Radioallergosorbent test (RAST)—reliable tool or poor substitute?[1]

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An in vitro method, the radioallergosorbent test (RAST) has been developed for the detection of allergen-specific antibodies of the IgE class. Review of the literature shows that in comparison to skin testing, the RAST has a high degree of correlation (60% to 90% depending on the antigen); however, this method is not as sensitive as other tests (50% false-negative). The RAST is affected by blocking antibodies (IgG), resulting in false-negative values and high levels of IgE that bind on the allergen discs, giving false-positive findings. Because of these problems, RAST is somewhat limited for use in the clinical setting.

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Skin testing has been the traditional method for diagnosis of IgE-mediated allergic disorders. This bioassay is highly sensitive, cost-effective, and safe when used by experienced personnel. In 1967, Wide et al,[1] in Sweden, reported a new technique capable of detecting the minute quantities of allergen-specific IgE antibodies that circulate in the serum of allergic patients. This laboratory procedure, called RAST (radioallergosorbent test), utilized a solid-phase radioimmunoassay method. During the last decade this in vitro test has been refined and is now a commercially available laboratory test for clinical laboratories and, in kit form, for physicians' offices. Proponents of this new method claim that its results are more objective, safer for the patient, and not affected by symptoms or medication.

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Mechanism of the radioallergosorbent test

The radioallergosorbent test is used to identify the presence of IgE antibodies against specific allergens. Allergens are coupled to solid-phase polysaccharides (Sephadex) or linked to filter paper discs by cyanogen bromide. These solid-phase allergens are then exposed to serum containing antibodies to the allergens; during incubation, antibodies to the allergen, including IgE antibodies, bind to the allergen. After being washed to remove unbound antibodies, the solid-phase allergen-antibody complex is exposed to radioiodinated, purified anti-IgE antibody. This complex is washed again, and the bound radioactivity is measured. The radioactivity bound to the disc is an indirect measurement of the amount of specific IgE present in the patient's serum.

One of the greatest difficulties with RAST has been understanding the confusing scoring systems for determining test results. The present Phadebas RAST (PRU) scoring system uses four reaginic reference standards: standard A, which is pooled serum from patients highly sensitive to birch pollen allergen; standard B, which is a fivefold dilution of standard A; standard C, which is a fivefold dilution of standard B; and standard D, which is a twofold dilution of standard C.[2] To determine the RAST units (PRU/ml), references A, B, C, and D are assigned values of 17.5, 3.5, 0.7, and 0.35 PRU/ml respectively. In accord with this assignment, the counts for each reference serum versus assigned PRU/ml are plotted logarithmically. The PRU of each unknown sample can be determined by noting where its count falls on this reference curve. The PRU is only a relative term and does not quantitate specific IgE; however, Lundkvist[3] observed that the Phadebas reference B had binding ability similar to 10 units of IgE in the PRIST system (*Fig. 1*).

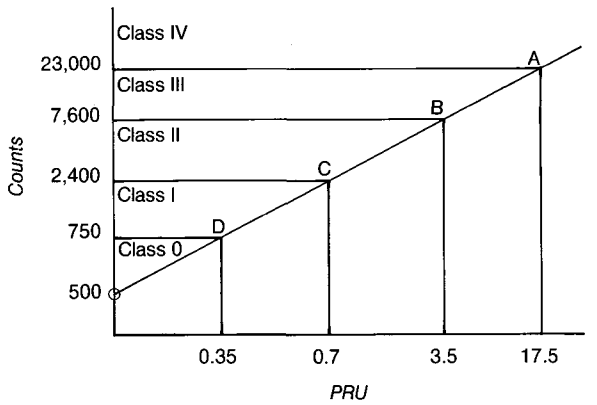

Fig. 1. Serial dilutions of reference standard A (PRU) are plotted against the radioactivity counts for each dilution.

In the Phadebas RAST scoring system, serum with bound radioactivity less than that obtained with standard D (which has been tested against its homologous birch disc) is considered to have an undetectable level of allergen-specific IgE. To correlate these values with results of allergy skin testing, the reference curve is divided into classes. Values ≤ 0.35 PRU/ml, class 0; values 0.35–0.7 PRU/ml, class I; 0.7–3.5 PRU/ml, class II; 3.5–17.5 PRU/ml, class III; ≥ 17.5 PRU/ml, class IV. The higher the score, the more specific IgE is present.

