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A simplified radioimmunoassay technique for measuring human IgE

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A simplified and sensitive solid-phase radioimmunoassay method for measuring IgE levels in human serum and tissue fluids is described. The test is accurate, reproducible, simple to use, and suitable for research as well as for routine clinical use. By appropriate dilutions of the test sera it is possible to achieve a working range from 1 to 10,000 U. of IgE per milliliter with the use of this test. Sera from non-allergic individuals gave a mean value of 80 U. of IgE per milliliter with 90 per cent having levels under 150 U. per milliliter, while an allergic group had a mean value of 290 U. per milliliter with 46 per cent having levels above 150 U. per milliliter.

There are a number of tests available for measuring IgE levels in human serum and various tissue fluids. The single radial diffusion technique¹ and the double diffusion technique² can be used to measure IgE levels in the microgram range. A further increase in sensitivity for measurement in the nanogram range can be achieved by use of a radioimmunoassay method. Gleich and associates³ have reported the use of a double antibody method for radioimmunoassay. Johansson⁴ used a radioimmunosorbent assay with the antibody combined to insoluble dextran, and McLaughlan and associates⁵ used the antibody coupled to cellulose. Although these radioimmunoassay methods are extremely sensitive, they are not easy to perform for routine clinical use.

Catt and Tregear⁶ found that plastic tubes can bind antibody by physical adsorption, and they utilized the ability of the adsorbed antibody to bind ¹²⁵I-labeled antigen as a simplified method of solid-phase radioimmunoassay. Bates and Garrison⁷ adapted this method, using disposable plastic plates to

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measure insulin levels in sera. We wish to describe a similar solid-phase radioimmunoassay method for measuring IgE levels in human sera with the use of antibodies adsorbed to the individual wells of a disposable plastic plate.

MATERIALS AND METHODS

Materials

Disposable flexible plastic microplates were obtained from the Linbro Chemical Co., Inc., New Haven, Conn. Each plate consisted of 96 individual wells with 0.2 ml. capacity.

Monospecific sheep antihuman IgE was purchased from Pharmacia Laboratories, Inc., Piscataway, N. J. The antiserum was prepared against Fc fragments and epsilon chains from E myeloma protein N.D. and absorbed with pooled human sera of low IgE concentration. It was found to be specific for IgE with the use of gel diffusion and immunoelectrophoresis and gave a precipitin reaction with E myeloma N.D., E myeloma P.S., and IgE from allergic sera. The antiserum contained 0.2 to 0.5 mg. of specific anti-IgE antibody per milliliter. The immunoglobulin fraction of the antiserum was prepared by precipitation at 33 per cent cold ammonium sulfate concentration, redissolved in 0.15M borate buffer, pH.9, and dialyzed against the buffer.

Plasma from human myeloma P.S. was kindly supplied by Dr. McIntyre and purified following the method of Ogawa and associates⁸ using DEAE cellulose chromatography and G-200 Sephadex gel filtration. The purified IgE was labeled with ¹²⁵I (Union Carbide Corp., Tuxedo, N. Y.) using chloramine-T after the method of Hunter.⁹ IgE, 10 μ g in 0.1 ml. of 0.5M phosphate buffer, pH. 7.5, was mixed with 1 mc. of ¹²⁵I in 0.1 ml. of buffer and 200 μ g of chloramine-T in 0.1 ml. of buffer for 3 minutes. Then 250 μ g/0.1 ml. sodium metabisulfite was added, and the mixture was diluted to 5 ml. with phosphate-buffered saline (PBS) and dialyzed against PBS. The efficiency of labeling was 50 per cent. The labeled IgE was diluted with one per cent human serum albumin (HSA) in PBS so that 0.05 ml. contained 1 ng. of IgE labeled with 0.05 μ c of ¹²⁵I.

Reference IgE standards prepared from pooled human sera were obtained from the WHO International Reference Centre for Immunoglobulins in Lausanne. Normal human IgE control serum was purchased from Pharmacia Laboratories, Inc.

The sheep anti-IgE globulin fraction was diluted 10 times with borate buffer, pH.9, to give a 1 mg. per milliliter approximate protein concentration, and 0.1 ml. aliquots were pipetted into the individual wells of plastic plates with the use of an Eppendorf micro-pipette with a disposable plastic tip. The plates were sealed with 3 inch wide cellophane tape and incubated overnight to allow adsorption to occur. The sheep anti-IgE was then removed and saved for future re-use. (We have used the antiserum up to 4 successive times with comparable results.) The plates were then washed 3 times with PBS, and the wells were filled with one per cent HSA in PBS and incubated for 6 hours to coat the remaining areas of the wells. After having been washed 3 times with PBS, the plates were either used immediately or sealed and stored at 4° C. for several weeks until needed.

