

Comparison of IgE values as determined by different solid phase radioimmunoassay methods

S. G. O. JOHANSSON,* ASTA BERGLUND† and
N.-I. M. KJELLMAN‡

* *The Blood Centre, University Hospital, and*

† *Research and Development, Pharmacia Diagnostics AB, Uppsala*

‡ *Department of Paediatrics, Linköping University, Linköping*

Summary

Accurate measurement of low IgE concentrations is technically difficult. In this paper results obtained by a direct sandwich and three inhibition methods of radioimmunoassays are compared. For values above 50 U/ml good correlation was obtained with all methods. Below 50 U/ml, however, the inhibition methods tended to yield falsely high values. For very low concentrations, 1-10 U/ml the best correlation was obtained between the direct sandwich test (PRIST*) and the inhibition test using a correction factor to allow for the non-specific effect of serum.

The four methods were used to quantify IgE in cord serum samples from healthy individuals. The mean value obtained by PRIST was 0.4 U/ml and by the inhibition test, using a correction factor, 0.6 U/ml respectively. Because of its greater simplicity the direct sandwich test is recommended.

Introduction

The first report of raised levels of IgE, at that time provisionally designated IgND, in atopic, extrinsic asthma but not in other types of asthma (Johansson, 1967) stimulated an interest in the measurement of serum IgE in immediate hypersensitivity disorders as well as other diseases with immunological engagements. To date well over a hundred papers have confirmed the initial findings and extended them to other atopic diseases, parasitic infections and certain immunodeficiency states (Johansson, Bennich & Berg, 1972).

It was recently found that a raised serum IgE level may be predictive of later onset of extrinsic asthma in children with wheezy bronchitis (Foucard, 1974) and of any type of atopic allergy in infancy (Hamburger *et al.*, 1974). To enable the accurate measurement of the very low IgE levels of early childhood sophisticated technology is necessary. In this paper two solutions are reported to the problems involved in

* Trade mark applied for by Pharmacia Diagnostics AB, Uppsala.

Correspondence: Dr N.-I. M. Kjellman, Department of Paediatrics, Regionsjukhuset, S-581 85 Linköping, Sweden.

quantification of low serum IgE concentrations. The results obtained by two different radioimmunoassays (RIA) in various modifications are compared.

Materials and methods

At the department of paediatrics, Linköping University, Sweden, 228 serum samples were collected from children aged 0 weeks (cord serum) to 15 years (for details regarding age and sex distribution see Kjellman, Johansson & Roth, 1976). Venous blood was collected and serum was separated after 1–2 hr at room temperature and stored at -20°C until analysed. The cord blood was stored in a refrigerator for about 1 day before serum separation and then kept deep frozen until analyses were performed.

IgE determination

The IgE determinations on the serum samples were performed at the Blood Centre, University Hospital, Uppsala, Sweden. The serum IgE concentrations were measured in duplicate in two independent experiments by means of two radioimmunoassay solid phase techniques and the results expressed in U/ml serum (1 U/ml = 1 kU/ml).

Inhibition technique (RIST). Cord blood serum and sera from children less than 1 year of age were diluted 1:5 in buffer before assay. The remaining serum samples were diluted 1:10.

Direct competitive method. The Phadebas® IgE Test kit (Pharmacia, Uppsala) was used for all serum samples collected. This radioimmunoassay method depends on the ability of an antibody to bind its antigen which is labelled with a radioactive isotope and the competitive inhibition of this reaction by the unlabelled antigen. By using antibodies coupled to a solid phase the antibody γ -bound labelled and unlabelled antigen can easily be separated from the unbound antigen.

The assays were set up according to the test procedure recommended by the manufacturer except that two additional IgE standard dilutions were prepared for the standard curve: 0.1 and 0.5 U/ml. IgE values were calculated according to instructions for the test.

Direct competitive method with modified calculations. In order to correct for possible serum interference in the test system where buffer was used as a diluent, the IgE levels were also calculated by means of a correction factor established in the following manner. The IgE standard of the Phadebas IgE kit was prepared in buffer according to the instructions in the manual and in IgE-free serum diluted five and ten times in buffer. The IgE-free serum was prepared by exposing human serum, containing less than 1 U IgE/ml to a temperature of 56°C for 4 hr. The assay was set up according to the recommended test procedure. To detect serum interference more easily, standard curves were constructed by plotting the bound activity as count rates per minute (c.p.m.) against the concentration of the IgE standard. By using IgE standards prepared in IgE-free serum the standard curve was shifted to the left. The results varied when different serum concentrations were used. The standard curve obtained by using IgE-free serum of the same concentration, both as diluent and zero sample, was found superimposable on a standard curve obtained by using buffer as a diluent. Both curves were constructed by plotting IgE concentrations *v.* the count rate for the standards as a percentage of the zero dose bound activity. Consequently, buffer can be used as diluent for the standards if the bound activity of the unknown sample is expressed as a percentage of the bound activity at zero dose in IgE-free serum rather than in buffer, as

