenges in patients with suspected AERD are typically done more than 3 days with the first day being performed with placebos to ensure baseline stability of asthma (ie, FEV_1 should change $<15\%$). A commonly used protocol begins with 15 to 30 mg of aspirin or acetylsalicylic acid on day 2 followed by doses of 45 to 60 mg and 100 mg in 3-hour intervals with serial measurement of FEV_1 hourly.[1060] On day 3 of the challenge, aspirin or acetylsalicylic acid doses of 150 mg, 325 mg, and 650 mg are given in 3-hour intervals. If 650 mg of aspirin or acetylsalicylic acid is administered and there is no reaction and the patient is not taking more than 10 mg of prednisone or a leukotriene modifier, the test result is determined to be negative. Reactions to aspirin or acetylsalicylic acid in patients with AERD typically occur within 15 minutes to 1 hour after ingestion of aspirin. Reactions include not only bronchospasm (which may be severe) but also naso-ocular symptoms and infrequently cutaneous and gastrointestinal symptoms. Physicians need to be prepared to treat these reactions aggressively.

Summary Statement 218. Urticaria, angioedema, and anaphylactic reactions to NSAIDs are distinctly different drug reactions from AERD reactions. In contrast to AERD reactions, anaphylactic reactions to NSAIDs are usually drug specific and patients typically tolerate other structurally dissimilar NSAIDs. (B)

Aspirin or acetylsalicylic acid and NSAIDs may also cause urticaria and angioedema or even anaphylaxis. The approach to these patients differs from that for patients with AERD. Patients with a history of urticaria or angioedema to NSAIDs may be challenged using a graded challenge (test dose) protocol similar to other drugs. For most patients with anaphylactic reactions to NSAIDs, these reactions are drug specific and challenging with a structurally different NSAID would be the preferred strategy.[1063]

Perioperative Anaphylaxis

Summary Statement 219. Skin testing is a useful diagnostic tool in cases of perioperative anaphylaxis, and when skin testing is used to guide subsequent anesthetic agents, the risk of recurrent anaphylaxis to anesthesia is low. (C)

Skin testing has been reported to be of diagnostic utility in identifying the causative agent in cases of anaphylaxis during general anesthesia in both retrospective and prospective studies.[1064,1065] Intracutaneous testing is the most widely used method and has been determined to be a valid and reproducible method in several studies.[1064,1065] Prick testing has also been evaluated, and 2 prospective studies confirmed that prick testing was a useful diagnostic tool and highly correlated with intracutaneous skin testing.[1036,1065] For propofol reactions, intracutaneous testing is more reliable.[1066,1067] Skin testing has not been applied to any gold standard for anesthetic allergy due to the inherent dangers with challenging a patient with a history of anaphylaxis and the inherent pharmacologic effects of the anesthetic. When skin testing is used to guide subsequent anesthetic agents, the risk of recurrent anaphylaxis to anesthesia is low.[1066,1068] Nevertheless, false-negative skin test results have been reported and the true-negative predictive value remains unknown.[1066]

The concentrations and dilutions for skin testing used in different studies is varied.[1069] One approach recommended by the French Society of Anesthesiology and used in a study of 789 patients being evaluated for allergic reactions to anesthetics uses a combination of prick and intracutaneous tests.[1070] The drugs tested in this study included neuromuscular blocking agents, antibiotics, hypnotics, opioids, and others. Prick tests are performed with undiluted drug, with the exception of atracurium, mivacurium, and morphine, which are tested using a 1:10 (wt/vol) dilution. Intracutaneous tests are performed with 0.02 to 0.05 mL of serial dilutions of the drug every 15 minutes. The initial dilution is 10^{-4} (wt/vol) if the prick test result is positive and 10^{-3} (wt/vol) when the prick test result is negative and subsequent intracutaneous tests are performed at 10-fold higher concentrations up to 10^{-1} (wt/vol) for most drugs. The final testing dilution for morphine, rocuronium, and cisatracurium is 10^{-2} (wt/vol), and for atracurium, and mivacurium a maximal dilution of 10^{-3} (wt/vol) is recommended.

Specific IgE tests for detecting sensitization to neuromuscular blocking agents and latex have been used before general anesthesia to prevent anaphylaxis during surgery.[1071] In this French study, specific IgE was positive in 79% of cases of anaphylaxis attributed to neuromuscular blocking agents and 88% of cases attributed to latex.[1071] For neuromuscular blocking agents, skin testing appears to have greater sensitivity; however, a few patients may have positive specific IgE test results with negative skin test results.

Tryptase has been evaluated for its diagnostic value in perioperative anaphylaxis. In the previously cited French study, 112 of 175 patients (64%) with anaphylaxis had a tryptase level of more than 25 μg/L, whereas only 9 of 84 anaphylactoid reactions (11%) had an elevated tryptase level.[1071] For diagnosis of perioperative anaphylaxis, tryptase measurements had a positive predictive value of 92.6% but a negative predictive value of 54%. The positive likelihood ratio for an anaphylactic event based on a tryptase level was 6.0 and the negative likelihood ratio was 0.4.

