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## Evaluation of In Vitro IgE Testing to Diagnose Atopic Diseases

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### INTRODUCTION

IgE is the most important mediator in atopic disease. Moreover, it is highly increased in some parasitic diseases. The clinical usefulness of IgE determination, however, is of limited value.

#### WHO Memorandum (5)

Diagnostic tests and procedures serve many purposes. They screen for the presence of occult disease. They help to confirm some diagnoses and reject others. Occasionally they uncover diagnostic possibilities that would explain elusive symptoms. Once clinical problems have been defined, they assist in treatment. They often provide helpful prognostic information. Beyond these explicit purposes, they have reassurance value both for the patient and the physician. They also generate income (1). That the use of diagnostic tests and procedures is excessive has been shown in numerous studies (2-4). The World Health Organization (WHO) has prepared a memorandum evaluating diagnostic tests in clinical immunology (5).

The recent expansion of clinical immunology has been accompanied by the introduction of a variety of immunological diagnostic tests in clinical laboratories. Owing to increasing demands from clinicians for such procedures, their use has often been exaggerated, and there is a general feeling that a better definition of the indication for such tests, made in relation to patients' needs, would be beneficial.

Obviously, the immunological tests, like any other diagnostic tests, can be graded according to their usefulness in the care of patients. Some tests are essential for diagnosis, prognosis, or monitoring of disease; many tests are useful but optional for routine investigations; other tests are of interest for research purposes. In addition, a number of immunological tests are useless in some circumstances. There is a consensus among immunologists that an effort should be made to reduce their share in the continually increasing cost of medical laboratory investigations. This requires self-limitation in the routine application of some immunological techniques without detriment to patient care.

## HISTORY

In 1921, Prausnitz and Küstner described the test that today bears their names, commonly referred to as the PK test. This test, similar to the passive cutaneous anaphylaxis test, consists of intradermally injecting 0.1 mL of whole or diluted serum from an allergic individual into an unsensitized individual and, after about 24 h, injecting the antigen into the same site. A positive test produces local itching and formation of a papule surrounded by a zone of erythema. The reaction reaches a maximum within 10 min, persists for about 20 min, and gradually disappears. The antibody responsible for the PK reaction was found to be thermolabile, losing its activity after heating to 56°C for 2 h, and, later, its sensitivity to reduction by sulfhydryl agents such as 2-mercaptoethanol, followed by alkylation, was observed (6). This serum component was—and still is—called “reagin.”

It was the work of Kimishige and Teruko Ishizaka, then located at the Children's Asthma Research Institute in Denver, that demonstrated in atopic individuals highly allergic to ragweed pollen that reaginic antibody must belong to a distinct immunoglobulin class. They named this class “gamma E globulin” be-

cause of its capability of producing erythema. Meanwhile, Hans Bennich and S. G. O. Johansson, in Uppsala (Sweden), isolated and described a previously unknown myeloma protein. Minute amounts of this immunoglobulin, labeled IgND after the patient's initials, could block the PK reaction. Raised levels of IgND were found in serum with reagin allergy. Later, Hans Bennich established the structure and amino acid sequence. In 1968 this immunoglobulin was officially designated IgE and recognized as a fifth class of human immunoglobulin at a conference at the WHO Reference Center for Immunoglobulins in Lausanne (Switzerland). IgE was also found in the serum of healthy individuals (7,8).

### STRUCTURE

IgE has the "Y"-shaped structure common to all classes of immunoglobulin (Fig. 1). It is composed of two light chains and two heavy chains that are covalently linked by disulfide bonds (Fig. 2). Light chains may be kappa or lambda and are indistinguishable from light chains of other Ig classes. Heavy chains, or epsilon chains, unique to IgE, contain five complete domains, each with an intradomain disulfide bond. Analysis of amino acid sequence provided evidence that a common ancestral gene duplicated to form an ancestral gene of mu and alpha chains, and a separate ancestral gene for epsilon and gamma chains. Because epsilon and gamma chains share a common ancestor, they lack the C-terminal octadecapeptide capable of binding J chain; thus IgE and IgG are always monomeric immunoglobulins. IgE has five domains with two interesting antigenic determinants: D1, which is heat stable, and D2, located closer to the carboxyl terminal, which is degraded by heating at 56°C for 2 h.

IgE contains 12% carbohydrate; this accounts for its molecular weight of 188,000–196,000. IgE is constrained by two widely spaced intraheavy chain bonds and two intradomain bonds with its C<sub>E</sub>1 domains; this is probably why IgG is more easily "salted out" of solution than IgE (9,10).

### BIOLOGIC PROPERTIES

These properties are summarized in Table 1. Of all the immunoglobulins, IgE is normally present in the smallest concentration, the nanogram range. Since it is capable of sensitizing individuals,

