

Methods for Quantifying Allergen-Specific IgE Antibodies: Solid-Phase and Liquid-Phase Techniques

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The relative procedures and merits of IgE measurement by solid-phase (RAST) and liquid-phase (AlaSTAT) assays for IgE measurement are described. The wide normal range of total IgE and the imprecision of the solid-phase IgE antibody assay make the decision for cut-off values for clinical significance both arbitrary and difficult. Based on information theory, the RAST procedure could be made more informative if an arbitrary boundary was set at approximately 4 PRU/mL in a clinically allergic patient group. With present knowledge, it is not valid to assume that these tests by themselves should carry much weight as screening tests in reaching a positive diagnosis of atopic allergy. Fully automated IgE assays have become a reality due to computerization of data processing and the advent of new reagent technology such as fluid-phase allergens, and computerization of data archiving and retrieval. These advances will allow better disease association of IgE in populations, better quality control and, ultimately, better understanding of the significance of measuring these antibodies.

Keywords: IgE; IgE antibody tests; Fluid/liquid-phase allergens; Solid-phase allergens; RAST; CAP; AlaSTAT; Quality control; Information theory.

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Introduction

A new human serum immunoglobulin class possessing reagenic antibody activity was first demonstrated in 1966 (1) and was termed immunoglobulin E (2). This was confirmed soon after by the finding of a myeloma protein composed of immunoglobulin E (IgE), that allowed detailed biochemical analysis (3,4). One immediate consequence of this work was the development of assays to measure this immunoglobulin. A radioimmunoassay (RIA) (the radioimmunosorbent test, RIST) (5) demonstrated that IgE was present in serum in low concentrations: 1 ng/mL compared with mg/mL amounts for the major antibody classes. This RIST method was adapted to demonstrate in vitro the antibody activity of IgE to particular allergens. This sandwich RIA was called the radioallergosorbent test

(RAST) (6). More recent methods for the detection of IgE antibody include the CAP RAST (called ImmunoCAP® in the USA to avoid confusion with the College of American Pathologists) system (7), the Multiple Antigen Simultaneous Test, MAST (8), the Quidel allergy screen (9), the enzyme immunoassay testing system (10) and the AlaSTAT® (11). This last system measures IgE antibody activity to allergens in liquid phase, whereas the others use allergen bound to a solid phase so as to immobilize and measure circulating IgE antibody. All of the scientifically proven tests available for IgE measurement in the UK will be referenced as an Appendix, in the form of a procedural summary and tabulation of the main features of each test, to a report by the British Society for Allergy and Clinical Immunology Working Group on In Vitro Allergy Testing (12). The aim of this current report will be to consider the solid-phase RAST and the liquid-phase AlaSTAT methods.

Assay for IgE antibody: Liquid phase

A fluid-phase IgE enzyme immunoassay has recently been introduced (AlaSTAT-EIA®, Diagnostic Products Corporation, USA). This method claims advantages over the solid-phase assays. Firstly, it can be fully automated because there are no solid-phase reagents to manipulate. Secondly, IgE antibody activity to both protein and nonprotein allergens can be measured, because the binding of allergen to carrier matrix is done by a variety of coupling agents (listed below). Thirdly, no potentially dominant epitope is masked in the coupling procedure, because the different coupling agents can react with different parts of the molecule.

Preparation of the allergen reagent: Allergens are bound to branched, soluble polymer matrices such as dextran (70-400 Kda) and remain in solution. The fluid-phase allergen reagent is then biotinylated. Biotin is bound to the matrix rather than to the allergen, thereby avoiding unnecessary blocking of allergenic epitopes. The following coupling agents were selected according to the properties of the allergens:

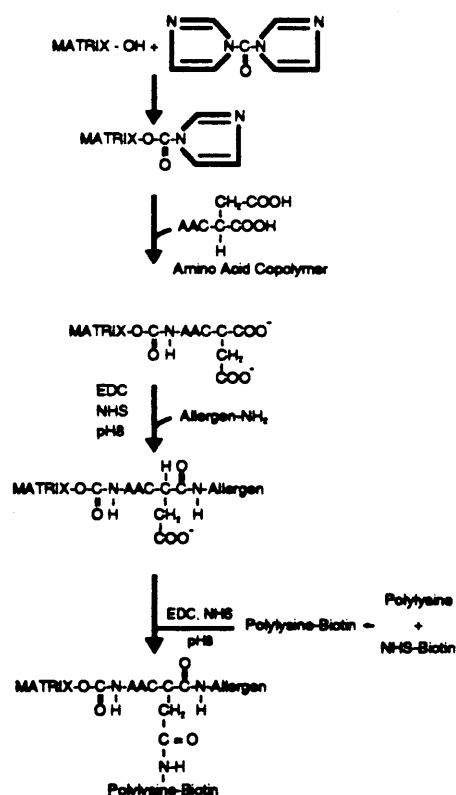
(a) 1,4-butanediol-diglycidyl ether (BDE) for proteins, nucleic acids, and carbohydrates.

(b) carbonyl diimidazole (CDI) for proteins and nucleic acids.

(c) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) for proteins and lipids.

Fig. 1 illustrates the method for preparing high concentrations of allergens bound with matrix-copolymers by CDI. The water-soluble dextran is first coupled to an amino acid copolymer (AAC), e.g., glutamic acid, ethyl glutamate. Then allergens are bound to this matrix by condensation reactions with EDC and N-hydroxysuccinimide. Lastly, the AAC part of the complex is biotinylated with polylysine-biotin. Once purified the Dextran-AAC.allergen-polylysine.biotin complex is stable for at least one year.

Procedure: The allergen reagent is allowed to react with the patient's serum in a test tube coated with biotinylated serum albumin. The purpose of the albumin coating is to provide a biotin anchor that will capture the biotinylated allergen reagent, together with any allergen-specific antibodies, when in the next step



EDC: N-ethyl-N-(3-dimethylaminopropyl) carbodiimide

NHS: N-hydroxysuccinimide Biotin

Fig. 1 The reaction sequence for 1,1'-carbonyldiimidazole (CDI) activation and coupling with amino acid copolymer and allergen. Reprinted from Ref. 11 with permission from Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, UK.

a streptavidin linker is added. After unbound serum components are washed away, any allergen-bound IgE is quantified by adding anti-IgE labelled with peroxidase and reacting with a suitable substrate (e.g., tetramethylbenzidine) to yield colored products. The total reaction time for this assay is 3 hours and the technique is very suitable for automation because of its simplicity.

Calibration system: A preparation of affinity-purified codfish-specific IgE antibody was used to standardize AlaSTAT semiquantitatively into Classes 0, 1, 2, 3, and 4. Class 4 corresponds to a concentration of at least 15 IU/mL of total IgE, calibrated against the WHO standard for total IgE 75/502. A secondary standard of grass pollen-specific IgE serum is used in the AlaSTAT assay to give an optical absorbance that is a linear function of IgE antibody concentration from 0.35 to over 60 IU/mL. A critical area of specific IgE measurement is at the low concentration range, and the AlaSTAT cut-off value of 0.35 IU/mL for Class 1 was determined by testing 40 definitely nonatopic serum samples. Two standard deviations above the mean value of the 40 sera corresponded to 0.2 IU/mL, but patients' samples with concentrations between 0.2 and 0.35 IU/mL are not considered by the manufacturer to be clinically relevant.

Assay for IgE antibody: Solid phase

The principle of the RAST test is that IgE antibodies in a serum react with an allergen that is covalently coupled by cyanogen bromide to an insoluble solid-phase matrix, such as a cellulose disc. The bound IgE is then quantified by binding radioiodinated anti-human IgE. The quantification of specific RAST IgE antibody is done by calibration against the PRIST total IgE assay system using the WHO reference 75/502. Five times the diluted RAST reference serum "A" should give the same counts as 10 IU IgE/mL in the PRIST (13). One Phadebas RAST Unit (PRU) is generally considered to be equal to one IU IgE/mL, i.e., 2.4 ng IgE/mL. The standard curve is constructed using a birch pollen reference serum and a birch pollen reference disc, and results are then expressed in PRU/mL.

