

Methods of quantifying circulating IgE

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Summary

Four radioimmunoassay techniques, two conventional and two sandwich, have been used to measure circulating IgE levels in 100 sera. The test sera had IgE levels ranging from 1.0 to 20,000 u/ml, and each was measured at five dilutions, ranging from three-fold to 400-fold. The same IgE standards were used throughout, and the optimal range for each assay was determined by assessing data for quality control sera and the WHO standard 69/204. To be of general use in the United Kingdom an IgE test must measure accurately levels as low as 20-30 u IgE/ml. The Phadebas RIST method failed to meet this criterion, and of the remaining tests the double antibody method had the most useful operating range and produced the most reliable results. However, the double antibody method is not available commercially and so, for the majority of laboratories, the Phadebas PRIST technique should be the method chosen.

Introduction

The discovery that immunoglobulin E (IgE) is the long-term sensitizing heat-labile antibody associated with atopic allergy (Ishizaka & Ishizaka, 1967; Stanworth *et al.*, 1967) has resulted in the development of total (Johansson, Bennich & Wide, 1968) and allergen-specific (Wide, Bennich & Johansson, 1967) IgE assays as aids to allergy diagnosis, allergen standardization and research. Circulating levels of IgE are elevated in diseases associated with a deficiency of suppressor T lymphocytes (de Weck, 1977) and IgE is routinely measured in most large pathology departments.

Normal circulating IgE levels in the U.K. were most frequently found to be 21 u/ml in a hospital population (Nye *et al.*, 1975) and 31 u/ml in an epidemiological survey (Burr *et al.*, 1975). These logarithmic mean values correspond to approximately 50 ng/ml (Bazaraal & Hamburger, 1972) and, since marked imprecision will occur with assays operating at their limits of sensitivity (Ekins, 1974), IgE is usually measured either by highly sensitive conventional or by sandwich radioimmunoassay (RIA) techniques. These techniques have been compared using the Phadebas RIST and

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PRIST commercial kits, albeit with discordant results (Johansson, Berglund & Kjellman, 1976; Bruynzeel, Houben & Berrens, 1977), and in addition the kits have been compared with the conventional double antibody RIA (Gleich & Dunnette, 1977).

We report in more detail on the quality control aspects of measuring total IgE levels with the commercial kits and compare their performance with that of the two tests routinely performed in our laboratory—the conventional double antibody technique (DAB) and the sandwich particle (CRIST) method.

Materials and methods

Assay buffer, diluent and double antibody reagents

Buffer. All antisera and ^{125}I -labelled tracers were diluted in 0.05 M phosphate, pH 7.4, containing 0.3% BSA (Armour Pharmaceutical Co. Ltd, Eastbourne, England), 0.5% Tween 20 (Sigma Chemical Co. Ltd, Kingston, England) and 0.5% sodium azide.

Diluent. IgE standards and patients' sera were diluted in a 1:1 mixture of horse serum (Gibco Biocult Ltd, Hounslow, England) and assay buffer.

Double antibody particles. A crude γ -globulin fraction of donkey anti-rabbit IgG (Benenden Chest Hospital, Cranbrook, England) was prepared by dissolving 0.18 g of anhydrous Na_2SO_4 in 1.0 ml of serum and mixing with 1.0 ml of 18% (w/v) Na_2SO_4 for 30 min at 37°C; the precipitate was collected by centrifuging at 2000 g for 10 min, washed twice with 2.0 ml of 18% Na_2SO_4 and then dissolved in 1.0 ml of 0.9% NaCl. 1.0 g of polymaleic anhydride (BDH Ltd, Poole, England) was suspended in 9.0 ml of 0.1 M NaHCO_3 and reacted overnight with 1.0 ml of γ -globulin solution. The immunosorbent was washed with 10 ml of 0.1 M NaHCO_3 , mixed for 3 hr with 10 ml of 1.0 M Tris, pH 8.5, and then washed twice with 10 ml of 0.05 M NaHCO_3 , twice with 10 ml of 0.1 M sodium acetate, pH 4.0, and twice with 10 ml of assay buffer, with continuous mixing for each wash. The final preparation was stored in 100 ml of assay buffer at 4°C, and was sufficient for 2000 assay tubes.

Decanting aid. The stock reagent, as supplied (Pharmacia G.B. Ltd, London, England), was diluted 1:100 in 0.9% saline.

