

REVIEW

Understanding the usefulness of specific IgE blood tests in allergy

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There is a need for accurate tools supporting clinical diagnosis of atopic allergy

Atopic allergic diseases and formation of specific immunoglobulin (Ig)E antibodies are increasingly common conditions that affect a large population in the western world. The reasons for this increased prevalence are only partly understood, and may relate to the lifestyle of the western world, including altered intestinal bacterial flora, use of antibiotics, pollution in the environment, obesity and physical inactivity compared with the past [1–4]. Allergic disorders consume substantial health-care expenditures and direct healthcare costs for illness such as allergic rhinitis and asthma has been estimated in the USA to more than 8 billion US dollars [5–8].

The difficulty in diagnosing atopic allergy consists of several other disease aetiologies being present with similar symptom profiles and no gold standard for true diagnosis exists [9]. A clinical diagnosis should be based on the patient's history, symptoms, findings at physical examination and laboratory test results, etc. Therefore, a definitive clinical diagnosis should not be given on the results of any single diagnostic method and should only be made by the physician when all clinical and laboratory findings have been evaluated.

From a clinical perspective, reliable and accurate assessment of specific IgE antibodies is critical for identification of patients with atopic allergy, also for the identification of the causative agents in patients with allergic disease [10]. Combined with clinical history and physical examination such determinations of specific IgE antibodies are used to establish the diagnosis of allergic disease. They are also used to make treatment decisions. At present, assays have been developed for specific IgE antibody determinations to more than 400 different allergens. With the recognition that allergic problems are increasing and that differential diagnosis by history and physical examination is difficult due to multiple causes of similar symptoms, well-defined blood assays for specific IgE antibodies form objective means of identifying specific allergic problems in different individual patients.

Historical perspectives of specific IgE blood tests

Since the discovery of the IgE molecule more than 30 years ago and the subsequent development of blood tests for allergen-specific IgE antibodies many controversies have arisen. The original Phadebas-RAST was introduced in 1974 [11].

Initially, the relative sensitivity and specificity of the tests was debated relative to skin testing and the assay was considered to suffer from lower sensitivity compared with skin tests. Alternative performances of Phadebas-RAST were devised using longer incubation times and alternative scoring system [12,13] to try to increase the sensitivity similar to skin testing, but resulting in loss of specificity instead. The trademark 'RAST' came to be used colloquially to describe all immunoassays for specific IgE. In reality, Phadebas-RAST and subsequently Pharmacia CAP System became a working standard against which all subsequent commercial tests were compared [14].

Since blood tests for specific IgE antibodies were introduced 25 years ago, development of the methodologies has been steady and there are a variety of different commercial tests available. They include the development of new test formats with different chemistries, reagent development and result presentation [10]. Many physicians are under the impression that specific IgE blood test results obtained from different laboratories are reliable and interchangeable. This is substantiated by the fact that other test systems largely adhere to the principal of using scoring systems giving seemingly similar or even identical results in classes or units. However, an apparent agreement in such classes or units can not always be supported by the true concentrations of the calibrators, making a direct comparison invalid [15] and giving evidence to the longstanding debate regarding the clinical value of blood tests for specific IgE antibodies.

Performance characteristics for blood tests for specific IgE antibodies

Specific recommendations for performing tests for specific IgE antibody determinations were recently detailed in a publication from the National Committee for Clinical Laboratory Standards (NCCLS) [16]. The recommendations include procedures for quality control for daily performance in a clinical laboratory setting and minimal performance targets of 15% Coefficient of Variation (CV) of IgE antibody assays. According to the guidelines by NCCLS quantitative assays shall meet criteria for quantification including recovery of antibodies, precision, linearity and parallelism over the measuring range. All assay designs include a solid phase for the separation of bound and unbound IgE antibodies and all allergen components used must be in access. The original Phadebas-RAST included a reference consisting of serial dilutions of a serum sample containing IgE antibodies to birch pollen. The dilutions were selected to construct a calibration curve giving results in arbitrary units (PRU/mL) and internally calibrated against the