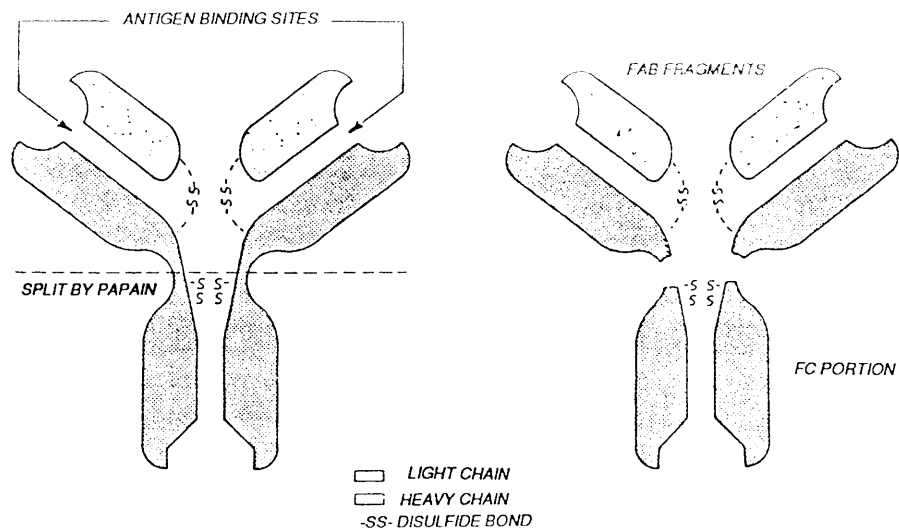


Fig. 1. Structural conception of immunoglobulin molecules, based on the work of Porter and Edelman. Reproduced with permission from Ishizaka, K. and Ishizaka, T. Structural and biologic characteristics of IgE, in *Allergy Unmasked: A New Understanding*. Pharmacia Diagnostics, 1979, Piscataway, NJ, p. 21.

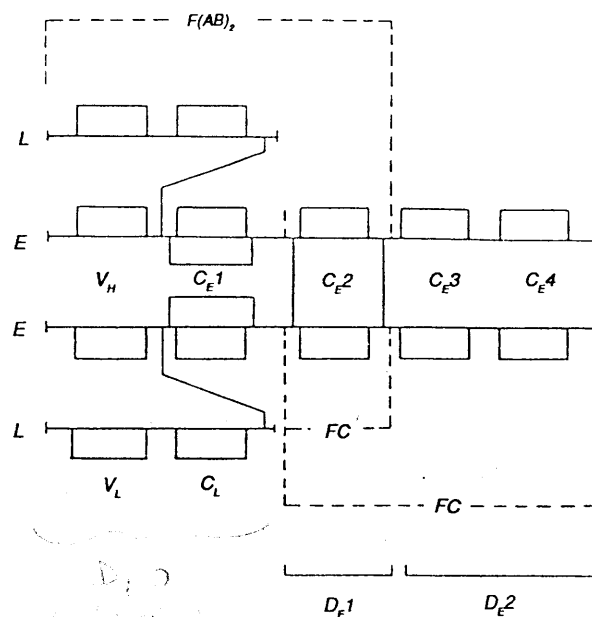


Fig. 2. Structure of IgE and sites of action of enzymes. Epsilon (heavy chains) have three major determinants. The class-specific  $\epsilon_2$  determinant is associated with the terminal portion of the Fc fragment, not included in the  $F(ab')_2$  fragment. The  $\epsilon_1$  determinant is present on the  $Fc'$  segment; the  $\epsilon_0$ , or idiotypic, determinant is in the  $Fd$  or N terminal segment. Reproduced with permission from Dorrington, K. J. and Bennich, H. H. *Physicochemical and conformational properties of immunoglobulin E*, in *Immediate Hypersensitivity: Modern Concepts and Developments*. Bach, M. K., ed., Dekker, New York, 1978, p. 47.

it has a high specific affinity for the cells involved in the allergic diathesis. Reaginic activity is dependent on the high affinity, but reversible interaction, of the Fc fragment with specific membrane receptors on the majority of tissue mast cells and circulating basophils. Cross-linking of bound IgE molecules (2 minimum, 3, or 5 optimum) by allergens initiates a chain of biochemical events that leads finally to the synthesis and/or release of pharmacological mediators that in turn give rise to the clinical symptoms of immediate hypersensitivity.

Not only is the affinity of the IgE molecules for basophils and mast cells highly specific, but it is extremely strong quantitatively; in other words, once an IgE molecule is bound to its target cell, it tends to remain so for a very long time. High affinity is a biologic compensation for the low concentration in serum and tissue fluids. This affinity has been quantified for IgE and basophils as  $10^9/M$ . Compared with  $10^5/M$  for IgG and monocytes, there is a 10,000-fold difference. Since the half-life of IgE in the circulation is only 2 to 3 d, IgE's high affinity also compensates for its rapid catabolism. Recently, IgE receptors have been described in both animals and humans on various other cells such as macrophages, monocytes, lymphocytes, and eosinophils (11,12) (Table 2).

Does IgE have a greater affinity or avidity (i.e., resistance to dissociation) in the allergic-atopic individual than in the nonatopic? Such a difference has not been found. There is an enhanced level of serum IgE in the atopic population that is related to increased synthesis. As a result, the target-cell receptor sites of allergic individuals are likely to be more highly saturated than those of normal individuals. In some nonatopic persons only about 20% of the basophil receptor sites ( $n = 40,000\text{--}100,000/\text{cell}$ ) are occupied by IgE molecules. In atopic individuals many more are occupied, approaching 100% saturation. This seems to be more a function of IgE levels than differences in affinity or avidity. However, there are some (20–30%) atopic persons with normal serum levels of IgE whose receptor sites are as highly saturated as those of individuals with elevated IgE levels (8).

Recently, high concentrations of salts and oses have been studied regarding the influence on denaturation of IgE owing to exposure to heat or acid pH (29). Results show that 50–100% of anaphylactic activity of rat and mouse IgE is conserved after heating at  $56^\circ\text{C}$  during 1 h and 30 min, and 5 h, respectively, if sera are treated in the presence of 1 M  $\text{MgSO}_4$  or 2 M mannose, glucose,