IgE standards and patients' sera

All IgE-containing standards and samples were prepared as 100 μl aliquots, and stored in stoppered 55/12 polystyrene tubes (Walter Sarstedt U.K. Ltd, Leicester) at -20°C until assayed.

IgE standards. The IgE standard supplied with the Phadebas RIST kit was prepared in horse serum diluent to provide standards of 0.5, 2.0, 5.0, 10, 20, 50, 100, 200 and 400 u IgE/ml.

Patients' sera. Sera from 100 adult patients were selected to provide a range of values from 1.0 to 20,000 u IgE/ml. All sera were diluted three, ten, twenty, fifty and 400 times with horse serum diluent. Reference serum 69/204 (NIBSC, Mill Hill, London) was diluted to 300 u IgE/ml in horse serum diluent. This stock was further diluted to provide reference sera with values of 1.0, 3.0, 10, 30 and 100 u IgE/ml. A human serum pool (1400 u IgE/ml) was diluted in a low titre human serum, containing less than 0.5 u IgE/ml, so as to provide quality control sera of 7.0, 35 and 140 u IgE/ml.

Table 1. Radioimmunoassay protocols

Order and volume of additions	Conventional RIA		Sandwich RIA	
	Double antibody	Phadebas RIST	CRIST	Phadebas PRIST
Test sample/standard ¹²⁵ I-labelled IgE	100 μ l 50 μ l (6000 ct/min)	100 μ l 100 μ l (17000 ct/min)	100 μ l —	100 μ l —
Rabbit anti-IgE	100 μ l	1.0 ml (Sephadex particles)	100 μ l (cellulose particles)	1 paper disc (sheep anti-IgE)
First incubation at room temperature	16 hr	16 hr (with vertical rotation)	3 hr	3 hr
Decanting aid	—	—	2.0 ml	—
Washing solid phase anti-IgE	—	—	Centrifuge/decant	Wash three times with 0.9% saline
¹²⁵ I-labelled anti-IgE	—	—	100 μ l (30000 ct/min)	50 μ l (50000 ct/min)
Donkey anti-rabbit IgG	50 μ l (polymaleic anhydride particles)	—	—	—
Second incubation at room temperature	10 min	—	16 hr (6 hr at 37°C)	16 hr (6 hr at 37°C)
Decanting aid	2.0 ml	—	2.0 ml	—
Separation of 'bound' and 'free' fractions	Centrifuge/decant	Centrifuge/washed three times with 0.9% saline	Centrifuge/decant	Wash three times with 0.9% saline
Total incubation time	20 hr	20 hr	20 hr	20 hr

Radioimmunoassays

Double antibody (DAB). The reagents were added to the pre-aliquotted IgE standards and serum samples in the order and volumes summarized in Table 1. The method was similar to that described previously (Merrett & Pantin, 1975), that is, when diluted in buffer one bottle of ^{125}I -labelled IgE from the Phadebas RIST kit was sufficient for 400 assay tubes, and 1.0 ml of rabbit anti-human IgE (Hoechst U.K. Ltd, Hounslow) was sufficient for 250,000 assay tubes. However, the separation of the 'antibody-bound' from 'free' tracer fractions has been made much more convenient because (a) the second incubation with double antibody particles has been reduced from 3 hr at 37°C to 10 min with agitation at room temperature, and (b) the radioactivity associated with the particles is measured after a single centrifugation and decantation step, using 2.0 ml of decanting aid and centrifuging for 2 min at 1500 g before decanting the supernatant solution.

Phadebas radioimmunosorbent test (RIST). This test was performed as recommended by the manufacturer (Pharmacia Diagnostics AB, Uppsala, Sweden), except that the IgE standards were prepared in horse serum diluent, as described above. This modification allowed the same standards to be used for all assay methods and, incidentally, obviated the need to multiply answers by the correction factors suggested for all sera diluted ten-fold or less.

Cellulose particle radioimmunosorbent test (CRIST). This test was carried out as previously described (Merrett & Merrett, 1978) and is identical in principle to the Phadebas PRIST, except that rabbit anti-IgE (Hoechst U.K. Ltd, Hounslow) was linked to cellulose particles (Wide, 1969) and the first incubation ended by a centrifugation/decantation step.

