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Introduction
Considerable interest has developed in the medical community and the general public concerning the diagnosis and management of allergic disease. Some of this interest is based on the great strides that have been made in the understanding of immunologic disorders over the past century. The scientific basis of the allergic process, beginning with conjunctival tests for allergy early in the last century, and continuing through the recognition of IgE as the antibody most commonly associated with allergic disease, has enabled physicians to more accurately direct the diagnostic process.

At the same time misuse of the term "allergy" has led to a variety of unexplained phenomena being labeled as "allergic" disorders, sometimes to the patient's disadvantage. While allergists have increasingly understood the mechanism of immediate hypersensitivity allergic disease, the American Academy of Asthma Allergy & Immunology (AAAAI) has recognized the need to help the wider community understand the correct methods for the diagnosis of these disorders. This document is the result. It was prepared by a subcommittee of the AAAAI, at the request of the Board of Directors to address this need for practitioners and patients.

The diagnostic algorithm for human allergic disorders (immediate or type 1 hypersensitivity) begins with an appropriate clinical history and physical examination. Once the medical, family and environmental histories identify a temporal association between allergic symptoms and allergen exposures, allergic disease may be suspected or confirmed, depending on the strength and consistency of the findings. Most often, however, diagnosis of an allergic disorder requires confirmation by selected tests that are performed to verify the patient's production of specific IgE antibody.

In vivo tests, most commonly skin tests, are viewed by many as the most relevant indicator of IgE antibody since they involve direct observation of a biological response in

the patient. It remains the primary method used by allergists to detect IgE antibody, because of its sensitivity, specificity, speed, cost effectiveness, and ease of performance. Skin tests may be performed by several methods including prick or puncture, and intradermal techniques. For this discussion, tests that involve placing the allergen on the skin or a device and pricking through or puncturing the skin are referred to as percutaneous or prick/puncture tests. Tests that involve injecting allergen intradermally are called intracutaneous tests. Adverse reactions, while not unheard of, are rare, especially with percutaneous testing.

In clinical practice, *in vitro* methods for the detection of allergen-specific IgE in serum offer different advantages when compared to *in vivo* tests.   They may offer potentially better quantitation, the ability to detect IgE antibody in the presence of dermatographism, a lack of drug interference by antihistamines and related medications, and the potential for long term storage of serum specimens for longitudinal testing. They offer somewhat greater safety than skin testing, although as will be discussed, the risk of *in vivo* testing, especially with a percutaneous technique, is extremely small.

This paper examines methods for diagnosing IgE mediated disease, beginning with the history and extending through the various confirmatory tests that are currently available. It is intended as a useful guide for interested parties but is not intended to establish an unswerving standard. The authors recognize that medicine is still part art and part science. While some of the recommendations reflect what has been successful in the hands of the authors, there may be other, equally valid ways to approach a patient's symptoms.

MEDICAL HISTORY

History taking is one of the most important diagnostic tools in medicine. In some cases it can be the most definitive one, especially in the field of allergy. In this paper, a succinct general outline is presented; more details relevant to specific allergic disorders can be found in special practice parameter publications.[1-9]

Under some circumstances, particularly involving standardization for clinical investigation, a structured questionnaire may be useful. Several examples of such questionnaires are available in allergy textbooks.[10-12] Completion by a physician, or by a skilled allied health professional, may be more reliable than having it filled out by the patient or relatives. The interaction involved in personal history taking by the physician can also reveal personality qualities of the informant that can be very helpful in interpreting the history, planning the management and in predicting compliance and outcome.

Most importantly, the practitioner needs to start with the patient's complaint, rather than what they think they are "allergic" to. The latter can lead to misdiagnosis, with potential serious adverse consequences.[13] Regardless of the method that the practitioner finds appropriate, a consistent historical association, as well as exclusion of confounding factors, remains fundamental.

In some instances it can be difficult to keep the historian on track while trying to obtain information in a systematic manner. Patients may be so convinced they know what is causing a problem that they refuse to consider alternative diagnoses, such as bacterial sinusitis, non-allergic adverse reactions to food or gastroesophageal reflux disease (GERD). Patients usually have little difficulty when they are told that they have developed allergy to grass pollen or a medication, but they will often resist the association if their difficulty is blamed on a favorite pet.

It should be noted that this discussion is directed at the initial evaluation of a patient's allergic complaint. It is impractical to conduct a complete and detailed history at each visit and that is not routinely expected.

Chief Complaint: The patient's chief complaint is the starting point and should be in the form of symptoms rather than a specific diagnosis, particularly when it is a self-claimed one. If there are multiple unrelated symptoms, they should be listed and addressed in the order of severity or importance to the patient.

History of Present Illness: In obtaining the history of the present illness, it is often best to begin with more open ended questions, such as, "When was your first respiratory complaint?", rather than simply asking "When did your allergies begin?" Details should be gathered about each chief complaint, such as nasal congestion, wheezing, pruritis, rash or systemic symptoms such as anaphylaxis. Whenever possible, details should be obtained about the specific symptoms when first noted, as well as their overall allergic history. Truly allergic patients will often have a history going back many years. Patients need to be encouraged to report even mild or trivial symptoms that did not require medical attention or even the use of over the counter medication, as these may reflect longstanding atopy. Recollection of factors associated with symptom development such as age of onset, suspected cause, specific situations or circumstances, geographical location where symptoms occur, seasonal pattern and response to prior therapy may all be helpful.

