

A Comparison of Acti*Tip™ Allerg*E™ and Allerg*ENS™ System to Phadebas RAST® and Phadebas IgE PRIST® in Assaying Total IgE and Specific IgE in Human Serum

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ABSTRACT

*Two in vitro systems of measuring total IgE and specific IgE antibodies were compared using serum of patients suspected of having allergic rhinitis due to inhalation of grass pollen. One in vitro system was the Phadebas PRIST and RAST. The accuracy of these tests has been well defined and they were used as the standard. The Acti*Tip Allerg*E and Allerg*ENS system is a new technique utilizing a similar solid-phase immunologic methodology with an enzyme-based detection system. It has some advantages primarily related to the mechanics of performing the test and marked reduction in time for obtaining results. The correlation between the Phadebas and Acti*Tip systems was highly significant for measuring total IgE and specific IgE antibodies for grasses in serum.*

Allergic rhinitis is often a diagnostic consideration in patients with nasal disease. A positive diagnosis of allergic rhinitis requires a history of exposure to antigen associated with symptoms compatible with allergic rhinitis and demonstration of specific IgE antibodies for the antigen. Traditionally, antibodies are indirectly

identified by a variety of skin testing procedures. Skin testing provides a rapid identification of the presence or absence of antibodies but carries some danger of serious reactions (anaphylaxis) or in very rare instances, death.¹ Proper use of the skin test requires considerable training and should be done only by those qualified through training.

In some patients skin testing is not feasible or desirable because of age (the very young or very old), poor quality of the skin (eczema or dermatitis), suspected marked sensitivity (potential threat of anaphylaxis), and personnel lacking proper training to do the testing. In these instances, an alternative method for identification of specific antibodies is desirable.

In 1967 Wide and associates² reported an *in vitro* solid-phase immunologic method for assaying specific IgE antibodies in serum. This method utilizes paper discs to which specific allergens are attached. The discs are incubated overnight with a small quantity of patient's serum. If there are IgE antibodies in the serum specific for the antigen on the disc, the antibodies become attached to the antigen on the disc. The discs are washed to eliminate the serum. An anti-IgE antiserum labeled with a radioactive isotope (anti-IgE-¹²⁵I) is then added to and incubated with the disc. The labeled anti-IgE antibodies added will attach to IgE

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bound to the antigen on the disc and because of the isotope, provide a means of measuring the amount of attached anti-IgE. The unattached anti-IgE-¹²⁵I is washed away so that the isotope bound to the disc can be counted in a gamma counter. The whole procedure including incubation times requires about 36–48 hours.

The usefulness of *in vitro* specific IgE assays was clarified by a position paper from the American Academy of Allergy and Immunology³ in which it was stated that for clinical purposes either *in vitro* or skin testing could be used to demonstrate the presence of IgE antibodies. Skin tests are recognized as being a more sensitive method of demonstrating IgE but also may be associated with more false positive reactions.

The major disadvantages of the PRIST (paper radioimmunosorbent test) for measuring total IgE and RAST (radio allergosorbent test) for detecting specific IgE methodology are that these laboratory procedures are time-consuming, results cannot be obtained for 36–48 hours, a considerable amount of laboratory space is required, the equipment is expensive, and disposal of radioactive material presents problems. I am reporting on a comparison of the Phadebas PRIST and RAST methodology to a new method, the Acti*Tip Allerg*E and Allerg*ENS system (Dexall Biomedical Laboratories, Gaithersburg, MD) for determining IgE antibodies which eliminates some of the disadvantages described above.

METHODS

Subjects

Serum was obtained from 35 individuals being enrolled in a pharmaceutical trial in the spring of 1988. All subjects had symptoms typical of allergic rhinitis occurring during the spring season. The antigens prevalent in the environment at the time were primarily grass and tree pollen. All sera were tested for anti-IgE antibodies to grass by the Fadal-Nalebuff modified RAST technique⁴ utilizing Pharmacia grass discs and Dexall's grass Acti*Tips. Thirty-three sera were examined for total IgE by the Pharmacia PRIST technique⁵ and Dexall's Allerg*E enzyme system.

