

# Radioimmunoassay of IgE and IgE antibody and its clinical application<sup>1</sup>

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For more than 50 years it has been known that some patients with asthma, allergic rhinitis and eczema possess antibodies with skin-sensitizing properties, so-called reagins. It is now well established that the reagins belong to a unique immunoglobulin class, IgE (Ishizaka and Ishizaka, 1967; Johansson and Bennich, 1967). The isolation of IgE and the raising of antibodies against IgE made possible the development of immunological assays for IgE and allergen-specific IgE antibody. The aim of this paper is to discuss the methodological aspects of the determination of IgE and IgE antibody as well as their clinical application.

## Methods for Quantitation of IgE

IgE is distributed both free in the body fluids and bound to blood basophils and tissue mast cells (Ishizaka, Ishizaka, Johansson, and Bennich, 1969). The amount of tissue-bound IgE cannot be routinely assessed at present. However, attempts to calculate the amount of cell-fixed IgE have resulted in figures of the order of 0.1% or less of the total body pool of IgE (Ishizaka and Ishizaka, 1973). (The levels of IgE given in the literature refer to the concentrations of unbound IgE present in serum and other body fluids.) In healthy adult individuals the level of IgE in serum is of the order of 100 µg/l. This means that gel diffusion techniques are not sensitive enough for IgE quantitation, unless the IgE level is at least 10 times higher than the normal mean for adults.

Several methods for quantitative determination of IgE have been described (Johansson, Bennich, and Berg, 1972). Radioimmunoassay (RIA) has been used almost exclusively, although a successful application of the enzyme-linked immunosorbent assay (Engvall and Perlmann, 1971) has been reported (Hoffman, 1973). Most of the RIA procedures used are conventional competitive binding assays. The radioimmunosorbent test (RIST, Johansson, Bennich, and Wide, 1968) is a solid phase-antibody

method; anti-IgE is covalently bound to CNBr-activated Sephadex particles for which labelled and unlabelled IgE compete. The separation of free from bound labelled IgE requires only centrifuging. In 'sequence-RIST' the sample is incubated with the solid phase antibody without labelled IgE, which is added later, ie, RIST with the late addition of the labelled antigen. In the double-antibody technique the antihuman IgE is in solution and separation of bound from free IgE is achieved by means of precipitation with a second antibody specific for the gamma globulin having the anti-IgE activity (Gleich, Averbek, and Swedlund, 1971).

A direct RIA has also been described for quantitation of IgE (Ceska and Lundkvist, 1972) with the following principle (fig 1). The gamma globulin fraction of an anti-IgE antiserum is coupled to CNBr-activated filter paper discs. One disc is incubated with 50 µl of a serum sample or a reference preparation. The IgE in the sample, or reference solution, will bind to the disc. After washing with buffer the amount of bound IgE on the disc is measured by immunosorbent-purified anti-IgE labelled with <sup>125</sup>I, which couples with bound IgE so that the amount of radioactivity in the solid phase is directly proportional to the amount of IgE.

Both competitive and direct radioimmunoassays

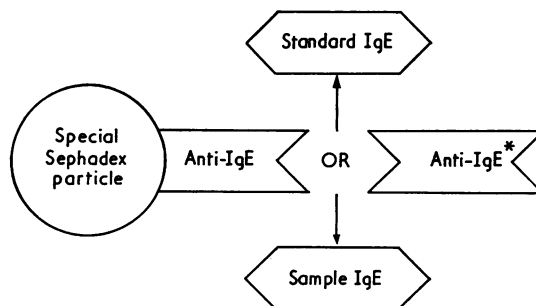


Fig 1 The principle of the direct radioimmunoassay used for determination of IgE.

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for IgE are highly sensitive. Under optimal conditions concentrations as low as 0.1-1.0  $\mu\text{g/l}$  of IgE in serum can be accurately determined. However, since all test reagents are mixed together in the competitive tests, they seem to be prone to the influence of non-specific factors. Any serum factor that interferes with the binding of labelled IgE to the anti-IgE will reduce the radioactivity bound to the antibody and hence give falsely high results. This effect, which appears to be somewhat more evident in RIST than in the double-antibody technique, can be decreased by making up the standards in IgE-free serum. An alternative is to compensate for the difference between the non-specific effects of serum and the standard, which is diluted in buffer, by means of an appropriate correction factor. In RIST this factor is 0.96 for serum samples tested in a dilution of 1/10 (Berglund, unpublished observation). However, the degree of non-specific interference is not constant from one serum to another, and to be certain that the unwanted effect is eliminated it is necessary to determine the factor for each individual sample (Bazara, Orgel, and Hamburger, 1971).