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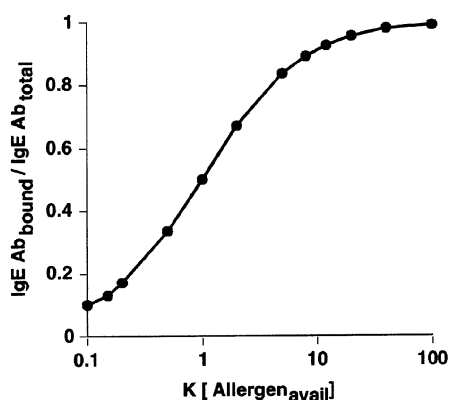


Fig. 1. The relation between equilibrium constant (K) and amount of allergen in Pharmacia CAP System[®], demonstrating antibody affinity independent of IgE antibodies. (Modified from [14]). The assay will be essentially affinity independent when the product of allergen concentration and affinity is greater than 10. Then more than 90% of the IgE antibody will be bound.

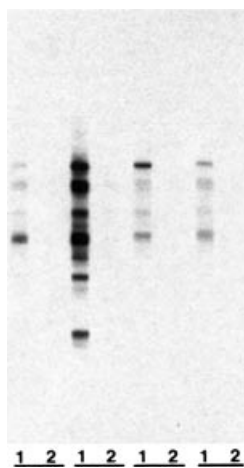


Fig. 2. Binding of IgE antibodies in Pharmacia CAP System[®] is complete as illustrated by subsequent immunoblot experiments. (Modified from [19]). Immunoblotting analysis of 4 sera with wheat-specific IgE antibodies before (1) and after (2) contact with a wheat-allergen ImmunoCAP. Antibodies of all detectable sub-specificities removed by the solid-phase allergens from [19]).

World Health Organisation (WHO) standard [17]. The new generations of Pharmacia CAP System and UniCAP replaced the IgE antibody birch pollen reference with a calibrator directly traceable to the WHO standard 75/502 for IgE [17]. Other systems have also largely adhered to this principle.

From the Law of Mass Action applied to a heterogeneous solid-phase immunoassay it can be concluded that when the concentration of the allergen multiplied with the equilibrium constant exceeds 10, more than 90% of the IgE antibodies are bound and the reaction becomes antibody affinity independent (Fig. 1) [14]. Therefore the solid-phase and all allergen components need to be at large excess to provide such a very high binding capacity binding all antibodies regardless of antibody affinity. This was not the situation in the past, and there are still systems not fulfilling such criteria. On the other hand, commercial assay systems exist that fulfil such criteria [15,18]. As a matter of fact, immunoblotting experiments have demonstrated

that virtually all IgE antibodies are bound in serum samples to the allergens on the ImmunoCAP (Fig. 2) [19].

Furthermore, this assay system utilizes the required highly specific, selected monoclonal and polyclonal anti-IgE reagents for quantitative detection of very small concentrations of specific IgE antibodies [14].

Together with the specific anti-IgE reagents, assays for the measurements of specific IgE antibodies require the inclusion of a standard curve. Calibrators should be traceable to the WHO International Reference Preparation for Human IgE, 75/502. For correct results, dilution of samples needs to be parallel to the standard curve (Fig. 3). Furthermore, specific absorption of antibodies should also decrease the content of total IgE [10,14,20] (Fig. 4).

Quality assurance and quality control

From the introduction of specific IgE antibody assays significant improvements have been achieved in the performance of the assays in the clinical laboratories. Quality assurance by manufacturers include specifications for all aspects of production to verify reagent performance. Clinical laboratories are encouraged to participate in interlaboratory proficiency testing. Independent programs for such testing exist in several countries. Proficiency testing provides a benchmark of test accuracy as performed by different clinical laboratories. The results enable the laboratory to assess their own performance as well as the performance of different commercial test systems [21–23].