Since most patients present with recurrent or chronic symptoms with periodic exacerbations, data should be gathered on the frequency of recurrence, average duration of symptoms or exacerbations, and relationship to specific activities, places, exposures, eating, infections, emotions, menstrual period, time of day, or season. Respiratory symptoms limited to or exacerbated during a particular time of the year strongly point to seasonal pollen allergy. Symptoms related to eating a particular food when it is commercially prepared but not when it is prepared at home might be caused by a food additive or by a hidden food allergen.

The use of air-conditioning, detergent, and fabric softener, and the presence of carpet, pets or other animals in the home or work environment may all be important, depending on the patient's history. Personal or passive tobacco exposure is also important to determine.

Inquiry should be made about sources of specific allergens or irritants at home, including articles that collect dust mites, tobacco smoke, fireplaces, wood-burning stoves, fragranced personal care products and strong cleaning agents. One must also include environmental related questions that encompass places that are frequently visited by the patient. If the symptoms worsen at work or in school, certain provoking or contributing factors may exist there. Adults should be asked about their occupations and exposures to allergens or irritants at work in a way that does not create in the patient the presumption they have a work related problem. Likewise, mold is ubiquitous in the environment. While it may be a factor in certain cases, physicians need to be careful to neither miss the diagnosis nor create a premature presumption that the patient's problem is caused by mold, latex or occupational exposures.

The importance of obtaining a thorough environmental history relevant to the symptoms cannot be overemphasized. It may reveal a culprit that may not be discovered by other tests, but it is also subject to observer bias. Patients will often more readily identify an odor at work than their cigarettes or dog as the cause of difficulty. Conversely, a self-employed individual may fail to consider evidence that adversely affects their livelihood.

An appropriately detailed review of previous or current evaluation and treatment as well as current management of the chief complaint should be obtained. Attention should be paid to the results and reliability of tests formerly completed, recognizing that the diagnostic value may vary depending on where and when they the tests were conducted.

The degree of impact of illness can be assessed by the number of lost days from work or school, social adjustments, limitation of activities, presence of nocturnal symptoms, and the frequency of unscheduled physician's visits, emergency department visits or hospitalizations. Providing teenagers with the opportunity to speak in the absence of parents may reveal valuable information, including emotional factors or the use of tobacco, alcohol and other recreational drugs.

Review of Systems: Although patients usually seek medical care for a particular complaint, they may have symptoms that can be related to other allergic or non-allergic conditions. Typically, the patient should be asked about symptoms related to the nose, eyes, ears, head, chest, skin, and gastrointestinal tract. The concomitant presence of atopic eczema in a child with lower respiratory tract symptoms should alert the physician to the possibility of asthma. Often complaints that the patient considers unrelated will provide clues to resolving their chief complaint.

Other organ-related diseases or medications might be the cause of the presenting symptoms. Examples include cough or angioedema in a patient taking ACE inhibitors, or nasal congestion secondary to the intake of estrogen or certain anti-hypertensive medications. Poorly controlled asthma may be attributed to the concomitant intake of beta-adrenergic blockers.

The history should always include the psychosocial setting; presence of perceived stress factors at work, school, and home; and any other non-medical concerns the patient feels

may be impacting his or her health. Emotional factors may alter both the manifestation and the perception of complaints.

Past Medical History: Previous non-allergic illnesses or surgical treatments may have relevance to the patient's current symptoms. For example, a child with a history of prematurity and prolonged oxygen therapy in the neonatal period may develop bronchopulmonary dysplasia that mimics asthma. Likewise, a problem feeding history during infancy might reveal the culprit to be a food allergy in a young child with chronic allergy symptoms. A physician treating adults with shortness of breath may need to consider a broad differential list of possible diagnoses, from coronary artery disease to a collagen disorder or cancer. However, the more consistent the patient's history and findings are for an allergic issue, the less that alternative explanations will need to be sought.

A complete list of medications needs to be obtained, including vitamins and herbal remedies. Prior drug allergies and intolerances need to be documented. Each practitioner will develop their own style of when and how to get various pieces of information. Depending on the patient's complaints, how they are as an historian, and how they answer initial questions will determine the best course to take. It is impossible to formulate in advance or in a paper such as this all the questions and issues that should be pursued. Likewise, additional questions may be suggested by the results of the physical examination or diagnostic studies.

Family History: It is established that the allergy trait in general, not necessarily the specific manifestation or sensitivity to a specific allergen, is inherited. However, a familial incidence of asthma is common. An allergy diagnosis would be supported by the presence of allergy in the family, particularly in parents or siblings. Such a history, however, may be inaccurate and the absence of overt allergies in the family should not exclude a possible allergy in the patient. Conversely, allergies are common. The presence of allergies in a close relation does not establish such a diagnosis. Depending on the presentation and complaints, inquiry may be made about problems such as immunodeficiency disorders and cystic fibrosis, heart disease, or connective tissue disorders.