Chemotherapeutics

Summary Statement 219. Skin testing is not helpful in cases of taxane-induced anaphylactoid reactions. (C)

Summary Statement 220. Skin testing to carboplatin yields favorable predictive values. (C)

Summary Statement 221. Skin testing with asparaginase before treatment is recommended but does not identify all patients at risk of reactions. (C)

Hypersensitivity reactions have been reported for virtually all commonly used chemotherapeutic agents. Reactions range from mild cutaneous eruptions to fatal anaphylaxis. In some cases, it is difficult to determine whether a reaction is anaphylactic (ie, mediated by drug specific IgE antibodies) or anaphylactoid (due to nonimmune degranulation of mast cells and basophils as occurs with Cremophor-EL, a solvent used for many cancer chemotherapy drugs). For some chemotherapeutics, skin testing may help identifying patients at high risk for allergic reactions to chemotherapy.

In the taxane family, paclitaxel and docetaxel produce anaphylactoid reactions in as many as 42% of patients on first administration and rarely (3%) with subsequent cycles.[1072] The pathophysiology of these reactions is unknown but unlikely to be IgE mediated because skin test results to taxanes are negative in patients with these anaphylactoid reactions[1062] and prophylactic therapy with antihistamines and corticosteroids reduces hypersensitivity reactions to approximately 4%.[1073] Test dose protocols have been used to reduce the incidence of reactions and cost of drug wastage. However, the largest study to date using test dosing of paclitaxel in 130 patients revealed no significant difference in hypersensitivity reactions compared with patients treated without using the test dosing protocol (2.3% in test dose group vs 6.2% in control group, $P < .20$).[1074] Of note, 1 severe reaction occurred in the non–test dose group, but none were observed in the test dose group. Finally, the test dose strategy was actually more expensive (increased cost of $6,100 for 130 patients).

Platinum compounds (cisplatin, carboplatin, and oxiplatin) typically cause hypersensitivity reactions after completion of several treatment courses, suggesting an immunologic mechanism.[1075,1076] Skin testing with carboplatin has been shown to help predict patients who will have allergic reactions to carboplatin. A study of 47 patients receiving carboplatin for gynecologic malignancies had intracutaneous tests with 0.02 mL of undiluted carboplatin and found that a negative skin test result accurately predicted the absence of an allergic reaction in 166 of 168 courses of therapy.[1077] This rate of reactivity was lower than historical controls who had a 27%

incidence of allergic reactions. A larger study of 126 women with gynecologic cancers performed intracutaneous skin tests with 0.02 mL of undiluted carboplatin in women who had received more than 6 courses of platinum-based chemotherapy.[1078] Of 668 negative skin test results, 10 were associated with hypersensitivity reactions (1.5% false negatives but none were severe. Although most patients with positive test results did not receive further carboplatin, 7 patients who had positive skin test results received carboplatin and 6 of 7 had allergic reactions. On the basis of this information, it has been recommended that skin testing to carboplatin be performed before the eighth cycle of chemotherapy.[1079]

Immediate-type reactions to asparaginase occur in as many as 43% of patients, and the reaction rate increases after the fourth weekly dose.[1080] It is unknown whether the mechanism is anaphylactic or anaphylactoid, and it may be different in different patients. Use of skin testing with asparaginase before treatment is recommended but does not identify all patients at risk of reactions.[1080] In addition to false-negative skin test results, false-positive skin test results may also occur.[1080] Polyethylene glycolated–asparaginase has also been reported to cause anaphylaxis, and skin test results to *Escherichia coli* derived granulocyte colony-stimulating factor was positive, suggesting an IgE-mediated mechanism involving bacteria-specific antigens.[1081] This report suggests that patients with allergic reactions to *E coli*–derived asparaginase should avoid other products synthesized with recombinant *E coli* systems and that skin testing may be helpful in confirming such cross-reactivity, although the predictive value of this testing is unknown.

Local Anesthetics

Summary Statement 223. Skin testing for diagnosis of local anesthetic allergy is limited by false-positive reactions. The gold standard for establishing a diagnosis of local anesthetic allergy is the provocative challenge. (C)

Local anesthetics are commonly used and adverse reactions to injections of local anesthetics may occur. Nearly all of these reactions are due to vasovagal reactions, anxiety, psychosomatic, or toxic effects. IgE-mediated or anaphylactoid reactions to local anesthetics are extremely rare and have been documented in only a few case reports.[1082,1083] The gold standard for establishing a diagnosis of local anesthetic allergy is the provocative challenge. Skin testing has also been used as a diagnostic tool; however, several studies have indicated false-positive intracutaneous skin test results.[1084–1086] In patients who subsequently tolerate a provocative challenge without an adverse reaction, false-positive intracutaneous test results occur in approximately 19% and 9% of history-negative and history-positive patients, respectively.[1087] On the basis of the low pretest probability of IgE-mediated local anesthetic allergy and the occurrence of false-positive results, it is unclear whether intracutaneous tests have any benefit in the diagnostic approach to local anesthetic allergy.[1088,1089] Rare patients may also have positive skin test results to methylparaben additives in the local anesthetics and some of these may be false-positive skin test results because subsequent subcutaneous challenge to local anesthetic with methylparabens are often negative.[1090] Subcutaneous local anesthetic challenges using a graded incremental approach after skin tests have been reported as a safe method in a study of 236 patients with histories of adverse reactions to local anesthetics.[1088] Rechallenge without prior skin tests was reported to be an easy and cheap alternative to skin testing.[1091] However, the possibility of a rare, systemic reaction must still be kept in mind.