Table 1  
Biologic Properties of Immunoglobulins

| Property                     | IgG1         | IgG2         | IgG3  | IgG4  | IgA1  | IgA2  | IgM   | IgD   | IgE     |
|------------------------------|--------------|--------------|-------|-------|-------|-------|-------|-------|---------|
| Antigen binding sites        | 2            | 2            | 2     | 2     | 2     | 2     | 5-10  | 2     | 2       |
| Serum, mg/ml                 | 5-12         | 2-6          | 0.5-1 | 0.2-1 | 0.5-2 | 0-0.2 | 0.5-2 | 0-0.4 | 0-0.002 |
| Distribution in secretions   | <sup>a</sup> | -            | -     | -     | +++   | +++   | ++    | -     | +       |
| Half-life in days            | 23           | 23           | 16    | 23    | 6     | 6     | 5     | 3     | 2       |
| Fractional, turnover percent | 7            | 7            | 17    | 7     | 25    | ?     | 18    | 37    | 89      |
| Synthesis: mg/kg/d           | 25           |              | 3.4   |       | 24    | ?     | 7     | 0.4   | 0.002   |
| Placental transfer           | <sup>b</sup> | +            | +     | +     | -     | -     | -     | -     | -       |
| P-K reactivity               | -            | -            | -     | -     | -     | -     | -     | -     | +       |
| Complement fixation          |              |              |       |       |       |       |       |       |         |
| Classic pathway              | +++          | +            | +++   | -     | -     | -     | +++   | -     | -       |
| Alternate pathway            | -            | -            | -     |       | +     | +     | -     | +     | +       |
| Receptor sites on            |              |              |       |       |       |       |       |       |         |
| Macrophages                  | +            | -            | +     | -     | -     | -     | -     | -     | +       |
| Basophils                    | -            | -            | -     | -     | -     | -     | -     | -     | +       |
| Mast cells                   | -            | -            | -     | -     | -     | -     | -     | -     | +       |
| Neutrophils                  | +            | -            | +     | -     | -     | -     | -     | -     | -       |
| Platelets                    | +            | +            | +     | +     | -     | -     | -     | -     | +       |
| Lymphocytes                  | +            | <sup>c</sup> | +     | ?     | -     | -     | -     | -     | +       |

<sup>a</sup>-Negative<sup>b</sup>+Positive<sup>c</sup>?Uncertain

Table 2  
IgE Receptors  
on Cells Other than Mast Cells and Basophils in Humans

|                                                                                                                        | References |
|------------------------------------------------------------------------------------------------------------------------|------------|
| Macrophages and monocytes                                                                                              |            |
| Fc <sub>ε</sub> on human macrophages                                                                                   | 13         |
| Release of lysosomal enzymes from alveolar macrophages stimulated with anti-IgE or specific antigen in atopic subjects | 14         |
| IgE receptor of human monocytes                                                                                        | 15         |
| IgE-mediated cytotoxicity of human Monocytes                                                                           | 16         |
| Lymphoid cells                                                                                                         |            |
| Fc <sub>ε</sub> receptor on lymphoblasted cells                                                                        | 17         |
| Fc <sub>ε</sub> receptor on 1.5–4% of peripheral lymphocytes: 35–60% B cells                                           | 18         |
| Fc <sub>ε</sub> receptor on 1.5–4% of peripheral lymphocytes: 20–25% T cells                                           | 19         |
| Fc <sub>ε</sub> receptor on 1.5–4% of peripheral lymphocytes: 20–40% Null cells                                        | 20         |
| Eosinophils                                                                                                            |            |
| IgE receptors on human eosinophils                                                                                     | 21         |

or fructose. Presence of 2 M glucose prevents loss by heating of specific antigenic determinants of human or rat IgE Fc fragment. Denaturation of rat IgE antibodies induced by strongly acid pH is reduced if the medium contains 1 M MgSO<sub>4</sub> or 2 M glucose.

The actual hypothesis to explain these results is that an interaction exists between IgE-IgE molecules to prevent denaturation by coating and infiltration of mineral salts in hyperconcentration or oses in molar concentration. Protection is proportional: at 1 M protection is weak, but full protection is obtained at 2 M for oses (23). These findings complement the studies of Darrington and Bennich (24), who examined the spectroscopic changes on heating of IgE and its various fragments and concluded that the C-terminal (C<sub>E</sub>4) domain and/or the penultimate (C<sub>E</sub>3) were involved in receptor binding. Takatsu et al. (25) correlated the reduction of the intraepsilon disulfide bond adjoining the C<sub>E</sub>3 domain with loss of receptor binding activity. More recently, Darrington and Bennich (26) proposed

Table 3  
Immunologic Properties of IgE

| Reactions                             | Minimum concentration<br>of antibody | Activity |
|---------------------------------------|--------------------------------------|----------|
|                                       | $\mu\text{g/ N/mL}$                  |          |
| In vitro                              |                                      |          |
| Agglutination                         | $10^{-2}$                            | +        |
| C activation (classical<br>pathway)   | $>800^a$                             | -        |
| C activation (alternative<br>pathway) | $80^a$                               | +        |
| In vivo                               |                                      |          |
| PK in human                           | $4 \times 10^{-5}$                   | +        |
| PCA in monkey                         | $10^{-3}$                            | +        |
| PCA in guinea pig                     | $>100$                               | -        |

<sup>a</sup>Aggregated IgE

a model for the IgE Fc structure based on the localization of disulfide bonds and on analogy with X-ray crystallographic data on IgG structure. Kulczycki noted that IgE molecules devoid of N-linked oligosaccharides (prepared by culture of IgE-producing cells with tunicamycin) demonstrated receptor binding activity (27). Agglutination is not an important biologic function of IgE antibodies. High concentrations of aggregated IgE are unable to activate the complement cascade by the classic pathway but do so by the alternative pathway. Aggregated Fc fragments also activate the alternative pathway, whereas F(ab<sup>1</sup>)<sub>2</sub> fragments are much less active (28) (see Table 3).

### CLINICAL IMPLICATIONS

The concentration of IgE in the serum of healthy individuals is extremely low; a geometric mean in adults of 13 kU/L\* has been reported (29). Even in allergic patients "classical methods" fail to detect minute amounts of reagin, and Teruko Ishizaka quotes, "since there was no in vitro test sensitive enough to detect a minute amount of reagin at that time (1965), the only way to detect the presence of reagin was to use our own skins for PK reactions" (30).

