

Measurement of serum IgE by a one-step single radial radiodiffusion method

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A relatively simple, reproducible method for the measurement of serum IgE has been described. It is practical and could be performed in most laboratories and the results obtained in a few days. A known reference serum standard (such as the WHO standard) could be employed, and IgE myeloma protein would not be needed. The IgE values in serum may possibly be of diagnostic value in differentiating atopic dermatitis from other dermatoses and atopic versus nonatopic rhinitis and asthma. The studies presented confirm the work of previous investigators as to elevated IgE serum levels in atopic eczema, parasitic infestations, and, to a lesser degree, respiratory allergies. The additional (coincidental?) findings of increased serum IgE levels in patients with chronic infection, eosinophilia, and trichinosis need further elaboration.

Since the detection of IgE as the carrier of reaginic activity,¹ two basic methods of measuring small quantities of IgE in serum have been reported. These techniques vary in sensitivity as well as in complexity. One technique, which employs inhibition of radiolabeled IgE binding with IgE protein, was first described by Johansson, Bennich, and Wide² and subsequently modified by Salmon³ and by Gleich, Averbek, and Swedlund.⁴ As little as 5 ng., 1 ng., and 0.1 ng. per milliliter, respectively, could be detected by these investigators. The classical Mancini single radial diffusion technique⁵ can detect levels of immunoglobulins of only 1,500 ng. per milliliter or greater. Rowe⁶ was able to increase the sensitivity of this method by covering the sheep antibody-containing plate after diffusion of the antigen with ¹³¹I-labeled rabbit globulin containing antibody to the sheep immunoglobulin; 40 ng. per milliliter or more of IgE could be detected by this technique. This method has been used by other investigators.⁷⁻⁹

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We have recently reported another method for measuring IgE.¹⁰ The details of this one-step single radial diffusion method using ¹²⁵I-labeled antiserum to human IgE will be described. This technique is relatively simpler than the other procedures, and the results are more quickly obtained. Results of the application of this technique will be presented.

MATERIALS AND METHODS

IgE protein

Plasma from Patient P. S. with IgE myeloma¹¹ was obtained through the courtesy of Dr. Ogawa. A WHO reference serum, Code No. 68/344, containing 20 μ g per milliliter of IgE* was supplied by Dr. D. S. Rowe, WHO International Reference Center for Immunoglobulins, Lausanne, Switzerland.

Fractionation of goat antiserum to Fc of IgE

The goat antiserum containing antibodies only to the class-specific antigen of IgE was the same as that described previously.¹² This antiserum was fractionated by DEAE-cellulose chromatography with the use of phosphate buffer of pH 8.0 and concentrations of 0.01 and 0.05M. Elution with the 0.01M buffer yielded a small amount of γ G₂, while the 0.05M buffer eluted most of the goat γ G₁ fraction. Both fractions were found suitable for quantitation of IgE, but for the experiments described in this report only the γ G₁ fraction was used.

Radiolabeling of γ G₁ fraction

The serum fraction was labeled with ¹²⁵I (International Chemical & Nuclear Corporation, Irvine, Calif.), using in principle the method described by Greenwood, Hunter, and Glover.¹³ In brief, 10 mg. of goat γ G₁ protein, containing approximately 10 per cent anti-IgE(Fc), was pipetted into a 10 ml. beaker together with 0.1 ml. of a solution of potassium iodide (1.66 mg. per 100 ml. borate buffer, pH 8.0). With the use of suitable precautions, 0.025 ml. of Na¹²⁵I (2.5 mc. of activity) was added. A solution of chloramine-T (70 mg. per 100 ml. of borate buffer), 0.1 ml., was added. The mixture was stirred for at least 5 minutes, but not more than 10 minutes, at 4 to 6° C. (coldroom).

Sodium metabisulfite solution (52 mg. per 100 ml. distilled water), 0.1 ml., was added to stop the reaction. After 2 minutes of stirring, free iodine was removed by passing the mixture through Sephadex G-25 equilibrated with phosphate buffer containing one per cent bovine serum albumin (BSA). The specific activity of the radiolabelled antiserum was about 200 μ c per milligram of protein.