Phadebas paper radioimmunosorbent test (PRIST). This test was performed as recommended by the manufacturer (Pharmacia Diagnostics AB), except that the kit IgE standards were not used. The test differed from CRIST in using sheep anti-IgE attached to the paper discs, which were washed three times with 0.9% saline to end both incubation steps.

Protocols. The volumes and order of adding the reagents in each assay are summarized in Table 1. Each test serum was assayed in duplicate at five different dilutions, in order to be sure of interpolating the IgE concentrations from a reliable part of the standard calibration curve, and as a check for parallelism.

Duplicate standard curves were arranged at the beginning and end of each assay; the three dilutions of the quality control serum were introduced after every seventeenth test serum, while the six reference serum (69/204) dilutions were placed after every thirty-fourth test serum.

Standard curve control points. Three sets of duplicate tubes preceded each assay to provide information about the total radioactivity added to each tube, and the maximum and minimum radioactivity that could be associated with the bound fractions.

Total counts were determined by adding tracer to one set of tubes, thus ^{125}I -labelled IgE was added in the conventional methods, and ^{125}I -labelled anti-IgE in the sandwich methods.

Maximum binding tubes were similar to other standard assay tubes, except that in the conventional methods 100 μl of horse serum diluent replaced the standard IgE sample, while in the sandwich methods a test sample containing 800 u IgE/ml was substituted.

Minimum (non-specific) binding tubes in the conventional methods were similar to

maximum binding tubes, except that assay buffer replaced rabbit anti-IgE (Hoechst U.K. Ltd) in the DAB test, and 1.0 ml of Sephadex-linked anti-T3 (Phadebas T3 RIA Kit, Pharmacia Diagnostics AB) replaced the Sephadex-linked anti-IgE in the Phadebas RIST technique. In the sandwich assays, minimum binding tubes contained horse serum diluent instead of the test sample.

Other standard curve parameters used in this laboratory are defined and illustrated in Fig. 1.

Calculation of results. The residual counts bound in the assay tubes were measured in an LKB Wallac 1270 automatic gamma scintillation counter equipped with a microprocessor for on-line data processing. The amounts of IgE in the test samples were interpolated automatically, using a straight line fit between each quadruplicate set of points on the standard calibration curve.

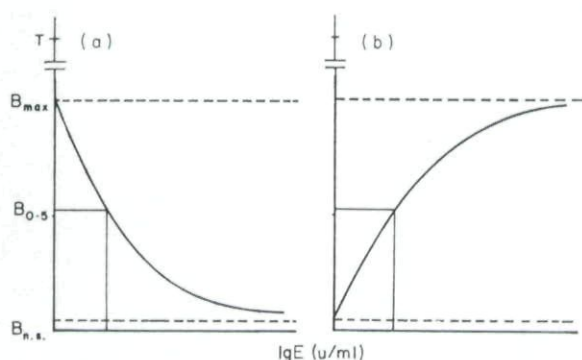


Fig. 1. (a) Conventional RIA; (b) sandwich RIA. Standard curve control points: T = total counts per assay tube; B_{max} = maximum binding of radiolabelled tracer in the assay; $B_{n.s.}$ = minimum (non-specific) binding of tracer in the assay; and $B_{0.5}$, the 50% intercept, yields the standard IgE concentration at half the binding range = $(B_{max} + B_{n.s.})/2$.

The confidence limits of the tests were determined by noting the accuracy with which the reference (69/204) and quality control serum dilutions were measured, and so the recorded IgE levels of the test samples are the average for those dilutions that were within the assay range (usually an average of three dilutions).

Results

The standard calibration curves for each assay are shown in Fig. 2 and the standard curve control points summarized in Table 2. It should be noted that although the double antibody method uses less ^{125}I -labelled IgE than does the RIST, its higher maximum binding results in similar amounts of tracer finally being measured.