The principal criticism of the Phadebas RAST method is that sensitivity is set too high, thereby missing a significant amount of antibody, which may be clinically important. Muittari[4] found positive nasal and bronchial provocation responses in 50% of the patients with negative RAST test results. Deuschal and Johansson[5] also described 18 patients with allergic rhinitis (positive by provocation, history, and skin tests) who failed to have detectable levels of allergen-specific IgE by the Phadebas RAST. By diluting reference D fivefold, (the cutoff point was lowered to 0.2 PRU/ml), they improved the sensitivity of the tests. Their test data showed significant skin reactivity in all these patients. Nalebuff[6] observed that a tenfold dilution of reference standard D with human cord serum (to 0.1 PRU/ml) still gave a binding value greater than twice that obtained from negative controls. As a result of these observations and in response to many criticisms leveled at the test for its allegedly low sensitivity, a new modified RAST system was developed by Fadal and Nalebuff.[2] This system extends the initial incubation period from three to 18 hours. It increases the test volume from 50 to 100 μl. The discs are removed from the original tubes and placed in fresh tubes before counting. To keep counts constant from day to day despite variation in temperature and isotope decay, a time control is incorporated using 25 units of IgE, which reacts with the RAST isotope, and the time required to reach 25,000 counts is determined. In this assay the lower limit of detectable levels of allergen-specific IgE, or the cutoff level, is 1.5 times the binding of negative control. Therefore, the cutoff point is 750 counts. Serum between 250 and 750 counts is recorded as negative, indicating allergen-specific IgE antibody below detectable levels. The lower cutoff point

of this system is 0.02 units of IgE (*Fig. 2*). Because the bound radioactivity varies in direct proportion to the square root of the change in serum concentration, a reference curve is established by using 5X multiples of the cutoff point. This reference curve is then divided into classes to simplify reporting. The negative control has a count of 750. It is felt that samples falling into class I or 0 contain insignificant amounts of specific IgE. The higher the class, the more specific IgE is present. Proponents of the modified RAST scoring system allege that the diagnostic sensitivity of the test has increased, thus permitting identification of patients with lower, but clinically significant IgE antibody levels. Clearly, a physician faces a dilemma in determining the significance of the laboratory test in relationship to the clinical condition of the patient and in choosing the appropriate test.

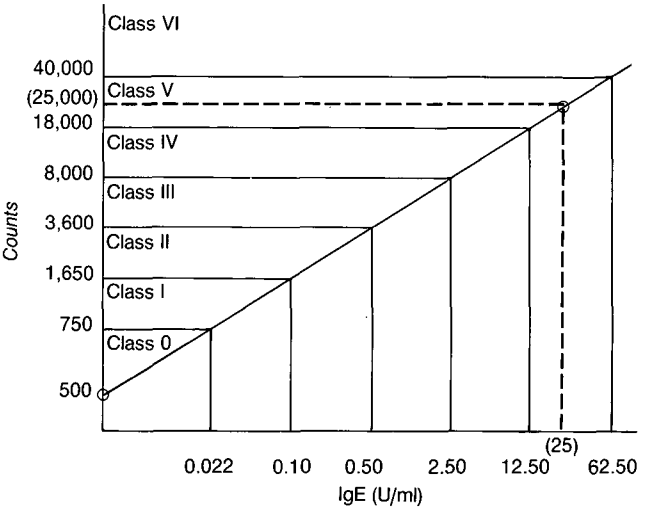

Fig. 2. The reference standard is a known serum sample containing 25 International units IgE per ml, which has been determined to have a radioactivity count of 25,000 cps. The lower cutoff point is set at 500 counts, which is a fivefold dilution of the reference standard. Serum samples with counts below 500 cps contain insignificant amounts of specific IgE. Test results are reported as classes, depending on the amount of specific IgE present.

Comparison between radioallergosorbent and other diagnostic tests for atopy

Initial studies of RAST have compared its ability to identify patients with allergy to danders, pollens, or foods with that of provocation tests and skin tests. Agreement between these procedures has ranged from 59% for allergies to house dust to 93% for fish allergies. Wide et al[1] showed a 68% agreement between results of 140 intradermal tests and RAST using 14 different antigens. Stenius and Wide[7] found 80% agreement between RAST and prick skin tests with extracts of house dust and house dust mite in patients with histories suggesting house dust allergy. In the Stenius study, patients with discordant prick test and RAST results usually had positive skin tests and negative RAST; in most cases the negative RAST results correlated with negative provocation results. These and other studies seem to indicate a high correlation between RAST and skin test procedures and/or provocation tests. However, agreement is not 100%, and herein lies the controversy. Proponents of skin testing maintain that RAST is not sensitive enough and will miss a significant number of allergies. Proponents of RAST maintain that skin testing is *too* sensitive and that the additional allergies identified by skin testing may not be clinically significant.

