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A comparison of the Phadebas® and Phadezym® IgE paper immunosorbent test kits

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Summary

The Phadebas (isotype label) and Phadezym (enzyme label) paper immunosorbent test kits have been compared. Precision and ease of operation are shown to be comparable for both kits, and the advantages and disadvantages of each are similar. The enzymatic labelled kit seems to provide a suitable alternative for laboratories wishing to measure IgE but who do not have isotope-counting facilities available.

Introduction

The measurement of total serum IgE is now a well-established procedure in the diagnosis of atopic allergy (Wraith *et al.*, 1979). The measurement is used either alone, or along with other procedures such as skin testing or specific allergen determination, to distinguish atopic from non-atopic sensitivity. The test can also be used as a guide to the number of allergens involved in a particular case (Merrett *et al.*, 1980).

The estimation of IgE usually involves a sandwich technique using isotopically labelled antibody, for example, the Phadebas IgE test, PRIST (Paper radioimmunosorbent test). The method involves the reaction of IgE in the patients serum, with immobilized antibody. After suitable time for reaction the immobilized complex is washed to remove the serum, and labelled antibody added. This labelled antibody reacts with the now immobilized IgE during incubation and after washing away excess labelled antibody the amount of IgE initially in the patients serum can be estimated by detection of the label.

Many small laboratories may not possess, or have easily available isotope-counting equipment, and in any case the necessary precautions when using radioactive material may deter some potential users. The introduction of enzymes as labels has reduced the need for isotope labelling. This report is concerned with the comparison of the Phadebas and Phadezyme kits for determination of IgE in serum. Information about precision, correlation, speed and relative merits and disadvantages is presented.

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Materials and method

The two products were used exactly as instructed by the manufacturer, the only variation occurring when the colour development was studied in the enzyme kit and various incubation times were used. For the enzymatic kit a Beckman model 25 spectrophotometer equipped with a flow-through microcuvette (400 μl) and automatic print-out was used to measure the colour produced. A Nuclear Enterprise N.E. 1600 gamma counter was used in the isotope studies. The tubes containing the discs were counted for a period of 100 sec. This gave a total activity (T) count in the range 70 000–80 000. The top standard gave a count in the range 35 000–45 000 counts. The ratio between the counts of the top standard and the counts of the total activity is $> 40\%$ and therefore meets the criteria specified in the instructions for use of the kit.

All comparisons were done on human serum specimens which arrived in the laboratory as part of a routine request, and routine analytical conditions were maintained as far as possible.

Results

The isotope kit has standards in the range 5–1000 u/ml, while the enzyme kit is 5–600 u/ml. For comparative purposes the 1000 standard was used in both methods so that equal ranges of analysis could be compared. Table 1 shows the two series of standards analysed by the enzyme method (see Discussion).

Because of the very wide range over which the kits can be used precision studies were difficult and for this reason two different ranges were considered. These are 0–150 and 150–> 1000. Values over 1000 were recorded as 1000 and values < 5 were recorded as 5. Since the isotope kit was in routine use the between batch precision was on record from controls analysed each batch, while within-batch precision was checked by duplicate analysis. The same specimens were used for duplicate analysis to check

Table 1. Colour development of the enzymatic kit

	Incubation period			
Standards (ku/l)	15 min	30 min	45 min	60 min
Phadezym				
5	0·030	0·050	0·054	0·065
15	0·041	0·063	0·084	0·103
40	0·064	0·106	0·145	0·183
100	0·125	0·220	0·299	0·380
250	0·222	0·397	0·559	0·730
600	0·336	0·578	0·857	1·079
Phadebas				
5	0·037	0·051	0·067	0·081
10	0·039	0·056	0·076	0·095
25	0·049	0·081	0·108	0·138
75	0·096	0·166	0·230	0·296
200	0·191	0·342	0·476	0·606
500	0·311	0·555	0·766	1·005
1000	0·349	0·679	0·938	1·201

within-batch precision of the enzyme kit, and between-batch for this method was studied using two operators analysing the same specimens in different batches.

Standard deviations of duplicates were calculated using the formula s.d. $= \sqrt{(\Sigma d.^2/2n)}$ where d = the difference between duplicates and n = number of specimens.

Within-batch precision

These results are shown in Table 2. The F values showed that the within-batch precision between Phadebas and Phadezym in the two different ranges was not significantly different.

Table 2. Within-batch precision of the two kits studied

	Range	n (pairs)	Mean	CV (%)	F
Phadebas	0–150	24	35	8·6	1·25
Phadezym	0–150	23	39	9·7	
Phadebas	150–>1000	19	466	7·3	1·50
Phadezym	150–>1000	20	454	5	

Between-batch precision

As the Phadebas kit was in routine use, between batch statistics were available from a precision control run in each batch. This control gave a between-batch CV of 6·7% at a mean value of 360 ($n = 17$). For the Phadezym method twenty-two pairs of sera were analysed by two operators in two different batches. This gave a between-batch CV of 13·7% at 360 ($n = 6$ pairs) and 16% at 69 ($n = 16$ pairs) (see Discussion).