Pharmacia Phadebas System

To measure PRIST technique, sera were diluted 1:9 with IgE-free diluent, and 100 μ l of each diluted serum was added to a test tube. A solid phase paper disc with covalently bound anti-IgE was added to each tube and incubated for 3 hours at room temperature. The tubes were washed three times with 2.5 ml of saline, and 100 μ l of anti-IgE-¹²⁵I was added to each tube and incubated overnight. The tubes were washed three times with 2.5 ml of saline to separate the bound from unattached radioactive isotope. The isotope bound to the

each disc was counted in a gamma counter for 1 minute. A standard curve for estimating IgE was made by preparing similar tubes containing standard solutions with known concentrations of IgE (1, 2, 10, 20, 50, and 100 IU/ml). To determine the total radioactivity added to each tube 100 μ l of the anti-IgE-¹²⁵I was counted.

For the RAST technique 100 μ l of each patient's serum was added to a tube. A mixed grass allergen paper disc (mixture of orchard, meadow fescue, perennial rye, timothy, and June/Kentucky blue grass covalently bound to the disc) was added to each serum tube. The mixture was incubated at room temperature overnight. The tubes were washed three times with 2.5 ml of saline, and 50 μ l of anti-IgE-¹²⁵I was added to each tube. The tubes were incubated overnight and washed three times with saline, and the bound isotope in each tube was counted in a gamma counter.

Dexall Acti*Tip System

The Acti*Tip System utilizes unique devices. The antigen (allergen for specific IgE antibodies or anti-IgE for total IgE measurements) is bound to the flared end of a plastic rod called Acti*Tip. Rods, which are about 3 inches long with a flared end 5 mm in diameter, are color-coded for the easy identification of different antigens (Fig. 1). Twelve of these rods fit in a holder in such a manner that each rod enters one well of a microstrip having an array of 12 wells (Fig. 2). Sera and reagents are added to the wells and the holders are placed over the strips so that the tips are immersed in

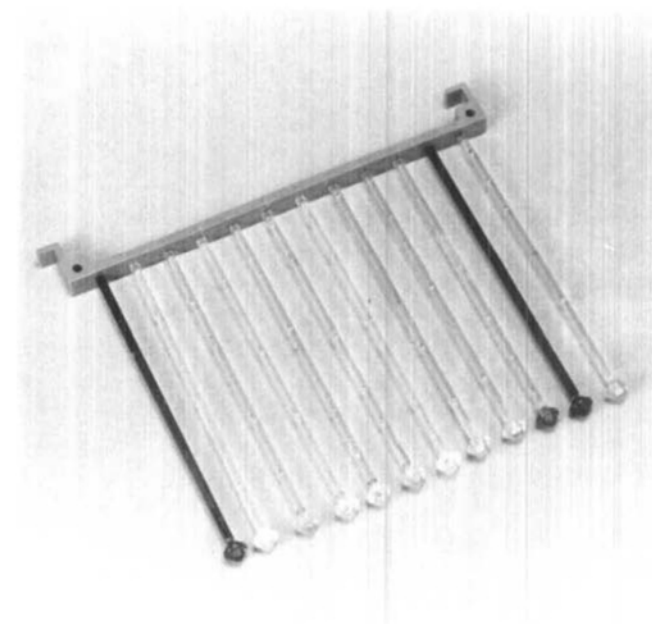


Figure 1. Acti*Tip rods displayed in a 12-unit holder. Rods are color-coded for different antigens, for a blank well, and for calibration procedures.

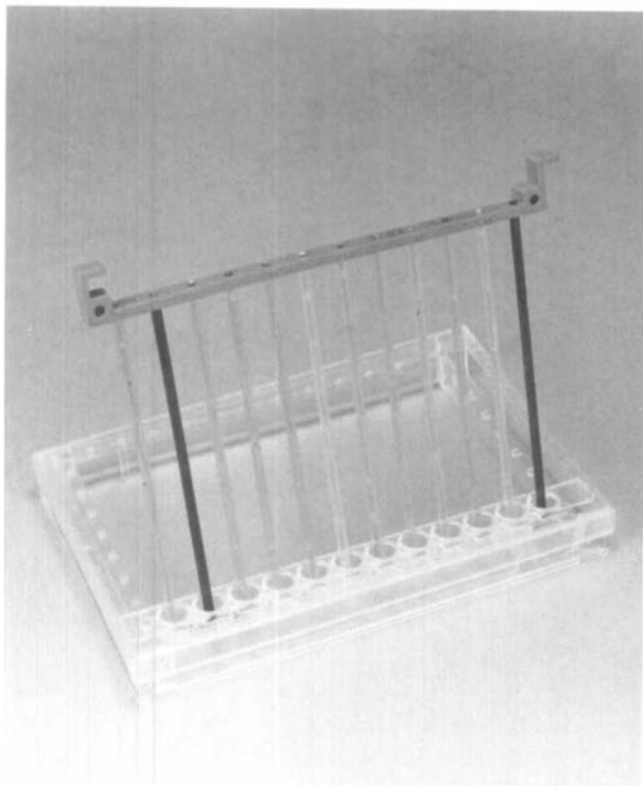


Figure 2. Acti-Tip rods and holder shown as they rest in a 12-well microstrip in the microstrip holder. Eight microstrips can be placed in the holder (for a total of 96 assays) as an aid in processing tests.

