

LABORATORY METHODS IN ALLERGOLOGY

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IDENTIFICATION AND QUANTIFICATION OF SPECIFIC IgE ANTIBODIES

This new regular feature will be focusing on the different methodologies used in the diagnosis of allergies, describing current assays and introducing novel approaches. Hypersensitivity reactions can be differentiated into IgE- and non-IgE-mediated allergic but also non-allergic reactions. Over the next issues the different *in vitro* and *in vivo* diagnostic assays will be explained and the advantages and limitations highlighted.

In this first article I will focus on established as well as more novel approaches to quantifying and detecting specific IgE antibodies *in vitro*.

Allergic disease is a rapidly growing health problem. A precise, reliable *in vitro* assay for IgE antibodies to specific substances is a valuable tool to support the clinician in making diagnosis of or excluding allergy, prescribing and following up treatment and predicting disease development (Fig. 1).

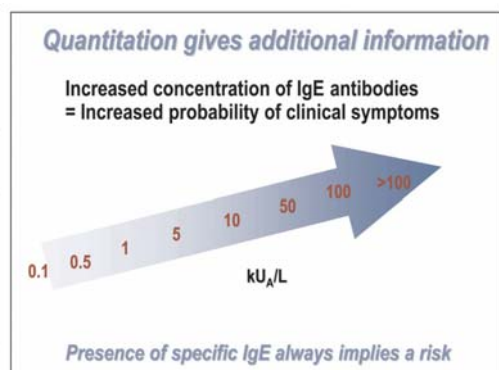


Fig. 1. Information about concentration of specific IgE will give more support to the clinician who evaluates the test results together with case history, physical examination and allergen exposure of the patient.

Assaying IgE antibodies is considerably more complicated than performing most other immuno-assays and requires considerable experience. There are a number of complicating factors:

- The concentration of IgE antibodies in blood is extremely low (several thousand times lower than IgG), even in highly sensitised individuals!

- Each main allergen contains a large number of different allergenic components (proteins). The assay must therefore be sensitive enough to capture antibodies to all relevant components, even if these are present only in very minute amounts.
- The assay must have a high enough capacity to bind all IgE antibodies to an allergen in competition with other antibodies with the same specificity from other immunoglobulin classes present in higher concentrations (e.g. IgG).
- To achieve a precise and reproducible test system, total control of the allergen source material is necessary, both in content and in allergenic activity, thus assuring lot-to-lot reproducibility.

There are several commercial tests to quantify specific IgE antibodies; however, the most prominent system is the ImmunoCAP. This system is highly automated allowing fast, accurate and reproducible results. The actual serum sample needed is only about 80 microlitres and the analysis time very short. Over 550 different allergens are available for allergy testing, as well as mixtures of different allergens in the fields of inhalant, food and occupational allergies (for more details see http://www.diagnostics.nu/upload/Allergy/Products/Files/Product_Catalogue_AL.pdf). For example the ImmunoCAP fx5 is a test for sensitisation to common food allergens (soy, peanut, milk, egg, wheat, fish) and is particularly useful in children. Other mixes are available, e.g. for moulds, pollens or mites. The clinical sensitivity and specificity have been established in many trials to be very high – 89% and 91% respectively.

A new tool for quantifying specific IgE antibodies to allergens where no commercial validated test system is available has been developed. Researchers can now couple their own allergens to the **Streptavidin ImmunoCAP** using the normal ImmunoCAP system.

In addition other ImmunoCAPs are available such as the quantification of **total IgE antibodies**, which is important in establishing possible parasitic infections. Normal values can vary from 10 to 100 kU/l, but reach several thousand kU/l in parasite-infected individuals.

The presence of atopic allergy to common inhalant allergens is established using the **ImmunoCAP Phadiatop**. The test comprises a mixture of over 15 inhalant allergens and a positive result is usually indicated when the IgE level exceeds the current cut-off of 0.35 kU/l. The upper limit of detection is 100 kU/l but very high levels can easily be quantified by diluting the serum up to 100-fold, without losing precision. ImmunoCAP gives precise and correct results throughout the conventional range, with a technical detection limit of less than 0.1 kU/l.

Is it clinically relevant to measure low levels of IgE antibodies?