PHYSICAL EXAMINATION
If the patient is asymptomatic, the physical examination may reveal no or minimal findings, yet the medical history can be compatible with allergy. If the history is vague and the physical findings are not convincing, the patient may be advised to return when symptomatic. Examination will need to be tailored to the patient's complaint, as well as their age and other factors. Examination items important in a sixty-five year old with shortness of breath thought possibly related to asthma will differ from those in a one year old with a rash.

Examination begins with vital signs, including height and weight. In children, this can be related to expected growth rates. In adults, weight gain or loss may impact on their

illness and provide important diagnostic clues. Important negatives may need to be noted.

The degree of documentation will depend on the circumstances and is increasingly dictated by third parties due to reimbursement issues. Patient evaluation is a diagnostic process where historical information will often determine clues that are sought during the examination. No patient should have "every test". Tests are chosen based on the patient's complaint, history and physical findings. Likewise, the details of the examination may be altered by the history and differential diagnosis under consideration at a given point in the evaluation process.

Some findings that may be important include the following:
 Eyes: excessive lacrimation, erythema of the bulbar conjunctiva, cobblestoning of the tarsal conjunctiva, dermatitis of the eyelids.
Nose: transverse crease, turbinate edema and pallor or bluish discoloration, discharge, polyps, nasal septal deviation or perforation.
Sinuses: tenderness, purulent drainage from the sinus ostia.
Oropharynx: mouth breathing, dental malocclusion or overbite or postnasal drip, cobblestoning of the oropharyngeal wall, halitosis, hypertrophied tonsils or adenoids.
Ears: tympanic membrane dullness, redness, retraction, perforation or lack of mobility.
Neck: neck vein distention, adenopathy or tenderness
Chest: deformity, altered percussion, egophony, audible wheezing, abnormal sounds by auscultation, chest wall tenderness.
Heart: gallops, rubs or murmurs
Abdomen: Tenderness, distention or mass
Extremities: tenderness, erythema, signs of a connective tissue disorder
Neurologic: weakness, impaired cognition or thought process, including difficulty in recall or understanding their disease
Skin: rashes (description and distribution), dermatographism, infection.

Other body systems should be included in a comprehensive physical examination and abnormal findings recorded.

Evaluation may include measurement of the peak expiratory flow rate, spirometry, tympanometry, and flexible rhinolaryngoscopic examination by those experienced in the procedure, as well as various radiographic studies, manometry and other tests. A full listing and description of studies and procedures is beyond the scope of this paper.

IN VIVO TESTING
Skin testing represents the primary diagnostic tool in allergy that is used to confirm that a specific allergen, suggested by medical history, has induced an IgE antibody response. Percutaneous and intradermal skin tests to determine IgE mediated immediate hypersensitivity are the most clinically applicable techniques in the assessment of allergic patients because of their simplicity, biological relevance in the patient's own skin, rapidity of performance, low cost and high sensitivity. A positive IgE mediated skin test manifests as a wheal arid flare reaction. However, skin tests as with other physiologic

measures, require a degree of expertise by the observer to both interpret the results and correlate with the history and physical findings.

As with other diagnostic studies, improperly performed or interpreted skin test results can lead to false positive or negative results. Even what appears to be a *bona fide* positive reaction does not necessarily mean that the symptoms are due to an IgE mediated allergy. Patients who are symptom free may have positive allergen specific IgE skin tests. This should not be considered a fundamental flaw of skin testing. While a positive skin test reflects the presence of IgE antibody, or "allergy", the development of an allergic disease represents the interaction between the allergic state and what has been recognized as an increasingly complex physiologic process. Proper interpretation of skin test results requires the clinician's careful correlation with the history and examination findings.

Settipane and Hagy reported on their follow-up of 903 college freshman whom they skin tested and interviewed when they started college.[14] As expected, some of the students had positive skin tests and some negative. Not all skin test positive patients had an allergic disease (allergic rhinitis or asthma), but the risk of developing an allergic disease was significantly greater in the skin test positive group over the next four years. As time went on, the predictive value waned between the skin test negative and positive groups, presumably representing the development of new allergy in those not previously found to be atopic. Others have reported similar results.[15]

A positive skin test may be helpful in confirming the history, while a negative percutaneous skin test, particularly when confirmed by a second technique such as intradermal testing or allergen-specific IgE test is strong suggestive evidence that the disease is not caused by the suspected allergen. This will vary somewhat between allergens, with some more stable and well characterized, such as pollen, mite or cat dander, than others, such as foods. Food testing is complicated by the fragile nature of many food antigens and the fact that patients may be reacting to digestion products or by other mechanisms not evaluated for by percutaneous prick tests. In a study comparing commercial food skin test extracts to fresh foods, followed by oral challenge, the overall concordance between a positive prick test with commercial extracts and oral challenge was 58.8%, while the concordance was 91.7% when fresh foods were used.[16]

Likewise, cutaneous responsiveness will vary between patients, so some patients will have a more clearly positive result than others. The practitioner should be aware of the stability and concentration of the allergy extracts used. While arbitrary measurement values to determine positive and negative are useful in research, they may not be optimal in the clinical setting, where strength of history and the patient's own cutaneous responsiveness to positive and negative controls, as well as to other antigens, may play a role in correct interpretation. Distinction between "allergic sensitization" and an "allergic disease" requires thoughtful interaction between the patient and a physician trained in evaluating allergic illness.