the wells. At the end of the procedure the 12-well strip is read in a spectrophotometer, a process taking about 15 seconds. The spectrophotometer (Fig. 3) is computer driven and a printout of the values of each well is obtained in either international units (IU) of IgE per milliliter or Allerg*ENS units (AU) per milliliter and classes of reactivity to allergens. Either total IgE or measures of specific IgE antibodies may be obtained by choosing the rods with the proper tips.

In the Allerg*E test for the measurement of total serum IgE 10 μ l of patient's serum was added to a well along with peroxidase enzyme-labeled anti-IgE antibody. The Acti-Tip rod tip coated with anti-IgE antibody was immersed in the well for 30 minutes at room temperature. The tips were washed for 1 minute three times in distilled water and then incubated for 15 minutes with a chromogenic substrate specific for the peroxidase enzymes. This resulted in the development of a green color in the test wells that was proportional to the amount of IgE in the sera. After removing the Acti-Tips from the wells, the microstrips were read in the spectrophotometer. Results in international units per milliliter were obtained by comparing the absorbance readings of unknown samples and of a single



Figure 3. Labdex ASRTM automated strip reader spectrophotometer for reading the 12-well microstrips. To illustrate size, a small micropipette is shown in front of the instrument. A 12-strip well is in place for assay in the front left-hand portion of the instrument. The paper printout is seen in the right rear area. The keypad contains labeled keys for initiating the various procedures.

standard calibrator with an IgE concentration of 100 IU/ml.

In the Allerg*ENS test for the measurement of allergen-specific antibodies 100 μ l of patient's serum was incubated for 2½ hours with mixed grass allergen (mixture of orchard, meadow fescue, perennial rye, timothy, and June/Kentucky blue grasses) coated to individual color-coded Acti-Tips. The tips were then washed for 1 minute in distilled water and then incubated for 1 hour with peroxidase enzyme-labeled anti-IgE antibody. Tips were washed again in distilled water for 1 minute three times and then incubated for 30 minutes with chromogenic substrate at room temperature. The intensity of the color developed was proportional to the amount of grass allergen-specific antibodies in the patient's serum. Quantitation of the antibodies was achieved spectrophotometrically, and results were expressed in arbitrary Allerg*ENS units and Allerg*ENS classes by comparing readings of the unknown sera to that of a reference standard calibrator (concentration of 10 AU/ml).

RESULTS

Sera from 33 patients were assayed for total serum IgE by two methods. Figure 4 compares the relationship between total IgE as measured by PRIST

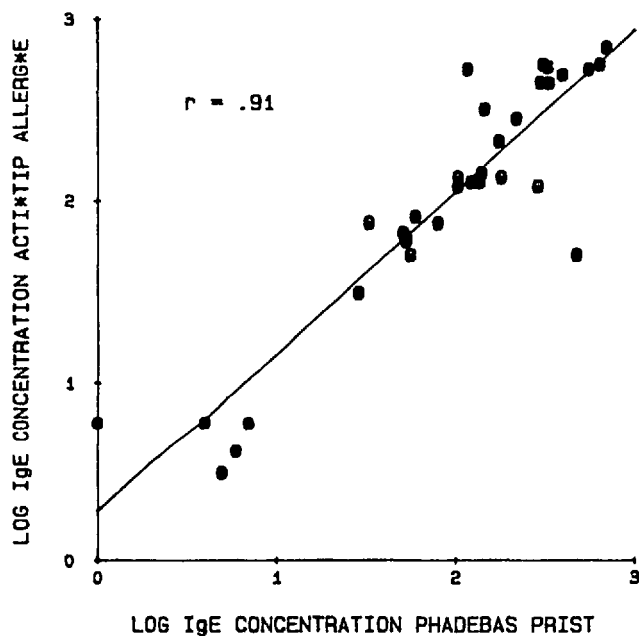


Figure 4. Comparison of PRIST total IgE and Allergy*E total IgE for 33 sera. The line of regression is shown.