It is important to note that even at very low levels of specific IgE there is still a certain degree of probability for a positive reaction; this may be particularly important in small children, as well as for testing sensitivity to venoms and drugs (Fig. 2).^{1,2} This is of particular importance as the trend is to replace the current class

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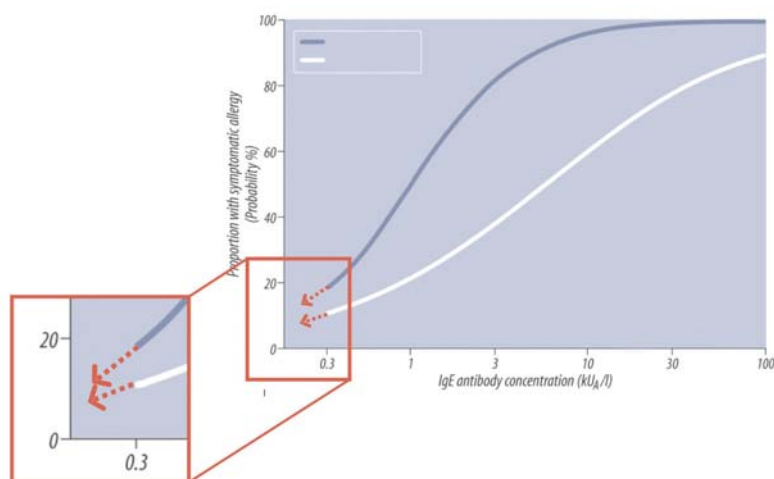


Fig. 2. The curves are generated with data obtained from 7 clinics in Europe and the USA (5 147 and 1 882 test results for inhalant and food allergens respectively). From Söderström et al.¹ and Sampson HA.²

system and use the actual quantity of specific IgE to a particular allergen instead. It has recently been highlighted that utilising the sum of IgE antibody levels in combination with the number of allergens represents a more efficient diagnostic tool than using individual positive IgE antibody results alone.^{1,3}

Currently we are evaluating in a long-term study the correlation of low IgE levels with onset of clinical symptoms. It will be very interesting to see what the future will bring in knowledge and possibilities when we have the chance to measure low levels of specific IgE antibodies routinely.

Cross-reactive IgE antibody detection

In some cases the quantification of specific IgE antibodies is directly linked to the presence and antibody recognition of carbohydrate. In most allergen sources, especially from the plant kingdom, glycan-related IgE-reactivities have been demonstrated. Since glycoepitopes can share significant structural homologies beyond the limits of protein families they are prone to extensive cross-reactivity and they have, consequently, been called **cross-reactive carbohydrate determinants (CCDs)**. Carbohydrate epitopes and CCDs add another level to IgE cross-reactivity mechanisms, in addition to cross-reactivity arising from taxonomy closeness of plants and from proteins belonging to the same family.

Furthermore, clinical data are emerging that these carbohydrate epitopes on glycoproteins have a clinical role in some cases⁴ (Fig. 3) as has been demonstrated in one-third of tomato-sensitive patients. Therefore, we must consider the sometimes confusing role of these epitopes in serum-based IgE antibody assays as these CCDs could have value when making a diagnosis in specific sub-groups of patients. In these groups, the use of natural allergens is preferable to the use of recombinant allergens often lacking these carbohydrates.

The risk for interference is greatest with food allergens of plant origin (e.g. celery), pollen, latex, and *Hymenoptera* venoms. Although generally of minor importance in everyday clinical practice, the *in vitro* reactivity of potential CCD specific IgE antibodies must be considered and can simply be measured *in vitro* using the ImmunoCAP-CCD; e.g. MUXF3 test.

Natural versus recombinant allergens

Naturally occurring allergens are routinely used in both *in vitro* and *in vivo* diagnostics, but vary significantly in their composition and allergenicity. Most patients do not raise specific IgE to all but only some allergenic components in an allergen source, which is of special importance for the correct diagnosis and composition of allergen vaccines for specific immunotherapy (SIT). To address this, recombinant allergens have recently been introduced into conventional testing, a strategy termed **component resolved diagnosis (CRD)**. Recombinant allergens are biotechnologically produced allergen molecules previously identified in whole allergen extracts. For instance, of the

hundreds of food allergens characterised, over 60 allergens have currently been produced as recombinant allergens (see <http://www.allergome.org/>). Subsequent to these developments about thirty **recombinant allergens** are already available in the ImmunoCAP system, particularly to latex, shrimp, *Aspergillus fumigatus* and pollen from birch and timothy grass.