\*One unit of IgE is approximately 2.4 ng.

Access to large amounts of isolated IgE, first from patient N. D. (31), allowed the production of specific antisera to IgE and the development of immunoassays for IgE and IgE antibody. The basis was laid for in vitro studies of the immune response in atopic allergy. The early discovery that patients with atopic asthma had on the average six to seven times higher IgE levels than nonatopic patients or healthy individuals (32) boosted the interest in studies of IgE levels in health and disease. Then RAST was developed for the determination of specific IgE antibodies and was found useful for allergy research as an in vitro diagnostic test in combination with case histories and skin tests (23).

### HIGH IgE IN SERUM INDICATES ATOPIC ALLERGY

Patients with atopic disease, including allergic asthma, and atopic dermatitis, commonly have moderately elevated IgE levels. In a few instances, most notably seasonal allergic rhinitis, it has been possible to demonstrate a significant positive correlation between the quantity of specific-IgE antibody and the intensity of patient symptoms (33). Such correlations provide indirect evidence that the IgE antibody plays a role in the pathogenesis of atopic respiratory diseases and suggest quantitation of IgE antibodies may be useful in the assessment of IgE-mediated respiratory symptoms. Serum-IgE levels are markedly elevated in approximately 90% of patients with atopic dermatitis (34). The degree of elevation correlates roughly with severity of disease (35). The massive elevations may result from nonspecific effects on IgE immunoregulation, since T cell dysfunction is also a feature of this disease (36). The contribution of IgE to the pathogenesis of eczema is unknown. IgE levels are higher in patients with atopic dermatitis and concomitant respiratory allergy than in those with eczema (34).

In patients with symptoms of chronic rhinitis or asthma, an elevated total serum-IgE level is compatible with an atopic etiology. Total serum-IgE levels are positively correlated with the duration, intensity, and multiplicity of allergic sensitivities. However, a normal serum-IgE level does not rule out a limited set of IgE-dependent allergies. In patients with rhinitis or asthma who have negative skin tests, a significantly raised IgE level supports a further search for allergic sensitivities. Conversely, a normal or low ser-

um-IgE level confirms that IgE-mediated mechanisms play little role in the pathogenesis of the patient's symptoms. There is appreciable overlap of serum IgE values taken from the individuals whose skin tests are positive and from those whose tests are negative. As a consequence, serum-IgE values less than 20 IU/mL or more than 250 IU/mL are the most useful in predicting the presence or absence of demonstrable IgE sensitivities to known aeroallergens (37).

Therefore, should total serum IgE determination be used as a routine screening procedure? In our increasingly cost-conscious world this does not appear to be warranted. However, in selected cases, it can be most valuable: in an infant with a positive family history of atopic allergy; in the child with troublesome symptoms that may or may not be caused by allergy. It can also be used to predict the risk of early onset allergic disease in asymptomatic infants. A serum IgE of 50 kU/L in a 1-y-old, asymptomatic child, for instance, would mean that the child has a 35% risk of developing serious allergy in the near future. If a child with a serum IgE of 50 kU/L already has had some possibly allergic symptoms (eczema, colic, diarrhea, wheezy bronchiolitis, recurrent URI, rhinitis), then the risk would be much higher (see Table 4).

### *IgE in Secretions*

The level of IgE in respiratory secretions is positively correlated with that found in serum (38). IgE detected in homogenized nasal polyps, and in most cases a higher IgE level is found in the polyp than in the serum from the same individual, suggests local synthesis. IgE is present in a variety of other tissue fluids, such as sputum, saliva, and gastrointestinal fluids, indicating that IgE is a widely distributed secretory antibody.

## METHODOLOGICAL CONSIDERATIONS

### *Total IgE (5)*

The recommended methods of measuring serum IgE (usually percent of U/L quantities) are ELISA techniques and solid-phase radioimmunoassay.

The common principle of the two methods is the use of insolubilized anti-IgE antibody. This reagent can be used either in a competitive-binding assay using radiolabeled IgE and IgE standard, or in a noncompetitive-binding assay using labeled anti-IgE. Like

Table 4  
Frequency of Major Allergy as a Function of Serum IgE Level  
in an "Unselected" Population of Children  
from Birth Through 2 Y of Age

| IgE<br>KU/L | Percent frequency <sup>a</sup><br>risk |
|-------------|----------------------------------------|
| <10         | 5                                      |
| 10-20       | 20                                     |
| 21-60       | 35                                     |
| >60         | 100                                    |

<sup>a</sup>In the absence of helminthiasis.

other competitive assays, the first one is subject to nonspecific inhibition by other serum factors, is of limited sensitivity, and is not recommended. The advantages of noncompetitive assays are increased sensitivity and precision, and the fact that they are usually free from interference by nonspecific serum factors. Although radioimmunoassays were used initially, ELISA has much potential and many advantages, especially for use in developing countries.

The main advantages of ELISA techniques are the avoidance of isotope markers, the long shelf-life of the reagents, and the evaluation by means of a photometer instead of the gamma-counter. The only limitation is that the ELISA techniques developed so far are not sufficiently sensitive to measure very low IgE levels.

Radioimmunoassays (and ELISA) are therefore the methods of choice in pediatric patients, in immunodeficiencies, and for analysis of cord blood, supernatants of cell cultures, and so on. For the higher sensitivity and reproducibility required for research purposes, suitable double-antibody assays have been described.

The values obtained must be compared with those of a control group matched according to age and geographical location. An International Reference Preparation is available from WHO.

I have tried to summarize in the Tables the practical characteristics of most Total IgE assays, presently commercially available (Table 5) (see pp. 14,15).