A number of studies have addressed whether different specific IgE antibody blood tests really give interchangeable results. The French quality assessment program [21] compared the performance of several commercial systems for the measurement of specific IgE antibodies to three different allergens. This showed a few systems demonstrating good performance characteristics, whereas several other systems and assays did not live up to an acceptable standard. A study from Germany evaluated 60 different assay runs performed during one year with one particular commercial test system and found excellent accuracy and reproducibility for ten different allergens [24]. The manufacturer of that particular system also has established a quality assurance program of its own with improving quality over the years since 1992, when it started up, until 2000 (Fig. 5). The conclusion has been that it is unlikely that the results from different assay systems are equivocal and interchangeable. Thus, while some procedures in different laboratories gave highly accurate, comparable and reproducible results, some others gave poor reproducibility and quantification of the antibodies. Allergens more difficult than others in this respect are, e.g. food, weeds and mould [15]. Some studies suggest that specific IgE antibody blood tests to allergens like latex may also vary between different systems [25]. For some systems several studies have demonstrated less than optimal performance for many allergens with several unexpected positive and negative results, both around the cut-off point between what is considered positive and negative, and over the total measuring range, suggesting imprecision of the calibrators [18]. Some procedures utilizing the so called modified RAST have tried to increase the sensitivity, but the results reported by such tests are becoming unexpectedly both positive and negative, indicating a high degree of imprecision [15,18,25].

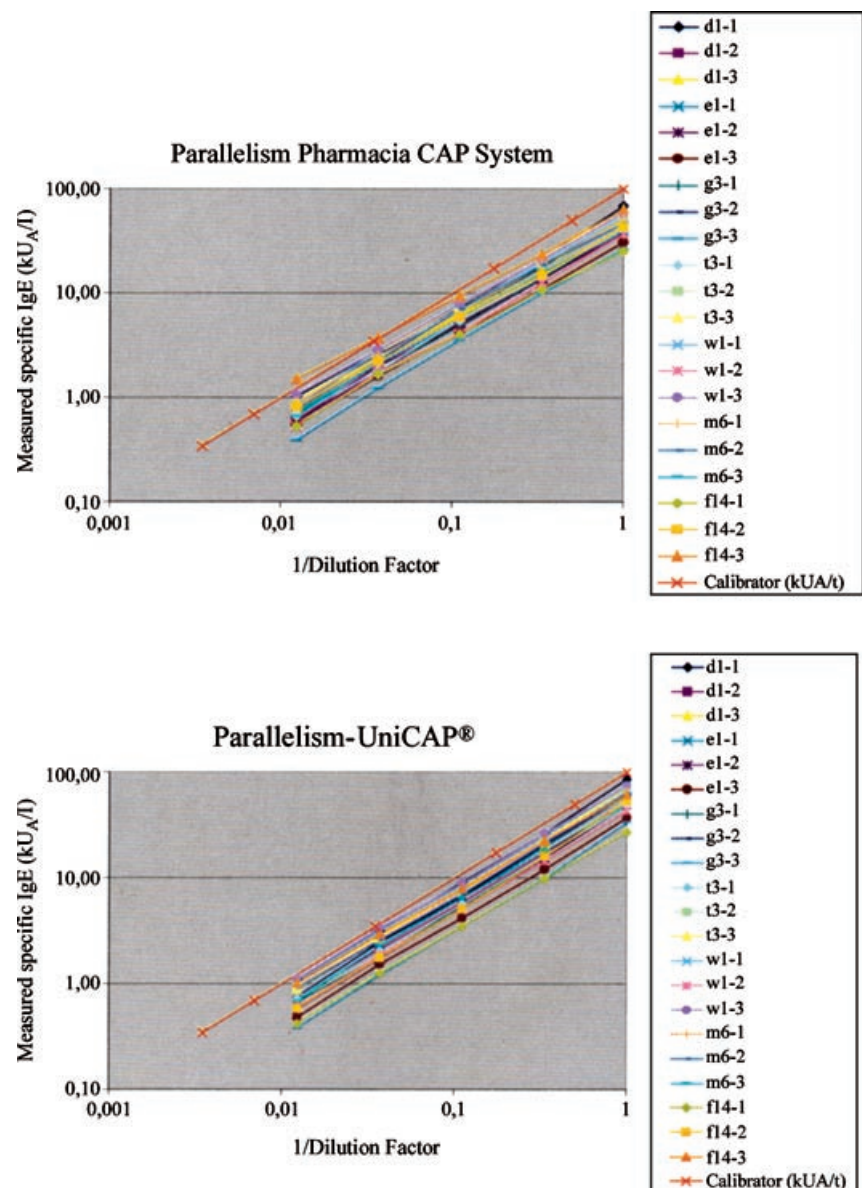


Fig. 3. Dilution of calibrator and serum containing specific IgE antibodies to different allergens in Pharmacia CAP and UniCAP Systems. Dilution curves are parallel, which is a prerequisite for adequate results over the measuring range.