A simpler procedure to avoid non-specific factors is to use a direct test (fig 1). Washing before the addition of the labelled anti-IgE will reduce the non-specific interference to a minimum. However, the possibility of a slight effect from other interfering factors in serum or secretions cannot be excluded.

A comparison of IgE levels in serum obtained by competitive binding and direct techniques shows a good correlation at concentrations higher than 100 units/ml (1 unit = 2 ng). At lower levels, the highest values are obtained by RIST, followed closely by sequence-RIST and double-antibody RIA. The lowest values are derived from the direct test. To illustrate these differences, the IgE concentrations found in cord serum using different methods are given in the table.

Radioimmunoassay	IgE	Significance of the Difference from Phadebas IgE Test
Phadebas <sup>†</sup> IgE test	3.8	
Phadebas IgE test (sequence)	3.1	P < 0.05
Phadebas IgE test (correction factor 0.96)	0.6	P < 0.001
Direct solid phase <sup>‡</sup>	0.4	P < 0.001

Table IgE concentration (geometric mean in units/ml)<sup>1</sup> in 26 normal cord serum samples analysed with various radioimmunoassays (data from Kjellman et al, in preparation)

<sup>1</sup>Geometric mean  $n = \sqrt{x_1 \times x_2 \times x_3 \times x_n}$

<sup>†</sup>Trade name for Pharmacia Diagnostics, Uppsala, Sweden

<sup>‡</sup>RIA described by Ceska and Lundkvist (1972).

### Serum IgE Levels in Clinical Disorders

The initial findings of raised serum IgE concentrations in patients with allergic asthma (Johansson, 1967) and hay fever (Berg and Johansson, 1969) have been confirmed by several workers (Johansson *et al*, 1972). About 50% of patients with 'extrinsic' asthma have significantly increased serum IgE concentrations, the highest levels being found in patients with hypersensitivity to many allergens and with combinations of asthma, hay fever, and eczema. Patients with hypersensitivity to only one or a few allergens to which they are exposed during only a limited time of the year quite often have normal IgE levels.

The short time of exposure to the allergens probably explains why patients with a monovalent pollen allergy tend to have normal IgE values. However, the reason can, at least partly, be technical, as the tendency for competitive binding tests to overestimate low IgE values will lead to poor discrimination of low levels. Recently a group of about 250 healthy, non-atopic adults and about 75 adults with a diagnosis of extrinsic asthma and/or hay fever were investigated for serum IgE levels using the paper disc direct IgE assay of Ceska and Lundkvist (1972). The geometric mean  $n = \sqrt{x_1 \times x_2 \times x_3 \times x_n}$  IgE value for the healthy group was 15 units/ml compared with 138 units/ml for the allergic patients, a difference of almost 10-fold. Of the healthy group, 57% had an IgE value below 20 units/ml but not a single allergic patient had such a low value (fig 2), whereas 63% of the allergic patients as compared

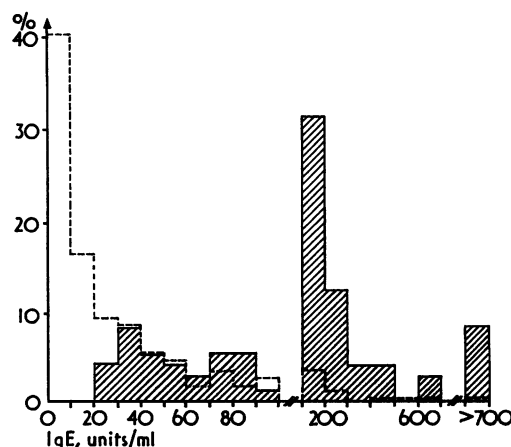


Fig 2 The percentage distribution of IgE values obtained by direct radioimmunoassay from 243 healthy adults (open area) and 73 adults suffering from asthma and/or hay fever (hatched area).

with 6% of the healthy ones had over 100 units/ml. These preliminary results suggest that the test may have considerable clinical value.

In atopic individuals the total serum IgE levels are not constant. Variations are seen with allergen exposure during the pollen season and early in the course of specific immunotherapy (Berg and Johansson, 1969). The presence of atopic eczema in addition to asthma and/or hay fever also seems to potentiate IgE production (Juhlin, Johansson, Bennich, Högman, and Thyresson, 1969) for reasons as yet unknown. Patients with 'atopic eczema' without any other atopic manifestation usually have normal IgE levels (Öhman and Johansson, 1974).