Skin testing should be performed with a physician available to treat adverse reactions, including anaphylaxis. Emergency equipment including epinephrine 1:1000 and

diphenhydramine should be available.   Positive and negative control reagents, such as histamine and saline, should be used. Skin testing should generally only be performed on normal skin. Antihistamines, tricyclic antidepressants,[17] and some tranquilizers suppress the wheal and flare response and should be discontinued prior to allergy testing. While discontinuation for 3-7 days will usually suffice, select agents in some patients may cause suppression for up to 10 days[18,19] or even more.[20]

Skin testing or the use of allergen immunotherapy in patients taking beta blocking agents has been considered potentially problematic, either because of risk of an exaggerated adverse reaction or a blunted response to treatment such as epinephrine.[21,22] Because reactions to immunotherapy are rare, and to skin testing even more uncommon, prospective randomized studies are not feasible, with a high enough reaction rate to detect a significant difference. Still, several authors have looked at this issue. A recent review of published evidence suggested that there was, in fact, no significant documented increased risk with concomitant beta-blocker therapy and allergy based diagnosis and treatment.[23] While physicians must always weigh risks against benefits, it would appear that prior concerns were overstated and that allergy directed treatment should not routinely be denied to patients because of the inability to discontinue beta-blocker therapy

The use of angiotensin-converting enzyme (ACE) inhibitors[24] and monoamine oxidase inhibitors, have also been questioned, based on similar concerns that these medications may increase the risk of systemic reactions or the response to treatment of a reaction. Anaphylaxis with patients taking ACE inhibitors has been shown to occur in those on dialysis,[25] but no increased risks have been associated with concomitant ACE inhibitor use in those receiving allergy directed diagnosis or treatment. Likewise, reviews of the relationship between MAO inhibitors and epinephrine suggests those concerns are also not clinically relevant.[26, 27]

Generally, drugs that inhibit coagulation do not interfere with skin testing. There are no age limitations for performance of skin tests, although patients need to be able to cooperate with testing. Special consideration should be given to pregnant patients with respect to whether the results will have substantial immediate and therapeutic implications.[28] While skin testing itself is unlikely to be harmful, an adverse reaction could adversely affect the developing fetus. Recording of the skin test reaction should be done at the appropriate time, usually 15 to 20 minutes after the allergen is applied. Repeat allergy skin testing should be done when changing symptoms or new exposures suggest the result may alter treatment. Some recommend repeat skin testing after three to five years of venom immunotherapy to evaluate the need for continued immunotherapy.[7]

TYPES OF SKIN TESTS

Percutaneous Tests: Prick or puncture tests, done by various techniques, are widely used because they are considered to be the most convenient and least expensive. When

they can be performed, they are usually the best screening method for detecting the presence of specific IgE antibodies in patients with appropriate exposure histories.

Percutaneous tests are performed by placing a known allergen on the skin or on a needle or alternative application device and either pricking through the allergen or applying it by puncturing the skin with the allergen coated device.   There are various devices available to perform these prick/puncture skin tests.   Practitioners should be familiar with the method selected.   Differences between the results obtained with various techniques have been examined.[29] Skin testing by percutaneous methods has been shown to be highly reproducible when carried out by trained individuals,[30] although that may not fully account for differences in antigens and patient characteristics.   Percutaneous allergy skin tests are dependent upon the skill of the individual tester, the device, the potency and stability of the allergen extracts, the depth of the needle puncture and force, and the duration and angle of the application device. This can result in variability in results of tests done by different technicians.[31] These tests are usually performed on the upper back or volar surface of the arm.[32] As noted results should be read at the peak of the reaction which usually is 15 to 20 minutes after application,[31] although delay may be appropriate if fading dermatographism suggests results will be easier to read after a few more minutes.   Both erythema and wheal diameter should be measured and recorded using a millimeter ruler.

A skin test wheal response of at least 3 mm greater than a diluent control has been suggested as proof of the presence of allergen specific IgE antibody.[33] However, as with other medical tests, the more abnormal, in this case larger, the test reaction the more likely it is to be clinically significant. Test results need to be interpreted in the context of the positive and negative controls, as well as the other antigens applied.

Percutaneous skin tests are generally thought to be more specific but less sensitive than intracutaneous or intradermal tests. While this is usually desirable, low potency or concentration extracts can produce false negative results.   In the absence of dermatographism, false positive irritant reactions are less likely with percutaneous than with intracutaneous tests. However, when dermatographism is present, percutaneous tests can provoke nonspecific erythema. In African American patients and others of dark skin, percutaneous tests can be more difficult to interpret. Percutaneous tests appear to be safe and can easily be completed on infants when necessary. While systemic reactions have been described; no fatalities have been reported.

Intracutaneous Tests: Intracutaneous tests are generally used when percutaneous tests are negative despite an adequate history of exposure and symptoms. They permit identification of a large number of clinically reactive patients, especially those with lower skin sensitivity,[34] and they provide a means to confirm a negative diagnosis for potentially important allergens. Unlike laboratory studies, replicate testing to ensure that each allergen is applied correctly and has appropriate determinates for a given patient on a given day is not practical. Intradermal testing, providing greater sensitivity than percutaneous testing can serve in effect, as an alternative way to confirm a negative diagnosis, or to find clinically relevant allergy missed by percutaneous testing.   In

addition, sensitivity to low potency allergenic extracts may best be evaluated by this method.