The clinical value of the RAST was established quickly and many routine clinical immunology laboratories worldwide provided this test as an adjunct to the clinical diagnosis of allergy. In our laboratory, the performance of the RAST was estimated from longitudinal quality control specimens (Table 1) and an estimate of measurement error was made. If RAST grades are used to describe semiquantitatively the IgE antibody activity, then we found that a single specimen (mean value 0.66 PRU/mL, Table 1) assayed repeatedly could give results varying between negative (less than 0.35 PRU/mL) and Class 2. This makes it hard to jus-

TABLE 1 Longitudinal quality control of IgE antibody titers PRU/mL (mean, standard deviation, and coefficient of variation) of 83 consecutive RAST assays using replicate aliquots of the same sera. The span of RAST grades this represents is listed

	RAST allergen discs (product code)					
	Milk (f2)	Grass (g8)	Cat (e1)	Egg (f1)	Dog (e5)	Mite (d1)
Mean	0.66	1.4	2.7	5.7	8.4	11.4
S.D.	0.23	0.4	0.7	1.6	3.1	2.7
C.V.%	34.8	27.9	28.1	37.1	23.7	
RAST Grade	0-2	1-2	2-3	2-3	2-3	3

tify the practice of reporting Class 2 results as clinically significant and Class 1 as borderline. If we report as "positive" only the sera of Class 3 and above, we would have more confidence in our diagnoses and also have the added benefit of highlighting the dominant allergens in a subject who was seropositive to several.

A major development of RAST technology was the introduction of the Pharmacia CAP RAST system (14). This consisted of new instrumentation and software but most importantly a new solid phase consisting of a flexible hydrophilic carrier polymer encased in a capsule that can bind at least three times as much allergenic protein as the RAST disc. This greater capacity and the beta-galactosidase labelled anti-IgE (a mixture of rabbit polyclonal and mouse monoclonal antibodies) allow the assay to proceed more quickly and to have a range extended to 100 IU/mL for specific IgE and 2,000 IU/mL for total IgE. Unlike RAST, CAP RAST uses a total IgE calibration curve and so the results for specific IgE are reported as IU/mL instead of PRU/mL. In comparison studies it seems that there is not a linear relationship between IU/mL and PRU/mL; this makes it difficult to compare results obtained between the two methods. An estimate of measurement error using the CAP total IgE system can be made from the performance of the assay measuring longitudinal quality control specimens (Table 2).

Relationship between IgE, IgE antibody, and allergic symptoms in an unselected young adult population: A clinical questionnaire (available on request from the authors) highlighting allergy, and blood samples were taken from a class of second-year dental students. Total IgE and IgE antibody to house-dust

mite, grass pollen, cat and dog dander, and egg-white were measured by Pharmacia PRIST and RAST, and two independent, experienced doctors classified the subjects into clinical groups according to their questionnaire responses. There were 159 complete data sets. The relationship between IgE, IgE antibody, and clinical group is shown in Table 4.

Criterion for distinguishing between positive and negative tests: Given that clinicians like to be told that a diagnostic test is either "positive" or "negative," either "clinically significant" or "not clinically significant," the only way to cater to this demand is to choose a particular concentration of IgE or of IgE antibody that will serve as an arbitrary boundary between positive and negative tests. We have studied the PRIST and the RAST data obtained from the dental students mentioned above to see whether our diagnostic results could be made more informative by choosing some arbitrary boundary for a total IgE (PRIST) test, or any detectable nonzero value (i.e., 0.35 PRU/mL, or over) in a RAST test.

For this purpose, we have applied a simple formula borrowed from information theory to measure how much information a positive or negative diagnosis will produce pertaining to the four clinical categories the subject would be found in (atopy excluded, unlikely, possible, or typical). The formula consists of calculating the probability of each clinical category, multiplying it by its own natural logarithm, adding together the four answers for the four clinical categories, and changing the sign to make the total positive. This gives a quantity known as the uncertainty. It measures our uncertainty about the clinical category of the patient. After a diagnostic result is found, the probabilities and the uncertainty change. The change in uncertainty, averaged over all the subjects in the study, is taken as a measure of the information content of the diagnostic result. The chief merit of using this measure of information is that it has a simple additive property. Independent, unrelated bits of information have a total information content that is simply the sum of their parts.