The reference serum and the quality control serum dilute parallel to the standard curve in all assays between the 80% and 20% intercepts of the binding range. This range for the DAB assay (2.0–200 u IgE/ml) compares favourably with the more limited ranges of the CRIST (2.0–110 u IgE/ml) and with the PRIST (3.0–40 u IgE/ml) assays, and also with the wider—but less usable—range of the RIST method (from 7.0 to about 400 u IgE/ml). The precision and accuracy with which the IgE values for the reference and quality control sera were obtained, both 'within assay'

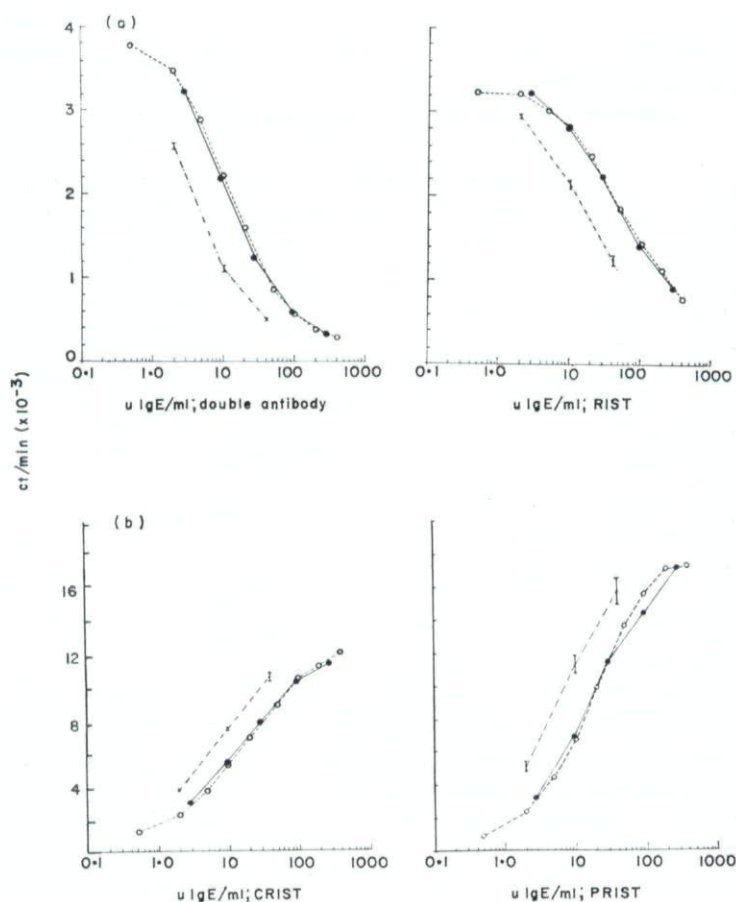


Fig. 2. (a) Conventional RIAs; (b) sandwich RIAs. Comparison of IgE standards ($\circ$), 69/204 reference serum dilutions ($\bullet$) and the quality control pool of human serum (— — —) used in the double antibody, RIST, CRIST and PRIST RIA methods for IgE estimation.

Table 2. Standard curve control points

Control points	Conventional RIA		Sandwich RIA	
	Double antibody	RIST	CRIST	PRIST
Total counts per tube (ct/min)	5800	16500	31500	50000
$B_{\max.}$ (%)	65.9	19.4	34.9	32.8
$B_{n.s.}$ (%)	2.1	1.6	3.1	1.1
50% intercept ($\mu\text{gE/ml}$)	12.5	56.0	17.0	16.0

Table 3. IgE control data: within assay precision

Sera	Dilution	Conventional RIA			Sandwich RIA		
		Double antibody	u IgE/ml $\pm$ c.v. (%)		CRIST	u IgE/ml $\pm$ c.v. (%)	
			RIST	Average u IgE/ml		PRIST	Average u IgE/ml
WHO 69/204 (300 u IgE/ml)	100	3.3 $\pm$ 0.3	1.8* $\pm$ 90.0	330	3.4 $\pm$ 4.0	3.2 $\pm$ 4.4	330
	30	10.7 $\pm$ 0.4	10.7 $\pm$ 11.1	321	10.9 $\pm$ 3.5	10.9 $\pm$ 9.1	327
	10	35.0 $\pm$ 3.1	31.8 $\pm$ 6.3	334	33.8 $\pm$ 6.3	32.8 $\pm$ 16.4	333
	3	101.0 $\pm$ 5.8	111.0 $\pm$ 16.8	318	94.0 $\pm$ 3.9	86.0* $\pm$ 33.3	282
	1	345.0 $\pm$ 5.7	334.0 $\pm$ 3.4	340	245.0* $\pm$ 18.2	159.0* $\pm$ 83.0	—
Human pool (1400 u IgE/ml)	200	7.5 $\pm$ 4.0	6.6 $\pm$ 8.1	1400	6.5 $\pm$ 3.7	6.5 $\pm$ 10.9	1300
	40	39.9 $\pm$ 3.7	35.8 $\pm$ 7.4	1516	34.4 $\pm$ 4.6	31.7 $\pm$ 12.8	1312
	10	130.0 $\pm$ 12.5	162.0 $\pm$ 12.6	1460	115.0* $\pm$ 19.8	124.0* $\pm$ 31.0	1324

c.v. = Coefficient of variation.