Intradermal testing has been criticized,[35,36] with one recent editorial going as far as to state, "Intradermal tests continue to be performed despite evidence that they add little to the diagnostic accuracy of skin tests."[37] Such conclusions are usually based on a small number of prior studies that used an arbitrary standard of a positive intradermal test as having a 5 or 6 mm wheal and "definite erythema."[38-40] They do not reflect contemporary clinical practice. A survey of practicing allergists in the United States showed that 85.2% performed intradermal skin tests in their practices, with nearly 60 percent using a 1:1000 dilution of extracts.[41]

This discordance concerning intracutaneous skin tests between critics and the vast number of practicing allergists who perform them likely reflects a difference in how the tests are interpreted and used. In reporting research results of skin test data from large numbers of patients, it is useful to have an absolute standard. Such a standard, however, cannot systematically account for individual differences in responsiveness, sensitivity, or even the ability to accurately measure small differences, with a change of as little as 1 mm in wheal diameter potentially changing a result between positive and negative. While measuring the response to acute allergen challenge is of demonstrated value, it does not directly measure the delayed effects of acute or chronic exposure on airway hyperreactivity.[42,43] A sustained adverse effect of chronic exposure on nasal responsiveness in allergic rhinitis has also been described.[44] As will be discussed subsequently, accuracy has been enhanced by quantitation of serologic specific IgE measurements, rather than converting results to either positive or negative at an arbitrary value.

As a physiologic parameter, skin test response to a percutaneous or intradermal antigen might be looked at as similar to spirometry. Pulmonary function testing's utility in evaluating obstructive lung disease is well accepted, despite the fact that in a given patient the use of an FEV1 of 80 percent to distinguish airway obstruction from no airway obstruction will not be uniformly reliable, especially when employed without taking into account that patient's history, treatment, and other aspects of their clinical context. For this reason, the FEV1 value itself is usually reported, allowing the clinician to interpret the result in the context of other findings.

Cytology evaluation, similar to skin testing because it is typically interpreted as positive or negative, also can have significant uncertainty. At a large university teaching hospital 6,117 non-gynecologic specimens were examined in 2005 (Marluce Bibbo, personal communication). While 55.13% were read as "negative" and 13.85 percent as "positive", 22.23% were "atypical", 5.05% "suspicious", and 3.74% "unsatisfactory", nearly a third of the specimens.

As with pulmonary function testing, cytology, and a host of other common ancillary studies, skilled clinicians need to properly select and evaluate intradermal allergy skin

tests and the results based on the patient's history, the strength of the reaction, and other skin test results including controls.

As a general rule, most allergists use a starting dose of intracutaneous extract solutions in patients with preceding negative prick skin tests of 1:500 to 1:1000. Intracutaneous tests elicit a low rate of systemic reactions,[45] and fatalities have only very rarely been reported during intracutaneous skin testing.[46] As with percutaneous testing erythema and wheal diameter should be measured with a millimeter ruler and recorded for allergens as well as positive and negative controls. It should be noted that the histamine intradermal skin test response peaks at 10 minutes while allergens peak at 15 to 20 minutes. Reproducibility of intracutaneous testing is affected by the same variables as those described above for percutaneous tests. Any reaction larger than a negative control may indicate the presence of specific IgE antibody. However, given the lower specificity of intracutaneous tests, small positive reactions may not be clinically relevant.[39]

Some patients will report a late phase response to intracutaneous testing with specific antigens at 24 hours or more after the test was applied. The significance of this reaction for clinical symptoms is unclear but should be reported and may also warrant investigation.

Number of tests: The number of skin tests varies according to the age of the patient and the allergens tested, whether there may be inhalant or food allergens. Generally, fewer prick skin tests need to be performed in infants and very young children because these age groups are less likely to be sensitized to as many allergens as older children and adults.[47] The evaluation of inhalant allergy may require up to 70 prick/puncture tests that may be followed by up to 40 intracutaneous tests which are ordinarily performed when percutaneous tests are negative.[48] For suspected food allergy the number of tests may vary from less than 20 to as many as 80 depending upon the clinical situation.

Factors Affecting All Skin Tests: Skin test reactions can vary with age, with infants and older adults having less reactivity. As noted, reactions may be more difficult to measure in patients with pigmented skin. Seasonal variations have been demonstrated in pollen allergy with increasing skin sensitivity after pollen seasons. Skin tests should not be performed in areas where any skin lesions are present that may interfere with the skin test reactivity. Some patients with chronic disease such as renal failure or patients with cancer, diabetes and spinal cord injuries may have a decrease in skin sensitivity. Short-term administration of corticosteroids does not modify the cutaneous sensitivity to allergy skin tests; however long-term corticosteroid therapy may modify the skin test sensitivity.[49]

Allergens: Accurate allergy diagnosis depends on the correct choice of allergens for testing. Selection of the number and type of allergens appropriate for testing depends upon their relevance to the patient's history, including significant exposure, objective proof of the allergenicity of the substances in question and the availability of suitable allergenic material for testing. Aeroallergens (including pollen, mite, fungi, animal dander), insect venoms, food protein, antibiotics, and occasionally occupational allergens

are well established as causes of IgE mediated disease. Allergens for drug allergy testing are less well characterized and in many cases are not available. This is particularly problematic when dealing with a risk of anaphylaxis, where a false negative diagnosis has greater significance.