used for the standard curve. An investigation was made to find the ratio between the bound activity of zero samples consisting of IgE-free serum and buffer. Human serum samples from twenty patients with IgE values less than 10 U IgE/ml were exposed to 56°C for 4 hr. The twenty 'IgE-free' serum samples were diluted five and ten-fold with buffer and were tested as zero samples in the IgE test. The ratio between the c.p.m. of the zero samples consisting of an IgE-free serum and a buffer respectively was found to be 0.96 at a serum dilution of 1:10 and 0.91 at a serum dilution of 1:5.

The unknown concentrations of IgE in serum samples (diluted 1/5 or 1/10), were read off from the standard curve using the bound activities of the samples expressed as percentage of the corrected zero dose bound activity (i.e. 0.91 or 0.96 times the buffer zero dose bound activity).

Sequential addition method was performed in the following way on all but one of the serum samples collected. The Sephadex-coupled anti-IgE from the Phadebas IgE Test kit was incubated at room temperature overnight with the patient sera. ¹²⁵I-labelled IgE, diluted according to instructions for the kit, was then added. The test tubes were subjected to a second incubation for 3–4 hours after which the tubes were washed five times with saline containing 0.1% Tween 20 before the final centrifugation and counting of the radioactivity bound to the Sephadex-anti-IgE complex was carried out. A standard curve was established in the same way as in the methods described above.

Sandwich technique.

Paper disc RIST (PRIST). The method described by Ceska & Lundkvist (1972) using anti-IgE antibody immobilized on paper discs as the solid phase was applied for total IgE determinations in 226 of the serum samples. The serum samples from children up to 4 years of age were generally tested undiluted while the remaining sera were tested diluted 1:5 in buffer. This system is a direct radioimmunoassay where the IgE to be assayed is first bound to the solid phase coupled antibody and after washing it is incubated with immunosorbent purified antibodies labelled with a radioactive isotope. The assay was performed in polystyrene test tubes. One paper disc to which anti-IgE was coupled was used per test tube. Fifty microlitres of serum or standard was added and incubated for 3 hr at room temperature, and then the discs were washed three times in saline with Tween 20. One hundred microlitres ¹²⁵I-anti-IgE representing approximately 100,000 c.p.m. was added and a second incubation overnight followed. Finally, the tubes were again washed and the radioactivity bound to the solid phase was determined in a gammacounter. Buffer and IgE standard used in this method were the same as that in the Phadebas IgE Test kit. The standard curve was prepared by means of the same standard dilutions as described above.

A certain serum effect was found with PRIST in the determination of IgE in sera diluted less than 1:5. This has to be compensated for by diluting the standards in an IgE-free serum.

Results

Radioimmunoassays based on two different principles have been used in this study. The results of an inhibition test, represented by the radioimmunosorbent test (RIST) and a direct sandwich test, the paper disc RIST (PRIST) have been compared (Fig. 1). The correlation is good for IgE concentrations above 50–60 U/ml but for values below that level there is an increasing tendency for RIST to give higher values than PRIST.

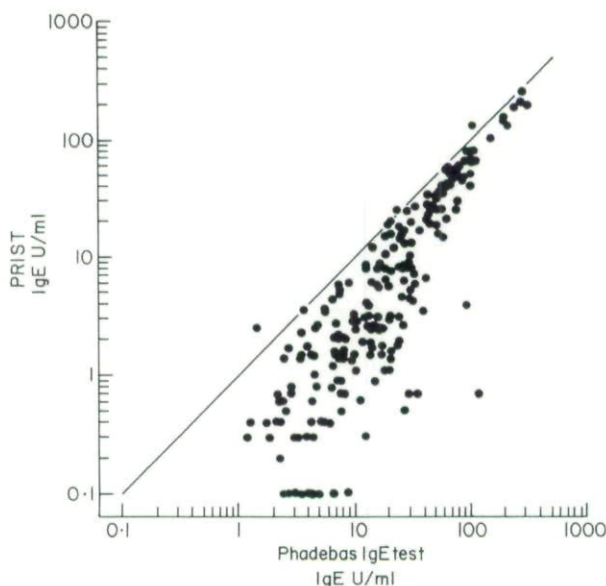


Fig. 1. Correlation between serum IgE values assayed with PRIST and the Phadebas IgE test.