#### ***Indications for Measuring Total IgE (5)***

Determination of total IgE is not essential except in the diagnosis of the rare hyper-IgE-syndrome associated with eosinophilia and recurrent infections described by Buckley.

Determination of total IgE may be useful in differentiation of IgE-mediated from non-IgE-mediated disorders when this cannot be done by clinical means. Such disorders include perennial rhinitis, bronchial asthma, dermatitis, chronic urticaria, and food intolerance. However, IgE levels are of limited value since total IgE level can be in the normal range in IgE-mediated disease (e. g., hay fever) and it can be increased by nonatopic mechanisms such as infestations with parasites. The result, therefore, should be interpreted with caution, taking into account all other pertinent clinical information. Serial determinations (e.g., during immunotherapy) are usually of limited value, with the possible exception of allergic bronchopulmonary aspergillosis.

In prospective investigations, IgE elevation in early childhood may be a useful indicator of high risk for atopic diseases.

Determination of total IgE can be considered as a tool for research in certain immunodeficiencies and in atopic families.

### *Specific IgE Antibodies in Allergy*

Although most normal adults are found with small amounts of specific IgE antibodies to environmental allergens (foods, inhalants, viruses), the presence of readily detectable specific IgE to allergens is clearly correlated to allergic symptoms. Among the many immunoassays for specific IgE determination (40), the radioallergosorbent test or RAST as a radioimmunoassay or an enzyme-linked immunosorbent assay, represents the assay most frequently used and widely available commercially (41). RAST is an antiglobulin technique in which the allergen is linked chemically (CnBr) to a solid matrix. The sensitivity is quite good; with slight modifications to the conventional protocol, 10–20 pg/mL of IgE antibody can be determined in tissue culture fluid (31). Several laboratories have compared the results of IgE antibody levels obtained by RAST and the concentration of reaginic antibodies by PK titration, passive sensitization of human lung tissue or human leukocytes for many kinds of allergens. Excellent correlations have been obtained and, for allergens available in pure form such as codfish allergen DS22, RAST was found to be as sensitive as the PK test (42).

However, the uncontrolled use of RAST, recently encouraged by the availability of commercial kits, requires a critical reevaluation of some methodological and clinical limits of this solid-phase test (43).

## METHODOLOGY

Bonini et al. (44) have recently drawn attention to the influence of nonspecific binding on the cutoff point of the RAST. Using different allergen discs, the nonspecific binding of sera containing different amounts of IgE was related to the solid-phase allergen used: false negative or false positive results were found if the commercially supplied birch pollen discs were used as the only reference. Several groups, expressing their results with different methods (45-47), suggest that it is necessary to have, for each allergen system, several control samples (BSA, HSA, cord serum, dilutions of IgE myeloma, monoclonal antibody), and to express results as positive or negative on the basis of these internal controls, and of a number of sera from normal subjects.

Another pitfall inherent to all solid-phase methods is the absence of discrimination for the avidity of IgE antibodies. Binaghi recently sensitized rodents to ovalbumin. Specific IgE antibodies repeatedly coated the ovalbumin discs, in the same serum samples, until the fifth to twelfth incubation (48). Therefore, the use of (IgE) RAST for monitoring specific immunotherapy should not be recommended; one does not know if a variation in the RAST value is owing to a change in the total amount of IgE antibodies or in the percentage of highly avid IgE antibodies. Cheung and Reid (49,50) considered this problem and suggested ways to minimize the consequences (53).

The nature of the solid phase is also essential. We have demonstrated (51) a variability in our results according to the nature of the plastic plate used in our amplified microELISA technique; polyvinylchloride (PVC, Dynatech) binds better with pure allergen (e.g., honeybee venom), whereas the binding of crude allergen is better on polystyrene (Immulon I, Dynatech).

There are also interfering factors in the RAST for the detection of IgE antibodies (52-55), mostly IgG antibodies, which can be present in huge amounts when compared to IgE titers, in untreated subjects (e.g., beekeepers) or in patients undergoing immunotherapy. Removal of IgG antibodies with protein A-sepharose caused an enhancement of as much as 66% in IgE-RAST binding (56), but 6-9% of polyclonal IgE molecules express a binding activity for SpA through the Fab fragment (58), therefore, this procedure cannot be widely recommended, although with a different matrix (56), interference can be minimized.