Corticosteroids

Summary Statement 224. The specificity and sensitivity of skin tests for systemic corticosteroid allergy are unknown, and cases of corticosteroid allergy with negative skin test results to the implicated corticosteroid have been reported. (D)

Immediate-type allergic reactions to corticosteroids are rare. The mechanisms of these reactions remain unclear, and both IgE- and non–IgE-mediated reactions have been proposed.[1092] Skin testing has been used in the diagnosis of corticosteroid hypersensitive reactions with variable results. Prick and intracutaneous tests have been used with a variety of concentrations[1093–1095] and have been found to be nonirritating in normal controls even up to undiluted concentrations.[1096] The specificity and sensitivity of skin tests for corticosteroid allergy are unknown, and cases of corticosteroid allergy with negative skin test results to the implicated corticosteroid have been reported, including a case with a positive provocative challenge.[1095] Finally, other components added to corticosteroid preparations (eg, carboxymethylcellulose) have been reported to be responsible for anaphylaxis after injection of parenteral corticosteroids.[1097]

Additives and Preservatives

Summary Statement 225. For most allergic reactions to additives, skin tests are of no diagnostic value, and placebo-controlled oral challenges are required. (C)

The number of additives used by the food and drug industries is extensive. Only a small number of additives have been implicated in IgE-mediated or other adverse reactions. For many additives, including tartrazine, aspartame, sodium benzoate, butylated hydroxyanisole, butylated hydroxytoluene, and FD&C dyes, skin tests are of no diagnostic value, and placebo-controlled oral challenges are required.[981] In rare cases of sulfite sensitivity, positive skin test results to sulfites have been described.[1098,1099] Natural food additives, such as annatto, saffron, carmine, and erythritol, have been described to rarely cause anaphylaxis and positive skin test results have been demonstrated.[1100–1103] Antibacterial additives such as parabens and benzylkonium chloride may also induce IgE-mediated symptoms.[909]

ASSESSMENT OF ALLERGIC CONTACT DERMATITIS

Summary Statement 226. Contact dermatitis is a common skin disorder seen by allergists and dermatologists and can

present with a spectrum of morphologic cutaneous reactions. (C)

Contact dermatitis is a common skin problem for which 5.7 million physician visits per year are made.[468,1104] All age groups are affected, with a slight female preponderance based on a large population-based survey of public health issues.[1105] The acute clinical expression of CD is characterized by redness, edema, papules, vesiculation, weeping, crusting, and pruritus most commonly recognized as eczema, a nonspecific term applied to a number of dermatitides, including atopic dermatitis. Prolonged persistence of this dermatitis may be associated with acneiform eruptions secondary to irritation of follicular function, hypopigmentation or hyperpigmentation due to alterations in melanocytic biology, skin thickening, lichenification, and fissuring. Exposure to UV light most commonly causes a phototoxic or sunburn type of reaction and less commonly a photoallergic reaction when the UV light interacts with chemical agents (ie, fragrances, PABA, plants, parsnips, figs, or several ingested drugs) inducing photosensitization of various forms.

Summary Statement 227. The initial approach to clinical diagnosis of CD is to distinguish between ACD and ICD. (C)

Contact dermatitis encompasses all adverse cutaneous reactions that result from the direct contact of an exogenous agent (a foreign molecule, UV light, or temperature) to the surface of the skin or mucous membranes. The skin can react immunologically and/or nonimmunologically to such exogenous agents. The inflammatory process resulting from an allergic substance is mediated through immunologic mechanisms, whereas irritant reactions result from direct tissue damage, which initiate alternative inflammatory reactions. However, the distinction between ACD and ICD has become increasingly blurred. Often these exogenous forms of dermatitis must be distinguished from endogenous dermatitis (ie, atopic dermatitis, nummular eczema, dyshidrosis).[1105] It is not unusual for an exogenous dermatitis to be superimposed on an endogenous eruption, most commonly encountered when compresses or topical antibiotics are used too long on barrier impaired skin.

Based on several studies, the bulk of exogenous cases are diagnosed as ICD. The appropriate diagnosis is made by evaluating the location and evolution of the inflammation, together with morphologic nuances, to arrive at a probable diagnosis. Patch testing remains the most useful method for confirming ACD. Irritant contact dermatitis is a diagnosis of exclusion without firm criteria or when patch test results for ACD are negative. However, if patch tests fail to test for the appropriate substance, an ICD diagnosis could be incorrect.