Thus no RIST value less than 1 U/ml was found, but as many as 22% of the PRIST values were that low.

The results of PRIST and the sequential addition modification of RIST were essentially similar in the low level region as were also results of PRIST and the original RIST (Fig. 2). The former good correlation seems to extend down to somewhat lower concentrations than in the case of the original RIST.

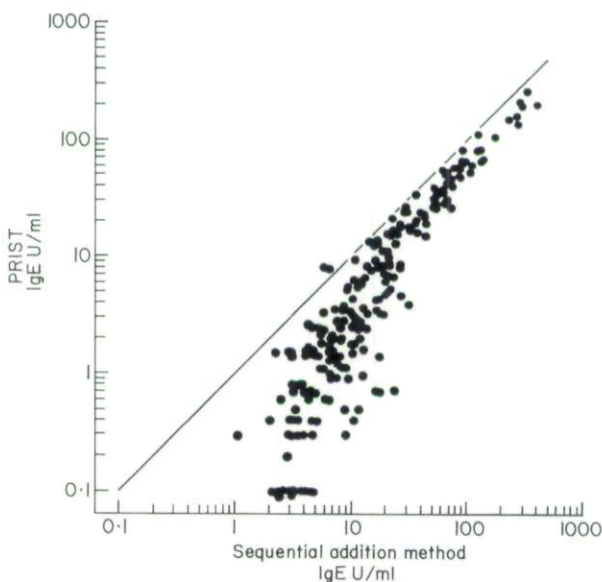


Fig. 2. Correlation between serum IgE values assayed with PRIST and the sequential addition method.

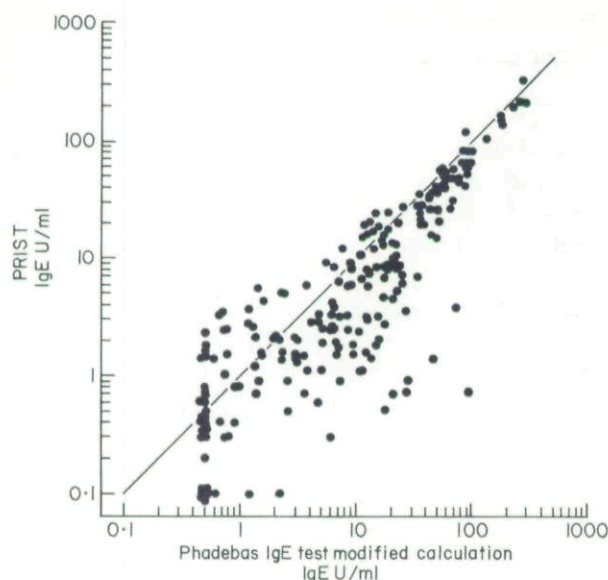


Fig. 3. Correlation between serum IgE values assayed with PRIST and the Phadebas IgE test with modified calculation.

The modified calculation procedure for RIST compensates for the average, non-specific influence of the serum milieu on the test system. When the results are compared with those of PRIST, this correlation seems to be very good for values above 30 U/ml (Fig. 3). Below this level an acceptable correlation is obtained but the scatter is considerable. Approximately the same number of values, 22%, were below 1 U/ml for both methods. However, 5.8% of the samples for which a value below 1 U/ml was obtained in one test were found to have a value higher than 1 U/ml in the other test.

Table 1. Comparisons by means of paired observations using Student's *t*-test of IgE levels in twenty-six cord blood sera obtained with different test methods

Test methods	$\bar{x}$ U/ml	$\bar{y}$ U/ml	p
x = Phadebas IgE Test (modified calculation)	0.64	3.79	<0.001
y = Phadebas IgE Test			
x = Phadebas IgE Test	3.79	0.39	<0.001
y = PRIST			
x = Sequential method	3.11	0.39	<0.001
y = PRIST			
x = Phadebas IgE Test (modified calculation)	0.64	0.39	<0.05
y = PRIST			

The mean IgE values found in cord serum using the four RIA methods (Table 1) illustrate the effect of the serum factor on the results obtained.