One-step method for determination of IgE

1. Agarose gel (Fisher Scientific, Rochester, New York), 0.85 per cent, was suspended in barbital buffer of pH 8.2, ionic strength 0.05, and heated until dissolved. Merthiolate was added to 0.01 per cent. A 30 ml. portion of the gel was allowed to cool to 56° C. in a water bath.
2. A selected dilution of the radiolabelled γ G₁ fraction of the goat antiserum in 1 per cent BSA in phosphate buffered saline (0.3 ml., pH 7.2), was mixed with the agarose solution. The mixture was allowed to stand at 56° C. for an additional 30 minutes to ensure homogeneity.
3. The agarose gel was then poured onto a 5 × 7 inch glass slide and allowed to set.
4. Twenty-eight wells, 5 mm. in diameter, were punched out of each plate. Serial dilutions with one per cent BSA of a reference serum (30 λ) were placed in ten of these wells to obtain a standard curve. The remaining wells were filled with various sera (30 λ) whose IgE values were to be measured.
5. The agarose gel plates were incubated for 2 days in a 37° C. moist chamber. The plates

*This value was checked through the courtesy of Dr. S. E. Salmon, University of California, San Francisco, Calif. 94122.

were washed with buffered saline, pH 8.0, for 2 days, and in distilled water for one hour. After drying, the plates were exposed to x-ray film (Kodak NS-54-T, National X-ray Products, Hackensack, N. J.) for 24 hours and the film developed.

6. Images on the x-ray film were projected onto rectangular coordinate paper by means of a photographic enlarger. Outer diameters (D) of the rings of the standards were measured and values of D^2 plotted on full log paper. The rings of the test sera were similarly measured and the values determined from the standard curve.

Sera selected for determination of the IgE levels

Sera were collected from 121 patients with the following allergic problems: atopic dermatitis, allergic rhinitis and/or bronchial asthma, drug reactions, serum sickness from antirabies horse serum, stinging insect sensitivity, and a variety of other allergic manifestations. None of the respiratory allergy patients had received specific immunotherapy. A random group of 183 sera was obtained from blood bank donors, the chemistry laboratory, and the prenatal clinic. No clinical information as to the prevalence of atopic disease in this group was available at the time the sera were collected.

Sera from hospitalized patients with other miscellaneous disorders were also collected for study. All sera were stored at -20° C. until used. Dr. Naspitz of Sao Paulo, Brazil, kindly sent us 9 lyophilized sera from patients infested with *Ascaris*.

RESULTS

One-step single radial immunodiffusion test

To determine the optimal conditions for measurements of IgE, agarose plates containing various dilutions of the labeled antiserum were prepared and known concentrations of myeloma IgE or the reference serum were applied. A 1:9 dilution of this antiserum produced the clearest diffusion rings, and it was chosen for preparation of plates for determination of IgE of unknown samples. Fig. 1 represents the contact print of a developed x-ray film. IgE in concentrations from 10,000 to 19 ng. per milliliter was used. The precipitation rings are clearly visible. The concentration of 39 ng. per milliliter is easily detectable with the use of the described conditions. The rings marked A to Q represent 17 sera taken at random. By using larger or deeper wells or further diluting the antiserum incorporated in the agarose, one could detect 20 ng. per milliliter. Below this value the diffusion rings were not clearly defined. Fig. 2 represents a standard curve of the diameter of the rings squared against known concentrations of IgE in nanograms per milliliter. This curve could be applied only for the determination of IgE in the conditions described, i.e., the antiserum diluted 1:9 and wells of 5 mm. Different dilutions of labeled antiserum or changes of the diameters of the wells would require other standard curves.

Concentrations of IgE in human sera

The range of IgE levels in various categories is summarized in Table I and diagrammatically shown on the scattergram (Fig. 3).

Random samples (age 18 to 67 years). One hundred and eighty-three sera were obtained from: blood bank donors (105), the chemistry laboratory (49), and the prenatal clinic (29). The IgE concentrations of this entire group were spread out over a range from < 20 to 8,850 ng. per milliliter. The mean value was 218 ng. per milliliter. One hundred and sixty-six of the 183 sera (90 per cent) had levels below 300 ng. per milliliter, and 176 (96.2 per cent) were under