*Excluded from average calculation.

Table 4. IgE control data: between assay precision

Sera	Dilution	Double antibody	Conventional RIA		Sandwich RIA		
			u IgE/ml $\pm$ c.v. (%)		u IgE/ml $\pm$ c.v. (%)		Average u IgE/ml
			RIST	Average u IgE/ml	CRIST	PRIST	
WHO 69/204 (300 u IgE/ml)	100	3.3 $\pm$ 1.6	4.0* $\pm$ 5.1	330	3.3 $\pm$ 4.4	3.3 $\pm$ 4.4	330
	30	10.7 $\pm$ 1.9	11.2 $\pm$ 9.9	327	10.4 $\pm$ 6.4	10.9 $\pm$ 8.1	321
	10	34.6 $\pm$ 2.3	33.8 $\pm$ 11.6	342	34.3 $\pm$ 9.1	33.2 $\pm$ 14.2	339
	3	98.0 $\pm$ 5.0	109.0 $\pm$ 13.7	312	94.0 $\pm$ 6.5	87.7* $\pm$ 28.1	282
	1	345.0 $\pm$ 15.7	336.0 $\pm$ 3.7	340	287.0* $\pm$ 28.4	236.0* $\pm$ 44.0	—
Human pool (1400 u IgE/ml)	200	7.4 $\pm$ 6.3	9.3* $\pm$ 34.2	1480	6.4 $\pm$ 4.7	6.4 $\pm$ 10.7	1280
	40	38.3 $\pm$ 6.8	39.1 $\pm$ 13.6	1548	35.8 $\pm$ 1.7	32.9 $\pm$ 14.1	1376
	10	136.0 $\pm$ 10.1	159.0 $\pm$ 9.0	1480	117.0* $\pm$ 20.2	97.0* $\pm$ 42.0	—

c.v. = Coefficient of variation.

*Excluded from average calculation.

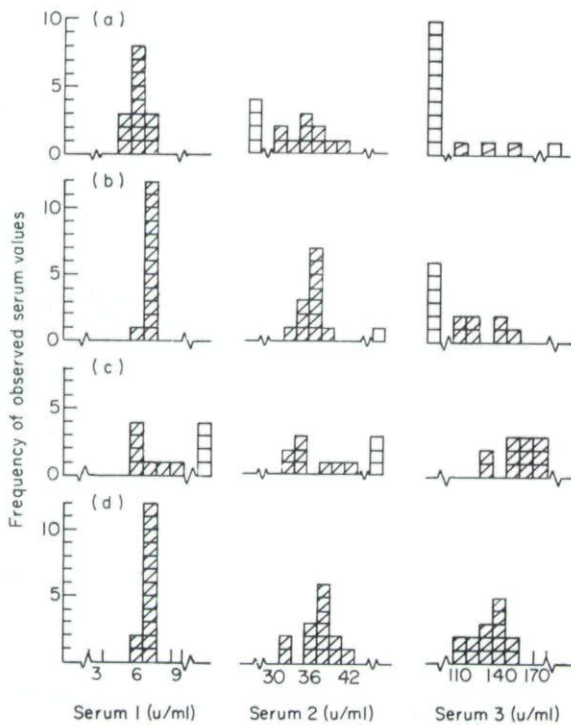


Fig. 3. Quality control data entry sheet (histogram format) comparing the between assay reproducibility of control serum estimations using the various procedures for IgE measurement. Each (■) represents an assay value plotted on-scale; (□) represents a value plotted offscale. (a) PRIST ($n = 14$); (b) CRIST ($n = 13$); (c) RIST ($n = 11$); (d) double antibody ($n = 14$).