Interpretation of results

The performance of the test is usually given as clinical sensitivity and specificity. Very good results of sensitivity and specificity for a variety of allergens have been documented [17]. For the early tests developed and marketed, data showed a good correlation between the levels of specific IgE antibodies and skin reactivity or symptom scores [26]. Values of more than 90% of sensitivity, specificity and predictive values have been obtained for certain test systems documented with several thousands patients [27].

Information of sensitivity, specificity and predictive values are highly dependent on the prevalence of disease in the population. Thus, for patients from a population with similar prevalence of atopic allergy to a given allergen, good predictive values could be obtained. Subsequent studies have confirmed the association between the levels of specific IgE antibodies and the

degree of allergen exposure as well as development of symptom [28]. However, to relate the presence or absence of precisely measured IgE antibodies to clinical disease is difficult, in particular since no gold standard exists for what is clinical allergic disease [9,29].

The information from tests should be given rather as Odds Ratios or risk assessment; assessing the risk of an individual to have or develop disease. This is further accentuated by the specific IgE antibodies developing before manifest and clear-cut clinical allergy symptoms have appeared, the so called Allergy March [30–32]. Mite sensitization early in infancy has been demonstrated to account for a 3.4–19.7-fold risk to develop asthma during childhood related to the degree of allergen exposure [33]. Consequently, allergen avoidance measures also significantly reduce the degree of sensitization and the development of symptoms [34–36]. Testing for IgE antibodies to a panel of inhalant allergens (Phadiatop) in children 0–1 and 2–4 years

of age allowed a risk assessment for subsequent asthma development of 7.4-fold and 17.2-fold, respectively [37].

One recent approach to this has been to associate the levels of specific IgE antibodies to foods with the probability of getting a clinical reaction upon exposure [38]. Thus, in a high-risk patient group, high specific IgE antibody levels were linked to a very high probability of reaction upon allergen exposure, whereas lower levels were associated with a lower probability. For different allergens different risks were associated with different levels of IgE antibody levels. At no level was the risk completely

negligible, even if it was small. A similar approach was tried with inhalant allergens from a trial with close to a thousand patients (Fig. 6). In other studies a high degree of correlation was found between the levels of mite-specific IgE antibodies and the concentrations of mite allergen in mattress dust. Subjects with more than 2 kUA/L of mite-specific IgE antibodies had a 77% probability of being exposed to more than 10 µg mite allergen per g dust. In contrast, those with less than 2 kUA/L had 77% probability of being exposed to lower concentrations than 10 µg mite allergen per g dust [39]. Such information may be of particular importance since it would allow the physician to identify not only patients who are sensitized but also those who need more environmental control measures.

Complicating factors in the understanding of IgE antibodies

The IgE antibodies are produced as a consequence of exposure to allergens in the environment. Regional differences result in different patterns of IgE antibody specificity. However, increasing international trade and use of tropical food, ornamental plants and herbs widens the exposure from what is common in the environment to something more universal. Proteins with similar functions in different plant species may have similar functions and structures. The similarities between allergenic structures in different plant species increase with increasing biological relation between the plants where they come from. The IgE antibodies may detect such similarities between allergens from different sources as a function of biology and chemistry. Allergen molecules like profilins have a relatively conserved structure and cross-react between species, whereas