Recently an independent evaluation of these RAST systems was completed at the Mayo Clinic by Santrach et al[8] (*Table*). Forty patients with allergic rhinitis to at least one of nine different pollens (ragweed, English plantain, orchard, timothy, alder, elm, oak, birch, and maple) were studied by provocation testing, skin testing (prick and intradermal), Phadebas RAST, Fadal/Nalebuff modified RAST, and an expanded Phadebas RAST, which extended the lower end point for the standards. In this study, results of 92% of the antigen tests were positive by prick testing, and 100% of the intradermal tests were

Table. Comparison of allergy skin tests to RAST by provocation challenge for 50 antigens*

Test		Provocation	False-pos	False-nég	Negative control
	%+	%+	%	%	%+
Prick	46/50 (92%)	ND	ND	ND	ND
ID	50/50 (100%)	42/50 (84%)	8/50 (16%)	0/50 (0%)	ND
Ph RAST	37/50 (74%)	34/50 (68%)	3/37 (8%)	8/13 (61%)	0
Modified RAST	42/50 (84%)	39/50 (78%)	3/42 (7%)	3/8 (38%)	7/21 (33%)
Expanded RAST	45/50 (90%)	40/50 (80%)	5/40 (13%)	2/5 (40%)	0

* From data published by Santrach et al.[8]

positive. In the Phadebas RAST 74% were positive, in the modified RAST 84% were positive, and in the expanded RAST 90% were positive. In these patients, provocation testing showed a false-positive result (negative challenge/positive RAST or skin test) of 16% for the intradermal group, 8% for the Ph RAST group, 7% for the modified RAST group, and 13% for the expanded RAST group. Incidence of false-negative results (positive challenge/negative RAST or skin test) was not reported for the skin testing group. The provocation test showed a false-negative result in 61% of the Ph RAST group, 38% in the modified RAST group, and 40% in the expanded RAST group. Of the negative controls tested in these three systems, 33% had a positive modified RAST result in class I. None of the negative controls were positive in the Phadebas RAST group. The conclusion of this study was that all these RAST systems had less than a 10% false-positive incidence and that there were no significant differences in these three systems for this factor. However, the false-negative incidence was 60% in the Phadebas RAST. When a lower reference point was established, this false-negative incidence was decreased to 40%, close to the false-negative incidence of the modified RAST (38%). Therefore, the modified RAST assay does not afford significant diagnostic advantages over the Phadebas RAST.

Advantages of the radioallergosorbent test

Although RAST is not as sensitive as intradermal skin testing, it has many advantages when used in conjunction with or as an alternative to conventional test procedures. The results of RAST are not affected by allergic symptoms or depressed by medications used for symptomatic treatment of allergic disease. Skin testing may worsen the condition of patients who have serious symptoms of asthma, urticaria, or atopic dermatitis. Patients using antihistamines cannot be skin tested because these medications will suppress the results. Since RAST is done outside the patient, it is not affected by illness or medication.

The small serum samples used in RAST can be stored for comparison studies. These samples are representative of the patient's IgE state at the time they are obtained and, therefore, results will not vary. In monitoring the patient's atopic problem, RAST may be an objective way of measuring disease progress.

In patients with skin disorders such as dermatographism or eczema and in elderly patients who have poor skin responses, allergy skin testing may not be helpful in identifying significant allergens. In dermatographism skin trauma alone is enough to induce a large wheal and flare, thus giving a false-positive skin test result. The RAST, done independently of these skin conditions, is therefore unaffected.

For many patients, especially younger children, allergy skin testing is traumatic. Occasionally these patients become so anxious that allergy skin testing cannot be done. The RAST may be a good alternative method for them.

The RAST method is safer for many patients because it does not expose them to hazardous antigens such as animal dander, insects, fish, and nuts, which cause a high incidence of systemic reactions when ingested or used in skin testing by people sensitive to them.