Correlation between kits

Correlation between the two methods (Fig. 1) gave an r value of 0·98 and a linear regression line of y(isotope $= -8{\cdot}62 + 1{\cdot}14\ x$(enzyme). These figures were calculated using the average of pairs of analysis and values above 1000 or below 5 were not included. A paired t-test on the specimens analysed by both methods gave a t value of 1·83 for 34 degrees of freedom. This is not a significant difference ($P = > 0{\cdot}05$).

Precision of isotope counting and absorbance readings

This was confirmed by counting one set of analyses twice for the isotopic method and measuring the absorbance twice for the enzymatic method. The correlations of variance were calculated from the duplicates and these were 0·8% and 1·4% for the counter and the spectrophotometer respectively.

Colour development in the Phadezym kit

The colour development in the enzymatic kit was studied at the same time as the standards supplied for the Phadezyme and Phadebas kits were compared. This involved studying colour development at 15, 30, 45 and 60 min, (60 min being the time recommended in the kit instructions). The result of this study is shown in Table 2.

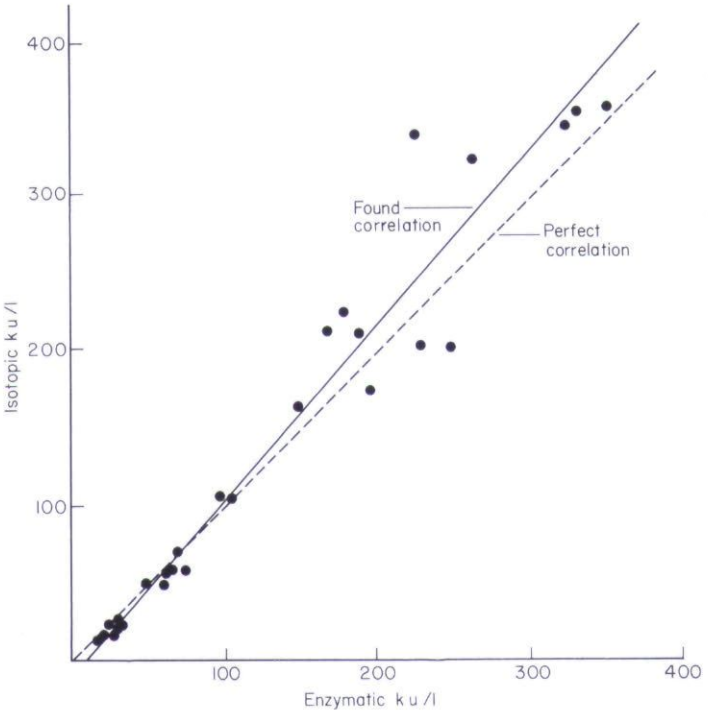

Figure 1. Correlation between the two kits

Discussion

Both kits performed well and as can be seen from the results there is little to choose in terms of within-batch precision. Between-batch precision is difficult to assess. The small numbers of comparisons undertaken, and the fact that duplicate analyses were done for the Phadezym method whilst replicate analyses were available for the Phadebas kit, makes comparison difficult. There is little reason to think that the enzyme kit would differ from the isotope kit on between-batch precision if replicate analyses were undertaken for both methods.

The standards supplied with the kits may have differed slightly particularly at the lower end, where the Phadezym standards were low compared to the Phadebas standards. This is also shown by the regression line which shows, that at the bottom of the range (about 20), the isotope method gave lower values for patients sera than the enzyme method. A better correlation line may have been obtained if the same standards had been used for each method.

A difficulty arising with both methods is the manual drawing of the standard curve. This may well be improved if a microprocessor is used, since there would be less chance of operator bias. Small changes in the position of the standard curve can cause quite large variation in the IgE value particularly at the top end of the range.

Because of the extra pipettings involved in the enzymatic kit and the extra incubation, this method takes approximately 2 hr longer per batch than the isotope kit. Also the extra pipettings and poorer CV of absorbance readings compared to isotope

counting could make the kit less precise. In fact in our hands precision (in the ranges studied) was not significantly different from the isotope kit.

Although the isotope kit is slightly faster and has less pipetting steps, it does involve the use of potentially dangerous radioactive material, and requires isotope-counting facilities. Whilst most laboratories may have a suitable spectrophotometer, many may not possess a gamma-counter. Consequently the Phadezym kit could be used in most laboratories without capital expenditure. Since a Phadezym RAST method is also available, the potential exists to provide an allergy service without use of radio-isotopes.

In our opinion the enzymatic kit provides a satisfactory alternative to the isotope-labelled method, and will give information of equal accuracy and usefulness.

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