Summary Statement 228. The inflammatory lesions of CD may result from either ACD or ICD mechanisms. Factors that affect response to the contact agent include the agent itself, the patient, the type and degree of exposure, and the environment. (A)

The potential for substances that could cause either ICD or ACD is variable. Thus, detergents have a higher irritancy index, whereas nickel is a major allergenic contactant chemical. The severity of the CD ranges from a mild, short-lived condition to a severe, persistent, but rarely life-threatening, disease. The thickness and integrity of the skin influence the potential for developing ICD or ACD. Thinner skin sites, such as the eyelids, ear lobes, and genital areas, are most vulnerable, whereas the thicker palms and soles are more resistant to injury induced by irritation or sensitization. Exposure time to allergenic contactants, which usually defines both induction and elicitation phases of ACD, varies from being brief (eg, poison ivy) to protracted (eg, nickel in jewelry or other chemicals in the work environment). Similarly, irritant substances may damage the skin in either the short or long term.

Summary Statement 229. Tissue reactions to contactants are attributable primarily to cellular immune mechanisms except for contact urticaria. (A)

Contact dermatitis reactions are noted almost exclusively at the site of exposure with the putative antigen. Most ACD antigens are small-molecular-weight molecules or haptens that become immunogenic after conjugation with proteins in the skin.[1106] Less commonly, large-molecular-weight peptides or proteins (eg, latex, cashew nuts) may both induce and elicit the classic inflammatory lesions of ACD.

Summary Statement 230. Irritant contact dermatitis is usually the result of nonimmunologic, direct tissue reaction and must be clearly differentiated from ACD. (A)

Irritant contact dermatitis is generally a multifactorial response that involves contact with a substance that chemically abrades, physically irritates, or damages the skin.[1107] Irritation is usually a direct cytotoxic reaction produced by a wide variety of agents (eg, chemicals, detergents, solvents, alcohol, creams, lotions, ointments, and powders) and by contributory physical factors that include excessive scrubbing, washing, overhydration, improper drying, perspiration, and temperature extremes. Any impairment to the epidermal barrier layer (eg, fissuring, superhydration) increases skin susceptibility to an irritant defect. The evolution and resolution of ICD are less predictable than those of ACD. The clinical presentation of ICD is more limited to the skin site directly in contact with the offending agent(s) with little or no extension beyond the site of contact.

Summary Statement 231. The diagnosis of ACD is suspected from the clinical presentation of the rash, which then must be supported by a history of exposure to a putative agent and subsequently confirmed by patch testing whenever this is possible. (C)

The suspicion of ACD is the first step in making the diagnosis. Thus, the history remains an essential part of the diagnosis and subsequent management of this disease. Although history can strongly suggest the cause of ACD, it has been reported that experienced physicians accurately predict the sensitizer in only 10% to 20% of patients with ACD when relying solely on the history and physical examination.[1108]

For ACD to occur, the site of inflammation must have come in direct contact with the offending agent. Initially, the area may itch, burn, or sting. The evolution of the lesion

depends on multiple factors, including the innate allergenicity or irritancy of the agent, the integrity of the involved skin, environmental conditions, a history of prior reactions, and immunocompetency status. Activities that involve exposure to sun, water, or airborne allergens may affect the skin distribution. Remissions and exacerbations may be related to weekends, vacations, and work schedule.

Work history must be carefully reviewed. The exact nature of the work duration of each activity and similar skin effects in coworkers may be relevant. Recent changes in procedure or chemical exposures, including vapors and fumes, must be probed. Protective wear and compliance with its use may give a clue as to the nature of the suspected allergen. Certain jobs require frequent hand washing and the use of special cleansing agents that not only may impair skin barrier but also may cause irritant hand dermatitis. Although moisturizers after hand washing may prevent dehydration, they may expose the patient to unsuspected allergens in the moisturizer preparation. Since the worker may be unaware of specific chemicals to which he/she is exposed, material safety data sheets may have to be obtained from the manufacturer.[1109] Chronologic exposure histories and other activities must be obtained.

Hobbies and nonwork activity, such as gardening, macramé, painting, ceramic work, carpentry, and photography, may be sources of exposure to other contactants. Obtaining a detailed history of animal exposure is essential. Pet dander, products used on pets, and traces of outdoor allergens all can cause ACD. The history should also include response to previous treatment. Many patients will have tried to eliminate multiple agents or have used various remedies before seeing a physician.