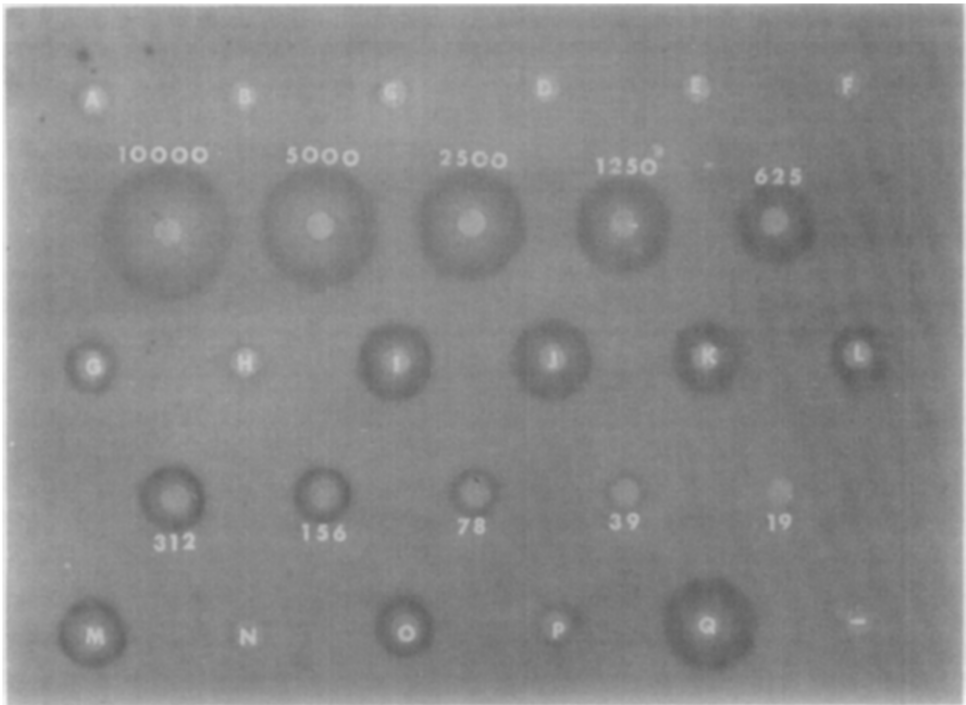


FIG. 1. Photograph of radioautograph of plate containing purified anti-IgE (Fc) goat serum incorporated in agarose. Wells in second and fourth rows from top filled with known amounts of IgE in nanograms per milliliter. Wells labeled **A** through **Q** were filled with randomly selected sera.

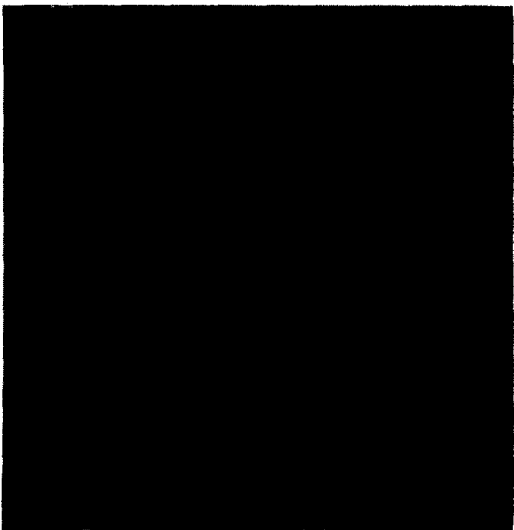


FIG. 2. Standard curve comparing amount of IgE (nanograms per milliliter) against square of ring diameters. This curve is useful only with purified G_1 fraction of anti-IgE (Fc) fragment of goat serum. A 1:9 dilution, 0.3 ml., in 30 ml. of agarose (a final dilution of 1:900) are employed.

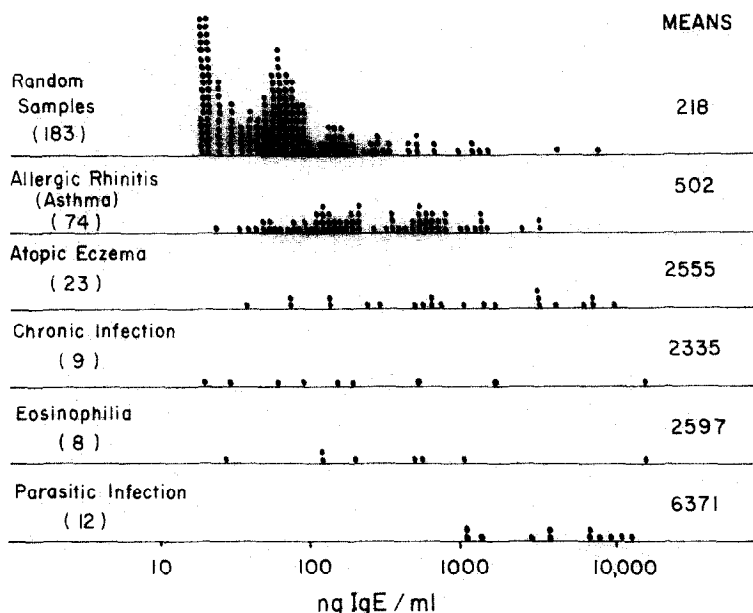


FIG. 3. Distribution of serum IgE levels of random population and of patients with various disease states.