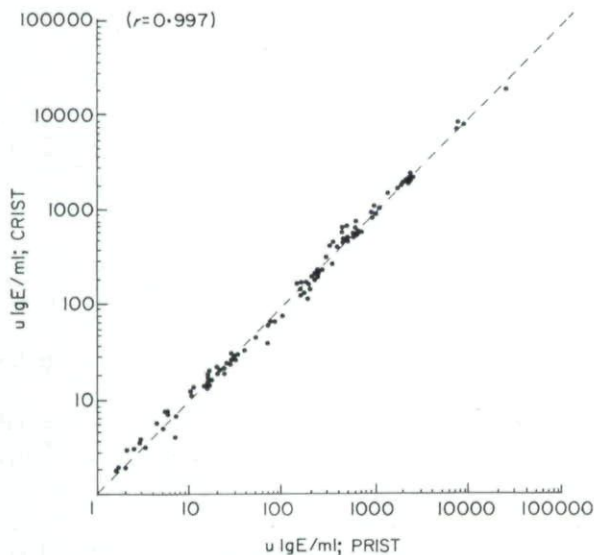


Fig. 4. Comparison of serum IgE levels measured by CRIST and PRIST sandwich RIA.

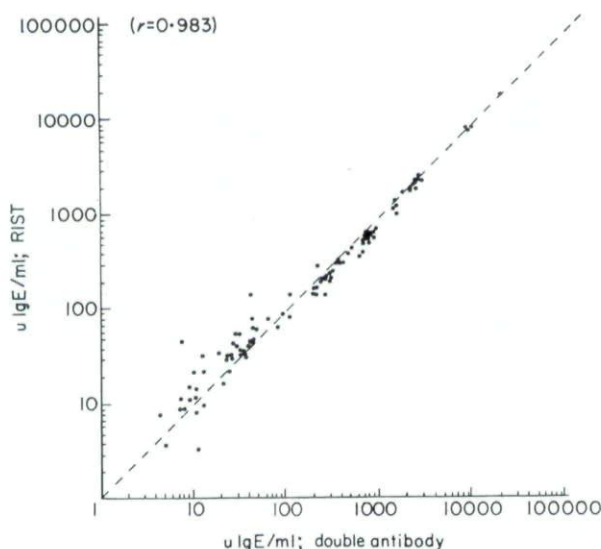


Fig. 5. Comparison of serum IgE levels measured by RIST and double antibody conventional RIA.

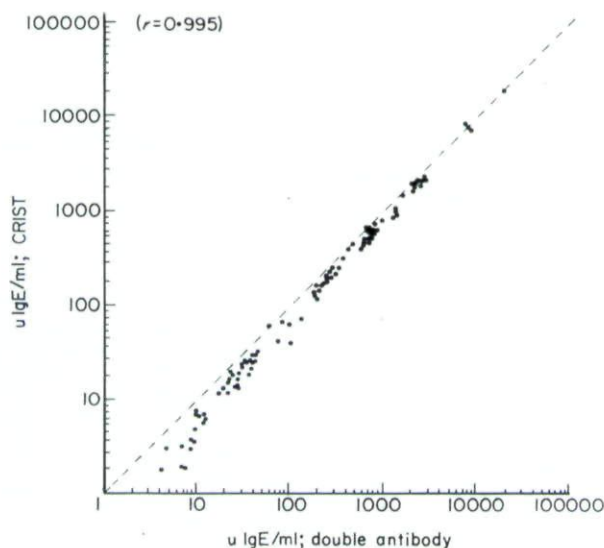


Fig. 6. Comparison of serum IgE levels measured by CRIST and double antibody RIA.

(Table 3) and 'between assay' (Table 4), confirm that the practical assay ranges correspond with the binding ranges of the 80% and 20% intercepts.

The quality control data were plotted on a frequency control chart (Fig. 3), and it can be seen that the best precision and accuracy of the results overall were obtained with the DAB assay, while the PRIST and CRIST performed well at the low IgE dose (serum 1) and the RIST at the high IgE dose (serum 3).