Summary Statement 232. The skin site of the dermatitis is important in the diagnosis of ACD because the area of predominant involvement and the regional distribution of the lesions often reflect the area of contact with the allergen. (A)

All inflammatory and spongiotic clinical reactions should include ACD as a possibility. Each lesional site usually corresponds to the site of contact with the putative allergen, and the physical appearance of the lesion may also suggest the potential for ACD. Particular attention should be given to certain anatomical sites, which include eyelids, face, neck, scalp, hands, axillae, lower extremities, and the anogenital area.[1110–1116]

Summary Statement 233. Epicutaneously applied patch tests are the standardized diagnostic procedures to confirm ACD. (A)

Patch testing is the gold standard for identification of a contact allergen.[1107] Although occlusive patch testing is the most common technique, open, prophetic (provocative), repeated insult, photopatch, and atopy patch tests are also available if special situations indicate their use. For example, open patch tests are preferred for potential photosensitizers, volatile substances, mascara, antiperspirants, shaving creams, dentifrices, and strong topical medicaments that could act as relative primary irritants. If photosensitization is suspected, photopatch tests should be performed by a physician with expertise in UV radiation. Duplicate applications of the suspected photocontactant(s) are placed on each side of the upper back. One side is irradiated with 5 J cm^{-2} of UV-A 24 to 48 hours later, and both radiated and unradiated sides are read 48 hours later.

The number of appropriate patch tests required to diagnose ACD may vary, depending on the nature of the clinical problem and the potential for significant allergen exposure. The value of a test depends on whether the clinical presentation warrants its use, the quality of reagents used, the timing of the application, an appropriate interpretation of the reaction, and establishing relevance for the benefit of the patient. Although the application of allergen patch testing is rather simple, allergen selection, the proper test concentration, and interpretation of the test require expertise. Clinical research defining the validity of each of these components has been extensive. Such data are well described in textbooks and previous practice parameters (Practice Parameter for Allergy Diagnostic Testing and Contact Dermatitis: A Practice Parameter).[235,1108–1120] These sources provide details for the purchase and/or preparation of allergens and materials for application, forms for record keeping, preparation of patch test sites, application of the allergens, times of reading, and interpretation according to internationally approved guidelines.[1117,1120] Because it is impractical to test an unlimited number of contactants, standardized sets have been designed and validated by collaborative dermatologic research societies.[469,481,482,1121,1122] These vary somewhat to reflect differences in exposure patterns in different parts of the world. New allergens are added from time to time, depending on changes of product utilization and exposure patterns. Since 2001, the North American Contact Dermatitis Group has enlarged its standard panel to 65 allergens and/or allergen mixes. However, use of the FDA-certified antigen panel available in the United States can fully evaluate approximately 25% to 30% of patients with ACD, especially those patients who are allergic to rubber, metals, fragrances, cosmetics, and medicaments.[469]

Summary Statement 234. Patch tests are indicated in any patient with a chronic, pruritic, eczematous, or lichenified dermatitis if underlying or secondary ACD is suspected. (C)

Virtually any eczematous lesion could be caused or aggravated by a contactant.[481,482,1107,1121–1124] The decision to patch test under these circumstances is often independent of the history because the patient may be unaware of any relevant exposure. Based on repetitive tests in patients with the angry back syndrome, it is recommended that patch testing should be deferred until the underlying dermatitis is no longer acute or severe.[507] Under such circumstances, the entire skin may be irritable and false-positive reactions may occur. There is always the possibility that a positive patch test result may trigger an exacerbation of the original dermatitis. In this situation, however, negative patch test results to a standard battery of allergens can be valuable in excluding a suspected agent.

Summary Statement 235. Patch test results are affected by oral corticosteroids but not by antihistamines. (A)

Immunocompromised adult patients, including those taking oral corticosteroids (>20 mg of prednisone per day or its equivalent) or those undergoing cancer chemotherapy, may show diminished or absent reactivity to the patch tests.[1125,1126] A multicenter, randomized, double-blind study revealed that systemic steroids in doses of less than 20 mg/d were not likely to suppress strongly positive patch test results, but they could suppress milder responses.[1126] The same study concluded that equivalent doses of prednisone did not affect irritant responses[1126] The effect of systemic corticosteroids on the results of patch testing is less understood for children.

The skin site where the patch tests are to be applied should have had no topical potent corticosteroid or calcineurin inhibitor applied for 5 to 7 days before testing since local anti-inflammatory effects of these agents can diminish or obliterate a possible positive test result. Systemic antihistamines do not affect the interpretation of patch tests. Surprisingly, patients who have late HIV disease are still reactive to contact allergens.[1127]

Summary Statement 236. Reading and interpretation of patch tests should conform to principles developed by the International Contact Dermatitis Research Group and the North American Contact Dermatitis Research Group. (A)

Summary Statement 237. A 96-hour reading may be necessary because 30% of relevant allergens that are negative at the 48-hour reading become positive in 96 hours. (A)

