The masks of allergy undone by IVT

By Connie Mardis, MEd; Tricia Bal, MD; and Roma Levy, MS

After traveling for more than a year and meeting with various lab directors, physicians, and outreach coordinators, we learned that exposure to allergens for individuals with asthma can cause an attack, and repeated exposure can lead to Eustachian-tube dysfunction, infections, and dermatological manifestations such as chronic rash. People often mistake allergies for a common cold because of the similarity of symptoms; however, when these symptoms persist, the chances are good that allergies are present and there is a need for testing. Early diagnosis and treatment options can improve an allergy sufferer’s quality of life. Allergies also affect families, and many people suffer from allergy because of genetic predisposition and underlying hereditary factors. This article examines the many masks of allergy and the testing solutions used to help resolve allergic suffering.

What is allergy?

Allergy is a hyperactive IgE immune-mediated response to a substance (allergen), which is not typically thought to be dangerous but is seen by the body’s immune system as an invader. Common allergens include pollens, animal dander, foods, molds, dust, insects, insect stings, metals (especially nickel), and drugs.

In the allergic cascade, when a B cell recognizes an allergen, it produces IgE antibodies. These antibodies circulate via the bloodstream; some of them attach to the surfaces of mast cells and basophils present in the nasal passages, lungs, skin, and digestive tract. When an allergen is encountered again, it binds to the IgE antibodies on a cell’s surface, causing the cell to release multiple inflammatory mediators including histamine. These

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inflammatory mediators produce allergy symptoms, which vary from mild to severe and life threatening (see sidebar on allergy symptoms, page 14).

How big is the problem?

Allergic diseases affect over 50 million Americans and costs the healthcare system over $18 billion annually.¹ Many patients do not have the opportunity for specialist care. In the United States, for example, one in seven is unlikely to receive specialist care because of the expense.²

The burden of allergy extends beyond the financial cost. According to the American Academy of Allergy, Asthma, and Immunology (AAAAI), allergies are responsible for 3.8 million lost work and school days each year. Allergy can lower the quality of life, restrict participation in outdoor sports, decrease on-the-job productivity, and unfavorably influence school performance. The time lost from school may negatively affect grades, academic achievement, self-esteem, and future life successes.³ Given the symptoms of allergy, it is not surprising schoolwork suffers. “Allergic kids may not sleep well, often sleeping with their mouths open,” says Terrence E. Zipfel, MD, East Liver- pool ENT and Allergy in Ohio. “It can be hard for them to stay attentive and focused on school when they are tired and itching, and have congested nasal and aural passages, and watery eyes.”³

The allergy march

The allergy march describes a characteristic progression of allergic diseases in atopic individuals (persons with a genetic predisposition for allergy) through childhood (see Figure 1). As shown in the figure, the allergy march frequently begins with atopic dermatitis, recurrent wheezing, and gastrointestinal distress during the first and second years of life. Upper-respiratory symptoms (sinusitis and/or rhinitis) can appear between ages three and seven, with otitis media and conjunctivitis as frequent co-morbidities. The allergy march culminates in asthma, which usually manifests between the ages of seven and 15.⁴⁻⁷

Figure 1 also illustrates why one mother’s description of her family’s experience is not uncommon: “My daughter was the colic queen. With all the ear infections she had, we were afraid to keep giving antibiotic courses, so we had ear tubes put in. I did not know those conditions [colic and ear infections] could be linked. But since she now has asthma, you could be on to something! Nobody ever suggested an allergy test.”⁴

The National Center for Health Statistics cites that the incidence of asthma increased 160% in children up to four years of age between 1980 and 1998. The incidence of asthma in all age groups increased 100% during that same period. Every day in America, 14 people die of asthma.⁴ As Möller, et al,⁹ showed in 2002, however, early identification of childhood allergies and immunotherapy may halt the allergy march and reduce morbidity due to allergies. Two other studies¹⁰,¹¹ demonstrated that early use of the anti-histamines cetirizine and ketotifen could also stop the onset of asthma in infants with atopic dermatitis. These three studies speak strongly to the importance of early intervention, which relies on early and accurate diagnosis.

Pathway to diagnosis

Although new allergies can arise at any age (even in adults), allergic disease is frequently observed as a common, chronic illness of childhood, and a primary-care physician or pediatrician is typically the first to evaluate potentially allergic children. The low allergist-to-patient ratio, the high cost of allergist care, and the increasing number of patients should provide strong incentive for primary-care physicians to be fully involved in allergy diagnosis and management.