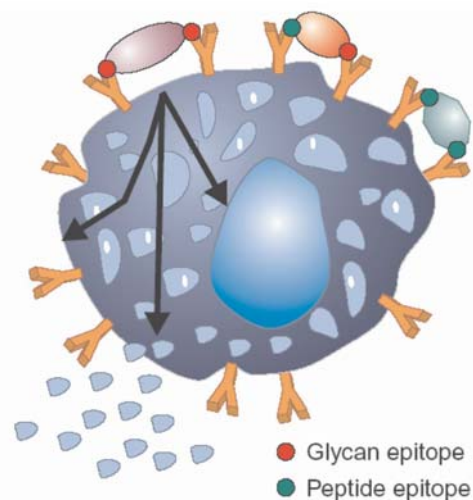


Fig. 3. Degranulation of mast cells requires the binding of at least two epitopes to two adjacent IgE antibody molecules. This cross-linking may be achieved by two peptide/protein epitopes (green), by one glycan (red) and one peptide epitope, but also by two glycan epitopes.

Micro-array technology

A novel antibody detection system, the **allergen micro-array** has emerged as a promising approach to high-throughput large-scale profiling of allergen interactions for simultaneous monitoring of IgE and IgG antibodies directed against a variety of allergy-eliciting molecules.⁵ A number of allergens are micro-arrayed onto a solid phase (e.g. modified glass slides or nitro-

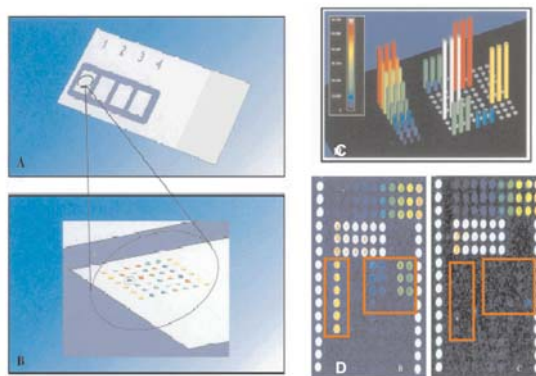


Fig. 4. Allergen micro-array: application of allergens (currently about 100) onto glass plates (A and B), detection of signal via laser (C) and analysis of allergen profile (D) (with positive patient indicated on the left panel).

cellulose membranes) and subsequently used to bind antibodies from the serum of allergic patients. Detection of allergen-specific antibody binding is accomplished by the addition of specific secondary antibodies that carry an appropriate label for the quantification using laser technology and are quantified in terms of g/l or IU/ml (Fig. 4).

The major benefit of this technology lies in its ability to screen for several hundred allergen molecules simultaneously while employing only minute amounts of the patient's serum (usually 20 microlitres). The capturing agents that can be used are either crude or partially purified allergen extracts, highly purified recombinant or natural allergenic components. Subsequently this will result in an optimal profiling of the patient's IgE response (in one analytical step), identifying major/minor allergens, pan-allergens and CCDs.

Immunoblot

Sometimes patients present with a clear history of allergic sensitisation but commercially available assays do not detect elevated specific antibodies. In this case when sensitisation to an unknown allergen source is suspected, immunoblotting (also called Western blotting) should be conducted. Protein extracts of the suspected allergen source are separated by gel-electrophoresis (in an electrical field) according to molecular size; the proteins are then transferred to a membrane (blotting) and the allergens detected with

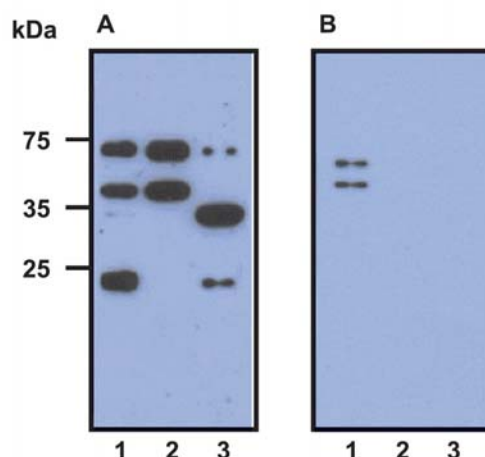


Fig. 5. Immunoblot of two patients (A and B) for three different crustacean species (1 = rock lobster; 2 = prawn; 3 = langoustine). Patient A demonstrates multiple reactivity to different allergens, whereas patient B reacts only to a few allergens in rock lobster.

serum IgE antibodies from sensitised patients (Fig. 5). This method can be very sensitive; however, the evaluation of the results is only qualitative and requires a sound knowledge of molecular allergens. It is advisable to compare results with non-sensitised individuals.

Declaration of conflict of interest

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