The initial reading of patch test results should be performed 48 hours after their application. Tests may need to be read 30 minutes after removal of the patch to allow resolution of erythema due to occluding pressure or the tape and/or chamber if present. Ideally, there should be an additional reading 3 to 4 days after the initial application and occasionally after 7 days for certain contactants.[483,1128,1129] A collaborative study documented that approximately 30% of relevant allergens that were negative at the 48-hour reading become positive at a 96-hour reading.[484] Conversely, some irritant reactions at 48 hours tended to disappear by 96 hours. The reading itself is based on a nonlinear, descriptive scale that was developed and validated by the International Contact Dermatitis Research Group.[1130] The details of this rating system and corresponding clinical interpretation with a visual key may be found in the parameter, Contact Dermatitis: A Practice Parameter. In general, there is good concordance of positive patch test results between individual Finn Chamber tests and the T.R.U.E. TEST technology and between different commercial manufacturers.[503–505] The relevance of positive reactions to clinical ACD can only be established by carefully correlating the history, including exposure to the allergen with the test results.[504,1135]Laser Doppler perfusion imaging of cutaneous blood flow has been proposed as an alternative to visual reading.[1131] This technique correlates with visual scoring but is not useful in distinguishing between allergic and irritant reactions.[1132,1133]

Summary Statement 238. Nonstandardized and customized patch testing is often required, depending on the patient's exposure history. (C)

When an agent not included in the standard set is suspected, kits for specific occupations (eg, beauty operators, machinists) and exposures (eg, shoes, plants, photoallergens) permit identification of many other significant allergens. Not infrequently, it may be necessary to customize patch tests in accordance with a patient's specific exposure history. "Leave-on" cosmetics (eg, nail polish, lipstick, rouge, foundation), clothing, gloves, and foods may be applied "as is." "Wash-off" cosmetics (eg, shampoos, conditioners, cleansers) should be diluted (10^{-2} or 10^{-3}) before application.[1118,1131] Other household and industrial products should only be tested after ascertaining their safety in material safety data sheet background information and in accord with an authoritative text on patch test concentrations.[1106] Even after this research, nonirritant concentrations may need to be performed in nonexposed controls if more precise toxic information cannot be obtained.

If photosensitization is suspected, photo patch tests should be performed by a physician with expertise in UV radiation. Duplicate applications of the suspected photo contactant(s) are placed on each side of the upper back. One side is irradiated with 5 J cm^{-2} of UV-A 24 to 48 hours later, and both irradiated and unradiated sides are measured 48 hours after irradiation.[1134]

Summary Statement 239. A problem-oriented approach to diagnostic patch testing using evidence-based principles of likelihood ratios and posttest probability is more likely to confirm clinical ACD than a randomly selected patch test approach. (B)

Recently, the question of proper pretesting probability measurement has been raised with the purpose of discouraging random patch testing, which has a low pretest predictive probability.[499] It is postulated that pretest probabilities can be estimated by the data of large-scale prevalence studies of contact allergy in the general population. Using these data, likelihood ratios and postpatch test probability of contact allergy can be ascertained.[499]

Summary Statement 240. Several in vitro procedures are being investigated for the diagnosis of ACD. (A)

The potential for induction and elicitation of sensitization is augmented if the allergen also has the ability to induce irritant signals, presumably through the innate immune system.[1135] Irritant signals may induce the synthesis and release of proinflammatory cytokines, such as TNF-α, IL-1, IL-8, and granulocyte-macrophage colony-stimulating factor.[1135] Thus, there is a rationale for developing alternative in vitro diagnostic tests. The lymphocyte transformation test is mostly used for research purposes.[235] Recently, an enzyme-linked immunospot (ELISPOT) assay, specifically designed to detect contactant-induced cellular release of cytokines (interferon-γ, IL-2, IL-4) by the patient's peripheral mononuclear cells, was compared with patch tests and lymphocyte transformation test. Overall, there was a statistically signifi-

Table 15. Common Non-*Rhus* Plant Contactants

Family	Common names	Antigen
Ambrosia	Giant and dwarf ragweed	Sesquiterpene lactones
Compositae	Chrysanthemums and daisies	Sesquiterpene
Liliaceae	Tulips, hyacinth, asparagus, garlic	Tuliposide
Amaryllidaceae	Daffodil and narcissus	Unknown
Primrose	Primula (a household plant)	Primin
Umbelliferae	Carrots, celery and parsnips	Unknown
Cannabinaceae	Nettles (hops)	Unknown
Rutaceae	Oranges, lemons, grapefruits	Unknown

cant relationship ($P < .05$) among the 3 tests.[1135] Several recent research methods for classifying allergenic potency of contact allergens could possibly facilitate the clinical utility and reliability of patch tests in the future.[1136–1139]

Summary Statement 241. The differential diagnosis for CD is influenced by many factors, such as clinical appearance of the lesions, distribution of the dermatitis, and associated systemic manifestations. (B)

Clinically, CD is an eczematous disease. Eczema encompasses a group of pleomorphic, cutaneous disorders (with or without identifiable exogenous causes) presenting with an inflammatory tissue response. The diagnosis of CD is based on the clinical appearance and the presence of intercellular edema of the epidermis known as spongiosis with varying degrees of acanthosis and superficial, perivascular, lymphohistiocytic infiltrate.[1105,1119] Clinically, the lesions of CD range from red clustered papules to vesicles and bullae. Scaling and pruritus are prominent features. There are many dermatologic entities that may simulate the clinical appearance of CD at various stages of their evolution. A summary of these conditions appeared in "Contact Dermatitis: A Practice Parameter."