Organ Challenge Test: There are occasions when results of primary *in vivo* and *in vitro* diagnostic tests (skin tests and IgE antibody serology) do not agree with each other or are not consistent with the history or other findings. In cases where there is a strongly suggestive clinical history and conflicting IgE confirmatory tests, *in vivo* provocation tests performed by a trained allergist may be appropriate to clarify the sensitivity status of the patient.

Patients practicing avoidance who have a positive skin test and a negative IgE antibody serology may be transitioning through the state where circulating IgE antibody has decreased below the serological assay's detection limits, but sufficient IgE still remains attached to effector cell receptors to generate a positive skin test reaction. The contrary has also been reported with Hymenoptera venom patients, in which a negative skin test has been observed in individuals who have a positive insect sting challenge and a positive IgE antibody serology.[50]

Organ challenge test material may be applied directly or by inhalation or ingestion to the mucosa of the conjunctivae, nares, bronchi or gastrointestinal tract. Considerable experience with these methods is required for proper testing, interpretation and analysis. All organ challenges should be preceded by a control test with a diluent. If possible, the procedure should be performed on a double blind or at least a single blind basis. Appropriate precautions should be taken in that fatalities have been reported.

A commonly used procedure is titrated food challenge testing, preferably in a double-blind or a single-blind manner.[51] Open challenges can be reliable in infants where symptoms are usually objective,[52] as well as in some adults, especially when they are negative or when there are objective signs of a reaction. The test should usually be avoided in patients who have had a well documented life-threatening reaction to a specific food, especially if sensitization can be confirmed by an *in vivo* or *in vitro* test for specific IgE. Obviously, the more difficult it is to avoid a food (e.g. milk or wheat as compared to shrimp or peanut), the more important it is to clarify the diagnosis.

In addition to experience in treating adverse reactions, appropriate resources should be available for treating symptoms that may occur. Inhalation and drug challenge tests have also been performed by a variety of methods, and may be useful, particularly in cases where occupational or other unavoidable environmental factors are thought to play a role, or the medication is essential, but the diagnosis remains unconfirmed.[53] Pulmonary and nasal function testing, as well as laryngoscopy, may be helpful in documenting objective evidence of the presence or absence of a response.   The decision to perform such testing requires careful evaluation of the history and other findings that is beyond the scope of this paper.

IN VITRO TESTING

Table 1 summarizes the principal analytes that may be measured in the clinical immunology laboratory. Some may be useful in diagnosis, others in the management of allergic diseases, and still others are limited in utility and are primarily used for research. The tests that are listed as having value in diagnosis and management do not have equal acceptance or importance in contemporary clinical practice.

Allergen-specific IgE antibody is the most important serological marker used in the diagnosis of allergic disease to confirm sensitization in an individual who has a positive history of exposure. Historically, the radioallergosorbent test (RAST) was used to detect allergen-specific IgE antibody in serum, however, radioactive isotopes are rarely used presently in clinical testing. Table 2 presents the advantages and limitations of the detection of IgE antibody in serum. IgE antibody may also be found bound to the surface of FcR1 bearing cells (basophils). While the basophil mediator release test involves the addition of allergen to whole blood or leukocyte preparations and the subsequent measurement of released mediator (histamine, LTC4),[54] technical problems with the storage and transportation of viable basophils limits its usefulness. The measurement of IgE antibody on basophils is considered a secondary *in vitro* IgE antibody measurement in clinical practice and it will not be discussed further. The report will focus on the diagnosis of the allergic patient using allergen-specific IgE antibody measurements in the serum. Other than IgE measurements, basophil mediator release and the analytes presented in Table 1 are only occasionally used in the diagnosis or management of allergic patients. They are not discussed further here. More information on them can be found in reviews cited above and elsewhere.[55,56]

Allergen-Specific IgE Antibody: Modern day diagnostic allergy serology began in 1968 with the determination that reaginic or skin sensitizing activity in human serum was associated with a new class of human immunoglobulin called IgE.[57] IgE was shown to have a molecular weight of 180,000 and to have a unique immunoglobulin epsilon heavy chain with antigenic light chain determinants in common with human IgG, IgA, IgM and IgD. The radioallergosorbent test or RAST was the first serological assay that was developed to detect allergen-specific IgE.[58] It used allergen bound to a cellulose solid phase to bind specific antibody from human serum. In a second incubation, radiolabeled anti-human IgE detected IgE antibody bound to the insolubilized antigen. The assay was calibrated with a reference serum such that the magnitude of the measured response (counts per minute or CPM bound) was proportional to the amount of IgE antibody in the original serum. IgE antibody results were initially reported as positive/negative or in arbitrarily defined antibody units or classes.