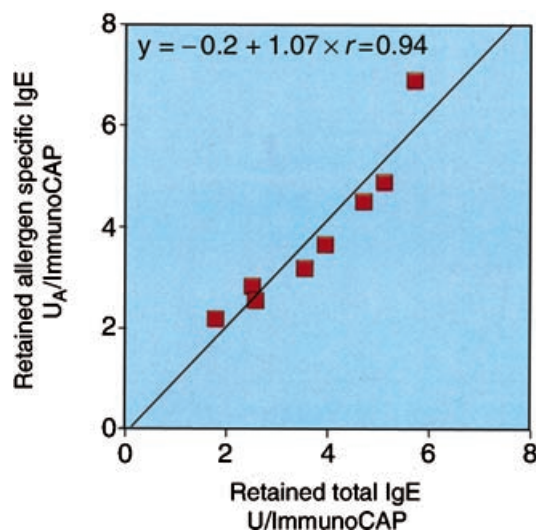


Fig. 4. Parallel change in IgE antibody and IgE protein in serum upon step-wise depletion of timothy-specific IgE antibody by timothy ImmunoCAP. (Modified from [14]).

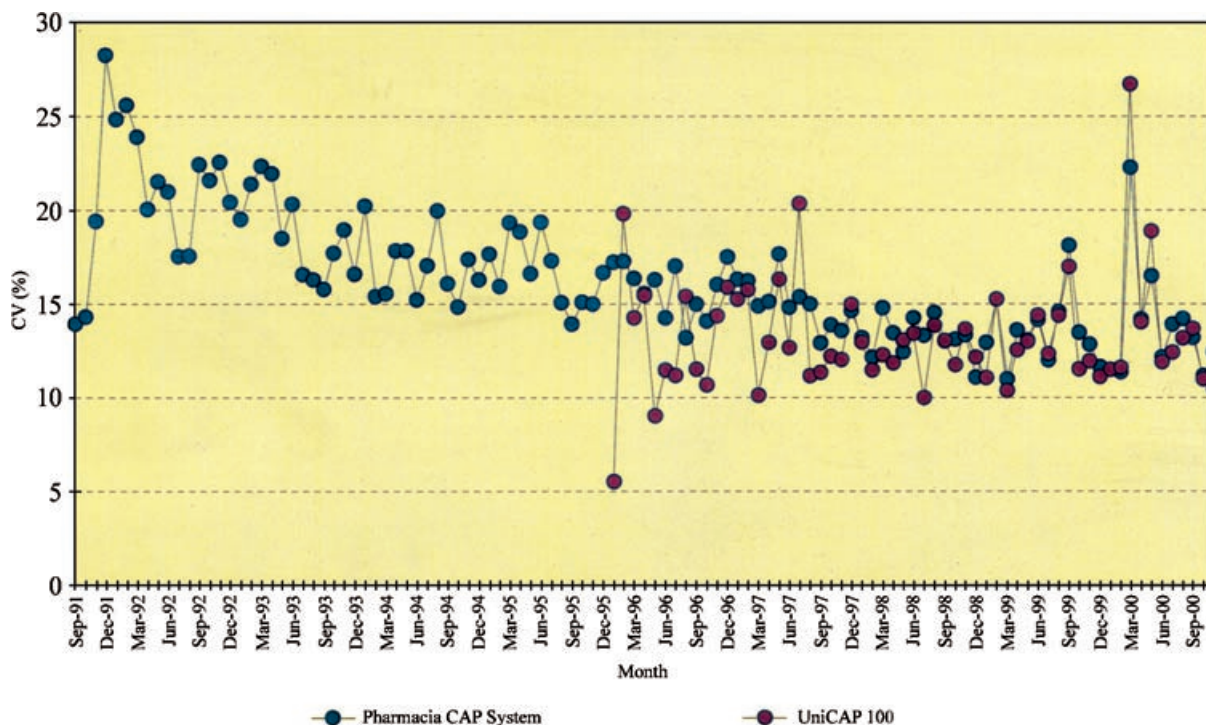


Fig. 5. Results from proficiency testing by one manufacturer during a nine year period with two assay systems. The results are expressed as Coefficient of Variation (CV) from more than 300 laboratories from the whole world.