Health-maintenance organizations (HMOs) mandate that primary-care physicians perform the initial patient evaluation (which may include diagnostic testing) to determine if referral to a specialist is needed. The primary-care physician frequently starts the diagnostic process that will differentiate allergic from non-allergic disease. In vitro testing (IVT) is a convenient, reproducible, and reliable tool for this process. It requires no special training for interpretation by primary-care physicians, and it agrees well with the results garnered from skin testing (ST) that would otherwise have to be performed in the specialist’s office.¹²⁻¹³

Considering the studies cited above, it is no wonder that the guidelines of both the AAAAI,¹⁶ and European Academy of Allergology and Clinical Immunology, or EAACI,¹⁷ recommend that diagnosis

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**Figure 1.** The allergy march (Adapted from Wahn).⁴
of allergy in children should occur as early as possible and that identification of the offending allergen(s) be achieved through diagnostic testing. In fact, the diagnostic guidelines mandate three specific elements:

- patient’s clinical history;
- clinical findings and symptoms; and
- diagnostic tests: in vivo (skin test, food challenge) and/or in vitro testing (IVT).

Once the patient’s allergies have been diagnosed, treatment may include one or more of the following:

- avoidance;
- pharmacotherapy; and
- immunotherapy (allergy shots).

According to the American Academy of Allergy, Asthma, and Immunology, allergies are responsible for 3.8 million lost work and school days each year.

Accurate diagnosis is a must. The hallmark symptoms of allergic rhinitis — sneezing, runny nose, watery and itchy eyes, and nasal congestion — also characterize other upper-respiratory diseases. One of the main reasons to diagnose and treat early allergies, such as sinusitis or rhinitis, is to prevent the development of asthma. Americans spend over $3 billion per year on antihistamines to treat the symptoms of allergy. Yet, a recent study found that 64% of the patients diagnosed with allergic rhinitis and treated with antihistamines had negative allergen-specific IgE tests. This study underscores that diagnostic testing is integral to the accurate diagnosis and treatment of allergy.

Improvements in testing

Traditionally in the United States, in vivo diagnostic tests (e.g., ST and food challenge) have been the preferred clinical choice used by the allergist for confirming the diagnosis of allergy and the identification of specific allergens. These methods have proven to be reliable in the hands of a trained allergist, but for certain types of allergies such as peanut or insect venoms (e.g., bee and wasp), in vivo tests may result in life-threatening anaphylaxis. In vitro tests can yield significant quantitative clinical data for most allergies without putting patients at risk.

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In general, *in vitro* allergen-specific IgE (sIgE) tests have been more readily accepted and widely used in Europe than in the United States, and the EACCI position is that IVT and ST are generally of equal diagnostic value.\(^9\) While many U.S. allergists still prefer skin tests, the technological improvements made to *in vitro* testing have made it a reliable test for identification of sIgE, and it is gaining increased acceptance, especially among primary-care physicians who may not have the specialist training necessary to perform skin testing.

Some doctors are still unfamiliar with advances in IVT for allergen-specific IgE. Improvements in the past two decades have transformed IVT to a sensitive and precise methodology for generating reliable results. Primary and continuing medical education are at least partly to blame as they often impart information drawn from literature that is one to two decades old. For example, a pediatric textbook\(^{20}\) published in 2000 states: “The radioallergosorbent test (RAST) determines antigen-specific IgE concentration in serum … *In vitro* methods of determining sIgE to several allergens simultaneously have been marketed as screening tests for allergy. The few published data evaluating such methods indicate that they are relatively insensitive and may fail to identify more than 30% of children with allergy.”\(^{20}\)

Although the text goes on to acknowledge that subsequent methodologies were somewhat improved, it illustrates the dissemination to physicians of out-of-date information that all current IVT methodologies are as clinically unreliable as the original commercial RAST test. Using RAST as a generic term for sIgE measurement by IVT has blurred the distinction between older and newer technologies and may be responsible for confusion that affects clinical practice and professional guidelines.\(^{21,22}\) Consequently, some doctors — unaware that earlier concerns have been ameliorated or resolved by modern technology — perpetuate misconceptions about the quality and clinical utility of currently available assays.

**Second-generation IVT**

Semiautomated, quantitative immunoassays, first marketed in the 1990s, greatly improved the quality and practicality of IVT. In some assays, sensitivity was improved by the replacement of paper disks with other substrates, making it possible to increase the amount of sIgE captured and boost the CPS,\(^{23}\) while chemical signal-detection methods (i.e., fluorescence) greatly improved assay sensitivity and time-to-first-result.