The IgE levels in the patients' sera were calculated using the dilution factors which allowed each serum to be measured accurately, and a high degree of correlation was

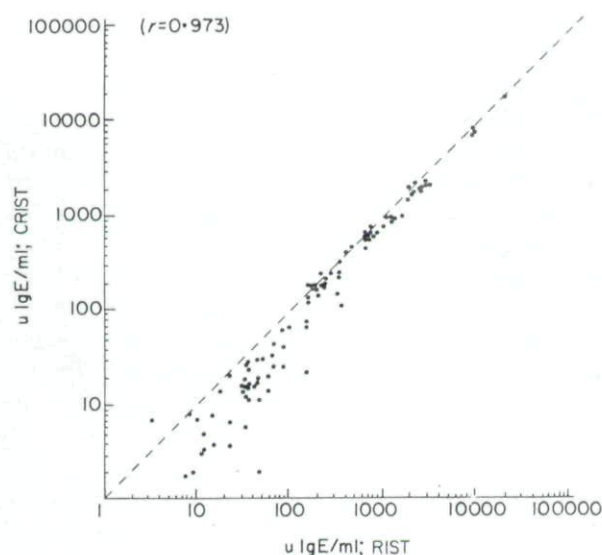


Fig. 7. Comparison of serum IgE levels measured by CRIST and RIST RIA.

Table 5. Precision of IgE measurements, expressed as the average coefficient of variation (c.v., %) for twenty sera (levels ranging from 20 to 100 u IgE/ml) assayed undiluted (1:1) and diluted five and ten-fold (1/5 and 1/10)

Serum dilutions			c.v. (%)	
			Double antibody	CRIST
1/5	1/10		2.9	9.3
1/1	1/5		8.8	13.8
1/1	1/5	1/10	7.3	15.7

found when the results obtained by each method were compared. However, while the sandwich methods yielded almost identical results (Fig. 4), the conventional tests showed an increasing scatter of points below 100 u IgE/ml because, at this level, the RIST generally produced higher values than the DAB method (Fig. 5). The sandwich technique, exemplified by the CRIST, consistently produced lower values than the conventional tests—especially below 10 u IgE/ml (Figs 6 and 7).

Twenty undiluted sera, with IgE levels ranging from 25 to 100 u IgE/ml, were assayed by the conventional double antibody and the sandwich CRIST methods. The results were compared with the same sera diluted five- and ten-fold, and Table 5 shows that the DAB test was less subject to non-specific serum effects than was the CRIST method.

Discussion

In this study we have measured circulating IgE levels ranging from 1.0 to 20,000 u/ml in 100 adults, using methods based on sandwich and conventional RIA principles. The

sandwich technique (Wide *et al.*, 1967) differs from the conventional method (Berson *et al.*, 1956) in being a non-competitive—and therefore a direct—assay. In theory it should be more sensitive than conventional methods, because it is an excess reagent disequilibrium system, i.e. an excess of insolubilized antibodies will bind the test antigen and, after an intermediate washing step, the excess of radiolabelled antibodies added will convert all the test antigen into a directly detectable product. The chance of non-specific serum effects is reduced because the second incubation is performed with the tracer in assay buffer, and so the sandwich system operates against a low background. The excess of reagents in the sandwich technique should also avoid some limitations in accuracy, since the addition of precise volumes of the labelled and unlabelled antibodies is less critical than in the conventional technique. In practice, these theoretical considerations do apply to the performance of the Phadebas PRIST and RIST methods.

Comparison of the Phadebas PRIST and RIST methods

The standard curve for the RIST is shifted more towards higher IgE concentrations and is less steep than the PRIST curve (Fig. 2). The 80% and 20% intercept values showed that the optimal range for the RIST standards is 7.0–400 u IgE/ml, while the PRIST range is 3.0–40 u IgE/ml; since the manufacturer recommends that all test sera are diluted ten times before assay, IgE levels below 70 u/ml are better measured by the PRIST, and levels above 400 u/ml by the RIST. Therefore, the PRIST performance is better suited to screening European (Nye *et al.*, 1975; Johansson *et al.*, 1976) and North American populations (Gleich *et al.*, 1971), whereas the RIST would be selected for those populations in whom high IgE levels are expected due to food allergies (Wraith *et al.*, 1978), aspergillosis (Rosenberg *et al.*, 1977) or to parasitic diseases (Johansson, Mellbin & Vahlquist, 1968; Merrett, Merrett & Cookson, 1976; Gerrard *et al.*, 1976).