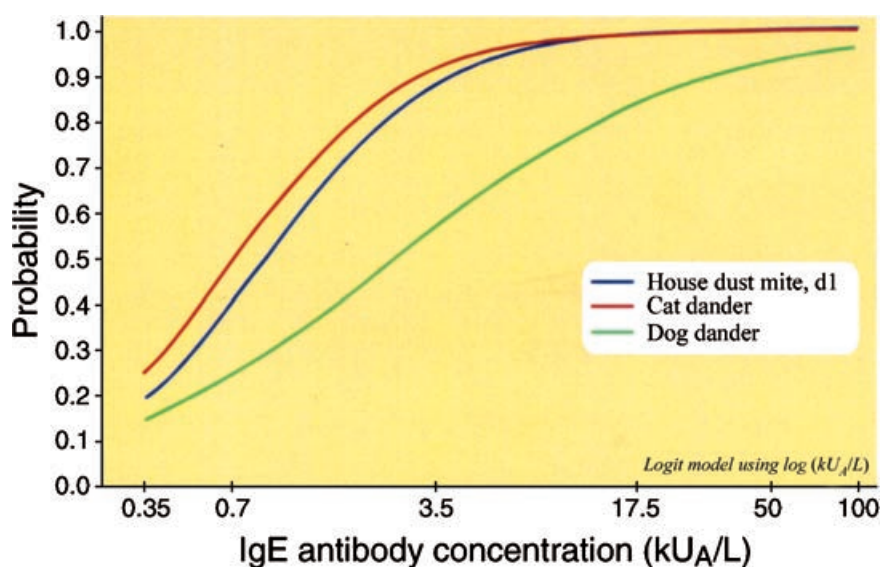


Fig. 6. Risk assessment curves for clinical reaction to a specific allergen at a given level of specific IgE antibodies, calculated from [27]. The results demonstrate different reactivity to different allergens or different precision in clinical diagnosis.

other structures are more species-specific, like Bet v 1 from birch tree showing, homologies only within the birch-related spring-pollen trees, like alder and hazel. The relations between different plant species have recently been classified by Takhtajan [40] and the amino acid sequence homologies retrieved by the National Centre for Biotechnology Information as recently described by Yman (E-mail: marketing.diagnostics@eu.pnu.com for further information). This provides a platform for predicting immunochemical relations and cross-reactivity between plant species. Therefore, IgE antibody formation may occur to proteins with the same function and similar structure in different plant species resulting in allergic reactions even if exposure to the allergen can not be identified.

Another important aspect which is widely debated is that IgE antibodies in a blood test may be bound to a univalent structure like a carbohydrate, whereas biological activity like skin test may be negative because of the univalency of the test material [41]. This does not permit the conclusion that the IgE antibodies are without biological and clinical significance. It does not certify that clinical reactions may not occur when the individual is exposed to allergenic material containing the carbohydrates in a different multivalent conformation, that induce the biological activation of cells and mediators triggering clinical reactions.

Consequences of using IgE antibody identification

Since allergic diseases have symptoms that can easily be confused with other disorders, even specialists may have difficulty in using only the case history and physical examination to distinguish the different disease aetiologies, without using 'tools' to aid in the identification of the atopic aetiology. Furthermore, specialists may experience difficulty in identifying the offending atopic allergen in a significant number of patients without using 'tools' to identify specific IgE antibodies [9]. However, the majority of allergic patients, maybe 80%, are seen by family physicians and primary care physicians or are not seeking medical care for their problems [42].

Allergist specialists have the education and training to take a good case history and make a physical examination often accompanied by special tests, like skin and blood tests. Primary care physicians have less training and expertise to use the sophisticated tools used by the specialists and then come to a final diagnosis. The practical value of any test resides in its ability to give accurate, consistent and thus reliable results. Clinically, the misclassification of patients by not using accurate blood tests for specific IgE antibodies could result in the risk of an incorrect clinical diagnosis [15,18,29] and the implementation of unnecessary treatments or the omission of effective clinical interventions. Very recent information from primary care in the USA suggest that non-allergic individuals may be misdiagnosed as allergic with poorer outcome and an increased cost due to the therapy they were given [43].

There are reports demonstrating that by using accurate and sensitive blood tests for the determination of the presence of specific IgE antibodies and atopic allergy, primary care can come close to the precision of allergist specialists [44]. The proper use of blood tests for IgE antibodies by family physicians and general practitioners would help in identifying patients to be kept in primary care from those who should be referred to specialists. The appropriate use of such tests would also help in establishing a good communication network between generalists and allergists and enhance the level of education [45].

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