The serum samples to be tested were diluted 10 times in PBS or 100 times in one per cent HSA in PBS, and 0.1 ml. aliquots were pipetted into individual wells and incubated for 6 hours. Then 0.05 ml. of ¹²⁵I-labeled IgE was added to each well, and the plates were sealed and incubated for a further 24 hours. They were washed 3 times with PBS, rinsed in cold tap water, and allowed to dry. Individual wells were cut out with scissors, and the bound radioactivity was estimated by use of a well-type gamma spectrometer.

A standard curve was prepared by testing doubling dilutions of WHO reference IgE serum of known concentration. Standard curves were also prepared for the Pharmacia control serum and for an allergic serum after calibration against the WHO IgE reference standard. The per cent inhibition was estimated from the counts per minute of standard samples of human IgE and the counts per minute of the control tests without cold IgE. All tests were done in quadruplicate and the mean value taken. The variation within each set of readings was generally less than 5 per cent. Rarely one reading will deviate markedly from the others, and in such cases the mean of the remaining 3 readings was used.

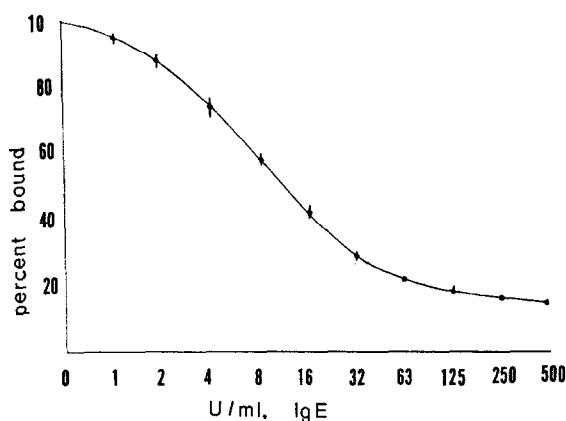


FIG. 1. Standard inhibition curve prepared using WHO reference IgE. The range of values for the Pharmacia standard and a known allergic serum is designated by the line through the result of WHO standard curve.

With the use of the technique described here, the IgE serum levels of 68 nonallergic and 54 known allergic individuals were tested. The allergic sera were obtained from the patients classified by the allergist as having bronchial asthma without apparent atopic dermatitis. The sera were tested undiluted or diluted 1:10 in PBS or 1:100 in one per cent HSA in PBS. When undiluted sera were used, the standards were made up in 6 per cent HSA to balance the protein concentration of undiluted serum samples.

RESULTS AND DISCUSSION

We observed that with the experimental method described here the absorbed sheep anti-IgE antibodies were able to bind up to 70 per cent of the added ^{125}I -labeled IgE. When IgE was present in the test serum, there was a decrease in the amount of ^{125}I -labeled IgE bound, and with increasing amounts of IgE present there was a corresponding progressive decrease in the amount of ^{125}I -labeled IgE bound. The standard curve showing the percentage inhibition of ^{125}I -labeled IgE is shown in Fig. 1. Under the conditions described here, there is a working range between 1 U. per milliliter to 100 U. per milliliter, IgE with the most sensitive range between 2 and 30 U. per milliliter. By testing the serum undiluted, and diluted 1:10 and 1:100, the working range is extended from 1 to 10,000 U. per milliliter.

The microplate test has a high degree of reproducibility, for standard curves prepared from different plates run simultaneously gave identical results. There were, however, slight differences between the standard curves estimated for plates prepared at different times, and hence a standard curve should be prepared for each batch of plates tested to ensure accurate readings. The inhibition curves prepared for the WHO reference serum, the Pharmacia control serum, and the allergic serum showed almost identical results, indicating that the test was measuring common determinants of the various IgE samples.