The PRIST standard curve was the steepest of all the methods, but the precision was the poorest, although certainly acceptable in the 3.0–40 u IgE/ml range (Tables 3 and 4, and Fig. 3). Ekins (1975) emphasized that assay precision depends on a number of independent factors which affect the slope of a standard curve, e.g. statistical counting errors, pipetting errors and misclassification errors of 'bound' and 'free' fractions in the separation step. An additional factor is the method of calculating results (Jeffcoate & Das, 1977), and so we have preferred the simple 'straight line between points' analysis because no convincing evidence has yet been produced that mathematical transformations can produce good test sample results from bad data (Challand, Spencer & Ratcliffe, 1976). We have noted that the precision is better if each disc is carefully dried after the first washing step, but in a routine IgE assay—which usually comprises more than 300 tubes—this is a tedious procedure and one that should be unnecessary in an excess reagent system ($B_{\max.} = 35\%$, Table 2).

The RIST is also tedious to perform because it is necessary to stopper the assay tubes prior to an overnight vertical rotation step. Furthermore, it produces spuriously high IgE values in agammaglobulinaemic sera (Polmar, Waldmann & Terry, 1973) and in breast milk (Underdown, Knight & Papsin, 1976). Nevertheless, the spuriously high IgE values we have recorded in this RIST study of adult sera probably occurred as a direct result of operating the test at, or beyond, its limits of sensitivity. In an investigation of children's IgE levels, this discrepancy between RIST and PRIST

results was also greater in the low IgE region (Johansson *et al.*, 1976). Thus we cannot agree with the interpretation by Bruynzeel *et al.* (1977) that the nature and concentration of serum-interfering factors may be age-dependent—a suggestion put forward to account for their discrepant RIST and PRIST values being found only in the high IgE region.

We conclude that the Phadebas PRIST operating range is more suited to the population of the United Kingdom than is the RIST, and so it should be preferred for routine estimations of circulating IgE levels.

Comparison of double antibody and CRIST methods

In this comparison the theoretical advantages of the sandwich assay system were not substantiated in practice. The conventional DAB method had a wider operating range (2.0–200 u IgE/ml) than the CRIST (2.0–110 u IgE/ml), and was more precise and accurate (Fig. 3). It was also less subject to non-specific serum effects (Table 5), and so it can be used to measure undiluted serum samples.

The lack of non-specific serum effects in the DAB assay suggests that the scatter of results seen in Fig. 6 is due to the CRIST (and, by implication, to the PRIST) yielding falsely low answers in this region. Careful inspection of Fig. 6 shows that the tendency for the sandwich assay to give lower answers than the double antibody test occurs throughout the assay range. We have no explanation as to why this result is contrary to an earlier report of Gleich & Dunnette (1977), who found an excellent agreement between the DAB and PRIST methods.

The solid phase double antibody technique we have used allows separation of the 'bound' from 'free' moieties in only 10 min, thus the DAB method is simpler to perform than the CRIST because only one washing/decanting step is needed instead of two. The radioactive tracer for the double antibody assay (^{125}I -labelled IgE) costs less than that for the CRIST (^{125}I -labelled anti-IgE) and is sufficient for twice as many assay tubes—however, the counting time is longer (Fig. 2).

We conclude that the double antibody technique is well suited to IgE measurement, and we find the CRIST is more valuable as a method for standardizing mixed allergen particles (Merrett & Merrett, 1978).

PRIST or double antibody?

The PRIST method has a restricted range and so for some purposes, such as monitoring hyposensitization therapy, it would be best to allow at least two different dilutions of the serum sample to be measured. We found the 'within assay' coefficient of variation for the PRIST in our typical 300 tube assays to be 4.4–16.4% in the sample region 32–330 u IgE/ml (Table 3). This figure should be improved in smaller assays, because the time it takes to add 300 anti-IgE discs may adversely affect the precision of the first 3 hr incubation. The manufacturer quotes the coefficient of variation to be 6.6–11.5% in the 20–500 u IgE/ml sample range and, since the assay procedure is very simple, we would expect this to be a typical result—even in relatively inexperienced hands.

The double antibody method produced the most reliable results in this study, and was the cheapest to perform. This result—at least to some extent—is probably due to our extreme familiarity with the method during 9 years, and also to the fact that it is a regularly performed routine research assay. Using the double antibody test requires a knowledge of applying RIA test procedures, and so we would advocate its

use only in laboratories where large numbers of IgE determinations are regularly performed. The Phadebas PRIST method is recommended for all other laboratories.

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