The World Health Organization International Reference Preparation was used to construct multipoint calibration curves, making standardized quantitative measurement possible. Results were reported in total serum IgE (kU/L). In the eyes of allergy specialists, this constellation of improvements justified designating these systems as second-generation.\(^{12,24}\)

Because many second-generation assays proved to have far better specificity and sensitivity than first-generation assays, researchers and allergists could finally recognize IVT’s potential for providing useful clinical information, especially because of its quantitative nature. Background noise, however, prevented the quantitative measurement of very low levels of sIgE below 0.35 kU/L (the technological limit of most second-generation systems), which presented an obstacle to further improvements in clinical sensitivity.

**Third-generation IVT**

Second-generation systems lacked the low-end precision required to quantify sIgE below 0.35 kU/L, and clinicians — of necessity — adopted this technological limitation as the *de facto* diagnostic cutoff. Consequently, a sample with an sIgE below this artificial cutoff was
considered to be negative. Researchers suspected that at least some allergens could evoke a response at much lower sIgE levels, although they were unable to investigate this hypothesis without an assay that could accurately and reliably measure sIgE below 0.35 kU/L. Obstacles to investigating the effects of lower levels of sIgE were removed with the introduction of an assay system which, among other advances, added a zero calibrator and optimized low-end precision. The assay runs on fully-automated, continuously loading, random access immunoassay systems. The assay employs a liquid-allergen matrix replacing the solid-phase capture media of all other sIgE assays. Use of a liquid matrix significantly enhances binding kinetics and sensitivity of the assay through the preservation of antigen conformation, and the reduction of non-specific binding. The liquid matrix improves reaction kinetics, sensitivity, low-end precision and diagnostic accuracy.

Because the assay has a detection limit of 0.1 kU/L and functional sensitivity of 0.20 kU/L, it has made it possible to study the significance of low levels of sIgE. Thus far, sIgE levels as low as 0.23 kU/L have been shown to evoke some venom allergy responses. Biagini, et al. showed that for the diagnosis of latex allergy, diagnostic performance at the 0.1 kU/L or greater cutoff level was superior over second-generation systems. In addition, Dr. Biagini’s study found the new assay system represented a technological advance, with enhanced speed and less operator intervention.

A case study by Grunwald, et al. demonstrates the potential clinical value of detecting very low levels of sIgE. In this study, a man who had suffered an anaphylactic response to the sting of an unknown insect had a negative ST for honeybee and wasp venom. Using the third-generation system, this patient was subsequently found to have honeybee venom sIgE present at a concentration of 0.23 kU/L (a level approximately 33% lower than the lowest readable level of sIgE in second-generation systems). He was diagnosed with and successfully treated for honeybee-venom allergy.

**The allergy march describes a characteristic progression of allergic diseases in atopic individuals (persons with a genetic predisposition for allergy) through childhood.**

Third-generation testing has three major improvements:
- It is quantitative — it can detect low levels.
- It is automated and accurate — similar to clinical chemistry immunoassay.
- Its test antigens are better defined and standardized.

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**Update:**

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Improvements in methodology, automation, and performance justify the classification of this system as a third-generation assay. Time to first result has been reduced to 65 minutes. Individual tests can be run on an as-needed basis, preventing batching delays. Automation and barcode scanning eliminate manual bench work, reducing human error to a minimum.

In vitro sIgE testing is a valuable, reliable tool for allergists and primary-care physicians because it facilitates early accurate diagnosis and appropriate therapeutic interventions with the capacity to serve the ever-increasing numbers of patients. Early diagnosis makes it possible to practice avoidance and begin treatment that can halt the progression of the allergy march, potentially preventing the development of asthma and improving the quality of life for countless children and adults. It also has the potential for reducing the cost-burden of allergy for patients, the healthcare industry, and the insurance industry. Third-generation testing is fully automated and quantitative, making it an excellent system for attaining these goals.

References
6. Sigurs N, Hattegård U, Kjellman B, Kjellman NI, Nisson L, Bjorksten B. Appearance of allergen-specific IgE measured by a fully automated and quantitative, making it an excellent system for reducing human error to a minimum.

Connie Mardis, head of Global Training at Siemens Medical Solutions Diagnostics, has 20+ years’ experience in cardiac perfusion, laboratory diagnostics, and education, with extensive working on promoting allergy diagnostic laboratories and with holding key marketing positions focused on hospital, reference, and physician-office laboratories, both domestically and internationally. Tricia Bal, MD, is a scientific writer at Siemens Medical Solutions Diagnostics. She received her degree from University of Michigan Medical School. Roma Levy, MS, is also a scientific writer for Siemens Medical Solutions Diagnostics, Los Angeles, CA. She holds a BA in biology from Northwestern University and an MA in biology from University of California, Santa Cruz.

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