Upon testing a randomly selected population of adult nonallergic individuals, we obtained IgE serum values ranging from an absence of detectable IgE to 1,000 U. per milliliter, with a mean of 80 U. per milliliter and a median value of 20 U. per milliliter. Adult allergic individuals showed an IgE range from 5

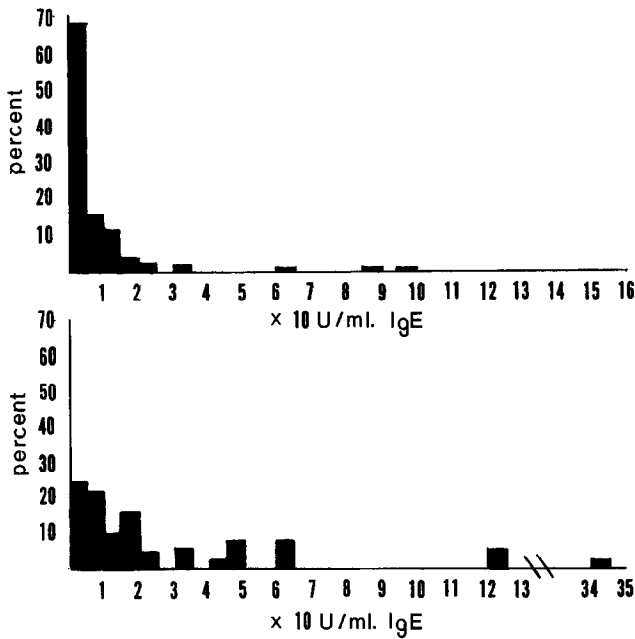


FIG. 2. Top, Distribution of IgE levels in a nonallergic population. Each bar represents an increment of 5 U. of IgE per milliliter. **Bottom,** Distribution of IgE levels in an allergic population.

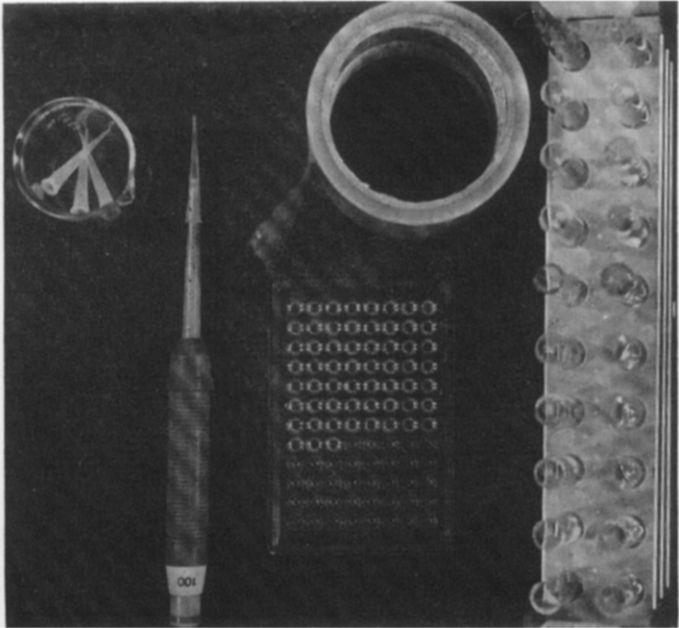


FIG. 3. Equipment used in the microplate test.

to 3,400 U. per milliliter, with a mean of 290 U. per milliliter and a median value of 130 U. per milliliter. The distribution of IgE levels in the two groups is shown in Fig. 2. There is some difference between the groups, as only 10 per cent of nonallergic individuals had IgE levels above 150 U. per milliliter, while 46 per cent of allergic individuals had IgE values above this level. Although it is generally believed that 1 U. will closely correspond to 1 ng. of IgE, Bazaral and Hamburger¹⁰ recently reported that 1 U. of IgE corresponded to 2.42 ng. of purified myeloma IgE. Using IgE myeloma protein as standard, Johansson⁴ has reported the mean concentration of IgE in normal serum to be 248 ng. per milliliter with confidence limits of 61.4 to 1,000 ng. of IgE per milliliter, and Gleich and associates³ reported the mean concentration of IgE in serum to be 179 ng. per milliliter with a 94 per cent confidence limit range of 6 to 780 ng. per milliliter. The different values obtained by various workers may be due to the different assay methods used. There is, however, fair agreement among the reports upon the general range and distribution between the allergic and nonallergic groups.

We have found the microplate method of IgE estimation described here to be accurate, reproducible, inexpensive, and simple to use. The actual handling time is short, and numerous samples can be tested simultaneously. We therefore consider this method to be suitable for research as well as for routine clinical use.

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