Table 5  
Comparative Testing for Total IgE

| Manufac-<br>turer          | Pharmacia                                                                      | Pharmacia                                                    | Pharmacia                                      | Ventrex                                                      | Ventrex                                                                | Kallestad                                   |
|----------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------|------------------------------------------------|--------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------|
| Product<br>name            | Phadebas<br>IgE<br>PRIST                                                       | Phadezym<br>IgE<br>PRIST                                     | Pharmacia<br>IgE<br>RIA                        | VAST™<br>Ventrex PD<br>IgE RIA                               | Ventrex/<br>Sep Solid<br>phase                                         | Quantitope<br>IgE Kit                       |
| Method-<br>ology           | Solid<br>phase<br>RIA I <sup>125</sup> ,<br>paper<br>disks,<br>poly-<br>clonal | Solid<br>phase<br>EIA,<br>paper<br>disks,<br>poly-<br>clonal | Double<br>antibody,<br>RIA,<br>poly-<br>clonal | Solid<br>phase<br>RIA,<br>paper<br>disks,<br>poly-<br>clonal | Solid<br>phase<br>RIA,<br>coated<br>stick and<br>tube, poly-<br>clonal | Double anti-<br>body RIA<br>poly-<br>clonal |
| Kit size                   | 60 and 180<br>tubes                                                            | 60 tubes                                                     | 100 tubes                                      | 50 tubes                                                     | 100 tubes                                                              | 100 and 300<br>tubes                        |
| Shelf life                 | 4 mo from<br>date of<br>mfg.                                                   | 6 mo from<br>date of<br>mfg.                                 | 4 mo from<br>date of<br>mfg.                   | (cannot be<br>longer<br>than<br>4 mo)                        | (cannot be<br>longer<br>than<br>4 mo)                                  | (cannot be<br>longer<br>than<br>4 mo)       |
| Sensitivity                | 0.5 KU/L                                                                       | 0.5 U<br>IgE/mL                                              | <1 IU/mL                                       | 0.1 U/mL                                                     | 0.5 U/mL                                                               | 2 IU/mL                                     |
| Incubation<br>time         | 3 h and<br>over-<br>night or<br>1.5 h<br>and<br>2.5 h on<br>a shaker           | 3 h and<br>over-<br>night<br>and 60<br>min<br>at 37°C        | 3 h +1 h                                       | 3 h and<br>over-<br>night                                    | 1 h 37°C<br>and 4 h<br>37°C                                            | 5 h                                         |
| Pipeting<br>steps          | Addition<br>of disks<br>and 2<br>pipeting<br>steps                             | Addition<br>of disks<br>and 4<br>pipeting<br>steps           | 3 pipeting<br>steps                            | Addition<br>of disks<br>and 2<br>pipeting<br>steps           | Addition<br>of stick<br>and 3<br>pipeting<br>steps                     | 5 pipeting<br>steps                         |
| Washing                    | Yes 2 × 3                                                                      | Yes 2 × 3                                                    | No-centri-<br>fugation                         | Yes 2 × 3                                                    | Yes 2 × 3                                                              | No-centri-<br>fugation                      |
| Sample<br>size             | 100 µL                                                                         | 100 µL                                                       | 100 µL                                         | 100 µL                                                       | 50 µL                                                                  | 100 µL                                      |
| Color<br>coding            | Yes                                                                            | Yes                                                          | Yes                                            | Yes                                                          | No                                                                     | Yes                                         |
| Dilutions                  | Yes 1:10                                                                       | Yes 1:10                                                     | No                                             | Yes 1:10                                                     | No —un-<br>less over<br>500 u/mL                                       | Yes 1:10                                    |
| Standard<br>curve<br>range | 0.5–100 IU<br>IgE/mL                                                           | 0.5–60 IU<br>IgE/mL                                          | 0–1000<br>IU/mL                                | 0–50<br>IU/mL                                                | 0–500<br>IU/mL                                                         | 0–200<br>IU/mL                              |
| Standards                  | 7                                                                              | 6                                                            | 7 including<br>0                               | 6                                                            | 7                                                                      | 5                                           |
| Instrument<br>required     | gamma<br>counter                                                               | photometer<br>420 nm                                         | gamma<br>counter                               | gamma<br>counter                                             | gamma<br>counter                                                       | gamma<br>counter                            |
| Sample                     | serum or<br>plasma                                                             | serum or<br>plasma                                           | only serum<br>mentioned                        | serum                                                        | serum                                                                  | serum                                       |

(Continued)

Table 5 (Continued)  
Comparative Testing for Total IgE

| Manufac-<br>turer          | Kallestad                                 | Kallestad                                 | Hybritech                                         | Hybritech                                                | Clinical<br>Assays                            | Calbiochem-<br>Behring                                                             | Leeco                                                           |
|----------------------------|-------------------------------------------|-------------------------------------------|---------------------------------------------------|----------------------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| Product<br>name            | Quanti-<br>coat IgE                       | Quanti-<br>zyme<br>IgE Kit                | Tandem<br>IgE Kit                                 | Tandem-E<br>IgE (im-<br>muno-<br>enzym-<br>atic assay    | Gamma<br>DabH                                 | Enzygnost<br>IgE                                                                   | IgE-Quant                                                       |
| Method-<br>ology           | Coated<br>tube<br>RIA,<br>poly-<br>clonal | Coated<br>tube<br>EIA,<br>poly-<br>clonal | Solid<br>phase<br>bead<br>RIA,<br>mono-<br>clonal | Solid<br>phase<br>bead,<br>EIA,<br>mono-<br>clonal       | Double<br>antibody<br>RIA,<br>mono-<br>clonal | Double<br>antibody<br>EIA,<br>solid<br>phase<br>coated<br>tube,<br>poly-<br>clonal | Double<br>antibody<br>IRMA,<br>RIA,<br>poly-<br>mono-<br>clonal |
| Kit size                   | 100 tubes                                 | 100 tubes                                 | 80 and<br>320 tubes                               | 100 tubes                                                | 125 tubes                                     | 100 tubes                                                                          | 100 and<br>200 tubes                                            |
| Shelf life                 | (no longer<br>than<br>4 mo)               | (at least<br>6 mo)                        | (cannot be<br>longer<br>than<br>4 mo)             | 6-12 mo                                                  | (cannot be<br>longer<br>than<br>4 mo)         | (at least<br>6 mo)                                                                 | (no longer<br>than<br>4 mo)                                     |
| Sensitivity                | 2 U/mL                                    | 5 IU/mL                                   | 2 IU/mL                                           | 2.4 IU/mL                                                | 1.1 IU/mL                                     | 0.4 IU/mL                                                                          | 1-3 IU/mL                                                       |
| Incubation<br>time         | Overnight                                 | 3 h                                       | Over-<br>night<br>or at 4 h<br>at 37°C            | Rotate 2 h<br>RT + 30<br>min or 4 h<br>at 37°C<br>30 min | Over-<br>night<br>and 15<br>min               | 2 h and<br>2 h and<br>30 min                                                       | 1 h 37°C                                                        |
| Pipeting<br>steps          | 2 pipeting<br>steps                       | 5 pipeting<br>steps                       | Addition of<br>bead and<br>2 pipet-<br>ing steps  | Addition of<br>bead and<br>4 pipet-<br>ing steps         | 4 pipeting<br>steps                           | 4 pipeting<br>steps                                                                | 3 pipeting<br>steps                                             |
| Washing                    | Yes                                       | Yes 2 x 3                                 | Yes 1 x 2                                         | Yes 1 x 3                                                | No-centri-<br>fugation                        | Yes 2 x 3                                                                          | No-centri-<br>fugation                                          |
| Sample<br>size             | 100 µL                                    | 50 µL                                     | 50 µL                                             | 10 µL                                                    | 100 µL                                        | 200 µL                                                                             | 0.05 µL                                                         |
| Color<br>coding            | Yes                                       | Yes                                       | No                                                | No                                                       | No                                            | No                                                                                 | No                                                              |
| Dilutions                  | Yes 1:10                                  | Yes 1:5                                   | No—unless<br>over 300<br>IU/mL                    | No—unless<br>over 300<br>IU/mL                           | No                                            | Yes 1:10                                                                           | No                                                              |
| Standard<br>curve<br>range | 0-200<br>IU/mL                            | 5-200<br>IU/mL<br>calibrator              | 0-300<br>IU/mL                                    | 0-200<br>IU/mL<br>low<br>control,<br>high<br>control     | Blank<br>5-400<br>IU/mL                       | Blank<br>10-1000<br>IU/mL                                                          | 0-500<br>IU/mL                                                  |
| Standards                  | 5                                         | 5 + zero                                  | 6                                                 | 4                                                        | 7                                             | 6                                                                                  | 7                                                               |
| Instrument<br>required     | gamma<br>counter                          | spectro-<br>photo-<br>meter               | gamma<br>counter                                  | spectro-<br>photo-<br>meter                              | gamma<br>counter                              | photometer<br>492 nm                                                               | gamma<br>counter                                                |
| Sample                     | serum                                     | serum                                     | serum                                             | serum                                                    | serum                                         | serum or<br>plasma                                                                 | serum or<br>plasma                                              |