With this standard to guide us, we tried 13 different criteria for deciding whether RAST tests should be reported as positive or negative, and we found that none of them were any more informative for the group as a whole than the customary criterion (i.e., greater than 0.35 PRU/mL). Similarly, we were unable to im-

TABLE 2 Longitudinal quality control (mean, standard deviation, and coefficient of variation) of 12 consecutive CAP RAST total IgE assays using a range of commercial standard sera with known IgE titers (IU/mL)

Standard sera (titers in IU/mL)	50	113	152	170	279	446	996
Mean	55.9	121.1	171.8	226.2	280.3	502.9	1176.3
S.D.	4.76	23.3	25.2	38.4	32.2	75.3	204.3
C.V.%	8.5	19.2	14.6	17.0	11.5	15.0	17.4

TABLE 3 The proportions of healthy nonallergic subjects and typically allergic subjects having A) total IgE levels, and B) RAST results, respectively greater and less than the values on the scale indicated

A) Total IgE IU/mL	% nonallergic subjects with results greater than indicated IgE levels	% typically allergic subjects with results less than indicated IgE levels
5	87	3
10	77	4
20	67	6
50	42	11
100	28	43
500	5	81
1000	3	92

B) RAST PRU/mL	% nonallergic subjects with results greater than indicated IgE levels	% typically allergic subjects with results less than indicated IgE levels
0	32	14
1	28	22
2	26	29
4	22	38
8	15	47
14	12	53

prove upon the criterion that a total IgE result should be reported as clinically significant if over about 56 IU/mL (i.e., the median value for the study group as a whole). If, as is likely in practice, the patients who clearly have no symptoms of atopy are excluded from diagnostic tests, then the calculations based on information theory indicated that the RAST test could, in fact, be made more informative if the arbitrary boundary was set at about 4 PRU/mL. This coincides approximately with the upper limit of the RAST Class 2 range (>3.5 PRU/mL) as defined by the manufacturers.

The fact that the existing criteria appeared to be fairly well chosen, however, does not mean that they are necessarily very useful or informative. In a sense, a positive RAST or a high IgE level in the absence of clinical information actually adds to our diagnostic uncertainty. Before we know anything about the patient, we can say that he is probably not atopic; after obtaining a positive PRIST or a high RAST, we are actually more equivocal: the probability of specific allergy is now close to 50 percent. Assays for specific or non-specific IgE no doubt have their uses, but we are doing a disservice to clinicians if we allow them to assume that these tests by themselves should carry much weight in reaching a positive diagnosis of specific allergy.

Application of IgE antibody measurement to clinical samples: Using the 3.5 PRU criterion, the distribution of positive IgE antibody (RAST) tests in a va-

riety of clinical conditions is shown in Fig. 2 and compared with that of the unselected young adult group. Clearly some of the disease groups have a high prevalence of associated IgE antibody, but some others, e.g., urticaria and irritable bowel syndrome (IBS), seem to have a lower than expected prevalence.

The extent to which IgE-mediated mechanisms play a role in the pathogenesis of urticaria and IBS is uncertain (15). Insofar as some nonatopic individuals (RAST-negative) can develop them whereas not all atopic individuals do, it would appear that IgE-mediated mechanisms are neither necessary nor sufficient for their development.

This lack of clear correlation in population studies is one source of frustration in our understanding of disease mechanisms. Despite this, we should be in a position to advise on the interpretation of these results. It might be appropriate at this point to highlight various other tests for allergy that have been discredited or have not been validated. These include the diagnostic tests used by practitioners of alternative allergy or clinical ecology. These practitioners are becoming more prevalent, which probably reflects increasing public awareness of allergy and the confusion or dissatisfaction of the general public with the current conventional management of allergy. Their methods include the provocation-neutralization test (the Miller technique), the leucocytotoxic test, hair analysis, the Vega test, applied kinesiology, and the auriculo-cardiac reflex method. These methods have been outlined in a report by the Royal College of Physicians Committee of Clinical Immunology and Allergy (16) which concluded that there is no convincing evidence for the value of alternative approaches. This should be brought to the attention of those interested in this field and the general public.