TABLE I. IgE levels

Patient status	Ages (yr.)	No. patients	Range (ng./ml.)	Mean	Median
Random samples	18 - 67	183	< 20 - 8,850	218	60
Allergic rhinitis/asthma	3 $\frac{1}{4}$ - 57	74	40 - 3,525	502	250
Atopic eczema	1 $\frac{1}{4}$ - 51	23	40 - 11,875	2,555	750
Chronic infection	3 - 50	9	< 20 - 18,000	2,335	156
Eosinophilia	24 - 56	8	28 - 18,000	2,594	207
				534	
Parasitic infestations	20 - 58	12	1,150 - 14,300	6,371	7,600

*Six patients were under age 12 years.

†Three patients were under age 12 years.

700 ng. per milliliter. Seven individuals of this group, whose serum IgE levels were higher than 700 ng. per milliliter, were interviewed for evidence of allergy, parasitic infestation, or any other medical abnormalities. Three of these 7 had evidence of allergies, one had cirrhosis of the liver with 8 per cent eosinophilia, another one had carcinoma of the larynx and a pyothorax, and two were apparently healthy individuals. One of these patients was pregnant and the other, a 19-year-old healthy individual, has a brother with bronchial asthma. The IgE levels of these latter two sera were 4,600 ng. per milliliter and 8,850 ng. per milliliter, respectively.

Allergic rhinitis and/or bronchial asthma. The range of IgE levels in the sera from 74 patients with positive skin tests and a definite diagnosis of either allergic rhinitis and/or bronchial asthma (but no atopic dermatitis) was from 40 to 3,525 ng. per milliliter (mean 502 ng. per milliliter). The patients' ages varied between 9 months to 57 years. Forty-two patients (57 per cent) had levels of less than

TABLE II. Miscellaneous group (adults)

Diagnosis	No. patients	IgE levels (ng./ml.)
Drug reactions	10	<20,* 30,* 65,* 115,* 300,* <20, <20, 30, 125, 160
Serum sickness	4	20, 295, 345, 470
Cold urticaria	4	60, 95, 130, 165
Stinging insects	3	50, 335,† 540†
Aspergillosis	2	820, 930
Scleroderma	1	200

*Five suspect ampicillin reactions (rash).

†Also had allergic rhinitis.

300 ng. per milliliter. Six of the 74 patients in this group were under 12 years of age. The serum IgE levels of these children ranged from 40 ng. per milliliter (in a 9-month-old baby) to 2,700 ng. per milliliter (in an 11-year-old child).

Atopic eczema. Twenty-three patients with classical atopic eczema had IgE levels from 40 to 11,875 ng. per milliliter, with a mean of 2,555 ng. per milliliter. Their ages ranged from 3 months to 51 years. Three patients were under 12 years of age. Their serum IgE levels were 140, 685, and 3,450 ng. per milliliter, respectively. Three of the remaining 20 patients had IgE serum levels < 300 ng. per milliliter.

Following the incidental finding that a patient with chronic osteomyelitis and a high eosinophilia had an IgE level of 18,000 ng. per milliliter, we studied patients with chronic infections and/or eosinophilia.

Chronic infection. The IgE values in the sera of 9 adult patients with chronic infections ranged from < 20 to 18,000 ng. per milliliter. The mean value was 2,335 ng. per milliliter.

Eosinophilia. Eight patients with 18 to 80 per cent eosinophilia and no evidence of allergies or parasitic infestations had IgE levels from 28 to 18,000 ng. per milliliter, with a mean value of 2,594 ng. per milliliter.

Parasitic infestations. Sera of 9 patients with ascariasis and one each with ancylostomiasis, strongyloidosis, and trichinosis were studied. The IgE values in these sera ranged from 1,150 to 14,300 ng. per milliliter, with a mean of 6,371 ng. per milliliter.

Miscellaneous allergic conditions. The sera IgE of 24 adult patients with a variety of other allergic reactions was also measured. The diagnosis and IgE values are listed in Table II. It will be noted that 2 patients with bronchopulmonary aspergillosis had IgE levels well above the mean value of the random population. Five of the 10 patients classified under drug reactions had diffuse maculopapular rashes from ampicillin. Although the sera of the 4 patients with serum sickness had positive cutaneous anaphylaxis (PCA) with titers of 80 to 160 in monkey skin, the IgE levels were not markedly elevated. Two of the three patients with documented systemic reactions to insect stings also had allergic rhinitis.