Even higher serum IgE levels are found in patients infected with parasites which enter the circulation (Johansson *et al.*, 1972) or with 'tropical eosinophilia' (Ezeoke, Perera, and Hobbs, 1973). Most of the IgE produced is probably not antibody to parasitic antigens although a significant part could be low affinity antibody to such antigens. Such non-specific stimulation of IgE synthesis might also occur in patients with atopic eczema. High IgE levels are also found in boys with Wiscott-Aldrich's syndrome (Berglund, Finnström, Johansson, and Möller, 1968), which includes increased susceptibility to infections, thrombocytopenia, and eczema. Whether the increased production of IgE is the result of the eczema or of some other factor basic to the disease is unknown. Great interest has been directed lately to a possible role of the T lymphocytes in the regulation of IgE synthesis. The very high IgE levels in some patients with T-lymphocyte deficiency, together with animal studies, support such a role. Patients with atopic eczema have been reported to have defective T-lymphocyte function (McGeady and Buckley, 1974).

#### Methods for the Determination of IgE Antibodies against Specific Allergens

Two approaches have been made to the problem of measuring specific antibodies in the IgE class. According to one principle, pure allergen is labelled with a radioactive isotope and added to a reaginic serum. The labelled IgE-allergen complex is precipitated with an antiserum to IgE and then measured by autoradiography. More precise measurement is possible by a solid phase modification (Zeiss, Pruzansky, Patterson, and Roberts, 1973). However, difficulties in the isolation, labelling and storage of the allergens have limited the usefulness of such methods.

The second principle used is the antiglobulin reaction of Coombs (Coombs, Howard, and Mynors,

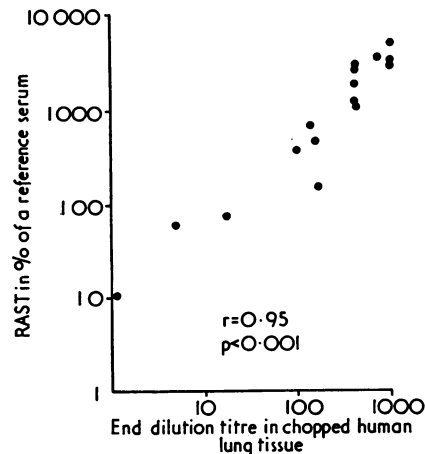


Fig 3 The correlation between IgE antibody levels in sera from patients allergic to Timothy pollen, measured by RAST and by reaginic end-dilution titration against passive sensitization of chopped human lung tissue (data from Foucard, 1972).

1953) which was found capable of detecting IgE antibody once specific anti-IgE became available (Coombs, Hunter, Jonas, Bennich, Johansson, and Panzani, 1968). A radioimmunoassay modification, the radioallergosorbent test (RAST), seems to be the most suitable large-scale test for IgE antibody: the allergen is covalently bonded to an insoluble matrix such as a filter paper disc. This allergen-polymer complex (APC) absorbs IgE antibodies specific for the allergen. After washing, the amount of IgE absorbed is measured by adding a highly purified, labelled anti-IgE (Johansson, Bennich, and Berg, 1971). Such solid-phase allergens are stable for months or years at 4°C.

To test the biological activity of the IgE antibody measured by RAST, comparisons have been made with *in-vivo* and biological *in-vitro* tests for reaginic activity. Excellent agreement was obtained between RAST and PK-titration<sup>1</sup> for cod fish (Foucard, Aas, and Johansson, 1973) and between RAST and histamine release from passively sensitized chopped human lung (fig 3) with allergens from horse dander, birch, and timothy pollen (Foucard, 1972). It is clear that RAST measures reaginic antibody and hence most, if not all, reaginic antibody is IgE. This does not exclude the possibility that tissue-sensitizing

<sup>1</sup>Prausnitz-Küstner (PK) titration consists of injecting dilutions of the patient's serum into the skin of another subject. Reagins in the patient's serum transfer the hypersensitivity to the recipient's skin. This is shown by the formation of a wheal on injecting the allergen into the sensitized area of skin. The reciprocal of the largest dilution which transfers hypersensitivity is the PK titre. — ED.

antibodies of other immunoglobulin classes occur, but those antibodies do not possess the physico-chemical properties characteristic of reagin (Parish, 1970).

#### IgE ANTIBODY IN SERUM

IgE antibody can rarely be detected in serum from healthy adults. In a study of blood donors selected for their freedom from allergy, positive RAST reactions were found in only 0.2% (Berg and Johansson, 1974).