Summary Statement 242. Occupational contact dermatitis is an inflammatory cutaneous disease caused or aggravated by workplace exposure. (B)

According to the US Bureau of Labor Statistics, occupational skin diseases (chiefly ICD and ACD) rank second only to traumatic injuries as the most common type of occupational disease. In 1999, the incidence rate of occupational skin disorders was 49 cases per 100,000 (http://www.cdc.gov/niosh/ocdrm1.htm). The OCD rate tends to be highest in small manufacturing plants (<500 workers) because they lack comprehensive health care programs. Chemical irritants such as solvents and cutting fluids account for most ICD cases.[1140,1141] More than 40% of Worker's Compensation cases involve the skin, and it is estimated that OCD constitutes 90% to 95% of all occupational skin diseases and that ICD is found in 70% to 80% of all OCD.[1142,1143] Of 5,839 patients tested in a collaborative study of the North American Contact Dermatitis Group, 1,097 (19%) were deemed to be occupationally related.[1144] Sixty percent were allergic in nature and 32% were irritant related. Hands were primarily affected in 64% of ACD and 80% of ICD. Carba mix, thiuram mix, epoxy resin, formaldehyde, and nickel were the most common allergens.[1144]

Reducing this cost to industry and preventing morbidity in workers should be the goal of occupational medical experts.[1145] Unfortunately, distinction rarely is made between ICD and ACD, either retrospectively or in ongoing surveillance programs.

Summary Statement 243. There are 7 generally acceptable criteria for establishing causation and aggravation of OCD. (C)

The responsibility for determining that dermatitis was caused or aggravated by employment is incumbent on the examining physician. As a practical guideline for this evaluation, Mathias proposed 7 criteria for confirming this judgment.[1145] These include (1) the clinical appearance is consistent with CD; (2) potential cutaneous irritants or allergens are present in the workplace; (3) the anatomic distribution of dermatitis is consistent with skin exposure to chemicals in the course of various job tasks; (4) the temporal relationship between exposure and onset of symptoms is consistent with CD; (5) nonoccupational exposures are excluded as probable causes of the dermatitis; (6) dermatitis improves away from work exposure and reexposure causes exacerbation; and (7) there are positive and relevant patch tests performed according to established guidelines. Four of the 7 criteria must be positive to conclude that dermatitis is OCD. The validity of the Mathias criteria was recently confirmed in a 2- to 5-year prospective study.[1146,1147]

Summary Statement 244. Among health care professionals, ACD may occur as part of the spectrum of immunoreactivity to NRL in latex gloves. (A)

With the advent of AIDS and consequent universal barrier control required for health care professionals, the repetitive use of latex gloves eventuated in a progressive increase in the prevalence of both occupational and nonoccupational reactions, both immune-mediated and irritant.[1148–1152] Clinical responses were chiefly IgE-mediated, including contact urticaria, rhinitis, asthma, and/or anaphylaxis. In most cases, these clinical events could be confirmed by specific prick or specific IgE tests.[1153, 1154] However, a large multicenter, prospective study conducted by the British Contact Dermatitis Group revealed that 1% of patients with hand eczema had positive patch test results to NRL.[1155] Health care workers

Table 16. Classification of Preservative Agents in Cosmetics

Formaldehyde releasers	Nonformaldehyde systems
Diazolidinyl urea	Parabens
Imidazolindinyl urea	Methylchloroisothiazolinone/ methylisothiazolinone
Quaternium-15	Methyldibromoglutaronitrile/ phenoxyethanol
DMDM hydration	PCMX/PCMC
Bromonitropropane	Benzalkonium chloride
	Thimerosal

may develop ACD to other chemicals in rubber gloves, including bisphenol A in vinyl gloves.[1156,1157] In such instances, patch tests to various rubber mix chemicals or the suspected article itself are appropriate. Patients with proven ACD may experience flares of generalized or localized dermatitis after ingestion of foods cross-reactive with NRL (see Practice Parameters on Food Allergy and Anaphylaxis).

Summary Statement 245. Allergic contact dermatitis from exposure to plants is the result of specific cell-mediated hypersensitivity induced by previous contact with that family of plants. (A)

Toxicodendron dermatitis (poison ivy) is the most common form of ACD and can be readily identified by its streak-like or linear papulovesicular presentation. While the poison ivy group of plants (Anacardiaceae) causes most cases of plant dermatitis, other plants that are common sensitizers are listed in Table 15. The sensitizing substances in most plants are present mainly in the oleoresin fraction; in some plants, the allergens are water-soluble glucosides. Most plants must be crushed to release the antigenic chemicals.