(shown as the log of radioactive counts in each serum) and by Allerg*E Acti*Tip System (log of IU of IgE). The serum values as determined by PRIST ranged from 1 to 699 IU/ml while those for Allerg*E were 1.3 to 700 IU/ml. The line of regression is shown. The degree of correlation was measured by the product moment correlation coefficient (r). The r value 0.91 indicated a high degree of positive correlation between the two methods.

Sera from 35 patients were assayed for grass allergen-specific IgE antibodies by two methods. The composition of the mixed grass used in both assays was identical, as specified above. Figure 5 illustrates the relationship between RAST and Allerg*ENS values of allergen-specific IgE antibodies to mixed grass in patient's serum. Values for RAST (shown as log of radioactive counts) ranged from 221 to 18,000 radioactive counts per minute and from 1.1 to 232 AU/ml (shown as log of AU/ml). Again, a high degree of correlation ($r = 0.89$) exists between the two methods.

DISCUSSION

Two methods for measuring total IgE and specific IgE antibodies in human sera were compared. Excellent correlation exists between the two methods. The laboratory procedural steps (adding reagents, washing, etc.) required in both systems are about equal. On the other hand, the Acti*Tip system offers a number of advantages in that once rods are placed in holders, they may be maneuvered in multiples of 12 or less (one

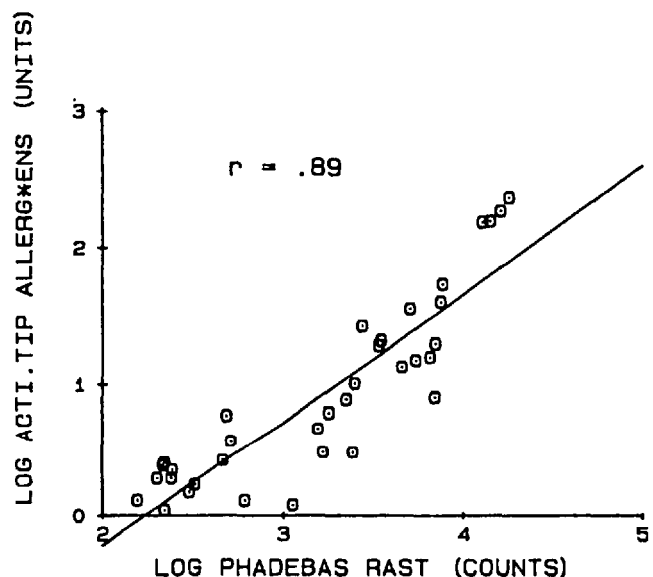


Figure 5. Comparison of mixed grass RAST and Allerg*ENS for 35 sera. The line of regression is shown.

Acti*Tip holder contains 12 rods) for the various steps saving a considerable amount of time. The washing of solid-phase Acti*Tips is simpler than filling and suctioning out individual test tubes. Although large automated gamma counters are comparable in speed to the automated spectrophotometer, they are much more expensive and much larger, and individual test tubes containing the discs to be read still have to be loaded into receptacles for counting. In the Acti*Tip system the microstrips (of 12 wells each) may be placed directly in the spectrophotometer for rapid reading. The space required for the Acti*Tip procedures is much smaller. The price of the computerized spectrophotometer is about one-tenth that of a comparable gamma counter. Another economy in time and expense is the use of a single standard calibrator for Allerg*E and one for Allerg*ENS at no sacrifice of accuracy as compared to multiple standards used in the Phadebas system. Perhaps, the greatest advantage of the Acti*Tip System is the marked reduction of the time required to obtain results. Total IgE (Allerg*E) can be performed in less than 1 hour. Results for specific allergens (Allerg*ENS) may be obtained in approximately 4 hours.

The value of determining total IgE and sensitivity to specific antigens is well recognized. The vast majority of patients avoid allergic workup because of cost, fear of skin testing, and in some cases because their disease does not seem significant enough to bother with choosing to simply rely on over-the-counter pharmaceuticals. On the other hand, many of these patients could be markedly benefitted by simple environmental control in addition to pharmacotherapy. Such control of necessity requires a knowledge of what sensitivities exist in a particular patient or even whether or not the patient is

atopic. These areas could better be defined by determining the presence or absence of IgE antibodies to environmental agents by such simple accurate noninvasive techniques.

ACKNOWLEDGMENT

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