Discussion

An inhibition RIA is based on the following principle: the non-labelled antigen (in our case IgE) in the sample or in the reference standard is quantified from its capacity to inhibit the reaction between the isolated, isotope-labelled antigen (IgE) and the specific antibody (anti-IgE). Separation of bound and non-bound tracer (labelled-IgE) can be accomplished by precipitation by a second antibody directed against the Fc part of the anti-IgE followed by washing of the precipitate, the so-called double-antibody technique (Gleich, Averbek & Swedlund, 1971). The separation can also be obtained by binding the first antibody (anti-IgE) to a solid phase, like the inner surface of a disposable polystyrene test tube (Catt & Tregear, 1967) or Sephadex particles (Wide & Porath, 1966). The latter system is usually designed so that the antibody can bind only 10–20% of added, labelled antigen, while for double antibody methods this figure is often considerably higher. This non-saturation of the system is necessary to ensure high sensitivity of an inhibition test. Any substance that interferes non-specifically with the reaction of IgE with anti-IgE will decrease the radioactivity bound to the particles and will thus be interpreted as IgE. The higher protein concentration of the serum sample compared to the standard diluted in buffer exerts such a non-specific effect.

Antibodies to the gamma globulin of the same animal origin as the anti-IgE used can, when bound to the antiserum, sterically interfere with the reaction between IgE and anti-IgE. Such antibodies with specificity for bovine gamma globulin occur in approximately 70% of the population (Foucard *et al.*, 1975). Due to cross reaction with sheep and goat gamma globulin, anti-IgE from these animals cannot be recommended in inhibition types of RIA.

The direct sandwich RIA (Wide, 1971), PRIST, should theoretically suffer from the same non-specific factors as inhibition techniques. The decreased amount of IgE bound to the solid phase anti-IgE which will lead to a decreased amount of radioactivity on the disc will in this case be interpreted as a falsely low IgE value. Experimental studies indicate that the influence of such activity on the IgE values is not so pronounced. However, a certain serum effect cannot be excluded when sera are tested undiluted and the standard is diluted in buffer.

The reason why PRIST is less influenced by non-specific effects than RIST is probably two-fold. The washing step performed before the addition of the labelled reagent will probably eliminate some serum factors. In addition, an excess of anti-IgE is bound to each disc and therefore much more animal gamma globulin is added to each test sample. This will make it less likely for antibodies to bovine gamma globulin to sterically interfere with the binding of IgE to anti-IgE.

The sequential modification of inhibition techniques has been reported to increase the sensitivity of the assay (Wide, 1971). However, in this study advantage was not taken of the somewhat higher sensitivity of the sequence test. The serum samples were tested at the same dilution as in the direct inhibition test leading to comparable serum effect in both methods. This probably explains why the mean IgE value for cord serum was found to be as high as 3.1 U/ml compared to 3.8 U/ml for the direct inhibition test.

It has been suggested that solid phase techniques should be more easily influenced

by non-specific factors than would a double-antibody method. This effect seems to be especially prominent when the solid phase consists of Sepharose or BAC-cellulose (Polmar, Waldmann & Terry, 1972) or of carbon-particles (McLaughlan *et al.*, 1974). Under certain conditions also the double-antibody method seems to give falsely high levels as judged from the mean of 39 ng/ml (approximately 16 U/ml) reported for cord serum by Stevenson *et al.* (1971). Our very first investigations into serum IgE levels in 1967 using RIST also seemed, in retrospect, to yield high values. However, the serum effect on solid phase inhibition techniques is usually of little clinical importance unless low levels are to be studied. In the present study the direct inhibition technique gave a figure for cord serum of 3.8 U/ml which is comparatively low.

When the serum effect was compensated for by the correction factor a significantly lower mean value was obtained and PRIST gave even lower levels. In fact, with PRIST, no IgE (less than 0.1 U/ml) was detected in nine out of twenty-six tested cord serum samples.

The double-antibody method seems sufficiently sensitive to detect IgE at concentrations below 10 U/ml and even below 1 U/ml (Gleich *et al.*, 1971). To reach such a high sensitivity with acceptable precision a prolonged incubation time, up to 96 hr, is necessary and the operation range is narrow (Nye *et al.*, 1975). Even under optimal conditions this method tends to give higher values in the low level region than PRIST (Lundkvist & Merrett, personal communication).

Several approaches have been tried to overcome the difficulties involved when low levels of IgE are to be assayed by inhibition techniques. Bazaral and co-workers suggested that the non-specific effect of each serum sample should be measured and subtracted from the results obtained (Bazaral, Orgel & Hamburger, 1971). However, this is a rather laborious procedure. It is possible to dilute the standard in an IgE-free serum, but large quantities of such sera are very difficult to obtain. The compensating factor suggested here, which takes into account the average serum factor effect of various dilutions of the tested serum samples is an attractive solution. However, the best solution would probably be to use a direct sandwich-type of RIA like the PRIST. This method is also a technically very simple method. A test procedure based on paper discs excludes many steps in laboratory routine. Stoppering of test tubes, rotation or shaking during incubation and centrifugation are not necessary.

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