Summary Statement 246. Contact dermatitis is commonly implicated after exposure to topical medications, including lanolin, PABA, caine derivatives, antihistamines, iodochlorhydroxyquin, NSAIDs, and corticosteroids. (A)

If an eruption worsens, rather than improves, after the application of lanolin, PABA (in sunscreens), caines (antiitch preparations), antibiotics, antihistamines, and/or corticosteroids, patch testing to the suspected topical agent should be considered.[1158–1163] Neomycin, bacitracin, and iodochlorhydroxyquin are well-known sensitizers. Preservative agents in cosmetics are often incriminated (Table 16). When a topical sensitizing agent is used systemically in sensitive individuals, CD can occur at the original site of sensitization.

Summary Statement 247. Allergic contact dermatitis due to topical corticosteroids may occur in up to 5% of patients with suspected CD. (A)

Corticosteroids are used extensively in all areas of medicine and are administered orally, parenterally, intralesionally, intra-articularly, intrathecally, by inhaled nasal/asthma dispensers, and topically to the skin.[486,1164] Certain groups of diseases put patients at increased risk of corticosteroid ACD. These include treatment of refractory eczema, leg ulcers, and stasis dermatitis.[486] The patient usually notes a failure to improve or experiences a flare-up of the underlying dermatitis being treated with the topical corticosteroid. Patch testing to corticosteroids is complicated by the therapeutic, antiinflammatory nature of the drug itself, which results in frequent false-negative results. Patch test readings should also be performed 7 days after application because of the immunosuppressant nature of the test reagent itself.[487]

The most commonly used screening agents in patch testing for topical corticosteroid allergy are budesonide and tixocortol tivalate 1% in petrolatum.[488] Because these allergens do not detect all cases of sensitivity, other screening agents have been suggested. Coopman et al have suggested that 4 major groups of corticosteroid preparations should suffice because there is considerable cross-reactivity within groups and possible cross-reactivity between them.[489] For budesonide testing, Rhinocort nasal formula can be sprayed onto a Finn Chamber and used as a patch test.[1165] Testing with the patient's own corticosteroid product may be required for definitive evaluation of possible corticosteroid allergy. Ferguson et al have reported that intracutaneous tests demonstrate allergic reactivity when corticosteroid patch test results are negative.[477] Sensitized patients must be instructed to avoid corticosteroid administration by nontopical (including inhalant and oral) routes, because such treatment may cause local and distant exacerbation of ACD.

Summary Statement 248. Simultaneous exposure to allergens and irritants may produce both additive and synergistic ACD responses due to their interaction. (A)

Up-regulation of TNF-α, IL-1, IL-8, and granulocyte-macrophage colony-stimulating factor by an irritant or the irritant domain of an allergen is important for initiation of ACD.[1166] Another possible interaction is that the irritant may facilitate penetration of the allergen. Conversely, patients with positive patch test results tend to have a lower irritant threshold and thus greater susceptibility to skin irritation.[1167] Several investigations have documented that exposure to irritants before or at the same time as allergen patch tests significantly decreased elicitation thresholds and concentration required for patch test reactivity.[1168,1169]

Summary Statement 249. The role of detergents in hand dermatitis is a reflection of their ability to disrupt the skin barrier. (A)

In a prospective, controlled study of consumers for evaluation of potential ACD to granular and liquid detergents, 0.7% had a positive patch test result.[1170] On further testing, these reactions either could not be replicated or were identical to control patch test sites. This apparent patch test positivity would suggest that this was due to an irritant rather than an allergic response. By contrast, other investigators have found evidence of ACD hand dermatitis. In a separate investigation of ACD in patients with hand dermatitis vs nonhand ACD, ACD was less common in hand dermatitis (47%) than nonhand dermatitis (63%).[1171] However, ACD was more common in vesicular and fissured forms than hyperkeratotic and pompholyx-like hand dermatitis. Taken together, these studies emphasize the important role of barrier injury as a prerequisite to ACD.

Summary Statement 250. Allergic contact dermatitis is a significant clinical problem in children. (A)

Although less frequent in the first years of life (ie, before the age of 10 years), the rate of occurrence beginning at this age and through teen years attains and even exceeds that observed in adults.[1172,1173] The order and prevalence of ACD to individual allergens are generally comparable to a general adult population with nickel, fragrances, and rubber chemicals being similar in occurrence in the 2 groups of patients.[1174] The influence of fashion trends, hobbies, and lifestyle activity, such as body piercing, decorative skin paintings (eg, black henna tattoo), natural remedies, and cosmetics (eg, tea tree oil)m or the use of products with fragrances and herbal ingredients are important determinants for ACD in this age group.[1174–1176]

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1. Practice parameters for the diagnosis and treatment of asthma. *J Allergy Clin Immunol.* 1995;96:S707–870.
2. Practice parameters for allergy diagnostic testing. *Ann Allergy.* 1995; 75:543–625.
3. Practice parameters for the diagnosis and management of immunodeficiency. *Ann Allergy.* 1996;76:282–294.
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These parameters are also available on the internet at: http://www.jcaai.org.

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