Over the years, IgE antibody assay technology has improved with new high binding capacity solid phase matrices, non-isotopic labels for detection antibodies and standards calibrated to the World Health Organization IgE reference preparation. These enhancements have led to an evolution in assay methods from the first generation qualitative assays (RAST, MAST, EAST), through the second generation semi-quantitative IgE assays (AutoCAP, Alastat, HYTech, Matrix, MagicLite), to the present state-of-the-art quantitative "third generation" autoanalyzers.[59] The two widely used third

generation immunoassays are the ImmunoCAP System (Phadia [formally Pharmacia], Uppsula, Sweden) and the Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA)]. Their chemistry is similar to the original RAST, but they employ non-isotopic labels and have more rapid throughput with improved precision, accuracy and analytical sensitivity. Their automated chemistries report out allergen-specific IgE antibody in quantitative kIU/L, traceable to the WHO IgE 75/502 total serum IgE reference preparation.[60] In the case of at least one assay (ImmunoCAP), 1 kIU/L of allergen-specific IgE antibody has been shown to be equivalent to 2.44 micrograms per liter of total serum IgE.

Effort has been made to enhance the utility of serologic tests, which are slower than skin tests in providing results in clinical practice, and are generally more expensive to perform on a specific allergen basis. The multi-allergen screen is a minor modification of the allergen-specific IgE assay that has been developed to measure IgE antibody to multiple allergen specificities in one analysis. Allergens from different groups (e.g., dust mites, pet epidermals, grass pollens, tree pollens, weed pollens, mold spores) or multiple specificities from the same allergen group (e.g., molds; *Penicillium, Cladosporium, Alternaria, Aspergillus*) are all attached to a single solid phase. The multi-allergosorbent binds IgE antibody to the allergen specificities represented on the solid phase. It is able to detect the presence of all specificities of IgE antibody in a single blood specimen analysis, subject to the sensitivity and specificity limitations of serologic testing.

The original multi-allergen screen was designed to detect the presence of IgE antibody to any of the major approximately 15 aeroallergens that drive the majority of adult aeroallergen-related disease.[61] This single test from several commercial sources displays a high negative predictive value, which may allow it to be used to rule out the presence of sensitization (atopy) in an individual whose clinical history does not suggest IgE-mediated allergic disease. More recently, the multi-allergen screen strategy has been modified to look at defined panels of aeroallergens and food allergens relevant to different aged groups, such as infants at 2 years of age.[62] If positive, a further clinical history and more extensive IgE antibody testing to individual allergens is required to identify the actual allergen specificities to which the individual is sensitized. While the multi-allergen screen is possibly the most cost effective allergy screening test, it produces only qualitative (positive or negative) results, and its exact role in patient evaluation has yet to be established.

More recently, third generation autoanalyzers have allowed the accurate, reproducible and quantitative measurement of the level of IgE antibody of a defined allergen specificity in a given patient's serum. This has permitted investigators to evaluate the relationship between a given level of allergen-specific IgE antibody in serum and the probability of a clinically-relevant allergic reaction in the patient following allergen exposure. The first application of probability disease prediction has been in the area of food allergy. Children with defined levels of specific IgE antibody in their serum to peanut, egg white, cow's milk and fish will have a defined probability of clinical sensitivity as assessed in a double-blinded placebo controlled food challenge.[63,64] Probability distributions for a positive food challenge as a function of food-specific IgE

antibody in serum have been determined using IgE antibody levels measured with the ImmunoCAP assay. Using published probability curves, IgE thresholds have been defined for provocative testing below which there is >95% probability that the challenge will be negative. The upper threshold limits define IgE levels above which a positive food challenge test is >95% likely. This predictive analysis may minimize the need for cumbersome, expensive and sometimes uncomfortable double-blind placebo-controlled food challenges in children. Since the published probability-based risk curves have been defined using data from the ImmunoCAP, food specific IgE levels measured by the Immulite 2000 cannot be interpreted from the same published prediction curves without further clinical validation studies, since the two assays can detect different populations of food-specific IgE antibodies.

Probability-based risk assessment has also been applied to respiratory allergy using quantitative allergen-specific IgE antibody data previously reported from four European laboratories.[65] Logistic regression was used to compare the relationship between the doctor's final diagnosis of allergic respiratory disease (positive or negative) based on a clinical history, physical examination and skin testing versus the quantitative level of serum IgE antibody alone. In this study, the shape of the IgE antibody level versus probability of clinical respiratory disease curves differed depending on the allergen specificity and the doctor's individual interpretation of the patient's clinical history of respiratory allergy. Use of specific IgE antibody levels to support the clinical diagnosis of allergic disease differed for the same allergist depending on the particular inhalant allergen and between allergists for the same allergen specificity. Thus, there appeared to be wide variance in the actual disease vs. serum IgE antibody level curves with the allergen specificity and doctor's interpretation of clinical disease. Despite this variance, evidence suggests that where appropriate, quantification of serum IgE antibody may improve the confidence of the clinical diagnosis of inhalant allergies.

In interpreting the significance of the level of specific IgE antibody, the total IgE level should be taken into consideration. Highly elevated total IgE levels can be associated with numerous "positive" specific IgE that may not be clinically relevant. On the other hand, a low specific IgE level may have clinical relevance when total IgE level is low.