RAST can be used to identify and quantitate allergens in complex mixtures. Two modifications of RAST have been used for allergen measurement, allergen extract standardization, or both.[9] In the first of these, termed the *direct* RAST, varying concentrations of allergens are attached to solid-phase particles and made to react with antibodies. As the quantity of solid-phase allergen increases, binding of labeled anti-IgE approaches a maximum. The potency of the extract is taken as the quantity needed for half-maximal binding in comparison with the arbitrary standard. The second procedure, termed RAST *inhibition*, establishes a competition between solid-phase and fluid-phase allergen in RAST. In the first step, fluid-phase allergen is added and competes with solid-phase allergen for IgE antibodies. As fluid-phase is increased, less IgE is bound to the solid-phase allergen and, therefore, less radioactivity is bound in the second step of the procedure. Analyses of short ragweed pollen, grass pollen, and Alternaria extracts have disclosed a 1000-fold difference in potency among materials claimed by manufacturers to have approximately the same potency. When RAST potencies were compared with potencies determined by skin-test end-point titration, positive correlations were obtained. Thus, RAST inhibition can be used to measure the potency of allergen extracts by comparing the inhibition produced by various extracts.

Lastly, a major advantage of RAST, a financial one, is that third party payers reimburse physicians for laboratory tests but not for skin testing. Since RAST requires little of the patient's and physician's time, the cost is relatively minimal.

Limitations of the radioallergosorbent test

Some major disadvantages of RAST severely limit its use in the majority of allergy patients. First, it is clearly less sensitive than allergy skin tests in detecting small amounts of clinically relevant IgE. Several studies have shown that false-negative results may be as high as 50% because the end-point sensitivity is set too high.[4,5,8] This problem is especially important in anaphylactic states such as penicillin or Hymenoptera allergy in which low amounts of IgE may be clinically serious for the patient.

A second problem is the limited availability of allergens for RAST. Although many antigens are listed, the actual number shown to be dependable and related to the atopic problem is small.[10] Many RAST laboratories offer antigens for foods (e.g., strawberries, lettuce) or drugs (e.g., codeine, contrast media), but there is no scientific proof that an IgE-mediated response is associated with adverse reactions to these materials.

A third problem is poor quality control of RAST and its use in many commercial laboratories. Hamburger[11] recently reported that measurements of IgE and RAST for grass pollen varied by as much as 200% for a known amount of test sample sent to 13 research and 10 commercial laboratories.

In patients receiving immunotherapy, Zeiss et al[12] found that blocking IgG antibodies bind to the allergen discs, giving artifically low values for RAST. This interference greatly limits use of RAST in monitoring the results of immunotherapy. Other studies have found that IgE levels > 500, IU/ml cause nonspecific binding of IgE to the allergen discs, resulting in a false elevation of RAST results.[13–15] Because of problems with IgG or nonspecific IgE, specialized laboratory procedures available to only a small number of sophisticated laboratories can be used to overcome these problems. For most RAST laboratories, these problems further narrow the spectrum of patients who can be helped by RAST methods.

Many patients and their referring physicians may find the waiting time for RAST results inconvenient. RAST done in a physician's office takes a minimum of two days to obtain results. If a serum sample is sent to an outside commercial laboratory, results may take as long as three to four weeks. By contrast, results of allergy skin tests are known immediately, alleviating much patient anxiety.

The last major objection to RAST is expense of the radiolabeled reagents, equipment, and laboratory technician's time, which can cost up to $3 per test antigen. Many commercial laboratories charge $12 per antigen. The recommended diagnostic screening panel of 20 RAST antigens can become a considerable expense for a patient or a third party payer. Thus RAST is not a cost-effective method for many atopic patients.

In summary, RAST, like skin testing, can only determine if specific IgE antibodies are present. All diagnostic procedures used in allergy testing must be correlated with the patient history and physical findings. Our responsibility must be to remain open-minded and informed about the advantages and limitations of each diagnostic allergy method. To remain dedicated to one method of diagnostic testing or to choose a less dependable diagnostic test solely because of its economic advantage does not provide the best in patient care. In some clinical situations RAST may be more helpful than other tests. In time, as solutions are found for problems with expense, sensitivity, and the availability of a wide range of dependable antigens, this method will become more effective. However, with present limitations, RAST may not be the first choice for diagnostic allergy evaluations.

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