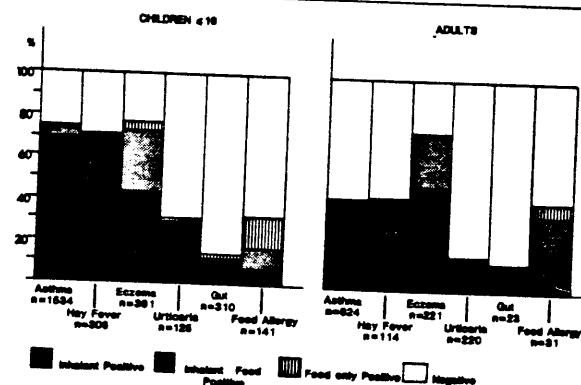


Fig. 2 The distribution of positive RAST (>3.5 PRU/mL) for any of four common inhalant (house-dust mite, grass pollen, cat and dog dander) or seven common food (egg-white, cows' milk, fish, wheat, oats, pea, peanut) allergens. The patient population was divided into adults or children at 16 years and the disease category and number of patients are listed under each column.

TABLE 4 Relationship between IgE level, IgE antibody (any positive RAST), and clinical group. The geometric mean total IgE level was 1.75 (55.9 IU/mL)

	RAST negative	RAST positive	All subjects	
Clinical group				
1. Nonallergic	53	25	78	Number of subjects
	1.39	2.09	1.61	Mean log IgE
	0.57	0.61	0.66	SD of log IgE
2. Allergy unlikely	12	7	19	Number
	1.30	1.84	1.5	Mean log IgE
	0.45	0.57	0.55	SD of log IgE
3. Allergy possible	9	17	26	Number
	1.40	2.03	1.81	Mean log IgE
	0.79	0.49	0.67	SD log IgE
4. Typically allergic	5	31	36	Number
	1.48	2.23	2.12	Mean log IgE
	0.76	0.42	0.54	SD of log IgE
All subjects	79	80	159	Number
	1.38	2.11	1.75	Mean log IgE
	0.58	0.52	0.66	SD of log IgE

Future considerations for IgE antibody measurement: An improved way to report RAST and total IgE results might be to append to each result a comment such as the following:

RAST Test Result:

House-dust mite. 2.0 PRU/mL.

Remarks: Not necessarily indicative of allergy: Among healthy subjects with no allergic symptoms, 26% gave one or more RAST results greater than 2.0 PRU/mL.

Tables from which statistical comments like this could be quoted are derived from the unselected population discussed above, as shown in Table 3. Other centers could derive their own data base for comparison.

Conclusion

The relative merits of the AlaSTAT and RAST have been considered (11,17) and both these correlated well with each other and with skin-prick tests, and showed good sensitivity and specificity. In both systems, the results are expressed as negative or Class 1-4 depending on binding relative to arbitrary divisions of the standard curve. This grading system is easily misinterpreted to imply corresponding severity of clinical disease. Such an inference is not proven. It remains that irrespective of how IgE is measured, it still does not appear to be absolutely necessary nor specific for allergic diseases; therefore, the relationship between IgE and disease still requires basic research. We believe that there is a need to collect a substantial archive of diagnostic RAST results, and to analyze these, relating them to clinical data where possible. To this end, an automated system that could incorporate a data handling and archive retrieval facil-

ity would make retrospective assessment, research, longitudinal quality control, and audit much easier.

The main developments of consequence in the future are likely to be fully automated systems for IgE measurement that can interface with a computerized reporting system. These developments are likely to be of value to the large central laboratories where results can be interpreted appropriately and proper advice and instruction can be given relating to IgE values in the local population incorporating the many other variables involved (18).

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