(Continued)

Table 5 (Continued)  
Comparative Testing for Total IgE

| Manufac-<br>turer          | 3MDS                                                   | Beckman                                            | Abbott<br>Labor-<br>atories                        | Staller-<br>genes                                       | Clinetics                                  | Nichols<br>Institute<br>Diagnostics                                                           | Scavo                                                 |
|----------------------------|--------------------------------------------------------|----------------------------------------------------|----------------------------------------------------|---------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------|
| Product<br>name            | Total IgE<br>FAST                                      | Epsilon<br>IgE                                     | Abbott<br>IgE<br>EIA                               | Staller-<br>stick                                       | <sup>125</sup> I IGE<br>RIA<br>Test Kit    | Alleagro<br>IgE                                                                               | UNI-<br>Fast                                          |
| Method-<br>ology           | Solid<br>phase<br>micro-<br>titer<br>fluoro-<br>metric | Solid<br>phase<br>beads,<br>EIA<br>mono-<br>clonal | Solid<br>phase<br>beads,<br>EIA<br>poly-<br>clonal | Nitro-<br>cellulose-<br>strip<br>EIA<br>poly-<br>clonal | Competi-<br>tion<br>RIA<br>poly-<br>clonal | Double<br>antibody<br>EIA solid<br>phase<br>bead<br>poly-<br>clonal<br>and<br>mono-<br>clonal | Coated<br>tip<br>EIA<br>poly-<br>clonal               |
| Kit size                   | 24 and 96<br>wells                                     | 100 tubes                                          | 100 tubes                                          | 10 strips                                               | 50-500                                     | 100                                                                                           | 48                                                    |
| Shelf life                 | 1 y                                                    | 1 y                                                | 1 y                                                | 1 y                                                     | cannot be<br>longer<br>than<br>4 mo        | 6 mo                                                                                          | 9 mo                                                  |
| Sensitivity                | 0.5 IU/mL                                              | 2.5 IU/mL                                          | 0.3 IU/mL                                          | 0.5 IU/mL                                               | 6.5 IU/mL                                  | 2.5 IU/mL                                                                                     | 5 IU/mL                                               |
| Incubation<br>time         | 1 h and<br>30 min                                      |                                                    | 1 h<br>30 min                                      | Over-<br>night on<br>shaker<br>+ 15 min                 | 90 min<br>+ 15 min                         | 20 min<br>on rotator<br>+ 20 min<br>or 40 min<br>+ 20 min                                     | 30 min<br>+ 20<br>min                                 |
| Pipeting<br>steps          | 4 pipeting<br>steps                                    | Addition<br>of beads<br>+ 4<br>pipeting<br>steps   | Addition<br>of beads<br>+ 5<br>pipeting<br>steps   | 2 (pipet-<br>provided)                                  | 3                                          | 2                                                                                             | 4                                                     |
| Washing                    | Yes 1 x 3                                              | Yes 1 x 3                                          | Yes 2 x 3                                          | Yes 2 x 3                                               | No-centri-<br>fugation                     | Yes 1 x 3                                                                                     | Yes 1 x 3                                             |
| Sample<br>size             | 20 µL                                                  | 20 µL                                              | 50 µL                                              | 1 µL                                                    | 20 µL                                      | 25 µL                                                                                         | 10 µL                                                 |
| Color<br>coding            | No                                                     | No                                                 | No                                                 | No                                                      | Yes                                        | Yes                                                                                           | Yes                                                   |
| Dilutions                  | No—un-<br>less<br>over<br>200<br>IU/mL                 | No—un-<br>less<br>over<br>400<br>IU/mL             | No—un-<br>less<br>over<br>200<br>IU/mL             | No                                                      | No                                         | No                                                                                            | No                                                    |
| Standard<br>curve<br>range | 0-3000<br>IU/mL                                        | 10-400<br>IU/mL                                    | 0-200<br>IU/mL<br>control                          | 0-10<br>IU/mL                                           | 0-600<br>IU/mL                             | 0-1000<br>IU/mL                                                                               | Blank +<br>100<br>IU/mL                               |
| Standards                  | 5                                                      | 5                                                  | 5                                                  | 5                                                       | 6                                          | 5                                                                                             | 1                                                     |
| Instrument<br>required     | fluoro-<br>meter                                       | spectro-<br>photo-<br>meter                        | spectro-<br>photo-<br>meter                        | none                                                    | gamma<br>counter                           | spectro-<br>photo-<br>meter                                                                   | spectro-<br>photo-<br>meter<br>or<br>micro-<br>reader |
| Sample                     | serum or<br>plasma                                     | serum                                              | serum/<br>plasma                                   | heparinized<br>plasma                                   | serum                                      | serum                                                                                         | serum                                                 |