In contrast, almost all patients with a manifest hypersensitivity have detectable amounts of IgE antibody in serum. The level does not remain constant. As stated earlier for total IgE, an increase in specific IgE antibody can be seen as a result of antigen stimulation during the pollen season in patients with hay fever or early in a course of allergen-specific immunotherapy. The long-term effect of desensitization is a decrease in IgE antibody, but it rarely disappears completely. A similar trend was found in some children spontaneously growing out of their allergy (Foucard, 1973). Other kinds of treatment, such as antihistamines, bronchial dilators, disodium cromoglycate and steroids in moderate dosage, do not depress the IgE antibody level.

The use of RAST as a serological test for atopic allergy has been intensively investigated during the past seven years. Good agreement with conventional diagnostic procedures has been obtained. Screening tests, such as skin tests performed with only one allergen dilution, do not correlate well with a quantitative test like RAST. For intradermal tests, the results agreed with RAST in 50 to 70% of cases (Johansson *et al.*, 1972). The scratch and prick tests, which are less sensitive, usually show a 5-10% better correlation (Berg and Johansson, 1974). Very good agreement has also been reported between RAST and leucocyte sensitivity and symptom score (Norman, Lichtenstein, and Ishizaka, 1973). However, the best agreement has been obtained with quantitative tests like skin test titration or provocation tests. Agreement between RAST and provocation tests of 75 to 100% has been reported (Johansson *et al.*, 1972; Wide, 1973).

From our experience we have evolved a protocol for the diagnosis of allergy (fig 4). Central to the application of this scheme are the patient's symptoms and case history. Any test result obtained, whether from skin and provocation testing or from RAST, must be interpreted by an experienced allergist in the light of clinical evidence. The case history, with results of prick or scratch tests, will help the allergist to determine whether the case is typical or atypical. In a typical case the patient is concluded to have an atopic disorder and the allergist suspects that the

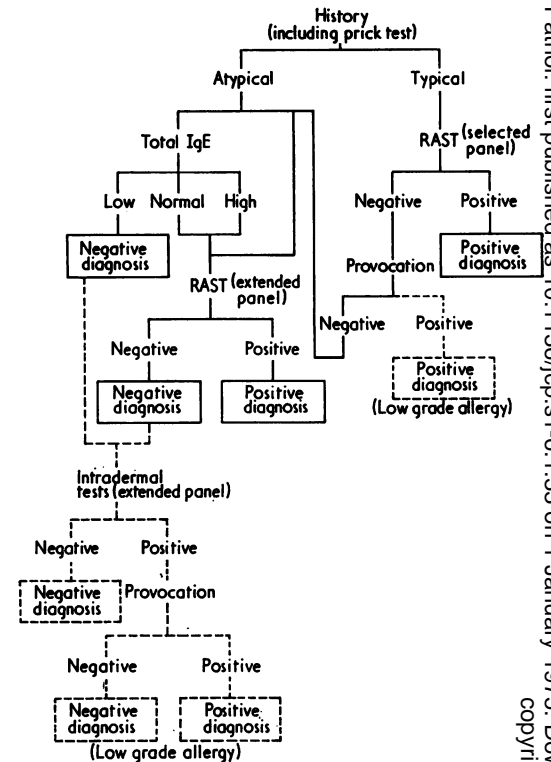


Fig 4 A schematic representation of the role of tests for total IgE and IgE antibody in the diagnosis of atopic allergy. The results of these *in vivo* and *in vitro* tests are valid only when compatible with the case history (from Johansson, 1974).

allergen causing the major symptoms is restricted to a fairly limited range of allergens. The radioallergo-sorbent test is performed with all these allergens and if found positive the diagnosis is made. A negative test does not exclude hypersensitivity because the patient may have a low-grade allergy. If the allergist is convinced that the considerable effort required to detect low-grade allergy is justified, provocation tests may be performed. An intradermal skin titration is probably a good substitute for the provocation test. If the latter tests are negative it is possible that the case history was misleading. In this situation quantitation of the total IgE level in serum would be useful, because an elevated IgE level is indicative of atopy if a parasitic infestation can be excluded. If a high concentration of IgE is found, RAST should be performed against a large panel of allergens. The number of allergens depends on such factors as the type of symptoms, the age of the patient, and the allergen-frequency profile of the local region. In



most instances, however, 15 to 20 allergens will probably suffice.

For an allergist with long experience, a large practice and good facilities who can standardize the qualitative and quantitative aspects of the allergen extracts he is using, RAST will be a useful complement in diagnostic testing. It has been estimated that RAST can reduce the number of provocation tests administered by 80%, thus providing the allergist with more time for complicated tests. For an allergist with limited facilities, RAST is the diagnostic test of choice. With minimal inconvenience to the patient, a small blood sample can yield as much information as 20 or more skin test endpoint titrations or provocation tests.

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