Quality Assurance of Allergen-Specific IgE Measurements: Allergen-specific IgE antibody measurements obtained from different diagnostic allergy laboratories may not be uniformly equivalent because they use different assays, methods of reporting and quality control procedures. This has significant impact when applying the results of studies involving small numbers of patients and a single laboratory to clinical practice. While clinicians can control the skin testing performed under their supervision, in many cases the choice of laboratory used for specific IgE testing will be determined by the patient's insurance carrier.

The clinician ordering an IgE antibody measurement should ensure that the laboratory to which they are sending their patient's serum is federally-licensed for highly complex immunology clinical testing under the Clinical Laboratory Improvement Act of 1988 (CLIA-88).[66] This is unlikely to be a problem with the large commercial laboratories that

are commonly used, or with specialty laboratories operated by major teaching centers. CLIA-88 certification insures that the laboratory personnel have the appropriate qualifications, that FDA-cleared assay methods are appropriately used, proper internal quality control procedures are in place and that the laboratory is successfully participating in relevant and valid tri-annual external proficiency testing surveys. The ideal would be to use a CLIA-88 certified laboratory that uses a third generation IgE antibody assay to report results in quantitative kIU/L.

Quantitative IgE antibody measured in the two most widely used third generation assays (ImmunoCAP and Immulite 2000) are not always directly equivalent because the allergens used in their allergen-containing reagents are not identical. They vary in the exact composition and quantity of individual allergenic components for any given allergen specificity. For example, the assays can differ in the relative amount of group 1 and group 2 dust mite (*Dermatophagoides pteronyssinus*) allergens. Within a given assay, however, there is excellent inter-laboratory agreement[67] based on data from the College of American Pathologists (CAP) Diagnostic Allergy (SE) Proficiency survey.

Ideally, those responsible for selecting laboratories for clinical studies will review a copy of the performance records of the laboratory's external proficiency survey each year. One widely subscribed external proficiency survey is the CAP SE survey that sends 5 or 6 sera every 17 weeks to over 150 clinical laboratories performing allergen-specific IgE antibody testing. Each serum is tested for IgE antibody to 5 allergen specificities, a multi-allergen screen and a total serum IgE. The data are reported back to the College within 10 working days. The SE survey participant summary is public domain information.

Occasionally, an FDA-cleared method such as the ImmunoCAP will have an infrequently requested allergosorbent specificity (e.g., human semen or blueberry) that has not passed FDA clearance due to the absence of the required number of sera from individuals confirmed clinically to be allergic to that specificity. Even though the assay itself (e.g., ImmunoCAP) is FDA-cleared, individual allergosorbents may not be FDA-cleared and these are provided by the manufacturer to laboratories as Analyte Specific Reagents or ASRs. A qualifying statement should be at the bottom of the report to indicate which allergosorbents are ASRs (not FDA cleared). ASR allergosorbents are quality controlled by the manufacturer with limited positive IgE antibody containing sera before release, but they should be treated as investigational measurements until such time as FDA clearance is obtained.

Table 1

Analytes Measured in the Diagnostic Allergy Laboratory

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1. Diagnosis

    Allergen-Specific IgE
        Individual allergen specificities
        Multi-allergen-specific IgE screen (adult and pediatric forms)
    Total serum IgE
    Precipitating antibodies specific for proteins in organic dusts
    Tryptase (α, β) (Mast cell protease used as a marker for mast cell mediated anaphylaxis)
    Complete blood cell count
    Sputum examination for eosinophils and neutrophils

2. Management

    Allergen-specific IgG [Hymenoptera]
    Free IgE (Monitoring patients receiving Xolair for non-Omalizumab bound [free] IgE)
    Indoor Environmental Aeroallergen quantitation in surface dust
        Der p 1/Der f 1 (Dust mite, Dermaphagoides)
        Fel d 1 (Cat, Felis domesticus)
        Can f 1 (Dog, Canis familaris)
        Bla g 1/Bla g 2 (Cockroach: Blattela germanica)
        Mus m 1 (Mouse: Mus musculus)
        Rat n 1 (Rat: Ratus norvegius)
    Cotinine (Metabolite of nicotine used as marker of smoke exposure)

Table 2

In vitro Clinical Diagnostic Tests for the Evaluation of IgE Mediated Disorders

Specimen Source	Analyte	Advantages	Limitations
Serum	IgE antibody assays for individual allergen specificities	1. Third generation immunoassays provide quantitative IgE antibody results 2. Automation: increased precision and shorter turnaround times 3. Miniaturization chip technology reduces serum requirement 4. Ability to repeat IgE analyses with stored serum for longitudinal assessment 5. Adaptable for use with purified native and recombinant allergens	1. Delayed results in comparison to skin testing (1+ day turn around) 2. Insufficient analytical sensitivity for some allergies (venoms, drugs and latex) 3. Potential antigenic competition and isotype (IgG) inhibition
Serum	Multi-allergen specific IgE antibody "screening" assays	single test for qualitative detection of IgE to principal aeroallergen and food specificities	1. Designed best to confirm non-atopic status 2. Allergen-specificities on multi-allergen screen not defined and different among various manufacturers 3. Different reagents required for children and adults and for individuals in different countries

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