## CLINICAL APPLICATIONS OF RAST

## A CAUTIOUS VIEW

The use of this test should be restricted to diseases in which a role for IgE has been well-documented, such as, allergic rhinitis, bronchial asthma, atopic dermatitis, anaphylaxis, and bronchopulmonary allergic aspergillosis. The analysis of data collected by 3076 RAST determinations (44) clearly shows the prevalence of a few allergens so that a wide RAST panel is not justified. The mean number of allergens tested in a series of 1443 patients referred to the Roman allergic clinics for "allergic symptoms" was 2.1 (45), it was 3.8 in the group of children studied by Foucard (57).

Inhalant allergens are readily implicated by RAST. There is a wide cross-reactivity between most grass pollen allergens (including the rye groups I, II, and III) (56); therefore, the study of multiple grass pollens is a waste of material. In most cases *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* crossreact with each other but not with house dust (60,61). For some antigens such as ragweed, a large amount of antigen-specific IgE antibody is found both because the total IgE level is elevated and because a large portion (13–50%) (62) of total IgE is antigen specific. A contrasting situation is present for some dust-sensitive asthmatics. Two studies (63,64) suggest that individuals predominantly allergic to dust may have relatively normal IgE levels. The fraction of the total IgE directed against the major antigen of the house dust mite averaged 12% (65). In many dust-sensitive asthmatics, the serum concentration of antigen-specific IgE is relatively low compared with patients with ragweed or grass allergy. An alternative assumption that will lead to a somewhat similar conclusion is that dust antigens bind to antigen-specific IgE antibodies with relatively low affinity (9). RAST with food allergens involves both methodological (poor purification, high nonspecific binding) and conceptual problems (allergenicity of digests, non-IgE-mediated mechanisms in food allergy) (66–68).

Usefulness of RAST in drug allergy is presently poor owing to the scanty number of allergens available. On the other hand, insect venom allergy has been an interesting field for detection of circulating specific IgE in patients with anaphylaxis, IgE antibodies to other allergens have also been detected, e. g., bacteria, azodyes, toluene diisocyanate (TDI), tetanus toxoid, and so on (45). RAST

Table 7 (Continued)  
Comparison of Tests for Specific IgE

| Manufac-<br>turer             | Kallestad                               | Fadal/Nale-<br>buff                        | 3 MDS                                                                              | MAST<br>immuno-<br>systems                           |
|-------------------------------|-----------------------------------------|--------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------|
| Color<br>coding               | Yes                                     | Depends<br>on manu-<br>facturer's<br>disks | No                                                                                 | No                                                   |
| Dilution<br>Standard<br>curve | No<br>A, B, C, D,<br>perennial<br>range | No<br>25 IU/mL<br>negative<br>ryegrass     | No<br>A, B, C, D,<br>perennial<br>control<br>0. 1, 0. 8,<br>4 & 20<br>IU/mL<br>IgE | No<br>No standard<br>curve<br>ryegrass;              |
| Standards                     | 4                                       | 1 + control                                | 4                                                                                  | no stand-<br>ards<br>only pos<br>and neg.<br>control |
| Instrument<br>required        | spectro-<br>photo-<br>meter             | gamma<br>counter                           | fluoro-<br>meter                                                                   | photo<br>cassette<br>and densi-<br>tometer           |
| Sample                        | serum                                   | serum                                      | serum                                                                              | serum                                                |

### FURTHER COMMENTS

The significance of changes in IgE, both total serum and specific antibodies, remains to be fully appreciated.

IgE might be an "innocent bystander" not directly involved in the disease. High IgE levels are found in patients with abnormalities of the T-cell system such as some infectious diseases, immunodeficiencies, Hodgkin's disease. The T-cell imbalance also involves subpopulations exerting helper or suppressor activity on IgE synthesis. The presence of specific IgE antibodies, even in large amounts, might be only a concomitant phenomenon of an imbalanced humoral response.

IgE has protective functions ("gatekeeper theory") (70). Specific IgE, mostly synthesized locally following stimulation by bacterial, parasitic, viral, or tumoral antigens, would induce the release of vasoactive mediators thereby "opening the doors" to a local influx

of other isotypic antibodies and actively involving lymphocytes, monocytes, macrophages, neutrophils, and eosinophils. IgE bound to basophils, macrophages, or mast cells (71) induces the release of platelet activating factor (PAF), increasing capillary permeability and tissue deposition of immune complexes (72). IgE, when aggregated, activates the alternative pathway of complement; antigen may be necessary to unmask the complement reactive piece on the Fc fragment of IgE (73). Complexed IgE has been found in the serum of patients with pollenosis and atopic dermatitis (74), and has been induced by challenge in patients with food allergy (75).

IgE is certainly the major serological marker of allergy in humans, but this phenomenon is probably one of its numerous—and fascinating—roles.

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