Sera from 19 children, 6 weeks to 2 years of age, with different diseases were examined. Two of 13 patients with bronchiolitis, ages 14 to 20 months, had IgE serum values of 156 ng. per milliliter. All the others were well below this value. The serum IgE levels of the 6 nonbronchiolitis patients were elevated in 3 pa-

tients; one (age 12 months) had frequent upper respiratory infections (210 ng. per milliliter), another (age 5 months) had recurrent bronchitis and pneumococcal infections (1,800 ng. per milliliter), and the third (age 4½ months) had a rash similar to atopic dermatitis (1,950 ng. per milliliter). A patient (age 12 months) with Swiss-type agammaglobulinemia and a normal 6-month-old infant had no detectable IgE (< 20 ng. per milliliter) in their sera.

DISCUSSION

The technique described above is relatively simple, regularly reproducible (variation 4 to 10 per cent), and the results are obtainable in 3 to 4 days. Levels from 20 ng. upward can be detected. Hence, this method would be readily applicable for measurements within the range of 20 to 10,000 ng. per milliliter. Our method has an advantage over the other suggested means of detecting IgE in that it does not require purified IgE and it avoids the additional step needed in the Rowe⁶ technique. The chief disadvantage is that even under special, ideal conditions, with the use of larger wells for the antigen and weaker concentrations of the antisera, the minimal amount that can be detected is 20 ng. per milliliter. When one must measure values below this level (as in secretions), the inhibition methods²⁻⁴ would have to be used.

The mean values that we have found in the sera of a random population and of patients with various disorders are well within the range of those reported in previously published articles.^{2-4, 7-9, 14-17}

The IgE levels are moderately increased in respiratory (atopic) allergies such as allergic rhinitis and/or bronchial asthma and markedly elevated in patients with atopic dermatitis.¹⁷ Four adult patients with severe generalized atopic eczema were treated with intensive corticosteroid therapy systemically and locally. Their skin lesions cleared almost completely, but their serum IgE levels prior to and 4 to 6 weeks after steroid therapy remained unchanged. This would suggest that there is no relationship between the serum IgE levels and the severity of the disease. As was previously reported,¹⁸⁻²⁰ patients with parasitic infestations had extremely high IgE serum levels. Our results confirm these studies.

Although there is a broad range of IgE levels in all these disorders, their mean values are much higher than those in the normal population (Table I). The mean IgE serum levels were highest in the parasite-infested patients among the various groups studied.

It is interesting that a very high IgE serum level was found in one patient with chronic osteomyelitis, but 3 others with the same disease had IgE serum levels in the normal range. One infant with recurrent pneumococcal infections and bronchitis and another (4½ months old) with minimal eczema and a failure to thrive also had high serum IgE levels. As will be noted on the scattergram (Fig. 3), there is a marked spread in values in the categories of chronic infection and high eosinophilia. In view of the small number of sera studied, the mean values may well be misleading.

Infants with bronchiolitis, ranging in age from 6 weeks to 20 months, had IgE levels from < 20 ng. per milliliter to 156 ng. per milliliter. These values

are similar to those reported by Johansson¹⁵ in normal infants and children. It has been previously reported²¹ that a certain percentage of patients with bronchiolitis develop allergic asthma later on in life, and we therefore examined the possibilities that infants might have somewhat higher IgE levels than normal.

Of the group of sera tested from adult patients allergic to drugs such as penicillin, aspirin, and insulin, there were no elevated γ E levels. Those who developed urticaria and systemic reactions and one with a probable penicillin-induced interstitial nephritis had IgE levels within the mean range. Two of three sera from patients with definite systemic reactions to stinging insects had IgE levels above the mean value, but both patients also had allergic rhinitis. Although the sera of 2 patients with bronchopulmonary aspergillosis had elevated IgE levels, they were also atopic individuals and had positive skin tests to many other allergens. Heiner and Rose¹⁹ have reported that patients with this condition have elevated serum IgE. It is interesting that the IgE serum levels of the 4 patients who developed classical serum sickness following antirabies horse serum were not markedly elevated, although each serum had reaginic activity. Likewise, the 4 patients with cold urticaria and reaginic activity in their sera had IgE levels within the range of the mean of the random population. One patient with trichinosis infestation proved by biopsy had a high level of IgE.

Elevated IgE serum levels in conditions other than atopy, such as cirrhosis, myeloma and larvae migrans,¹⁹ and celiac disease²² have been reported. Patients with chronic infections, eosinophilia, and trichinosis may now be added to this list.

Many more sera must be studied with the widest variety of diseases, and then hopefully the role of IgE in man in sickness and health may be clarified. Until such large-scale investigations can be made, one must consider the mere coincidence of elevated IgE levels in various disease states.

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